Category: Biological Sciences

Title: BTK inhibitor compound X reprograms myeloid derived suppressor cells into dendritic cells

Student Presenter: Pablo Alarcon

Faculty Advisor: Satoskar, Abhay

Abstract: It is widely accepted that the immune system has a role in controlling and eliminating cancers. The tumor microenvironment, however, can suppress the immune response. Myeloid-Derived Suppressor Cells (MDSC) are immature immune myeloid cells that can subdue the development of important tumoricidal immune cell populations that are present in tumors. The accumulation of these MDSCs has been linked with poor prognosis in humans and tumor models. Compound X is currently used to treat B-cell malignancies, as it inhibits Bruton’s Tyrosine Kinase (BTK), an enzyme necessary for B-cell development. However, X has also been reported to affect other populations, including dendritic cells, a group that activates tumoricidal cells. The literature suggests that X accelerates the rate of maturation in these cells. It has also been observed that treating mice with X decreases the MDSC population, however, the fate of these cells remains unclear. In this study, we aim to address this issue and hypothesize that inhibiting BTK reprograms immature MDSCs into dendritic cells. Bone marrow progenitor cells were cultured and differentiated into MDSCs using growth factors and tumor supernatants. Experimental groups were then treated with X on either day three or days one and three. On day five, samples were collected for cytokine release, gene/protein expression, and phenotype evaluation via ELISA, RTPCR, western blot, and flow cytometry assays. Samples were also co-cultured with splenocytes to measure T-cell proliferation. Our results thus far suggest that X-treated MDSCs acquired a phenotype similar to fully differentiated dendritic cells, consistent with our hypothesis. Understanding X’s effects on non-cancer cells is crucial to being able to effectively implement the drug in patients. Our next objective is to identify the signaling pathway following BTK inhibition that leads to dendritic cell development, giving us a better understanding of these mechanisms that have significant impacts on a patient's prognosis.
Category: Biological Sciences

Title: Measuring membrane repair provides a road map for therapeutic strategy in heart failure

Student Presenter: Kenan Alzouhayli

Faculty Advisor: Weisleder, Noah

Abstract: Plasma membrane repair is a highly conserved process that operates in nearly all eukaryotic cell types to restore membrane integrity after injury of the plasma membrane. Repair of the membrane is essential to restore cellular homeostasis, and compromised membrane repair can contribute to a number of disease states, including heart failure, muscular dystrophy and other neuromuscular diseases. Previous studies indicate that compromised repair capacity can exacerbate cardiac injury while enhancing membrane repair capacity can reduce cardiac pathology. In this project, we are standardizing the methodology employed for the direct measurement of the repair capacity by cardiac tissue. Thick slices of myocardium from explanted human and mouse hearts were probed using multi-photon microscopy to determine the membrane repair capacity. In these experiments we use a tissue slicer to cut thick sections (~1 mm) from an ex vivo heart while in a physiologic buffer that allows for resealing of injured cells. These tissue sections are damaged using infrared multi-photon confocal microscopy and then FM4-64 dye entry is recorded over a defined time course to measure membrane resealing. FM4-64 dye only fluoresces once it enters the cell making it a specific readout. When the membrane reseals, dye entry ceases and the fluorescent signal stabilizes. Currently, we are refining the data acquisition and processing for high throughput assay protocol.
Title: A forward genetic screen in Arabidopsis identifies several new genes involved in formation of distinct cellular domains on pollen surface

Student Presenter: Prativa Amom

Faculty Advisor: Dobritsa, Anna

Abstract: Deposition of extracellular materials next to a cell can protect this cell, change its growth and morphology, or help it to move and communicate with other cells. To perform such important functions, extracellular materials must be deposited very precisely, but how such precision is achieved is mostly unknown. A beautiful example of an extracellular structure with very precise deposition is the wall surrounding pollen grains. Pollen from different species often look remarkably different - in part, because wall materials are deposited at some regions on pollen surface and absent from the others. The regions where the wall is absent are called apertures, and these structures help pollen perform its reproductive function. Across species, pollen apertures often differ in shape, number, and positions. Within a species, aperture patterns are usually the same suggesting that developing pollen consistently marks specific surface regions as different from the rest of the surface, ensuring that only they will develop into apertures. Previously, only one gene, INP1, was known to influence pollen aperture formation. In order to identify other players involved in this process, we performed a mutagenesis screen in Arabidopsis. Aperture mutants forming five new complementation groups were isolated by microscopy. We then used positional cloning to identify genes affected in four of these mutants. To do this, mutants in the Landsberg erecta background were crossed with wild-type plants from another Arabidopsis accession, Columbia, and the genes were mapped using PCR markers that could distinguish between Landsberg and Columbia genomic sequences. Four genes potentially involved in pollen aperture formation were identified: MACAROON (encoding an ELMO/CED-12 domain protein), SRF2 (a receptor-like kinase), and two novel genes, INP2 and DONUT. Further characterization of these genes can provide important clues for understanding the molecular mechanism of pollen aperture development and generation of distinct cellular and extracellular domains.
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 from Dipteran insects: Lachesin (Lac) and Amalgam (Ama). Ama arose as a duplication of Lac in early Dipteran evolution, and both proteins still share extensive amino acid similarity. Lac, which is membrane-linked, homophilically binds itself. Ama, secreted into the extracellular matrix, has both a homophilic binding property as well as the ability to heterophilically bind 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 an interaction with Nrt. Therefore, I am identifying 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) cells. The S2 cells are then to be used for aggregation assays, which allow us to observe the binding properties of the chimeric proteins. In order to test for the S2 cell expression of these proteins, I am adding epitope tags to each chimera, making them detectable in a Western blot. Additionally, I am cloning the Ama and Lac orthologues from other Dipteran species to better understand how the unique binding properties of these two proteins have evolved. This project will help to further characterize the complex series of intercellular interactions during nervous system development.
Abstract: Toxin-producing Escherichia coli (Shiga Toxin-producing, STEC; Enterotoxigenic, ETEC) causes severe dysentery and gastrointestinal disease in children, the elderly, and immunocompromised people. Antibiotic treatment places cellular stress on the pathogen, which can result in toxin release. Phage therapy, the use of specific viruses to eradicate a bacterial infection, is an alternative solution to antibiotics. Each bacteriophage (phage) has evolved to attach to a specific bacterial cell. The phages then replicate interiorly, eventually bursting the cell and releasing progeny phages. To investigate potential candidates for phage therapy, our study characterized phage-host interactions of four phages that infect STEC and one phage that infects ETEC. Properties of the phage-host interactions were determined via i) genomic analysis to identify various gene functions and their locations in the phage genomes; ii) adsorption kinetics to determine attachment efficiency of phages to their host; iii) one-step growth curves to measure the latent period and burst size of the phage infection; and iv) plaque morphology comparisons. One STEC phage’s gene functions were annotated as follows: 36% host takeover; 11% DNA replication and repair; 17% structural proteins; and 2% DNA packaging and cell lysis. The five phages have similar adsorption rates (29-63%) and latent periods (20-30 minutes). While the STEC phages have low burst sizes (2.15-3.80 virions/infected cell), we anticipate the burst size of the ETEC phage to be larger due to the increased size and reduced turbidity of plaques compared to STEC phages. This indicates that compared to the STEC phages, the ETEC phage is a more competitive candidate for phage therapy. We will continue to characterize its properties, aspiring to engineer an efficient infection to eradicate specific Escherichia coli bacterial pathogens.
Abstract: Everyday, we depend on our auditory system to perceive sound through mechanotransduction within our ears. When sound enters the cochlea, it deflects microscopic hair bundles made of stereocilia located at the top of sensory hair cells, causing protein filaments called tip links to pull open ion channels. Opening of these channels results in hair-cell depolarization that triggers sound perception. The tip link filaments are made of Cadherin 23 (CDH23) and Protocadherin (PCDH15), which are involved in hereditary deafness and interact tip-to-tip to form a molecular handshake essential for hearing. Here I show our work on a deafness related mutation, R113G in PCDH15, that replaces a charged arginine (R) with a glycine (G). The overall aim of the project was to perform protein crystallization on the mutated PCDH15 R113G + CDH23 complex to determine the effects of the mutation on the tip link's functionality. I started by transforming the DNA plasmids of CDH23 WT and PCDH15 R113G to express, purify, and refold the complex. The vapor-diffusion sitting-drop method was used to attempt its crystallization. After 3 weeks, crystals were obtained and "fished" for X-ray exposure. One of them was shot with X-rays to obtain a diffraction data set. Wild-type versions of the PCDH15 and CDH23 tips were used to solve for the structure of the new mutant complex using molecular replacement and the refinement of the structure is currently in progress. Once the complete crystal structure of the complex is obtained, I will use molecular dynamics simulations to characterize the change in interactions and predict the complex's strength. I expect less force should be needed to unbind the mutated protein complex as the interactions are weaker and results should show how the R113G mutation affects the PCDH15 and CDH23 interaction leading to deafness in humans.
Abstract: Iron-sulfur (Fe-S) clusters are essential for cell life and are required in all parts of the cell. In eukaryotes, the export of Fe-S clusters from the mitochondria to other parts of the cell is an important and currently ill-understood process. This project studies the role of Dre2, an Fe-S cluster protein, as a proposed part of the Fe-S cluster assembly machinery that transports Fe-S clusters out of the mitochondria. Dre2 has previously been shown to hold two clusters—a 4Fe-4S cluster and a 2Fe-2S cluster. In this study, UV-Vis and circular dichroism (CD) have shown Dre2 to take up a 4Fe-4S cluster through chemical reconstitution and release Fe-S clusters in glutathione extractions, supporting their potential to transport clusters. Incubation of Dre2 with the scaffold protein Isu was monitored by circular dichroism (CD) and demonstrated to transfer an Fe-S cluster from Isu to Dre2. Incubation of reconstituted Dre2 with Nfu also demonstrated transfer of a second cluster, likely a 2Fe-2S cluster, to Dre2. Dre2 was also reconstituted following incubation with Atm1p, a proposed mitochondrial Fe-S exporter, and a glutathione-coordinated Fe-S cluster. Transfer chemistry was again monitored by CD, showing that Atm1p may facilitate uptake of a second, likely 2Fe-2S, cluster to Dre2. Additional incubations with holo yeast Nfu, Grx3, and Isu are currently under study to elucidate the mechanism of 2Fe-2S and 4Fe-4S cluster transfer to Dre2. This preliminary data suggests Dre2 to play an important role in cellular Fe-S cluster assembly, potentially linking mitochondrial and cytosolic assembly pathways. If this transport role is confirmed, Dre2 could be further studied for links to human disease. Since Dre2 has been implicated in neurodegenerative conditions, there may be a previously unidentified link between cluster transport and diseases such as Alzheimer's and Parkinson's.
Category: Biological Sciences

Title: Defects in tRNA intron turnover create novel small RNAs: possible consequences for cell growth

Student Presenter: Alicia Bao

Faculty Advisor: Hopper, Anita

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 mRNA codons on 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 yeast, these account for 20% of pre-tRNAs. Normally, free introns are barely detectable, indicating that intron degradation occurs efficiently and rapidly. Through an unbiased screen of the entire 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. Rlg1 then ligates the two halves of the mature tRNA. Surprisingly, my studies have showed that there are at least 3 more possible mechanisms by which tRNA introns can be destroyed for the other intron-containing pre-tRNAs, and that particular introns can form circles. The fact that cells have evolved multiple tRNA intron turnover mechanisms strongly suggests that increased intron levels are unhealthy for cells. To test this, we devised a genetic approach to determine the biological consequences of aberrant accumulation of pre-tRNA introns. I introduced a multi-copy plasmid encoding a bacterial ligase, RtcB, into yeast, replacing Rlg1 in tRNA half ligation, but not phosphorylation of the intron 5' termini. As a result, the cells generate large quantities of introns that cannot be degraded, causing growth defects and cellular stress. This raises the question of potential molecular interactions of tRNA introns, as well as whether certain conditions favor cells to accumulate free introns.
Title: Using molecular dynamics simulations to investigate the dynamics of human skeletal troponin C

Student Presenter: Matthew Belardo

Faculty Advisor: Lindert, Steffen

Abstract: The activity of the troponin protein complex, which is composed of three separate subunits (TnC, TnI, and TnT) is an important component of both muscular and skeletal contraction: conformational changes in this complex that occur upon calcium binding cause a shift in the location of tropomyosin, exposing myosin binding sites on the actin filament. The key to this process is the interaction between the troponin I subunit and a hydrophobic patch on troponin C. Experimental data suggests that this "sticky" patch is opened when calcium binds to the two EF-hand domains of troponin C. However, exposure of a large hydrophobic patch is extremely energetically unfavorable. Studies of cardiac troponin C suggested that the opening was not a single event, and that the open conformation was only occasionally reached by TnC without the subsequent binding of TnI, leading to the notion that the opening event is a more dynamics-based process. To investigate this, models of human skeletal troponin C were constructed using Rosetta Comparative Modelling with a number of other species' skeletal troponin as base models. Simulations were carried out in cellular conditions using NAMD, the molecular dynamics simulation program. Preliminary results suggest that, similar to cardiac troponin, calcium-bound skeletal troponin does not remain open, but instead remains in a mostly closed state for the majority of the time, while becoming more dynamic and sampling more varied conformations, but further simulations are still being run to see if this holds for >100ns. This research has the potential to change our understanding of muscle contraction at the cellular level, and has further implications for investigating the energy associated with contraction.
Abstract: Few diseases in public health are as threatening as influenza, a viral disease with a history of crippling global populations due to the virus' ability to mutate and introduce novel pathogenic strains. The CDC reported more than 300,000 hospitalizations in the 2015-2016 influenza season for the United States alone. Vaccines are developed annually against influenza, yet no effective treatment exists for severe cases. Previous findings show an increase of certain microRNAs (miRs) in response to influenza infection. Examining the role of these molecules, specifically miR-155, may reveal critical knowledge on disease development. This small nucleic acid's expression increases consistently over a 6 day course of influenza infection in a murine model (C57BL/6). Therefore, we believe miR-155 plays a key role in the inflammatory response to influenza infection, leading to acute respiratory distress syndrome (ARDS). Alveolar type 2 (ATII) cells are respiratory cells which produce surfactant lipid and proteins in the lung. As a primary target of influenza, these cells express high concentrations of inflammatory markers upon infection, making their isolation essential. Isolated populations are flow-sorted to obtain infected cells, recognizable due the red fluorescence of the PR8/mCherry/NS1 virus. Subsequent real-time PCR allows for quantification of miR-155 and comparison to other experimental parameters. We observed that miR-155 levels are higher in mCherry-positive ATII cells compared to mCherry-negative cells from the same lung. Target genes of miR-155, including antiviral factors such as IFNγ receptor and NF-kB regulators, were shown to differ in expression between virally infected and uninfected ATII cells within the same lung. The information drawn from this project may lead to investigating new targets for therapeutic agents which enhance the host's immune capability to fight off newly emerging strains of influenza.
Abstract: \(\delta\beta\alpha\)–thalassemia is an inherited form of anemia that results from insufficient expression of \(\delta\beta\alpha\)–globin. It is caused by a change in reading frame or a mutation that results in the premature termination of the translation of the gene. The presence of the premature termination codon initiates the degradation of \(\delta\beta\alpha\)–globin mRNA through a process known as nonsense mediated decay (NMD). Accumulation of decay intermediates corresponding to the 3’ ends of thalassemic RNAs can be detected in erythroid cells. These 3’ fragments containing either exon III or exons II and III have a structure on the 5’ end seemingly identical to a typical m7GpppX cap. The purpose of this project was to evaluate if these stable mRNA fragments are translated. To test this I used site-directed mutagenesis to introduce multiple copies of the hemagglutinin (HA) tag to the very 3’ end of the wild type and thalassemic \(\delta\beta\alpha\)–globin gene. The genes for both wild type and thalassemic beta globin containing two HA tags immediately before the normal stop codon have been inserted in the CMV-driven pcDNA3 plasmid and confirmed to contain the tags in-frame by sequencing. The goal is to use the HA tag to demonstrate expression of full-length \(\beta\)–globin protein from the wild-type gene and to determine if shortened forms of this protein are expressed from genes containing a premature termination codon. Expression and evaluation of these plasmids via western blotting is underway. Identification of HA-tagged, shortened beta globin products would show that these stable, capped mRNA fragments are in fact translated into truncated proteins, which have the potential to disrupt the oxygen-binding capacity of the hemoglobin complex and result in the anemic condition of the blood. This finding could lead to new therapies for treating persons afflicted with this form of anemia.
Abstract: Trypanosoma cruzi (T. cruzi), the causative agent of Chagas disease, is a protozoan parasite that infects 6-7 million people worldwide. Parasite cyclophilin 19 (Cyp19), is a member of the family of peptidyl-prolyl isomerases (PPIase), which catalyzes the interconversion of proteins from cis to trans conformation around proline residues. These abundant enzymes are critical to protein trafficking, cellular activation and signaling. Secreted T. cruzi Cyp19 neutralizes the anti-parasitic activity of cationic antimicrobial peptides secreted by the insect vector of T. cruzi, and plays a role in modulating parasite virulence. Our data indicates that Cyp19 is expressed in all life cycle stages of the parasite, suggesting a broader role of this protein in the biology of the parasite. The goal of the present study is to establish the biological role(s) of Cyp19 in the parasite life-cycle, and identify the residues of Cyp19 critical for enzymatic activity aiding in the development of small molecule inhibitors. Comparison of the Cyp19 sequence with cyclophilin A, the closest human homolog, highlights a unique 10 residue N-terminal domain (NTD) which we hypothesize is important for Cyp19 regulation/secretion. Both wild-type and an NTD deletion mutant of Cyp19 have similar levels of PPIase activity which is disrupted through using cyclosporine A and MM284 inhibitors. Analysis of small peptide inhibitors, based on the NTD sequence, identify promising potent inhibitors of Cyp19 and human CypA. We have also identified several point and deletion mutations which are critical to Cyp19 activity. Furthermore, we have constructed Cyp19 knock-out (KO) parasites and compared the growth rate between KO and WT parasites. KO parasites grow much slower than the wild-type indicating Cyp19's role in the viability of T. cruzi. These results provide a platform for additional studies into the role of Cyp19 function within parasite biology and the development of small molecule inhibitors.
Category: Biological Sciences

Title: Plasmid addiction system in hydrogen bacteria for sustainable production of biofuels/chemicals from carbon dioxide (CO2)

Student Presenter: Tejaswanni Bolineni

Faculty Advisor: Tabita, F. Robert

Abstract: The Plasmid Addiction System (PAS) may be used to obtain desired gene expression without the need for co-inducers or antibiotics to maintain plasmid stability. Experiments have previously demonstrated the utility of PAS for biofuel production using Esherichia coli, but this technology has not yet been employed for the industrially significant organism Ralstonia eutropha H16. The benefit of using R. eutropha is its ability to utilize gases such as CO2, H2 and O2 to support chemoautotrophic (CA) growth. The organism employs the Calvin-Benson-Bassham (CBB) cycle to convert CO2 to all the building blocks necessary for life, which can be advantageous for removing this greenhouse gas from the environment. Our experiments will use the strain H16^LS::mpspec. The genes encoding the subunits of RubisCO, the key enzyme for CO2 fixation, have been deleted in this strain. Without RubisCO, the cells cannot grow on CO2; however, the RubisCO genes may be incorporated into a plasmid for gene expression, with the result that CO2-dependent growth is restored. In addition, experiments were performed to determine whether R. eutropha PAS can express non-essential genes in addition to the RubisCO genes. Plasmid p90:LS contains the cbb promoter and RubisCO genes from R. eutropha. p90:LS and p90:LS,lacZ were cloned and conjugated into H16^LS::mpspec. These cells were plated onto bromochloroindoxyl galactoside (Xgal) and stored in a chemoautotrophic environment with 5% CO2. The cells retaining the non-essential lacZ gene turned blue in the presence of Xgal. LacZ assays showed enzymatic activity in R. eutropha. Since PAS works with lacZ, a heterologous two-gene ethanol PAS was tested in R. eutropha. Cultures demonstrated activity and initial ethanol titers of 2.0 mg/L. Experiments are currently underway to construct a longer five-gene heterologous butanol PAS. Making these alcohols in a sustainable manner could change how fuels and chemicals are made in many industries.
Abstract: One of the important aspects of modern ecological research is determining the effects of global warming on ecosystems. Specifically, this includes understanding the complex relationships among intrinsic environmental factors, trophic relationships, and the biodiversity and abundance of the species living in the environment. The purpose of this research was to determine levels of periphyton and allochthonous organic matter in relation to physical characteristics (flow velocity, substrate type) of glacial stream microhabitats (riffles, runs, rapids, and pools), and assess how these parameters affect insect community structure during summer and winter seasons in a glacier stream in Yunnan, China. The amount of periphyton and organic carbon present is hypothesized to have a positive relationship with the abundance of primary feeders and shredder functional feeder groups, respectively. The abundance of primary feeders and shredders will positively affect the abundance of predators. Periphyton will be sorted from microhabitat samples and volume estimated by water displacement. Organic carbon was measured using ash free dry mass. Taxonomy and macroinvertebrate abundance data was provided for the analysis. It is predicted that the macroinvertebrate biodiversity and abundance will be higher in surber samples where periphyton and organic carbon levels are high. Understanding baseline trophic structure is paramount to determining stream health as it is impacted by climate change. Even though macroinvertebrate diversity is a common bioindicator of stream health, this project aims to contribute further understanding to the structure of macroinvertebrate communities as related to climate change in glacier streams, which are critical but lesser explored ecosystems.
Category: Biological Sciences

Title: Evaluation of human NKG2D signaling pathway functionality in mice to create human NKG2D KI mouse model

Student Presenter: Alexandria Carter

Faculty Advisor: Chan, Wing

Abstract: Novel target development for immune-based cancer therapies is an active area of clinical interest. NKG2D is a natural killer (NK) cell receptor that can be targeted with a bispecific antibody (BiKE) targeting multiple myeloma. In humans, NKG2D (hNKG2D) signals through the adaptor protein DAP10 to initiate cell cytotoxicity and IFN-γ release. We seek to develop an immunocompetent mouse system with hNKG2D knocked-in (KI) to determine the efficacy of human BiKE for the treatment of multiple myeloma. Studies show human NKG2D colocalizes with murine and human DAP10, but pathway functionality is unknown. We provide evidence that despite 76% homology between mouse and human DAP10 and sharing the same PI3K activation domain, hNKG2D cannot initiate downstream signaling with murine DAP10. In mice, DAP10 controls cell cytotoxicity while adaptor protein DAP12 controls IFN-γ release. Murine adaptor protein specificity of mNKG2D has been traced to the transmembrane domain, so chimeric constructs were created to evaluate the importance of the murine transmembrane domain for human NKG2D signaling. Three NKG2D+ cell lines were created by viral transduction of murine NK cells with cDNA from human and murine NKG2D utilizing: 1) hNKG2D DNA, 2) hNKG2D ectodomain with murine NKG2D transmembrane and intracellular domains, and 3) hNKG2D DNA with the murine NKG2D transmembrane domain. NKG2D signaling was evaluated via cytotoxicity and IFN-γ assays. We hypothesize our human NKG2D cell line will initiate cell cytotoxicity while our chimera cell lines will initiate cell cytotoxicity and IFN-γ release. Our results confirmed hNKG2D cannot signal through murine DAP10/12 as there was no IFN-γ release upon anti-human NKG2D activation, and no significant difference in cell cytotoxicity (hNKG2D KI: 11.5% versus negative control: 11.6%). Chimeric cell line testing continues and further research is needed to determine the specific NKG2D signaling pathway defects to test BiKE effectiveness in vivo.
Category: Biological Sciences

Title: Investigating a link between RNA metabolism and endogenous gene silencing

Student Presenter: Alissa Cullen

Faculty Advisor: Slotkin, Keith

Abstract: Transposable elements (TEs) are segments of DNA that are able to duplicate in or move throughout a host genome creating mutations and posing a threat to that organism’s genome. Consequently, organisms have evolved defense mechanisms to silence TEs. Silencing of TE activity occurs post-transcriptionally through TE mRNA degradation and transcriptionally through modification of the TE chromatin. This transcriptional silencing is mediated by small RNA-directed DNA methylation (RdDM), which establishes and maintains the silencing of TEs. Previously, our lab discovered that the post-transcriptional regulation of TEs is connected to the establishment of TE transcriptional silencing through a pathway we named RDR6-RdDM. Understanding how this pathway functions will allow us to target RdDM and silencing to any region of the genome. Two key proteins in this pathway that have been identified are ARGONAUTE6 (AGO6) and RNA-DEPENDENT RNA POLYMERASE 6 (RDR6) and have been the subject of detailed investigation. To develop a further understanding of the precise mechanism and all proteins involved in RDR6-RdDM, first a reverse genetics screen was completed of known AGO6 interacting proteins to identify two new proteins involved in the RDR6-RdDM pathway. Data will be presented on the progress of this reverse genetics screen, which identified two new key proteins involved in distinct aspects of RdDM. Data will also be presented connecting RDR6-RdDM to mRNA quality control. In quality control mRNA decapping mutants, many mRNAs feed into RNAi and we believe RdDM. Data from this project has further characterized proteins involved newly the discovered RDR6-RdDM pathway, and links the TE entry into RDR6-RdDM to mRNA quality control mechanisms.
Category: Biological Sciences

Title: Molecular investigation of honey bee foraging on soybean, Glycine max, in Ohio, USA

Student Presenter: Hailey Curtis

Faculty Advisor: Johnson, Reed

Abstract: We previously surveyed Ohio honey samples and quantified soybean content using traditional microscopic palynology. We found the proportion of soybean pollen to be related to the amount of soybean cultivation surrounding the apiary. However, given the difficulty of identifying pollen to the species level using microscopy, we applied a molecular method to confirm the presence of soybean pollen. We extracted and analyzed pollen DNA from eight honey samples and a positive control sample of soybean (Glycine max) leaf tissue. PCR products amplified with soybean-specific primers and gel electrophoresis was used to confirm the presence of G. max in honey. Our results indicate that soybean pollen is present in some honey samples. However, we detected G. max in the sample from which the most honey, 21 grams, was extracted. Thus, pollen extracted from a larger volume of honey may be more likely to yield G. max DNA.
Abstract: Cardiovascular disease is the leading global cause of death, claiming upwards of 17.3 million lives each year. Vascular-associated diseases such as diabetes and hypertension exhibit defects in blood vessel formation and function. Currently scientists are attempting to define these blood vessel anomalies at the molecular level to identify key mechanisms in disease progression and offer avenues for novel treatment. Blood vessels are comprised of two types of cells, endothelial cells and smooth muscle cells. Proper communication between these cells is vital for ensuring normal vessel formation and function. The Notch signaling pathway has been implicated in this cell-cell communication. The Notch pathway is activated when smooth muscle cells are cultured in the presence of endothelial cells. My preliminary studies demonstrate that the protein Cytoglobin, which is implicated in stress regulation, is upregulated in co-culture conditions as well. I hypothesize that Cytoglobin is regulated by Notch signaling and functions in maintaining vascular homeostasis. To test this, I have shown that blocking the intracellular signaling of the Notch pathway inhibits Cytoglobin expression. Currently, I am testing whether Notch is necessary for Cytoglobin expression by chemically deleting the Notch receptor. Next, I will test whether Notch activity is sufficient for Cytoglobin expression by introducing an activated form of the Notch receptor into smooth muscle cells. I will then test the function of Cytoglobin using proliferation, differentiation, and contractile assays to determine its role in vascular function. Smooth muscle cell phenotypes, such as proliferation, contribute to disease conditions. Therefore, evaluating the function and regulation of Cytoglobin in the context of smooth muscle cell function will contribute to our current understanding of cellular communication in the vasculature and provide new insight into therapies for vascular diseases.
Investigating the functional relationships between three novel microtubule-associated proteins during intracellular transport in Aspergillus nidulans cells.

Student Presenter: Angela Davis

Faculty Advisor: Osmani, Stephen

Abstract: Many cell types, like neurons, exhibit highly polarized growth, generating long cells that require efficient intracellular trafficking systems to transport proteins, mRNA and vesicles over long distances. Transport of many of these cargoes occurs via motor proteins that move along tracks called microtubules (MTs). The cells of filamentous fungi, like Aspergillus nidulans, are also very long and highly polarized and provide a sophisticated experimental system for studying transport within elongated cells. Our lab uses A. nidulans to discover and study the roles of new microtubule-associated proteins (MAPs) in subcellular transport. Previously, we were investigating two novel proteins (MAPA and MAPB) that move bidirectionally on MT's via MT-based motors dynein and kinesin. Using biochemical purifications we identified a third previously unstudied protein (MAPC) that purifies with both MAPA and MAPB. Because the relationships between these three proteins are unknown, the focus of my research is to understand the functional relationships of MAPC with MAPA and MAPB. Using live cell confocal microscopy to follow fluorescently-tagged proteins, I have determined that MAPC locates and travels on MTs and localizes with MAPA and MAPB. We then used a genetic deletion approach to define how those two proteins influence MAPC. We found that in the absence of MAPB, MAPC fails to locate to mobile foci and disperses in the cytoplasm, suggesting that MAPB could act as a linker between MAPC and MT motors. We further found that in the absence of MAPA, a subset of MAPC accumulates at immobile foci, indicating that MAPA plays a role in the mobilization of MAPC onto MT's. My research therefore provides new insight into the potential roles of three previously unstudied mobile proteins.
Abstract: HIV is a deadly virus that is constantly changing and integrating itself into the host genome which can cause lifelong infection as the virus lies in latency. In response to this global health issue, we hope to find new treatments that target a host pathway called de novo fatty acid synthesis. This fatty acid synthesis pathway is typically not turned on in healthy adult cells. However, a recent study obtained results showing a positive correlation between the expression of fatty acid synthase (key enzyme used in fatty acid synthesis) and HIV. Our hypothesis stems from the fact that fatty acid synthase utilizes important molecules in biochemical pathways to make palmitic acid into myristic acid, a fatty acid with a long hydrophobic carbon chain that is used for many biochemical pathways, which can then attach to an HIV protein called gag. It is important to understand that when myristic acid (long non-polar chain) is added to gag (small polar protein), the myristoylated non-polar gag protein relocates itself to the lipid bilayer so that a new HIV molecule can aggregate, assemble, and bud off. The hypothesis is that if fatty acid synthase is inhibited, myristoylation of gag will be prevented and localize it in the cytoplasm. Currently we are testing this by using immunofluorescence microscopy to localize the fluorescence of gag tagged with GFP that is transfected into our MDCK cell line. We are in the process of testing two fatty acid synthase inhibitors on this cell line after transfecting gag GFP and the positive control plasmid gag G2A GFP to observe a difference in fluorescence localization. We expect that gag GFP treated with inhibitors will localize primarily in the cytoplasm, indicating that fatty acid synthase plays a role in HIV replication.
A set of fossils from Pakistan, particularly the 12 million year old Progonomys, have traditionally been interpreted as representing the point in time when laboratory mice (Mus) and lab rats (Rattus) last shared a common ancestor. This interpretation of their evolutionary history has been used extensively among biomedical researchers working with these model organisms. However, subsequent molecular results conflict with the evolutionary tree that forms the foundation for interpreting the fossil record. This in turn has now forced paleontologists to re-evaluate the Siwalik fossils. With this new information, a new Mus-Rattus date needs to be determined for use by biomedical researchers. We analysed a four gene nuclear dataset within the family Muridae and incorporated information from fossils involving several locations and evolutionary events. We conclude with recommendations to researchers looking to calibrate the Mus-Rattus divergence.
Category: Biological Sciences

Title: Quorum sensing autoinducer impact biofilm formation in a hydraulically fractured environment

Student Presenter: Kevin Eberle

Faculty Advisor: Wilkins, Michael

Abstract: The hydraulic fracturing (HF) process for hydrocarbon extraction involves pumping millions of gallons of water mixed with biocides and sand into deep subsurface shale formations. The addition of water increases the pressure in the formation, fracturing the shale and allowing for natural gas extraction. Following HF, the high salinity flow-back fluids (“produced fluids”) are stored in tanks prior to disposal. Issues arising with disposal of these fluids are linked to high concentrations of subsurface radioactive elements, salts, and chemical additives. One method to remove these contaminants utilizes microbial fuel cells (MFCs). MFCs can oxidize organic pollutants under anaerobic conditions using a steel casing as an electron acceptor. Degradation of hydrocarbons requires halotolerant organisms with the ability to form biofilms, enabling the degradation of organic contaminants while generating harvestable power. Through this project, I attempt to chemically induce biofilm formation of known bacterial halophiles isolated from produced fluids obtained from a HF well located in Morgantown, West Virginia. The genera of identified isolates include Halolactobacillus, Orenia, Marinilabilia, Frackibacter, Desulfobrivio, and Halanaerobium. Using a high salt media representative of subsurface fluids, I tested biofilm formation at two cell concentrations: 1.0 x 10^7 and 1.0 x 10^8 with the addition of two different chemical autoinducers: 2-heptyl-3-hydroxy-4-quinolone and N-(3-oxo-dodecanoyl)-homoserine lactone at concentrations of 1 μM and 100 nm. I added these to potentially enhance biofilm formation by acting as quorum sensing autoinducers. Tests were conducted using a 96-well plate biofilm assay coupled with a crystal violet staining method, and revealed that 1 μM and 100 nm concentrations of 2-heptyl-3-hydroxy-4-quinolone had an increase of on the biofilm-forming potential of Orenia, Marinilabilia, and Desulfobrivio strains. Next, I plan to measure the MFC capability of Orenia, Marinilabilia, and Desulfobrivio within the reservoir environment which would alleviate an economic and environmental challenge for the oil and gas industry.
Category: Biological Sciences

Title: Mapping of Plk4 phosphorylation sites in Mps1 potentially involved in the regulation of centrosome and cancer biology

Student Presenter: Alexis Espinal

Faculty Advisor: Fisk, Harold

Abstract: The centrosome is composed of two centrioles surrounded by a proteinaceous pericentriolar material. It is the major microtubule-organizing center, and plays a role in the equal segregation of chromosomes and maintaining proper cell division. Typically, the centrosome is regulated such that it is duplicated only once every cell cycle. However, in some cases, dysfunction of centrosome duplication, such as an overabundance or deficit of chromosomes, can then lead to aneuploidy and genetic instability, which is a hallmark of cancer. Additionally, previous research has demonstrated a positive correlation between the rate and severity of cancer development and centrosome abnormality. Thus, studying the regulation of centrosome duplication allows us to discover potential mechanisms regarding the morphology of cancer and potential for its metastasis, as well as uncover potential drug targets to begin development of cancer-treating therapeutics. Mps1, a protein kinase, is seen in elevated amounts in the event of centrosome over-duplication. This means that proper management of Mps1 ensures a lower potential for chromosomal abnormality. Our studies investigate the prospective regulation of Mps1. Preliminary data suggests that Mps1 is phosphorylated by Plk4, a protein kinase that controls centriole biogenesis to begin centrosome duplication. To further investigate this, we performed site-directed mutagenesis on multiple Plk4 phosphorylation sites in Mps1, mutating them into a non-phosphorylatable Alanine form and a phosphor-mimetic Aspartic Acid form. Successful mutants were verified by sequencing. We validated four mutant constructs via a western blot analysis for protein expression and indirect immunofluorescence microscopy to indicate localization to the centrosome. Thus, our mutant constructs have the ability to serve as a tool to further understanding of how Mps1 is regulated and provide additional insights for centrosome biology.
Abstract: Cutaneous Squamous Cell Carcinoma (cSCC) is the second most common cancer diagnosed in individuals with fair complexions, with around 700,000 cases treated each year. Although most cSCC tumors are superficial and are removed by surgical excisions, about 2-6% of the tumors will metastasize to other regions of the body, leading to a 44% mortality rate in individuals with distant metastatic tumors. A microRNA expression assay was performed in the Toland laboratory on 48 samples including primary and metastatic cSCC to see which microRNAs were differentially expressed between metastatic versus non-metastatic cSCCs. The novel microRNA-4516 (miR-4516) was shown to be downregulated by about 50% in metastatic tumors compared to non-metastatic tumors. Work by others showed that miR-4516 directly targets Signal transducer and activator of transcription 3 (STAT3), a known oncogene. The goal of this study is to determine how differential expression of miR-4516 affects tumor and metastatic phenotypes of cSCCs and the role of STAT3 in this process. Initial studies using quantitative PCR show that a metastatic cSCC cell line, COLO-16 expresses less miR-4516 than non-metastatic cSCC cell lines (SCC13 and SRB-12) by 50%. However, Western analyses on these untreated cell lines do not show differences in STAT3 expression. Ongoing studies will assess the effects of re-expressing or knocking down miR-4516 in cSCC cell lines on invasion, migration, proliferation, apoptosis, and expression of candidate miR-4516 targets. The results of this study could lead to an increased understanding of the metastasis of cSCCs and could also identify potential therapeutic targets in patients, leading to less metastasis and better outcomes in the clinic.
Abstract: The small noncoding RNAs known as microRNAs play a critical role in gene regulation, being implicated in processes ranging from development and cell differentiation to cancer. This project aims to determine the role of the microRNA mir-125a-5p in mouse skeletal development and infertility. We have previously identified a functional role for this miRNA in the regulation of Lfng glycosyltransferase during chick somitogenesis. Somitogenesis is the process by which somites, the precursors of ribs and vertebrae, form. This requires oscillatory expression of Lfng. In chick, somitogenesis and Lfng oscillations require binding between Lfng and mir-125a-5p, but it is unknown if this regulatory interaction is conserved in mammals. To investigate the role of mir-125a-5p in mouse somitogenesis, we generated mutant mice lacking this miRNA. Mutant and wildtype mouse embryos were inspected for differences in Lfng expression, somite formation, and skeletal development. Neither somitogenesis nor skeletal development was altered in mutants. This demonstrates that mir-125a-5p is dispensable for mouse somitogenesis, possibly due to redundancy with similar miRNAs, or because of differences in the regulation of somitogenesis in chick and mouse. Interestingly, homozygous mutant males of one mir-125a-5p-deficient line, called 125a^11, are infertile. I will identify the cause of this infertility, which is likely related to dysfunction of the host gene of mir-125a-5p, Spaca6, a gene required for sperm-egg fusion. mir-125a-5p lies within an intron of Spaca6. 125a^11 likely inhibits mir-125a-5p function by preventing Drosha cleavage of the miRNA. Since Drosha has been shown to affect splicing of miRNA-containing introns, 125a^11 may interfere with splicing of the host gene. Spaca6 is unusually spliced in 125a^11 mutants. This could also be caused by an off site mutation within Spaca6. Current work will distinguish between these possibilities.
Abstract: Tuberculosis (TB), caused by Mycobacterium tuberculosis (M.tb), leads to about 1.5 million deaths annually. M.tb is transmitted through aerosols and infects lung macrophages, altering the host immune response for survival. MicroRNAs (miRs) are small noncoding RNAs that regulate gene expression by binding to the 3'UTR region of mRNA for degradation. We have previously demonstrated that miR-125b expression is increased in macrophages by virulent M.tb but not by the non-virulent M. smegmatis. Increased miR-125b expression decreases host-protective cytokines during M.tb infection in human monocyte-derived macrophages (hMDMs). The major goal herein was to determine the transcription factors mediating miR-125b expression in response to M.tb. In silico promoter analysis revealed that Nrf2, PPAR&gamma;, and STAT-1 are major transcription factors for miR-125b. Thus we hypothesized that M.tb activates several transcription factors to regulate miR-125b expression. Focusing on Nrf2, we incubated hMDMs with a Nrf2 activator (AI-1) and determined miR-125b expression by qRT-PCR. We found that Nrf2 activation enhances miR-125b expression. To determine whether M.tb differentially activates Nrf2 compared to M. smegmatis, hMDMs were infected with M.tb or M. smegmatis and Nrf2 phosphorylation was determined by Western blot. Our results indicate that M.tb infection causes greater Nrf2 phosphorylation than M. smegmatis infection. To confirm the involvement of Nrf2 in miR-125b expression, we transfected macrophages with Nrf2 siRNA or scramble control by nucleofection and then infected with M.tb. Interestingly our results showed that Nrf2 is a contributor to the induction of miR-125b expression, but suggest that Nrf2 deficiency is compensated for by other transcription factors such as PPAR&gamma; and STAT-1. We conclude that M.tb activates Nrf2 which partially regulates miR-125b expression during M.tb infection. Determining the molecular mechanism(s) underlying miR-125b expression, which influences M.tb pathogenesis by regulating host-protective cytokines, will help us to develop new host-directed therapeutic approaches for TB.
Category: Biological Sciences

Title: Identifying novel protein components of the cytoplasmic capping complex using proximity-dependent biotinylation

Student Presenter: Andrew Giltmier

Faculty Advisor: Schoenberg, Daniel

Abstract: mRNAs that appear without a 5' cap in the cytoplasm are readily identified and degraded by cytoplasmic enzymes. However, a smaller set of uncapped mRNAs can be recapped in the cytoplasm, protecting them from degradation by exonucleases, allowing them to return to a translationally active state. The cytoplasmic capping complex consists of cytoplasmic capping enzyme (cCE), RNA-guanine-7methyltransferase (RNMT), and a 5' RNA kinase that assemble on adapter protein Nck1. This complex is the set of proteins responsible for replacing the 5' cap that provides a unique form of post-transcriptional regulation. Although the basic structure of the cytoplasmic capping complex is established, many details remain unknown. Our goal is to further our understanding of the structure of this complex using an in vivo approach for proximity-dependent labeling of proteins called BioID (proximity-dependent biotin identification). Using a promiscuous biotin ligase bound to cCE, we are able to identify known and unknown candidates of the cytoplasmic capping complex by biotin tagging proteins as they interact with the complex. These tagged candidate proteins are isolated via streptavidin affinity chromatography and identified with proteomic mass spectrometry analysis. Not only will these results allow for a more comprehensive explanation of the cytoplasmic capping process, but they also open the door for the further characterization of newly identified candidate proteins.
Abstract: The Patterning Cascade Model (PCM) accounts for the establishment of tooth shape during development. In this model, each tooth cusp forms at a specialized site on the dental epithelium, an enamel knot, which is a gene signaling center that stimulates downward epithelial growth while simultaneously inhibiting other enamel knots from forming nearby. For a new cusp to form, a new enamel knot must form beyond this zone of inhibition. Humans have a typical number of principal cusps (4-5) on their upper and lower first molars, but some individuals also express smaller, accessory cusps, either peripheral or central in position. According to the PCM, small inhibition zones, protracted growth, or both promote the formation of peripheral enamel knots. Accordingly, small intercusp spacing relative to tooth size should be associated with greater peripheral cusp expressions. In contrast, centrally located accessory cusps, according to the PCM, may be associated with larger distance between the principal cusps that surround them, giving them space to form. Previous research has found that the presence of the Carabelli's cusp is associated with greater expression of peripheral accessory cusps. This study analyzes the rest of the individual's dentition to see if accessory cusps are more likely to appear on all first molars if found on any first molar. Data from first molars of 37 individuals from a Seminole dental cast collection were collected by measuring the degree of expression of cusps and intercusp distances. Preliminary analysis suggests that in almost all cases, accessory cusps are more prevalent with smaller intercusp distances relative to tooth size regardless of their position on the tooth. This study gives insight into the developmental mechanisms that underlie cusp formation in humans and other mammals. Understanding these patterns will ultimately refine the understanding of evolutionary patterns in cusp formation and loss.
Category: Biological Sciences

Title: Polymorphic variation alters midline axon guidance in Drosophila melanogaster

Student Presenter: Maya Gosztyla

Faculty Advisor: Seeger, Mark

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 Drosophila melanogaster. However, no studies thus far have attempted to utilize the polymorphic variation that exists in natural populations to study axon guidance. This approach was only recently made possible by the creation of the D. melanogaster Genetic Reference Panel (DGRP), which consists of more than 200 genomically sequenced strains derived from an outbred population. In the present study, embryos from 42 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 identified 11 strains with commissural defects and 11 additional strains with ectopic midline crossovers, with different lines varying in the penetrance and expressivity of the observed phenotypes. This demonstrates that significant 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. Through a series of crosses, we will generate DGRP strains in a background deficient for Abelson (Abl) tyrosine kinase, a known regulator of midline axon guidance. By assessing percent viability at the pupal and adult stages, we will observe whether any strains deviate from expected survival rates, indicating the presence of a polymorphic variant potentially influencing axon guidance through the Abl signaling pathway. In the long-term, this research will provide insight into the complex network of ligands, receptors, and signaling molecules that regulate axon guidance.
Category: Biological Sciences

Title: BRD4 cooperates with TWIST1 to drive oncogenic microRNA-214 expression in cutaneous T-cell lymphoma

Student Presenter: Leah Grinshpun

Faculty Advisor: Mishra, Anjali

Abstract: Cutaneous T-cell lymphoma (CTCL) is a non-Hodgkin lymphoma of skin-homing malignant CD4+ T-cells. Long-term survival in advanced-stage patients is extremely poor, highlighting the need to identify novel therapies to inhibit key oncogenic processes. Previous studies have identified bromodomain-containing protein-4 (BRD4) as a key regulator of several oncogenic expression in CTCL. In this study, we demonstrated that BRD4, in complex with aberrantly expressed TWIST1, upregulates microRNA-214 (miR-214), a key oncomiR which is known to target several tumor-suppressor genes. Patient CD4+ T-cells treated with a bromodomain inhibitor, JQ1, showed significantly decreased expression of miR-214 (p). In order to test these findings in vivo we utilized our IL-15 transgenic mice which develops CTCL by 4 weeks of age. We treated 10 week old mice with JQ1 or vehicle control for 3 days and collected spleen and blood for evaluation of miR-214 and its target genes. Our findings suggest that the BRD4-TWIST1 complex serves as co-activators for transcription of oncogenic miR-214. This study identifies BRD4, TWIST1, and miR-214 as potential novel therapeutic targets in CTCL.
Identification of miR-122 targetome by HITS-CLIP analysis revealed its regulation of WNT/B-catenin activity through BCL9.

Student Presenter: Maria Jose Guerrero

Faculty Advisor: Ghoshal, Kalpana

Abstract: Liver cancer has the highest mortality rate among all malignancies and hepatocellular carcinoma makes up over 90% of all liver neoplasms. The observed poor patient prognosis is due to the inadequate mechanistic understanding of disease initiation and progression. To expand the working knowledge of HCC, we have worked with a liver-specific miRNA 122 (miR-122), that is responsible for the post-transcriptional regulation of gene expression mediated by 3'UTR binding after incorporation into Argonaute (Ago) containing miRNA-induced silencing complex (miRISC). We found that our miR-122 knockout (KO) mice displayed liver pathology such as altered lipid metabolism, inflammation, fibrosis, and spontaneous HCC development that mimics different stages of nonalcoholic fatty liver induced HCC in humans. In addition to this, it has been found that patients with this type of cancer generally have lower levels of miR-122 and have worse overall prognoses. To have a better understanding of the miR-122 function in the liver, we identified miR-122 targets in the whole liver using Argonaute high-throughput sequencing of RNA isolated by UV-crosslinking and immunoprecipitation with an Argonaute antibody (Ago-HiTS-CLIP) in collaboration with the lab of Dr. Robert Darnell in WT and miR-122 KO mice. RNA-seq analysis revealed functional de-repression of both 3'-UTR and Coding DNA Sequence (CDS) targets in KO mice livers. We looked at these targets in human liver cancer patients' data (n = 373) in The Cancer Genome Atlas (TCGA), that revealed alterations in BCL9, a β-catenin co-factor critical for Wnt-signaling. Its dysregulation was found to be associated with poor patient outcome and it negatively correlated with miR-122 expression. We were able to validate BCL9 as a novel, conserved miR-122 target using Dual Luciferase Reporter Assay. Finally, we validated BCL9 CDS as a miR-122 target using wild-type and mutated miR-122 binding sites. Our collective data identified a novel mechanism connecting miR-122 to Wnt/b-Catenin signaling, frequently dysregulated in HCC patients.
Title: Using plumage reflectance to distinguish between Greater and Lesser Prairie-Chickens in an area of sympatry

Student Presenter: Erica Harvey

Faculty Advisor: Augustine, Jacqueline

Abstract: Identification of conspecific individuals is necessary to prevent hybridization in areas where two closely-related species coexist. Greater and Lesser Prairie-Chickens (Tympanuchus cupido and T. pallidicinctus) are two closely-related species, whose ranges have expanded recently, and now overlap. There is behavioral evidence that the two species may be hybridizing. We sought to examine whether feather coloration could be used to differentiate between species, and to estimate the hybridization rate. Our hypothesis was that Greater Prairie-Chickens and Lesser Prairie-Chickens differed in feather coloration and patterning, and that birds of hybrid origin would show intermediate feather characteristics. Spectroscopy readings and feather measurements were taken from both Greater and Lesser Prairie-Chicken reference populations and compared to the birds in the area of overlap to determine species identity. We conducted two discriminant analyses. The first utilized feather measurements such as feather length, subterminal bar length, number of bars, and brightness of the light and dark bars. The second had all of the same measurements except it excluded the brightness of the dark and light bars. Hybrids were defined as individuals that were not assigned to either parental species. The discriminant analysis was able to differentiate between Greater and Lesser Prairie-Chickens with a high degree of certainty. We found a hybridization rate of between 4.1 and 7.3%. The analysis with color was able to determine species identity with higher confidence than the analysis without color. Our hypothesis that Greater Prairie-Chickens and Lesser Prairie-Chickens differ in feather coloration and patterning was supported. This study demonstrates that plumage on the prairie-chickens could potentially be used for species differentiation, and can be used by researchers to identify the species. Although plumage differentiates Greater and Lesser Prairie-Chickens, the hybridization rate suggests that plumage alone is not sufficient to prevent interspecific mating.
Abstract: The morphology and physiology of the gastrointestinal tract becomes increasingly complex the more that it is researched, and model systems are often helpful in understanding the processes that can be confounding in more complex systems, like humans. The adaption to utilize gastrointestinal symbiotic microbes allows animals to extract nutrients that the organism cannot obtain on its own. Cockroaches are used as model systems for studying the gut because of their sustained symbiotic ties to microbes. Previous work examining the effects of bacteria in the development of the cockroach gut has shown that the gut develops differently in germ-free and germ-typical (normal gastrointestinal microflora) cockroaches. Another detail of physiology that has not yet been explored is the effect that normal microflora has on the transit rate of food through the gut as compared to germ-free guts, which is the purpose of this project. Bacteria help break down substrates that are not digestible by the host, allowing the host to obtain a greater amount of nutrients. Other studies show that longer residence time of food in the gut increases nutrient uptake in guts with normal microflora. By feeding cockroaches fluorescent microbeads and dissecting after set time intervals, the transit rate of food through the gut can be compared between germ-typical and germ-free cockroaches. The results show the opposite of the expected results: germ-typical cockroaches showed a significant pattern of slower velocity though the gut at the four-hour mark as compared to their germ-free counterparts. The unexpected results build on our previous understanding of the gut and rise new questions about how the microflora of the GI tract effects gut transit.
Abstract: In recent years, numerous defects in tRNA biogenesis and related processes, including tRNA aminoacylation, have been linked to human disease. As direct mediators of translational fidelity, aminoacyl-tRNA synthetases (aaRS) are responsible for the correct pairing of an amino acid with its cognate tRNA. Mispaired aminoacyl-tRNA (aa-tRNA) species occur due to a lack of amino acid discrimination within the aaRS active site. In Saccharomyces cerevisiae, abolishing the editing activity of misacylated Tyr-tRNAPhe in the phenylalanyl-tRNA synthetase (PheRS) leads to severe growth defects in the presence of high concentrations of the near-cognate amino acid, Tyr. Historically, misincorporation of Tyr into the proteome at Phe codons has been considered a major contributor to an overall decrease in cell viability. Looking at the accumulation of mutations over time through a yeast-based mutation rate analysis is useful for identifying factors that contribute to disease progression. We hypothesized that changes in proteome fidelity caused by aaRS mediated mistranslation lead to dramatic genomic alterations through an increase in mutation rate. A mutation fluctuation assay first described by Luria and Delbruck was utilized, and yeast cells were modified to remove endogenous copies of phenylalanyl-tRNA synthetases. Mutation rate was monitored by measuring cell survival, which reflects the frequency of inactivation of the CAN1 locus. CAN1 is responsible for importing canavanine, a toxic analog of arginine. Data obtained from the mutation rate analysis has provided insight into the direct contribution of aaRS mediated mistranslation to genome stability. Initial experiments suggest that increased error rates in protein synthesis are an important facet of stress response during amino acid limitation. Furthermore, disruption of the proteome has been previously shown to directly cause tumorigenesis and increase susceptibility to pro-tumorigenic stress. Therefore, our results could be further applied to human cell lines using the endogenous yeast enzyme.
Abstract: The &gamma;-crystallins are the major structural proteins in the vertebrate eye lens. To maintain lifelong transparency and high refractive index without protein renewal, the &gamma;-crystallins have evolved to retain high solubility and stability under an extremely crowded environment. It has been suggested that highly concentrated &gamma;-crystallins can form short ordered array in lenses, and molecular dipole has been hypothesized to contribute to the ordered "crystal-like" arrangement. In addition, &gamma;-crystallins have been proposed to possess a thinner hydration shell compared to the average values for water soluble proteins based on analytical ultracentrifugation (AUC) results. In this study, we used both NMR and femtosecond (fs) resolved laser approaches to investigate the protein and water dynamics of the lens protein &gamma;M7-crystallin from zyberfish. H/D exchange experiment by NMR showed that &gamma;M7 exhibits two dynamically different sides, which is also observed in WT &gamma;S-crystallin from mice. We hypothesize that two dynamically different protein sides may be a prevalent property in all &gamma;-crystallins and it may contribute to ordered protein arrangement at high concentration. In order to probe the water dynamics around the surface of &gamma;M7-crystallin, about 20 mutants with a surface residue mutated to Tryptophan (Trp) were identified to have stable emission lifetime for fluorescence measurement. The preliminary fluorescence result from &gamma;M7 Y56W was best fitted to a double exponential decay, suggesting two slow conformational fluctuations with the miss of a very fast decay due to bulk water motion. The data agreed well with the local environment around Y56, which is located in the dimer interphase and not fully exposed to bulk water. Further study will be performed to other mutants containing more exposed Trp to provide a complete description of the hydration shell around &gamma;M7-crystallin.
Abstract: Transfer RNAs (tRNA) are extensively modified during RNA processing. One such modification performed by the tRNA methyltransferase Trm10 is the N-1 methylation of guanosine at the G9 position (m1G9) in 14 types of yeast tRNA. The Trm10 gene is widely conserved in Eukarya and Archaea. Mutations that decrease expression or catalytic activity of the Trm10 human homolog (TRMT10A) can cause microcephaly, short stature, and glucose sensitivity. In yeast, the trm10^ strain grows normally on rich media, but is sensitive to growth on media containing 5-fluorouracil (5FU). We hypothesize the lack of Trm10-catalyzed modification in trm10^ yeast renders a specific tRNA less functional when grown on 5FU media. We hypothesized that if more of that tRNA is expressed, it may allow cells to overcome the deficiency by maintaining a sufficient pool for translation. Vectors for tRNA overexpression were transformed into trm10^ strains and plated on media containing 5FU. Compared to controls, increased growth was seen with overexpression of tRNATrp. Interestingly, decreased growth was seen in strains overexpressing tRNASe, indicating the increase in tRNA abundance could be interfering with other cellular processes. Overexpression of tRNATrp in wild-type yeast had no observable effect on growth, suggesting that the poor growth of yeast in the absence of Trm10 is related specifically to the presence of 5FU. Growth curve assays showed an increase in growth compared to control for strains overexpressing tRNATrp. Similarly, these assays showed a dampening of growth for strains overexpressing tRNASe after initial growth. Northern blot assays of tRNATrp and tRNASe will be performed to assess the levels of tRNA compared to controls. Further study of the role of tRNATrp in trm10^ yeast in the presence of 5FU could lead to further insight into the role of the m1G9 modification in normal cells and how its absence leads to a diseased state.
Category: Biological Sciences

Title: The development and characterization of neural-specific continuous cell lines in Drosophila melanogaster

Student Presenter: Nanki Hura

Faculty Advisor: Simcox, Amanda

Abstract: Neurodegenerative diseases affect millions of Americans and are caused by the deterioration of neurons in the central nervous system. The mutations that cause these neurodegenerative diseases have been modeled in Drosophila (fruit fly) transgenic lines and have been used to produce flies with neurological defects to mirror the human pathology. Meanwhile, continuous cell lines have transformed biomedical research, making it possible to study biological systems on a scale requiring large numbers of similar cells. However, there is a lack of continuous cell lines in Drosophila melanogaster available in the scientific community that would allow for the study of specific mechanisms within the nervous system. The objective of this study was to utilize the expression of oncogenic Rasv12 to produce neuronal cell lines in Drosophila melanogaster; neuronal lineage markers were then used to characterize the cell lines. The GAL4/UAS system was used to induce the tissue-specific expression of a given gene of interest. Fly "drivers" expressing the GAL4 protein in neuroblasts were crossed with flies containing upstream activating sequences (UAS) upstream of Rasv12, leading to the proliferation of neuroblasts in progeny. Both pan-neuronal and motoneuron-specific drivers were used. Embryos were collected from the cross and grown in vitro as a primary culture. Cell cultures were stained with the neuronal cell-type specific markers: HRP, Fasciclin-II, and 22C10. Cell lines previously established in the lab from constitutive Rasv12 expression, and that appeared to exhibit neuronal properties, were also characterized with the neuronal lineage markers. This study tests the efficacy of utilizing Rasv12 for the production of continuous cell lines of neuronal lineage to model and develop therapies for neurodegenerative diseases.
Abstract: Adult stem cells are essential for the normal homeostasis of many body tissues and play critical roles in tissue regeneration following damage. In order to remain undifferentiated yet continue to give rise to differentiating progeny, stem cells must constantly balance self-renewal and differentiation. One critical transition during stem cell differentiation is the switch from dividing progenitor cells to post-mitotic differentiating cells. For some genes, this process is characterized by a shortening of the 3' translated region (3'UTR). In male germline stem cells, we have identified a widespread shortening of the 3'UTR in over 600 mRNAs during the switch from proliferation to differentiation. Using the established model of stem cell differentiation in the Drosophila male germline we are studying a subset of 60 mRNAs, to observe the function of these genes whose 3'UTRs shorten during differentiation. Through these studies, we have discovered that the gene Chromator, previously not studied in the male germline, is required for proper germ cell differentiation. The continued study of these genes will shed light on the functions of the many genes whose 3'UTRs shorten during male germline stem cell differentiation.
Abstract: This project aims to illustrate the relationship between protein sequence, structure, and stability in the context of a protein Er-23, a pheromone secreted by the protozoan Euplotes raikovi. Er-23 is 51 amino acids long and has 10 cysteine residues in 5 disulfide bonds, whose role in the stability and structure of the protein are of particular interest. The literature suggests that the structure of the protein is driven by the formation of disulfide bonds, and is supported by the currently accepted 3-D structure of the protein. Our working hypothesis is that the folding of the protein is driven primarily by the amino acid sequence, while the disulfide bonds play a role in the overall stability of the protein. We have successfully expressed Er-23 in E. coli, a heterologous expression system, and will use solution state NMR Spectroscopy, Mass Spectrometry, X-Ray Crystallography and CD Spectroscopy to elucidate the precise structure and compare it to that of the homologously expressed protein. Our current data suggest that the protein spontaneously adopts its native conformation in E. coli, and we aim to corroborate this through denaturation and refolding experiments. Additionally, our NMR and Mass Spectrometry data suggest that the disulfide bonds and overall structure of the protein may differ from that of the reported structure. This fundamental information about the interplay between protein fold, stability, and sequence can be useful in the future investigation and design of novel proteins.
Abstract: Glioblastoma multiforme (GBM) is one of the most common and aggressive human brain tumors, accounting for 52% of all primary brain tumors. The median survival for a person diagnosed with GBM is 15 months. GBM originates in glial cells, cells that provide support and protection to neurons in the nervous system. The current treatments for glioblastomas include surgery and chemotherapy, but prolonged remission is rare with the median time for tumor recurrence being about 6.9 months. GBM is currently being studied in human cells and animal models, including the fruit fly, Drosophila melanogaster. Fly glioblastoma models have been developed, but there is no equivalent cell culture model of the glioblastoma in flies. Developing a fly cell model of glioblastoma is the goal of my project. I propose to generate cell culture models of glioblastoma, by expression of an oncogene and by repression of a tumor suppressor. In the models, glial cells will be induced to express RasV12, an oncogene known to be involved in human GBM. In addition, the tumor suppressor called brain tumor (brat) will be repressed. Loss of function of TRIM3, the human homolog of brat, has been implicated in human GBM. To determine if the cell lines I generate are of glial-cell type, I will analyze the cells using a specific cell marker. To test the tumorigenicity of the cells, I will perform cell transplant assays to determine if injected cells form tumors in the flies. Once established, these models of the glioblastoma will be useful in understanding the basic mechanisms of the disease and for testing therapies.
Category: Biological Sciences

Title: Determining the antimicrobial activities of ant-derived natural products

Student Presenter: Emily Katula

Faculty Advisor: Adams, Rachelle

Abstract: Fungus-farming ants cultivate and consume fungi as their primary source of food. This relationship represents a classic example of mutualism and, to date, more than 250 New World species across 16 genera have been identified. As social insects with highly organized societies, these ants depend upon a system of communication mediated by pheromones and other chemical substances derived from biosynthetic pathways and released via the numerous exocrine glands throughout their bodies. Previous research has demonstrated the various communicative roles these natural products serve; however, recent investigations have shown these compounds may also function as antimicrobial agents used to defend the colony’s fungal garden from various pathogenic microorganisms. Protection and maintenance of their fungal symbiont is paramount to the survival and success of a colony. By utilizing these compounds, they are able to eliminate parasites and ensure the continuation of this vital mutualism. In this study, we determined the antimicrobial activities of three ant-derived natural products through standardized disc diffusion susceptibility procedures with two species of bacteria, *Escherichia coli* and *Staphylococcus saprophyticus*, with assays of 8 other species forthcoming. Areas of zones of inhibition were measured using ImageJ software and the mean values compared using a parametric F test and a Tukey test (P ≤ 0.05). This study demonstrates the potency and antimicrobial effectiveness of 2-methyl-2-decenal and 2-methyl-2-nonenal, both newly discovered natural products identified as the major components of the mandibular gland extract of *Apterostigma dentigerum*, as well as cis-3-butyl-5-hexylpyrrolizidine, a venom alkaloid from the social parasite *Megalomyrmex symmetochus* which parasitizes colonies of fungus-farming ants. These results shed further light on the complex systems of fungus-farming ant mutualisms, as well as the various means in which they are able to employ their own chemistry to transform their environments.
Title: Characterization and target recognition of Salmonella enterica biofilm inhibitors T315 and JK-1

Student Presenter: Darpan Kaur

Faculty Advisor: Gunn, John

Abstract: Salmonella enterica serovar Typhi (S. Typhi) is the disease-causing agent of typhoid fever. Post-treatment of acute infection, S. Typhi colonizes the gallbladder of 3-5% of hosts in a chronic carriage state. In the chronic carriage state, S. Typhi forms aggregations of bacteria, known as biofilms, on the surface of gallstones and the gallbladder epithelium allowing S. Typhi to persist in the host. As traditional antibiotics are ineffective in treating Salmonella chronic carriage, new drugs are desperately needed. From a large ATP-mimetic (kinase inhibitor) compound screen, two compounds, T315 and JK-1, were identified to possess anti-biofilm characteristics against S. Typhi and S. Typhimurium. The effect of both compounds on biofilm formation has been evaluated using rapid attachment, viability, EC50, and dispersal assays. The respective EC50 values for T315 and JK-1 were calculated to be 4.61 μM and 7.27 μM, and both work similarly in inhibiting the early attachment of bacteria in a non-bactericidal manner. The compounds have been/are being derivatized and screened to identify those that exhibit efficient anti-biofilm properties with decreased EC50 values. Previous JK-1 experiments identified DeoD and GroEL as potential targets, which are being characterized. The lack of synergistic or additive activity of the two compounds added together suggests they may have a common target. Pull-down assays using biotinylated T315 synthesized utilizing click-chemistry are underway to confirm the target(s). Characterization of promising derivatives of T315 and JK-1 and their target identification will further assist in the development of anti-biofilm compounds to treat Salmonella chronic carriers.
Abstract: Mycobacterium tuberculosis (M.tb) infects 1/3 of the world's population. Upon deposition of M.tb in the airways, bacteria are bathed in the lung mucosa, a.k.a. alveolar lining fluid (ALF). Although ALF is primarily composed of surfactant lipids, approximately 15% of ALF are proteins, many with enzymatic hydrolytic capabilities. Our data indicate that ALF hydrolases alter the cell wall of M.tb by releasing soluble bioactive bacterial fragments (ALF released fragments) into the milieu. These M.tb fragments are capable of influencing the macrophage function, thus modifying the lung microenvironment that M.tb encounters as it infects host cells. Lung macrophages are the body’s first line of defense against M.tb, either by ingesting M.tb and triggering an immune response to contain the infection. Receptors on the surface of macrophages play an important role in determining the course of the infection. Some receptors trigger a pro-inflammatory response, while others trigger an anti-inflammatory response. Macrophages have receptors on their surface called pattern recognition receptors (PRRs) that can identify microbial-associated molecular patterns on M.tb. Some PRRs have a phagocytic function while others have a signaling function. Toll-like receptors (TLRs) are signaling PRRs. Our data show that ALF released fragments induce IL-10 secretion allowing macrophages to control better M.tb infection. We hypothesize that TLR2 and TLR4 play a role in this IL-10 secretion and the resulting observed macrophage response. To evaluate this hypothesis, we assessed the production of IL-10, IL-6, and TNF through using wild type, TLR-2 KO, and TLR-4 KO murine macrophage cell lines. Cells were grown as per protocol and plated at a quantity of 3x10^5 in 24-well tissue culture plates. Cells were allowed to adhere for 16 h and were then washed and stimulated with the ALF-fragments (obtained from exposure of M.tb to human ALF from three different donors) using proper positive and negative controls. After 48 h, supernatants were collected and the release of TNF, IL-10, and IL-6 was quantified by ELISA. Our results show no significant production of TNF, IL-10, and IL-6 when exposed to ALF fragments, when compared to our positive controls. This leads us to conclude that other receptors (i.e. the phagocytic mannose receptor) may play an integral role in the secretion of IL-10 involved in the better control of the M.tb infection.
Title: Dual-specificity phosphatase 4 (DUSP4) over-expression in cells prevents H/R-induced apoptosis via the up-regulation of eNOS

Student Presenter: Joanna Kilbane Myers

Faculty Advisor: Chen, Chun-An

Abstract: MAPK signaling cascades regulate several cellular functions, including differentiation, proliferation, and apoptosis. The duration and magnitude of phosphorylation of these MAPKs are critical determinants of their physiological effects. The kinetic control of these MAPK signal cascades is modulated by DUSPs. Previously, we demonstrated that DUSP4-/- hearts sustain a larger infarct and have poor functional recovery, when isolated hearts were subjected to ischemia/reperfusion (I/R). Uncontrolled p38 activation and up-regulation of Nox4 expression are the main effectors for this functional alteration. Here, DUSP4 over-expression in endothelial cells was used to investigate the role of DUSP4 on the modulation of ROS generation and vascular function, when cells were subjected to hypoxia/re-oxygenation (H/R) insult. Immunostaining with cleaved caspase-3 revealed that DUSP4 over-expression prevents caspase-3 activation and apoptosis after H/R. The beneficial effects occur via modulating p38 activity, increased NO bioavailability, and reduced oxidative stress. More importantly, DUSP4 overexpression up-regulates eNOS protein expression during H/R induced stress. NO is a critical small molecule involved in regulating vascular tone, vascular growth, platelet aggregation, and modulation of inflammation. The level of NO generation determined using DAF-2 fluorescence demonstrated that DUSP4 over-expression augments NO production, and thus improves vascular function. The level of superoxide generated from cells after being subjected to H/R was determined using DHE-HPLC method. The results suggested that DUSP4 over-expression in cells decreases H/R-induced superoxide generation and thus reduces oxidant stress. This also correlates to the reduction in the total protein S-glutathionylation, an indicator of protein oxidation. These results further support our hypothesis that DUSP4 is an antioxidant gene and a key phosphatase in modulating MAPKs, especially p38, during oxidative stress; which regulates ROS generation and eNOS expression and thus protects against oxidant-induced injury or apoptosis. Overall, DUSP4 may serve as an excellent molecular target for the treatment of ischemic heart disease.
Category: Biological Sciences

Title: The identification and validation of ASD-causal copy-number variants in a familial dataset.

Student Presenter: Alexander Lacey

Faculty Advisor: Bartlett, Christopher

Abstract: Autism Spectrum Disorder (ASD) is a heterogeneous group of related developmental disorders, and it is estimated to affect ~1.5% of children. The cause of ASD has been shown to contain a strong genetic component; however, currently-acknowledged susceptibility genes only account for ~20-25% of ASD cases. Some of the unexplained causality could be related to copy-number variants (CNVs), which are deviations from the standard two alleles at a given location in the genome. We hypothesize that rare, de novo CNVs play a causal role in some of the ASD-affected individuals in a familial dataset from the New Jersey Language and Autism Genetics Study (NJLAGS). In our investigation, we create a filtered list of potentially-causal copy-number variants, and then we individually investigate each case within the ranked list in an attempt to validate each one. This research will contribute to the growing body of knowledge regarding the genetic underpinnings of ASD, which we hope will someday provide physicians with the ability to quantify risk of the disorder—or maybe even provide a diagnosis-based on genetic factors.
Title: Accessory cusp expression in upper and lower human molars: testing the patterning cascade mode

Student Presenter: Jacob Lawrence

Faculty Advisor: Guatelli-Steinberg, Debra

Abstract: The Patterning Cascade Model (PCM) accounts for the establishment of tooth shape during development. In this model, each tooth cusp forms at a specialized site on the dental epithelium, an enamel knot, which is a gene signaling center that stimulates downward epithelial growth while simultaneously inhibiting other enamel knots from forming nearby. For a new cusp to form, a new enamel knot must form beyond this zone of inhibition. Humans have a typical number of principal cusps (4-5) on their upper and lower first molars, but some individuals also express smaller, accessory cusps, either peripheral or central in position. According to the PCM, small inhibition zones, protracted growth, or both promote the formation of peripheral enamel knots. Accordingly, small intercusp spacing relative to tooth size should be associated with greater peripheral cusp expressions. In contrast, centrally located accessory cusps, according to the PCM, may be associated with larger distance between the principal cusps that surround them, giving them space to form. Previous research has found that the presence of the Carabelli’s cusp is associated with greater expression of peripheral accessory cusps. This study analyzes the rest of the individual’s dentition to see if accessory cusps are more likely to appear on all first molars if found on any first molar. Data from first molars of 37 individuals from a Seminole dental cast collection were collected by measuring the degree of expression of cusps and intercusp distances. Preliminary analysis suggests that in almost all cases, accessory cusps are more prevalent with smaller intercusp distances relative to tooth size regardless of their position on the tooth. This study gives insight into the developmental mechanisms that underlie cusp formation in humans and other mammals. Understanding these patterns will ultimately refine the understanding of evolutionary patterns in cusp formation and loss.
Category: Biological Sciences

Title: Rop as a model protein to further understand structure and function

Student Presenter: Sheridan Leistikow

Faculty Advisor: Magliery, Thomas

Abstract: Linking the sequence of a protein to its stability and function and predicting how a protein folds from its sequence is a goal of biochemical researchers. A particular region of interest in a protein is its core. The composition of a protein core, among other elements, determines and drives the topological formation of the entire molecule. Previous work has been done concerning how mutations within the core of Rop, an alpha-helical protein composed of 63 amino acids that forms an anti-parallel homodimer, changes the structure and stability. Through characterization of a set of Rop variants, we observed that repacking Rop's core leads to variable outcomes. Generally, the protein core is relatively intolerant to mutation, but how resilient are loops to mutation, and what amino acid sequence makes the 'best' loop? Using Rop, we can model how randomization of a loop region alters the biophysical characteristics of a protein. First, we have randomized the canonical four residues of Rop's loop using degenerate codons to create a protein library in both wild-type Rop and a variant engineered to remove a potentially stabilizing ionic interaction between the monomers. Two other protein libraries were also created with an additional residue in the loop. These four protein libraries are being subjected to a cell-based screen that connects phenotype to activity in vivo. High Throughput Thermal Scanning will be used to study the stability of active variants. Additionally, through an enrichment process, we will observe the composition of the most favorable Rop loops. Future studies include characterization of a few interesting variants by thermal and chemical denaturation and NMR. From these data, we will draw conclusions about how tolerant Rop's loop is to mutation. These conclusions may lend insight into the relationship between sequence and fold and provide useful knowledge for therapeutic development and industrial processes.
Abstract: All eukaryotic cells require an intracellular transport system to move cargo like organelles, vesicles, and proteins over large distances. This transport occurs on long tracks called microtubules (MTs) and is governed by a set of rules that is not well understood. MTs are polar cables that are anchored at the MT-organizing centers at their minus ends and grow at their plus-ends. Our lab uses the filamentous fungus A. nidulans to study the role of MT-associated proteins (MAPs) in subcellular travel. We are currently investigating the localization and dynamics of three novel proteins, MAPA, MAPB, and MAPC, that move bi-directionally on MTs and interact with each other. The focus of my research was to utilize quantitative analyses on existing microscopy data to investigate the role of MAPA and MAPC in regulating the dynamics of MAPB. I used the kymograph tool and derived the rate of movement of MAPB foci in wildtype cells. I found that the rate of movement of MAPB towards the plus-end of MTs is similar to that towards the minus-end of MTs. Furthermore, the rate of movement of MAPB is comparable to that of MAPA and MAPC in both directions. My ongoing analyses are targeted towards computing the rates of movement of MAPB in the absence of MAPA and MAPC and compare them to rates in wildtype. Overall, my research is aimed to provide a more quantitative understanding of the movement of novel dynamic proteins, ultimately providing a more thorough insight into the rules governing intracellular transport.
Abstract: Primates, relative to other mammals, often have extended periods of infancy and adolescence in order to learn and develop the skills needed to survive in adulthood. Therefore, the time from birth to sexual maturity is an important area of study to understand primate behavioral development. Although this has already been explored in several primate species, little has been published on the behavior of infant Asian colobines. The birth of two silvered langurs (Trachypithecus cristatus) to different mothers within the same breeding group at the Columbus Zoo and Aquarium offered a unique chance to study infancy in this species and uncover possible differences related to sex, age, and mothering style. From January to August 2016, this group underwent daytime observation at various times for one to three hours. For each individual, a range of behaviors was recorded, including activities such as feeding, sleeping, and locomoting, and social behaviors such as grooming, supplanting, and proximate location. In addition, specific behaviors relative to the offspring were recorded, such as nursing, holding, and carrying by their mothers. Analysis of the data showed little variation between the two offspring in locomotive activities and maternal contact, suggesting little difference in social or active behaviors. The results are different from those found in studies of other primate species, and indicate a need for continued study of silvered langur infancy and adolescence in order to inform how they are kept and bred in captivity.
Abstract: RNAs perform an array of functions in all life (e.g., catalysis, chromatin remodeling, structural scaffolds for large assemblies). Understanding this versatility of RNAs requires knowledge of their structure-function relationships. Probing RNA structure often requires spectroscopic methods, which in turn necessitates strategies for post-synthetic, site-specific incorporation of chemical probes into target RNAs. One method to achieve this goal is through in vitro transcription (IVT) of RNAs by T7 RNA polymerase and a GTP-initiating class III &Phi;6.5 promoter. In addition to GTP, T7 RNA polymerase can incorporate 5'-modified guanosine analogs during transcriptional priming. Because the nucleoside/nucleotide monophosphate guanosine analog cannot be used in elongation, it can only serve as the initiator. By using a 4- to 10-fold excess of the 5'-modified guanosine analog:GTP in the IVT, we and others have successfully generated RNAs with 5'-modifications. We have now rigorously examined the extent of 5'-modification with RNAs of different sizes and with 5'-deoxy-5'-azidoguanosine (Az-G) as the modifier. For small RNAs (5 nts), T7 RNA polymerase indeed generates mostly 5'-Az-G-modified RNAs (~90%). With tRNAs (150 nts), we determined that there is an unexpected maximum threshold (}
Category: Biological Sciences

Title: Non-canonical 5'-end maturation pathways in Caenorhabditis elegans

Student Presenter: Nicholas Mitchell

Faculty Advisor: Jackman, Jane

Abstract: Histidyl-tRNA (tRNAHis) has the essential role of acting as an adaptor molecule in translation and is required for addition of histidine to growing protein chains. To maintain fidelity of protein synthesis, the histidyl-tRNA synthetase (HisRS) must ensure the correct attachment of the histidine residue to its cognate tRNAHis. For nearly all eukaryotic organisms, the identifying element on a tRNAHis utilized by HisRS to ensure fidelity is a guanosine nucleotide at the minus one position (G-1) that is present on the 5' end of a mature tRNAHis, but not on any other tRNA. G-1 incorporation in many eukaryotes occurs by a two step mechanism, where Ribonuclease P (RNase P) cleaves to remove the 5'-leader sequence from pre-tRNAHis, followed by tRNAHis guanylyltransferase (Thg1) catalyzing the unusual 3' to 5' addition of a guanosine nucleotide to the 5' end of tRNAHis. Consistent with the importance of this role, Thg1 is widely conserved among eukaryotes. Recently, we identified an exception to this pathway in Caenorhabditis elegans (C. elegans). C. elegans tRNAHis contains a G-1 residue that is still the identifying element for C. elegans HisRS. However, the genome of C. elegans does not encode any identifiable Thg1 enzyme, raising the question of how this G-1 element is incorporated into tRNAHis in this species. Interestingly, tRNAHis genes in C. elegans contain an encoded G-1 nucleotide in their 5' leader sequences, suggesting that G-1 might be incorporated during transcription, and retained by an altered activity of RNase P, constituting a mechanism for incorporation of G-1 that has so far only been observed in Bacteria. To test whether this altered RNase P cleavage activity might be responsible for retention of G-1 on tRNAHis, we performed in vitro 5'-maturation assays using transcripts from C. elegans. Results implying non-canonical recognition of tRNAHis by C. elegans RNase P will be presented.
Category: Biological Sciences

Title: Role of canonical and variant androgen receptors in promoting hepatocellular carcinoma proliferation

Student Presenter: Riley Mullins

Faculty Advisor: Coss, Christopher

Abstract: Hepatocellular carcinoma (HCC) is the most common primary liver cancer and second highest cause of cancer related deaths. Curative treatments, such as hepatic resection, are effective in early stage HCC. However, HCC is often diagnosed during the late stage. Available systematic therapy at this stage extends survival only by months. HCC is proposed to be promoted by the transcription factor androgen receptor (AR), yet not androgens. Indeed, men are diagnosed at a rate 2- to 4-fold higher than women. In both sexes, HCC incidence and mortality rates are increasing. AR antagonists are an established drug class used to treat prostate cancer, an androgen dependent malignancy. Unfortunately, clinical trials using anti-androgens to treat HCC did not demonstrate efficacy. Our lab has shown that HCC cells express AR splice variants that lack the ligand binding domain essential for current AR antagonist drugs. These variants are thought to be constitutively active and have been identified in prostate cancer, in which they may confer drug resistance. Despite these evidences, the functional role in HCC of full-length AR and AR splice variants, that is, the macrocellular effects of the gene programs they regulate, is not understood. It is hypothesized that AR splice variants can function as a surrogate of full-length AR through redundant activation of proliferative genes. This will be tested by assessing the consequence to in vitro HCC proliferation of suppressing either full-length AR, AR splice variants, or both. Suppressing both full-length AR and AR splice variants is expected to decrease proliferation more than inhibiting either alone. The results of this project will inform future studies of the genes regulated by full-length AR and AR splice variants. Ultimately, elucidating the functional role of AR in HCC tumor growth will offer insight into the potential utility of novel AR-targeted anti-cancer therapeutics.
Abstract: Proteins adopt well defined tertiary structures and form quaternary assemblies via non-covalent interactions. Understanding protein structures and protein interactions is necessary to understand how proteins accomplish their physiological functions. One important tool for protein structure analysis is native mass spectrometry (MS) with surface induced dissociation (SID) as gas phase activation method. In native MS, protein complex ions can be directly generated from non-denaturing aqueous solutions. Those protein complex ions can then be collided into a surface to generate sub-complexes during the SID process. Based on the occurrence of sub-complexes, information on the stoichiometry and topology of a protein complex is easily obtainable. In this work, we analyze how the abundance of generated sub-complexes correlates with the collision energy and how this relates to the number and nature of non-covalent interactions within a protein complex. For this study, we analyzed hetero-tetrameric a&beta;&beta;a tryptophan synthases (TS) from different organisms by SID MS. We found significant differences in the abundance of observed sub-complexes (a, a&beta;,, a&beta;&beta;, &beta;&beta;,, &beta;) at different SID collision energies for different TS. As the analyzed TS share the same quaternary structure, our data shows that protein-protein interactions with distinct non-covalent connections can be differentiated by SID MS. We are currently working on understanding how the different types of non-covalent interactions effect the energy required for complex disruption in the gas phase to make SID-MS an even more powerful tool for the study of protein interactions.
Title: Horizontal gene transfer of nitrate assimilation genes may facilitate shifts in the ecology of fungi.

Student Presenter: Nic Petrykowski

Faculty Advisor: Slot, Jason

Abstract: The goal of the research was to determine the role, evolutionary history, and ecology associated with nitrate assimilation in fungi. Previous studies have suggested the gain or loss of nitrate assimilation genes may be responsible for ecological shifts in fungi. Protein sequences of nitrate reductase (NAR), nitrate transporter (NRT2), and nitrite reductase (NADPHnir) were obtained by blastp of a local database of 612 fungal proteomes. Phylogenetic analysis was performed on the Ohio Supercomputer Cluster by 1) aligning sequences using mafft 2) curating the alignment using TrimAl 3) searching for the best evolutionary model using Prottest and 4) conducting Maximum Likelihood analysis using RaxML. Gene clusters made up of 2 or more nitrate assimilation genes were then mapped to the resulting phylogenetic trees utilizing the ETE toolkit. Horizontal gene transfers were identified by observing the trees for clusters inconsistent with expected species relationships. In order to test for additional genes involved in these transfers, a protein blast search of NCBI nonredundant proteins was performed using 5 genes up- and downstream of horizontally transferred genes. Two new cases of Horizontal Gene Transfer (HGT) were identified. First, HGT of NAR & NRT2 into a putative neurotransmitter synthesis cluster in the insect pathogen Cordyceps militaris which may play a role in parasitism of insects. Second, HGT of a partial nitrate assimilation cluster to Amanita muscaria may be related to its recent ecological shift from saprotrophic to mycorrhizal nutrition. These findings call for further study in order to determine the specific roles of nitrate in fungus-plant and fungus-insect interactions.
Category: Biological Sciences

Title: Topological and statistical analyses of gene regulatory grids reveal unifying emergent properties

Student Presenter: Katja Pogacar

Faculty Advisor: Grotewold, Erich

Abstract: Most complex biological systems have interacting components that can be modelled graphically using networks. Among them are gene regulatory grids (GRGs) that are made up of connections between transcription factors (TFs) and target genes, i.e. protein-DNA interactions (PDIs). In general, the connectivity of such GRGs can be described using a power law function of the degree, whereby few TFs have a significantly higher number of interactions than others. In order to understand the complexity of GRGs, we studied the topological properties of GRGs of four organisms: Caenorhabditis elegans (worm), Drosophila melanogaster (fly), Saccharomyces cerevisiae (yeast) and Arabidopsis thaliana (plant). Preliminary results revealed the exponent parameter (alpha) of the fitted power law function on these PDIs were different for each organism, suggesting that the exponent is an intrinsic organism-specific property. Due to the incomplete nature of the observed grids, we created synthetic complete networks resembling biological properties we had previously observed. Firstly, we predicted the total number of interactions which also showed the percentage of the complete GRG that is observed. Secondly, this helped us evaluate smaller subgrids of the GRGs and to determine the largest size of a grid for which the parameters of interest retained reliability. This is the first time a rigorous evaluation of the correlation between the parameter alpha and few other properties of these GRGs has been performed. These findings suggest that GRGs connectivity is organism-specific, however they seem to follow a unifying emergent property. The analyses reported herein can be extended to networks of other species. Additionally, more interactions are needed for further analysis of the other three model organisms.
Abstract: Bacteroidetes is a large, diverse phylum with representative species inhabiting a wide variety of niches; many of which are associated with other organisms as pathogens or mutualists. Genome size and G-C content are characteristics that have been shown to have a loose positive correlation in Bacteria. This project seeks to determine if this relationship is stronger within host-associated groups compared to free-living Bacteroidetes, and if less variability of genomic content is seen within the host-associated groups. To investigate this, genomic data was obtained from sequenced Bacteroidetes genomes from NCBI, which were subsequently classified according to their corresponding ecological niche at the genus level. Statistical analysis revealed a stronger correlation between G-C content and genome size within each niche than within the phylum as a whole, and more variability was observed in the genome sizes of free-living or facultative host-associated bacteria than in groups intimately associated with a host. Contrary to previous studies, aerobic members of Bacteroidetes were found to have a higher median G-C content as well as a significantly stronger G-C content to genome size correlation as compared to anaerobes. Deviation from genus and niche norms facilitated identification of incorrectly classified sequences and unique lifestyles within groups. This project demonstrates how ecological classification of large-scale public access genomic data can be used to contribute to a better understanding of the relationship between bacterial genomic content and corresponding lifestyle.
Category: Biological Sciences

Title: Physiological role and mutational analysis of BOLA3 protein in iron-sulfur cluster biogenesis

Student Presenter: Brian Rao

Faculty Advisor: Cowan, James

Abstract: Iron-sulfur (Fe-S) clusters are integral protein co-factors that are responsible for many metabolic processes. These ubiquitous clusters are one of the most versatile prosthetic groups and function in electron transfer and storage, donation of sulfur in liopate synthesis, regulation of enzyme activity, and regulation of gene expression. These roles are all vital for a number of essential physiological processes, such that genetic mutations to Fe-S cluster proteins can result in a variety of human diseases, due to defects in Fe-S cluster biogenesis and the proteins involved therein. A mutation in one of the Fe-S cluster binding proteins known as BOLA3 has recently been discovered to cause multiple mitochondrial dysfunction syndrome 2 (MMDS2), a condition that causes severe weakness, respiratory failure, impairment of neurological development, hyperglycinemia, and overall failure to thrive. Biochemical features of this disease include a fatal deficiency of multiple respiratory chain complexes and 2-oxoacid dehydrogenase enzymes as well as lipoic acid synthesis defectiveness. However, the cellular role of BOLA3 as well as its functional role in Fe-S biogenesis and its relationship with protein partners, NFU1, glutaredoxins, and final target proteins remains unclear. To explore the specific functions of BOLA3 in Fe-S cluster biogenesis and why mutations to it have such severe effects, we have isolated the protein and are characterizing its properties of cluster binding and possible protein partners through the use of circular dichroism, UV-Visible spectroscopy, gel filtration, ultracentrifugation, and isothermal titration calorimetry. By identifying the preferred protein partners and kinetic transfer properties, we will be able to understand the physiological role of BOLA3 and begin to work towards analysis of the disease condition to develop treatment options.
Category: Biological Sciences

Title: Regulatory elements in the HIV-1 5' UTR modulate Gag binding specificity

Student Presenter: Joshua-Paolo Reyes

Faculty Advisor: Musier-Forsyth, Karin

Abstract: The 5' untranslated region (5'UTR) of the human immunodeficiency virus type 1 (HIV-1) genomic RNA (gRNA) contains a structured RNA element (termed Psi) that is specifically recognized by the HIV-1 Gag polyprotein, ensuring that two strands of gRNA are packaged into newly assembled virions. However, the mechanism by which Gag recognizes gRNA over other cellular RNAs and spliced viral RNAs is not well understood. A recent study suggested that a negative regulatory element upstream of Psi reduces high-affinity Gag binding, and a positive regulatory element downstream of Psi counteracts the upstream element and restores high-affinity binding. The aim of this study is to determine how these elements affect the specificity and mode of Gag binding. Using a fluorescence anisotropy-based salt-titration binding assay, the electrostatic and nonelectrostatic (i.e., specific) components of binding were measured. We have previously shown that Gag interacts with a 109-nucleotide (nt) Psi RNA construct that lacks the putative regulatory elements with high specificity and relatively few electrostatic interactions. Using a 356-nt RNA construct that includes the negative regulatory element in addition to Psi, we observed a loss in Gag binding specificity and an increase in electrostatic interactions. Interestingly, a 400-nt construct that contains the positive and negative elements flanking Psi restored highly specific binding and reduced the electrostatic interactions made with the RNA. Furthermore, a construct wherein the 40-nt positive regulatory element was appended to Psi, demonstrated the same specificity as Psi alone. Taken together, these data are consistent with a mechanism whereby the negative and positive regulatory elements flanking Psi modulate Gag binding mode and specificity.
Abstract: Ovarian clear cell carcinoma (OCCC) is the second leading cause of death from ovarian cancer because of its poor response to treatment. Chemo-resistance of OCCC leads to a worse prognosis compared to that of high grade serous ovarian cancer (HGSOC). To improve the outcomes for patients with OCCC, it is critical to find ways to bypass chemo-resistance. Therefore, identifying novel unique proteins involved in OCCC chemo-resistance and designing a drug to selectively target those proteins would make a significant impact on therapy. Transmembrane protein 205 (TMEM205) is one such protein which has been linked to cisplatin resistance in epidermoid carcinoma, however the association in OCCC chemo-resistance has not been elucidated. In order to determine the significance of TMEM205 in OCCC, we have evaluated the TMEM205 expression in 6 OCCC patient tissue samples and 8 HGSOC samples by Western Blot and immunohistochemistry. This revealed highly elevated TMEM205 expression in all the OCCC samples, but absent or lowered TMEM205 expression in HGSOC human tissue samples. Four OCCC cell lines also showed high expression of TMEM205 concentrated in the extranuclear space. In order to determine if TMEM205 is linked to chemo-resistance, we created TMEM205 knocked down OCCC cells. The wild type OCCC and TMEM205 knockdown OCCC cells were treated with cisplatin (10µM) for 3 hours, collected, and the exosomes were isolated from the conditioned culture media for inductively coupled plasma mass spectrometry (ICP-MS) analysis. It was found that intracellular cisplatin increased in the TMEM205 knockdown cells, and the exosomes released from these cells showed decreased cisplatin concentration. In conclusion, this study indicates that TMEM205 expression plays a key role in chemo-resistance in OCCC and is mediated by the exosome efflux pathway.
Category: Biological Sciences

Title: Using affinity chromatography and mass spectrometry to discover diagnostic protein biomarkers of invasive Aspergillosis

Student Presenter: Cory Roeth

Faculty Advisor: Wysocki, Vicki

Abstract: Invasive Aspergillosis (IA) is a fungal disease commonly caused by Aspergillus fumigatus that occurs in individuals with suppressed innate immunity. Other IA causing pathogens are A. flaveus, A. niger, and A. terreus. IA is problematic because of the mortality rate, which can be as high as 90% in high-risk populations, with the occurrence rate doubling in the past 20 years. A positive outcome is more likely when treatment is started earlier, which requires early diagnosis. Currently, definitive diagnosis requires biopsy, an invasive procedure. While adjunctive diagnostic methods, such as CT scans, culture, and the use of galactomannan as a biomarker, are noninvasive, their reliability are often questioned. Protein biomarkers offer the best chance for early diagnosis, thus this project is aimed at identifying multiple protein biomarkers for IA. One method uses affinity chromatography with mass spectrometry to detect low abundance Aspergillus proteins. The affinity column has a polyclonal antibody to Aspergillus immobilized to the resin. The antibody is reactive with proteins of the four common IA causing species of Aspergillus, but the specific antigens are unknown. Probable and possible IA patient bronchoalveolar lavage (BAL) fluid samples were run separately on the affinity column. After running the fractions on sodium dodecyl sulfate polyacrylamide electrophoresis and completing an in-gel digestion with trypsin, the elution fractions were analyzed by 1-dimensional liquid chromatography-mass spectrometry (1DLC-MS). In addition to human samples, paraffin-tissue samples from Aspergillus infected mice were tested. These samples were deparaffinated, subjected to a cell lyses buffer, digested with trypsin and then analyzed with 2DLC-MS. Statistical analysis of direct 2DLC-MS runs of human BAL fluid samples has revealed a potential human protein biomarker. Future work entails further optimizing the current sample preparation techniques, in addition to obtaining and analyzing BAL fluid samples from a new Aspergillus mouse model.
Abstract: Toxin-producing Escherichia coli (Shiga Toxin-producing, STEC; Enterotoxigenic, ETEC) causes severe dysentery and gastrointestinal disease in children, the elderly, and immunocompromised people. Antibiotic treatment places cellular stress on the pathogen, which can result in toxin release. Phage therapy, the use of specific viruses to eradicate a bacterial infection, is an alternative solution to antibiotics. Each bacteriophage (phage) has evolved to attach to a specific bacterial cell. The phages then replicate interiorly, eventually bursting the cell and releasing progeny phages. To investigate potential candidates for phage therapy, our study characterized phage-host interactions of four phages that infect STEC and one phage that infects ETEC. Properties of the phage-host interactions were determined via i) genomic analysis to identify various gene functions and their locations in the phage genomes; ii) adsorption kinetics to determine attachment efficiency of phages to their host; iii) one-step growth curves to measure the latent period and burst size of the phage infection; and iv) plaque morphology comparisons. One STEC phage's gene functions were annotated as follows: 36% host takeover; 11% DNA replication and repair; 17% structural proteins; and 2% DNA packaging and cell lysis. The five phages have similar adsorption rates (29-63%) and latent periods (20-30 minutes). While the STEC phages have low burst sizes (2.15-3.80 virions/infected cell), we anticipate the burst size of the ETEC phage to be larger due to the increased size and reduced turbidity of plaques compared to STEC phages. This indicates that compared to the STEC phages, the ETEC phage is a more competitive candidate for phage therapy. We will continue to characterize its properties, aspiring to engineer an efficient infection to eradicate specific Escherichia coli bacterial pathogens.
Category: Biological Sciences

Title: The role of the sodium-glutamate transporter in ischemic stroke

Student Presenter: Monica Sarkar

Faculty Advisor: Terman, David

Abstract: Stroke is a major cause of morbidity and mortality in the United States, with one stroke occurring every forty seconds. About eighty-seven percent of all strokes are ischemic, in which a blood vessel to the brain is blocked, resulting in an energy crisis and subsequent disruption of ion gradients. Eventually, extracellular glutamate builds up and leads to excitotoxicity, swelling and death of brain cells. The goal of this research project is to better understand the role of the sodium-glutamate transporter during ischemic stroke. The sodium-glutamate transporter is located in both astrocytes and neurons. Under physiological conditions, it releases sodium into and removes glutamate from the extracellular space. However, under pathological conditions, the transporter can reverse and lead to glutamate accumulation in the extracellular space, further contributing to cell death. This project examines how the location of the sodium-glutamate transporter - within the astrocyte, neuron or both cells - affects the electrical activity of the cells, particularly the waves of depolarization that characterize ischemia. With the computational program XPPAUT and literature review, we have constructed a functioning model of one astrocyte and one neuron that incorporates voltages, ion currents, membrane potentials, cell receptors, sodium-glutamate transporters and sodium-potassium pumps. Preliminary simulations have suggested that the model best replicates experimental results, such as neuronal and astrocytic firing patterns, when the sodium-glutamate transporter is located in the neuron alone, rather than in the astrocyte alone or in both cells. Further simulations and analysis will be carried out to determine the reason for this discrepancy. This mathematical model simulates multiple biological processes that occur rapidly, which can be difficult to test and measure experimentally. Our findings may also suggest possible avenues to explore in future experiments. By more fully illuminating the dynamics of ischemic stroke, medicines and therapies can be developed and improved.
Category: Biological Sciences

Title: Self-organization and organogenesis program during the neurosphere life cycle

Student Presenter: Austin Schoeffler

Faculty Advisor: Imitola, Jaime

Abstract: Neurospheres are defined as self-renewing, heterogeneous aggregates of free-floating neural progenitor cells and their progeny. These spherical conglomerates are utilized in a variety of applications from modeling the in vitro growth patterns of neural stem cells and neurological diseases, to neurotransplantation clinical trials. While essential to the study of neurogenesis, it is currently unknown how these cells retain their proliferative abilities among multiple generations in culture, and how neurospheres behave upon heterogenous fusion. In this study, we analyzed the dynamics of neurosphere development by live imaging single cell conglomeration into neurospheres, fusion of neurospheres, spontaneous generation of organoids in high-density cultures and finally death of the organoids (Neurosphere life-cycle). Furthermore, we observed a degree of self-organization in these structures by using GFP reporter stem cells. We computationally modeled the evolution of neurospheres from single cells in vitro. Finally, we studied the effects of clonal density on spontaneous organoids formation during the life span of a neurosphere culture. We conclude that by characterizing the intercellular dynamics of the Neurosphere Life cycle, we hope to elucidate more efficient techniques to culture tissues for neural stem cell transplantation, while minimizing the risks of tumorigenesis. Furthermore, our model can be useful to characterize the encoded program of tissue formation of neurospheres by restricted neural progenitors.
Abstract: The Notch signaling pathway is a fundamental cellular communication mechanism that is conserved across all metazoans. Notch activation occurs when a ligand on a signal-sending cell physically interacts with a receptor in a signal-receiving cell. In contrast, when Notch ligands and receptors are expressed in the same cell, their interactions inhibit the ability of the cell to send or receive signals. This process is called "cis-inhibition", and is a critical mechanism of pathway regulation. Because aberrant activation of Notch has been demonstrated in many diseases, understanding the mechanisms that regulate cis-inhibition is important. One such regulator of Notch activity is glycosylation of the EGF (epidermal growth factor-like) repeats of receptors. This mechanism begins with the addition of an O-linked fucose, which can then be extended by FRINGE family glycosyltransferases. This specific glycosylation modifies the strength of ligand/receptor interactions in cis and in trans. Ligands may also be glycosylated, but the functional relevance of this modification is not known. To examine the importance of ligand glycosylation, we utilized sequence analysis to identify ligand EGF repeats that contain consensus sequences for fucosylation. We identified two potential targets on the ligand DLK (delta-like) 1, on EGF3 and EGF6. DLK2 contains consensus sites on EGF2 and EGF5. We also identified several potential glycosylation motifs within JAGGED1 and are focusing on EGF repeats 4-6, which have been shown to be critical for cis-inhibition. After subcloning the relevant repeats, click chemistry utilizing a bioactive fucose demonstrated that fucosylation occurs on DLK1, DLK2, and JAGGED1. We are currently examining extension of the saccharide by FRINGE and the functional relevance of ligand glycosylation using mass spectroscopy and luciferase assays, respectively. Glycosylation is a relatively understudied idea in Notch signaling, this research could open the door for new insights into cell communication.
Abstract: The HP1 ortholog, Swi6, is found in Schizosaccharomyces pombe (S. pombe), and provides a relatively simple system with which to observe HP1 binding effects. Heterochromatin protein 1 (HP1) is an essential, highly conserved protein associated with heterochromatic spread and gene silencing. Heterochromatin is a tightly packed form of DNA, and its spread is known to contribute to gene silencing. HP1 proteins specifically recognize histone H3 lysine 9 tri-methylation (H3K9me3) and bind to nucleosomes with this modification. The mechanism for this binding in Swi6 and its role in heterochromatin spread is still largely unknown. We demonstrate that Swi6 increases H3K9me3-modified nucleosome accessibility via the model transcription factor LexA. By comparing the Förster Resonance Energy Transfer (FRET) efficiency for titrations of modified and unmodified nucleosomes with Swi6 and LexA, we compared changes in nucleosome accessibility for varying protein concentrations. We propose that at higher concentrations, Swi6 will no longer increase accessibility potentially due to oligomerization.
Category: Biological Sciences

Title: KMT2D as a candidate gene for metastatic squamous cell carcinoma

Student Presenter: Sharon Shim

Faculty Advisor: Toland, Amanda

Abstract: Every year, around 3900-8800 individuals die due to metastatic cutaneous squamous cell carcinomas (cSCC), though only a small percentage (roughly 5%) actually metastasize. In our lab’s previous exome sequencing studies, 67% of metastatic cSCC were found to have a mutated KMT2D gene. Only 31% of primary cSCCs have a mutation in this gene. KMT2D, also known as MLL2, belongs to a family of chromatin-remodeling enzymes and is responsible for the production of an enzyme which functions as a histone methyltransferase. Based on literature, KMT2D is thought to function as a tumor suppressor. Due to the fact that a higher percentage of the metastatic cSCCs contained a mutated KMT2D gene, I hypothesized that mutations in the gene directly impact metastasis and promotion of cancer cell growth. To test this hypothesis, we knocked down expression of the KMT2D gene using CRISPR/Cas-9 and then evaluated tumor phenotypes such as migration, proliferation, and growth in soft agar between wild-type and knock-down cell lines. Initial results do not show large differences. In addition to these functional studies, an interesting direct correlation was found between the KMT2D and TP53 gene in which knock-down of KMT2D resulted in lower expression of TP53, suggesting a relationship between this gene of interest and a well-known mutated gene in many cancers. Learning more about the direct effects of loss of KMT2D and the connection with TP53 will result in a more profound and accurate knowledge of not only its influence on metastatic cSCC, but also on other SCCs due to high mutation frequencies in SCCs in the head, neck, and esophagus.
Abstract: One of the important aspects of modern ecological research is determining the effects of global warming on ecosystems. Specifically, this includes understanding the complex relationships among intrinsic environmental factors, trophic relationships, and the biodiversity and abundance of the species living in the environment. The purpose of this research was to determine levels of periphyton and allochthonous organic matter in relation to physical characteristics (flow velocity, substrate type) of glacial stream microhabitats (riffles, runs, rapids, and pools), and assess how these parameters affect insect community structure during summer and winter seasons in a glacier stream in Yunnan, China. The amount of periphyton and organic carbon present is hypothesized to have a positive relationship with the abundance of primary feeders and shredder functional feeder groups, respectively. The abundance of primary feeders and shredders will positively affect the abundance of predators. Periphyton will be sorted from microhabitat samples and volume estimated by water displacement. Organic carbon was measured using ash free dry mass. Taxonomy and macroinvertebrate abundance data was provided for the analysis. It is predicted that the macroinvertebrate biodiversity and abundance will be higher in surber samples where periphyton and organic carbon levels are high. Understanding baseline trophic structure is paramount to determining stream health as it is impacted by climate change. Even though macroinvertebrate diversity is a common bioindicator of stream health, this project aims to contribute further understanding to the structure of macroinvertebrate communities as related to climate change in glacier streams, which are critical but lesser explored ecosystems.
Category: Biological Sciences

Title: Development of a CRISPR/Cas9 mutation system in Caenorhabditis briggsae and use in altering the Notch signaling pathway

Student Presenter: Robert Smith

Faculty Advisor: Chamberlin, Helen

Abstract: Notch and the Epidermal Growth Factor (EGF) are crucial growth pathways found across almost all species of animals. Many types of cancers develop from malignant mutations affecting these pathways; some can even result from negative distortions in both. In order to further understand the basic operation of Notch and EGF, research on lower model organisms is necessary. The nematode vulva provides an ideal case to study where the two pathways interrelate since Notch interacts with the EGF pathway in order to specify different types of vulval cells. Current research focuses on comparing this interaction to the process in a related species, C. briggsae. We find that in C. elegans, the vulval development is entirely dependent on EGF signaling, while in C. briggsae vulval cells divide even if EGF signaling is blocked. Comparison of the signal interactions in these two species provides a model for how different genetic backgrounds may impact cell division regulation. This project tested the hypothesis that these cell divisions result from EGF-independent Notch signaling. To better understand Notch signaling in C. briggsae, the LIN-12/Notch receptor was genetically manipulated in order to constitutively activate and block this signaling pathway in the vulval precursor cells (VPCs). This was done by creating sgRNAs and specific repair templates that induced mutations in the Cbr-lin-12 gene through the CRISPR/Cas9 system. We are currently using these sgRNAs and repair templates to introduce specific mutations into the C. briggsae genome. For this project we also developed novel CRISPR/Cas9 reagents and methods for use in C. briggsae, and demonstrated their efficacy, Worms were observed throughout the process to provide evidence of successful CRISPR-based transformations. By understanding these interactions more in depth, pathway interplays evidenced by nematodes can be extrapolated to orthologous pathways in humans in order to more fully comprehend the signals that lead to growth in human cells.
Category: Biological Sciences

Title: Characterization of reactive intermediates generated during cofactor assembly in a ribonucleotide reductase-like ligand-binding oxidase

Student Presenter: Zachary Smith

Faculty Advisor: Shafaat, Hannah

Abstract: Non-heme diiron carboxylate proteins play important roles in diverse biological functions, ranging from hydrocarbon oxidation to DNA synthesis to intracellular signaling. Ribonucleotide reductase-like ligand-binding oxidase (R2lox) is a member of this family that performs multi-electron oxidative chemistry via a metallic binuclear active site. R2lox incorporates manganese and iron cofactors in two distinct configurations: one utilizing a heterobinuclear manganese-iron active site and another incorporating a homobinuclear diiron cofactor. However, the mechanism of assembly of these bimetallic cofactors has not been identified. To better understand the chemical processes underlying the assembly of R2lox, UV-visible spectroscopy was used to evaluate the kinetics of assembly of an R2lox mutant strain as well as identify potential intermediates in the reaction. Stopped-flow (SF) UV-visible and rapid freeze-quench electron paramagnetic resonance (EPR) spectroscopies were used to further characterize reaction intermediates. Preliminary results suggest that the kinetics of assembly and intermediate formation in the mutant protein are markedly different from wild-type (WT) R2lox. Compared to the WT protein, the Y175F variant assembles with slower rates, and the optical signatures of intermediates are blue-shifted relative to WT. Data collected from the UV-vis will resolve the kinetics of these processes down to the millisecond timescale, which will help distinguish similarities and differences of assembly, in addition to highlighting the intrinsic properties of Y175F. EPR spectroscopy will help identify reaction pathways and intermediates, depending on the magnetic properties of the resulting species generated. Incorporating new kinetic information along with identities of distinct reactive intermediates in Y175F in addition to other R2lox mutants will reveal the mechanism of incorporation of binuclear cofactors in R2lox.
Abstract: Increasing sea surface temperatures, a result of anthropogenic global change, is causing an increase in the frequency and severity of mass coral bleaching events. When heat stressed, corals expel their photosynthetic algal endosymbionts that provide them with fixed carbon to meet metabolic energy requirements. In the absence of endosymbionts, corals with high levels of stored energy reserves (lipids, carbohydrates, and protein) and corals that acquire energy through heterotrophy are known to have increased survival and resilience potential. To evaluate how lipid management can infer resilience, I measured changes in lipid class composition in repeatedly bleached (treatment) and non-bleached corals (control) of three species (O. faveolata, P. astreoides, and P. divaricata) across several recovery time points over two years. In experimentally bleached O. faveolata, phospholipid concentrations decreased by about half, which might correspond to cell loss associated with bleaching. However, after repeated bleaching, O. faveolata had phospholipid concentrations comparable to controls and cholesterol concentrations increased steadily, results that perhaps are associated with increased heterotrophic feeding on zooplankton. Baseline concentrations of cholesterol were higher in P. astreoides than in O. faveolata and P. divaricata. These initial findings suggest that these three species manage their lipid reserves differently under heat stress, possibly a reflection of switches between photoautotrophy and heterotrophy. We are currently measuring more lipid classes (tri- di- & mono-acylglyceride, free fatty acid, glycerol and wax ester) to have a comprehensive record of coral lipid management under repeated heat stress, to describe resilience strategies following annual bleaching events.
Abstract: Deposition of extracellular materials next to a cell can protect this cell, change its growth and morphology, or help it to move and communicate with other cells. To perform such important functions, extracellular materials must be deposited very precisely, but how such precision is achieved is mostly unknown. A beautiful example of an extracellular structure with very precise deposition is the wall surrounding pollen grains. Pollen from different species often look remarkably different - in part, because wall materials are deposited at some regions on pollen surface and absent from the others. The regions where the wall is absent are called apertures, and these structures help pollen perform its reproductive function. Across species, pollen apertures often differ in shape, number, and positions. Within a species, aperture patterns are usually the same suggesting that developing pollen consistently marks specific surface regions as different from the rest of the surface, ensuring that only they will develop into apertures. Previously, only one gene, INP1, was known to influence pollen aperture formation. In order to identify other players involved in this process, we performed a mutagenesis screen in Arabidopsis. Aperture mutants forming five new complementation groups were isolated by microscopy. We then used positional cloning to identify genes affected in four of these mutants. To do this, mutants in the Landsberg erecta background were crossed with wild-type plants from another Arabidopsis accession, Columbia, and the genes were mapped using PCR markers that could distinguish between Landsberg and Columbia genomic sequences. Four genes potentially involved in pollen aperture formation were identified: MACAROON (encoding an ELMO/CED-12 domain protein), SRF2 (a receptor-like kinase), and two novel genes, INP2 and DONUT. Further characterization of these genes can provide important clues for understanding the molecular mechanism of pollen aperture development and generation of distinct cellular and extracellular domains.
Abstract: Plasmodium parasites, the causative agent of malaria, were responsible for 438,000 deaths in 2015, Plasmodium falciparum causing the most deadly form. Atg8, a small, ubiquitin-like protein, is commonly the central marker of autophagy, a cellular recycling pathway. While the autophagy pathway is limited in Plasmodium, having only five associated genes, Atg8 appears to have a novel function in these parasites, localizing to the apicoplast, a remnant plastid organelle. In both autophagy and localization to the apicoplast, Atg8 is conjugated to the lipid phosphatidylethanolamine (PE) at a conserved C-terminal glycine. Using the CRISPR/Cas9 system, this essential glycine was mutated to alanine, disrupting lipidation and impairing Atg8’s normal function. Data from the related parasite, Toxoplasma gondii, suggest an essential role for Atg8 in autophagy, as well as in homeostasis of both the apicoplast and the mitochondrion. The glycine to alanine mutant displays a slow-growth phenotype, loss of the apicoplast, and fragmentation of the mitochondrion. Our P. falciparum data are consistent with these findings, showing a dramatic slow-growth phenotype and morphological abnormalities. Metabolically, mutants appear to be unaffected, displaying similar growth patterns with or without supplementation of the key apicoplast metabolite, isopentenyl pyrophosphate (IPP). Mutant parasites appear to form fewer daughter merozoites during schizogony, potentially attributed to impaired expansion and segmentation of the apicoplast. These data suggest that Atg8 plays an important role in ensuring normal apicoplast division into merozoites, but further characterization of these morphological differences is needed. Apicoplast expansion and segmentation appears physically coupled with the same processes in the mitochondrion. Apicoplast-deficient parasites may also show similar mitochondrial defects. These defects will be explored using drug sensitivity assays targeting mitochondrial (atovoquone/proguanil) and apicoplast (tetracycline) functions. Definition of the role of Atg8 in P. falciparum could elucidate new therapeutic avenues, potentially slowing parasite growth within the realm of immune system control.
Category: Biological Sciences

Title: The role of fast muscle myosin light chain mylpfa in muscle function and integrity

Student Presenter: Emily Teets

Faculty Advisor: Amacher, Sharon

Abstract: Structural proteins expressed in muscle fibers vary among muscle types; this allows for variability in muscle contraction strength and endurance. Different myosin light chains, proteins key to muscle contraction, are expressed in fast and slow muscle fibers, large multinucleated muscle cells. This suggests that the light chains may help tune the function of these fiber types. The function of these proteins has been previously studied in vitro and in vivo using mouse and Drosophila models; however, the developmental role of myosin light chain genes hasn't been closely examined, nor the specific functions of the four zebrafish fast muscle-expressed myosin light chain genes. We hypothesized that mylpfa, one of these four genes, is necessary for formation of normal intracellular muscle contractile units (sarcomeres), and thus muscle function, but not for muscle fiber formation. Using CRISPR-mediated mutagenesis, we created a null mutation in mylpfa. Consistent with our hypothesis, fast muscle fibers form in mylpfa mutants, though the sarcomeres within these fibers do not form normally. In contrast, mylpfa mutant slow muscle fibers appear normal. Muscle function in mylpfa mutants is differentially affected depending upon myofiber composition; the pectoral fin, composed primarily of fast fibers, is paralyzed, whereas the trunk, comprised of both fast and slow fibers, are still contractile. As the embryo grows, fast fibers degenerate in mylpfa mutants, first becoming evident by 76 hours post fertilization (hpf). Our results demonstrate that mylpfa is dispensable for initial muscle fiber formation but essential for assembly of the sarcomeres in these fibers. The degeneration of mylpfa mutant fast muscle fibers indicates that mylpfa promotes muscle fiber integrity. Our studies of mylpfa mutants indicate a previously unappreciated cellular mechanism that preserve muscle integrity, via the sarcomere. Further investigation into this mechanism may help explain degenerative muscle diseases, particularly those caused by defects in sarcomeric proteins.
Abstract: Polyploidy is a phenomenon that involves the duplication of an organism's entire genome and occurs most commonly in plants. These duplication events make it especially difficult to understand the genetic ancestry among closely related polyploids. Penstemon (Plantaginaceae) is the largest genus of plants native to North America, and has several sections of species that are predominantly polyploid. Our study group, the P. attenuatus species complex, contains three regional varieties distributed in the Pacific Northwest of North America. Each of these varieties (var. attenuatus, var. militaris, and var. pseudoprocerus) is hypothesized to be a polyploid hybrid (allopolyploid) between two other species. To evaluate these hypotheses, we collected genetic data using amplified fragment length polymorphism (AFLP) DNA fingerprinting techniques for all members of the complex. For each variety and its putative parents, we evaluated their hypothesized relationships using clustering analyses based on genetic distances (principle coordinates analysis, neighbor joining trees) and genetic ancestry mixture models (STRUCTURE). Our pilot study tested one of the triplets involving P. attenuatus var. pseudoprocerus. Our results showed that it is more closely related to one putative parent (P. procerus) than the other parent (P. albertinus), which does not support our original hypothesis. We have expanded our study to include over three hundred individuals from thirty localities comprising all varieties plus their purported parental species. Testing these hypothesis will reveal the relationships among the species in our study group and will also demonstrate the analytical tools that can be used to understand the origins of allopolyploid species.
Abstract: Salmonella is a foodborne pathogen that causes millions of cases of salmonellosis globally every year. Because Salmonella-specific drugs are not available, there has been a search for unique metabolic pathways in this pathogen. It was recently discovered that Salmonella could utilize fructose-asparagine (F-Asn), a naturally occurring Amadori product, as its sole carbon and nitrogen source. Utilization of F-Asn requires an asparaginase, a kinase, and a deglycase. In addition to Salmonella, these enzymes appear to be present only in Citrobacter and Clostridia, indicating a limited distribution in the animal gut microbiome. Salmonella mutants lacking the deglycase (encoded by FraB) are up to 100,000-fold attenuated in mouse models of intestinal inflammation. This phenotype has been attributed to the build-up of 6-phospho-fructose-aspartate (6-P-F-Asp), which is typically converted to glucose-6-phosphate and aspartate by FraB. Collectively, these findings highlight the value of FraB as a potential Salmonella-specific drug target. Towards this goal, we initiated biochemical characterization of recombinant FraB. We first determined the optimal pH and temperature for its activity, and the Michaelis-Menten kinetic parameters for the conversion of 6-P-F-Asp to glucose-6-phosphate and aspartate. To understand the substrate-recognition determinants of FraB, we then compared the deglycase activity towards 6-P-F-Asp (L-isomer) vs. 6-P-F-Glu or 6-P-F-Asn, as well as the D-isomer of 6-P-F-Asp. To test a catalytic mechanism that we postulated, we chose for site-directed mutagenesis eight residues guided by sequence alignment of FraB homologs and spatial proximity of residues in a homology-based tertiary-structure model. Activity assays of these mutants revealed amino acid residues in the putative active site of FraB that are essential for activity in vitro. To gain detailed structural insights, we have initiated FraB crystallization. Results from these ongoing studies represent the first analysis of the biochemical properties of the Salmonella FraB deglycase, and provide a framework for exploring FraB as a drug target.
Abstract: The cell cycle is the process through which cells divide and either produce two identical cells (mitosis) or four gametes (meiosis). The expression of the major factors that drive the cell cycle is largely regulated by the E2F family of transcription factors. An essential group of E2F targets are the Cyclins, a family of regulatory proteins that activate cyclin-dependent kinases and control the progression of the cell cycle. This includes Cyclin A2 (Ccna2/CCNA2), an important cyclin in the S-phase of the cell cycle that has been found to be misregulated in certain types of cancer. In order to study the importance of E2F-mediated regulation of Ccna2 in vivo, we generated mice in which a key E2F site in the Ccna2 promoter is mutated and E2F binding is abolished, creating a Ccna2 promoter that is not responsive to E2F regulation. Although mice were viable and appeared healthy, males exhibited testicular atrophy and were infertile. Examination of the testes revealed a three-fold decrease in testes weight in adult mice, while histological analysis of the testes revealed a progressive loss of differentiating spermatocytes in juvenile mice and a partial recuperation in adults. Using quantitative PCR and immunohistochemistry, we measured the expression of CCNA2 and stage-specific markers of spermatogenesis during the first weeks of development and in adult mice. This revealed abnormal expression of Ccna2 and downregulation of late meiotic markers. Collectively, the data suggests that E2F regulation of Ccna2 is important for the proper progression of the 1st round of spermatogenesis and consequentially, fertility. This brings us closer to obtaining a better understanding of spermatogenesis and how the cell cycle is regulated, which could lead to personalized gene therapies that can aid those with many genetic diseases (including infertility and cancer).
Category: Biological Sciences

Title: Targeting p38 MAPK mediated phosphorylation of MITF results in reduced osteoclast differentiation due to down regulated Vav3 expression

Student Presenter: Anh Tran

Faculty Advisor: Sharma, Sudarshana

Abstract: Bone, a dynamic tissue, undergoes constant remodeling throughout the lifetime of the vertebrate organism. Bone remodeling occurs through balanced actions of bone formation by osteoblasts and bone resorption by osteoclasts. Osteoclasts are formed from myeloid precursors. Two cytokines, colony-stimulating factor-1 (CSF-1) and receptor activator of NF-kB ligand (RANKL) are sufficient for the differentiation of osteoclasts from myeloid precursors in vitro. The downstream cascade results in the expression of effector genes through transcription factors (TFs). One main TF is Microphthalmia-associated transcription factor (MITF), which is phosphorylated at serine 307 by p38 MAPK. Inhibition of p38 MAPK also inhibits osteoclast differentiation. An imbalance in bone formation and resorption mainly due to excessive osteoclast differentiation and function leads to bone diseases like osteoporosis. Approximately 2/3 of adult female population is susceptible to osteoporosis in the US. Available therapeutics target osteoclasts and cause osteoclast apoptosis, which again results in inadequate bone remodeling. We used mouse genomics and genetics to evaluate the RANKL-specific target of MITF. The MITFce mouse model, without the p38 MAPK phosphorylation and binding site of MITF, showed age resolved osteopetrosis as a result of defective osteoclast differentiation and function. To evaluate whether over-expression of p38 MAPK docking site with a mutation in the phosphorylation site could sequester available p38 MAPK from phosphorylating endogenous MITF, we over-expressed a short plasmid construct encoding the p38 MAPK binding domain of MITF along with a control plasmid. This mimicked the in vitro differentiation program from the MITFce mouse model. Our result indicates that by ablating MITF phosphorylation alone by p38 MAPK, we could modulate and reduce excessive osteoclast differentiation and function. This could be due to the down regulation of Lcp2 and Vav3 genes that are osteoclast specific regulators or Rac1. With further research, the short peptide could potentially act as a therapeutic intervention for osteoporosis.
Abstract: While the system for texture perception on the dermis has been well researched, there has been little work done on texture perception in the oral cavity. This study seeks to compare edge and point sensitivity of the anterior tongue to the fingertip by using a simple letter identification task. It is hypothesized that the tongue will be better at edge and point detection as the ability to evaluate objects within the oral cavity in the absence of visual cues plays a vital role in chewing and swallowing. Small, plastic alphabet letters of various sizes were created and bound to tongue depressors. During the experiment, thirty subjects were asked to close their eyes, assess a letter stimulus using either the fingertip or the anterior tongue, and respond with the identity of the letter they perceived. In the first part, they were presented with a stimulus and asked to lick it with the anterior one third of their tongue. This process was repeated with their fingertip. If correct, subjects were given the next smallest letter size in the next round; if incorrect, they were given the next largest letter. In order to analyze the data collected, the detection threshold will be calculated as the mean of eight reversals along the ladder. Binomial statistics will be used to determine if people are significantly more accurate with their tongue than with their fingers. This data will confirm whether the tongue or the fingertip has a greater ability to detect edges and points. Results from this study, combined with data from future comparative tactile sensitivity studies using other stimuli and oral surfaces, will help to build a better picture of the mechanisms of texture perception of oral tissues. This knowledge can, in turn, provide insight into how texture perception may influence food preferences.
Category: Biological Sciences

Title: Geographic patterns of floral morphology change in Penstemon albidus and Penstemon whippleanus (Plantaginaceae)

Student Presenter: Morgan VanDeCarr

Faculty Advisor: Wolfe, Andrea

Abstract: Pollinators are one of the major selective agents in the evolution of flower morphology. Numerous studies have shown that changes in floral morphology are related to changes in pollinators. We used digital herbarium specimens to measure nine floral characters indicative of pollinator specificity (plant height, basal leaf length, mid leaf length, tip length, corolla length, corolla tube width, corolla throat width, corolla mouth width, and inflorescence length) in Penstemon albidus and P. whippleanus. To assess the level of correlation for geographic distribution and morphological data, we used several R software packages (gstst, lattice,spatial,sp,rgdal). The most variable characters in both P. albidus and P. whippleanus were inflorescence length and plant height. Corolla tube width and corolla mouth width were the least variable in P. albidus while in P. whippleanus, corolla throat width and corolla length showed the least amount of variation. The analysis of spatial correlation of morphological data with geographic distribution indicated that in P. albidus corolla mouth width, tube width, and throat width sizes increase as individuals move from East to West and from South to North. The other characters showed a slightly less pronounced trend. Environmental factors such as temperature influence flowering time so that plants with northern distributions flower later than those in the south. This results in a more limited time for pollinator visits and consequentially reduces gene flow among northern populations. In P. whippleanus, the Rocky Mountains played an important role in the distribution of floral characters. West of the Rockies, individuals are larger than in the East for most characters. This trend is less obvious in the corolla length and tip leaf length. These preliminary results suggest abrupt changes in topography represent a stronger barrier to gene flow than flowering time and pollinator interaction.
Abstract: Melanoma, the deadliest form of skin cancer, has a 5-year survival rate of 15% for stage IV patients. Melanoma mortality is largely attributable to its metastatic potential. Thus, understanding the molecular mechanisms that drive melanoma metastasis is particularly important. P21 Activated Kinases (PAKs) are commonly overexpressed and altered in a variety of cancers and regulate mechanisms important for cancer development including: cell movement, growth, and apoptotic resistance. There are two types of PAK proteins: group I (PAK1, 2, and 3) and group II (PAK4, 6, and 7), divided based on sequence homology and distinct regulatory properties. PAK7 is the most frequently mutated PAK in melanoma, yet, the role of this protein in melanoma progression is unknown. We tested the hypothesis that PAK7 mutants promote melanoma initiation and metastasis through inappropriate activation of downstream signaling pathways. We generated stable melanocyte cell lines expressing melanoma-associated PAK7 mutants, kinase-dead, and kinase-hyperactive mutants. We tested the effects of these alterations on proliferation, migration, and downstream signaling. We observed no changes in signaling through the mitogenic MAPK pathway and proliferation was unaltered. By contrast, we observed a correlation between PAK7 kinase activity and migration. This lead us to perform mass spectrometry to identify PAK7 interacting partners involved in cell motility. This work identified GEF-H1, a guanine nucleotide exchange factor that activates Rho and Rac-GTPases, both major players in cell motility. We validated this interaction in our melanocyte stable lines and observed increased PAK7 kinase activity resulted in increased phosphorylated GEF-H1. Further, we identified the region within PAK7 necessary for GEF-H1 interaction. Together, this work suggests a model wherein PAK7 functions through GEF-H1 to influence cell motility. Future work will explore the role of PAK7:GEF-H1 interaction in melanoma metastasis. Understanding the role of PAK7 in melanoma metastasis will improve diagnostic capabilities and provide novel therapeutic targets.
Abstract: ETS-1 is a transcription factor known to play an important regulatory role in lymphoid cells. In humans, ETS-1 is most highly expressed at the mature stage of Natural Killer (NK) cell development. This stage is characterized by the acquisition of several activating receptors including NKp46. Previously, global ETS-1 knockout models have been used to show a reduced and defective NK cell population compared to wildtype. However, this model leaves out whether ETS-1 functions in an extrinsic or intrinsic manner and does not address the differential expression at different stages in development. This project aims to determine if there is an intrinsic role of ETS-1 in the development and function of NK cells with an NKp46-Cre conditional ETS-1 knockout mouse model. A multi-color flow cytometry panel will allow for the dissection of NK cell populations at all stages of development. Differential gene expression will be examined for a mechanistic analysis of the role of ETS-1. Results from these assays will be a starting point for further evaluating the defects caused by ETS-1 deficiency at the mature NK cell stage. Taken together this project aims to elaborate on the role of ETS-1 in NK cell development. This research will constitute a critical step forward in understanding the biology and mechanisms underlying mature NK cell regulation of development. Furthermore, continuing to better understand transcription factor ETS-1 is essential when dealing with diseases mediated by lymphoid cells. Unique to this project is the development of a stage and cell specific deletion of ETS-1 in maturing NK cells. The use of an NKp46 Cre promoter ensures that only NK cells that have reached the mature stage become deficient in ETS-1. This presents the opportunity to analyze how ETS-1 regulates mature NK cells unlike previous studies.
Category: Biological Sciences

Title: Examining the origins of the polyploid Penstemon attenuatus species complex

Student Presenter: Emiko Waight

Faculty Advisor: Wolfe, Andrea

Abstract: Polyploidy is a phenomenon that involves the duplication of an organism's entire genome and occurs most commonly in plants. These duplication events make it especially difficult to understand the genetic ancestry among closely related polyploids. Penstemon (Plantaginaceae) is the largest genus of plants native to North America, and has several sections of species that are predominantly polyploid. Our study group, the P. attenuatus species complex, contains three regional varieties distributed in the Pacific Northwest of North America. Each of these varieties (var. attenuatus, var. militaris, and var. pseudoprocerus) is hypothesized to be a polyploid hybrid (allopolyploid) between two other species. To evaluate these hypotheses, we collected genetic data using amplified fragment length polymorphism (AFLP) DNA fingerprinting techniques for all members of the complex. For each variety and its putative parents, we evaluated their hypothesized relationships using clustering analyses based on genetic distances (principle coordinates analysis, neighbor joining trees) and genetic ancestry mixture models (STRUCTURE). Our pilot study tested one of the triplets involving P. attenuatus var. pseudoprocerus. Our results showed that it is more closely related to one putative parent (P. procerus) than the other parent (P. albertinus), which does not support our original hypothesis. We have expanded our study to include over three hundred individuals from thirty localities comprising all varieties plus their purported parental species. Testing these hypotheses will reveal the relationships among the species in our study group and will also demonstrate the analytical tools that can be used to understand the origins of allopolyploid species.
Abstract: High throughput next generation gene expression analysis (RNA-seq) has been widely accepted by basic/translational scientists and physicians in bench top research, drug development and patient diagnosis and prognosis. With this wide acceptance, this research space is filled with new kits for RNA isolation and for sequencing library generation. Often times, different protocols were used to generate RNA-seq data across vast time spans and by different staff members or from different labs/cores/companies. Before one commits to perform computation resource intensive analyses, it is important to establish that the RNA-seq data of interest are comparable in qualities and overall profiles. QuaCRS II fills this gap by employing a database of computed quality control (QC) metrics for this important assessment. This allows users to interactively drill down on QC and read distribution parameters from disparate sets of data via a web-based viewer with ease in real time. In addition to enabling on-demand statistical comparison between groups, QuaCRS II is now supported by a configurable data model and newly updated tools for QC generation. The utility of QuaCRS II is demonstrated with data released in a recent multi-center RNA-seq standardization study conducted by the Association of Biomolecular Resource Facilities (ABRF).
Category: Biological Sciences

Title: Update on conservation genetics of Penstemon caryi (Plantaginaceae) around the Tensleep Preserve (Wyoming) and Pryor Mountains (Montana)

Student Presenter: Alexander Ward

Faculty Advisor: Wolfe, Andrea

Abstract: Penstemon caryi is a rare plant species endemic to two mountain ranges in northern Wyoming and southern Montana. It exhibits a fragmented distribution, appearing only on limestone outcrops and talus slopes, making it a target for potential conservation efforts. Our previous work in the Tensleep Preserve area highlighted the need to continue monitoring these populations. Recently, new samples of P. caryi have been collected from the Pryor Mountains of Montana. We assayed these new samples using seven microsatellite markers as well as two AFLP primer sets in order to estimate population genetic diversity, differentiation, and gene flow compared to the previous nine populations studied. The data collected were analyzed using the programs GenAlEx and STRUCTURE. Results of the STRUCTURE analysis show that the Pryor Mountains population is more genetically distinct compared to the Tensleep populations. Analyses with GenAlEx also show that the Pryor Mountains population has a greater number of unique genetic variants compared to the rest of the populations. Furthermore, the Pryor Mountains population has the lowest average heterozygosity (Ho=.449) across all loci, suggesting that those plants may be at greater risk of extirpation due to low levels of genetic diversity. While all populations of P. caryi should be monitored and protected due to the rarity of the species, these analyses suggest that conservation efforts for the Pryor Mountains populations need to be made a priority due to the population being a source of distinct genetic diversity for the species.
Category: Biological Sciences

Title: Understanding the molecular basis of multiple mitochondrial dysfunctions syndrome 1: impact of mutation on the structure and function of NFU1

Student Presenter: Nathaniel Wesley

Faculty Advisor: Cowan, James

Abstract: Studying novel proteins and their behavior in disease states often leads to elucidation of their function in vivo and provides a platform to guide drug development and treatment efforts. Recent patient studies have shown that a fatal mitochondrial disease, multiple mitochondrial dysfunctions syndrome 1 (MMDS1) arises from two distinct genetic mutations near the active site of the essential iron-sulfur (Fe/S) protein NFU1. Symptoms of MMDS1 include impairment of neurological development, lactic acidosis, failure to thrive, and ultimately death in infancy - all indicative of a general failure of the metabolic system. Reduced function of target Fe/S proteins lipoic acid synthetase (LIAS) and succinate dehydrogenase (SDH) in the MMDS1 disease state suggests that multiple metabolic pathways require proper function of the native NFU1 protein through Fe/S cluster delivery and trafficking. However, the precise function of NFU1 remains unknown, and there is no treatment or cure for MMDS1. To investigate the molecular consequences of the disease-causing Gly208Cys and Gly189Arg mutations, the structure and function of the mutant human NFU1 protein forms were analyzed in vitro using a suite of biochemical techniques and compared to data obtained for the native construct. In the case of G208C NFU1, the mutation initiates a global structural change, which alters the monomer-dimer equilibrium such that it is unable to receive an Fe/S cluster from physiologically-relevant sources. Preliminary data suggests a similar phenomenon could be occurring in the case of G189R NFU1. In order to complement study of these mutant constructs, selective mutations at positions 189 and 208, along with cluster-binding residues at positions 210 and 213 have provided an in-depth investigation into the effects of mutation on Fe/S cluster coordination and transfer, and corroborated conclusions drawn from study of the G208C and G189R constructs.
Category: Biological Sciences

Title: Functional selection of methylthioribulose-1-phosphate aldolase genes for anaerobic methionine salvage

Student Presenter: John Wildenthal

Faculty Advisor: Tabita, F. Robert

Abstract: In cell division, methionine is used in polyamine synthesis, resulting in a toxic, sulfur-containing byproduct, 5-methylthioadenosine (MTA). As biologically available sulfur is typically limiting, many organisms possess a Methionine Salvage Pathway (MSP) to detoxify MTA and recycle the sulfur back to methionine. Nearly all eukaryotes and many prokaryotes employ the “universal” MSP, which requires molecular oxygen. Recently, our group discovered the first oxygen-independent MSP in Rhodospirillum rubrum that functions both aerobically and anaerobically, as well as a second, strictly anaerobic MSP. The strictly anaerobic MSP utilizes in part an operon encoding three enzymes that sequentially metabolize MTA to methylthioacetaldehyde (MTAdh). A yet to be elucidated enzyme(s) then converts MTAdh to methionine, creating ethylene gas (C2H4) as a byproduct. The third enzyme, a novel methylthioribulose-1-phosphate (MTRu-1P) aldolase cleaves MTRu-1P (an MTA-derived metabolite) to form MTAdh. Based on amino acid sequence similarity, at least 320 additional bacterial species contain a putative MTRu-1P aldolase that may function as part of an anaerobic MSP. In this study we have explored the functionality of MTRu-1P aldolase homologs from enteric bacteria Eubacterium limosum and Morganella morganii via a gene complementation in R. rubrum. The native MTRu-1P aldolase in R. rubrum was knocked out, disrupting the strictly anaerobic MSP, thus causing ethylene to be marginally produced. The putative aldolases of interest were then cloned into complementation plasmids able to express the provided gene. These plasmids were mated into the R. rubrum aldolase knockout strain, and ethylene production was measured. The E. limosum aldolase could not restore ethylene production, indicating a lack of MTRu-1P aldolase activity in E. limosum. However, the M. morganii aldolase restored ethylene production, suggesting a similar strictly anaerobic MSP may exist in M. morganii. Further studies will determine if MTRu-1P aldolase homologs from other organisms can function in a similar manner.
Title: The inhibition of WH2 domain containing proteins by ACD actin-crosslinked oligomers

Student Presenter: Blake Williams

Faculty Advisor: Kudryashov, Dmitri

Abstract: The actin crosslinking domain (ACD) is an actin-specific toxin produced by Vibrio and Aeromonas spp that catalyzes the formation of a covalent bond between actin monomers, forming non-polymerizable oligomers. Recently, our group demonstrated that these oligomers amplify their toxicity by potently inhibiting formins - important actin nucleators. Because such potent inhibition is enabled by the abnormally high affinity of oligomers for tandem actin binding domains of formins, we hypothesized that oligomers may also target other proteins with multiple actin binding domains, and thus further amplify the toxicity. One conserved G-actinâ€’binding domain found in an array of actin mediating proteins is the WASPâ€’homology 2 (WH2) domain. WH2â€’domains are found in Spire, Arp2/3 complex activators (WASP, WAVE, etc.), and bacterial toxins VopF/L. In these proteins, WH2 domains contribute to nucleation of new filaments by organizing actin monomers into filament-like orientations, either on their own (tandem WH2 domain of Spire, VopL/F) or in concert with other proteins (Arp2/3 complex). To test the hypothesis, we assessed the effects of the ACD toxin-produced actin oligomers on their nucleation activity. We employed Total Internal Reflection Fluorescence (TIRF) microscopy and pyrene actin polymerization methods to assess the effects of oligomers on the single filament and bulk levels, respectively. We found by TIRF that sub-stoichiometric concentrations of oligomers efficiently blocked VopF/L and Spire mediated nucleation. The dose-dependent inhibition of Spire, Arp2/3 activator WASP, and VopF/L-mediated actin nucleation was further confirmed by bulk solution assays. We found that actin oligomers inhibit Spire, and VopF/L with low nanomolar affinities likely owing to the tandem organization and unique geometry of the later. In contrast, the inhibition of Arp2/3 complex activation requires higher concentrations of oligomers. In conclusion, we have now identified new members of the toxicity cascade that ACD initiates by converting actin monomers into potent secondary toxins.
Category: Biological Sciences

Title: Mechanics of cadherin unbinding using coarse-grained models

Student Presenter: Lahiru Wimalasena

Faculty Advisor: Sotomayor, Marcos

Abstract: Cell-cell adhesion is mediated by calcium-dependent proteins called cadherins, which are important in neuronal connectivity and tissue integrity. Cadherins are modular proteins with large extracellular domains that have typically been modeled using all-atom molecular dynamics (MD) simulations. However, these simulations are computationally expensive and most of them only include small fragments of these cadherin extracellular domains. To overcome these limitations, we use a coarse-grained (CG) model with the MARTINI force field to study large cadherin complexes over long time scales. All-atom MD simulations were used to find optimal parameters for an elastic network model that stabilized the protein secondary structure. The CG model allowed for a 5x increase in timestep and a 10-fold reduction of system sizes. Using this model we studied the dynamics and elastic response of classical cadherins and clustered protocadherins. In these simulations, the extracellular domains of classical cadherins straighten before unbinding, while protocadherins slip past each other during unbinding. Overall, our results confirm that our model is an effective simulation tool for studying of the mechanics of cadherin complexes.
Abstract: Understanding how patterns of energy and resource allocation are related to lifetime reproductive output propel the conceptualization of evolution of life history in organisms. Animals allocate energy and resources to maintenance, growth and reproduction. One mechanism that might drive allocation of resources is phenotypic plasticity of organ size. Animals can increase or decrease the size of metabolically active organs in response to certain environmental conditions, and that might have consequences in overall energy expenditure and allocation. We studied phenotypic plasticity of organ size in females of Diploptera punctata, a viviparous cockroach. Female D. punctata feed their nymphs with a secretion produced by the abdominal epithelium, and give birth 9-12 nymphs after a gestational period of 55-60 days. We measured metabolic rate (MR) in four groups of females: (1) pregnant females at day 55 fed with a high quality (HQ) diet, (2) pregnant females at day 55 fed with a low quality (LQ) diet, (3) non-pregnant females in which we switched diets from LQ to HQ, then back to LQ in periods of 30 days, and (4) non-pregnant females in which we switched diets from HQ to LQ then to HQ again in periods of 30 days. We measured mass of digestive tract, abdominal organs, embryos in the pregnant females, and carcass. We found a positive relationship between gut mass and MR and a negative association between the mass of abdominal organs and MR. There seems to be a trade-off between gut mass and abdominal organ mass, and gut mass and reproductive output. Interestingly, females acclimated to different diets tend to adjust their physiological traits to a given environment in a manner dependent on the previous event. We suggest that changes in organ mass in response to environmental conditions determines complex patterns of allocation of resources to reproduction in D. punctata.
Category: Biological Sciences

Title: CRISPR/Cas9 genomic modification reveals effects of CD79B phosphorylation in B-Cell receptor signaling of DLBCL cell lines

Student Presenter: Allen Yi
Faculty Advisor: Davis, Richard

Abstract: Diffuse large B-cell Lymphoma (DLBCL) has two subtypes: activated B-cell (ABC), which depends on antigen-driven signals from its B-cell receptor (BCR), and germinal center B-cell (GCB), which uses its BCR in tonic signaling. A single amino acid (Y196) of CD79B, one of four proteins comprising the BCR, is mutated in 18% of ABC-DLBCL primary tumors, implying positive selection for a functional consequence. This project aims to study the mutation's effects on survival and BCR signaling by creating "isogenic" pairs of DLBCL cell lines, with vs. without CD79B mutation. The CRISPR/Cas9 system was used for precise genomic modifications. Two "cutting" plasmids expressing Cas9 protein and specificity for CD79B, and one "repair" plasmid bearing the desired CD79B sequence plus a reporter (CFP), were introduced to human GCB-DLBCL cell lines by electroporation. Knock-in created CD79B sequences that were wild-type (WT) or had Y196F, Y207F (in the same "ITAM" signaling domain as Y196), or both mutations. Flow cytometry was used to detect modified cells and measure size, growth, and antigen-driven BCR signaling (by ratiometric calcium flux). As expected, a fraction of cells (~15%) were BCR+/CFP+, indicating the desired knock-in. Initial experiments on two GCB-DLBCL cell lines showed that double-mutant cells were smaller, exhibited reduced relative growth, and had amplified calcium flux after BCR cross-linking compared to WT cells. Single mutants gave mixed results, requiring more experiments to clarify. Since F is similar to Y but cannot be phosphorylated, phosphorylation of CD79B appears to reduce antigen-driven BCR signaling, potentially providing a reason why CD79B Y196 mutations are frequent in ABC-DLBCL; this will be more directly tested by reverting Y196-mutant ABC-DLBCL lines to WT. The negative growth effect of double-mutant CD79B in GCB-DLBCL lines was unexpected, but additional techniques will be used to study these modified cells and may provide insights in tonic signaling.
Abstract: It is widely accepted that the immune system has a role in controlling and eliminating cancers. The tumor microenvironment, however, can suppress the immune response. Myeloid-Derived Suppressor Cells (MDSC) are immature immune myeloid cells that can subdue the development of important tumoricidal immune cell populations that are present in tumors. The accumulation of these MDSCs has been linked with poor prognosis in humans and tumor models. Compound X is currently used to treat B-cell malignancies, as it inhibits Bruton's Tyrosine Kinase (BTK), an enzyme necessary for B-cell development. However, X has also been reported to affect other populations, including dendritic cells, a group that activates tumoricidal cells. The literature suggests that X accelerates the rate of maturation in these cells. It has also been observed that treating mice with X decreases the MDSC population, however, the fate of these cells remains unclear. In this study, we aim to address this issue and hypothesize that inhibiting BTK reprograms immature MDSCs into dendritic cells. Bone marrow progenitor cells were cultured and differentiated into MDSCs using growth factors and tumor supernatants. Experimental groups were then treated with X on either day three or days one and three. On day five, samples were collected for cytokine release, gene/protein expression, and phenotype evaluation via ELISA, RTPCR, western blot, and flow cytometry assays. Samples were also co-cultured with splenocytes to measure T-cell proliferation. Our results thus far suggest that X-treated MDSCs acquired a phenotype similar to fully differentiated dendritic cells, consistent with our hypothesis. Understanding X's effects on non-cancer cells is crucial to being able to effectively implement the drug in patients. Our next objective is to identify the signaling pathway following BTK inhibition that leads to dendritic cell development, giving us a better understanding of these mechanisms that have significant impacts on a patient's prognosis.
Abstract: Plasma membrane repair is a highly conserved process that operates in nearly all eukaryotic cell types to restore membrane integrity after injury of the plasma membrane. Repair of the membrane is essential to restore cellular homeostasis, and compromised membrane repair can contribute to a number of disease states, including heart failure, muscular dystrophy and other neuromuscular diseases. Previous studies indicate that compromised repair capacity can exacerbate cardiac injury while enhancing membrane repair capacity can reduce cardiac pathology. In this project, we are standardizing the methodology employed for the direct measurement of the repair capacity by cardiac tissue. Thick slices of myocardium from explanted human and mouse hearts were probed using multi-photon microscopy to determine the membrane repair capacity. In these experiments we use a tissue slicer to cut thick sections (~1 mm) from an ex vivo heart while in a physiologic buffer that allows for resealing of injured cells. These tissue sections are damaged using infrared multi-photon confocal microscopy and then FM4-64 dye entry is recorded over a defined time course to measure membrane resealing. FM4-64 dye only fluoresces once it enters the cell making it a specific readout. When the membrane reseals, dye entry ceases and the fluorescent signal stabilizes. Currently, we are refining the data acquisition and processing for high throughput assay protocol.
Category: Biological Sciences

Title: A forward genetic screen in Arabidopsis identifies several new genes involved in formation of distinct cellular domains on pollen surface

Student Presenter: Prativa Amom

Faculty Advisor: Dobritsa, Anna

Abstract: Deposition of extracellular materials next to a cell can protect this cell, change its growth and morphology, or help it to move and communicate with other cells. To perform such important functions, extracellular materials must be deposited very precisely, but how such precision is achieved is mostly unknown. A beautiful example of an extracellular structure with very precise deposition is the wall surrounding pollen grains. Pollen from different species often look remarkably different - in part, because wall materials are deposited at some regions on pollen surface and absent from the others. The regions where the wall is absent are called apertures, and these structures help pollen perform its reproductive function. Across species, pollen apertures often differ in shape, number, and positions. Within a species, aperture patterns are usually the same suggesting that developing pollen consistently marks specific surface regions as different from the rest of the surface, ensuring that only they will develop into apertures. Previously, only one gene, INP1, was known to influence pollen aperture formation. In order to identify other players involved in this process, we performed a mutagenesis screen in Arabidopsis. Aperture mutants forming five new complementation groups were isolated by microscopy. We then used positional cloning to identify genes affected in four of these mutants. To do this, mutants in the Landsberg erecta background were crossed with wild-type plants from another Arabidopsis accession, Columbia, and the genes were mapped using PCR markers that could distinguish between Landsberg and Columbia genomic sequences. Four genes potentially involved in pollen aperture formation were identified: MACAROON (encoding an ELMO/CEC-12 domain protein), SRF2 (a receptor-like kinase), and two novel genes, INP2 and DONUT. Further characterization of these genes can provide important clues for understanding the molecular mechanism of pollen aperture development and generation of distinct cellular and extracellular domains.
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 from Dipteran insects: Lachesin (Lac) and Amalgam (Ama). Ama arose as a duplication of Lac in early Dipteran evolution, and both proteins still share extensive amino acid similarity. Lac, which is membrane-linked, homophilically binds itself. Ama, secreted into the extracellular matrix, has both a homophilic binding property as well as the ability to heterophilically bind 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 an interaction with Nrt. Therefore, I am identifying 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) cells. The S2 cells are then to be used for aggregation assays, which allow us to observe the binding properties of the chimeric proteins. In order to test for the S2 cell expression of these proteins, I am adding epitope tags to each chimera, making them detectable in a Western blot. Additionally, I am cloning the Ama and Lac orthologues from other Dipteran species to better understand how the unique binding properties of these two proteins have evolved. This project will help to further characterize the complex series of intercellular interactions during nervous system development.
Title: Candidate bacteriophages for phage therapy against pathogenic E. coli

Student Presenter: Paige Anderson

Faculty Advisor: Sullivan, Matthew

Abstract: Toxin-producing Escherichia coli (Shiga Toxin-producing, STEC; Enterotoxigenic, ETEC) causes severe dysentery and gastrointestinal disease in children, the elderly, and immunocompromised people. Antibiotic treatment places cellular stress on the pathogen, which can result in toxin release. Phage therapy, the use of specific viruses to eradicate a bacterial infection, is an alternative solution to antibiotics. Each bacteriophage (phage) has evolved to attach to a specific bacterial cell. The phages then replicate interiorly, eventually bursting the cell and releasing progeny phages. To investigate potential candidates for phage therapy, our study characterized phage-host interactions of four phages that infect STEC and one phage that infects ETEC. Properties of the phage-host interactions were determined via i) genomic analysis to identify various gene functions and their locations in the phage genomes; ii) adsorption kinetics to determine attachment efficiency of phages to their host; iii) one-step growth curves to measure the latent period and burst size of the phage infection; and iv) plaque morphology comparisons. One STEC phage’s gene functions were annotated as follows: 36% host takeover; 11% DNA replication and repair; 17% structural proteins; and 2% DNA packaging and cell lysis. The five phages have similar adsorption rates (29-63%) and latent periods (20-30 minutes). While the STEC phages have low burst sizes (2.15-3.80 virions/infected cell), we anticipate the burst size of the ETEC phage to be larger due to the increased size and reduced turbidity of plaques compared to STEC phages. This indicates that compared to the STEC phages, the ETEC phage is a more competitive candidate for phage therapy. We will continue to characterize its properties, aspiring to engineer an efficient infection to eradicate specific Escherichia coli bacterial pathogens.
Abstract: Everyday, we depend on our auditory system to perceive sound through mechanotransduction within our ears. When sound enters the cochlea, it deflects microscopic hair bundles made of stereocilia located at the top of sensory hair cells, causing protein filaments called tip links to pull open ion channels. Opening of these channels results in hair-cell depolarization that triggers sound perception. The tip link filaments are made of Cadherin 23 (CDH23) and Protocadherin (PCDH15), which are involved in hereditary deafness and interact tip-to-tip to form a molecular handshake essential for hearing. Here I show our work on a deafness related mutation, R113G in PCDH15, that replaces a charged arginine (R) with a glycine (G). The overall aim of the project was to perform protein crystallization on the mutated PCDH15 R113G + CDH23 complex to determine the effects of the mutation on the tip link's functionality. I started by transforming the DNA plasmids of CDH23 WT and PCDH15 R113G to express, purify, and refold the complex. The vapor-diffusion sitting-drop method was used to attempt its crystallization. After 3 weeks, crystals were obtained and "fished" for X-ray exposure. One of them was shot with X-rays to obtain a diffraction data set. Wild-type versions of the PCDH15 and CDH23 tips were used to solve for the structure of the new mutant complex using molecular replacement and the refinement of the structure is currently in progress. Once the complete crystal structure of the complex is obtained, I will use molecular dynamics simulations to characterize the change in interactions and predict the complex's strength. I expect less force should be needed to unbind the mutated protein complex as the interactions are weaker and results should show how the R113G mutation affects the PCDH15 and CDH23 interaction leading to deafness in humans.
Abstract: Iron-sulfur (Fe-S) clusters are essential for cell life and are required in all parts of the cell. In eukaryotes, the export of Fe-S clusters from the mitochondria to other parts of the cell is an important and currently ill-understood process. This project studies the role of Dre2, an Fe-S cluster protein, as a proposed part of the Fe-S cluster assembly machinery that transports Fe-S clusters out of the mitochondria. Dre2 has previously been shown to hold two clusters—a 4Fe-4S cluster and a 2Fe-2S cluster. In this study, UV-Vis and circular dichroism (CD) have shown Dre2 to take up a 4Fe-4S cluster through chemical reconstitution and release Fe-S clusters in glutathione extractions, supporting their potential to transport clusters. Incubation of Dre2 with the scaffold protein Isu was monitored by circular dichroism (CD) and demonstrated to transfer an Fe-S cluster from Isu to Dre2. Incubation of reconstituted Dre2 with Nfu also demonstrated transfer of a second cluster, likely a 2Fe-2S cluster, to Dre2. Dre2 was also reconstituted following incubation with Atm1p, a proposed mitochondrial Fe-S exporter, and a glutathione-coordinated Fe-S cluster. Transfer chemistry was again monitored by CD, showing that Atm1p may facilitate uptake of a second, likely 2Fe-2S, cluster to Dre2. Additional incubations with holo yeast Nfu, Grx3, and Isu are currently under study to elucidate the mechanism of 2Fe-2S and 4Fe-4S cluster transfer to Dre2. This preliminary data suggests Dre2 to play an important role in cellular Fe-S cluster assembly, potentially linking mitochondrial and cytosolic assembly pathways. If this transport role is confirmed, Dre2 could be further studied for links to human disease. Since Dre2 has been implicated in neurodegenerative conditions, there may be a previously unidentified link between cluster transport and diseases such as Alzheimer's and Parkinson's.
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 mRNA codons on 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 yeast, these account for 20% of pre-tRNAs. Normally, free introns are barely detectable, indicating that intron degradation occurs efficiently and rapidly. Through an unbiased screen of the entire 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. Rlg1 then ligates the two halves of the mature tRNA. Surprisingly, my studies have showed that there are at least 3 more possible mechanisms by which tRNA introns can be destroyed for the other intron-containing pre-tRNAs, and that particular introns can form circles. The fact that cells have evolved multiple tRNA intron turnover mechanisms strongly suggests that increased intron levels are unhealthy for cells. To test this, we devised a genetic approach to determine the biological consequences of aberrant accumulation of pre-tRNA introns. I introduced a multi-copy plasmid encoding a bacterial ligase, RtcB, into yeast, replacing Rlg1 in tRNA half ligation, but not phosphorylation of the intron 5' termini. As a result, the cells generate large quantities of introns that cannot be degraded, causing growth defects and cellular stress. This raises the question of potential molecular interactions of tRNA introns, as well as whether certain conditions favor cells to accumulate free introns.
Category: Biological Sciences

Title: Using molecular dynamics simulations to investigate the dynamics of human skeletal troponin C

Student Presenter: Matthew Belardo

Faculty Advisor: Lindert, Steffen

Abstract: The activity of the troponin protein complex, which is composed of three separate subunits (TnC, TnI, and TnT) is an important component of both muscular and skeletal contraction: conformational changes in this complex that occur upon calcium binding cause a shift in the location of tropomyosin, exposing myosin binding sites on the actin filament. The key to this process is the interaction between the troponin I subunit and a hydrophobic patch on troponin C. Experimental data suggests that this "sticky" patch is opened when calcium binds to the two EF-hand domains of troponin C. However, exposure of a large hydrophobic patch is extremely energetically unfavorable. Studies of cardiac troponin C suggested that the opening was not a single event, and that the open conformation was only occasionally reached by TnC without the subsequent binding of TnI, leading to the notion that the opening event is a more dynamics-based process. To investigate this, models of human skeletal troponin C were constructed using Rosetta Comparative Modelling with a number of other species' skeletal troponin as base models. Simulations were carried out in cellular conditions using NAMD, the molecular dynamics simulation program. Preliminary results suggest that, similar to cardiac troponin, calcium-bound skeletal troponin does not remain open, but instead remains in a mostly closed state for the majority of the time, while becoming more dynamic and sampling more varied conformations, but further simulations are still being run to see if this holds for >100ns. This research has the potential to change our understanding of muscle contraction at the cellular level, and has further implications for investigating the energy associated with contraction.
Category: Biological Sciences

Title: Influence of miR-155 on murine ATII cell gene expression during influenza infection

Student Presenter: Adam Bercz

Faculty Advisor: Davis, Ian

Abstract: Few diseases in public health are as threatening as influenza, a viral disease with a history of crippling global populations due to the virus' ability to mutate and introduce novel pathogenic strains. The CDC reported more than 300,000 hospitalizations in the 2015-2016 influenza season for the United States alone. Vaccines are developed annually against influenza, yet no effective treatment exists for severe cases. Previous findings show an increase of certain microRNAs (miRs) in response to influenza infection. Examining the role of these molecules, specifically miR-155, may reveal critical knowledge on disease development. This small nucleic acid's expression increases consistently over a 6 day course of influenza infection in a murine model (C57BL/6). Therefore, we believe miR-155 plays a key role in the inflammatory response to influenza infection, leading to acute respiratory distress syndrome (ARDS). Alveolar type 2 (ATII) cells are respiratory cells which produce surfactant lipid and proteins in the lung. As a primary target of influenza, these cells express high concentrations of inflammatory markers upon infection, making their isolation essential. Isolated populations are flow-sorted to obtain infected cells, recognizable due the red fluorescence of the PR8/mCherry/NS1 virus. Subsequent real-time PCR allows for quantification of miR-155 and comparison to other experimental parameters. We observed that miR-155 levels are higher in mCherry-positive ATII cells compared to mCherry-negative cells from the same lung. Target genes of miR-155, including antiviral factors such as IFNγ receptor and NF-kB regulators, were shown to differ in expression between virally infected and uninfected ATII cells within the same lung. The information drawn from this project may lead to investigating new targets for therapeutic agents which enhance the host's immune capability to fight off newly emerging strains of influenza.
Abstract: \(\delta^{\beta_{-}}\)thalassemia is an inherited form of anemia that results from insufficient expression of \(\delta^{\beta_{-}}\)globin. It is caused by a change in reading frame or a mutation that results in the premature termination of the translation of the gene. The presence of the premature termination codon initiates the degradation of \(\delta^{\beta_{-}}\)globin mRNA through a process known as nonsense mediated decay (NMD). Accumulation of decay intermediates corresponding to the 3' ends of thalassemic RNAs can be detected in erythroid cells. These 3' fragments containing either exon III or exons II and III have a structure on the 5' end seemingly identical to a typical m7GpppX cap. The purpose of this project was to evaluate if these stable mRNA fragments are translated. To test this I used site-directed mutagenesis to introduce multiple copies of the hemagglutinin (HA) tag to the very 3' end of the wild type and thalassemic \(\delta^{\beta_{-}}\)globin gene. The genes for both wild type and thalassemic beta globin containing two HA tags immediately before the normal stop codon have been inserted in the CMV-driven pcDNA3 plasmid and confirmed to contain the tags in-frame by sequencing. The goal is to use the HA tag to demonstrate expression of full-length \(\beta\)-globin protein from the wild-type gene and to determine if shortened forms of this protein are expressed from genes containing a premature termination codon. Expression and evaluation of these plasmids via western blotting is underway. Identification of HA-tagged, shortened beta globin products would show that these stable, capped mRNA fragments are in fact translated into truncated proteins, which have the potential to disrupt the oxygen-binding capacity of the hemoglobin complex and result in the anemic condition of the blood. This finding could lead to new therapies for treating persons afflicted with this form of anemia.
Abstract: Trypanosoma cruzi (T. cruzi), the causative agent of Chagas disease, is a protozoan parasite that infects 6-7 million people worldwide. Parasite cyclophilin 19 (Cyp19), is a member of the family of peptidyl-prolyl isomerases (PPIase), which catalyzes the interconversion of proteins from cis to trans conformation around proline residues. These abundant enzymes are critical to protein trafficking, cellular activation and signaling. Secreted T. cruzi Cyp19 neutralizes the anti-parasitic activity of cationic antimicrobial peptides secreted by the insect vector of T. cruzi, and plays a role in modulating parasite virulence. Our data indicates that Cyp19 is expressed in all life cycle stages of the parasite, suggesting a broader role of this protein in the biology of the parasite. The goal of the present study is to establish the biological role(s) of Cyp19 in the parasite life-cycle, and identify the residues of Cyp19 critical for enzymatic activity aiding in the development of small molecule inhibitors. Comparison of the Cyp19 sequence with cyclophilin A, the closest human homolog, highlights a unique 10 residue N-terminal domain (NTD) which we hypothesize is important for Cyp19 regulation/secretion. Both wild-type and an NTD deletion mutant of Cyp19 have similar levels of PPIase activity which is disrupted through using cyclosporine A and MM284 inhibitors. Analysis of small peptide inhibitors, based on the NTD sequence, identify promising potent inhibitors of Cyp19 and human CypA. We have also identified several point and deletion mutations which are critical to Cyp19 activity. Furthermore, we have constructed Cyp19 knock-out (KO) parasites and compared the growth rate between KO and WT parasites. KO parasites grow much slower than the wild-type indicating Cyp19's role in the viability of T. cruzi. These results provide a platform for additional studies into the role of Cyp19 function within parasite biology and the development of small molecule inhibitors.
Abstract: The Plasmid Addiction System (PAS) may be used to obtain desired gene expression without the need for co-inducers or antibiotics to maintain plasmid stability. Experiments have previously demonstrated the utility of PAS for biofuel production using Esherichia coli, but this technology has not yet been employed for the industrially significant organism Ralstonia eutropha H16. The benefit of using R. eutropha is its ability to utilize gases such as CO2, H2 and O2 to support chemosynthetic (CA) growth. The organism employs the Calvin-Benson-Bassham (CBB) cycle to convert CO2 to all the building blocks necessary for life, which can be advantageous for removing this greenhouse gas from the environment. Our experiments will use the strain H16^LS::mpspec. The genes encoding the subunits of RuBisCO, the key enzyme for CO2 fixation, have been deleted in this strain. Without RuBisCO, the cells cannot grow on CO2; however, the RuBisCO genes may be incorporated into a plasmid for gene expression, with the result that CO2-dependent growth is restored. In addition, experiments were performed to determine whether R. eutropha PAS can express non-essential genes in addition to the RuBisCO genes. Plasmid p90:LS contains the cbb promoter and RuBisCO genes from R. eutropha. p90:LS and p90:LS,lacZ were cloned and conjugated into H16^LS::mpspec. These cells were plated onto bromochloroindoxyl galactoside (Xgal) and stored in a chemosynthetic environment with 5% CO2. The cells retaining the non-essential lacZ gene turned blue in the presence of Xgal. LacZ assays showed enzymatic activity in R. eutropha. Since PAS works with lacZ, a heterologous two-gene ethanol PAS was tested in R. eutropha. Cultures demonstrated activity and initial ethanol titers of 2.0 mg/L. Experiments are currently underway to construct a longer five-gene heterologous butanol PAS. Making these alcohols in a sustainable manner could change how fuels and chemicals are made in many industries.
Category: Biological Sciences

Title: Periphyton and organic matter as an indicator of functional macroinvertebrate feeder group abundance in glacial streams

Student Presenter: Anneliese Bonn

Faculty Advisor: Lanno, Roman

Abstract: One of the important aspects of modern ecological research is determining the effects of global warming on ecosystems. Specifically, this includes understanding the complex relationships among intrinsic environmental factors, trophic relationships, and the biodiversity and abundance of the species living in the environment. The purpose of this research was to determine levels of periphyton and allochthonous organic matter in relation to physical characteristics (flow velocity, substrate type) of glacial stream microhabitats (riffles, runs, rapids, and pools), and assess how these parameters affect insect community structure during summer and winter seasons in a glacier stream in Yunnan, China. The amount of periphyton and organic carbon present is hypothesized to have a positive relationship with the abundance of primary feeders and shredder functional feeder groups, respectively. The abundance of primary feeders and shredders will positively affect the abundance of predators. Periphyton will be sorted from microhabitat samples and volume estimated by water displacement. Organic carbon was measured using ash free dry mass. Taxonomy and macroinvertebrate abundance data was provided for the analysis. It is predicted that the macroinvertebrate biodiversity and abundance will be higher in surber samples where periphyton and organic carbon levels are high. Understanding baseline trophic structure is paramount to determining stream health as it is impacted by climate change. Even though macroinvertebrate diversity is a common bioindicator of stream health, this project aims to contribute further understanding to the structure of macroinvertebrate communities as related to climate change in glacier streams, which are critical but lesser explored ecosystems.
Title: Evaluation of human NKG2D signaling pathway functionality in mice to create human NKG2D KI mouse model

Student Presenter: Alexandria Carter

Faculty Advisor: Chan, Wing

Abstract: Novel target development for immune-based cancer therapies is an active area of clinical interest. NKG2D is a natural killer (NK) cell receptor that can be targeted with a bispecific antibody (BiKE) targeting multiple myeloma. In humans, NKG2D (hNKG2D) signals through the adaptor protein DAP10 to initiate cell cytotoxicity and IFN-γ release. We seek to develop an immunocompetent mouse system with hNKG2D knocked-in (KI) to determine the efficacy of human BiKE for the treatment of multiple myeloma. Studies show human NKG2D colocalizes with murine and human DAP10, but pathway functionality is unknown. We provide evidence that despite 76% homology between mouse and human DAP10 and sharing the same PI3K activation domain, hNKG2D cannot initiate downstream signaling with murine DAP10. In mice, DAP10 controls cell cytotoxicity while adaptor protein DAP12 controls IFN-γ release. Murine adaptor protein specificity of mNKG2D has been traced to the transmembrane domain, so chimeric constructs were created to evaluate the importance of the murine transmembrane domain for human NKG2D signaling. Three NKG2D+ cell lines were created by viral transduction of murine NK cells with cDNA from human and murine NKG2D utilizing: 1) hNKG2D DNA, 2) hNKG2D ectodomain with murine NKG2D transmembrane and intracellular domains, and 3) hNKG2D DNA with the murine NKG2D transmembrane domain. NKG2D signaling was evaluated via cytotoxicity and IFN-γ assays. We hypothesize our human NKG2D cell line will initiate cell cytotoxicity while our chimera cell lines will initiate cell cytotoxicity and IFN-γ release. Our results confirmed hNKG2D cannot signal through murine DAP10/12 as there was no IFN-γ release upon anti-human NKG2D activation, and no significant difference in cell cytotoxicity (hNKG2D KI: 11.5% versus negative control: 11.6%). Chimeric cell line testing continues and further research is needed to determine the specific NKG2D signaling pathway defects to test BiKE effectiveness in vivo.
Category: Biological Sciences

Title: Investigating a link between RNA metabolism and endogenous gene silencing

Student Presenter: Alissa Cullen

Faculty Advisor: Slotkin, Keith

Abstract: Transposable elements (TEs) are segments of DNA that are able to duplicate in or move throughout a host genome creating mutations and posing a threat to that organism's genome. Consequently, organisms have evolved defense mechanisms to silence TEs. Silencing of TE activity occurs post-transcriptionally through TE mRNA degradation and transcriptionally through modification of the TE chromatin. This transcriptional silencing is mediated by small RNA-directed DNA methylation (RdDM), which establishes and maintains the silencing of TEs. Previously, our lab discovered that the post-transcriptional regulation of TEs is connected to the establishment of TE transcriptional silencing through a pathway we named RDR6-RdDM. Understanding how this pathway functions will allow us to target RdDM and silencing to any region of the genome. Two key proteins in this pathway that have been identified are ARGONAUTE6 (AGO6) and RNA-DEPENDENT RNA POLYMERASE 6 (RDR6) and have been the subject of detailed investigation. To develop a further understanding of the precise mechanism and all proteins involved in RDR6-RdDM, first a reverse genetics screen was completed of known AGO6 interacting proteins to identify two new proteins involved in the RDR6-RdDM pathway. Data will be presented on the progress of this reverse genetics screen, which identified two new key proteins involved in distinct aspects of RdDM. Data will also be presented connecting RDR6-RdDM to mRNA quality control. In quality control mRNA decapping mutants, many mRNAs feed into RNAi and we believe RdDM. Data from this project has further characterized proteins involved newly the discovered RDR6-RdDM pathway, and links the TE entry into RDR6-RdDM to mRNA quality control mechanisms.
We previously surveyed Ohio honey samples and quantified soybean content using traditional microscopic palynology. We found the proportion of soybean pollen to be related to the amount of soybean cultivation surrounding the apiary. However, given the difficulty of identifying pollen to the species level using microscopy, we applied a molecular method to confirm the presence of soybean pollen. We extracted and analyzed pollen DNA from eight honey samples and a positive control sample of soybean (Glycine max) leaf tissue. PCR products amplified with soybean-specific primers and gel electrophoresis was used to confirm the presence of G. max in honey. Our results indicate that soybean pollen is present in some honey samples. However, we detected G. max in the sample from which the most honey, 21 grams, was extracted. Thus, pollen extracted from a larger volume of honey may be more likely to yield G. max DNA.
Abstract: Cardiovascular disease is the leading global cause of death, claiming upwards of 17.3 million lives each year. Vascular-associated diseases such as diabetes and hypertension exhibit defects in blood vessel formation and function. Currently scientists are attempting to define these blood vessel anomalies at the molecular level to identify key mechanisms in disease progression and offer avenues for novel treatment. Blood vessels are comprised of two types of cells, endothelial cells and smooth muscle cells. Proper communication between these cells is vital for ensuring normal vessel formation and function. The Notch signaling pathway has been implicated in this cell-cell communication. The Notch pathway is activated when smooth muscle cells are cultured in the presence of endothelial cells. My preliminary studies demonstrate that the protein Cytoglobin, which is implicated in stress regulation, is upregulated in co-culture conditions as well. I hypothesize that Cytoglobin is regulated by Notch signaling and functions in maintaining vascular homeostasis. To test this, I have shown that blocking the intracellular signaling of the Notch pathway inhibits Cytoglobin expression. Currently, I am testing whether Notch is necessary for Cytoglobin expression by chemically deleting the Notch receptor. Next, I will test whether Notch activity is sufficient for Cytoglobin expression by introducing an activated form of the Notch receptor into smooth muscle cells. I will then test the function of Cytoglobin using proliferation, differentiation, and contractile assays to determine its role in vascular function. Smooth muscle cell phenotypes, such as proliferation, contribute to disease conditions. Therefore, evaluating the function and regulation of Cytoglobin in the context of smooth muscle cell function will contribute to our current understanding of cellular communication in the vasculature and provide new insight into therapies for vascular diseases.
Category: Biological Sciences

Title: Investigating the functional relationships between three novel microtubule-associated proteins during intracellular transport in Aspergillus nidulans cells.

Student Presenter: Angela Davis

Faculty Advisor: Osmani, Stephen

Abstract: Many cell types, like neurons, exhibit highly polarized growth, generating long cells that require efficient intracellular trafficking systems to transport proteins, mRNA and vesicles over long distances. Transport of many of these cargoes occurs via motor proteins that move along tracks called microtubules (MTs). The cells of filamentous fungi, like Aspergillus nidulans, are also very long and highly polarized and provide a sophisticated experimental system for studying transport within elongated cells. Our lab uses A. nidulans to discover and study the roles of new microtubule-associated proteins (MAPs) in subcellular transport. Previously, we were investigating two novel proteins (MAPA and MAPB) that move bidirectionally on MT's via MT-based motors dynein and kinesin. Using biochemical purifications we identified a third previously unstudied protein (MAPC) that purifies with both MAPA and MAPB. Because the relationships between these three proteins are unknown, the focus of my research is to understand the functional relationships of MAPC with MAPA and MAPB. Using live cell confocal microscopy to follow fluorescently-tagged proteins, I have determined that MAPC locates and travels on MTs and localizes with MAPA and MAPB. We then used a genetic deletion approach to define how those two proteins influence MAPC. We found that in the absence of MAPB, MAPC fails to locate to mobile foci and disperses in the cytoplasm, suggesting that MAPB could act as a linker between MAPC and MT motors. We further found that in the absence of MAPA, a subset of MAPC accumulates at immobile foci, indicating that MAPA plays a role in the mobilization of MAPC onto MT's. My research therefore provides new insight into the potential roles of three previously unstudied mobile proteins.
Abstract: HIV is a deadly virus that is constantly changing and integrating itself into the host genome which can cause lifelong infection as the virus lies in latency. In response to this global health issue, we hope to find new treatments that target a host pathway called de novo fatty acid synthesis. This fatty acid synthesis pathway is typically not turned on in healthy adult cells. However, a recent study obtained results showing a positive correlation between the expression of fatty acid synthase (key enzyme used in fatty acid synthesis) and HIV. Our hypothesis stems from the fact that fatty acid synthase utilizes important molecules in biochemical pathways to make palmitic acid into myristic acid, a fatty acid with a long hydrophobic carbon chain that is used for many biochemical pathways, which can then attach to an HIV protein called gag. It is important to understand that when myristic acid (long non-polar chain) is added to gag (small polar protein), the myristoylated non-polar gag protein relocates itself to the lipid bilayer so that a new HIV molecule can aggregate, assemble, and bud off. The hypothesis is that if fatty acid synthase is inhibited, myristoylation of gag will be prevented and localize it in the cytoplasm. Currently we are testing this by using immunofluorescence microscopy to localize the fluorescence of gag tagged with GFP that is transfected into our MDCK cell line. We are in the process of testing two fatty acid synthase inhibitors on this cell line after transfecting gag GFP and the positive control plasmid gag G2A GFP to observe a difference in fluorescence localization. We expect that gag GFP treated with inhibitors will localize primarily in the cytoplasm, indicating that fatty acid synthase plays a role in HIV replication.
A set of fossils from Pakistan, particularly the 12 million year old Progonomys, have traditionally been interpreted as representing the point in time when laboratory mice (Mus) and lab rats (Rattus) last shared a common ancestor. This interpretation of their evolutionary history has been used extensively among biomedical researchers working with these model organisms. However, subsequent molecular results conflict with the evolutionary tree that forms the foundation for interpreting the fossil record. This in turn has now forced paleontologists to re-evaluate the Siwalik fossils. With this new information, a new Mus-Rattus date needs to be determined for use by biomedical researchers. We analysed a four gene nuclear dataset within the family Muridae and incorporated information from fossils involving several locations and evolutionary events. We conclude with recommendations to researchers looking to calibrate the Mus-Rattus divergence.
Category: Biological Sciences

Title: Quorum sensing autoinducers impact biofilm formation in a hydraulically fractured environment

Student Presenter: Kevin Eberle

Faculty Advisor: Wilkins, Michael

Abstract: The hydraulic fracturing (HF) process for hydrocarbon extraction involves pumping millions of gallons of water mixed with biocides and sand into deep subsurface shale formations. The addition of water increases the pressure in the formation, fracturing the shale and allowing for natural gas extraction. Following HF, the high salinity flow-back fluids ("produced fluids") are stored in tanks prior to disposal. Issues arising with disposal of these fluids are linked to high concentrations of subsurface radioactive elements, salts, and chemical additives. One method to remove these contaminants utilizes microbial fuel cells (MFCs). MFCs can oxidize organic pollutants under anaerobic conditions using a steel casing as an electron acceptor. Degradation of hydrocarbons requires halotolerant organisms with the ability to form biofilms, enabling the degradation of organic contaminants while generating harvestable power. Through this project, I attempt to chemically induce biofilm formation of known bacterial halophiles isolated from produced fluids obtained from a HF well located in Morgantown, West Virginia. The genera of identified isolates include Halolactobacillus, Orenia, Marinilabilia, Frackibacter, Desulfobrivio, and Halanaerobium. Using a high salt media representative of subsurface fluids, I tested biofilm formation at two cell concentrations: 1.0 x 10^7 and 1.0 x 10^8 with the addition of two different chemical autoinducers: 2-heptyl-3-hydroxy-4-quinolone and N-(3-oxo-dodecanoyl)-homoserine lactone at concentrations of 1 μM and 100 nm. I added these to potentially enhance biofilm formation by acting as quorum sensing autoinducers. Tests were conducted using a 96-well plate biofilm assay coupled with a crystal violet staining method, and revealed that 1 μM and 100 nm concentrations of 2-heptyl-3-hydroxy-4-quinolone had an increase of on the biofilm-forming potential of Orenia, Marinilabilia, and Desulfobrivio strains. Next, I plan to measure the MFC capability of Orenia, Marinilabilia, and Desulfobrivio within the reservoir environment which would alleviate an economic and environmental challenge for the oil and gas industry.
Abstract: The centrosome is composed of two centrioles surrounded by a proteinaceous pericentriolar material. It is the major microtubule-organizing center, and plays a role in the equal segregation of chromosomes and maintaining proper cell division. Typically, the centrosome is regulated such that it is duplicated only once every cell cycle. However, in some cases, dysfunction of centrosome duplication, such as an overabundance or deficit of chromosomes, can then lead to aneuploidy and genetic instability, which is a hallmark of cancer. Additionally, previous research has demonstrated a positive correlation between the rate and severity of cancer development and centrosome abnormality. Thus, studying the regulation of centrosome duplication allows us to discover potential mechanisms regarding the morphology of cancer and potential for its metastasis, as well as uncover potential drug targets to begin development of cancer-treating therapeutics. Mps1, a protein kinase, is seen in elevated amounts in the event of centrosome over-duplication. This means that proper management of Mps1 ensures a lower potential for chromosomal abnormality. Our studies investigate the prospective regulation of Mps1. Preliminary data suggests that Mps1 is phosphorylated by Plk4, a protein kinase that controls centriole biogenesis to begin centrosome duplication. To further investigate this, we performed site-directed mutagenesis on multiple Plk4 phosphorylation sites in Mps1, mutating them into a non-phosphorylable Alanine form and a phosphor-mimetic Aspartic Acid form. Successful mutants were verified by sequencing. We validated four mutant constructs via a western blot analysis for protein expression and indirect immunofluorescence microscopy to indicate localization to the centrosome. Thus, our mutant constructs have the ability to serve as a tool to further understanding of how Mps1 is regulated and provide additional insights for centrosome biology.
Abstract: Cutaneous Squamous Cell Carcinoma (cSCC) is the second most common cancer diagnosed in individuals with fair complexions, with around 700,000 cases treated each year. Although most cSCC tumors are superficial and are removed by surgical excisions, about 2-6% of the tumors will metastasize to other regions of the body, leading to a 44% mortality rate in individuals with distant metastatic tumors. A microRNA expression assay was performed in the Toland laboratory on 48 samples including primary and metastatic cSCC to see which microRNAs were differentially expressed between metastatic versus non-metastatic cSCCs. The novel microRNA-4516 (miR-4516) was shown to be downregulated by about 50% in metastatic tumors compared to non-metastatic tumors. Work by others showed that miR-4516 directly targets Signal transducer and activator of transcription 3 (STAT3), a known oncogene. The goal of this study is to determine how differential expression of miR-4516 affects tumor and metastatic phenotypes of cSCCs and the role of STAT3 in this process. Initial studies using quantitative PCR show that a metastatic cSCC cell line, COLO-16 expresses less miR-4516 than non-metastatic cSCC cell lines (SCC13 and SRB-12) by 50%. However, Western analyses on these untreated cell lines do not show differences in STAT3 expression. Ongoing studies will assess the effects of re-expressing or knocking down miR-4516 in cSCC cell lines on invasion, migration, proliferation, apoptosis, and expression of candidate miR-4516 targets. The results of this study could lead to an increased understanding of the metastasis of cSCCs and could also identify potential therapeutic targets in patients, leading to less metastasis and better outcomes in the clinic.
Category: Biological Sciences

Title: The role of the microRNA mir-125a-5p in mouse skeletal development and fertility

Student Presenter: Sophia Friesen

Faculty Advisor: Cole, Susan

Abstract: The small noncoding RNAs known as microRNAs play a critical role in gene regulation, being implicated in processes ranging from development and cell differentiation to cancer. This project aims to determine the role of the microRNA mir-125a-5p in mouse skeletal development and infertility. We have previously identified a functional role for this miRNA in the regulation of Lfng glycosyltransferase during chick somitogenesis. Somitogenesis is the process by which somites, the precursors of ribs and vertebrae, form. This requires oscillatory expression of Lfng. In chick, somitogenesis and Lfng oscillations require binding between Lfng and mir-125a-5p, but it is unknown if this regulatory interaction is conserved in mammals. To investigate the role of mir-125a-5p in mouse somitogenesis, we generated mutant mice lacking this miRNA. Mutant and wildtype mouse embryos were inspected for differences in Lfng expression, somite formation, and skeletal development. Neither somitogenesis nor skeletal development was altered in mutants. This demonstrates that mir-125a-5p is dispensable for mouse somitogenesis, possibly due to redundancy with similar miRNAs, or because of differences in the regulation of somitogenesis in chick and mouse. Interestingly, homozygous mutant males of one mir-125a-5p-deficient line, called 125a^11, are infertile. I will identify the cause of this infertility, which is likely related to dysfunction of the host gene of mir-125a-5p, Spaca6, a gene required for sperm-egg fusion. mir-125a-5p lies within an intron of Spaca6. 125a^11 likely inhibits mir-125a-5p function by preventing Drosha cleavage of the miRNA. Since Drosha has been shown to affect splicing of miRNA-containing introns, 125a^11 may interfere with splicing of the host gene. Spaca6 is unusually spliced in 125a^11 mutants. This could also be caused by an off site mutation within Spaca6. Current work will distinguish between these possibilities.
Abstract: Tuberculosis (TB), caused by Mycobacterium tuberculosis (M.tb), leads to about 1.5 million deaths annually. M.tb is transmitted through aerosols and infects lung macrophages, altering the host immune response for survival. MicroRNAs (miRs) are small noncoding RNAs that regulate gene expression by binding to the 3'UTR region of mRNA for degradation. We have previously demonstrated that miR-125b expression is increased in macrophages by virulent M.tb but not by the non-virulent M. smegmatis. Increased miR-125b expression decreases host-protective cytokines during M.tb infection in human monocyte-derived macrophages (hMDMs). The major goal herein was to determine the transcription factors mediating miR-125b expression in response to M.tb. In silico promoter analysis revealed that Nrf2, PPAR&gamma; and STAT-1 are major transcription factors for miR-125b. Thus we hypothesized that M.tb activates several transcription factors to regulate miR-125b expression. Focusing on Nrf2, we incubated hMDMs with a Nrf2 activator (AI-1) and determined miR-125b expression by qRT-PCR. We found that Nrf2 activation enhances miR-125b expression. To determine whether M.tb differentially activates Nrf2 compared to M. smegmatis, hMDMs were infected with M.tb or M. smegmatis and Nrf2 phosphorylation was determined by Western blot. Our results indicate that M.tb infection causes greater Nrf2 phosphorylation than M. smegmatis infection. To confirm the involvement of Nrf2 in miR-125b expression, we transfected macrophages with Nrf2 siRNA or scramble control by nucleofection and then infected with M.tb. Interestingly our results showed that Nrf2 is a contributor to the induction of miR-125b expression, but suggest that Nrf2 deficiency is compensated for by other transcription factors such as PPAR&gamma; and STAT-1. We conclude that M.tb activates Nrf2 which partially regulates miR-125b expression during M.tb infection. Determining the molecular mechanism(s) underlying miR-125b expression, which influences M.tb pathogenesis by regulating host-protective cytokines, will help us to develop new host-directed therapeutic approaches for TB.
Category: Biological Sciences

Title: Identifying novel protein components of the cytoplasmic capping complex using proximity-dependent biotinylation

Student Presenter: Andrew Giltmier

Faculty Advisor: Schoenberg, Daniel

Abstract: mRNAs that appear without a 5' cap in the cytoplasm are readily identified and degraded by cytoplasmic enzymes. However, a smaller set of uncapped mRNAs can be recapped in the cytoplasm, protecting them from degradation by exonucleases, allowing them to return to a translationally active state. The cytoplasmic capping complex consists of cytoplasmic capping enzyme (cCE), RNA-guanine-7methyltransferase (RNMT), and a 5' RNA kinase that assemble on adapter protein Nck1. This complex is the set of proteins responsible for replacing the 5' cap that provides a unique form of post-transcriptional regulation. Although the basic structure of the cytoplasmic capping complex is established, many details remain unknown. Our goal is to further our understanding of the structure of this complex using an in vivo approach for proximity-dependent labeling of proteins called BioID (proximity-dependent biotin identification). Using a promiscuous biotin ligase bound to cCE, we are able to identify known and unknown candidates of the cytoplasmic capping complex by biotin tagging proteins as they interact with the complex. These tagged candidate proteins are isolated via streptavidin affinity chromatography and identified with proteomic mass spectrometry analysis. Not only will these results allow for a more comprehensive explanation of the cytoplasmic capping process, but they also open the door for the further characterization of newly identified candidate proteins.
Abstract: The Patterning Cascade Model (PCM) accounts for the establishment of tooth shape during development. In this model, each tooth cusp forms at a specialized site on the dental epithelium, an enamel knot, which is a gene signaling center that stimulates downward epithelial growth while simultaneously inhibiting other enamel knots from forming nearby. For a new cusp to form, a new enamel knot must form beyond this zone of inhibition. Humans have a typical number of principal cusps (4-5) on their upper and lower first molars, but some individuals also express smaller, accessory cusps, either peripheral or central in position. According to the PCM, small inhibition zones, protracted growth, or both promote the formation of peripheral enamel knots. Accordingly, small intercusp spacing relative to tooth size should be associated with greater peripheral cusp expressions. In contrast, centrally located accessory cusps, according to the PCM, may be associated with larger distance between the principal cusps that surround them, giving them space to form. Previous research has found that the presence of the Carabelli’s cusp is associated with greater expression of peripheral accessory cusps. This study analyzes the rest of the individual’s dentition to see if accessory cusps are more likely to appear on all first molars if found on any first molar. Data from first molars of 37 individuals from a Seminole dental cast collection were collected by measuring the degree of expression of cusps and intercusp distances. Preliminary analysis suggests that in almost all cases, accessory cusps are more prevalent with smaller intercusp distances relative to tooth size regardless of their position on the tooth. This study gives insight into the developmental mechanisms that underlie cusp formation in humans and other mammals. Understanding these patterns will ultimately refine the understanding of evolutionary patterns in cusp formation and loss.
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 Drosophila melanogaster. However, no studies thus far have attempted to utilize the polymorphic variation that exists in natural populations to study axon guidance. This approach was only recently made possible by the creation of the D. melanogaster Genetic Reference Panel (DGRP), which consists of more than 200 genomically sequenced strains derived from an outbred population. In the present study, embryos from 42 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 identified 11 strains with commissural defects and 11 additional strains with ectopic midline crossovers, with different lines varying in the penetrance and expressivity of the observed phenotypes. This demonstrates that significant 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. Through a series of crosses, we will generate DGRP strains in a background deficient for Abelson (Abl) tyrosine kinase, a known regulator of midline axon guidance. By assessing percent viability at the pupal and adult stages, we will observe whether any strains deviate from expected survival rates, indicating the presence of a polymorphic variant potentially influencing axon guidance through the Abl signaling pathway. In the long-term, this research will provide insight into the complex network of ligands, receptors, and signaling molecules that regulate axon guidance.
Abstract: Cutaneous T-cell lymphoma (CTCL) is a non-Hodgkin lymphoma of skin-homing malignant CD4+ T-cells. Long-term survival in advanced-stage patients is extremely poor, highlighting the need to identify novel therapies to inhibit key oncogenic processes. Previous studies have identified bromodomain-containing protein-4 (BRD4) as a key regulator of several oncogenic expression in CTCL. In this study, we demonstrated that BRD4, in complex with aberrantly expressed TWIST1, upregulates microRNA-214 (miR-214), a key oncomiR which is known to target several tumor-suppressor genes. Patient CD4+ T-cells treated with a bromodomain inhibitor, JQ1, showed significantly decreased expression of miR-214 (p). In order to test these findings in vivo we utilized our IL-15 transgenic mice which develops CTCL by 4 weeks of age. We treated 10 week old mice with JQ1 or vehicle control for 3 days and collected spleen and blood for evaluation of miR-214 and its target genes. Our findings suggest that the BRD4-TWIST1 complex serves as co-activators for transcription of oncogenic miR-214. This study identifies BRD4, TWIST1, and miR-214 as potential novel therapeutic targets in CTCL.
Category: Biological Sciences

Title: Identification of miR-122 targetome by HITS-CLIP analysis revealed its regulation of WNT/B-catenin activity through BCL9.

Student Presenter: Maria Jose Guerrero

Faculty Advisor: Ghoshal, Kalpana

Abstract: Liver cancer has the highest mortality rate among all malignancies and hepatocellular carcinoma makes up over 90% of all liver neoplasms. The observed poor patient prognosis is due to the inadequate mechanistic understanding of disease initiation and progression. To expand the working knowledge of HCC, we have worked with a liver-specific miRNA 122 (miR-122), that is responsible for the post-transcriptional regulation of gene expression mediated by 3'UTR binding after incorporation into Argonaute (Ago) containing miRNA-induced silencing complex (miRISC). We found that our miR-122 knockout (KO) mice displayed liver pathology such as altered lipid metabolism, inflammation, fibrosis, and spontaneous HCC development that mimics different stages of nonalcoholic fatty liver induced HCC in humans. In addition to this, it has been found that patients with this type of cancer generally have lower levels of miR-122 and have worse overall prognoses. To have a better understanding of the miR-122 function in the liver, we identified miR-122 targets in the whole liver using Argonaute high-throughput sequencing of RNA isolated by UV-crosslinking and immunoprecipitation with a Argonaute antibody (Ago-HiTS-CLIP) in collaboration with the lab of Dr. Robert Darnell in WT and miR-122 KO mice. RNA-seq analysis revealed functional de-repression of both 3'-UTR and Coding DNA Sequence (CDS) targets in KO mice livers. We looked at these targets in human liver cancer patients' data (n = 373) in The Cancer Genome Atlas (TCGA), that revealed alterations in BCL9, a β-catenin co-factor critical for Wnt-signaling. Its dysregulation was found to be associated with poor patient outcome and it negatively correlated with miR-122 expression. We were able to validate BCL9 as a novel, conserved miR-122 target using Dual Luciferase Reporter Assay. Finally, we validated BCL9 CDS as a miR-122 target using wild-type and mutated miR-122 binding sites. Our collective data identified a novel mechanism connecting miR-122 to Wnt/b-Catenin signaling, frequently dysregulated in HCC patients.
Abstract: Identification of conspecific individuals is necessary to prevent hybridization in areas where two closely-related species coexist. Greater and Lesser Prairie-Chickens (Tympanuchus cupido and T. pallidicinctus) are two closely-related species, whose ranges have expanded recently, and now overlap. There is behavioral evidence that the two species may be hybridizing. We sought to examine whether feather coloration could be used to differentiate between species, and to estimate the hybridization rate. Our hypothesis was that Greater Prairie-Chickens and Lesser Prairie-Chickens differed in feather coloration and patterning, and that birds of hybrid origin would show intermediate feather characteristics. Spectroscopy readings and feather measurements were taken from both Greater and Lesser Prairie-Chicken reference populations and compared to the birds in the area of overlap to determine species identity. We conducted two discriminant analyses. The first utilized feather measurements such as feather length, subterminal bar length, number of bars, and brightness of the light and dark bars. The second had all of the same measurements except it excluded the brightness of the dark and light bars. Hybrids were defined as individuals that were not assigned to either parental species. The discriminant analysis was able to differentiate between Greater and Lesser Prairie-Chickens with a high degree of certainty. We found a hybridization rate of between 4.1 and 7.3%. The analysis with color was able to determine species identity with higher confidence than the analysis without color. Our hypothesis that Greater Prairie-Chickens and Lesser Prairie-Chickens differ in feather coloration and patterning was supported. This study demonstrates that plumage on the prairie-chickens could potentially be used for species differentiation, and can be used by researchers to identify the species. Although plumage differentiates Greater and Lesser Prairie-Chickens, the hybridization rate suggests that plumage alone is not sufficient to prevent interspecific mating.
Abstract: The morphology and physiology of the gastrointestinal tract becomes increasingly complex the more that it is researched, and model systems are often helpful in understanding the processes that can be confounding in more complex systems, like humans. The adaption to utilize gastrointestinal symbiotic microbes allows animals to extract nutrients that the organism cannot obtain on its own. Cockroaches are used as model systems for studying the gut because of their sustained symbiotic ties to microbes. Previous work examining the effects of bacteria in the development of the cockroach gut has shown that the gut develops differently in germ-free and germ-typical (normal gastrointestinal microflora) cockroaches. Another detail of physiology that has not yet been explored is the effect that normal microflora has on the transit rate of food through the gut as compared to germ-free guts, which is the purpose of this project. Bacteria help break down substrates that are not digestible by the host, allowing the host to obtain a greater amount of nutrients. Other studies show that longer residence time of food in the gut increases nutrient uptake in guts with normal microflora. By feeding cockroaches fluorescent microbeads and dissecting after set time intervals, the transit rate of food through the gut can be compared between germ-typical and germ-free cockroaches. The results show the opposite of the expected results: germ-typical cockroaches showed a significant pattern of slower velocity though the gut at the four-hour mark as compared to their germ-free counterparts. The unexpected results build on our previous understanding of the gut and rise new questions about how the microflora of the GI tract effects gut transit.
Abstract: In recent years, numerous defects in tRNA biogenesis and related processes, including tRNA aminoacylation, have been linked to human disease. As direct mediators of translational fidelity, aminoacyl-tRNA synthetases (aaRS) are responsible for the correct pairing of an amino acid with its cognate tRNA. Mispaired aminoacyl-tRNA (aa-tRNA) species occur due to a lack of amino acid discrimination within the aaRS active site. In Saccharomyces cerevisiae, abolishing the editing activity of misacylated Tyr-tRNAPhe in the phenylalanyl-tRNA synthetase (PheRS) leads to severe growth defects in the presence of high concentrations of the near-cognate amino acid, Tyr. Historically, misincorporation of Tyr into the proteome at Phe codons has been considered a major contributor to an overall decrease in cell viability. Looking at the accumulation of mutations over time through a yeast-based mutation rate analysis is useful for identifying factors that contribute to disease progression. We hypothesized that changes in proteome fidelity caused by aaRS mediated mistranslation lead to dramatic genomic alterations through an increase in mutation rate. A mutation fluctuation assay first described by Luria and Delbruck was utilized, and yeast cells were modified to remove endogenous copies of phenylalanyl-tRNA synthetases. Mutation rate was monitored by measuring cell survival, which reflects the frequency of inactivation of the CAN1 locus. CAN1 is responsible for importing canavanine, a toxic analog of arginine. Data obtained from the mutation rate analysis has provided insight into the direct contribution of aaRS mediated mistranslation to genome stability. Initial experiments suggest that increased error rates in protein synthesis are an important facet of stress response during amino acid limitation. Furthermore, disruption of the proteome has been previously shown to directly cause tumorigenesis and increase susceptibility to pro-tumorigenic stress. Therefore, our results could be further applied to human cell lines using the endogenous yeast enzyme.
Abstract: The $\gamma$-crystallins are the major structural proteins in the vertebrate eye lens. To maintain lifelong transparency and high refractive index without protein renewal, the $\gamma$-crystallins have evolved to retain high solubility and stability under an extremely crowded environment. It has been suggested that highly concentrated $\gamma$-crystallins can form short ordered array in lenses, and molecular dipole has been hypothesized to contribute to the ordered "crystal-like" arrangement. In addition, $\gamma$-crystallins have been proposed to possess a thinner hydration shell compared to the average values for water soluble proteins based on analytical ultracentrifugation (AUC) results. In this study, we used both NMR and femtosecond (fs) resolved laser approaches to investigate the protein and water dynamics of the lens protein $\gamma$M7-crystallin from zyberfish. H/D exchange experiment by NMR showed that $\gamma$M7 exhibits two dynamically different sides, which is also observed in WT $\gamma$S-crystallin from mice. We hypothesize that two dynamically different protein-sides may be a prevalent property in all $\gamma$-crystallins and it may contribute to ordered protein arrangement at high concentration. In order to probe the water dynamics around the surface of $\gamma$M7-crystallin, about 20 mutants with a surface residue mutated to Tryptophan (Trp) were identified to have stable emission lifetime for fluorescence measurement. The preliminary fluorescence result from $\gamma$M7 Y56W was best fitted to a double exponential decay, suggesting two slow conformational fluctuations with the miss of a very fast decay due to bulk water motion. The data agreed well with the local environment around Y56, which is located in the dimer interphase and not fully exposed to bulk water. Further study will be performed to other mutants containing more exposed Trp to provide a complete description of the hydration shell around $\gamma$M7-crystallin.
Category: Biological Sciences

Title: Exploring the role of tRNA modifications in cytotoxicity of the anti-cancer drug 5-fluorouracil

Student Presenter: Abigail Hubacher

Faculty Advisor: Jackman, Jane

Abstract: Transfer RNAs (tRNA) are extensively modified during RNA processing. One such modification performed by the tRNA methyltransferase Trm10 is the N-1 methylation of guanosine at the G9 position (m1G9) in 14 types of yeast tRNA. The Trm10 gene is widely conserved in Eukarya and Archaea. Mutations that decrease expression or catalytic activity of the Trm10 human homolog (TRMT10A) can cause microcephaly, short stature, and glucose sensitivity. In yeast, the trm10^ strain grows normally on rich media, but is sensitive to growth on media containing 5-fluorouracil (5FU). We hypothesize the lack of Trm10-catalyzed modification in trm10^ yeast renders a specific tRNA less functional when grown on 5FU media. We hypothesized that if more of that tRNA is expressed, it may allow cells to overcome the deficiency by maintaining a sufficient pool for translation. Vectors for tRNA overexpression were transformed into trm10^ strains and plated on media containing 5FU. Compared to controls, increased growth was seen with overexpression of tRNATrp. Interestingly, decreased growth was seen in strains overexpressing tRNAser, indicating the increase in tRNA abundance could be interfering with other cellular processes. Overexpression of tRNATrp in wild-type yeast had no observable effect on growth, suggesting that the poor growth of yeast in the absence of Trm10 is related specifically to the presence of 5FU. Growth curve assays showed an increase in growth compared to control for strains overexpressing tRNATrp. Similarly, these assays showed a dampening of growth for strains overexpressing tRNAser after initial growth. Northern blot assays of tRNATrp and tRNAser will be performed to assess the levels of tRNA compared to controls. Further study of the role of tRNATrp in trm10âˆ† yeast in the presence of 5FU could lead to further insight into the role of the m1G9 modification in normal cells and how its absence leads to a diseased state.
Abstract: Neurodegenerative diseases affect millions of Americans and are caused by the deterioration of neurons in the central nervous system. The mutations that cause these neurodegenerative diseases have been modeled in Drosophila (fruit fly) transgenic lines and have been used to produce flies with neurological defects to mirror the human pathology. Meanwhile, continuous cell lines have transformed biomedical research, making it possible to study biological systems on a scale requiring large numbers of similar cells. However, there is a lack of continuous cell lines in Drosophila melanogaster available in the scientific community that would allow for the study of specific mechanisms within the nervous system. The objective of this study was to utilize the expression of oncogenic Rasv12 to produce neuronal cell lines in Drosophila melanogaster; neuronal lineage markers were then used to characterize the cell lines. The GAL4/UAS system was used to induce the tissue-specific expression of a given gene of interest. Fly "drivers" expressing the GAL4 protein in neuroblasts were crossed with flies containing upstream activating sequences (UAS) upstream of Rasv12, leading to the proliferation of neuroblasts in progeny. Both pan-neuronal and motoneuron-specific drivers were used. Embryos were collected from the cross and grown in vitro as a primary culture. Cell cultures were stained with the neuronal cell-type specific markers: HRP, Fasciclin-II, and 22C10. Cell lines previously established in the lab from constitutive Rasv12 expression, and that appeared to exhibit neuronal properties, were also characterized with the neuronal lineage markers. This study tests the efficacy of utilizing Rasv12 for the production of continuous cell lines of neuronal lineage to model and develop therapies for neurodegenerative diseases.
Abstract: Adult stem cells are essential for the normal homeostasis of many body tissues and play critical roles in tissue regeneration following damage. In order to remain undifferentiated yet continue to give rise to differentiating progeny, stem cells must constantly balance self-renewal and differentiation. One critical transition during stem cell differentiation is the switch from dividing progenitor cells to post-mitotic differentiating cells. For some genes, this process is characterized by a shortening of the 3' untranslated region (3'UTR). In male germline stem cells, we have identified a widespread shortening of the 3'UTR in over 600 mRNAs during the switch from proliferation to differentiation. Using the established model of stem cell differentiation in the Drosophila male germline we are studying a subset of 60 mRNAs, to observe the function of these genes whose 3'UTRs shorten during differentiation. Through these studies, we have discovered that the gene Chromator, previously not studied in the male germline, is required for proper germ cell differentiation. The continued study of these genes will shed light on the functions of the many genes whose 3'UTRs shorten during male germline stem cell differentiation.
Abstract: This project aims to illustrate the relationship between protein sequence, structure, and stability in the context of a protein Er-23, a pheromone secreted by the protozoan Euplotes raikovi. Er-23 is 51 amino acids long and has 10 cysteine residues in 5 disulfide bonds, whose role in the stability and structure of the protein are of particular interest. The literature suggests that the structure of the protein is driven by the formation of disulfide bonds, and is supported by the currently accepted 3-D structure of the protein. Our working hypothesis is that the folding of the protein is driven primarily by the amino acid sequence, while the disulfide bonds play a role in the overall stability of the protein. We have successfully expressed Er-23 in E. coli, a heterologous expression system, and will use solution state NMR Spectroscopy, Mass Spectrometry, X-Ray Crystallography and CD Spectroscopy to elucidate the precise structure and compare it to that of the homologously expressed protein. Our current data suggest that the protein spontaneously adopts its native conformation in E. coli, and we aim to corroborate this through denaturation and refolding experiments. Additionally, our NMR and Mass Spectrometry data suggest that the disulfide bonds and overall structure of the protein may differ from that of the reported structure. This fundamental information about the interplay between protein fold, stability, and sequence can be useful in the future investigation and design of novel proteins.
Abstract: Glioblastoma multiforme (GBM) is one of the most common and aggressive human brain tumors, accounting for 52% of all primary brain tumors. The median survival for a person diagnosed with GBM is 15 months. GBM originates in glial cells, cells that provide support and protection to neurons in the nervous system. The current treatments for glioblastomas include surgery and chemotherapy, but prolonged remission is rare with the median time for tumor recurrence being about 6.9 months. GBM is currently being studied in human cells and animal models, including the fruit fly, Drosophila melanogaster. Fly glioblastoma models have been developed, but there is no equivalent cell culture model of the glioblastoma in flies. Developing a fly-cell model of glioblastoma is the goal of my project. I propose to generate cell culture models of glioblastoma, by expression of an oncogene and by repression of a tumor suppressor. In the models, glial cells will be induced to express RasV12, an oncogene known to be involved in human GBM. In addition, the tumor suppressor called brain tumor (brat) will be repressed. Loss of function of TRIM3, the human homolog of brat, has been implicated in human GBM. To determine if the cell lines I generate are of glial-cell type, I will analyze the cells using a specific cell marker. To test the tumorigenicity of the cells, I will perform cell transplant assays to determine if injected cells form tumors in the flies. Once established, these models of the glioblastoma will be useful in understanding the basic mechanisms of the disease and for testing therapies.
Abstract: Fungus-farming ants cultivate and consume fungi as their primary source of food. This relationship represents a classic example of mutualism and, to date, more than 250 New World species across 16 genera have been identified. As social insects with highly organized societies, these ants depend upon a system of communication mediated by pheromones and other chemical substances derived from biosynthetic pathways and released via the numerous exocrine glands throughout their bodies. Previous research has demonstrated the various communicative roles these natural products serve; however, recent investigations have shown these compounds may also function as antimicrobial agents used to defend the colony's fungal garden from various pathogenic microorganisms. Protection and maintenance of their fungal symbiont is paramount to the survival and success of a colony. By utilizing these compounds, they are able to eliminate parasites and ensure the continuation of this vital mutualism. In this study, we determined the antimicrobial activities of three ant-derived natural products through standardized disc diffusion susceptibility procedures with two species of bacteria, Escherichia coli and Staphylococcus saprophyticus, with assays of 8 other species forthcoming. Areas of zones of inhibition were measured using ImageJ software and the mean values compared using a parametric F test and a Tukey test (P \leq 0.05). This study demonstrates the potency and antimicrobial effectiveness of 2-methyl-2-decenal and 2-methyl-2-nonenal, both newly discovered natural products identified as the major components of the mandibular gland extract of Apterostigma dentigerum, as well as cis-3-butyl-5-hexylpyrrolizidine, a venom alkaloid from the social parasite Megalomyrmex symmeochus which parasitizes colonies of fungus-farming ants. These results shed further light on the complex systems of fungus-farming ant mutualisms, as well as the various means in which they are able to employ their own chemistry to transform their environments.
Abstract: Salmonella enterica serovar Typhi (S. Typhi) is the disease-causing agent of typhoid fever. Post-treatment of acute infection, S. Typhi colonizes the gallbladder of 3-5% of hosts in a chronic carriage state. In the chronic carriage state, S. Typhi forms aggregations of bacteria, known as biofilms, on the surface of gallstones and the gallbladder epithelium allowing S. Typhi to persist in the host. As traditional antibiotics are ineffective in treating Salmonella chronic carriage, new drugs are desperately needed. From a large ATP-mimetic (kinase inhibitor) compound screen, two compounds, T315 and JK-1, were identified to possess anti-biofilm characteristics against S. Typhi and S. Typhimurium. The effect of both compounds on biofilm formation has been evaluated using rapid attachment, viability, EC50, and dispersal assays. The respective EC50 values for T315 and JK-1 were calculated to be 4.61 μM and 7.27 μM, and both work similarly in inhibiting the early attachment of bacteria in a non-bactericidal manner. The compounds have been/are being derivatized and screened to identify those that exhibit efficient anti-biofilm properties with decreased EC50 values. Previous JK-1 experiments identified DeoD and GroEL as potential targets, which are being characterized. The lack of synergistic or additive activity of the two compounds added together suggests they may have a common target. Pull-down assays using biotinylated T315 synthesized utilizing click-chemistry are underway to confirm the target(s). Characterization of promising derivatives of T315 and JK-1 and their target identification will further assist in the development of anti-biofilm compounds to treat Salmonella chronic carriers.
Abstract: Mycobacterium tuberculosis (M.tb) infects 1/3 of the world's population. Upon deposition of M.tb in the airways, bacteria are bathed in the lung mucosa, a.k.a. alveolar lining fluid (ALF). Although ALF is primarily composed of surfactant lipids, approximately 15% of ALF are proteins, many with enzymatic hydrolytic capabilities. Our data indicate that ALF hydrolases alter the cell wall of M.tb by releasing soluble bioactive bacterial fragments (ALF released fragments) into the milieu. These M.tb fragments are capable of influencing the macrophage function, thus modifying the lung microenvironment that M.tb encounters as it infects host cells. Lung macrophages are the body's first line of defense against M.tb, either by ingesting M.tb and triggering an immune response to contain the infection. Receptors on the surface of macrophages play an important role in determining the course of the infection. Some receptors trigger a pro-inflammatory response, while others trigger an anti-inflammatory response. Macrophages have receptors on their surface called pattern recognition receptors (PRRs) that can identify microbial-associated molecular patterns on M.tb. Some PRRs have a phagocytic function while others have a signaling function. Toll-like receptors (TLRs) are signaling PRRs. Our data show that ALF released fragments induce IL-10 secretion allowing macrophages to control better M.tb infection. We hypothesize that TLR2 and TLR4 play a role in this IL-10 secretion and the resulting observed macrophage response. To evaluate this hypothesis, we assessed the production of IL-10, IL-6, and TNF through using wild type, TLR-2 KO, and TLR-4 KO murine macrophage cell lines. Cells were grown as per protocol and plated at a quantity of 3x10^5 in 24-well tissue culture plates. Cells were allowed to adhere for 16 h and were then washed and stimulated with the ALF-fragments (obtained from exposure of M.tb to human ALF from three different donors) using proper positive and negative controls. After 48 h, supernatants were collected and the release of TNF, IL-10, and IL-6 was quantified by ELISA. Our results show no significant production of TNF, IL-10, and IL-6 when exposed to ALF fragments, when compared to our positive controls. This leads us to conclude that other receptors (i.e. the phagocytic mannose receptor) may play an integral role in the secretion of IL-10 involved in the better control of the M.tb infection.
Category: Biological Sciences

Title: Dual-specificity phosphatase 4 (DUSP4) over-expression in cells prevents H/R-induced apoptosis via the up-regulation of eNOS

Student Presenter: Joanna Kilbane Myers

Faculty Advisor: Chen, Chun-An

Abstract: MAPK signaling cascades regulate several cellular functions, including differentiation, proliferation, and apoptosis. The duration and magnitude of phosphorylation of these MAPKs are critical determinants of their physiological effects. The kinetic control of these MAPK signal cascades is modulated by DUSPs. Previously, we demonstrated that DUSP4-/- hearts sustain a larger infarct and have poor functional recovery, when isolated hearts were subjected to ischemia/reperfusion (I/R). Uncontrolled p38 activation and up-regulation of Nox4 expression are the main effectors for this functional alteration. Here, DUSP4 over-expression in endothelial cells was used to investigate the role of DUSP4 on the modulation of ROS generation and vascular function, when cells were subjected to hypoxia/re-oxygenation (H/R) insult. Immunostaining with cleaved caspase-3 revealed that DUSP4 over-expression prevents caspase-3 activation and apoptosis after H/R. The beneficial effects occur via modulating p38 activity, increased NO bioavailability, and reduced oxidative stress. More importantly, DUSP4 overexpression up-regulates eNOS protein expression during H/R-induced stress. NO is a critical small molecule involved in regulating vascular tone, vascular growth, platelet aggregation, and modulation of inflammation. The level of NO generation determined using DAF-2 fluorescence demonstrated that DUSP4 over-expression augments NO production, and thus improves vascular function. The level of superoxide generated from cells after being subjected to H/R was determined using DHE-HPLC method. The results suggested that DUSP4 over-expression in cells decreases H/R-induced superoxide generation and thus reduces oxidant stress. This also correlates to the reduction in the total protein S-glutathionylation, an indicator of protein oxidation. These results further support our hypothesis that DUSP4 is an antioxidant gene and a key phosphatase in modulating MAPKs, especially p38, during oxidative stress; which regulates ROS generation and eNOS expression and thus protects against oxidant-induced injury or apoptosis. Overall, DUSP4 may serve as an excellent molecular target for the treatment of ischemic heart disease.
Category: Biological Sciences

Title: The identification and validation of ASD-causal copy-number variants in a familial dataset.

Student Presenter: Alexander Lacey

Faculty Advisor: Bartlett, Christopher

Abstract: Autism Spectrum Disorder (ASD) is a heterogeneous group of related developmental disorders, and it is estimated to affect ~1.5% of children. The cause of ASD has been shown to contain a strong genetic component; however, currently-acknowledged susceptibility genes only account for ~20-25% of ASD cases. Some of the unexplained causality could be related to copy-number variants (CNVs), which are deviations from the standard two alleles at a given location in the genome. We hypothesize that rare, de novo CNVs play a causal role in some of the ASD-affected individuals in a familial dataset from the New Jersey Language and Autism Genetics Study (NJLAGS). In our investigation, we create a filtered list of potentially-causal copy-number variants, and then we individually investigate each case within the ranked list in an attempt to validate each one. This research will contribute to the growing body of knowledge regarding the genetic underpinnings of ASD, which we hope will someday provide physicians with the ability to quantify risk of the disorder—or maybe even provide a diagnosis-based on genetic factors.
Title: Accessory cusp expression in upper and lower human molars: testing the patterning cascade mode

Student Presenter: Jacob Lawrence

Faculty Advisor: Guatelli-Steinberg, Debra

Abstract: The Patterning Cascade Model (PCM) accounts for the establishment of tooth shape during development. In this model, each tooth cusp forms at a specialized site on the dental epithelium, an enamel knot, which is a gene signaling center that stimulates downward epithelial growth while simultaneously inhibiting other enamel knots from forming nearby. For a new cusp to form, a new enamel knot must form beyond this zone of inhibition. Humans have a typical number of principal cusps (4-5) on their upper and lower first molars, but some individuals also express smaller, accessory cusps, either peripheral or central in position. According to the PCM, small inhibition zones, protracted growth, or both promote the formation of peripheral enamel knots. Accordingly, small intercusp spacing relative to tooth size should be associated with greater peripheral cusp expressions. In contrast, centrally located accessory cusps, according to the PCM, may be associated with larger distance between the principal cusps that surround them, giving them space to form. Previous research has found that the presence of the Carabelli’s cusp is associated with greater expression of peripheral accessory cusps. This study analyzes the rest of the individual’s dentition to see if accessory cusps are more likely to appear on all first molars if found on any first molar. Data from first molars of 37 individuals from a Seminole dental cast collection were collected by measuring the degree of expression of cusps and intercusp distances. Preliminary analysis suggests that in almost all cases, accessory cusps are more prevalent with smaller intercusp distances relative to tooth size regardless of their position on the tooth. This study gives insight into the developmental mechanisms that underlie cusp formation in humans and other mammals. Understanding these patterns will ultimately refine the understanding of evolutionary patterns in cusp formation and loss.
Abstract: Linking the sequence of a protein to its stability and function and predicting how a protein folds from its sequence is a goal of biochemical researchers. A particular region of interest in a protein is its core. The composition of a protein core, among other elements, determines and drives the topological formation of the entire molecule. Previous work has been done concerning how mutations within the core of Rop, an alpha-helical protein composed of 63 amino acids that forms an anti-parallel homodimer, changes the structure and stability. Through characterization of a set of Rop variants, we observed that repacking Rop's core leads to variable outcomes. Generally, the protein core is relatively intolerant to mutation, but how resilient are loops to mutation, and what amino acid sequence makes the 'best' loop? Using Rop, we can model how randomization of a loop region alters the biophysical characteristics of a protein. First, we have randomized the canonical four residues of Rop's loop using degenerate codons to create a protein library in both wild-type Rop and a variant engineered to remove a potentially stabilizing ionic interaction between the monomers. Two other protein libraries were also created with an additional residue in the loop. These four protein libraries are being subjected to a cell-based screen that connects phenotype to activity in vivo. High Throughput Thermal Scanning will be used to study the stability of active variants. Additionally, through an enrichment process, we will observe the composition of the most favorable Rop loops. Future studies include characterization of a few interesting variants by thermal and chemical denaturation and NMR. From these data, we will draw conclusions about how tolerant Rop's loop is to mutation. These conclusions may lend insight into the relationship between sequence and fold and provide useful knowledge for therapeutic development and industrial processes.
Category: Biological Sciences

Title: Quantitative analyses of the localization and dynamics of novel microtubule-associated proteins in intracellular trafficking in Aspergillus nidulans cells.

Student Presenter: Dale Lingo

Faculty Advisor: Osmani, Stephen

Abstract: All eukaryotic cells require an intracellular transport system to move cargo like organelles, vesicles, and proteins over large distances. This transport occurs on long tracks called microtubules (MTs) and is governed by a set of rules that is not well understood. MTs are polar cables that are anchored at the MT-organizing centers at their minus ends and grow at their plus-ends. Our lab uses the filamentous fungus A. nidulans to study the role of MT-associated proteins (MAPs) in subcellular travel. We are currently investigating the localization and dynamics of three novel proteins, MAPA, MAPB, and MAPC, that move bi-directionally on MTs and interact with each other. The focus of my research was to utilize quantitative analyses on existing microscopy data to investigate the role of MAPA and MAPC in regulating the dynamics of MAPB. I used the kymograph tool and derived the rate of movement of MAPB foci in wildtype cells. I found that the rate of movement of MAPB towards the plus-end of MTs is similar to that towards the minus-end of MTs. Furthermore, the rate of movement of MAPB is comparable to that of MAPA and MAPC in both directions. My ongoing analyses are targeted towards computing the rates of movement of MAPB in the absence of MAPA and MAPC and compare them to rates in wildtype. Overall, my research is aimed to provide a more quantitative understanding of the movement of novel dynamic proteins, ultimately providing a more thorough insight into the rules governing intracellular transport.
Abstract: Primates, relative to other mammals, often have extended periods of infancy and adolescence in order to learn and develop the skills needed to survive in adulthood. Therefore, the time from birth to sexual maturity is an important area of study to understand primate behavioral development. Although this has already been explored in several primate species, little has been published on the behavior of infant Asian colobines. The birth of two silvered langurs (Trachypithecus cristatus) to different mothers within the same breeding group at the Columbus Zoo and Aquarium offered a unique chance to study infancy in this species and uncover possible differences related to sex, age, and mothering style. From January to August 2016, this group underwent daytime observation at various times for one to three hours. For each individual, a range of behaviors was recorded, including activities such as feeding, sleeping, and locomoting, and social behaviors such as grooming, supplanting, and proximate location. In addition, specific behaviors relative to the offspring were recorded, such as nursing, holding, and carrying by their mothers. Analysis of the data showed little variation between the two offspring in locomotive activities and maternal contact, suggesting little difference in social or active behaviors. The results are different from those found in studies of other primate species, and indicate a need for continued study of silvered langur infancy and adolescence in order to inform how they are kept and bred in captivity.
Category: Biological Sciences

Title: Utilization of an RNase P-based assay for accurate measurement of 5'-modification in tRNAs

Student Presenter: Seth Lyon

Faculty Advisor: Gopalan, Venkat

Abstract: RNAs perform an array of functions in all life (e.g., catalysis, chromatin remodeling, structural scaffolds for large assemblies). Understanding this versatility of RNAs requires knowledge of their structure-function relationships. Probing RNA structure often requires spectroscopic methods, which in turn necessitates strategies for post-synthetic, site-specific incorporation of chemical probes into target RNAs. One method to achieve this goal is through in vitro transcription (IVT) of RNAs by T7 RNA polymerase and a GTP-initiating class III &Phi;6.5 promoter. In addition to GTP, T7 RNA polymerase can incorporate 5'-modified guanosine analogs during transcriptional priming. Because the nucleoside/nucleotide monophosphate guanosine analog cannot be used in elongation, it can only serve as the initiator. By using a 4- to 10-fold excess of the 5'-modified guanosine analog:GTP in the IVT, we and others have successfully generated RNAs with 5'-modifications. We have now rigorously examined the extent of 5'-modification with RNAs of different sizes and with 5'-deoxy-5'-azidoguanosine (Az-G) as the modifier. For small RNAs (5 nts), T7 RNA polymerase indeed generates mostly 5'-Az-G-modified RNAs (~90%). With tRNAs (150 nts), we determined that there is an unexpected maximum threshold (
Abstract: Histidyl-tRNA (tRNAHis) has the essential role of acting as an adaptor molecule in translation and is required for addition of histidine to growing protein chains. To maintain fidelity of protein synthesis, the histidyl-tRNA synthetase (HisRS) must ensure the correct attachment of the histidine residue to its cognate tRNAHis. For nearly all eukaryotic organisms, the identifying element on a tRNAHis utilized by HisRS to ensure fidelity is a guanosine nucleotide at the minus one position (G-1) that is present on the 5' end of a mature tRNAHis, but not on any other tRNA. G-1 incorporation in many eukaryotes occurs by a two step mechanism, where Ribonuclease P (RNase P) cleaves to remove the 5'-leader sequence from pre-tRNAHis, followed by tRNAHis guanylyltransferase (Thg1) catalyzing the unusual 3' to 5' addition of a guanosine nucleotide to the 5' end of tRNAHis. Consistent with the importance of this role, Thg1 is widely conserved among eukaryotes. Recently, we identified an exception to this pathway in Caenorhabditis elegans (C. elegans). C. elegans tRNAHis contains a G-1 residue that is still the identifying element for C. elegans HisRS. However, the genome of C. elegans does not encode any identifiable Thg1 enzyme, raising the question of how this G-1 element is incorporated into tRNAHis in this species. Interestingly, tRNAHis genes in C. elegans contain an encoded G-1 nucleotide in their 5' leader sequences, suggesting that G-1 might be incorporated during transcription, and retained by an altered activity of RNase P, constituting a mechanism for incorporation of G-1 that has so far only been observed in Bacteria. To test whether this altered RNase P cleavage activity might be responsible for retention of G-1 on tRNAHis, we performed in vitro 5'-maturation assays using transcripts from C. elegans. Results implying non-canonical recognition of tRNAHis by C. elegans RNase P will be presented.
Abstract: Hepatocellular carcinoma (HCC) is the most common primary liver cancer and second highest cause of cancer related deaths. Curative treatments, such as hepatic resection, are effective in early stage HCC. However, HCC is often diagnosed during the late stage. Available systematic therapy at this stage extends survival only by months. HCC is proposed to be promoted by the transcription factor androgen receptor (AR), yet not androgens. Indeed, men are diagnosed at a rate 2- to 4-fold higher than women. In both sexes, HCC incidence and mortality rates are increasing. AR antagonists are an established drug class used to treat prostate cancer, an androgen dependent malignancy. Unfortunately, clinical trials using anti-androgens to treat HCC did not demonstrate efficacy. Our lab has shown that HCC cells express AR splice variants that lack the ligand binding domain essential for current AR antagonist drugs. These variants are thought to be constitutively active and have been identified in prostate cancer, in which they may confer drug resistance. Despite these evidences, the functional role in HCC of full-length AR and AR splice variants, that is, the macrocellular effects of the gene programs they regulate, is not understood. It is hypothesized that AR splice variants can function as a surrogate of full-length AR through redundant activation of proliferative genes. This will be tested by assessing the consequence to in vitro HCC proliferation of suppressing either full-length AR, AR splice variants, or both. Suppressing both full-length AR and AR splice variants is expected to decrease proliferation more than inhibiting either alone. The results of this project will inform future studies of the genes regulated by full-length AR and AR splice variants. Ultimately, elucidating the functional role of AR in HCC tumor growth will offer insight into the potential utility of novel AR-targeted anti-cancer therapeutics.
Category: Biological Sciences

Title: Investigation of protein-protein interactions using surface induced dissociation (SID) MS

Student Presenter: Stacey Nash

Faculty Advisor: Wysocki, Vicki

Abstract: Proteins adopt well defined tertiary structures and form quaternary assemblies via non-covalent interactions. Understanding protein structures and protein interactions is necessary to understand how proteins accomplish their physiological functions. One important tool for protein structure analysis is native mass spectrometry (MS) with surface induced dissociation (SID) as gas phase activation method. In native MS, protein complex ions can be directly generated from non-denaturing aqueous solutions. Those protein complex ions can then be collided into a surface to generate sub-complexes during the SID process. Based on the occurrence of sub-complexes, information on the stoichiometry and topology of a protein complex is easily obtainable. In this work, we analyze how the abundance of generated sub-complexes correlates with the collision energy and how this relates to the number and nature of non-covalent interactions within a protein complex. For this study, we analyzed hetero-tetrameric αβαβα tryptophan synthases (TS) from different organisms by SID MS. We found significant differences in the abundance of observed sub-complexes (α, αβαβ, αβαβ, βαβα, βαβα, βαβα) at different SID collision energies for different TS. As the analyzed TS share the same quaternary structure, our data shows that protein-protein interactions with distinct non-covalent connections can be differentiated by SID MS. We are currently working on understanding how the different types of non-covalent interactions effect the energy required for complex disruption in the gas phase to make SID-MS an even more powerful tool for the study of protein interactions.
Category: Biological Sciences

Title: Horizontal gene transfer of nitrate assimilation genes may facilitate shifts in the ecology of fungi.

Student Presenter: Nic Petrykowski

Faculty Advisor: Slot, Jason

Abstract: The goal of the research was to determine the role, evolutionary history, and ecology associated with nitrate assimilation in fungi. Previous studies have suggested the gain or loss of nitrate assimilation genes may be responsible for ecological shifts in fungi. Protein sequences of nitrate reductase (NAR), nitrate transporter (NRT2), and nitrite reductase (NADPHnir) were obtained by blastp of a local database of 612 fungal proteomes. Phylogenetic analysis was performed on the Ohio Supercomputer Cluster by 1) aligning sequences using mafft 2) curating the alignment using TrimAl 3) searching for the best evolutionary model using Prottest and 4) conducting Maximum Likelihood analysis using RaxML. Gene clusters made up of 2 or more nitrate assimilation genes were then mapped to the resulting phylogenetic trees utilizing the ETE toolkit. Horizontal gene transfers were identified by observing the trees for clusters inconsistent with expected species relationships. In order to test for additional genes involved in these transfers, a protein blast search of NCBI nonredundant proteins was performed using 5 genes up- and downstream of horizontally transferred genes. Two new cases of Horizontal Gene Transfer (HGT) were identified. First, HGT of NAR & NRT2 into a putative neurotransmitter synthesis cluster in the insect pathogen Cordyceps militaris which may play a role in parasitism of insects. Second, HGT of a partial nitrate assimilation cluster to Amanita muscaria may be related to its recent ecological shift from saprotrophic to mycorrhizal nutrition. These findings call for further study in order to determine the specific roles of nitrate in fungus-plant and fungus-insect interactions.
Title: Topological and statistical analyses of gene regulatory grids reveal unifying emergent properties

Student Presenter: Katja Pogacar

Faculty Advisor: Grotewold, Erich

Abstract: Most complex biological systems have interacting components that can be modelled graphically using networks. Among them are gene regulatory grids (GRGs) that are made up of connections between transcription factors (TFs) and target genes, i.e. protein-DNA interactions (PDIs). In general, the connectivity of such GRGs can be described using a power law function of the degree, whereby few TFs have a significantly higher number of interactions than others. In order to understand the complexity of GRGs, we studied the topological properties of GRGs of four organisms: Caenorhabditis elegans (worm), Drosophila melanogaster (fly), Saccharomyces cerevisiae (yeast) and Arabidopsis thaliana (plant). Preliminary results revealed the exponent parameter (alpha) of the fitted power law function on these PDIs were different for each organism, suggesting that the exponent is an intrinsic organism-specific property. Due to the incomplete nature of the observed grids, we created synthetic complete networks resembling biological properties we had previously observed. Firstly, we predicted the total number of interactions which also showed the percentage of the complete GRG that is observed. Secondly, this helped us evaluate smaller subgrids of the GRGs and to determine the largest size of a grid for which the parameters of interest retained reliability. This is the first time a rigorous evaluation of the correlation between the parameter alpha and few other properties of these GRGs has been performed. These findings suggest that GRGs connectivity is organism-specific, however they seem to follow a unifying emergent property. The analyses reported herein can be extended to networks of other species. Additionally, more interactions are needed for further analysis of the other three model organisms.
Category: Biological Sciences

Title: Application of large-scale genomic and ecological data in the bacterial phylum Bacteroidetes

Student Presenter: Victoria Ramsey

Faculty Advisor: Sabree, Zakee

Abstract: Bacteroidetes is a large, diverse phylum with representative species inhabiting a wide variety of niches; many of which are associated with other organisms as pathogens or mutualists. Genome size and G-C content are characteristics that have been shown to have a loose positive correlation in Bacteria. This project seeks to determine if this relationship is stronger within host-associated groups compared to free-living Bacteroidetes, and if less variability of genomic content is seen within the host-associated groups. To investigate this, genomic data was obtained from sequenced Bacteroidetes genomes from NCBI, which were subsequently classified according to their corresponding ecological niche at the genus level. Statistical analysis revealed a stronger correlation between G-C content and genome size within each niche than within the phylum as a whole, and more variability was observed in the genome sizes of free-living or facultative host-associated bacteria than in groups intimately associated with a host. Contrary to previous studies, aerobic members of Bacteroidetes were found to have a higher median G-C content as well as a significantly stronger G-C content to genome size correlation as compared to anaerobes. Deviation from genus and niche norms facilitated identification of incorrectly classified sequences and unique lifestyles within groups. This project demonstrates how ecological classification of large-scale public access genomic data can be used to contribute to a better understanding of the relationship between bacterial genomic content and corresponding lifestyle.
Category: Biological Sciences

Title: Physiological role and mutational analysis of BOLA3 protein in iron-sulfur cluster biogenesis

Student Presenter: Brian Rao

Faculty Advisor: Cowan, James

Abstract: Iron-sulfur (Fe-S) clusters are integral protein co-factors that are responsible for many metabolic processes. These ubiquitous clusters are one of the most versatile prosthetic groups and function in electron transfer and storage, donation of sulfur in lipoprotein synthesis, regulation of enzyme activity, and regulation of gene expression. These roles are all vital for a number of essential physiological processes, such that genetic mutations to Fe-S cluster proteins can result in a variety of human diseases, due to defects in Fe-S cluster biogenesis and the proteins involved therein. A mutation in one of the Fe-S cluster binding proteins known as BOLA3 has recently been discovered to cause multiple mitochondrial dysfunction syndrome 2 (MMDS2), a condition that causes severe weakness, respiratory failure, impairment of neurological development, hyperglycinemia, and overall failure to thrive. Biochemical features of this disease include a fatal deficiency of multiple respiratory chain complexes and 2-oxoacid dehydrogenase enzymes as well as lipoic acid synthesis defectiveness. However, the cellular role of BOLA3 as well as its functional role in Fe-S biogenesis and its relationship with protein partners, NFU1, glutaredoxins, and final target proteins remains unclear. To explore the specific functions of BOLA3 in Fe-S cluster biogenesis and why mutations to it have such severe effects, we have isolated the protein and are characterizing its properties of cluster binding and possible protein partners through the use of circular dichroism, UV-Visible spectroscopy, gel filtration, ultracentrifugation, and isothermal titration calorimetry. By identifying the preferred protein partners and kinetic transfer properties, we will be able to understand the physiological role of BOLA3 and begin to work towards analysis of the disease condition to develop treatment options.
Abstract: The 5' untranslated region (5'UTR) of the human immunodeficiency virus type 1 (HIV-1) genomic RNA (gRNA) contains a structured RNA element (termed Psi) that is specifically recognized by the HIV-1 Gag polyprotein, ensuring that two strands of gRNA are packaged into newly assembled virions. However, the mechanism by which Gag recognizes gRNA over other cellular RNAs and spliced viral RNAs is not well understood. A recent study suggested that a negative regulatory element upstream of Psi reduces high-affinity Gag binding, and a positive regulatory element downstream of Psi counteracts the upstream element and restores high-affinity binding. The aim of this study is to determine how these elements affect the specificity and mode of Gag binding. Using a fluorescence anisotropy-based salt-titration binding assay, the electrostatic and nonelectrostatic (i.e., specific) components of binding were measured. We have previously shown that Gag interacts with a 109-nucleotide (nt) Psi RNA construct that lacks the putative regulatory elements with high specificity and relatively few electrostatic interactions. Using a 356-nt RNA construct that includes the negative regulatory element in addition to Psi, we observed a loss in Gag binding specificity and an increase in electrostatic interactions. Interestingly, a 400-nt construct that contains the positive and negative elements flanking Psi restored highly specific binding and reduced the electrostatic interactions made with the RNA. Furthermore, a construct wherein the 40-nt positive regulatory element was appended to Psi, demonstrated the same specificity as Psi alone. Taken together, these data are consistent with a mechanism whereby the negative and positive regulatory elements flanking Psi modulate Gag binding mode and specificity.
Category: Biological Sciences

Title: Role of TMEM205 in ovarian clear cell carcinoma chemo-resistance

Student Presenter: Maria Riley

Faculty Advisor: Karuppaiyah, Selvendiran

Abstract: Ovarian clear cell carcinoma (OCCC) is the second leading cause of death from ovarian cancer because of its poor response to treatment. Chemo-resistance of OCCC leads to a worse prognosis compared to that of high grade serous ovarian cancer (HGSOC). To improve the outcomes for patients with OCCC, it is critical to find ways to bypass chemo-resistance. Therefore, identifying novel unique proteins involved in OCCC chemo-resistance and designing a drug to selectively target those proteins would make a significant impact on therapy. Transmembrane protein 205 (TMEM205) is one such protein which has been linked to cisplatin resistance in epidermoid carcinoma, however the association in OCCC chemo-resistance has not been elucidated. In order to determine the significance of TMEM205 in OCCC, we have evaluated the TMEM205 expression in 6 OCCC patient tissue samples and 8 HGSOC samples by Western Blot and immunohistochemistry. This revealed highly elevated TMEM205 expression in all the OCCC samples, but absent or lowered TMEM205 expression in HGSOC human tissue samples. Four OCCC cell lines also showed high expression of TMEM205 concentrated in the extranuclear space. In order to determine if TMEM205 is linked to chemo-resistance, we created TMEM205 knocked down OCCC cells. The wild type OCCC and TMEM205 knockdown OCCC cells were treated with cisplatin (10μM) for 3 hours, collected, and the exosomes were isolated from the conditioned culture media for inductively coupled plasma mass spectrometry (ICP-MS) analysis. It was found that intracellular cisplatin increased in the TMEM205 knockdown cells, and the exosomes released from these cells showed decreased cisplatin concentration. In conclusion, this study indicates that TMEM205 expression plays a key role in chemo-resistance in OCCC and is mediated by the exosome efflux pathway.
Category: Biological Sciences

Title: Using affinity chromatography and mass spectrometry to discover diagnostic protein biomarkers of invasive Aspergillosis

Student Presenter: Cory Roeth

Faculty Advisor: Wysocki, Vicki

Abstract: Invasive Aspergillosis (IA) is a fungal disease commonly caused by Aspergillus fumigatus that occurs in individuals with suppressed innate immunity. Other IA causing pathogens are A. flaveus, A. niger, and A. terreus. IA is problematic because of the mortality rate, which can be as high as 90% in high-risk populations, with the occurrence rate doubling in the past 20 years. A positive outcome is more likely when treatment is started earlier, which requires early diagnosis. Currently, definitive diagnosis requires biopsy, an invasive procedure. While adjunctive diagnostic methods, such as CT scans, culture, and the use of galactomannan as a biomarker, are noninvasive, their reliability are often questioned. Protein biomarkers offer the best chance for early diagnosis, thus this project is aimed at identifying multiple protein biomarkers for IA. One method uses affinity chromatography with mass spectrometry to detect low abundance Aspergillus proteins. The affinity column has a polyclonal antibody to Aspergillus immobilized to the resin. The antibody is reactive with proteins of the four common IA causing species of Aspergillus, but the specific antigens are unknown. Probable and possible IA patient bronchoalveolar lavage (BAL) fluid samples were run separately on the affinity column. After running the fractions on sodium dodecyl sulfate polyacrylamide electrophoresis and completing an in-gel digestion with trypsin, the elution fractions were analyzed by 1-dimensional liquid chromatography-mass spectrometry (1DLC-MS). In addition to human samples, paraffin-tissue samples from Aspergillus infected mice were tested. These samples were deparaffinated, subjected to a cell lyses buffer, digested with trypsin and then analyzed with 2DLC-MS. Statistical analysis of direct 2DLC-MS runs of human BAL fluid samples has revealed a potential human protein biomarker. Future work entails further optimizing the current sample preparation techniques, in addition to obtaining and analyzing BAL fluid samples from a new Aspergillus mouse model.
Abstract: Toxin-producing Escherichia coli (Shiga Toxin-producing, STEC; Enterotoxigenic, ETEC) causes severe dysentery and gastrointestinal disease in children, the elderly, and immunocompromised people. Antibiotic treatment places cellular stress on the pathogen, which can result in toxin release. Phage therapy, the use of specific viruses to eradicate a bacterial infection, is an alternative solution to antibiotics. Each bacteriophage (phage) has evolved to attach to a specific bacterial cell. The phages then replicate interiorly, eventually bursting the cell and releasing progeny phages. To investigate potential candidates for phage therapy, our study characterized phage-host interactions of four phages that infect STEC and one phage that infects ETEC. Properties of the phage-host interactions were determined via i) genomic analysis to identify various gene functions and their locations in the phage genomes; ii) adsorption kinetics to determine attachment efficiency of phages to their host; iii) one-step growth curves to measure the latent period and burst size of the phage infection; and iv) plaque morphology comparisons. One STEC phage's gene functions were annotated as follows: 36% host takeover; 11% DNA replication and repair; 17% structural proteins; and 2% DNA packaging and cell lysis. The five phages have similar adsorption rates (29-63%) and latent periods (20-30 minutes). While the STEC phages have low burst sizes (2.15-3.80 virions/infected cell), we anticipate the burst size of the ETEC phage to be larger due to the increased size and reduced turbidity of plaques compared to STEC phages. This indicates that compared to the STEC phages, the ETEC phage is a more competitive candidate for phage therapy. We will continue to characterize its properties, aspiring to engineer an efficient infection to eradicate specific Escherichia coli bacterial pathogens.
Abstract: Stroke is a major cause of morbidity and mortality in the United States, with one stroke occurring every forty seconds. About eighty-seven percent of all strokes are ischemic, in which a blood vessel to the brain is blocked, resulting in an energy crisis and subsequent disruption of ion gradients. Eventually, extracellular glutamate builds up and leads to excitotoxicity, swelling and death of brain cells. The goal of this research project is to better understand the role of the sodium-glutamate transporter during ischemic stroke. The sodium-glutamate transporter is located in both astrocytes and neurons. Under physiological conditions, it releases sodium into and removes glutamate from the extracellular space. However, under pathological conditions, the transporter can reverse and lead to glutamate accumulation in the extracellular space, further contributing to cell death. This project examines how the location of the sodium-glutamate transporter - within the astrocyte, neuron or both cells - affects the electrical activity of the cells, particularly the waves of depolarization that characterize ischemia. With the computational program XPPAUT and literature review, we have constructed a functioning model of one astrocyte and one neuron that incorporates voltages, ion currents, membrane potentials, cell receptors, sodium-glutamate transporters and sodium-potassium pumps. Preliminary simulations have suggested that the model best replicates experimental results, such as neuronal and astrocytic firing patterns, when the sodium-glutamate transporter is located in the neuron alone, rather than in the astrocyte alone or in both cells. Further simulations and analysis will be carried out to determine the reason for this discrepancy. This mathematical model simulates multiple biological processes that occur rapidly, which can be difficult to test and measure experimentally. Our findings may also suggest possible avenues to explore in future experiments. By more fully illuminating the dynamics of ischemic stroke, medicines and therapies can be developed and improved.
Category: Biological Sciences

Title: Self-organization and organogenesis program during the neurosphere life cycle

Student Presenter: Austin Schoeffler

Faculty Advisor: Imitola, Jaime

Abstract: Neurospheres are defined as self-renewing, heterogeneous aggregates of free-floating neural progenitor cells and their progeny. These spherical conglomerates are utilized in a variety of applications from modeling the in vitro growth patterns of neural stem cells and neurological diseases, to neurotransplantation clinical trials. While essential to the study of neurogenesis, it is currently unknown how these cells retain their proliferative abilities among multiple generations in culture, and how neurospheres behave upon heterogenous fusion. In this study, we analyzed the dynamics of neurosphere development by live imaging single cell conglomeration into neurospheres, fusion of neurospheres, spontaneous generation of organoids in high-density cultures and finally death of the organoids (Neurosphere life-cycle). Furthermore, we observed a degree of self-organization in these structures by using GFP reporter stem cells. We computationally modeled the evolution of neurospheres from single cells in vitro. Finally, we studied the effects of clonal density on spontaneous organoids formation during the life span of a neurosphere culture. We conclude that by characterizing the intercellular dynamics of the Neurosphere Life cycle, we hope to elucidate more efficient techniques to culture tissues for neural stem cell transplantation, while minimizing the risks of tumorigenesis. Furthermore, our model can be useful to characterize the encoded program of tissue formation of neurospheres by restricted neural progenitors.
Abstract: The Notch signaling pathway is a fundamental cellular communication mechanism that is conserved across all metazoans. Notch activation occurs when a ligand on a signal-sending cell physically interacts with a receptor in a signal-receiving cell. In contrast, when Notch ligands and receptors are expressed in the same cell, their interactions inhibit the ability of the cell to send or receive signals. This process is called "cis-inhibition", and is a critical mechanism of pathway regulation. Because aberrant activation of Notch has been demonstrated in many diseases, understanding the mechanisms that regulate cis-inhibition is important. One such regulator of Notch activity is glycosylation of the EGF (epidermal growth factor-like) repeats of receptors. This mechanism begins with the addition of an O-linked fucose, which can then be extended by FRINGE family glycosyltransferases. This specific glycosylation modifies the strength of ligand/receptor interactions in cis and in trans. Ligands may also be glycosylated, but the functional relevance of this modification is not known. To examine the importance of ligand glycosylation, we utilized sequence analysis to identify ligand EGF repeats that contain consensus sequences for fucosylation. We identified two potential targets on the ligand DLK (delta-like) 1, on EGF3 and EGF6. DLK2 contains consensus sites on EGF2 and EGF5. We also identified several potential glycosylation motifs within JAGGED1 and are focusing on EGF repeats 4-6, which have been shown to be critical for cis-inhibition. After subcloning the relevant repeats, click chemistry utilizing a bioactive fucose demonstrated that fucosylation occurs on DLK1, DLK2, and JAGGED1. We are currently examining extension of the saccharide by FRINGE and the functional relevance of ligand glycosylation using mass spectroscopy and luciferase assays, respectively. Glycosylation is a relatively understudied idea in Notch signaling, this research could open the door for new insights into cell communication.
Abstract: The HP1 ortholog, Swi6, is found in Schizosaccharomyces pombe (S. pombe), and provides a relatively simple system with which to observe HP1 binding effects. Heterochromatin protein 1 (HP1) is an essential, highly conserved protein associated with heterochromatic spread and gene silencing. Heterochromatin is a tightly packed form of DNA, and its spread is known to contribute to gene silencing. HP1 proteins specifically recognize histone H3 lysine 9 tri-methylation (H3K9me3) and bind to nucleosomes with this modification. The mechanism for this binding in Swi6 and its role in heterochromatin spread is still largely unknown. We demonstrate that Swi6 increases H3K9me3-modified nucleosome accessibility via the model transcription factor LexA. By comparing the Förster Resonance Energy Transfer (FRET) efficiency for titrations of modified and unmodified nucleosomes with Swi6 and LexA, we compared changes in nucleosome accessibility for varying protein concentrations. We propose that at higher concentrations, Swi6 will no longer increase accessibility potentially due to oligomerization.
Abstract: Every year, around 3900-8800 individuals die due to metastatic cutaneous squamous cell carcinomas (cSCC), though only a small percentage (roughly 5%) actually metastasize. In our lab's previous exome sequencing studies, 67% of metastatic cSCC were found to have a mutated KMT2D gene. Only 31% of primary cSCCs have a mutation in this gene. KMT2D, also known as MLL2, belongs to a family of chromatin-remodeling enzymes and is responsible for the production of an enzyme which functions as a histone methyltransferase. Based on literature, KMT2D is thought to function as a tumor suppressor. Due to the fact that a higher percentage of the metastatic cSCCs contained a mutated KMT2D gene, I hypothesized that mutations in the gene directly impact metastasis and promotion of cancer cell growth. To test this hypothesis, we knocked down expression of the KMT2D gene using CRISPR/Cas-9 and then evaluated tumor phenotypes such as migration, proliferation, and growth in soft agar between wild-type and knock-down cell lines. Initial results do not show large differences. In addition to these functional studies, an interesting direct correlation was found between the KMT2D and TP53 gene in which knock-down of KMT2D resulted in lower expression of TP53, suggesting a relationship between this gene of interest and a well-known mutated gene in many cancers. Learning more about the direct effects of loss of KMT2D and the connection with TP53 will result in a more profound and accurate knowledge of not only its influence on metastatic cSCC, but also on other SCCs due to high mutation frequencies in SCCs in the head, neck, and esophagus.
Abstract: One of the important aspects of modern ecological research is determining the effects of global warming on ecosystems. Specifically, this includes understanding the complex relationships among intrinsic environmental factors, trophic relationships, and the biodiversity and abundance of the species living in the environment. The purpose of this research was to determine levels of periphyton and allochthonous organic matter in relation to physical characteristics (flow velocity, substrate type) of glacial stream microhabitats (riffles, runs, rapids, and pools), and assess how these parameters affect insect community structure during summer and winter seasons in a glacier stream in Yunnan, China. The amount of periphyton and organic carbon present is hypothesized to have a positive relationship with the abundance of primary feeders and shredder functional feeder groups, respectively. The abundance of primary feeders and shedders will positively affect the abundance of predators. Periphyton will be sorted from microhabitat samples and volume estimated by water displacement. Organic carbon was measured using ash free dry mass. Taxonomy and macroinvertebrate abundance data was provided for the analysis. It is predicted that the macroinvertebrate biodiversity and abundance will be higher in surber samples where periphyton and organic carbon levels are high. Understanding baseline trophic structure is paramount to determining stream health as it is impacted by climate change. Even though macroinvertebrate diversity is a common bioindicator of stream health, this project aims to contribute further understanding to the structure of macroinvertebrate communities as related to climate change in glacier streams, which are critical but lesser explored ecosystems.
Category: Biological Sciences

Title: Development of a CRISPR/Cas9 mutation system in Caenorhabditis briggsae and use in altering the Notch signaling pathway

Student Presenter: Robert Smith

Faculty Advisor: Chamberlin, Helen

Abstract: Notch and the Epidermal Growth Factor (EGF) are crucial growth pathways found across almost all species of animals. Many types of cancers develop from malignant mutations afflicting these pathways; some can even result from negative distortions in both. In order to further understand the basic operation of Notch and EGF, research on lower model organisms is necessary. The nematode vulva provides an ideal case to study where the two pathways interrelate since Notch interacts with the EGF pathway in order to specify different types of vulval cells. Current research focuses on comparing this interaction to the process in a related species, C. briggsae. We find that in C. elegans, the vulval development is entirely dependent on EGF signaling, while in C. briggsae vulval cells divide even if EGF signaling is blocked. Comparison of the signal interactions in these two species provides a model for how different genetic backgrounds may impact cell division regulation. This project tested the hypothesis that these cell divisions result from EGF-independent Notch signaling. To better understand Notch signaling in C. briggsae, the LIN-12/Notch receptor was genetically manipulated in order to constitutively activate and block this signaling pathway in the vulval precursor cells (VPCs). This was done by creating sgRNAs and specific repair templates that induced mutations in the Cbr-lin-12 gene through the CRISPR/Cas9 system. We are currently using these sgRNAs and repair templates to introduce specific mutations into the C. briggsae genome. For this project we also developed novel CRISPR/Cas9 reagents and methods for use in C. briggsae, and demonstrated their efficacy, Worms were observed throughout the process to provide evidence of successful CRISPR-based transformations. By understanding these interactions more in depth, pathway interplays evidenced by nematodes can be extrapolated to orthologous pathways in humans in order to more fully comprehend the signals that lead to growth in human cells.
Abstract: Non-heme diiron carboxylate proteins play important roles in diverse biological functions, ranging from hydrocarbon oxidation to DNA synthesis to intracellular signaling. Ribonucleotide reductase-like ligand-binding oxidase (R2lox) is a member of this family that performs multi-electron oxidative chemistry via a metallic binuclear active site. R2lox incorporates manganese and iron cofactors in two distinct configurations: one utilizing a heterobinuclear manganese-iron active site and another incorporating a homobinuclear diiron cofactor. However, the mechanism of assembly of these bimetallic cofactors has not been identified. To better understand the chemical processes underlying the assembly of R2lox, UV-visible spectroscopy was used to evaluate the kinetics of assembly of an R2lox mutant strain as well as identify potential intermediates in the reaction. Stopped-flow (SF) UV-visible and rapid freeze-quench electron paramagnetic resonance (EPR) spectroscopies were used to further characterize reaction intermediates. Preliminary results suggest that the kinetics of assembly and intermediate formation in the mutant protein are markedly different from wild-type (WT) R2lox. Compared to the WT protein, the Y175F variant assembles with slower rates, and the optical signatures of intermediates are blue-shifted relative to WT. Data collected from the UV-vis will resolve the kinetics of these processes down to the millisecond timescale, which will help distinguish similarities and differences of assembly, in addition to highlighting the intrinsic properties of Y175F. EPR spectroscopy will help identify reaction pathways and intermediates, depending on the magnetic properties of the resulting species generated. Incorporating new kinetic information along with identities of distinct reactive intermediates in Y175F in addition to other R2lox mutants will reveal the mechanism of incorporation of binuclear cofactors in R2lox.
Abstract: Increasing sea surface temperatures, a result of anthropogenic global change, is causing an increase in the frequency and severity of mass coral bleaching events. When heat stressed, corals expel their photosynthetic algal endosymbionts that provide them with fixed carbon to meet metabolic energy requirements. In the absence of endosymbionts, corals with high levels of stored energy reserves (lipids, carbohydrates, and protein) and corals that acquire energy through heterotrophy are known to have increased survival and resilience potential. To evaluate how lipid management can infer resilience, I measured changes in lipid class composition in repeatedly bleached (treatment) and non-bleached corals (control) of three species (O. faveolata, P. astreoides, and P. divaricata) across several recovery time points over two years. In experimentally bleached O. faveolata, phospholipid concentrations decreased by about half, which might correspond to cell loss associated with bleaching. However, after repeated bleaching, O. faveolata had phospholipid concentrations comparable to controls and cholesterol concentrations increased steadily, results that perhaps are associated with increased heterotrophic feeding on zooplankton. Baseline concentrations of cholesterol were higher in P. astreoides than in O. faveolata and P. divaricata. These initial findings suggest that these three species manage their lipid reserves differently under heat stress, possibly a reflection of switches between photoautotrophy and heterotrophy. We are currently measuring more lipid classes (tri- di- & mono-acylglyceride, free fatty acid, glycerol and wax ester) to have a comprehensive record of coral lipid management under repeated heat stress, to describe resilience strategies following annual bleaching events.
Abstract: Deposition of extracellular materials next to a cell can protect this cell, change its growth and morphology, or help it to move and communicate with other cells. To perform such important functions, extracellular materials must be deposited very precisely, but how such precision is achieved is mostly unknown. A beautiful example of an extracellular structure with very precise deposition is the wall surrounding pollen grains. Pollen from different species often look remarkably different - in part, because wall materials are deposited at some regions on pollen surface and absent from the others. The regions where the wall is absent are called apertures, and these structures help pollen perform its reproductive function. Across species, pollen apertures often differ in shape, number, and positions. Within a species, aperture patterns are usually the same suggesting that developing pollen consistently marks specific surface regions as different from the rest of the surface, ensuring that only they will develop into apertures. Previously, only one gene, INP1, was known to influence pollen aperture formation. In order to identify other players involved in this process, we performed a mutagenesis screen in Arabidopsis. Aperture mutants forming five new complementation groups were isolated by microscopy. We then used positional cloning to identify genes affected in four of these mutants. To do this, mutants in the Landsberg erecta background were crossed with wild-type plants from another Arabidopsis accession, Columbia, and the genes were mapped using PCR markers that could distinguish between Landsberg and Columbia genomic sequences. Four genes potentially involved in pollen aperture formation were identified: MACAROON (encoding an ELMO/CED-12 domain protein), SRF2 (a receptor-like kinase), and two novel genes, INP2 and DONUT. Further characterization of these genes can provide important clues for understanding the molecular mechanism of pollen aperture development and generation of distinct cellular and extracellular domains.
Category: Biological Sciences

Title: Definition of the function of the Atg8 protein in the malaria parasite, Plasmodium falciparum

Student Presenter: Kieran Tebben

Faculty Advisor: Drew, Mark

Abstract: Plasmodium parasites, the causative agent of malaria, were responsible for 438,000 deaths in 2015, Plasmodium falciparum causing the most deadly form. Atg8, a small, ubiquitin-like protein, is commonly the central marker of autophagy, a cellular recycling pathway. While the autophagy pathway is limited in Plasmodium, having only five associated genes, Atg8 appears to have a novel function in these parasites, localizing to the apicoplast, a remnant plastid organelle. In both autophagy and localization to the apicoplast, Atg8 is conjugated to the lipid phosphatidylethanolamine (PE) at a conserved C-terminal glycine. Using the CRISPR/Cas9 system, this essential glycine was mutated to alanine, disrupting lipidation and impairing Atg8's normal function. Data from the related parasite, Toxoplasma gondii, suggest an essential role for Atg8 in autophagy, as well as in homeostasis of both the apicoplast and the mitochondrion. The glycine to alanine mutant displays a slow-growth phenotype, loss of the apicoplast, and fragmentation of the mitochondrion. Our P. falciparum data are consistent with these findings, showing a dramatic slow-growth phenotype and morphological abnormalities. Metabolically, mutants appear to be unaffected, displaying similar growth patterns with or without supplementation of the key apicoplast metabolite, isopentenyl pyrophosphate (IPP). Mutant parasites appear to form fewer daughter merozoites during schizogony, potentially attributed to impaired expansion and segmentation of the apicoplast. These data suggest that Atg8 plays an important role in ensuring normal apicoplast division into merozoites, but further characterization of these morphological differences is needed. Apicoplast expansion and segmentation appears physically coupled with the same processes in the mitochondrion. Apicoplast-deficient parasites may also show similar mitochondrial defects. These defects will be explored using drug sensitivity assays targeting mitochondrial (atovoquone/proguanil) and apicoplast (tetracycline) functions. Definition of the role of Atg8 in P. falciparum could elucidate new therapeutic avenues, potentially slowing parasite growth within the realm of immune system control.
Abstract: Structural proteins expressed in muscle fibers vary among muscle types; this allows for variability in muscle contraction strength and endurance. Different myosin light chains, proteins key to muscle contraction, are expressed in fast and slow muscle fibers, large multinucleated muscle cells. This suggests that the light chains may help tune the function of these fiber types. The function of these proteins has been previously studied in vitro and in vivo using mouse and Drosophila models; however, the developmental role of myosin light chain genes hasn't been closely examined, nor the specific functions of the four zebrafish fast muscle-expressed myosin light chain genes. We hypothesized that mylpfa, one of these four genes, is necessary for formation of normal intracellular muscle contractile units (sarcomeres), and thus muscle function, but not for muscle fiber formation. Using CRISPR-mediated mutagenesis, we created a null mutation in mylpfa. Consistent with our hypothesis, fast muscle fibers form in mylpfa mutants, though the sarcomeres within these fibers do not form normally. In contrast, mylpfa mutant slow muscle fibers appear normal. Muscle function in mylpfa mutants is differentially affected depending upon myofiber composition; the pectoral fin, composed primarily of fast fibers, is paralyzed, whereas the trunk, comprised of both fast and slow fibers, are still contractile. As the embryo grows, fast fibers degenerate in mylpfa mutants, first becoming evident by 76 hours post fertilization (hpf). Our results demonstrate that mylpfa is dispensable for initial muscle fiber formation but essential for assembly of the sarcomeres in these fibers. The degeneration of mylpfa mutant fast muscle fibers indicates that mylpfa promotes muscle fiber integrity. Our studies of mylpfa mutants indicate a previously unappreciated cellular mechanism that preserve muscle integrity, via the sarcomere. Further investigation into this mechanism may help explain degenerative muscle diseases, particularly those caused by defects in sarcomeric proteins.
Abstract: Polyploidy is a phenomenon that involves the duplication of an organism's entire genome and occurs most commonly in plants. These duplication events make it especially difficult to understand the genetic ancestry among closely related polyploids. Penstemon (Plantaginaceae) is the largest genus of plants native to North America, and has several sections of species that are predominantly polyploid. Our study group, the P. attenuatus species complex, contains three regional varieties distributed in the Pacific Northwest of North America. Each of these varieties (var. attenuatus, var. militaris, and var. pseudoprocerus) is hypothesized to be a polyploid hybrid (allopolyploid) between two other species. To evaluate these hypotheses, we collected genetic data using amplified fragment length polymorphism (AFLP) DNA fingerprinting techniques for all members of the complex. For each variety and its putative parents, we evaluated their hypothesized relationships using clustering analyses based on genetic distances (principle coordinates analysis, neighbor joining trees) and genetic ancestry mixture models (STRUCTURE). Our pilot study tested one of the triplets involving P. attenuatus var. pseudoprocerus. Our results showed that it is more closely related to one putative parent (P. procerus) than the other parent (P. albertinus), which does not support our original hypothesis. We have expanded our study to include over three hundred individuals from thirty localities comprising all varieties plus their purported parental species. Testing these hypotheses will reveal the relationships among the species in our study group and will also demonstrate the analytical tools that can be used to understand the origins of allopolyploid species.
Title: Uncovering the biochemical properties of the Salmonella deglycase FraB, a potential drug target

Student Presenter: Nicholas Thomsen

Faculty Advisor: Gopalan, Venkat

Abstract: Salmonella is a foodborne pathogen that causes millions of cases of salmonellosis globally every year. Because Salmonella-specific drugs are not available, there has been a search for unique metabolic pathways in this pathogen. It was recently discovered that Salmonella could utilize fructose-asparagine (F-Asn), a naturally occurring Amadori product, as its sole carbon and nitrogen source. Utilization of F-Asn requires an asparaginase, a kinase, and a deglycase. In addition to Salmonella, these enzymes appear to be present only in Citrobacter and Clostridia, indicating a limited distribution in the animal gut microbiome. Salmonella mutants lacking the deglycase (encoded by FraB) are up to 100,000-fold attenuated in mouse models of intestinal inflammation. This phenotype has been attributed to the build-up of 6-phospho-fructose-aspartate (6-P-F-Asp), which is typically converted to glucose-6-phosphate and aspartate by FraB. Collectively, these findings highlight the value of FraB as a potential Salmonella-specific drug target. Towards this goal, we initiated biochemical characterization of recombinant FraB. We first determined the optimal pH and temperature for its activity, and the Michaelis-Menten kinetic parameters for the conversion of 6-P-F-Asp to glucose-6-phosphate and aspartate. To understand the substrate-recognition determinants of FraB, we then compared the deglycase activity towards 6-P-F-Asp (L-isomer) vs. 6-P-F-Glu or 6-P-F-Asn, as well as the D-isomer of 6-P-F-Asp. To test a catalytic mechanism that we postulated, we chose for site-directed mutagenesis eight residues guided by sequence alignment of FraB homologs and spatial proximity of residues in a homology-based tertiary-structure model. Activity assays of these mutants revealed amino acid residues in the putative active site of FraB that are essential for activity in vitro. To gain detailed structural insights, we have initiated FraB crystallization. Results from these ongoing studies represent the first analysis of the biochemical properties of the Salmonella FraB deglycase, and provide a framework for exploring FraB as a drug target.
Abstract: The cell cycle is the process through which cells divide and either produce two identical cells (mitosis) or four gametes (meiosis). The expression of the major factors that drive the cell cycle is largely regulated by the E2F family of transcription factors. An essential group of E2F targets are the Cyclins, a family of regulatory proteins that activate cyclin-dependent kinases and control the progression of the cell cycle. This includes Cyclin A2 (Ccna2/CCNA2), an important cyclin in the S-phase of the cell cycle that has been found to be misregulated in certain types of cancer. In order to study the importance of E2F-mediated regulation of Ccna2 in vivo, we generated mice in which a key E2F site in the Ccna2 promoter is mutated and E2F binding is abolished, creating a Ccna2 promoter that is not responsive to E2F regulation. Although mice were viable and appeared healthy, males exhibited testicular atrophy and were infertile. Examination of the testes revealed a three-fold decrease in testes weight in adult mice, while histological analysis of the testes revealed a progressive loss of differentiating spermatocytes in juvenile mice and a partial recuperation in adults. Using quantitative PCR and immunohistochemistry, we measured the expression of CCNA2 and stage-specific markers of spermatogenesis during the first weeks of development and in adult mice. This revealed abnormal expression of Ccna2 and downregulation of late meiotic markers. Collectively, the data suggests that E2F regulation of Ccna2 is important for the proper progression of the 1st round of spermatogenesis and consequentially, fertility. This brings us closer to obtaining a better understanding of spermatogenesis and how the cell cycle is regulated, which could lead to personalized gene therapies that can aid those with many genetic diseases (including infertility and cancer).
Abstract: Bone, a dynamic tissue, undergoes constant remodeling throughout the lifetime of the vertebrate organism. Bone remodeling occurs through balanced actions of bone formation by osteoblasts and bone resorption by osteoclasts. Osteoclasts are formed from myeloid precursors. Two cytokines, colony-stimulating factor-1 (CSF-1) and receptor activator of NF-κB ligand (RANKL) are sufficient for the differentiation of osteoclasts from myeloid precursors in vitro. The downstream cascade results in the expression of effector genes through transcription factors (TFs). One main TF is Microphthalmia-associated transcription factor (MITF), which is phosphorylated at serine 307 by p38 MAPK. Inhibition of p38 MAPK also inhibits osteoclast differentiation. An imbalance in bone formation and resorption mainly due to excessive osteoclast differentiation and function leads to bone diseases like osteoporosis. Approximately 2/3 of adult female population is susceptible to osteoporosis in the US. Available therapeutics target osteoclasts and cause osteoclast apoptosis, which again results in inadequate bone remodeling. We used mouse genomics and genetics to evaluate the RANKL-specific target of MITF. The MITFce mouse model, without the p38 MAPK phosphorylation and binding site of MITF, showed age resolved osteopetrosis as a result of defective osteoclast differentiation and function. To evaluate whether over-expression of p38 MAPK docking site with a mutation in the phosphorylation site could sequester available p38 MAPK from phosphorylating endogenous MITF, we over-expressed a short plasmid construct encoding the p38 MAPK binding domain of MITF along with a control plasmid. This mimicked the in vitro differentiation program from the MITFce mouse model. Our result indicates that by ablating MITF phosphorylation alone by p38 MAPK, we could modulate and reduce excessive osteoclast differentiation and function. This could be due to the down regulation of Lcp2 and Vav3 genes that are osteoclast specific regulators or Rac1. With further research, the short peptide could potentially act as a therapeutic intervention for osteoporosis.
Title: Evaluation of alphabet stimuli using anterior tongue and fingertip

Student Presenter: Karli Van Simaeys

Faculty Advisor: Simons, Christopher

Abstract: While the system for texture perception on the dermis has been well researched, there has been little work done on texture perception in the oral cavity. This study seeks to compare edge and point sensitivity of the anterior tongue to the fingertip by using a simple letter identification task. It is hypothesized that the tongue will be better at edge and point detection as the ability to evaluate objects within the oral cavity in the absence of visual cues plays a vital role in chewing and swallowing. Small, plastic alphabet letters of various sizes were created and bound to tongue depressors. During the experiment, thirty subjects were asked to close their eyes, assess a letter stimulus using either the fingertip or the anterior tongue, and respond with the identity of the letter they perceived. In the first part, they were presented with a stimulus and asked to lick it with the anterior one third of their tongue. This process was repeated with their fingertip. If correct, subjects were given the next smallest letter size in the next round; if incorrect, they were given the next largest letter. In order to analyze the data collected, the detection threshold will be calculated as the mean of eight reversals along the ladder. Binomial statistics will be used to determine if people are significantly more accurate with their tongue than with their fingers. This data will confirm whether the tongue or the fingertip has a greater ability to detect edges and points. Results from this study, combined with data from future comparative tactile sensitivity studies using other stimuli and oral surfaces, will help to build a better picture of the mechanisms of texture perception of oral tissues. This knowledge can, in turn, provide insight into how texture perception may influence food preferences.
Category: Biological Sciences

Title: Geographic patterns of floral morphology change in Penstemon albidus and Penstemon whippleanus (Plantaginaceae)

Student Presenter: Morgan VanDeCarr

Faculty Advisor: Wolfe, Andrea

Abstract: Pollinators are one of the major selective agents in the evolution of flower morphology. Numerous studies have shown that changes in floral morphology are related to changes in pollinators. We used digital herbarium specimens to measure nine floral characters indicative of pollinator specificity (plant height, basal leaf length, mid leaf length, tip length, corolla length, corolla tube width, corolla throat width, corolla mouth width, and inflorescence length) in Penstemon albidus and P. whippleanus. To assess the level of correlation for geographic distribution and morphological data, we used several R software packages (gstst, lattice, spatial, sp, rgdal). The most variable characters in both P. albidus and P. whippleanus were inflorescence length and plant height. Corolla tube width and corolla mouth width were the least variable in P. albidus while in P. whippleanus, corolla throat width and corolla length showed the least amount of variation. The analysis of spatial correlation of morphological data with geographic distribution indicated that in P. albidus corolla mouth width, tube width, and throat width sizes increase as individuals move from East to West and from South to North. The other characters showed a slightly less pronounced trend. Environmental factors such as temperature influence flowering time so that plants with northern distributions flower later than those in the south. This results in a more limited time for pollinator visits and consequentially reduces gene flow among northern populations. In P. whippleanus, the Rocky Mountains played an important role in the distribution of floral characters. West of the Rockies, individuals are larger than in the East for most characters. This trend is less obvious in the corolla length and tip leaf length. These preliminary results suggest abrupt changes in topography represent a stronger barrier to gene flow than flowering time and pollinator interaction.
Abstract: Melanoma, the deadliest form of skin cancer, has a 5-year survival rate of 15% for stage IV patients. Melanoma mortality is largely attributable to its metastatic potential. Thus, understanding the molecular mechanisms that drive melanoma metastasis is particularly important. P21 Activated Kinases (PAKs) are commonly overexpressed and altered in a variety of cancers and regulate mechanisms important for cancer development including: cell movement, growth, and apoptotic resistance. There are two types of PAK proteins: group I (PAK1, 2, and 3) and group II (PAK4, 6, and 7), divided based on sequence homology and distinct regulatory properties. PAK7 is the most frequently mutated PAK in melanoma, yet, the role of this protein in melanoma progression is unknown. We tested the hypothesis that PAK7 mutants promote melanoma initiation and metastasis through inappropriate activation of downstream signaling pathways. We generated stable melanocyte cell lines expressing melanoma-associated PAK7 mutants, kinase-dead, and kinase-hyperactive mutants. We tested the effects of these alterations on proliferation, migration, and downstream signaling. We observed no changes in signaling through the mitogenic MAPK pathway and proliferation was unaltered. By contrast, we observed a correlation between PAK7 kinase activity and migration. This lead us to perform mass spectrometry to identify PAK7 interacting partners involved in cell movement. This work identified GEF-H1, a guanine nucleotide exchange factor that activates Rho and Rac-GTPases, both major players in cell motility. We validated this interaction in our melanocyte stable lines and observed increased PAK7 kinase activity resulted in increased phosphorylated GEF-H1. Further, we identified the region within PAK7 necessary for GEF-H1 interaction. Together, this work suggests a model wherein PAK7 functions through GEF-H1 to influence cell motility. Future work will explore the role of PAK7:GEF-H1 interaction in melanoma metastasis. Understanding the role of PAK7 in melanoma metastasis will improve diagnostic capabilities and provide novel therapeutic targets.
Abstract: ETS-1 is a transcription factor known to play an important regulatory role in lymphoid cells. In humans, ETS-1 is most highly expressed at the mature stage of Natural Killer (NK) cell development. This stage is characterized by the acquisition of several activating receptors including NKp46. Previously, global ETS-1 knockout models have been used to show a reduced and defective NK cell population compared to wildtype. However, this model leaves out whether ETS-1 functions in an extrinsic or intrinsic manner and does not address the differential expression at different stages in development. This project aims to determine if there is an intrinsic role of ETS-1 in the development and function of NK cells with an NKp46-Cre conditional ETS-1 knockout mouse model. A multi-color flow cytometry panel will allow for the dissection of NK cell populations at all stages of development. Differential gene expression will be examined for a mechanistic analysis of the role of ETS-1. Results from these assays will be a starting point for further evaluating the defects caused by ETS-1 deficiency at the mature NK cell stage. Taken together this project aims to elaborate on the role of ETS-1 in NK cell development. This research will constitute a critical step forward in understanding the biology and mechanisms underlying mature NK cell regulation of development. Furthermore, continuing to better understand transcription factor ETS-1 is essential when dealing with diseases mediated by lymphoid cells. Unique to this project is the development of a stage and cell specific deletion of ETS-1 in maturing NK cells. The use of an NKp46 Cre promoter ensures that only NK cells that have reached the mature stage become deficient in ETS-1. This presents the opportunity to analyze how ETS-1 regulates mature NK cells unlike previous studies.
Abstract: Polyploidy is a phenomenon that involves the duplication of an organism's entire genome and occurs most commonly in plants. These duplication events make it especially difficult to understand the genetic ancestry among closely related polyploids. Penstemon (Plantaginaceae) is the largest genus of plants native to North America, and has several sections of species that are predominantly polyploid. Our study group, the P. attenuatus species complex, contains three regional varieties distributed in the Pacific Northwest of North America. Each of these varieties (var. attenuatus, var. militaris, and var. pseudoprocerus) is hypothesized to be a polyploid hybrid (allopolyploid) between two other species. To evaluate these hypotheses, we collected genetic data using amplified fragment length polymorphism (AFLP) DNA fingerprinting techniques for all members of the complex. For each variety and its putative parents, we evaluated their hypothesized relationships using clustering analyses based on genetic distances (principle coordinates analysis, neighbor joining trees) and genetic ancestry mixture models (STRUCTURE). Our pilot study tested one of the triplets involving P. attenuatus var. pseudoprocerus. Our results showed that it is more closely related to one putative parent (P. procerus) than the other parent (P. albertinus), which does not support our original hypothesis. We have expanded our study to include over three hundred individuals from thirty localities comprising all varieties plus their purported parental species. Testing these hypotheses will reveal the relationships among the species in our study group and will also demonstrate the analytical tools that can be used to understand the origins of allopolyploid species.
Title: QuaCRS (Quality Control for RNA-Seq) II: A user-friendly web-based tool for assessing RNA-seq data quality by samples, groups, or projects

Student Presenter: Logan Walker

Faculty Advisor: Yan, Pearly

Abstract: High throughput next generation gene expression analysis (RNA-seq) has been widely accepted by basic/translational scientists and physicians in bench top research, drug development and patient diagnosis and prognosis. With this wide acceptance, this research space is filled with new kits for RNA isolation and for sequencing library generation. Often times, different protocols were used to generate RNA-seq data across vast time spans and by different staff members or from different labs/cores/companies. Before one commits to perform computation resource intensive analyses, it is important to establish that the RNA-seq data of interest are comparable in qualities and overall profiles. QuaCRS II fills this gap by employing a database of computed quality control (QC) metrics for this important assessment. This allows users to interactively drill down on QC and read distribution parameters from disparate sets of data via a web-based viewer with ease in real time. In addition to enabling on-demand statistical comparison between groups, QuaCRS II is now supported by a configurable data model and newly updated tools for QC generation. The utility of QuaCRS II is demonstrated with data released in a recent multi-center RNA-seq standardization study conducted by the Association of Biomolecular Resource Facilities (ABRF).
Category: Biological Sciences

Title: Update on conservation genetics of Penstemon caryi (Plantaginaceae) around the Tensleep Preserve (Wyoming) and Pryor Mountains (Montana)

Student Presenter: Alexander Ward

Faculty Advisor: Wolfe, Andrea

Abstract: Penstemon caryi is a rare plant species endemic to two mountain ranges in northern Wyoming and southern Montana. It exhibits a fragmented distribution, appearing only on limestone outcrops and tallus slopes, making it a target for potential conservation efforts. Our previous work in the Tensleep Preserve area highlighted the need to continue monitoring these populations. Recently, new samples of P. caryi have been collected from the Pryor Mountains of Montana. We assayed these new samples using seven microsatellite markers as well as two AFLP primer sets in order to estimate population genetic diversity, differentiation, and gene flow compared to the previous nine populations studied. The data collected were analyzed using the programs GenAlEx and STRUCTURE. Results of the STRUCTURE analysis show that the Pryor Mountains population is more genetically distinct compared to the Tensleep populations. Analyses with GenAlEx also show that the Pryor Mountains population has a greater number of unique genetic variants compared to the rest of the populations. Furthermore, the Pryor Mountains population has the lowest average heterozygosity (Ho=.449) across all loci, suggesting that those plants may be at greater risk of extirpation due to low levels of genetic diversity. While all populations of P. caryi should be monitored and protected due to the rarity of the species, these analyses suggest that conservation efforts for the Pryor Mountains populations need to be made a priority due to the population being a source of distinct genetic diversity for the species.
Abstract: Studying novel proteins and their behavior in disease states often leads to elucidation of their function in vivo and provides a platform to guide drug development and treatment efforts. Recent patient studies have shown that a fatal mitochondrial disease, multiple mitochondrial dysfunctions syndrome 1 (MMDS1) arises from two distinct genetic mutations near the active site of the essential iron-sulfur (Fe/S) protein NFU1. Symptoms of MMDS1 include impairment of neurological development, lactic acidosis, failure to thrive, and ultimately death in infancy - all indicative of a general failure of the metabolic system. Reduced function of target Fe/S proteins lipoic acid synthetase (LIAS) and succinate dehydrogenase (SDH) in the MMDS1 disease state suggests that multiple metabolic pathways require proper function of the native NFU1 protein through Fe/S cluster delivery and trafficking. However, the precise function of NFU1 remains unknown, and there is no treatment or cure for MMDS1. To investigate the molecular consequences of the disease-causing Gly208Cys and Gly189Arg mutations, the structure and function of the mutant human NFU1 protein forms were analyzed in vitro using a suite of biochemical techniques and compared to data obtained for the native construct. In the case of G208C NFU1, the mutation initiates a global structural change, which alters the monomer-dimer equilibrium such that it is unable to receive an Fe/S cluster from physiologically-relevant sources. Preliminary data suggests a similar phenomenon could be occurring in the case of G189R NFU1. In order to complement study of these mutant constructs, selective mutations at positions 189 and 208, along with cluster-binding residues at positions 210 and 213 have provided an in-depth investigation into the effects of mutation on Fe/S cluster coordination and transfer, and corroborated conclusions drawn from study of the G208C and G189R constructs.
Abstract: In cell division, methionine is used in polyamine synthesis, resulting in a toxic, sulfur-containing byproduct, 5-methylthioadenosine (MTA). As biologically available sulfur is typically limiting, many organisms possess a Methionine Salvage Pathway (MSP) to detoxify MTA and recycle the sulfur back to methionine. Nearly all eukaryotes and many prokaryotes employ the “universal” MSP, which requires molecular oxygen. Recently, our group discovered the first oxygen-independent MSP in Rhodospirillum rubrum that functions both aerobically and anaerobically, as well as a second, strictly anaerobic MSP. The strictly anaerobic MSP utilizes in part an operon encoding three enzymes that sequentially metabolize MTA to methylthioacetaldehyde (MTAdh). A yet to be elucidated enzyme(s) then converts MTAdh to methionine, creating ethylene gas (C2H4) as a byproduct. The third enzyme, a novel methylthioribulose-1-phosphate (MTRu-1P) aldolase cleaves MTRu-1P (an MTA-derived metabolite) to form MTAdh. Based on amino acid sequence similarity, at least 320 additional bacterial species contain a putative MTRu-1P aldolase that may function as part of an anaerobic MSP. In this study we have explored the functionality of MTRu-1P aldolase homologs from enteric bacteria Eubacterium limosum and Morganella morganii via a gene complementation in R. rubrum. The native MTRu-1P aldolase in R. rubrum was knocked out, disrupting the strictly anaerobic MSP, thus causing ethylene to be marginally produced. The putative aldolases of interest were then cloned into complementation plasmids able to express the provided gene. These plasmids were mated into the R. rubrum aldolase knockout strain, and ethylene production was measured. The E. limosum aldolase could not restore ethylene production, indicating a lack of MTRu-1P aldolase activity in E. limosum. However, the M. morganii aldolase restored ethylene production, suggesting a similar strictly anaerobic MSP may exist in M. morganii. Further studies will determine if MTRu-1P aldolase homologs from other organisms can function in a similar manner.
Abstract: The actin crosslinking domain (ACD) is an actin-specific toxin produced by Vibrio and Aeromonas spp that catalyzes the formation of a covalent bond between actin monomers, forming non-polymerizable oligomers. Recently, our group demonstrated that these oligomers amplify their toxicity by potently inhibiting formins - important actin nucleators. Because such potent inhibition is enabled by the abnormally high affinity of oligomers for tandem actin binding domains of formins, we hypothesized that oligomers may also target other proteins with multiple actin binding domains, and thus further amplify the toxicity. One conserved G-actin-binding domain found in an array of actin mediating proteins is the WASP-homology 2 (WH2) domain. WH2 domains are found in Spire, Arp2/3 complex activators (WASP, WAVE, etc.), and bacterial toxins VopF/L. In these proteins, WH2 domains contribute to nucleation of new filaments by organizing actin monomers into filament-like orientations, either on their own (tandem WH2 domain of Spire, VopL/F) or in concert with other proteins (Arp2/3 complex). To test the hypothesis, we assessed the effects of the ACD toxin-produced actin oligomers on their nucleation activity. We employed Total Internal Reflection Fluorescence (TIRF) microscopy and pyrene actin polymerization methods to assess the effects of oligomers on the single filament and bulk levels, respectively. We found by TIRF that sub-stoichiometric concentrations of oligomers efficiently blocked VopF/L and Spire mediated nucleation. The dose-dependent inhibition of Spire, Arp2/3 activator WASP, and VopF/L-mediated actin nucleation was further confirmed by bulk solution assays. We found that actin oligomers inhibit Spire, and VopF/L with low nanomolar affinities likely owing to the tandem organization and unique geometry of the later. In contrast, the inhibition of Arp2/3 complex activation requires higher concentrations of oligomers. In conclusion, we have now identified new members of the toxicity cascade that ACD initiates by converting actin monomers into potent secondary toxins.
Abstract: Cell-cell adhesion is mediated by calcium-dependent proteins called cadherins, which are important in neuronal connectivity and tissue integrity. Cadherins are modular proteins with large extracellular domains that have typically been modeled using all-atom molecular dynamics (MD) simulations. However, these simulations are computationally expensive and most of them only include small fragments of these cadherin extracellular domains. To overcome these limitations, we use a coarse-grained (CG) model with the MARTINI force field to study large cadherin complexes over long time scales. All-atom MD simulations were used to find optimal parameters for an elastic network model that stabilized the protein secondary structure. The CG model allowed for a 5x increase in timestep and a 10-fold reduction of system sizes. Using this model we studied the dynamics and elastic response of classical cadherins and clustered protocadherins. In these simulations, the extracellular domains of classical cadherins straighten before unbinding, while protocadherins slip past each other during unbinding. Overall, our results confirm that our model is an effective simulation tool for studying of the mechanics of cadherin complexes.
Category: Biological Sciences

Title: Phenotypic plasticity of organ size and allocation of energy and resources to reproduction in the viviparous cockroach Diploptera punctata.

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Abstract: Understanding how patterns of energy and resource allocation are related to lifetime reproductive output propel the conceptualization of evolution of life history in organisms. Animals allocate energy and resources to maintenance, growth and reproduction. One mechanism that might drive allocation of resources is phenotypic plasticity of organ size. Animals can increase or decrease the size of metabolically active organs in response to certain environmental conditions, and that might have consequences in overall energy expenditure and allocation. We studied phenotypic plasticity of organ size in females of Diploptera punctata, a viviparous cockroach. Female D. punctata feed their nymphs with a secretion produced by the abdominal epithelium, and give birth 9-12 nymphs after a gestational period of 55-60 days. We measured metabolic rate (MR) in four groups of females: (1) pregnant females at day 55 fed with a high quality (HQ) diet, (2) pregnant females at day 55 fed with a low quality (LQ) diet, (3) non-pregnant females in which we switched diets from LQ to HQ, then back to LQ in periods of 30 days, and (4) non-pregnant females in which we switched diets from HQ to LQ then to HQ again in periods of 30 days. We measured mass of digestive tract, abdominal organs, embryos in the pregnant females, and carcass. We found a positive relationship between gut mass and MR and a negative association between the mass of abdominal organs and MR. There seems to be a trade-off between gut mass and abdominal organ mass, and gut mass and reproductive output. Interestingly, females acclimated to different diets tend to adjust their physiological traits to a given environment in a manner dependent on the previous event. We suggest that changes in organ mass in response to environmental conditions determines complex patterns of allocation of resources to reproduction in D. punctata.
Abstract: Diffuse large B-cell Lymphoma (DLBCL) has two subtypes: activated B-cell (ABC), which depends on antigen-driven signals from its B-cell receptor (BCR), and germinal center B-cell (GCB), which uses its BCR in tonic signaling. A single amino acid (Y196) of CD79B, one of four proteins comprising the BCR, is mutated in 18% of ABC-DLBCL primary tumors, implying positive selection for a functional consequence. This project aims to study the mutation's effects on survival and BCR signaling by creating "isogenic" pairs of DLBCL cell lines, with vs. without CD79B mutation. The CRISPR/Cas9 system was used for precise genomic modifications. Two "cutting" plasmids expressing Cas9 protein and specificity for CD79B, and one "repair" plasmid bearing the desired CD79B sequence plus a reporter (CFP), were introduced to human GCB-DLBCL cell lines by electroporation. Knock-in created CD79B sequences that were wild-type (WT) or had Y196F, Y207F (in the same "ITAM" signaling domain as Y196), or both mutations. Flow cytometry was used to detect modified cells and measure size, growth, and antigen-driven BCR signaling (by ratiometric calcium flux). As expected, a fraction of cells (~15%) were BCR+/CFP+, indicating the desired knock-in. Initial experiments on two GCB-DLBCL cell lines showed that double-mutant cells were smaller, exhibited reduced relative growth, and had amplified calcium flux after BCR cross-linking compared to WT cells. Single mutants gave mixed results, requiring more experiments to clarify. Since F is similar to Y but cannot be phosphorylated, phosphorylation of CD79B appears to reduce antigen-driven BCR signaling, potentially providing a reason why CD79B Y196 mutations are frequent in ABC-DLBCL; this will be more directly tested by reverting Y196-mutant ABC-DLBCL lines to WT. The negative growth effect of double-mutant CD79B in GCB-DLBCL lines was unexpected, but additional techniques will be used to study these modified cells and may provide insights in tonic signaling.