Biology with Microbiology and Molecular Biology

SHIGA TOXIN PATHOGENESIS IN THE GASTROINTESTINAL TRACT. Abigail J. Lenz & Andrew J. Fabich. Dept. of Biology & Chemistry, Liberty Univ., Lynchburg, VA 24515. Enterohemorrhagic *Escherichia coli* (EHEC) is a gram negative bacteria that is the leading cause of infectious kidney failure worldwide; a primary mechanism of EHEC infection is Shiga toxin (stx), which is carried by Golgi phosphoprotein 4 (GPP130) and binds globotriaosylceramide (Gb3) receptors on the endothelial cell surface, initiating a cascade that ultimately leads to apoptosis. *Citrobacter rodentium* genetically engineered with stx (CR) has successfully been used in mice to model the effects of EHEC in humans. We show that competition drives the pathogenic effects of CR infection.

ENTRAINMENT OF SYNTHETIC GENE OSCILLATORS BY A NOSIY STIMULUS. N. C. Butzin, P. L. Hochendoner, C. T. Ogle, P. Hill & W. Mather, Dept. of Physics, Virginia Tech, Blacksburg, VA. Modulation of biological oscillations by external stimuli lies at the root of many phenomena, including maintenance of circadian rhythms, propagation of neural signals, and somatogenesis. While it is well established that regular periodic modulation can entrain an oscillator, a curious phenomenon is that a noisy (rugged) modulation can also robustly entrain oscillations. This latter scenario may describe, for instance, the effect of irregular weather patterns on circadian rhythms, or why irregular neural stimuli can still reliably transmit information. A synthetic biology approach has already proven useful in understanding the entrainment of oscillators by periodic signaling, which can mimic the response of a number of noisy oscillating systems: cell cycles, NF-*k*B response, etc. We similarly seek to use synthetic biology as a platform to understand how aperiodic signals can strongly correlate the behavior of cells. This study should lead to a deeper understanding of how fluctuations in our environment and even within our body may promote substantial synchrony between our cells. We investigate experimentally and theoretically the entrainment of an ensemble of synthetic gene oscillators by a noisy stimulus. Stochastic simulations suggested that a synthetic gene oscillator would be strongly entrained by two aperiodic signals: telegraph noise and phase noise. This simulation-based prediction was tested by a combination of microfluidic and microscopy using a real synthetic circuit in *Escherichia coli*. We use delayed feedback models to analyze these cells. We show that cells are entrained by two noisy signals: telegraph and phase noise. Cells are entrained when either signal period or amplitudes are varied.

EFFECT OF CONVERTING TO ORGANIC TURKEY REARING PRACTICES ON ANTIBIOTIC RESISTANCE OF ENTEROCOCCI FROM USED LITTER. Steven G. McBride, Lindsey Toothman, Pradeep Vasudevan & Joanna B. Mott. Dept. of Biol., James Madison Univ., Harrisonburg VA. 22807. Conventional turkey production employs antimicrobial compounds for prophylaxis, treatment, and growth promotion. Conversely, organically raised turkeys are not administered antimicrobial compounds. Increasing public interest in organic and antibiotic free foods has led to a growing

market for their production. In this study the prevalence of antibiotic resistance in *Enterococcus* spp. isolated from the turkey litter of adult tom turkeys after they were sent to market, was compared before and after the farm transitioned to organic rearing practices. *Enterococcus* spp. were isolated on m-EI agar and identified to species phenotypically. Susceptibility to 12 antimicrobial compounds was determined using the Kirby-Bauer disc diffusion technique. The proportions of resistant bacteria were compared using chi-square test and Fischer's exact test. Isolates from the litter of organic birds differed in species composition and resistance profiles from those isolated from the conventionally farmed litter. Isolates from organic litter exhibited a reduction in the percentage of isolates resistant to gentamicin (- 47%), tetracycline (- 42%), and doxycycline (- 25%). These results suggest that changing to organic rearing practices affects the antibiotic resistance of bacteria in turkey litter.

EFFECTS OF SYNTHETHETIC ANDROGEN ON P21 EXPRESSION IN MCF-7 CELLS. Tawany C. Almeida & Rosemary Barra, Department of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401. In the United States, breast cancer is the most prevalent form of cancer in women and is second only to lung and bronchial cancer as the leading cause of cancer death. A number of factors have been identified that increase the risk of developing breast cancer, including specific gene expression, age, estrogen exposure, and obesity. It has also been demonstrated by both *in vivo* and *in vitro* studies, that some breast cancer cells express both androgen and estrogen receptors. These studies suggest that breast cancer may be linked to a hormonal imbalance between estrogens and androgens. The physiological effects of androgens on breast cancer cells have not been clearly described. However, the hyperactivation of the mitogen-activated protein kinase (MAPK) pathway has been shown to result in a growth-inhibitory response. Recent studies have also shown that androgen receptor signaling/activation of the MAPK pathway is dependent on the cyclin dependent inhibitor, p21. For this study, we hypothesized that androgens would have an inhibitory effect on the growth of MCF-7, androgen receptor positive breast cancer cells, and that p21 would mediate this effect. In the first phase of this study, we determined the effects of androgens on cell viability using the MTT assay. At an androgen concentration of 10^{-6} M, the cell viability was decreased 12% in comparison to the control cells. A p21 ELISA assay was used to detect and quantify the level of p21 activity in MCF-7 cells treated with 10^{-6} M androgen. The results of three experiments indicated that p21 activity was increased 34.7% by exposing the cells to androgens for 24 hours. These preliminary studies suggest that p21 plays a role in the observed anti-proliferative effect, however additional studies are needed to confirm this observation and to elucidate the mechanism.

QUORUM SENSING EFFECTS BASIC BACTERIAL PHYSIOLOGY. R. A. Betar, R. N. Montalvo, A. J. Fabich PhD. Department of Biology, Liberty University, Lynchburg, VA 24502. Bacterial populations communicate to each other through Quorum sensing (QS). This form of communication is based on genetic induction by a two component regulatory system, which has been associated with the bacterial stress

response. The protein QseC is the receptor sensory kinase that phosphorylates the response regulator QseB and induces transcription of specific genes. QseC typically responds to autoinducers released from bacteria and also responds to intestinal to epinephrine and norepinephrine. QS is known to cause induction of both virulence and motility genes. Previous research has been performed in pathogens, associating QS with virulence. To understand the basis of QS more *E. coli* MG1655 wild type and *E. coli* MG1655?*qseC* were colonized within the murine intestine. The results indicated that without the sensory kinase, there was a competitive advantage for colonization. Since no one had previously measured bacterial physiology of *E. coli* MG1655?*qseC*, we measured basic physiology on the strain to determine the cause of the greater advantage on colonization. We found that QS is not limited to the induction of virulence and motility as shown in previous studies, and that downstream carbon metabolism is heavily influenced. Specifically, the *E. coli* MG1655?*qseC* strain has a quicker generation time than the wild type on both catabolite-repressing and non-catabolite repressing sugars.

STABLE ISOTOPE ANALYSES OF DIETARY DIFFERENTIATION AMONG FREE-RANGING SMALL RUMINANT BREEDS. Pieter A.P. deHart & Julie A. Lozier, Department of Biology, Virginia Military Institute, Lexington VA 24450. As society strives to adapt to a rapidly changing economic and environmental climate, families and farmers are exploring opportunities for increased self-reliance and sustainable practices. These explorations have been particularly evident in small- and medium-sized farms throughout the United States, as there have been systematic increases in abundance of free-ranging dairy goat (*Capra hircus*) and sheep (*Ovis aries*) production throughout the country. Concomitant with this growth have been new research efforts aimed at assisting producers in refining herds and their diets to increase output and efficiency. One popular approach has centered on both optimal breed selection and an increase in breed diversification. Little scientific evidence exists, however, on innate physiological differences of dietary uptake between breeds, which may dictate herd health, as well as milk, meat, and fleece quality. Given inherent differences in size, behavior, and diet selectivity between breeds, there are likely breed-specific and dietary-independent physiological differences dictating overall herd characteristics in both these species. To determine what specific differences may exist, and bridge a critical knowledge gap, we examined the carbon and nitrogen isotopic signatures from hairs of six breeds of dairy goats (n=123) and from six breeds of multi-purpose sheep (n=56) across a total of ten farms exhibiting similar vegetation profiles within Appalachian Virginia. Our results indicate that while the mean (±SD) values obtained for all breeds within each species varied widely, the differences between goat breeds ($\leq$2.3‰ δ^{15}N, $\leq$1.7‰ δ^{13}C) and sheep breeds ($\leq$2.8‰ δ^{15}N, $\leq$5.7‰ δ^{13}C) are much greater than those between farms ($\leq$£0.3‰ for both δ^{15}N and δ^{13}C). For goats, the expected ontogenetic differences yielded more exaggerated distinctions between breeds, with variations between kids of different breeds $\leq$3.8‰ and $\leq$2.2‰ for δ^{15}N and δ^{13}C, respectively. This variation in δ^{15}N and δ^{13}C of kids is likely due to differential protein investment in milk by does, which could have implications for milk production

rate and quality, and ultimately breed selection for specific environments. For sheep, greatest variation was apparent between genders, as males of most breeds had signatures differing by ~2.1‰ and ~1.7‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. These sex-mediated differences in sheep are likely driven by behavioral differences, and so producers selecting breeds which exhibit minimal gender differences could yield more consistent meat and fleece quality throughout the flock. This work suggests that future research on free-ranging ruminants, their diet, and health effects of dietary supplementation should be performed at breed-specific levels.

GENOME-WIDE RNA-SEQ ANALYSIS OF CHANGING GENE EXPRESSION IN BRAIN AND BLOOD IN AN ALZHEIMER'S DISEASE MOUSE MODEL. A. Házy, M. Dalton & G. D. Isaacs, Dept. of Biology and Chemistry, Liberty University, Lynchburg VA 24515. Previous studies have established a causative role for altered gene expression in development of Alzheimer's disease (AD). These changes can be affected by methylation and miRNA regulation. In this study, genome-wide expression changes in AD hippocampus and blood were determined by RNA-sequencing of mRNA from control and AD mice. Epigenetic changes contributing to AD were also investigated via qPCR to determine expression changes of miRNA known to change methylation status in AD. The qPCR data showed significantly increased expression of Mir 17 in AD. Sequencing data revealed 729 genes in hippocampus and 152 genes in blood showing significant differential expression (q value < 0.05). Changing genes in hippocampus were compared to changing genes in blood to identify 75 genes as candidates for development of an expression-based diagnostic blood test for AD.

ANATOMY OF AN INVASION: THE EFFECTS OF HOST PREFERENCE ON THE BIOGEOGRAPHY OF TICKS. Robyn M. Nadolny, Dept. of Biological Sciences, Old Dominion University, Norfolk, VA 23529. Ticks depend on the movements of their hosts to transport them across a landscape, and host specificity is key to any parasite's ability to disperse and invade new areas. Ticks that show preference for specific hosts may move across a landscape in different and potentially unexpected ways. We documented the northward expansions into Virginia of two tick species, *Ixodes affinis* and *Amblyomma maculatum,* both of which carry pathogens that have been linked to human and wildlife disease. To assess population connectivity and ancestry, we sequenced the 16S mitochondrial rRNA gene from a representative sample of individuals of both species from populations throughout the eastern US. We found that despite overlapping host preferences throughout ontogeny, each species exhibited very different genetic and geographic patterns of population establishment and connectivity. We suggest that tick life stage, host, and habitat preferences are all important in understanding and predicting tick range expansions.

Posters

GENETIC AND PHENOTYPIC CHARACTERIZATION OF TWO EXTANT RHOA ALLELES IN DROSOPHILA MELANOGASTER. Laura A. Johansen & Susan R.

Halsell, Dept. of Biol., James Madison Univ., Harrisonburg, VA 22807. *Drosophila melanogaster*, the fruit fly, is a powerful model for genetic studies into developmental biology. Function of the Rho signal transduction pathway is critical during development, including in humans. In *Drosophila*, mutant alleles coding for the RhoA protein have been implicated in causing morphogenetic errors in fruit fly embryos. These characterized *RhoA* mutant alleles are homozygous lethal with characteristic defects in head involution. Genetic and phenotypic characterization of the $RhoA^{3.5.1}$ and $RhoA^{4.4.2}$ alleles involved an examination of the severity of defects in homozygous embryos. Since $RhoA^{3.5.1}$ showed a complete loss of function as its lethality rate is 100% of all homozygous embryos and trended towards the more severe phenotype defects, it is likely that the mutation is consistent with previously found *RhoA* loss-of-function mutant alleles. For $RhoA^{4.4.2}$, the results strongly suggest it is a partial loss-of-function mutation known as a hypomorph. Homozygous $RhoA^{4.4.2}$ embryos showed only 58% lethality at the embryonic stage of development. However, when one of the $RhoA^{4.4.2}$ alleles is replaced with a deficiency that completely removes the entire *RhoA* gene, $RhoA^{4.4.2}$*/Df(2R)Jp8* embryos showed an increased embryonic lethality of 84%. This means that each $RhoA^{4.4.2}$ allele provides some functional protein, but when placed in trans to the deficiency encoding no protein, less than half of the protein is made, resulting in the more severe phenotype.

CELL PHONE INDUCED GENE EXPRESSION IN HUMAN GLIOBLASTOMA CELLS. Virginia L. King & Deborah A. O'Dell. Dept. of Biol., Univ. of Mary Washington. Fredericksburg VA 22401. The link between cell phone radiation and the production of brain cancers has not been firmly established, although many governments regulate the amount of radiation permitted in cell phones. Previous studies in Dr. O'Dell's lab indicate an increase in certain oncogenes and a decrease in certain tumor suppressor genes up to 24 hours after a 25 minute exposure to cell phone radiation, followed by a decline in expression by 36 hours, indicating that cell phone radiation can lead to cell cycle changes. Although the changes were reversed, they were not reversed back to pre-exposure levels. We were interested in determining the length of time it took to completely reverse gene expression changes induced by cell phone radiation. We exposed cultured glioblastoma cells to 25 minutes of continuous cell phone radiation. The cells were separated from the cell phone by skeletal, muscle and skin tissue approximating the human skull structure. Whole cell RNA was extracted from the cultured at 24 and 48 hours after exposure and the change in expression was measured using RT-PCR using a commercially available array assessing 84 oncogenes and tumor suppressor genes (Qiagen). The changes in gene expression were analyzed using 2^dCt to measure fold changes. Any gene which showed a change in expression >2 is considered to have significant changes in expression. These results confirm previous results that a brief exposure to cell phone radiation temporarily increases gene activity. These results also showed that cell phone radiation is not impeded by structures of the human head. The types of changes indicate that cell activity may be altered in such a way to promote a cancerous condition. This study was funded in part by the VA. Academy of Science and the Univ. of Mary Washington.

TRANSCRIPTIONAL REGULATORS OF EPIGENETICALLY ALTERED GENES IN ALZHEIMER'S DISEASE. John T. Lawson & Gary D. Isaacs, Dept. of Biol. and Chem., Liberty Univ., Lynchburg VA 24515. Most cases of Alzheimer's disease (AD) are sporadic-not clearly genetic in nature. Because of this, epigenetics, which deals with factors that modify DNA expression without altering the DNA sequence, may help explain how the environment could interact with genes to promote AD. Epigenetic changes have been observed in AD and the Isaacs lab at Liberty previously determined genes that increase in methylation (hypermethylated) and decrease in methylation (hypomethylated). Methylation can silence genes by inhibiting transcription factor binding. To look into what transcription factors may regulate these genes, bioinformatics methods and tools including the AME program from the MEME suite were used to test for enrichment of transcription factor motifs in promoters and "peaks" (areas of epigenetic changes) of genes that were differentially methylated in AD. Out of 591 motifs from the Jaspar and Uniprobe Mouse databases, 16 motifs were enriched in genes that were hypermethylated in AD and 8 motifs in genes that were hypomethylated in AD. Out of these, 14 of 16 motifs from hypermethylated genes and 8 of 8 motifs from hypomethylated genes were also specifically enriched in the "peak" regions of epigenetic change in each promoter compared to adjacent regions in the same promoters. The transcription factors for the enriched motifs, many of which were involved in processes that could be relevant to AD, may be able to provide a link between epigenetic changes and changes in gene expression in AD as well as offer prospects for future research. (Supported by: the Jeffress Memorial Trust, the Virginia Academy of Science, and the ARDRAF grant from the Virginia Center on Aging).

SIGNIFICANT UP-REGULATION OF MIR-17 IN AN ALZHEIMER'S DISEASE MOUSE MODEL. M. R. Dalton, A. D. Házy & G. D. Isaacs, Dept. of Biol. and Chem., Liberty University, Lynchburg VA 24515. Previous work by our group has demonstrated a correlation between AD pathology and changes in epigenetic markers, including cytosine methylation of gene promoter regions. Several genes determined to have AD-related changes in methylation code for miRNA, which is known to regulate gene expression at a post-transcriptional level. Research suggests that miRNA play a key role in AD development by alteration of gene products and transcription factors, particularly in that of amyloid-ß (Aß) production and apoptosis of postmitotic neurons. This analysis shows a significant up-regulation of demonstrated epigenetically modified miR-17 in the hippocampi of transgenic AD mice when compared to that of non-AD mice. MiR-17 belongs to the polycistronic cluster miR-17-92 and is believed to be involved in normal neuronal cell proliferation and ultimately the regulation of Beclin-1, which has been shown to modulate amyloid beta accumulation in mice.

VALIDATION OF PREDICTED TRANSCRIPTION FACTOR BINDING SITES IN ALZHEIMER'S DISEASE. Bria E. Johnston & Rachel C. Bordelon, Dept. of Biology and Chemistry, Liberty University, Lynchburg VA 24515. Alzheimer's Disease (AD) is a neurodegenerative disorder affecting over five million Americans as of 2015. Symptoms include severe memory loss, confusion, and gradual loss of the ability to

self-care. While the underlying causes of the disease are yet unknown, what is clear is that there is no single mutation that causes AD. Rather, changes to the genome that affect gene expression but do not alter DNA sequence may be more likely to blame. These epigenetic changes include the chemical modification of bases, such as the methylation of certain cytosine bases. Methylation within the promoter region of a gene can change the expression levels of that gene by preventing transcription factors from binding and recruiting RNA polymerase. Using preliminary data from another student generated using various bioinformatics programs, the transcription factor binding sequences within the promoter regions of eight genes of interest will be predicted. Whether these sequences actually bind to the corresponding transcription factors will be tested using an electrophoretic mobility shift assay (EMSA), commonly used to show DNA-protein interaction. Once these sequences are known, the methylation status within the transcription factor binding site can be compared between the AD state and wild type. For a given gene, if the methylation state changes between healthy and diseased state, the expression of that gene is likely changing as well, potentially contributing to AD pathology.

CORRELATION OF METHYLATION ASSAY AND BISULFITE SEQUENCING DATA TO DETERMINE THE EPIGENETIC REGULATION OF MIR-17 IN AN ALZHEIMER'S DISEASE MODEL. John D. Arza, Nathan J. MacGilvary & Gary D. Isaacs, Liberty University. Previous research done by our team indicated that there are epigenetic modifications on genes coding for miRNAs in an Alzheimer's disease (AD) model. miRNAs are involved in the post-transcriptional regulation of genes and may play a role in the pathology of AD. miR17-1 showed epigenetic significance from a methylation sensitive microarray analysis. However, the base pair resolution of these epigenetic changes has not been achieved. This study incorporates bisulfite sequencing to analyze the promoter region of miR17-1 for site-specific CpG methylation to further the understanding of AD pathology. In addition, this method makes up for the weaknesses of the methylation microarray in order to give a more holistic picture of the genomic epigenetic changes in AD.

GENERAL METHOD FOR VALIDATION OF PRIMER PAIRS FOR MEASURING INFLAMMATORY GENE EXPRESSION IN NAFLD SUBJECTS BY REAL-TIME Q-PCR. Anna Zhang[1], Edgar Rodriquez[2], Ancha Baranova[2,3], & Aybike Birerdinc[2,3], [1]Chemistry (Biochemistry) Department, College of Science, George Mason University, Fairfax, VA, [2]Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA and [3]Center for the Study of Chronic Metabolic Diseases, School of Systems Biology, George Mason University, Fairfax, VA. Real-time polymerase chain reaction (q-PCR) is a popular tool used in a variety of settings to amplify, detect and quantify specific nucleotide sequences, or genes. In order to achieve reliable and reproducible results from q-PCR, it is important to take initial steps to ensure specificity of primer pairs used to initiate the amplification of nucleotide sequences. Primer validation utilizes qPCR and gel electrophoresis to ensure primer specificity. Primer pairs were synthesized using Primer-BLAST tool provided by

NCBI, and validated for the following target genes: *CTGF* (connective tissue growth factor), *IL13* (interleukin-13), *IL17* (interleukin-17), *CSF2* (colony stimulating factor-2) and *CSF3* (colony stimulating factor 3). All targeted genes when elevated, with the exception of *IL13*, are noted to correlate with markers of inflammation and have strong association with the advanced progression of non-alcoholic fatty liver disease (NAFLD) based on previous studies. Prior to designing specific primers, a literature search was done for genes with more than one transcript variant in order to determine the most common transcript variants discovered in visceral adipose tissue. For one such gene, *CSF3*, transcript variant 1 was determined to be the common variant found in adipose tissue. This project aims to document the materials and methods used for primer validation.

THE EXPRESSION OF A DOUBLE GREEN FLUORESCENT PROTEIN-NUCLEAR LOCALIZATION SIGNAL FUSION PROTEIN IN TRANSFECTED HUMAN EMBRYONIC KIDNEY CELLS. Alexander Bond, Kristina Krumpos, Deborah Zies & Stephen Gallik, University of Mary Washington. Green Fluorescent Protein (GFP) is a preferred reporter protein for cellular localization studies, but for nuclear localization studies, a fusion protein containing multiple copies of GFP must be used to eliminate simple diffusion as a mechanism through which the protein can enter the nucleus. Our long-term goal is to create a plasmid expression vector containing four copies of the GFP gene linked to a single monopartite nuclear localization signal (4GFP-1NLS). The specific objective of the research study reported here is to create an intermediate plasmid, containing two copies of the GFP gene, from which the 4GFP-1NLS plasmid could be eventually produced. Using site-directed mutagenesis techniques, the parent plasmid used in this study, the pCMV/myc/nuc/GFP pShooter plasmid (Life Technologies, Inc.), was modified to create 2 intermediate plasmids, one serving as a vector and the other serving as a source for a single copy GFP insert. The restriction enzyme *PstI* was then used to open the vector and isolate the insert. Attempts at ligating the insert into the vector have been unsuccessful. It is hoped that future attempts at the ligation will be successful, leading to the eventual production of the final 4GFP-1NLS plasmid. Once produced, the final plasmid could be used to study various aspects of NLS-dependent nuclear import.

SUPPRESSION OF PYRUVATE KINASE DELETION PHENOTYPES IN *CRYPTOCOCCUS NEOFORMANS*. Nicolas Terreri[1], Joshua Sellwood[1], John R. Perfect[2], and Michael S. Price[1,2], [1]Dept. of Biology and Chemistry, Liberty University, Lynchburg, VA, [2]Div. of Infectious Diseases, Duke University Medical Center, Durham, NC. *Cryptococcus neoformans* is a fungal pathogen that affects immunocompromised individuals such as AIDS patients. With the high prevalence of HIV in Africa, *Cryptococcus neoformans* and relative *Cryptococcus gattii* cause over 600,000 deaths every year just in Africa alone. Unlike many fungal pathogens, *Cryptococcus* migrates from the lungs, usually after causing pneumonia, to the brain into the cerebrospinal fluid (CSF). Once in the brain, it causes fungal meningitis and encephalitis which are life threatening diseases [3]. Carbon utilization is an important

part of the persistence of *Cryptococcus neoformans* because glucose utilization is required for CNS disease, and deletion of pyruvate kinase (*PYK1*) inhibits glucose utilization and CNS disease. *pyk1?* deletion mutants have been identified that are able to grow on glucose. We are identifying the gene(s) responsible for this phenotype rescue, which appears to be correlated with increased hyphal growth likely due to activation of the MAT locus located on Chromosome 5.

ESTABLISHMENT OF A CELL CULTURE SYSTEM FOR MEASURING THE EXPRESSION OF THE LONG NON-CODING RNA GAS5. J. Shahid[1], K. Solocinski[2], M. L. Gumz[2] & D. Zies[1], [1]Department of Biological Sciences, University of Mary Washington and [2]Department of Medicine, University of Florida College of Medicine. Aldosterone, a primary regulator of blood pressure in mammals, functions by complexing with the mineralocorticoid receptor and altering the expression of blood pressure associated genes. It is my hypothesis that Gas5, a newly discovered long non-coding RNA, is also a negative regulator of the aldosterone response. The overall goal of this project is to test this hypothesis by treating mouse IMCD3 cells with aldosterone and measuring changes in Gas5 mRNA expression by real-time PCR. Through my research experience at the University of Florida (UF) in summer 2014, I was able to learn about long noncoding RNAs, aldosterone, transporter proteins, cell signaling pathways in the kidneys and the molecular techniques used to study them. I gained hands-on experience managing and treating cell cultures in order to perform RNA isolations, RT-PCR, and semi-quantitative and real-time PCR and also western blots. My specific goal here, at UMW, has been to establish the use of these skills and extend the work started at UF. Here, I show results from successful RNA isolation from aldosterone treated IMCD3 cells and preliminary real-time PCR experiments. The establishment of these protocols will help future students investigate the link between Gas5 and Aldosterone. If Gas5 is involved in regulating the aldosterone response then understanding more about its expression may reveal its effects in blood pressure regulation and facilitate the treatment of cardiovascular disease.

TXL INDUCED APOPTOSIS IN JURKAT E6-1 CELLS. Claire R. Harrington & Rosemary Barra, Depart. of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401. The herbal dietary supplement, Tian Xian Liquid (TXL), has been reported to be an effective treatment for some forms of cancer. The mechanism of this effect is unknown, however, studies using a related herbal extract, Tien-Hsien liquid, showed an increase in apoptosis in cancer cells. The activation of p53 is a major regulator of cellular apoptosis. In this study, the anti-proliferative effects of TXL were determined by utilizing a MTT Assay. An ELISA assay was subsequently used to quantify p53 levels in TXL-treated cells. TXL, at concentrations ranging from .002 to .2 mg/mL, showed significant anti-proliferative effects in a dose-dependent manner on transformed cells. As the concentration of TXL increased, the number of viable cells decreased. At .2 mg/mL, the viability expressed as a percent of control values was 54.5%. There was an increased level of p53 activity in the TXL-treated cells. Interestingly, the cells treated with .002 mg/mL of TXL showed the greatest

absorbance, indicating the greatest activity of p53 in those cells, compared to the control and the higher concentrations of TXL. This may be due to the fact that there are a greater number of viable cells at lower concentrations of TXL, allowing the p53 levels to be observed more readily. This is preliminary data and more research is needed to confirm the role of p53 in the anti-proliferative effects of TXL.

INVESTIGATING PHENOTYPIC DIFFERENCES BETWEEN STP1 K/O AND WT *ARABIDOPSIS THALIANA* IN RESPONSE TO VARIOUS SALT CONCENTRATIONS. Katie McCullar, Dustin Phillips, Maria Wilkins & Janet Daniel, Department of Biology, James Madison University, Harrisonburg, VA 22807. *Arabidopsis thaliana* is a commonly used model organism in the study of plants and is described as a glycophytic plant. In this experiment, the role of sugar transport protein-1 (STP1) in relation to salt resistance is investigated by observing phenotypic differences in the growth of wild type (WT) and STP1 k/o plants when exposed to varying concentrations of sodium chloride using hydroponics and agar plates. The hydroponics system is used to expose the plants to 0mM or 50mM concentrations and measurements are taken of root length, stalk length and number of leaves at four weeks. The average root lengths of STP1 k/o were longer than WT at 50mM after four weeks when grown using hydroponics. We also investigated a dose response using agar plate concentrations from 0-200mM. Root length was not correlated with NaCl concentration. While increased average root lengths were seen at 25mM and 100mM NaCl, at concentrations greater than 100mM, both WT, and STP1 k/o decreased in average root lengths. Overall, STP1 k/o average root lengths grown on plates were longer than WT root lengths, at two and four weeks. In total, results suggest that the STP1 k/o plant exhibits an alternate phenotype when grown in higher NaCl concentrations. This may indicate that STP1 k/o phenotype includes a resistance to increased salt concentrations.

DETERMINATION OF THE IMPACT OF *PYK1* DELETION ON INTERACTIONS OF *C. NEOFORMANS* WITH THE HOST IMMUNE SYSTEM. Elizabeth Rasmussen[1], Yansirre Aviles[1], Michal Olszewski[2], and Michael Price[1], [1]Dept. of Biology & Chemistry, Liberty University, Lynchburg. VA, [2]Div. of Pulmonary and Critical Care Medicine, University of Michigan, Ann Arbor, MI. *Cryptococcus neoformans* is an important fungal pathogen of immunocompromised individuals. During initial infection, *C. neoformans* colonizes the airspaces of the lungs, resulting in pneumonia, and subsequently migrates to the central nervous system (CNS). There is also epidemiological evidence for dormancy of cryptococcal infections. To greater understand fungal carbon utilization (particularly gluconeogenesis and glycolysis) during colonization of these fundamentally different niches within the host, mutants were created at key points in these carbon metabolic pathways. Our objective is to develop a model that will allow for the effective studying of dormancy in this important human pathogen by quantifying cytokine expression in macrophages exposed to these pyruvate kinase mutants that fail to elicit inflammation in the lung.

OPTIMIZING METAGENOMICS ANALYSIS. Vhuthuhawe T. Madzinge & Andrew J. Fabich, Dept. of Biology and Chemisty, Liberty Univ., Lynchburg, VA 24515. *Escherichia coli* is a micro-organism that is common in the gastrointestinal tract. It is found in normal microbiota. While most *E. coli* are harmless, some are equipped with toxins that are harmful to humans. The focus of this study in this experiment uses non-pathogenic *E.coli* studied in streptomycin-treated mice. *E.coli* was used as a model to observe the changes that are likely to occur in the GI when it is introduced in streptomycin-treated mice. *E.coli* was chosen because previous studies have shown it to become an efficient colonizer. Such advantageous colonization is achieved by its ability to compete for nutrients efficiently and maintain a growth rate of 2-hours per turnover of the intestinal contents. These advantageous abilities are influenced by the deletion of *flhDC* operon, also known as the master regulator of flagella synthesis. Its deletion saves energy and thus causes an up-regulation/hyper-expression of carbon and energy metabolizing genes therefore increasing the population of *E.coli*. However, how do these changes affect the community of the normal microbiota? Is this advantageous or disadvantageous? Does this improve or decrease the health of the streptomycin-treated mice? First, knowing what the microbial community that interact with *E.coli* are made of will assist in answering some of these questions. Streptomycin-treated mice colonized with different *E. coli* demonstrated similar metagenomic signatures with notable differences in the Bifidobacteria. Studying these interactions will help us understand the value of bacterial interaction in mammals.

INDUCING *CITROBACTER RODENTIUM* VIRULENCE IN STREPTOMYCIN-TREATED MICE. Kaitlyn A. Shondelmyer and Andrew J. Fabich, Dept. of Biology and Chemisty, Liberty Univ., Lynchburg, VA 24515. *C. rodentium* causes disease in conventional mice; however, it does not in streptomycin-treated mice. We know, therefore, that colonization is not sufficient for pathogenesis, so there must be other facultative anaerobes driving pathogenesis. Competition with certain *E. coli* strains causes pathogenesis by an unknown mechanism. If the competition-induced disease state is similar to disease caused by *C. rodentium* in conventional mice, inducing pathogenesis via competition will be relevant to EHEC modeling. Streptomycin-treated mice colonized with *C. rodentium* and laboratory strains of *E. coli* did not induce pathogenesis. However, pathogenesis returned with *C. rodentium* colonized with either human commensal or probiotic *E. coli*.

TAU AND BETA-AMYLOID INTERACTIONS IN ALZHEIMER'S DISEASE. Tyrrell C. Graham & Deborah A. O'Dell, Dept. Biological Sciences, UMW, Fredericksburg, VA 22401. In Alzheimer's Disease, changes in the phosphorylation of Tau protein and the presence of b-amyloid plaques are seen. The stimulus for changing Tau phosphorylation is unknown. The effect of the presence on beta-amyloid aggregates on the phosphorylation state of Tau was examined by exposing cultured neurons to beta-amyloid aggregates and using immunohistochemistry. Microscopic examination of cells showed that cultured cells exposed to aggregates had increased levels of pThr231 Tau protein compared to control cells. This suggests that the

presence of beta-amyloid plaques stimulates the changes in the phosphorylation of Tau. This work was supported by an Undergraduate Research Grant from UMW.

RHO-GDP DISSOCIATION INHIBITOR AFFECTS GROWTH AND AFLATOXIN PRODUCTION IN *ASPERGILLUS FLAVUS*. Stephen Matherlee[1], Claudia Bernaschina[1], Gregory OBrian[2], Gary A. Payne[2], and Michael S. Price[1, 2], [1]Dept. of Biology & Chemistry, Liberty University, Lynchburg. VA, [2]Dept. of Plant Pathology, NC State University, Raleigh, NC. Regulation of aflatoxin (AF) production is complex, involving transcriptional and post-transcriptional regulation focused mainly through the pathway specific transcriptional regulator *aflR*. An investigation into the nature of the transcriptional regulation of AF production by comparing conducive and non-conducive culture conditions revealed a clade of genes with a similar transcription profile to that of *aflR*. One of these genes, a putative Rho-GDP dissociation inhibitor, was characterized by gene deletion and shown to regulate AF production in *Aspergillus flavus*. The protein encoded by this gene, Afrdi1, showed 45% identity to Rdi1p in *S. cerevisiae*. The Δ*Afrdi1* mutant exhibits a severe growth defect on minimal medium, a moderate growth defect on complete medium, and a temperature sensitive phenotype. Moreover, the Δ*Afrdi1* mutant produces 97.3% less toxin than wild type. Inferences from *S. cerevisiae* reveal a possible link between AfRdi1 and RasA, which has been shown to regulate sterigmatocystin production in *A. nidulans*.

Biomedical and General Engineering

FEMTOSECOND LASER USE IN MEDICAL THERAPEUTICS: KEY ADVANTAGES AND LIMITATIONS OF NONLINEAR OPTICAL TISSUE INTERACTIONS. William R. Calhoun III[1,2] & Ilko K. Ilev[2], [1]Virginia Commonwealth University, Richmond VA 23298 and [2]Department of Biomedical Physics, Office of Science and Engineering Labs, U.S. Food and Drug Administration, Silver Spring MD 20993. Some of the most commonly performed surgical operations in the world, including laser-assisted in-situ keratomileusis (LASIK), lens replacement (e.g. cataract surgery), and keratoplasty (cornea transplant), now employ Femtosecond Lasers (FSLs) for their extreme precision, low energy and ablation characteristics. The application of FSLs in medical therapeutics is a recent development, and although they offer many benefits, FSLs also stimulate nonlinear optical effects (NOEs), many of which were insignificant with previously developed lasers. In order to improve the understanding of FSL-tissue interactions related to NOEs stimulated during laser beam propagation though corneal tissue, research investigations were conducted to determine how corneal tissue properties including corneal layer, collagen orientation and collagen crosslinking, and laser parameters including pulse energy, repetition rate and numerical aperture affect second and third-harmonic generation (HG) intensity, duration and efficiency. The results of these studies revealed that all laser parameters and tissue properties had a substantial influence on HG. The dynamic relationship between optical breakdown and HG was responsible for many observed changes in HG metrics. The results also