

Our results indicate that increasing temperatures will be devastating for the melanic mosquitofish population, resulting in extinction within 1000 years. Despite these findings, our simulations also indicate that if the temperature stabilizes before the melanic mosquitofish becomes extinct the melanic population will also stabilize near the frequencies present at the time the temperature stabilizes. (Supported by Old Dominion University Spring 2013 Undergraduate Research Grant.)

Biology with Microbiology and Molecular Biology

GENOME MUTATION MAPPER: NEXT-GEN BIOINFORMATICS APPLICATIONS FOR VIRAL PATHOGENS DETECTION ESPECIALLY HUMAN ADENOVIRUSES. Amirhossein Shamsaddini, Donald Seto, & Ancha Baranova, School of Systems Biology, College of Science, George Mason University. To date, applying high-resolution genomics and bioinformatics approaches has yielded in-depth and better views of the natural variation of Viral Pathogens. Emerging next-generation sequencing technologies have revolutionized the collection of genomic data for applications in bioforensics, biosurveillance, and for use in clinical settings. However, to make the most of these new data, new methodology needs to be developed that can accommodate large volumes of genetic data in a computationally efficient manner. We present Two Different Applications of Computational Framework to analyze Sequences for rapid species identification and Evolution Patterns Analysis. Software for our approaches is available at <http://ssb.gmu.edu/gmm>.

TRACKING BROWN ADIPOSE TISSUE IN VISCERAL FAT TISSUE OF PATIENTS WITH OBESITY. E. Dadkhah², R. Mehta^{1,2}, K. Doyle^{1,2}, A. Baranova,^{1,2} A. Birerdinc^{1,2} & Z. Younossi^{1,2,3}, Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA, ²Center for the Study of Chronic Metabolic Diseases, School of Systems Biology, George Mason University, Fairfax, VA, & ³Center for Liver Diseases, Inova Fairfax Hospital. The Prevalence of obesity is going to increase and is estimated to reach 42% of the population by 2030. Several factors are related to obesity. One of the attractive subjects of researches which is even trying to use as a therapy for obesity these days is brown adipose tissue (BAT). The imbalance between BAT and white adipose tissue (WAT) can induce obesity. BAT promotes energy burning that avoids obesity. In this study, the proportion of BAT to WAT DNA was studied in visceral adipose tissue to find out any relation between the amount of BAT and BMI. Ninety eight fresh fat tissues were collected from obese patients at Inova Fairfax Hospital after collecting consent forms. PCR was performed with 15ng of extracted DNA. As BAT differs from WAT by the large amount of mitochondria, the ratio of mitochondrial DNA to genomic DNA considered as the ratio of BAT to WAT in the fat tissue. Two Cyclin B1 primers (CCNB1a & CCNB1C) and two mitochondrial primers (M12 & M13) were selected as genomic and mitochondrial DNA indicators respectively. C(t) values were normalized using CCNBb (IPC) for genomic and M14 for mitochondrial DNA. Technique optimization efforts are ongoing.

IN VIVO FLORESCENT IMAGING OF HOLLOW POLYMERIC NANOCAPSULES LOADED WITH ALEXAFLOUR 750. Venkata Suresh Patthipati¹, Christopher Osgood¹, James Swanson¹, Ramjee Balasubramanian², Kalpana Mahadevan², & Sangbum Han², ¹Department of Biological Sciences, ODU, Norfolk, VA, ²Department of Chemistry and Biochemistry, ODU, Norfolk, VA. Nanoparticle based imaging strategies hold significant promise in addressing current diagnostic and therapeutic challenges. However, previous nanomaterials failed to clear effectively from the circulation via the renal route. This work demonstrates the use of highly fluorescent, water-soluble, resorcinarene nanocapsules (122 nm) and their effectiveness in biomedical imaging. These hollow polymeric nanocapsules loaded with Alexa Fluor 750 fluoresce brightly in the urinary bladder of mice under the *in vivo* fluorescent imager indicating that their major route of clearance is through the kidney. TEM analysis of the urine recovered from the mice showed the presence of intact nanocapsules. Such florescent nanocapsules can be very effective in visualizing the parts of the urinary tract. They are particularly useful in abdomino-pelvic surgeries ranging from caesarian delivery to laparoscopy. The use of these nanocapsules are not restricted to the imaging as they could be loaded with a host of molecules making them efficient drug delivery vehicles in treating cancer and other pathologies.

THE DIFFERENTIAL ROLE OF CBP UBIQUITIN LIGASE ACTIVITIES IN p53 REGULATION. Oluwatoyin E. Akande & Steven R. Grossman, Dept. of Microbiology & Immunology, Virginia Commonwealth Univ., Richmond, VA 23298. The acetyltransferase CREB-Binding Protein (CBP) is a known transcriptional co-activator involved in p53 regulation and has been shown to encode cytoplasmic, but not nuclear, E3 autoubiquitination and p53-directed E4 ubiquitin ligase activities. In this work, we sought to determine the regulation of differential ubiquitin ligase activities between nuclear and cytoplasmic CBP. Understanding how CBP regulates p53 ubiquitination and stability may lead to potential therapeutics that can modulate p53 stability in cancer cells. We show that a nuclear interacting factor represses cytoplasmic CBP E3 (autoubiquitination) ligase activity in unstressed U2OS cells. We incubated immunoprecipitated cytoplasmic CBP from U2OS cells with either nuclear lysates or CBP- immunodepleted nuclear fraction. Our results showed reduced CBP E3 autoubiquitination in the mixtures when compared to purified cytoplasmic CBP alone. We further showed that DNA damage by doxorubicin (dox) in U2OS cells activated E3 ligase activity in the nuclear fraction. These observations may provide a possible explanation for the inactive nuclear E3 ubiquitin ligase activity but active cytoplasmic E3 and p53-directed E4 ubiquitin ligase activities of CBP in unstressed cells. The dox dependent activation of nuclear CBP E3 autoubiquitination may result from dox dependent inactivation or re-localization of the nuclear inhibitor to other cellular compartments. Further work will identify the nuclear interacting factor and in parallel, determine if the differential ubiquitin ligase activity observed between cytoplasmic and nuclear CBP is otherwise dependent on post-translational modifications of CBP itself.

ENRICHMENT OF CAPTIVE SQUIRREL MONKEYS. LaCheryl A. Ball & Eric L. Walters, Department of Biology, Old Dominion University, Norfolk, VA 23529. Food enrichment is a technique used by the zoo industry to promote overall wellness of animals in captivity. I measured responses of captive, *Saimiri sciureus* squirrel

monkeys to food enrichment at the Virginia Zoo (Norfolk, VA). The research involved determining pre-treatment activity levels in order to test the effect of food enrichment on post-treatment activity levels. I hypothesized that foraging and active behaviors would increase as follows: baseline < post-enrichment < treatment. The experiment was divided into three phases: the first of which provided baseline data on the population's behaviors and activity levels prior to enrichment. The second phase involved the introduction of enrichment feeders on alternating treatment and control days. The third phase involved gathering post-treatment behavioral data, which determined if there were any protracted effects of food enrichment on behavior after the feeders were removed. Ultimately, introduction of food enrichment resulted in a 21% increase in foraging behaviors of both the adult male and juvenile males and a 16% increase in the adults females. In conclusion, food enrichment was a successful method of promoting foraging behaviors and increasing activity levels in captive squirrel monkeys and has important implications for increased health and well-being of captive primates.

EPIGENETICS AND ALZHEIMER'S DISEASE: DISTINCT PATTERN SHIFTS IN DNA METHYLATION. Noor M. Taher, Courtney A. McKenzie, Rebecca C. Garrett, Matthew S. Baker & Gary D. Isaacs, Department of Biology and Chemistry, Liberty University, Lynchburg VA 24502. Amyloid beta (A β) plaques are one hallmark of Alzheimer's disease. Despite ongoing research, there remains some ambiguity surrounding the role of A β in the pathogenesis of this neurodegenerative disease. Even more obscure, however, are the epigenetic changes these plaques cause to neurons. To that end, we wanted to shed more light on the changes in DNA methylation neurons incur when treated with A β in vitro. In order to accomplish this, we isolated DNA from A β -treated and control neurons and differentially digested the two samples with either a methylation-sensitive or a methylation-insensitive restriction endonuclease. Amplified fragments were then co-hybridized to a commercial promoter microarray. Data analysis revealed a subset of genomic loci that shows a significant change in DNA methylation following A β treatment. After mapping these loci to nearby genes, we discovered high enrichment for cell-fate genes that control apoptosis and neuronal differentiation. Finally, we incorporated these genes in a possible model suggesting the means by which A β contributes to the brain shrinkage and memory loss seen in Alzheimer's disease.

CHARACTERIZATION OF *CITROBACTER RODENTIUM* TRANSMISSIBLE COLONIC HYPERPLASIA AND IMMUNE RESPONSES IN STREPTOMYCIN-TREATED AND CONVENTIONAL MOUSE MODELS. M. W. Canfarotta¹, M. H. VanTil¹, D. A. DeWitt¹, T. A. Snider² & A. J. Fabich¹, ¹Department of Biology and Chemistry, Liberty University, Lynchburg VA 24502 & ²Center for Veterinary Health Sciences, Oklahoma State University. *Citrobacter rodentium* is an enteric murine pathogen similar to the human pathogen enterohemorrhagic *Escherichia coli* (EHEC) as it forms an attaching and effacing (A/E) lesion during gastrointestinal infection. Previous studies have utilized *C. rodentium* as a mouse model for EHEC disease in humans, however there is currently no comprehensive study characterizing the course of infection in specific mouse models with or without streptomycin treatment. The use of streptomycin effectively depletes the facultative anaerobic niche allowing for the direct study of *C. rodentium* colonization, whereas a model lacking antibiotic treatment

studies pathogenesis since *C. rodentium* must overcome colonization resistance. In this study, *in vivo* colonization assays of *C. rodentium* were performed in CD-1, BALB/c, C57Bl/6, and C3H/Hen mice with and without streptomycin treatment and colon health was monitored histologically and quantitatively by measuring empty colon weight to identify edema and hyperplasia. Male CD-1 mice with streptomycin treatment were colonized by *C. rodentium* at stable levels without severe colonic inflammation, whereas male C3H/Hen mice without antibiotic treatment displayed the most significant colonic hyperplasia, suggesting that these models are best utilized to study colonization and pathogenesis respectively. This study was funded in part by The Virginia Academy of Science.

SYNERGISTIC AND ANTAGONISTIC EFFECTS OF PESTICIDE MIXTURES TO BEES. J. R. Williams, R. D. Fell & T. D. Anderson, Dept. of Entomology, Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. Pollinators are a critical component to plant health and production of agricultural landscapes. Pesticide exposures to honey bees, *Apis mellifera* L., are implicated in the decline of these pollinators and their ecosystem services. Knowledge of the toxicological consequences of these pesticide exposures, alone and in combination, to bees and their decline is limited. In the present study, we will show the: 1) acute toxicities of in-hive pesticides to bees; 2) binary interactions of in-hive pesticides to bees; and 3) metabolic activities of bees towards in-hive pesticides. Select pesticides (*tau*-fluvalinate, coumaphos, coumaphos-oxon) are acutely toxic to bees at environmentally relevant levels of parts per billion (ppb), an order of magnitude lower than those concentrations reported in bee colonies. The combination of *tau*-fluvalinate and antibiotics increases bee mortality *ca.* 50%; however, a combination coumaphos-oxon and antibiotics reduces bee mortality *ca.* 20% relative to the miticide-only treatments. In contrast, the combination of *tau*-fluvalinate and chlorothalonil reduces bee mortality *ca.* 27%; whereas, a combination of coumaphos-oxon and chlorothalonil increases bee mortality *ca.* 30% relative to the miticide-only treatments. The exposure of bees to antibiotics or chlorothalonil reduces cytochrome P450 monooxygenase activity *ca.* 50%; whereas, esterase and glutathione *S*-transferase activities increase *ca.* 26% relative to the untreated bees. This information will be utilized for the development of ecologically- and chemically-based strategies to improve pollinator health and reduce pesticides exposures to bee colonies in southwest Virginia.

EVIDENCE OF P-GLYCOPROTEIN MODIFICATION OF INSECTICIDE TOXICITY IN VECTOR MOSQUITOES. Ngoc N. Pham & Troy D. Anderson, Department of Entomology, Virginia Tech, Blacksburg, VA 24061. Mosquitoes affect human health worldwide as a result of their ability to vector multiple diseases. Widespread resistance is a serious public health challenge that limits the use of high efficacy insecticides to reduce the risk of mosquito-vectored diseases. P-glycoprotein (P-gp) is an efflux transporter that assists in maintaining the blood-brain barrier interface of insects and may serve as a first line of defense to insecticide exposures. Previous studies have demonstrated the blood brain-barrier of mosquitoes to interfere with target-site action of established and experimental insecticides; however, the interaction of P-gp toward these chemistries is unclear. In this study, we provide a: 1) toxicological analysis of tacrine-based anticholinesterases for mosquitoes, alone and

in combination, with the P-gp inhibitor verapamil and 2) biochemical analysis of acetylcholinesterase for mosquitoes exposed to these compounds.

E3-MEDIATED DOWN-REGULATION OF THE CIRCADIAN FACTOR PERIOD 2. Jing-Jing Liu, Tetsuya Gotoh, Marian Vila-Caballer, Carlo S. Santos, Jianhua Yang, & Carla V. Finkielstein, Department of Biological Sciences, Virginia Tech, Blacksburg, VA. The circadian rhythm and cell cycle are the two main oscillatory systems in cells. How cells sense time and decide what is the best time for growing, proliferating or apoptosis? One possibility is that there are crosstalks between these two systems. Based on the fact that Period 2 (Per2) also plays essential role in DNA damage response, Per2 is supposed to connect circadian rhythm and cell cycle, which makes Per2 work as a tumor suppressor. We found Per2 regulating p53 pathway but little is known about how Per2 itself is regulated. One interesting finding is that independent of transcriptional regulation, overexpressed Per2 protein also oscillates; this implies posttranslational modifications are essential for sustaining Per2 protein oscillation. Per2 binds to Mdm2, a well-known E3 ubiquitin ligase, both *in vitro* and *in vivo*. Mdm2 induces Per2 ubiquitination *in vitro*, but further experiments are needed to verify Mdm2 is an E3 ligase for Per2 *in vivo*.

CIRCADIAN MODULATION OF CELL CYCLE PROGRESSION. Samuel Schiffhauer, Marian Vila-Caballer, Tetsuya Gotoh, Jianhua Yang & Carla V. Finkielstein, Department of Biology, Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061. Individuals who have prolonged disrupted circadian rhythms, such as shift workers and flight attendants, display higher rates of cancer development. Yet the impact of the body's circadian rhythm on critical cellular processes such as cell division is complex and poorly understood. We aim to develop an overall representation of the influence circadian proteins have on normal cell cycle progression. To achieve this, cytosolic levels of cell cycle proteins were monitored over a 48 hour period in a human cancer cell line (HCT116), then compared to an identical experiment in which circadian regulator protein Period 2 was overexpressed. Shifts in both amplitude and timing were observed in Cyclin E, Cyclin B, phospho-Cdc2, Wee1, and p21, supporting our hypothesis that circadian proteins are capable of direct control over cell cycle progression in unstressed cells. Furthermore, we showed critical circadian oscillator proteins CLOCK, BMAL1, Per2 and Cry1 have direct transcriptional activation activity on the promoter regulatory elements of the kinase inhibitor p21, a protein with well established roles in cellular senescence, polyploidy, and differentiation. The consequences of this activation in a cancer cell model will be the focus of future experiments, to better understand the molecular underpinnings of cancerous phenotypes.

PRIMER DESIGN AND VALIDATION FOR REFERENCE GENES AND ALTERNATIVELY SPLICED MRNAS ENCODING FOR RAT CGRP AND CALCITONIN. Caitlin Koob^{1,2,3}, Katie Doyle^{1,2}, Rohini Mehta^{1,2}, Aybike Bireddinc^{1,2}, Margaret Slavin⁴ & Ancha Baranova^{1,2}, ¹Center for the Study of Chronic Metabolic Diseases, School of Systems Biology, College of Science, George Mason University, Fairfax, VA. ²Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, ³Chemistry Department, College of Science, George Mason

University, Fairfax, VA, & ⁴Department of Nutrition and Food Studies, College of Health and Human Services George Mason University, Fairfax, VA. Peripheral CGRP release is a marker for neurogenic inflammation and a contributor to migraine headache. Orthologous *Cgrp* and calcitonin encoding mRNAs expressed in rat thyroid parafollicular CA77 cells may provide a model for the screening of migraine-provoking food compounds. Designing primers for alternatively spliced *Cgrp* variants and normalizing a set of reference genes specific to this cell line is necessary to measure expression of *Cgrp* by qPCR. In this study, all reference mRNAs were successfully validated by qPCR using Rat Universal and rat thyroid parafollicular CA77 cDNA as templates and yielded predicted product size. The *Cgrp* primers did not show amplified product in Rat Universal, this may be because it does not contain the mRNA sequence for *Cgrp* that is known for its expression in only certain types of cells. *Cgrp* variants did show amplification in rat thyroid parafollicular CA77 cDNA, but optimization of primer annealing temperature and concentration is necessary. Future work will include normalizing validated reference mRNAs and selecting two genes that will serve as internal controls when measuring *Cgrp* expression in migraine models by qPCR.

THE KCTD FAMILY OF GENES AS POSSIBLE REGULATORS OF THE ADIPOSE TISSUE FUNCTION IN MORBID OBESITY. Mariam Hashemi^{1,2,3}, Ancha Baranova^{1,2} & Aybike Biredinc^{1,2}.¹Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA, ²Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, & ³Biology Department, College of Science, George Mason University, Fairfax, VA. KCTD is a family of tetramerization (T1) domain proteins that are similar to T1 domains of the voltage-gated channels. It has been predicted that KCTD may interfere with the assembly of the K⁺ channels by binding to their own T1 domain. A previous study has demonstrated KCNRG, a KCTD protein member, causes voltage-gated suppression. Visceral adipose tissue (VAT) samples were used in this study due to its endocrine-signaling characteristics. The purpose of this study is to determine if there are any genes in the KCTD protein family that are involved in diet-induced obesity. A literature research was conducted using PubMed to obtain any data on association between KCTD proteins and obesity or liver function. Primers were designed for the following KCTD proteins: KCTD9, KCTD12, and KCTD15. Using the VAT samples RNA extraction was conducted and converted to cDNA and stored for further use of qPCR and gene expression.

A STUDY OF FROZEN WHOLE TISSUE PRESERVATION FOR CELL SURVIVABILITY. Scarlett Koga^{1,2,3}, Ancha Baranova^{1,2} & Aybike Biredinc^{1,2},¹Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA, ²Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, & ³Biology Department, College of Science, George Mason University, Fairfax, VA. Visceral fat (visceral obesity) is a main contributor to the development of metabolic syndrome. Metabolic syndrome is a variety of medical disorders that may increase an individual's chance of developing cardiovascular disease alongside with other health complications such as diabetes or stroke. One of the major limitations in the study of human adipose sample is a variation of the tissue preservation techniques which may cause various degrees of RNA degradation, thus

limiting the value of downstream gene expression studies. For the purpose of this study, a literature survey of various methods for whole tissue preservation was conducted to find techniques to maximize the potential shelf-life of human adipose tissue to validate preservation techniques, we will use pre-existing adipose tissue samples that differ in the time of their storage at -80°C. An online survey with PubMed and Google was completed to find methods of preservation offered commercially. Hypothermic and Cryogenic preservations are common methods to preserve human adipose tissue. These preservation methods will be used to store samples and PCRs will be run to determine the degree of RNA degradation between the two techniques.

Posters

EFFECTS OF BRAIN DERIVED NEUROTROPHIC FACTOR ON Y79 RETINOBLASTOMA CELLS. Andrew N. Hogan & Rosemary Barra, Dept. of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22405. Retinoblastoma is a rare form of cancer that affects the retina of the eye, occurring in approximately 1 in 15,000 live births, and it is also the most common type of inherited malignancy. Although current treatments including chemotherapy and surgical procedures have been successful, there is still a high rate of reoccurrence in patients. Recent research on neural cancers has focused on the neurotrophin receptors and their corresponding ligands. This study was designed to evaluate the role of the p75 neurotrophin receptor (p75NTR) in retinoblastoma cells. The receptor and its ligand are known to play a role in the regulation of programmed cell death in neural tissues. Brain derived neurotrophin factor (BDNF) is a neural ligand typically associated with neurotrophin receptors. Retinoblastomas have been found to express low levels of p75NTR, leading to an increase in cell growth. The primary cell line used in this study was the Y79 human retinoblastoma cell line. The cells were cultured in RPMI media with 10% fetal bovine serum, and 1% penicillin-streptomycin. Initial immunocytochemical studies using an anti-p75 NGF receptor antibody (ab8874) demonstrated the presence of the p75NTR on the retinoblastoma cells. The Y79 cells were incubated for 24 hours at 37°C with various concentrations of BDNF (0.02ng/mL, 0.2ng/mL, and 2.0ng/mL) and a MTT viability assay was performed. Contrary to current literature, the MTT assay showed a decrease in overall cell viability at all concentrations tested. Treatment with 2.0 ng/ml BDNF decreased cell viability by 60% compared to the control cultures. A human 3 – caspase ELISA assay was conducted to determine whether the decrease in viability was caused by caspase 3 mediated apoptosis. Preliminary results indicate that BDNF treatment does affect caspase 3 activity. Cells incubated with 4 ng/ml of BDNF for 24 hrs showed a 30% decrease in caspase-3 activity.

INSIGHTS INTO THE COMPLEX FORAGING ECOLOGY OF THE APPALACHIAN COYOTE (*CANIS LATRANS*) USING STABLE ISOTOPE ANALYSES. P. A. P. deHart, C. B. Shutt, & R. Scruggs, Department of Biology, Virginia Military Institute, Lexington, VA 24450. The coyote (*Canis latrans*) has increased in population throughout the east coast of North America, especially in the recent decade. As a consequence of this range expansion, human-coyote interactions have increased, directly through livestock predation and indirectly through resource

competition with local hunters (primarily deer). Effective management of these interactions has been hampered by a significant lack of knowledge of the basic foraging ecology of coyotes. To investigate the specific trophic role of the coyote in this ecosystem, we examined the carbon and nitrogen isotopic signature from the hairs of wild coyotes and potential prey throughout Appalachia in multiple habitats. We found that coyotes varied widely in both $\delta^{15}\text{N}$ (5.9-10.6‰) and $\delta^{13}\text{C}$ (-25.5-21.1‰), highlighting foraging on multiple trophic levels and likely dependent upon regional differences in prey availability. Additionally, while concurrent studies suggest a seasonally shifting dependence on deer, mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values from coyotes captured over all seasons are consistent with a diet more predictably dependent on communities of small mammals. This new evidence suggests that while some coyotes may be occasionally utilizing select livestock as prey, they display greater foraging dependence on available wild species found in forested habitats. This study yields some of the first evidence into the true diet of coyotes in this region and also provides essential baseline values for multiple mammals in Virginia. This research suggests that regional variations in coyote predation strategy be considered to both refine current management plans and more efficiently implement the conservation plans for endangered competitors and prey.

NKG2D RECEPTOR ACTIVATION OF MTOR AND NFKB ENHANCES PROINFLAMMATORY CYTOKINE SECRETION IN CD8⁺ T CELLS. E. A. Whitman, T. Bedsworth & A. Barber, Department of Biological and Environmental Sciences, Longwood University, Farmville, VA, 23909. To induce strong anti-tumor immune responses, CD8⁺ T cells require stimulation through the TCR and costimulatory receptors. Each costimulatory molecule can have a unique effect on the function of T cells due to differential activation of downstream signaling pathways and gene expression. One costimulation molecule that is likely to be engaged in the tumor microenvironment is NKG2D since the ligands for this receptor are expressed in over 80% of tumors. In order to determine how activation of costimulatory receptors in the tumor microenvironment affects CD8⁺ T cell functions, this study investigated the differential activation of signaling pathways by two costimulatory receptors, CD28 and NKG2D. Specifically, this study focused on the activation of mTORC1 and mTORC2 along with NFκB, all of which have been shown to have important roles in anti-tumor functions of CD8⁺ T cells. Activation of CD8⁺ T cells through CD3 and NKG2D lead to an increased secretion of proinflammatory cytokines and a decrease in anti-inflammatory cytokines compared to CD3 or CD3/CD28 stimulation. These data show that stimulation through NKG2D leads to the differential activation of signaling pathways and alters the anti-tumor effector functions of CD8⁺ T cells.

IMPROVING EFFICIENCY OF GENOMIC SEQUENCING FROM DIVERSE NEMATODE SPECIES. Anna K. Kania, Daniel E. Browne, & Theresa M. Grana, Department of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401. Even though nematodes are one of the most abundant phyla on the planet, their phylogenetic history is lacking a lot of information due to the fact that only few thousand out of the estimated million species have been identified and characterized. Thus to truly understand the evolutionary patterns that have taken place in the very large and diverse phylum of Nematoda, more species need to be described and

sequenced. The primary goal of this project is to improve the efficiency and quality of genomic sequencing of new nematode species. The DNA sequences obtained through the process are used to place those new species in context of their evolutionary history on the phylogenetic tree. The genomic sequencing process is challenging, however. It consists of multiple steps, each with alternative ways of accomplishing the same task. The principal objective is to determine the optimal way of accomplishing each and every step and therefore maximize the overall efficiency. While progress has been made and now we are able to obtain sequences from 60% of strains, further improvements are necessary. Our goal is to obtain a good DNA sequence from 90% of the new nematode species collected.

A STUDY OF CHLOROQUINE'S ANTIRETROVIRAL CHARACTERISTICS. Ryan S. Green & Lynn O. Lewis, Dept. of Biological Sciences, Univ. of Mary Washington, Fredericksburg, VA, 22401. In this study a procedure to induce maximal viral replication was tested, in addition to testing chloroquine as a possible treatment. This research attempted to activate Murine Mammary Tumor Virus (MMTV) using progesterone, as opposed to the standard synthetic activator that is used, to increase viral yield (Ross, 2010). Additionally viral infections are difficult to treat. This research examined the effects of chloroquine, an anti-malarial drug, as a retroviral treatment. Chloroquine has been shown to have antiviral properties; however, its effects have not been adequately studied in retroviruses and never with MMTV (Campitelli, et al., 2007). It was hypothesized that if chloroquine were to be administered to MMTV infected tissue culture, then the cytopathic effects (CPE) due to MMTV would be reduced. During this study it was found that progesterone, corticosterone, and dexamethasone did increase CPE (visible morphological changes which can indicate viral replication and transformation) at high concentrations, but the different treatments had no significant difference. The lack of difference was possibly due to the range between concentrations being too large to pinpoint the differences between the similar chemicals. Chloroquine had no significant effect on dexamethasone activated cells, possibly due to too few trials to demonstrate the expected trends, or viral budding masking CPE due to viral integration. This research was funded by the University of Mary Washington and the Virginia Academy of Science.

OBJECT NEOPHILIA IN PUREBRED DOMESTIC DOGS. Lydia B. Kniowski & Gene Sattler, Dept. of Biology & Chemistry, Liberty Univ., Lynchburg, VA 24502. Neophilia is defined as a preference for novelty, and has been studied in a variety of animal species. We tested two purebred dog breeds for neophilia with inanimate objects. Observations of dogs' selection when presented with two familiar toys and a novel toy were analyzed. Novel toys were preferred in 60% of selections presenting a significant neophilic trend ($p=0.002$). Of the breeds analyzed, Labradors selected novel toys 53% of the time, while Brittanys preferred them in 67% of selections. Although both breeds showed a neophilic trend, only in Brittanys was it significant ($p=0.009$). Differing degrees of neophilic tendency may exist among breeds as a result of either past or current selective regimes. These tendencies may have played a role in the domestication of dogs, and could lend insight into breed characteristics.

PROGRESS ON THE RAI1 BINDING SITE IN *CLOCK*. K. Belrose-Ramey¹, C. Bax¹, S. Williams², S. Mullegama², S. Elsea^{2,3}, & D. Zies¹, ¹Dept. of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401, ²Virginia Commonwealth University, Richmond VA 23284, ³Baylor College of Medicine, Houston, TX 77030. Smith-Magenis Syndrome (SMS) is a neurobehavioral disorder characterized by mental retardation, sleep disturbances, obesity and attention seeking behavior. The major symptoms of SMS are caused by haploinsufficiency of the *retinoic acid-induced 1 (RAI1)* gene, a transcription factor involved in the regulation of many genes. Previously, a Chromatin Immunoprecipitation-microarray chip (ChIP-chip) experiment was conducted to determine genes regulated by RAI1. One gene identified was *Circadian Locomotor Output Cycles Kaput (CLOCK)*. The region of *CLOCK* present in the microarray chip was used to create a pGL3 luciferase reporter gene construct. Previous studies showed that transcription from this construct was increased when cotransfected with RAI1, suggesting RAI1 binding site was located within the fragment. Site directed mutation, serial deletions, and luciferase analysis led to the identification of a 50bp region containing the RAI1 binding site. Possible false positives with the pGL3 reporter gene led to the decision to move an updated reporter system creating pGL4-*CLOCK*(-). The goals of my project have been to re-create constructs in the pGL4 system and compare the results between the two systems. Once completed, I will continue with serial deletions and perform single-base mutations to identify the minimal binding sequence for RAI1. The identification of the RAI1 binding site would enable researchers to identify other genes regulated by RAI1 and could lead to new treatments for SMS.

THE EFFECTS OF BISPHENOL A ON Wnt GENE EXPRESSION IN DEVELOPING RATS. Kara Arbogast, Virginia L. King & Deborah A. O'Dell, Dept. of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401. Wnt proteins are involved in development of the nervous and reproductive systems in vertebrates and aberrant expression of certain Wnt proteins has been linked to cancers of the reproductive systems in males and females. The Wnt pathway has been demonstrated to interact with the estrogen pathway, indicating that stimulation of the estrogen pathways may affect the transcription of Wnt proteins. Bisphenol A (BPA) is a xenoestrogen which interacts with estrogen receptors, and has been linked to abnormal development and cancers of the reproductive systems. We examined whether the Wnt pathways could be affected by exposure to BPA during development. Tissues from neonates from mothers fed BPA(100 mcg/L) during gestation were compared with tissues from neonates of untreated mothers. Brains, gonads and reproductive tracts were analyzed for levels of Wnt7a and Wnt 5a, using Sandwich ELISA and for levels of BPA using HPLC. BPA levels in embryonic tissues in general were higher in treated than in untreated tissues. ANOVA analysis did not show any significant differences in Wnt proteins between treated and control tissues. Post-hoc analysis using Fishers LSD test did show significant differences in Wnt 7a levels in the male reproductive tract and brain and in Wnt 5a levels in the male reproductive tract. BPA can therefore interfere with normal reproductive tract formation by altering Wnt gene activity.

EPIGENETIC REMODELING AT DISCRETE GENOMIC LOCI IN A β -TREATED NEURONS. Matthew Baker, Noor Taher, Courtney McKenzie, Rebecca Garret & Gary D. Isaacs, Dept. of Biology and Chemistry, Liberty University, Lynchburg VA. Genetic contributions leading to the development of Alzheimer's disease are mostly obscure, however evidence suggests that epigenetic modifications may play a role. Cytosine methylation has been shown to play a role in several biological disease states, and several AD-associated genes are regulated through this mechanism. Therefore, we set out to determine if methylation can provide genetic markers for AD development. DNA was isolated from A β -induced neuronal cells from an IMR-32 cell line, which serves as the AD model, as well as non-induced control neuronal cells. Using methylation sensitive (HpaII) and insensitive (MspI) restriction endonucleases, which both target the sequence 5'-CCGG-3', we determined the methylation status of genomic promoter regions through LM-PCR and subsequent microarray analysis. Genomic regions showing A β -induced methylation modifications were then narrowed down to the 0.1% most changing regions for stringency purposes. The regions showing significant change were linked to their ontological function, revealing significant correlation with ontologies related to neurogenesis and apoptosis. These findings support the idea that AD pathology is associated with specific epigenetic changes, and that these changes influence specific cellular pathways. This research was funded by the Jeffress Memorial Trust [J-998].

ANALYSIS OF 5-HYDROXYMETHYLCYTOSINE DISTRIBUTION IN THE HIPPOCAMPUS OF AN ALZHEIMER'S MOUSE MODEL. Rebecca Haraf, Deanna Harrison, Matthew Baker, Noor Taher, & Gary D. Isaacs, Dept. of Biology and Chemistry, Liberty University, Lynchburg VA. Methylated and hydroxymethylated cytosines are epigenetic modifications known to directly affect gene expression. Although methylated cytosines have previously been associated with Alzheimer's Disease (AD), they have been primarily studied on a gene by gene basis. The genome-wide roles of the modifications of cytosine, specifically hydroxymethylation, are still unclear. DNA from the hippocampus of an AD mouse model was purified and a DNA Immunoprecipitation (DIP) assay done using antibodies specific to pull down to hydroxymethylated cytosines (hmCs). The DNA was hybridized to a microarray with 20,404 promoter regions and 15,980 CpG islands. This gave significant peaks of hmC gain or loss in the experimental mouse when compared to the control model. We used the data to determine regions that had a change in hmC content when an AD state was established. Our confirmation DIP will show that AD is an epigenetic disease specifically affected by the distribution and levels of hmC in the hippocampus of a mouse model. We will analyze these changes to determine the specific cell functions and biological pathways affected by hmCs in Alzheimer's Disease. Funded by Jeffress Memorial Trust [J-998].

BACTERIOPHAGE-MEDIATED ANTIBIOTIC RESISTANCE. Kathleen O. Blevins & Lynn O. Lewis, Dept. of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401. Bacteriophages, viruses that infect a specific host range of bacteria, can have one of two different replication cycles. The lysogenic cycle incorporates the phage genome into the host cell's genome after infection, while the lytic cycle produces more viruses and kills the host cell by lysis. As phages are

particular to only one type of bacteria, they could be used as an alternative method to treat antibiotic resistant bacterial infections, which is known as phage therapy. However, during the lysogenic process, phage genes could be transferred from phage to bacteria, making phage therapy risky. Phages that were isolated on *Bacillus thuringiensis* Kurstaki were sequenced, and a beta-lactamase gene was identified. These phages were used to lysogenize the nonpathogenic *Bacillus anthracis* Delta Sterne and tested for the transfer of antibiotic resistance by the Kirby Bauer test. No antibiotic resistance was noted in the lysogens. Because we have now isolated a number of *Bacillus* phages, a simple method for classifying them was attempted by utilizing PCR and primers designed to match several specific phages. The 37 phages isolated by the 2012-13 Phage Hunters class were separated into 15 subcategories based on the primer reactions, and the phages which had been sequenced were verified with BLAST. Further, one of the sequenced genomes, belonging to *Bacillus thuringiensis* Kurstaki phage JPB9, was annotated.

IN VITRO SELECTION OF *E. COLI* MG1655 *flhDC* MUTANTS. Ryan N. Montalvo, Edward Dunn Richards & Andrew J. Fabich, Department of Biology and Chemistry, Liberty University. Leatham *et al.* previously reported that *Escherichia coli* strain MG1655 loses motility after three days in the streptomycin-treated mouse intestine. MG1655 has been previously evaluated for motility but HS and Nissle human commensal strains have not. Motility research carried out by Fabich, Leatham, Gauger *et al.* has been known to last for a period of 15 days, while our experiment is ongoing past 60 days. In order to evaluate motility the mechanism by which motility is lost needed to be identified. When sequenced, these intestine adapted strains (*) displayed deletions of various lengths downstream of the *IS1* element within the regulatory region of the *flhDC* operon, which encode the master regulator for flagellar synthesis. In our study of this mutation we observed trends within the data where the strains exhibit gains or losses in motility on similar days, specifically with MG1655 and HS. MG1655 is an average of 33.7% less motile than the other strains consistently. On day 60, the three strains were less than 60% motile and ranged within 36.11% of one another. This data at 60 days would suggest that the strains may reach a relative equilibrium when examined for further months. The similar trends in fluctuation of motility amongst the individual strains supports the idea that motility within the intestine is a dynamic contributing factor to *E. coli* intestinal colonization niches.

THE GUT REACTION: HOW THE INTESTINAL MICROBIOTA RESPOND TO COLONIZATION OF *CITROBACTER RODENTIUM*. C. E. Black & A.J. Fabich. Department of Biology and Chemistry, Liberty University, Lynchburg VA 24502. Pathogenic *Escherichia coli* O157:H7 is a global health threat. The microbial response to enteric colonization of *E. coli* O157:H7 is not well understood, nor the potential role the intestinal microbiota in preventing its disease. By using the streptomycin-treated mouse model, streptomycin-resistant bacteria *Citrobacter rodentium*, as well as commensal *E. coli* strains MG1655 Str^r Nal^r and probiotic Nissle1917 Str^r Nal^r, were colonized for community analysis. We hypothesize that *Bacteroidales* spp. and *Clostridium* spp. will have significantly altered population abundances when streptomycin-treated mice are co-colonized with *C. rodentium* and either commensal *E. coli* strain MG1655 Str^r Nal^r or the probiotic Nissle 1917 Str^r Nal^r. Quantitative PCR

was used to determine the bacterial abundance and the complex interactions occurring with inoculation of *C. rodentium* and either MG1655 or Nissle. The results indicate an increase in the population abundance of *Bacteroidales spp.* with the co-colonization of Nissle but not MG1655; an increase in the population abundance of *Clostridium spp.* with the co-colonization of MG1655 and Nissle; and a 5-fold increase of *Lactobacilli spp.* with the co-colonization of MG1655. These results shed light on the competitive relationship between pathogenic *E. coli* O157:H7 and specific enteric microbes as well as the protective roles MG1655 and Nissle may have against the pathogen.

CHICKENS ARTIFICIALLY SELECTED FOR HIGH (HWS) AND LOW (LWS) BODY WEIGHT DIFFER IN PANCREATIC EXPRESSION OF GLUCOSE REGULATORY GENES. L. H. Sumners¹, X. Zhao², W. Zhang¹, C. F. Honaker¹, P. B. Siegel¹ & E. R. Gilbert¹, ¹Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA 24061 and ²Sichuan Agriculture University, Ya'an, China. Now in the 55th generation of selection, chickens genetically selected for high (HWS) and low (LWS) juvenile (8 wk) body weight differ in weight by 12-fold at selection age. Because HWS chickens display impaired glucose tolerance and a difference in threshold response to insulin, compared to LWS, we hypothesized that the lines differ in mRNA abundance of glucose regulatory genes. Pancreas head and body were sampled from male and female HWS and LWS chickens (day 65; n = 10/line), and mRNA of preproinsulin (*PPI*), preproglucagon (*PPG*), glucose transporter 2 (*GLUT2*), and pancreatic and duodenal homeobox 1 (*Pdx1*) were measured by real-time PCR (2^{- $\Delta\Delta C_t$} method). Effects of line, tissue, sex and the interactions between them were analyzed by ANOVA, and means were separated by Tukey's HSD. For all genes, mRNA was greater (p < 0.01) in LWS. *PPI* mRNA was greatest in the pancreas body of LWS males (p = 0.009), whereas *PPG* mRNA levels were lowest in the pancreas body of all male chickens (p = 0.01). Abundance of *GLUT2* mRNA was greater (p = 0.001) in males than females. *Pdx1* mRNA was more abundant in the pancreas body of females than of males, and the pancreas head of females (p = 0.002). There was also greater *Pdx1* mRNA in both the pancreas head and body of LWS, than HWS chickens (p = 0.04).

DEFINING DIRECT TARGETS OF OpaR IN THE QUORUM-SENSING REGULON OF *VIBRIO PARAHAEMOLYTICUS*. A. L. Kernell¹, L. T. C. Guthrie¹, R. V. Jensen¹, L. L. McCarter², & A. M. Stevens,¹ ¹Department of Biological Sciences (MC0910), 1070 Washington St. SW /Rm 222 Life Sciences I, Virginia Tech, Blacksburg, Virginia 24061; ²University of Iowa, 3-430 BSB Iowa City, IA 52242. *Vibrio parahaemolyticus* (VP) is an emerging pathogen that is associated with food-borne illnesses worldwide, including developed countries, such as the United States and Japan. It is acquired through consuming raw or undercooked seafood. The pathogenesis of this organism is controlled by the phenomenon known as quorum sensing and the master regulator of quorum sensing, in (VP), is OpaR. OpaR controls the virulence of (VP), as well as the colony and cellular morphology associated with growth on a surface. RNA-Seq, whole transcriptome Next Generation sequencing, was utilized to determine all the direct and indirect targets controlled by OpaR and revealed that over 10% of the genome is regulated by quorum sensing. Eleven transcription factors under OpaR control were identified and further studied using qRT-PCR and the

data collected confirmed the RNA-Seq results. ChIP-Seq methods are being optimized to identify the direct targets of OpaR. Select targets were confirmed direct targets via *in vitro* electrophoretic mobility shift assays (EMSA) with purified hexahistidine tagged OpaR. A previously published position specific weighted matrix (PSWM) will be utilized to assist in identification of possible OpaR binding sites.

DIVERGENCE OF X CHROMOSOME HETEROCHROMATIN AMONGST SPECIES OF THE ANOPHELES GAMBIAE COMPLEX. Atashi Sharma, Maria Sharakhova & Igor V. Sharakhov, Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA - 24061. Malaria is a scourge infecting 215 million people annually. The vector mosquito *Anopheles gambiae* along with its six sibling species forms the *gambiae* complex. Major vector *Anopheles gambiae* sensu stricto (s.s.) is currently undergoing incipient speciation into two molecular forms, designated M and S. Differences between these forms were determined on the basis of heterochromatin (repetitive DNA) present in X chromosomes using light microscopy and Fluorescence *in situ* hybridisation (FISH). In addition, 18S rDNA (responsible for protein synthesis) probe and species specific satellite DNA (important part of heterochromatin) was labelled and mapped to *An. gambiae*. We found significant differences in both the patterns and intensity of heterochromatin between the M and S forms. The 18S rDNA probe mapped adjacent to the heterochromatin in all the laboratory strains tested. Overall, our study indicates differences in heterochromatin between and within the species of *gambiae* complex and suggests a possible role of heterochromatin in speciation and protein synthesis.

Biomedical and General Engineering

ELECTROSPINNING AND CHARACTERIZATION OF LINEAR-DENDRITIC COPOLYMERS AS A NEW TISSUE ENGINEERING SCAFFOLD. Donald Jr C Aduba¹, Jefferson W. Overlin¹, Gary L. Bowlin¹ & Hu Yang^{1,2}, ¹Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284 & ²Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298. Polyamidoamine dendrimers (PAMAM) are an important class of macromolecules, which are known for their highly branched nanoscale structures and high density of surface groups. Numerous studies have been conducted to develop PAMAM dendrimer-based nanocarriers to deliver a broad spectrum of functional moieties such as drugs, imaging agents, and ligands. The purpose of this work is to explore a new way of using PAMAM dendrimers for wound healing and drug delivery applications. Particularly, we have developed novel electrospun nanofiber scaffolds made of linear-dendritic copolymer comprised of methoxy-polyethylene glycol (mPEG) as modifier to couple (PEGylate) dendrimer in modulating their biological and surface properties while facilitating electrospun nanofiber formation. In our study, we adjusted the degree of PEGylation of the dendrimer surface groups (100%, 50%, and 12.5%) by attaching PEG at 32:1, 16:1 and 4:1 ideal molar ratios respectively. Crosslinking reagents Eosin Y and 2,2-Dimethoxy-2-phenyl-acetophenone (DMPA) were used to help stabilize the electrospun scaffold. For each treatment, the scaffold's physical properties were evaluated. Ongoing studies include cell viability assays evaluating cell-scaffold