Acknowledgment
Poster 13:
An insertional trap for conditional gene expression in *Toxoplasma gondii*

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Boston College Biology Department

Undergraduate Research Celebration - May 7, 2010

Schedule of Events

2:00-3:00: Student Poster Presentations

3:00-3:30: Awards Presentations

Welcome
Prof. Kathy Dunn

Introductory Remarks
Dean David Quigley

Women in Science & Technology Recognition
Prof. Clare O’Connor

Recognition of Students Writing Senior Theses
Prof. Charles Hoffman

Presentation of Balkema Prize
Prof. Tom Chiles
Women in Science & Technology Participants

Laura Barrett ('11) - Biology
Susan Barton ('11) - Biology
Meg Cells ('10) - Chemistry / Education
Katie Doyle ('10) - Biology
Mary Ann Gallup ('10) - Biology
Lauren Jammallo ('11) - Biology
Catherine Macek ('12) - Chemistry
Courtney McKee ('11) - Biology
Cristina Olcese ('10) - Biology
Rachel Riccardi ('10) - Biochemistry
Lauren Ritter ('11) - Biology
Sara Samaha ('11) - Biology
Danielle Sanchez ('11) - Biology
Janine Sanderman ('10) - Biology / Italian
Caroline Sullivan ('10) - Biology
Meredith Taylor ('11) - Biology
Jacqueline Valenza ('12) - French
Amber Williams ('10) - Biology / English

Senior Thesis Students

Marcus Basiri (Senior Thesis)
Andrew Breglio (Advanced Independent Research)
Michelle Crowther (Bio Honors Program)
Brian Currie (Advanced Independent Research)
Megan Decoteau (Senior Thesis)
Edmundo Feris (Senior Thesis)
Andrew Gregg (Advanced Independent Research)
Erin Groden (Bio Honors Program)
Erin Hannah (Senior Thesis)
Xibei Jia (Bio Honors Program)
Innessa Kipnis (Senior Thesis)
Danielle Larson (Bio Honors Program)
Joshua Meidenbauer (Senior Thesis)
Derek Missert (Senior Thesis)
Cassandra Neitzel (Senior Thesis)
Krystyna Orzechowski (Advanced Independent Research)
Natalie Pham (Senior Thesis)
Chiara Rivas-Morello (Senior Thesis)
Natalie Pham (Senior Thesis)
Natasha Romero (Senior Thesis)
Kathleen Soltis (Senior Thesis)
Amber Williams (Advanced Independent Research)
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**POSTER 1**

Undergraduate Presenter: Marcus Basiri (‘10)

*Drosophila* Poc1 Forms a Cytoplasmic Complex with Core Centriolar Proteins that is Essential for the Formation of an Early Centriole Precursor and Proper Centriole Maturation

Tomer Avidor-Reiss Laboratory (Harvard Medical School, Dept. of Cell Biology)

Centrioles are cylindrical, structurally intricate, microtubule-rich organelles that display nine-fold radial symmetry and exist in a pair inside the amorphous centrosomal space. Centrosomes are the major microtubule-organizing centers of eukaryotic cells and centriolar defects often result in mitotic spindle instability and cancers. Perhaps most importantly, centrioles are required for the formation of cilia. During ciliogenesis, centriole elongation produces a basal body that ultimately gives rise to the ciliary axoneme. Occurring during each cell division or in response to a high cellular demand for centrioles, centriole duplication is a unique process in which each centriole facilitates the production of a new centriole at a defined site orthogonal to the original centriole. It involves the establishment of a centriole precursor including an early “cartwheel” structure followed by an elongation phase in which the centriole grows to maturity. Proteome of the Centriole 1 (Poc1) is a conserved centriolar WD40 protein identified in a basal body proteomics analysis. Poc1 is a very early marker of centriole duplication but its role in centriole biogenesis has remained unclear. Mutations in Poc1 result in centrioles that fail to elongate and in the loss of an early centriole intermediate termed the proximal centriole-like structure (PCL). Using biochemical approaches complemented with genetic considerations, the current study demonstrates that Poc1 exists in a cytoplasmic complex with core centriolar proteins, and provides insight into the role of Poc1 in stabilizing early centriole precursors and in ensuring proper centriole maturation.
Centrioles are critical cellular structures that give rise to the centrosomes and cilia. Centriolar abnormalities are implicated in cancer, ciliopathies, and fertility defects; yet, centriolar formation is poorly understood on multiple levels, including how it is initiated. Centriole formation is a multi-step process that begins with an amorphous intermediate in which an internal structure resembling a cartwheel later forms. Our lab has shown that the Proximal Centriole-Like (PCL) structure of the fly spermatid is a novel model for centriolar initiation and resembles the amorphous phase. Poc1 is a conserved cartwheel protein that is necessary for normal PCL formation; we have previously reported that, in Poc1 mutants, a centriolar protein fails to label the PCL. This abnormality suggests that Poc1 is a building block of the PCL. One expectation from this model is that Poc1 is a component of the PCL. To test Poc1 localization, transgenes using GFP and MYC tags were used, as well as antibodies against Poc1. This dual approach was meant to provide information regarding the native protein without having the results be complicated by specificity and difficulty in centriole penetration, as well as to overcome issues introduced by large markers. Poc1 was found to be enriched along the basal body and not the PCL, suggesting that it is not a constitutive component of the PCL but leaves the possibility that Poc1 is a transient player in earlier PCL formation. Analysis of a null mutant for Poc1 allowed for further understanding of its role as a recruiter of core centriolar proteins to the PCL and the elongating centriole; this complete absence of Poc1 also opens new opportunities for considering how Poc1 may be involved in post-fertilization centriolar development as a recruiter or even platform for important centriolar proteins to the PCL, an established early centriolar structure.
Tumor-derived Neuropilin-2 acts as a reservoir for VEGF-A and VEGF-C and increases angiogenesis and lymphangiogenesis in a breast cancer model

*Diane Bielenberg (Children’s Hospital, Department of Surgery)*

Vascular endothelial growth factor (VEGF-A), an angiogenic factor, binds to VEGF receptor-2 (VEGFR-2) and promotes the proliferation and migration of endothelial cells (EC). Neuropilins (NRP1, NRP2), co-receptors for VEGF-A, enhance VEGF activity but lack kinase activity. Neuropilin-2 (NRP2) is expressed on EC and tumor cells and binds VEGF-A and VEGF-C (lymphangiogenic protein). Tumor cells lack VEGFR-2 and therefore cannot directly signal upon VEGF binding. Our goal is to understand the contribution of tumor-derived NRP2 toward tumor progression. We hypothesize that NRP2 will act as a reservoir for VEGF-A and VEGF-C thus increasing angiogenesis and lymphangiogenesis in breast carcinoma models. Methods: Breast carcinoma cells, MCF7MFP1, were transfected with NRP2 in vitro. RT-PCR and western blot were used to determine the expression of endogenous VEGF ligands and receptors in these breast cancer cells. Binding assays were used to determine whether NRP2 enhanced the binding of VEGF-A or VEGF-C to the tumor cells. The tumorigenicity and metastatic potential of MCF7MFP1NRP2 and MCF7MFP1Mock were compared by injecting the cells orthotopically into immunodeficient mice. Results: Immunoblotting confirmed the overexpression of NRP2 in MCF7MFP1NRP2 cells. RT-PCR and western blot showed expression of VEGF-A and VEGF-C in tumor cell lysates and conditioned media. Expected Results: It is anticipated that these studies will demonstrate that tumor cells overexpressing NRP2 are able to bind more VEGF-A and VEGF-C than untransfected cells. These results may suggest that tumors expressing NRP2 will sequester VEGF-A and VEGF-C ligands in the tumor microenvironment and stimulate (lymph)angiogenesis.
Oxidative stress and cellular death in the brain is positively correlated with exposure to heavy metals such as iron and lead. The HFE protein influences cellular iron concentration by modulating the affinity of the major physiological iron carrier in the body, transferrin, for its receptor. While the C282Y and H63D variants of HFE are associated with hereditary hemochromatosis, the most common form of iron overload disease, aged individuals who express these variants also have increased levels of lead accumulation and cognitive decline. There is no known mechanism for this association. Our results demonstrate that human neuroblastoma cells transfected with the C282Y variant of the HFE protein have decreased levels of viability after lead treatment compared to cells that express the wild type variant of HFE, as measured by the MTT assay. These results correlate well with the decreased lead-mediated cognition associated with the C282Y variant in humans.
Undergraduate Presenters:
Bethel Belai ('11), Justin Chien ('11) and Jeremiah Wang ('11)

Research in Neuroscience Lab (Prof. Burdo)

Neuroprotection by EGCG during excitotoxicity in neuronal HT-22 cells

Excitotoxicity is the pathological process that leads to neuronal cell death due to glutamate and/or other similar neurotoxins. Excessive glutamate stimulation on neuronal cells interferes with the cystine-glutamate antiporter (xCT) and inhibits glutathione production, an extremely important antioxidant in the protection of cells. This influx of glutamate leads to cell death, which contributes to stroke, traumatic brain injury, and many other neurodegenerative diseases of the central nervous system (CNS). Previous studies have shown flavonoids to have neuroprotective abilities against excitotoxicity and cell death. In the present study, mouse hippocampal cell line, HT-22, was used to determine the effect of Epigallocatechin gallate (EGCG), a flavonoid commonly found in green tea, on glutamate neurotoxicity. Following the Method of Transcriptional and Translational (MTT) Assay protocol, it was possible to measure the amount of cell death through absorbance. After 24 hours of incubation, it was found that EGCG protected HT-22 cells against glutamate neurotoxicity when administered as a pre-treatment for 15 minutes before glutamate addition. The results indicate that EGCG is neuroprotective against glutamate-induced cell death at certain concentrations.
Research in Neuroscience Lab (Prof. Burdo)

The Effects of Mangiferin on Glutamate Toxicity in HT22 Neurons

Excessive extracellular glutamate concentration, or glutamate toxicity, leads to neuronal cell death associated with various neurodegenerative insults and diseases such as stroke, Parkinson’s disease and Alzheimer’s disease. Glutamate is the main excitatory neurotransmitter in neurons of the central nervous system. Since the HT22 immortal neurons used lack ionotropic glutamate receptors such as NMDA, glutamate-induced toxicity is caused by the inhibition of cystine uptake via the cystine/glutamate antiporter xCT. This inhibition leads to decreased levels of the antioxidant glutathione, subsequently resulting in oxidative stress and apoptosis. Mangiferin is a flavonoid antioxidant polyphenol that has been observed to have neuroprotective effects in ischemia-induced neurons. As mangiferin is able to traverse the blood-brain barrier, it has potential to attenuate the oxidative stress observed in neurodegenerative disorders by direct scavenging of reactive oxygen species (ROS).

We tested the effects of DMSO-dissolved mangiferin and a phosphodiesterase inhibitor, #27, in 96-well plates. The cells were pretreated with 10mM of each chemical for 15 minutes, followed by addition of 2.5mM glutamate for 24 hours. Cell viability was assessed and quantified using an MTT assay of mitochondrial function. Based on the results, mangiferin and #27 are able to diminish neuronal loss in glutamate-treated HT22 neurons. Although mangiferin may be of value in treating acute neuronal damage, the increase in cell viability in this experiment is not statistically significant.

Figure 1. 96-well Plate- MTT Assay Results
POSTER 7
Undergraduate Presenters:
Kristaq Koci ('10), Stephen Lo ('11), and Toan Phan ('10)

Research in Neuroscience Lab (Prof. Burdo)

The Role of L-theanine in promoting glutamate excitotoxicity on Hippocampal HT-22 Cells

Brain ischemia is strongly associated with increased levels of glutamate in the extracellular (EC) space of neurons. This increase in glutamate levels results in the reversal of the cystine-glutamate exchanger, which results in cystine efflux and glutamate influx in neurons. In absence of Cystine the production of glutathione is halted, resulting in oxidative glutamate toxicity and subsequent cell death. In this experiment, HT-22 cell cultures were used to test the effect that L-theanine, an antioxidant, can have in attenuating cell deaths caused by oxidative glutamate toxicity. In addition we tried to determine the correlation between glutamate levels and cell death. We used 96 well plating techniques, and MTT Assay to conclude that, L-theanine was not significant in attenuating HT-22 cell deaths. Nevertheless, increasing glutamate levels are directly correlated to increased cell death.
POSTER 8
Undergraduate Presenters:
Falen Demsas (’10), Krystal Marquis (’11), and Jessica Pierre Francois (’10)

Research in Neuroscience Lab (Prof. Burdo)

Neuroprotective Effects of Genistein Treatment of Neuronal HT22 Cells

Flavonoids are naturally occurring polyphenol antioxidants found in various foods that have been shown to reduce or prevent the oxidation of other molecules. In this study, the flavonoid, genistein was tested to ascertain whether it had neuroprotective antioxidative properties. Genistein is a strong antioxidant that removes damaging free radicals and reduces lipid peroxidation. Gensistein prevents heart attacks and strokes by acting as an anticlotting agent and increases the activity of other antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and glutathione reductase.

The HT22 cells, a model system for oxidative stress was used in order to determine the potential protective mechanisms of genistein in cell death. Glutamate is the most abundant excitatory neurotransmitter found in the nervous system. It is maintained at relatively low levels in the extracellular fluid to preserve the appropriate signal-to-noise ratio and prevent the excessive activation of glutamate receptors, such as the NMDA receptors, which may result in apoptosis. This type of cell death, termed glutamate excitotoxicity, is a pathological series of events in which high levels of glutamate over activate NMDA and AMPA receptors allowing high levels of calcium to enter the cell. As a result, apoptotic enzymes are activated and reactive oxygen species are released causing nerve damage and death. Glutamate excitotoxicity is believed to be responsible for the occurrence of cerebral ischemia and the development of many neurodegenerative diseases.
Dissecting the roles of CENP-B proteins in *S. Pombe*
Authors: Rafee Talukder, Dr. Hugh Cam
Department of Biology, Boston College

In humans, the CENP-B protein facilitates heterochromatin formation at centromeres, the sites of kinetochore assembly critical for proper chromosome segregation. This protein is highly conserved in mammals, and has three homologues in *Schizosaccharomyces pombe*: Abp1, Cbh1 and Cbh2. *S. pombe* CENP-B proteins have roles in centromeric heterochromatin formation as well as silencing of retrotransposons. It is not clear, however, how *S. pombe* CENP-B proteins contribute to these processes. To better understand the roles among the individual *S. pombe* CENP-Bs, biochemical and genetic approaches will be used to dissect their distinct functions.

In vivo binding experiments suggest that Abp1 and Cbh1 colocalize to repetitive elements including centromeric repeats and transposable elements. To identify putative DNA binding sequences of *S. pombe* CENP-B proteins, Bacterial expression vectors will be constructed for these proteins. Recombinant CENP-B proteins will be purified from *E. coli* and used for in vitro DNA assay against putative DNA binding sequences (Figure 2).

In spite of high sequence similarity among *S. pombe* CENP-B proteins, only single *abp1Δ* mutant exhibits defects in growth, chromosome segregation, and retrotransposon silencing. It is possible that the inabilities of Cbh1 and Cbh2 to compensate for the loss of Abp1 are due to their relatively much lower expression levels. To test this hypothesis, a strain will be constructed by standard yeast transformation assay whereby a *cbh1 ORF* will be inserted into the *abp1* locus replacing Abp1. We will assess whether the transgene *cbh1* driven from the strong Abp1 promoter will be sufficient to alleviate some or all of the defects associated with the loss of Abp1.
Cardiolipin (1,3-diphosphatidylglycerol) is a class of phospholipids that is specific to the mitochondrial inner membranes of eukaryotes and roughly consists of 20% of the lipid contents of the inner membranes. Cardiolipin is involved in insulation and in maintaining the stability of the protein complexes important to the electron transport chain. Modifications in cardiolipin content and molecular species composition have been identified in a variety of disease including diabetes, spontaneous heart failure, cancer, syphilis, and Barth syndrome. The modification of the four acyl chains in cardiolipin species is associated with the inability of the electron transport chain to effectively produce ATP through oxidative phosphorylation.

Cardiolipin is synthesized in the inner membrane of mitochondria by the condensation of two phosphatidylglycerols (PGs) and Cytidine diphosphate- diaclylglycerol (CDP-DAG) to form the immature cardiolipin with four acyl groups. The immature cardiolipin is remodeled accordingly using enzymes acyltransferases and/or transacylases. Cardiolipin remodeling utilizes acyl CoA and acyl chains at sn-2 position of glycerophospholipid choline (PC) and glycerol-phospholipid ethanolamine (PE).

Based on these biological parameters and shotgun lipidomic analysis, we have developed a web-based java applet simulation that allows for the construction of immature cardiolipin species followed by the progression of cardiolipin remodeling based on the concentration of the acyl chains available. Alterations in cardiolipin profile associated with disease progression can be demonstrated with this program through altered cardiolipin remodeling enzymatic activities or acyl selectivity.

The rates and extent to which cardiolipin species are remodeled can be modified in these simulations to fit to the experimental data obtained from shotgun lipidomics. By providing reference values for experimental measurements of cardiolipin, PG, PC, PE and acyl CoA, this JAVA dynamic simulator and viewer will allow insights into the mechanisms regulating cardiolipin molecular species composition in normal and disease states.
Endosomal Escape of Nanosensors

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Nanosensors are an up incoming technology capable of detecting small chemical fluctuations inside a cell. These sensors can provide quick and reliable fluorescent readings, which indicate the level of the desired chemical present. This allows for a variety of clinical and pharmaceutical uses for these nanosensors, whether it be drug creation, glucose level detections or monitoring sodium fluctuations. These nanosensors get endocytosed by the cell but then continue to remain trapped inside the endosome, a small membrane bound vesicle. This entrapment renders the nanosensors non-functional, as they are not able to detect cytoplasmic chemical fluctuations while in the endosome. These experiments attempt to coat the nanosensor with a bicarbonate buffer, which will react with the acidic atmosphere of the endosome to ultimately produce carbon dioxide. In theory, these carbon dioxide bubbles will cause the endosome to burst, allowing the nanosensor to be free and functional in the cytoplasm. The sensors are coated with the buffer and then injected into HEK cells, along with a red FM-143 endosomal dye. The cells are allowed to sit overnight and then are imaged on a Confocal microscope. The sensors fluoresce green and the endosomes are dyed red; using the microscope, we can visualize where in the cell the sensors are and if they have successfully escaped the endosome by using the production of carbon dioxide.
Structure Bioinformatics uses algorithms and computer science to predict the structure of molecular compounds such as proteins and RNA. The accessibility and ease of use of such programs is important for efficient research, development, and dissemination of knowledge. RNASuite provides researchers a unifying interface and database for RNA Secondary Structure research accessible through the Internet. RNASuite allows input through either an input RNA sequence or standardized EMBL identification number and returns pre-computed output from three programs: related to secondary structure of the input. Currently the programs used are RNAmutants, RNAbor, and RNAsat. Each provides information with regards to the secondary structure. RNAmutants returns a mutational landscape of the RNA sequence. RNAbor computes the number of secondary structures of the given RNA sequence at a base pair distance away from the structure. Finally RNAsat provides information on the saturated structures of the sequence where saturated is defined at the point no base pairs can be added without violating the definition of secondary structure. These three programs provide comprehensive data regarding the input sequence. RNASuite was developed using a Python based web development framework, Spyce, and mySQL in addition to newer web design technologies such as AJAX. The framework and foundation RNASuite is built in is comprehensibility, ease of use, and most importantly extensibility. As newer programs and algorithms are designed in the field of RNA Secondary Structure, the design of RNASuite allows inclusivity to further use to researchers. The database can be accessed at http://bioinformatics.bc.edu/clotelab/RNAsuite.
POSTER 13
Undergraduate Presenter: Lauren Jammallo (’11)

An insertional trap for conditional gene expression in *Toxoplasma gondii*

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During the lytic cycle of the apicomplexan parasite *Toxoplasma gondii*, tachyzoites make initial contact with, invade, and then undergo several rounds of intracellular replication within host cells. Upon maturation of the vacuole, the intracellular tachyzoites egress from and consequently destroy the host cell. Tachyzoites are then free to invade and repeat this destructive lytic cycle on neighboring host cells. In order to better elucidate the biological mechanisms that underlie the various steps of the lytic cycle, we sought to identify and characterize essential genes that play important roles in the lytic cycle of *Toxoplasma gondii*. To this end, we used a random insertional mutagenesis approach with a tetracycline-based regulatable promoter. Upon addition of tetracycline with the tetracycline inducible system, transcription of an essential gene is shut down. Two conditional mutants were generated that had lethal growth defects upon addition of tetracycline (4.3B13 and 3.3H6). These two mutants have contrasting phenotypes; 3.3H6 has a gross morphological defect upon addition of tetracycline, whereas 4.3B13 appears to die without a morphological defect. In both mutants, breakpoint mapping of inserts has been accomplished. In 3.3H6, one gene (77.m00088) was identified to contain an insert, but was not found to be essential. Another gene in 3.3H6 (72.m00683) was found to be able to restore the growth phenotype under tetracycline but contains no insert, thus inferring a suppressor role. In 4.3B13, one gene (64.m00349) has been found to be responsible for the lethal phenotype as shown by complementation with a covering cosm id in the presence of tetracycline. This particular gene has a conserved bromodomain and is thought to be involved in transcription. Herein, we developed the technical capabilities to generate conditional mutants via the tetracycline-based regulatable promoter system by random insertional mutagenesis. It should be feasible in the future to generate and identify essential genes involved in any of the steps involved in the lytic cycle of *Toxoplasma gondii*. 
POSTER 14
Undergraduate Presenter: Benjamin Hall ('12)

Genetic Diversity of Ecuadorian Mangroves Using Genomic and Expressed (EST-SSR) Microsatellites and Survey of Heavy Metals on Sediments and Mangrove Roots Along the Coast of Ecuador

Authors: Lauren Johnston, Amber Williams, Dr. Laura Hake, Dr. Acacia Alcivar-Warren
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Mangrove ecosystems are diverse wetlands occurring both in estuaries and along open coastlines in tropical, sub-tropical and temperate environments (Polidoro et al., 2010; MacFarlane et al. 2007). The plants prove to be of economical and ecological importance for they serve as nurseries and habitats to many juvenile fish, mollusks, and crustaceans, including the seafood most consumed by Americans, shrimp. Their presence directly influences the livelihood of the local communities for they can adapt to different degrees of salinity and mitigate natural disasters including storms, typhoons, and tsunamis (Lewis et al. 2006). In addition, mangroves have a staggering ability to sequester carbon from the atmosphere, and serve as both a source and repository for nutrients and sediments for other inshore marine habitats, such as seagrass beds and coral reefs. These forests provide at least $1.6 billion each year in ecosystem services. Protection and replanting of mangroves is necessary and people need to be educated about their importance in order to conserve biodiversity and maintain long lasting animal and public health. Despite their vital role, mangroves are being cleared at an alarming rate. The primary threats to all mangrove species are habitat destruction and removal of mangrove areas for conversion to aquaculture, agriculture, urban and coastal development, and overexploitation. In addition, pollutants threaten the conservation and sustainability of natural shrimp resource. More than one in six mangroves species worldwide are in danger of extinction due to coastal development and other factors including climate change, logging and agriculture, according to the first-ever global assessment on the conservation status of mangroves for the IUCN Red List of Threatened Species™ (Polidoro et al. 2010). As a result, 11 out of 70 mangrove species (16%) which were assessed will be placed on the IUCN Red List. The Atlantic and Pacific coasts of Central America, where as many as 40 percent of mangrove species are considered threatened, are particularly affected.

Ecuadorian mangroves have decreased from 202,201 hectares in 1969 to 148,230 hectares in 2006. It is estimated that 27% of coastal mangroves and salinas have disappeared since 1969 according to CLIRSEN (2007). No studies on the genetic diversity of mangroves species of Ecuador have been published. There is a study on genetic diversity of mangroves (Rhizophora mangle L.) from Colombia’s Punta Soldado, Colombian Pacific Coast, and 10 microsatellite sequences are deposited in the GenBank database (Rosero-Galindo et al. 2002). The specific objectives of this study are: (a) to collect samples of sediment and roots and/or leaves of all species of mangroves along the coast of Ecuador and isolate DNA and RNA from mangrove roots and/or leaves, (b) to clone a cDNA library from an Ecuadorian mangroves species to establish an expressed sequence tags (ESTa) database for microarrays and marker development (EST-SSRs) for population diversity analysis, and (c) to determine the levels of heavy metals in mangrove roots, sediments and water along the coast of Ecuador. Information will be presented on the current mangrove species along the five coastal provinces of Ecuador, and the microsatellite primer sequences available for other mangroves species that will be used for initial cross-species amplification of microsatellite alleles by PCR.
POSTER 15
Undergraduate Presenter: Aleksandra Jachotorowicz ('12)

Development of *Litopenaeus vannamei* cell line for in-vitro screening assays

Authors: Aleksandra Jachotorowicz, Dr. Laura Hake, Dr. Acacia Alcivar-Warren (Boston College, Biology Department)

Shrimp are the favorite seafood of Americans. Although endocrine-disrupting chemicals (EDCs) have been identified in seafood for human consumption, little shrimp endocrinology and toxicogenomics research has been completed. Despite much effort, a continuous *in vitro* cell line from the economically important shrimp, *Litopenaeus vannamei*, has not been established. Various attempts have been made to immortalize a shrimp cell line, with little to no success. A range of causes for the inability to maintain viable cells from a primary culture over an extended period of time has been suggested. The failure of shrimp cells to adhere to the culture surface after passage may be the single most important factor in this lack of success. A review of 30 articles indicates that the oka organ (lymphoid tissue) may be the most promising cell type as, consistently through the literature, it demonstrated the longest viability, greatest proliferation, fastest migration times, and best re-attachment results upon passage. It was also determined that shrimp cells exhibit extreme sensitivity to both trypsin-induced or mechanical detachment, failing to either re-attach or form monolayers after passage attempts. My goal is to investigate a suitable media and method of detachment and passage in order to develop an *in vitro* screening assay of *L.vannamei* for EDCs.
Shrimp is the favorite seafood of Americans and heavy metals are some of the most toxic and wide spread contaminants in estuarine systems. Important sources of these metals are solid wastes and waste water as well as byproducts of industrial development. Given the constant exposure that Penaeid shrimp have to these metals through direct contact, water absorption and diet it is important to analyze how much these metals accumulate in shrimp tissues as well as effects that the contaminants have on shrimp development. Previous work has detected trace concentrations of heavy metal in both wild and cultured Litopenaeus vannamei shrimp, the most economically important species of which $3.5$ billion worth are imported each year (see figure below). Bioaccumulation of heavy metals could be dangerous for both the Penaeid shrimp and the humans who consume them.

Experimental observations have implied that heavy metal accumulation can be toxic to shrimp and result in death, but even at sub lethal levels it can affect tissue health, metabolism, growth, molting and reproduction. It is necessary to determine the effects that metal exposure can have on development for commercial reasons as well as for human safety and health. For commercial reasons, breeding success is very important. Evidence has shown that success is dependant on water quality (Bray et al. 1992). For human health purposes, it has been shown that exposure to heavy metals can be both toxic and may interfere with hormonal systems even at low levels. The heavy metals in which most current research has focused on are mercury, lead, and cadmium, known endocrine disrupting chemicals in humans.

The specific objectives of this study are: 1) to establish a protocol for isolation of total RNA using various commercial RNA extraction kits, and 2) to measure concentrations of heavy metals in wild shrimp of Ecuador collected in 2000. We will present a comprehensive review of the literature regarding effects of heavy metals in growth, molting, and development of Penaeid shrimp, in addition to results on quality of RNA obtained from tail muscle of wild shrimp from Ecuador stored at -80C for many years.
Food allergies are the most frequent cause of allergic reactions in Americans; millions ever year are affected by related allergic reactions, and the prevalence of such reactions has been increasing, doubling between 1997 and 2002 alone. Shrimp is both the favorite seafood of Americans and one of the most frequent causes of allergic reactions in hypersensitive people. Most of the shrimp that Americans consume is imported from developing countries such as Ecuador, and as a result is often loaded with various viruses and many EDCs (endocrine-disrupting chemicals), including heavy metals such as cadmium, that are known to have negative effects in most living organisms.

Marine shrimp are especially sensitive to cadmium, which bioaccumulates in their bodies in proportion to the concentration of the metal in the surrounding environment. In humans, cadmium has been linked to kidney damage, prostate cancer and diabetes, and may also affect immune and allergic responses. Repeated exposure—in this case, likely a result of consuming imported shrimp—to even trace amounts of the metal could produce very harmful effects.

Little is known about the initiation of seafood allergic responses, but the four genes in the shrimp genome that have been confirmed as common allergens are tropomyosin, arginine kinase, myosin light chain and sarcoplasmic calcium-binding protein. Some of these allergens contain repeats that resemble the Transposable Elements of vertebrates, which could play a role in the body's recognition of foreign DNA and/or the overexpression of the aforementioned genes.

The overall goal of this project is to develop the essential knowledge base in shrimp genomics to develop biomarkers for susceptibility to allergy associated with consumption of the economically important Pacific whiteleg shrimp, *Litopenaeus vannamei*. The specific aims of the project are: 1. To isolate good-quality RNA from wild *L. vannamei* samples collected from 5 provinces of Ecuador in July 2010; 2. To study changes in mRNA expression of the aforementioned allergen genes in muscle tissue of the 2010 wild shrimp using quantitative RT-PCR, and compare these with the mRNA levels in shrimp collected in 2000; 3. To examine the association between mRNA expression levels and pollutant load (three shrimp viruses and cadmium). Information will be presented on preliminary results to isolate total RNA from shrimp muscle, total RNA quality criteria, and RT-PCR protocol being established for mRNA analyses. Research will be continued in summer 2010 and into the 2010-2011 school year.
Taura Syndrome Virus originated in Litopenaeus vannamei, the common white shrimp, of the Taura River in Ecuador. Now, the virus has been seen in shrimp in South and Central America, Mexico, the United States, and even Southeast Asia (Navararro et al., 2009). Shrimp farms have seen the development of new strains of TSV as the virus mutates and causes the death of farm stocks and the loss of billions of dollars.

As an RNA virus that replicates with RNA-dependent RNA polymerase, TSV undergoes frequent errors (as often as $10^2$-$10^4$ nucleotides polymerized) (Cote et al., 2009). Growth and distribution of “Specific Pathogen-Free” (SPF) shrimp produced by the US Marine Shrimp Farming Program (USMSFP) has been an effort to alleviate the losses, yet new strains of TSV still appear across the world from China and Indonesia to Hawaii and Nicaragua (Wertheim et al., 2009).

Further analysis by reviewing TSV nucleotide sequences in GenBank of different isolates from recent studies has pointed to possible DNA transposable sequences and retro transposable sequences using CENSOR (www.girinst.org). These matches start inquiry into the possibility of insertion of the virus into different genes of other genomes besides that of L. vannamei.

I hypothesize that TSV is present in frozen shrimp sold in MA supermarkets and restaurants. The specific objectives of this study are: (1) to survey the prevalence or lack of TSV in frozen commodity shrimp consumed by humans in restaurants and supermarkets in the Boston, MA, area and information on where these shrimp originate, (2) measure trace concentrations of cadmium (Cd), a heavy metal known as an endocrine-disrupting chemical in humans, in the same samples, and (3) compare the prevalence of TSV in frozen commodity shrimp of MA, with TSV prevalence of wild shrimp of Ecuador and (4) to determine the strain of TSV present in these samples.

![Figure 1: The Genome of the ssRNA virus Taura Syndrome Virus](Dhar et al., 2010)
POSTER 19
Undergraduate Presenter: Jeremy Vincent (‘11)

Detection of Infectious Hematopoietic Necrotic Virus (IHHNV) and
Survey of Endocrine Disrupting Chemicals in Shrimp Sold in Massachusetts
Supermarket Chains and Restaurants

Authors: Amber Williams, Lauren Johnston, Acacia Alcivar-Warren, and Laura Hake

In 2005, Reville et al. published results of a study that measured the prevalence of White Spot Syndrome Virus (WSSV) in frozen shrimp sold in Massachusetts supermarkets. A significant percentage of the shrimp that were tested, imported from areas including Thailand, Honduras, India and Belize, contained WSSV. This study is important because local crustaceans have not been exposed to WSSV, and as seen in other areas of the world, naive crustaceans are prone to a viral outbreak that could have drastic effects on the local aquatic ecosystem. One other highly prevalent virus found in imported shrimp that could have similar effects on local aquatic environments is infectious hypodermal and hematopoietic necrosis virus (IHHNV). While previous studies have failed to test for this virus in frozen shrimp sold in Massachusetts supermarkets and restaurants, it is found in high prevalence in shrimp farms all over the globe. I hypothesize that IHHNV is present in the shrimp that are sold for human consumption in supermarkets and restaurants across Massachusetts. In addition to carrying this virus, these imported shrimp may also contain other pollutants, including endocrine disrupting chemicals (EDCs) such as heavy metals, polychlorinated biphenols (PCBs) and polychlorinated hydrocarbons (PAHs). These pollutants could have negative hormonal affects in humans, as well as increase the risk of allergy in people who consume shrimp.

The specific aims of this project are (1) to survey the prevalence of IHHNV in frozen shrimp sold in Massachusetts supermarkets and restaurants, and (2) to measure concentrations of the heavy metal cadmium, a known endocrine disruptor in humans, in the same frozen shrimp. Preliminary information will be presented on the protocol being developed to isolate DNA from the shrimp sample, protocol for the DNA quality controls, and the primers used for PCR detection of IHHNV.
The effects of cadmium (Cd), one of the most hazardous heavy metals in aquatic environments, on gene expression of the commercially important shrimp species *Litopenaeus vannamei* are unknown. The long term goal of this study is to examine if Cd induces changes in gene expression associated with endocrine- [MIH, MF, vasa-like and CCH], immune- [LGBP, laminin receptor, antiviral AV, crustin] and allergy- [tropomyosin, arginine kinase, myosin light chain, sarcoplasmic calcium-binding protein] related shrimp genes by testing whether the concentration of Cd within the tail muscle has a relationship with these levels of gene expression. In order to perform analysis via real-time PCR, it is crucial to begin with suitable mRNA. After isolating total RNA from shrimp tail muscle, we observed substantial variation in the quality of RNA between and within sample groups (~160 shrimp collected from the Ecuadorian provinces of Manabi, Guayas, and Esmeraldas). As such, this current project developed a reliable protocol to isolate RNA from shrimp tail muscle using TRI Reagent and also created criteria on which to judge the quality of RNA individually, critical before performing real-time PCR. The criteria was based on the presence, and intensity of, the 18s rRNA band seen via quality control (QC) gel, and the amount of degradation, represented below the 18s rRNA band by a smear. On a scale of 1 to 5, where 1 is “good RNA” and 5 is “very poor RNA,” most of our samples were labeled a 3: “acceptable RNA,” showing an 18s rRNA band of low intensity with a degradation smear below. Twenty samples of comparable RNA quality were chosen and sent to Frontier Geosciences Inc. for cadmium analysis. A real-time PCR protocol has been established and is being used to estimate differential expression of the listed genes with the chosen pool of samples. Out of b-actin, EF-1a, GAPDH, and 18s rRNA, the genes EF-1a and GAPDH will be used as endogenous controls for differentially expressed target genes.
The Inhibition of Various Phosphodiesterase (PDE) Strains

Authors: Kristina Cotter, Didem Demirbas, Charles Hoffman  
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The inhibition of phosphodiesterase enzymes has been the subject of research due to its widespread clinical implications. PDE cleaves the second messenger molecules cyclic-AMP (cAMP) and cyclic-GMP (cGMP) into AMP. Inhibition therefore causes cAMP levels in cells to rise. A target of cAMP is Protein Kinase A (PKA), which has several significant roles concerning signal transduction. PDE inhibition therefore serves to regulate cell activity. The connection of PDE to various conditions including cancers, depression, and neurodegenerative diseases has made them a desirable target for drug development. Many tested inhibitors, such as Rolipram (a PDE4 inhibitor) have shown to have anti-inflammatory effects. Research this semester focused on yeast-based screening with a 5-fluoroorotic acid (5FOA) assay that detected inhibitors by their ability to raise cAMP levels in cells. PDE cell strains were cultured in a medium that repressed fbp1-ura4 transcription. Effectively, cells did not grow in media lacking uracil but were able grow in a 5FOA medium after PKA activation due to increasing amounts of cAMP. Growth in 5FOA was therefore indicative of PDE inhibition and was measured using spectrophotometry. Eight known PDE4 and PDE7 inhibitors (BC12, 28, 30, 35, 54, 58, and 76) were tested against a variety of PDE strains. Previous studies have indicated that BC27, 35, 54, 58, and 76 are PDE4 inhibitors while BC12, 28, 30, and 54 are PDE7 inhibitors. Strains from the PDE1, PDE4, and PDE7 families were subjected to this assay in order to reproduce these findings as well as analyze the effects of the compounds on different strains.
Studied of transcriptional regulators of {\textit{fbp1}} (italicized) in {\textit{Schizosaccharomyces pombe}} (italicized): New roles for Hsr1 and Rsv1

Authors: Brian Currie and Charles Hoffman
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Transcription of the fission yeast {\textit{Schizosaccharomyces pombe fbp1}} gene is regulated by glucose sensing mechanisms. {\textit{fbp1}} encodes fructose-1, 6-bis-phosphatase, an integral protein in gluconeogenesis, which is expressed in response to glucose starvation. Two zinc finger proteins, Rst2 and Scr1, regulate {\textit{fbp1}} transcription in a positive and negative manner, respectively. A search of the {\textit{S. pombe}} protein database identified two additional zinc finger proteins, Rsv1 and Hsr1, which closely resemble Rst2 and Scr1. To ascertain whether these proteins provide redundant or novel functions in {\textit{fbp1}} regulation, a variety of experiments were performed to evaluate the consequences of deleting the {\textit{hsr1}} and {\textit{rsv1}} genes. Our results suggest that Hsr1 and Rsv1 are partially responsible for {\textit{fbp1}} transcription and in the case of Hsr1 this regulation may be mediated via direct binding of the UAS2 region of the {\textit{fbp1}} promoter. During the course of these studies, an unusual growth defect was observed in certain strains possessing a deletion of {\textit{scr1}} and a disruption of the cAMP/PKA signaling pathway. These strains exhibit markedly slow growth on solid media, excessive flocculation in liquid media and other morphological characteristics reminiscent of stationary phase cells. To elucidate the biological mechanisms underlying this phenotype, a multicopy suppressor screen has been carried out to identify {\textit{S. pombe}} genes whose over-expression can compensate for this growth defect and restore normal growth rates. The results of this screen were inconclusive but it was independently discovered that the additional deletion of {\textit{rsv1}} in these strains results in a synthetic lethality while the deletion of {\textit{rst2}} alleviates the observed growth defect. These studies have therefore advanced our understanding of the regulatory strategies of {\textit{S. pombe}} involving the zinc-finger proteins Scr1, Rst2, Hsr1 and Rsv1, both at the level of {\textit{fbp1}} transcription and in terms of cellular-wide responses to stress. Given that mechanisms for transcriptional control are highly conserved in eukaryotic organisms, our studies of {\textit{fbp1}} could provide insights into the complex regulatory networks of higher eukaryotes.
Determining if kinase activity of Sck1 and Sck2 is required to regulate Gpa2 in *S. pombe*

Authors: Dayna Mudge and Charles Hoffman  
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*Schizosaccharomyces pombe* regulates *fbp1* gene transcription by a glucose signaling pathway. This pathway uses a G-protein coupled receptor to recognize the presence of extracellular glucose in order to regulate the expression of fructose-1, 6-bis-phosphatase, a gluconeogenic enzyme, by regulating the cAMP levels within the cell. Sck1 is a protein involved in this pathway that interacts with the Gpa2 alpha subunit of the heterotrimetric G-protein when the subunit is activated. Deletion of the *sck1* gene appears to elevate Gpa2-mediated signaling to suggest that Sck1 is a negative regulator of Gpa2. The Sck1 protein is a homologue of the Sch9 kinase in *Saccharomyces cerevisiae*, but it is not known whether Sck1 kinase activity is required for its role in glucose signaling. To study the nature of this interaction, an allele of the *sck1* gene is to be generated using recombinant DNA techniques to make an Sck1 protein that lacks kinase activity, but is otherwise structurally intact. As Gpa2-mediated signaling is also regulated by the Sck2 kinase, which is closely related to Sck1, a similar kinase-dead allele of *sck2* will be constructed. This study will further our understanding of the complex regulatory mechanisms that allow fission yeast cells to vary levels of gene expression through glucose signaling. This project also aims to study the potential technical benefit of using two selectable markers in a knockout cassette rather than one marker, to identify homologous recombination events that knock-in an unmarked allele of a gene of interest. This technique could be used to increase the efficiency of identifying successful knock-in strains in future experiments.
The role of Nucleophosmin 1 (NPM1) in influencing the transcriptional activity of CCAAT enhancer binding protein alpha (C/EBPα p42)

Authors: Michelle Levine and Arati Khanna-Gupta, Ph.D. (Brigham & Women’s Hospital)

CCAAT enhancer binding protein α (C/EBPα) is a member of a family of transcription factors that recognizes the sequence 5’ATTGCGCAAT3’ in the promoters of target genes and modulates their expression. C/EBPα plays a vital role in granulopoiesis and has been shown to be mutated in Acute Myeloid Leukemias (AMLs). Two forms of C/EBPα resulting from translation from alternative start codons result in the formation of two proteins, p42 and p30 (see Figure 1). Increased expression of p30 has been observed in AML. Nucleophosmin (NPM1) is a ubiquitously expressed nucleolar phosphoprotein that has been implicated in many different cellular pathways including ribosome biogenesis, chromosome stability, and nucleocytoplasmic transport. NPM1 is one of the most frequently mutated genes in AML. Previous studies in the Berliner lab had shown an association between NPM1 and C/EBPα at the protein level. In this study, I examined the role of NPM1 in influencing the transcriptional activity of C/EBPα. My approach was to determine if C/EBPα p42 transactivated C/EBP binding sites in reporter plasmids and then to examine if NPM1 altered this reporter gene activity in transient transfection assays. This was achieved by transfecting HEK293T cells with C/EBPα p42 alone, or C/EBPα p42 with the Lactoferin 89 (LF89-Luc) or 2x C/EBP-Luciferase (2x) reporter plasmids. The LF89 and 2x plasmid constructs contain the Luciferase reporter gene, which allowed for C/EBPα activity to be quantitatively measured through luciferase assays. A β-galactosidase plasmid was included to normalize for transfection efficiency. I showed in three separate experiments that C/EBPα upregulated promoter gene activation an average of 25 fold. These preliminary experiments will form the basis for further investigation of C/EBPα activity with NPM1 wild-type and with the mutant form of NPM1 (NPM C+).
The myelin “radial component,” a junction complex unique to the central nervous system, consists of linear, particulate strands running parallel to the nerve fibre axis and radially through the myelin sheath. The tetraspan protein claudin-11/Oligodendrocyte-Specific-Protein (OSP) has recently emerged as a major component of the radial component, though its role is yet to be fully elucidated. We employed x-ray diffraction to study the potential role of claudin-11/OSP as an adhesive protein and a diffusion barrier within central nervous system myelin. X-ray diffraction of fresh nerves from claudin-11/OSP +/- mice showed no evidence of hypomyelination or deviation from wildtype myelin organization. Conditions of electrostatic stressing were used to disrupt the balance of attractive and repulsive forces existing within the myelin from claudin-11/OSP +/-, claudin-11/OSP +/-, and claudin-11/OSP '-' mice. X-ray analysis of the myelin period in these nerves showed highly similar periods amongst the three genotypes across the spectrum of conditions tested, demonstrating that claudin-11/OSP is not involved in determining the predominant period of myelin. A solution of glycerol in saline, which is more electron dense than normal saline and can thus serve as a tracer, was used to examine if claudin-11/OSP helps to prevent diffusion through the myelin sheath. Our results show that diffusion through the myelin sheath occurs by the same process and at a nearly identical rate in claudin-11/OSP knockout myelin as it does in wildtype myelin. The electron density levels between the membranes, however, were higher in the wildtype myelin than in knockout myelin, due to more hydrated protein in the wildtype inter-membrane space.
Charcot-Marie-Tooth disease type 1B (CMT1B), a peripheral neuropathy, is caused by mutations in gene MPZ, which encodes PNS myelin protein zero (P0). P0 mutation Arg69Cys (R69C) causes a severe early-onset form of CMT1B. To elucidate the pathogenesis of this neuropathy, an Arg69Cys knock-in mouse was generated by targeting this mutation to one MPZ allele by homologous recombination in ES cells. Here we report our x-ray diffraction (XRD) measurements on the periodicity, membrane structure, and amount of myelin in unfixed, freshly-dissected nerves from wildtype (WT or +/+), heterozygous (R69C/+), and homozygous (R69C/R69C) mice. The XRD patterns showed decreasing strength of scattering intensity from myelin: WT > R69C/+ > R69C/R69C, indicating decreasing relative amounts of myelin. By contrast, optic nerves exhibited no such differences. The myelin periods of sciatic but not optic nerves were found to differ among the genotypes: 177.0 ± 0.4 Å for WT, 178.4 ± 0.5 Å for R69C/+, and 193.1 ± 4.2 Å for R69C/R69C. The membrane profiles showed R69C/R69C’s wider period derived from ~20 Å-swelling at the extracellular apposition. The extent of membrane packing distortion (D/d) in PNS myelin was 25% greater in R69C/+ and doubled in R69C/R69C compared to WT. Differences in amount of myelin, period, and D/d among the genotypes were statistically significant at p < 0.001. Comparison of R69C/+ with P0+/− and R69C/R69C with P0+/− suggested the small amount of mutant P0 that enters the myelin may detrimentally affect myelin-myelin interactions to produce less regular/unstable packing. Finally, CHOP-null mice on three backgrounds (WT, R69C/+, and R69C/R69C) were analyzed to detect possible reduced demyelination in the heterozygote and mutant. However, unlike the S63del mutant which is rescued, all three genotypes exhibited a slight decrease in relative amounts of myelin.
To develop therapeutic and diagnostic probes for Alzheimer’s disease, we are using polarized light microscopy to examine amyloid fibril formation and fibril inhibition in 0.5 mL drops deposited onto siliconized glass slides. Single sessile drops of amyloidogenic peptides—Ab 40 and tau peptide fragment acetyl-VQIVYK-amide, or “PHF6”—at 2-10 mg/mL formed spherical caps. We measured the intensity of the light transmitted through the drop as a function of dehydration and of incubation time of the peptide solution ± small molecules. The intensity is related to the volume fraction of the crystallites and the orientation function. After several minutes in air at room temperature, drops dried such that solute concentrated along the circumference of the circle defined by the initial drop size. X-ray diffraction of the same dried drops showed that the hydrogen-bonding direction for the cross-beta arrangement of fibrils was along the slow optic axis of the birefringent deposits—i.e., the fibrils were aligned tangential along the circumference of the drop. Optically, this was also shown by the birefringence color when using a first order red compensator plate, which gives positive birefringence for an elongated object with respect to its long axis. The transmitted intensity from the analyzer increased as a function of dehydration and with longer incubation time. Peptide solutions containing small molecules, such as tannic acid for Ab 40 and tosylate for PHF6, and large proteins such as MBP for Ab 40 showed the least intensity. With a total of 0.5 µg of peptide used for each sample at each condition and with a fast drying time of up to 2 min, this technology can prove to be a very fast and reliable screening method for amyloidogenic peptide inhibitors.
PMD (Pelizaeus-Merzbacher Disease) is a neurological disease of Central Nervous System (CNS) myelin that leads to reduced motor and balance skills for patients (Osaka et. al, 2009) and severe dysmyelination or amylination. To elucidate the myelin abnormality, a mouse model of a mild form of PMD was generated that contains a deletion of the intronic splicing enhancer (ISE). This deletion creates a molar ratio inversion PLP1/DM20 from 3:1 to 1:3 by inducing a preferential splicing of DM20 5'splice site (Wang et. al, 2008).

To explore myelin characteristics, optic and sciatic nerves from control and transgenic mice (PLP1-ISEdel) at ages 3.8 and 7.8 months were subjected to x-ray diffraction (XRD). All nerves were observed “fresh” after dissection, in physiological saline solution. Sciatic nerves were used as an internal control because they lack PLP1/DM20 in the compact myelin. Compared to wildtype controls, the transgenic mice showed an expansion by 2-3 Å in CNS myelin period for both ages. Relative myelin levels increased with age, but were similar between genotypes of same age groups. No trends were observed for changes in the packing disorder of myelin membranes.

The experiment illustrates the necessity of ISE and correct ratio of PLP1 and DM20 because altered ratios show a small, but significant expansion of myelin period. This may contribute to lower conduction speed of neural signals and also result in reduced motor and balance skills for patients diagnosed with PMD.
Further Characterization of the Nuclear Localization Signal of the Human Papillomavirus 16 E2 Protein

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The E2 protein of high risk human papillomavirus type 16 (HPV16) has many important functions in the viral life cycle, including playing a crucial role in the replication of the HPV genome upon infection. This E2 protein contains an amino-terminal (N) domain, a flexible hinge (H) region, and a carboxyl-terminal (C) DNA binding domain, also functioning in dimer formation. The wild-type form of this protein is localized almost exclusively in the nucleus, suggesting that it must pass the nuclear pore complex (NPC) to fulfill its role in the infected cell. Previous work in the Moroianu lab led to the identification and partial characterization of a nuclear localization signal (NLS) in the C-terminal domain of HPV16 E2, 298LKCLRYRFKK308, which has an a-helical structure and contains several highly conserved lysine and arginine residues, some of which have been determined critical for NLS function via mutational analysis (Klucevsek et al., 2007). The purpose of this project was to further characterize this cNLS as well as the flanking regions. This was carried out through the creation of five enhanced green fluorescent protein (EGFP) HPV16 E2 or –cE2 fusion plasmids with specific mutations either within or downstream of the NLS. The mutations studied in this project were two alanine (A) substitutions at L301 and F305 within the NLS, two stop codon substitutions at V326 and A331 downstream of the NLS, and a triple alanine substitution at 327KHK329 between the locations of the two stop mutations. The localization of these mutant proteins in vivo was observed using fluorescence microscopy. In these studies, both the L301A and F305A mutations were found to have no effect on the nuclear localization of the protein. The V326A and A331A mutations, however, each disrupted nuclear localization, causing pancelular localization of the truncated E2 proteins. The KHK327AAA mutation had no effect and the localization of this mutated protein was mostly nuclear.
The zinc-binding domain is essential for nuclear import of E7 protein of low risk Human Papillomavirus 11

Authors: Erin C. Hannah, Shahan Mamoor, Lauren Crosby and Junona Moroianu

Human papillomaviruses (HPVs) are classified as either high-risk or low-risk based on their oncogenic potential. The HPV E7 protein acts as one of the virus major oncoproteins that bind tumor suppressor proteins like pRB, p107 and p130, contributing to the oncogenic potential of the virus. HPV11 is a low-risk type that causes genital warts; however E7 relaxes the G1 to S phase cell-cycle check point allowing viral DNA amplification in the differentiated keratinocytes. Previous studies have shown that HPV11 E7 localizes to the nucleus in vivo suggesting that the protein contains an NLS. The nuclear localization is mediated by a cNLS containing a unique zinc-binding domain (Piccioli et al., 2010, submitted). The purpose of this study was to further characterize the nuclear import pathway of HPV11 E7 and the role of the zinc-binding domain in nuclear import. We found that mutations of Cys residues in the zinc-binding domain of the 11cE7 primarily inhibited nuclear import suggesting that the zinc-binding domain is essential in its nuclear import. We also found through Phenyl-Sepharose binding assays that the 11cE7 protein interacts with Phenyl-Sepharose beads which mimic the FG-containing nucleoporins. This suggests that HPV11 E7 protein may also be imported into the nucleus through direct hydrophobic interactions with FG-nucleoporins at the nuclear pore complex.
Further Characterization of the Nuclear Import Mechanism of Human Papillomavirus 16 E7 Oncoprotein

Authors: Danielle Larson, Jeremy Eberhard, Dr. Junona Moroianu

The E7 protein of human papillomavirus is one of the major transforming proteins in high-risk types, as it inhibits cell-cycle arrest by binding and inactivating tumor-suppressor protein pRb, as well as cyclin-dependent kinase inhibitors. As the E7 protein must be localized to the nucleus to affect these targets, the nuclear import and export of the E7 protein is essential for the viral life cycle and pathogenesis of high risk HPV16. Previous research in the Moroianu lab found that HPV16 E7 is imported into the nucleus via a novel Ran-dependent pathway independent of karyopherin import receptors (Angeline et al., 2003). It was also discovered that the carboxyl-terminal domain (16cE7, aa 38-98), of HPV16 E7, which contains a zinc-finger domain (aa 58-98), contains a nuclear localization signal, termed the cNLS, which mediates this pathway (Knapp et al., 2009). It is suggested that the zinc-finger domain of the 16cE7 may facilitate the nuclear import of HPV16 E7 via direct interactions with nucleoporins containing phenylalanine-glycine (FG) repeats. This study is concerned with further characterizing the nuclear import pathway for the HPV16 E7 oncoprotein, with specific focus on the role of the protein’s cNLS. In solution binding assays were performed with Phenyl Sepharose beads, which mimic the binding selectivity of FG repeats, and the full-length 16E7 protein, the C-terminus, 16cE7, and the N-terminus, 16nE7. We found specific binding of 16cE7 to Phenyl Sepharose, which suggests that the HPV16 E7 protein may be imported into the nucleus via direct low-affinity hydrophobic interactions between the protein’s cNLS and FG-containing nucleoporins. We also mutated Cys residues involved in zinc-binding in the 16cE7 for future analysis in binding assays and nuclear import experiments, in order to examine the role of the zinc binding domain in nuclear import of HPV16 E7.
The Zinc-binding Domain is Essential for the Nuclear Localization of E7 Protein of Low Risk Human Papillomavirus Type 11 in Vivo

Authors: Courtney H. McKee, Shahan Mamoor, Lauren Crosby and Dr. Junona Moroianu

Human papillomaviruses (HPVs) infections cause hyperproliferative lesions of mucosal and cutaneous epithelial tissues. Depending on their oncogenic activity mucosal HPVs have been classified into high risk types (such as 16 and 18), and low risk types (such as 6 and 11).

The E7 protein of low risk HPV11 is essential for viral DNA amplification in the differentiated epithelial cells leading to generation of new virions. Previous studies in the Moroianu Lab have shown that HPV11 E7 protein enters the nucleus via a Ran-dependent pathway independent of nuclear import receptors, and this pathway is mediated by a nuclear localization signal located in the C-terminal domain (cNLS) (Piccioli et al., 2010, submitted). This cNLS contains a unique Zinc-binding domain consisting of two copies of Cys-X-X-Cys sequence motif separated by 29 amino acids. To examine the role of the Zinc-binding domain in the nuclear localization mediated by the cNLS we mutated Cys residues in each of the two copies of Cys-X-X-Cys, and after transient transfection in HeLa cells we analyzed the localization of the resultant EGFP-11cE7 mutants and EGFP-11E7 mutants in comparison with the corresponding wild type proteins using confocal fluorescence microscopy. We discovered that mutations of Cys residues in the two Cys-X-X-Cys motifs which affect the zinc binding, clearly disrupted the nuclear localization of the resultant EGFP-11cE7 and EGFP-11E7 mutants. These data suggest that the integrity of the Zinc-binding domain is essential for the nuclear localization of 11cE7 and 11E7 mediated by the cNLS. In addition, we examined if CKII phosphorylation plays a role in the nuclear localization of HPV11 E7 protein. Analysis of the localization of an EGFP-11E7 (S2SS/AA) mutant, which cannot be phosphorylated by CKII, in comparison with the EGFP-11E7 wild type revealed that CKII phosphorylation is not required for the nuclear localization of 11E7 protein.

We will continue our mutational-function analysis to determine other critical amino acid residues involved in the nuclear localization of 11E7 protein mediated by its zinc-binding domain.
Global climate change and increasing sea surface temperatures pose major threats to aquatic organisms such as dinoflagellates. We are interested in how environmental changes affect an organism that seems to be left out of many discussions when scientists talk about the future of our planet—the dinoflagellate, *Amphidinium carterae* Hulburt (CCMP 121). This species is in the class Dinophyceae, and is found near North America in the Caribbean Sea. In one experiment, we studied how cultures of this species react under increased temperature and decreased salinity that would occur in the ocean if glacial ice melts at either pole of the earth. We tested this by running a lab experiment with four conditions (each condition had two replicates) for dinoflagellate cultures: high temperature with high salinity, high temperature with low salinity, low temperature with high salinity, and low temperature with low salinity. Our hypothesis is that dinoflagellates will experience a high mortality rate in the high temperature treatment. We expect that the low salinity and high temperature will be the condition with the least amount of cells (indicating lowest growth rate). Three cell counts will be performed using a hemocytometer over the course of 2 weeks. N2O production will also be measured using gas chromatography and compared in cultures with and without nitrate additions at two temperatures.

In that experiment, we hypothesize that there will be an increase in the production of nitrous oxide in the higher temperature and nitrate treatments. These results are important because dinoflagellates are primary producers and symbionts with coral reefs and anemones, making their maintenance a critical concern for their ecological value. The production of nitrous oxide, furthermore, could contribute to the warming of the planet in addition to the destruction of the ozone layer, posing serious health hazards to life on earth.
Methods in Ecology Lab (Prof. Moseman)

The effects of greenhouse gas production on the addition of iron and sulfate rich fertilizers to wetland systems

Ammonium sulfate is used largely as an artificial fertilizer in agricultural systems. In the soil the sulfate ion is released and forms bisulfate, lowering the pH balance of the while contributing essential nitrogen for plant growth. Among other fertilizers, ferrous sulfate is used for treating iron chlorosis a lawn conditioner, and moss killer and in specific wetland systems iron addition is experimentally used to combat eutrophication and sulphide toxicity. Although much is known about the direct effects of these chemicals in plant biology, not much is investigated into their impact on the soil cycles. Focusing on the denitrifying bacteria we examine the effects of these two fertilizers on the production of the prominent greenhouse gas nitrous oxide. Using slurry technique we examine different areas at Plum Island, Massachusetts and ran the samples through a gas chromatograph. Denitrifying bacteria work at sensitive levels of pH so we predict the change of pH in the addition of sulfate will interfere with the bacteria’s ability to denitrify the nitrogen in the soil sample. Highest levels of nitrous oxide would be predicted in the ammonium sulfate where an addition of nitrogen is combined with the sulfate. As for the ferrous sulfate, iron oxidizing bacteria was found in the soil sediment so closer examination of this process in its contribution to greenhouse gases can be examined when iron is deposited in surplus amounts. By examining the effects of fertilizers on the wetlands, better management and control on a quantitative level can help preserve our mashes as well as reduce the rate of global warming on a global scale.
A characteristic feature of marshes is zonation, where plants will grow in specific areas that maximize their growth and productivity, these areas being influenced by factors such as soil salinity and inundation frequency. The growth rate and productivity of these plants is critical to the stability of the marsh itself. Marshes around the world are degrading due to increased sea levels, which in turn are caused by a global rise in atmospheric temperature. While much research has been conducted relating the adverse effect of sea level rise on marsh plants, there is little research correlating the effect of atmospheric temperature rise in conjunction with this projected rise in sea level. Through this experiment, I attempted to find out what effect a higher atmospheric temperature (up to 30 deg. C) and sea level would have on the growth and productivity of Spartina patens, an important higher zone marsh plant. There are two parts to this experiment: In the field portion, S. patens from the high marsh zone were moved to the low marsh zone normally dominated by S. alterniflora in order to simulate the effect of sea level rise. In the lab portion, S. patens were grown at 25°C and 30°C in order to simulate global atmospheric temperature rise. I hypothesize that S. patens will have increased growth and productivity at the higher temperature and that it will have decreased growth in the lower marsh zone. The results could help in predicting how global climate change will affect the stability and hence viability of northern hemisphere salt marshes.
15N uptake by *S. patens* and *P. australis* in the presence and absence of PbNO3

As scientists and policy makers continue to examine restoration methods to remediate polluted and degraded sites, marshlands have been increasingly studied and recommended as integral tools for resource management and remediation action plans. Marshland vegetation species, while adapted to a high-stress environment, are also capable of storing heavy metals and other toxic substances within above- and below-ground biomass, making these species ideal candidates for remediation projects. This experiment examines the nutrient uptake of two marshland species, *Spartina patens* and *Phragmites australis*, as well as resident algae species, in the presence and absence of PbNO₃ in order to elucidate not only the effects of heavy metals on nutrient uptake, but also to discern relative competitive abilities of marshland species in the presence of pollutants. Isotopic enrichments of ¹⁵N were applied to 6 sites within a marsh in Revere, MA, and a 1mmol solution of PbNO₃ was applied to 2 additional sites at the same location. Additionally, a greenhouse experiment was completed where ¹⁵N and three varying concentrations of PbNO₃ (0 mmol, 0.5 mmol, 1mmol) were applied to *S. patens* replicates. I predict that my field data will demonstrate differences in nutrient uptake between the two marsh species in the absence of PbNO₃, which would suggest that certain marsh species are stronger competitors for nutrients. In the presence of PbNO₃, I predict that nutrient uptake will be diminished in both marsh species. Differences in nutrient uptake will reflect relative abilities of marsh species to retain nutrients and thrive within polluted and contaminated sites. Ultimately, the results of this study may be used to guide managers in the decision-making process regarding marshland remediation projects.
Our primary goal was to investigate the effects that increased nitrate levels have on dinoflagellate growth rates and how the combination of increased temperature and increased nitrate levels affect growth rates. Since the dinoflagellate *Symbiodinium* maintains a symbiotic relationship with the anemone *Aiptasia pallida*, identifying factors that determine its growth rates can help identify factors that lead to higher risk of coral bleaching. Two different types of *Symbiodinium* sp. belonging to two clades A4 and B1 (CCMP 2456 and CCMP 2460, respectively) were each grown in four different conditions: (1) 25°C with 0 μM nitrate, (2) 25°C with 200 μM nitrate, (3) 30°C with 0 μM nitrate, and (4) 30°C with 200 μM nitrate. The growth rates of dinoflagellates were measured using a hemocytometer taking measurements on 4 separate occasions over a 23 day period. Chla was also measured twice during this period. Additionally, the nitrous oxide (N₂O) levels emitted by a sample of the dinoflagellates was measured, as it may play an important role in the expulsion of dinoflagellates from corals and anemones.
Eutrophication and Flooding Effects on Greenhouse Gas Flux in Belle Isle Salt Marsh

Studying greenhouse gas flux is an important aspect of salt marsh ecology, as marshes are a significant location of carbon and nitrogen cycling. This study looked at the effects of eutrophication and flooding on gas production in Belle Isle salt marsh. Using flux chambers, production of carbon dioxide, methane, and nitrous oxide was measured in plots treated with either a sodium nitrate saltwater solution or just saltwater. The treatments simulated eutrophication and flooding conditions while background data, as well as control plots, were used to analyze the effects of the added nitrogen and saltwater on gas flux. Eutrophication normally increases the production of nitrous oxide and other byproducts of plant growth and metabolism, like carbon dioxide. I hypothesize that the added nitrogen will increase the flux of the three greenhouse gases in question, since plant and cyanobacteria production are known to increase under these conditions. Flooding promotes methane production in plants as well as anaerobic processes in marsh soil that lead to nitrous oxide production. This means it is likely that flooding also will increase greenhouse gas flux. These results would be significant as salt marshes are projected to face rising seas in the upcoming decades due to global warming. If flooding could increase the production of greenhouse gases, potentially accelerating the rate of global warming. At the same time, these results may show that greenhouse gas flux might be diminished by reducing eutrophication in the salt marsh. This would provide an important avenue to combat the cycle of growing greenhouse gas production in the salt marsh due to flooding and human activity.
Sal marsh communities are critical because they support a broad range of species and provide important ecosystem services. Currently, however, global climate change threatens to alter the structure and function of these marshes. Within salt marsh ecosystems, plant species exhibit consistent patterns in zonation determined by water level, salinity, and competition. It is predicted that an increase in sea level, due to global warming, will cause inundation, and changes in salinity and soil chemistry. Sulfide build up, associated with sea level rise, can be toxic to wetland plants. These changes will likely effect the zonation of wetlands. The goal of this study is to investigate the effects of sea level rise on salt marsh communities, using a three-tiered approach. Field studies completed at Belle Isle Marsh, Winthrop, MA, investigated plant zonation and inundation. Transect studies yielded data on the location and approximate percent biomass of plant species, allowing for analysis of the zonation of species in the marsh. Transplantation studies relocated Spartina patens from the high marsh to the low marsh in order to simulate inundation conditions predicted for future sea level rise. In the laboratory, a greenhouse study investigated inundation and soil chemistry. S. patens plants in the greenhouse study received the treatments of either low or high water level and either the presence or absence of sulfide. It is predicted that plants exposed to low water levels and no sulfide will have the largest final biomass, while plants exposed to high water levels and sulfide will have the smallest. Data obtained in this experiment is important for understanding the sensitivity of salt marsh ecosystems in response to sea level rise.
POSTER 40
Undergraduate Presenter: Philip Eliades ('11)

The effects of retinoic acid on regulatory T cell generation as a means to achieve allograft prolongation

Authors: Philip Eliades, Matthew O'Connor, BA, Patrick Duff, BS, James F. Markmann, MD, PhD, James I. Kim, PhD: Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA

The conversion of naïve T cells into regulatory T cells (Tregs) is an important mechanism for self-tolerance. Tregs act to regulate the adaptive immune response by suppressing the proliferation of antigen-reactive T cells. The differentiation of the Treg lineage is controlled by Foxp3, a transcriptional regulator. Due the central role Foxp3 plays in the delineation of Tregs, Foxp3 expression is the best way to identify Tregs. It has been shown that helper T cells up regulate Foxp3 upon exposure to TGF-β, and that this TGF-β mediated conversion from Foxp3- cells to Foxp3+ cells can be intensified by retinoic acid, a metabolite of vitamin A. While Tregs are important for tolerance, the role of conversion in transplant tolerance is unclear. Foxp3 expression is down regulated by counter-regulation, a mechanism that results from the transplant procedure and challenges the regulatory abilities of Tregs in vivo; however, the mechanisms of counter-regulation are not well defined. Expanding on the previous findings mentioned above, we want to examine if retinoic acid is valuable in the conversion of Foxp3+ cells in a transplant setting and if it promotes graft prolongation by mediating counter-regulation. Before conducting mouse skin graft survival experiments, preliminary experiments testing for the conversion of helper T cells to regulatory T cells in a transplant setting have been performed both in vitro and in vivo. Here we show that retinoic acid amplifies TGF-β dependent up regulation of Foxp3 in vitro, yet the in vivo conversion was not significantly enhanced by retinoic acid injections alone. Perhaps the injection of retinoic acid with TGF-β will be more promising. This still demonstrates the possibility of implementing retinoic acid as a way to prolong allograft survival.

Retinoic acid boosts TGF-β-induced expression of Foxp3 in antigen-activated T cells
Purified FeNos was digested with trypsin, and the tryptic peptides were analyzed by MALDI-TOF mass spectroscopy. On the mass spectrum at the right, peptides with the predicted masses for FeNos are indicated with asterisks.
Recovery of novel zebrafish blood mutants from forward genetic screens has revealed new genetic understandings of hematopoietic pathways and can serve as models of human blood disease. The novel zebrafish mutant, *malbec* (*mlb*), was identified from a large-scale mutagenesis screen. *Mlb* presents with anemia and severe pericardial edema by day 3 post fertilization (dpf). Characterization of *mlb* by whole mount *in situ* hybridization (WISH) revealed conservation of lymphoid and myeloid lineages, but demonstrated an inability to maintain erythropoiesis for long-term survival. Transgenic lines Tg(*cd41:egfp*) and Tg(*gata1:egfp*) crossed into the *mlb* background exhibit conservation of thrombocyte cells and a complete loss of gfp positive erythrocytes in *mlb* positive fish, respectively. High resolution mapping, to identify the gene mutated in *mlb*, has been restricted to a critical BAC clone containing only a few annotated genes. Future investigative studies will elucidate the critical role of *mlb* in hematopoiesis and provide insight into human congenital blood disorders.
IL-10 induction by Anti-thymocyte Globulin in a Mouse Model

Authors: Megan Decoteau, Kathleen Phillips, John P. Wing, and Melanie Ruzek

(Boston College Biology Department and Genzyme Corporation, Framingham, MA)

Polyclonal rabbit anti-human thymocyte globulin (Thymoglobulin®; Genzyme Corporation) is used clinically to prevent acute rejection in solid organ transplantation. Thymoglobulin depletes T cells, but additional mechanisms of action have also been suggested. To investigate whether cytokines are induced by a murine counterpart of Thymoglobulin (mATG), these studies investigated the in vitro and in vivo cytokine responses following mATG treatment. A specific focus was put on the production of the anti-inflammatory cytokines with the hope that the results may further explain the immunosuppressive function of Thymoglobulin/mATG. In vitro and in vivo experiments were conducted to study cytokine levels in splenocytes and blood serum, and anti-MHC-II column cell separation was utilized to determine which cells were responsible for the production. The addition of other immunosuppressive cytokines to cultures was also tested to see what effects, if any, the addition of these cytokines had on the cytokines produced following mATG treatment. This study found that mATG-treatment in vivo showed increased IL-10 production by splenocytes from these mice as well as when splenocytes were cultured in the presence of IL-10. In addition, we found that the IL-10 was produced primarily by non-MHC-II positive cells, which are primarily T cells. This data gives further insight into a possible pathway of immunosuppressive function that mATG may possess in its ability to effect cytokine levels and specifically induce the anti-inflammatory cytokine IL-10.
POSTER 44

Undergraduate Presenter: Xibei Jia (’10)

Changes in the fatty acid composition of brain cardiolipin following hypoxia treatment and calorie restriction in C57BL/6J, VM/DK, and F1 hybrid mice

Authors: Xibei Jia and Thomas Seyfried

Cardiolipin is an important lipid component of the brain mitochondria. It stabilizes the electron transport chain complexes and is integral to the functionality of respiration. Recently, the fatty acid composition of brain cardiolipin in the VM/DK (VM) mice was found to be altered compared to that of the C57BL/6J (B6) mice. In this study, we examined the potential inheritance of the altered fatty acid composition of brain cardiolipin in the F1 hybrids, and the changes in the fatty acid composition of brain cardiolipin following mitochondrial and metabolic stresses (i.e. hypoxia treatment and calorie restriction) in VM, B6 and F1 hybrids. Our study showed both reciprocal hybrids (VM×B6 F1, B6×VM F1), males and females, had fatty acid composition of brain cardiolipin similar to that of the VM mice. An increase in shorter chain fatty acids, C16:0, C18:0, C18:1 and a concomitant decrease in longer chain fatty acids, C20:4 and C22:6 was observed in the VM and F1 hybrid mice in comparison to the B6 mice. Under stressful environments of calorie restriction (CR) and hypoxia treatment (HT), changes in the percent distribution of fatty acids were similar among B6, VM and F1 hybrid mice with the exception of fatty acid 18:0. The percent distribution of C18:0 was decreased in calorie restricted VM and F1 hybrid mice, while increased in the calorie restricted B6 mice, compared to the control (normaxia and ad libitum) mice. In addition, the phenotypic inheritance was observed in an autosomal dominant manner. Calorie restricted VM and F1 hybrid mice showed a significantly lower hypoxic tolerance over three days than the B6 mice. We hypothesized that the changes in fatty acid composition of brain cardiolipin following HT and CR might be related to the phenotypic behaviors observed in B6, VM and F1 hybrid mice.
POSTER 45
Undergraduate Presenter: Joshua Meidenbauer (‘10)

The EL Mouse: A Natural Model of Autism and Epilepsy
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Purpose: Autism is a multifactorial disorder that involves impairments in social interactions and communication, as well as restricted and repetitive behaviors. About 30% of individuals with autism develop epilepsy by adulthood. The EL mouse has long been studied as a natural model of multifactorial idiopathic generalized epilepsy. Because epilepsy is a co-morbid trait of autism, we evaluated the EL mouse for behaviors associated with autism.

Methods: The neurobiological and behavioral abnormalities previously reported in EL mice were evaluated in light of similar abnormalities previously reported in persons with autism. We compared the behavior of EL mice (juvenile and adult) to age-matched control DDY mice, a genetically related non-epileptic strain. The mice were compared in the open-field and in the light-dark compartment tests to measure activity, exploratory behavior, and restricted and repetitive behavior. The social transmission of food preference test was employed to evaluate social communication. Home-cage behavior was also evaluated in EL and DDY mice as a measure of repetitive activity.

Results: We show that EL mice, besides developing adolescent-onset seizures, share several neurobiological and behavioral abnormalities with persons with autism. Both EL mice and individuals with autism exhibit increased microglial activation in the cerebellum as well as increased GFAP expression throughout the brain. Activity, exploratory behavior, and restricted behavior were significantly ($p < 0.01$) greater in EL mice than in DDY mice. EL mice exhibited impairment in the social transmission of food preference assay ($p < 0.05$). Also, a stereotypic myoclonic jumping behavior was observed in EL mice, but was not seen in DDY mice.

Conclusions: These findings suggest that the EL mouse expresses behavioral abnormalities similar to those seen in persons with autism. We propose that the EL mouse can be utilized as a natural model of autism and epilepsy.

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Obesity is considered a chronic low-grade inflammatory disease, which strongly associates with an increased risk for Type 2 Diabetes, hypertension, and hyperlipidemia. Obesity leads to hypertrophy and hyperplasia of adipocytes in adipose tissue (AT). AT is recognized as an active metabolic and endocrine organ that produces a variety of substances, known as adipokines. Epididymal fat pads are a large source of the AT in mice and have been examined extensively as visceral AT for the study of obese mice, which has been linked to insulin resistance and the metabolic syndrome. Our lab previously showed that the distribution of macrophages in epididymal fat varies greatly among the three main sections of this tissue, which are designated tip, body, and base. It is important to study adipocytes as this allows us to correlate the data more closely with visceral fat. Our main goal was to determine the cross-sectional area of adipocytes and crown-like structures (CLS) in tip, body, and base parts of epididymal fat pads of both diet induced obese (DIO) mice and lean mice. The CLS consists of adipocytes surrounded by macrophages and this indicates the inflammation in AT. Furthermore, we determined the adipocyte stem cell populations in the three major segments of epididymal fat pads by flow cytometry. Our data showed tip parts of epididymal fat pads had significantly more large adipocytes with CLS. On the other hand, flow cytometry analysis showed the adipocyte stem cells were highly concentrated in base parts of epididymal fat. These results indicate that there is a unique difference among the three parts of epididymal fat.
POSTER 47
Undergraduate Presenter: Krystyna Orzechowski (‘10)

Satellite cells as agents of pathogenesis in propagating dorsal root ganglia damage in SIV-infected rhesus macaques
Authors: Krystyna Orzechowski1, Tricia H. Burdo2, Jessica Button3, Andrew Miller2, and Kenneth Williams1

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HIV-related sensory neuropathy (HIV-SN) is currently the most common neurologic complication of HIV infection in the developed world, although the mechanisms underlying peripheral neuropathogenesis remain poorly understood. The most obvious histological changes in HIV-SN involve the peripheral nerves, but inflammatory infiltrates and neuronal injury have also been observed in the dorsal root ganglia (DRG) of HIV-infected individuals. DRGs are surrounded by a distinct sheath of satellite cells, considered resident macrophages of the PNS, which normally function to provide physical and chemical support for the neurons. However, during HIV infection, activated satellite cells and infiltrated monocytes have been shown to invade neuronal spaces in a process known as neuronophagia, resulting in the formation of Nageotte nodules. Ultimately, it is the dysregulation of monocytes and macrophages that is thought to be responsible for neurologic dysfunction with HIV infection.

The lack of an effective animal model of HIV-SN has hampered research in understanding the pathogenic mechanisms of the disease, as well as in identifying potential therapies that can enhance peripheral nerve regeneration and restore function. This study aimed to develop a model of HIV-SN, using simian immunodeficiency virus (SIV)-infected CD8+ T lymphocyte depleted rhesus macaques. DRG tissues were analyzed by immunohistochemical staining methods, and were examined for resident macrophage activation, monocyte/macrophage infiltration, and degree of productive infection, in order to define the role of satellite cells in producing DRG damage during SIV-infection. Pathological findings of peripheral DRG sections were also compared to CNS pathology in AIDS, to determine if a correlation exists between the incidence of peripheral neuropathy and the incidence of encephalitic lesions in the CNS. Our results showed that while there was no consistent correlation between CNS and PNS pathology, DRG damage and neuronal loss was significantly associated with satellite cell activation and an increase in monocyte trafficking from the bone marrow. Furthermore, the SIV-inoculated CD8+ T lymphocyte depleted rhesus macaque serves as an excellent rapidly-progressing model to study peripheral neuropathy, with both a higher incidence of SIVE and more severe DRG disease than seen in non-depleted animals.
Monocytes and macrophages are significant reservoirs of human immunodeficiency virus (HIV), contributing to viral load throughout disease progression, and delivering virus to various organs such as the brain. With chronic HIV infection, the ability of mucosal barriers in the gut to prevent pathogenic microbes from translocating into systemic circulation is critically impaired. These products, most notably lipopolysaccharide (LPS), readily enter circulation, and are hypothesized to cause widespread immune activation of monocytes and macrophages, a hallmark of HIV infection, that potentially drive macrophage mediated pathogenesis in AIDS. It has also been hypothesized that cells of the innate immune system can become sensitized to LPS and HIV due to prolonged exposure. However, mechanisms for this have not been elucidated. In this thesis, we aimed to test the responses of monocytes and macrophages to LPS and HIV in vitro, utilizing the rhesus macaque model and the simian immunodeficiency virus (SIV). Our goal was to define whether stimulation with LPS and SIV drives activation and maturation of monocytes thus providing a mechanism for monocyte/macrophage effects on microbial translocation and systemic immune activation observed in AIDS. We observed that LPS and SIV drives the activation and maturation of monocytes in cultured blood monocytes and ex vivo blood. Additionally, we found evidence of in vitro sensitization of these LPS and SIV mediated effects. Monocytes also became polarized to an anti-inflammatory phenotype after the initial pro-inflammatory response. We therefore provide a mechanism of microbial translocation and systemic immune activation of monocytes and macrophages in HIV and AIDS pathogenesis.
Through the process of cellular respiration of aerobic organisms, cells generate reactive oxygenated species. These play a role in the degradation of cellular lipids, DNA, and proteins. In order to repair oxidized methionine in both free and peptide forms, yeast have evolved the methionine sulfoxide reductases, Msra, Msrb, and frMsr, to reduce oxidized methionine before it is irreversibly oxidized into methionine sulfone. Our lab has repeatedly demonstrated resistance of the Dmsr knockout strains on hydrogen peroxide concentrated media. It is known that the Msra and Gpx3 proteins physically interact and act as a sensor for oxidative stress. Under conditions of oxidative stress, this link dissociates, allowing Gpx3 to activate transcription factor Yap1, resulting in the transcription of a number of genes involved in the oxidative stress response. In our proposed model, the Dmsra resistant phenotype is a result of unbound Gpx3 and constitutive activation of the transcription factor Yap1. We have focused on the involvement of two peroxiredoxins, Tsa1, which is a main component of the thioredoxin pathway, and Ahp1, an activator of transcription factor Cad1 (Yap2), a homologue of Yap1. DmsraTsa1 and DmrsaDaph1 have also repeatedly demonstrated additional resistance to oxidative stress of hydrogen peroxide, indicating that DmsrA resistance does not require Tsa1 or Ahp1. We propose that Tsa1 and Ahp1 may function as sensors to oxidative stress in a manner similar to the Msra::Gpx3 complex, though the specifics of these pathways are not yet known.
POSTER 50
Undergraduate Presenters: Michael Chen (‘10), David Faugno-Fusci (‘10) and Faith Goronga (‘11)

Methionine Sulfoxide Reductases in Oxidative Stress Pathways of S. Cerevisiae.

Authors: Michael Chen*, David Faugno-Fusci*, and Faith Goronga*, Dr. Clare O'Connor and Dr. John Wing, Boston College Biology Dept, Chestnut Hill, MA.

The release of oxygen radicals into cells results in oxidative stress, which has been linked to aging as well as DNA mutation, protein inactivation and degradation, and changes to lipid membrane permeability. Methionine Sulfoxide Reductases reduce free and protein bound Methionine and Methionine-Sulfoxide and are central to the oxidative stress response in yeast, Saccharomyces cerevisiae. We have identified a novel phenotype in Msr knockouts that is resistant to oxidative stress. To identify pathways required for this resistant phenotype, we examined the roles of ZWF1 and STB5, two proteins involved in the pentose phosphate pathway. The techniques utilized in order to examine the effects of these proteins were generation of double knockouts, solid media stress spot assays, liquid growth curve analysis, and RT-PCR. Double knockouts of DZWF1 and DSTB5 with DMSRA showed similar levels of sensitivity to the single knockouts of each and a loss of the resistant phenotype that the DMSRA strain shows. This data indicates that both ZWF1 and STB5 genes are required for the resistant phenotype. Our study shows that there is still a lot of potential for further research on these pathways in order to fully comprehend MSRA, an essential figure in the oxidative stress response, and to use this gained understanding to create a pharmaceutical utility applicable to people suffering from the negative effects of oxidative stress in their biological system.
A family of intermediate filament-like proteins is sequentially assembled into the cytoskeletal scaffold of *Toxoplasma gondii*. Submitted. (April 2010).

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SCAP is required for timely and proper myelin membrane synthesis.

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COMIT: Identification of Noncoding Motifs under Selection in Coding Sequences

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