Biology with Microbiology and Molecular Biology

MOLECULAR BASIS OF EXOSOMAL FUNCTION DURING RIFT VALLEY FEVER VIRUS INFECTION. N. A. Ahsan1, G. Sampey1, B. Lepene2, R. Barclay1, S. Iordanskiy1, F. Kashanchi1 & R. M. Hakami1, 1George Mason University, Manassas, VA, and 2Ceres Nanosciences, Inc., Manassas, VA. Exosomes are small “bioactive” extracellular vesicles that play a central role in intercellular communication and have garnered tremendous interest given the recent discovery of their critical role in a variety of diseases, including infectious diseases. Among these are immunomodulation, alteration of dissemination/infectivity during infection, and modulation of pathogenesis. However, their mechanisms of action remain largely unknown and their role during infections with biodefense agents remains unexplored. We have examined the role of host exosomes during infection with the Rift Valley Fever Virus (RVFV), a Category A priority Pathogen that carries the potential for both devastating public health and agricultural impacts. To assess the role of exosomes, clones of Vero cells that show resistance to RVFV infection and are unable to release functional virions were first generated. Exosomes from these clones contained exosomal markers such as CD63 and were able to activate the TLR3 pathway in recipient reporter cells. Interestingly, these exosomes contained viral RNA (signal for L, M, and S segments). Furthermore, exosomes derived from some of the resistant clones contained viral proteins such as N. Finally, treatment of immune recipient cells (T cells and monocytic cells) with some of the exosome preparations showed a drastic rate of apoptosis through PARP cleavage and caspase-3 activation. Collectively, our data suggest that exosomes from RVFV-infected cells alter the dynamics of the neighboring cells and may contribute to disease pathology.

S1P INHIBITS IL-1-INDUCED CCL5 EXPRESSION. Sabrina Andaluz, Debolina Biswas, Angela Gupta, Tomasz Kordula, Dept. of Biochemistry & Molecular Biology, VCU School of Medicine, Richmond, VA 23298. Both interleukin-1 (IL-1) and sphingosine-1-phosphate (S1P) are critical mediators of inflammation. However, it is unclear at the moment whether S1P promotes or inhibits IL-1-induced inflammatory responses. In preliminary studies we found that one of the five S1P cell surface receptors, S1PR2, may regulate inflammatory responses. To address this question, whether S1PR2 effects IL-1 signaling, we analyzed infiltration of macrophages into sites of sterile inflammation in wild type and S1PR2-/- mice. We employed a turpentine model of irritant-induced inflammation and found increase infiltration into the wound of S1PR2-/- mice. Accordingly, expression...
of CCL5 chemokine, which attracts macrophages, was elevated in S1PR2/-/- mice. We concluded that S1PR2 limits IL-1-induced expression CCL5, and recruitment of macrophages into sites of inflammation.

DETERMINATION OF THE IMPACT OF PYK1 DELETION ON INTERACTIONS OF C. NEOFORMANS WITH THE HOST IMMUNE SYSTEM. Yansirre Aviles & Elizabeth Rasmussen, Dept. of Biol., Liberty Univ., Lynchburg VA 24502. Cryptococcus neoformans is an important fungal pathogen of immune-compromised individuals that initially colonizes in the lungs and eventually migrates to the cerebral spinal fluid, eventually causing the death of about 625,000 people a year. Epidemiological evidence for the dormancy of cryptococcal infections exists, and our objective is to develop a model that allows for the effective study of said dormancy in such an important human pathogen. This study will be facilitated by quantifying cytokine expression in macrophages that have been exposed to pyruvate kinase mutants that fail to elicit inflammation in the lung. Initial results from Dr. Price’s lab suggest that the removal of the pyruvate kinase gene in H99 wild type strains results in a lack of the immune system’s ability to recognize the yeast. This dormancy phenomenon will be examined by analyzing the effects that alterations in carbon metabolism have on host immune cell cytokine production and cell signaling. C. neoformans wild type, pyk1Δ, pyk1Δ PYK1, and hxk1Δ hxk2Δ strains will be co-incubated with harvested primary BALB-C macrophages in conditions that simulate the interior of human lungs. Subsequently, the culture broth and macrophages themselves will be saved for ELISA analysis of cytokine production. To confirm compatibility between in-vitro studies of this culture data and actual in-vivo scenarios, live mice will be infected with both wild type and previously mentioned pyk1Δ mutant strains of C. neoformans. At set points post-infection, the spleens, lungs, and brains of the infected murine specimens will be harvested and used for cytokine and gene expression analysis.

MOLECULAR REGULATION OF TRKA TRAFFICKING IN SPACE AND TIME. Kelly A. Barford1, Christopher D. Deppmann2 & Bettina Winckler1, 1Department of Neuroscience, University of Virginia, Charlottesville VA 22908 and 2Department of Biology, University of Virginia, Charlottesville VA 22908. Protein trafficking is involved in all aspects of neuronal function, including development, axon and dendrite growth, and synaptic function. Presumably due to cellular complexity, neurons have acquired special endosomal machinery to deal with protein trafficking. One such protein is neuron enriched endosomal protein of 21kDa (Neep21). Neep21 is involved in the trafficking of proteins involved in synaptic function and disease
including GluR2, βAPP, and neurotensin receptor 1. Our lab has recently shown that Neep21 is involved in the axonal specification of the cell adhesion protein L1/NgCAM through transcytotic trafficking and avoidance of the lysosome. Another protein that undergoes both transcytosis and lysosomal evasion is the neurotrophin receptor TrkA. TrkA undergoes many different trafficking events both on its way to the axon and in a signaling endosome travelling retrogradely back to the soma. TrkA has the ability to undergo signaling transcytosis and to enter into the dendrites and affect synapse formation. However, the trafficking steps and molecules involved in the transport of TrkA have not been fully elucidated. Neep21’s involvement in transcytosis and lysosomal avoidance make it an ideal candidate for involvement in TrkA signaling, and its placement in the somatodendritic region primes it to interact with TrkA during distinct spatiotemporal trafficking events. Indeed, Neep21 is a novel effector for the signaling endosome, and we are currently investigating the role of Neep21 in TrkA-dependent processes.

DOMINANCE BEHAVIOR IN THE TUBE TEST, AND ITS RELATIONSHIP TO HOME CAGE BEHAVIOR IN MICE. Hannah M. Belski, Meagan T. Darling & R. Parrish Waters, Dept. of Biology, University of Mary Washington. In previous studies, scientists have used mice for their versatility as a whole animal model in examining human conditions. In many of these studies, the mouse’s behavior is the output and ultimately determines the result of the experiment. However, the mouse’s natural ethology is rarely prioritized in interpreting these results. What this experiment aims to do is develop an ethologically valid model to more fully understand the mouse’s behavior and, in turn, its response to stressful stimuli. Primarily, this study focuses on social stress; the most potent stressor animals encounter. To induce this social stress, we house five male CD-1 mice together in a cage in which close proximity elicits competition for resources. The design of this experiment consists of two components: behavioral and physiological. We performed several established tests of dominance, including a Tube Test for which we constructed an algorithm to generate a quantitative score of dominance, called a TDR (Total Dominance Ratio). To support these data, we determined fecal steroid hormone concentrations weekly and measured monoamines from several critical regions of the brain. We uncovered correlation between our TDR and other behavioral measures in several of the cages. When steroid hormones are compared to TDR, there is no significant correlation. However, emerging patterns of amygdalar dopamine and norepinephrine suggest depressive physiology in subordinate mice. Through both their behavioral and physiological measures, we hope to see consistent patterns that characterize dominant
or subordinate mice. Future directions of this project will include incorporating RFID technology as well as additional social stressors to model human pathologies.

SKELETAL MUSCLE MAY INTERACT WITH VASCULATURE THROUGH O-GlcNAc TRANSFERASE (OGT). Joel A. Brenny¹, Emily R. Berguson¹, David E. Gerrard², Hao Shi² & Pei Zhang¹, ¹Department of Biology and Chemistry, Liberty University, Lynchburg, VA, 24502 and ²Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. Cellular communication can occur through post-translational modification, the modification of proteins by enzymes such as O-linked N-Acetylglucosamine Transferase (OGT). OGT adds an N-acetylglucosamine (O-GlcNAc) moiety to a serine or threonine residue of the protein. O-GlcNAcylation is important because it can affect various functions of proteins. O-GlcNAcylation is also implicated in metabolic syndromes such as diabetes. At the center of metabolism, vasculature not only distributes nutrients and metabolites, but also actively interacts with surrounding tissue and remodels itself in response to physiologic and pathophysiologic stimuli. Many myokines, secreted by muscle cells, are involved in inflammation, and could be markers of vascular disease such as atherosclerosis. Using an OGT skeletal muscle knock-out murine model, we are examining the effects of OGT on vasculature with the goal of identifying basic mechanisms underlying interactions between skeletal muscle and vasculature. This study may shed new light on heterocellular metabolism and present new therapeutic potential for metabolic syndromes.

ALTERED MITOCHONDRIAL DYNAMICS IN VENEZUELAN EQUINE ENCEPHALITIS VIRUS INFECTED CELLS. Taryn Brooks-Faulconer, Moushimi Amaya, Forrest Keck, Charles Bailey & Aarthi Narayanan, National Center for Biodefense and Infectious Diseases, George Mason University, 10650 Pyramid Place, Manassas VA 20110. To establish productive infection, viruses profoundly alter both the intracellular environment and the cellular function. Mitochondria are critically important cellular organelles that generate energy and ensure cell survival. Mitochondria are also crucial for innate immunity response as they serve as the sentinels that sense infection and initiate host responses. For many viruses, the changes in mitochondrial dynamics were documented to occur early in infection. Venezuelan Equine Encephalitis Virus (VEEV) is a New World alphavirus that infects neuronal cells and produces an encephalitic phenotype. In this study, we demonstrate that VEEV infection results in mitochondrial alterations that include changes in the morphology and intracellular distribution of mitochondria, reduction in mitochondrial membrane potential and localization of their enzymatic components. In
particular, we report perinuclear accumulation of mitochondria in infected cells and partial co-localization of the viral capsid proteins with mitochondrial membranes. The pronounced changes to the mitochondria observed in VEEV infected cells probably play a role in the development of the virus-specific cytopathic effects. Our studies demonstrate that the mitochondria are critical intracellular platforms affected by alphavirus infections.

THE EFFECTS OF ROSMARINIC ACID ON EWING’S SARCOMA CELL VIABILITY. Samuel J. Clark & Rosemary Barra, Dept. of Biol., Univ. of Mary Washington, Fredericksburg VA 22401. Botanical and herbal medications are among the most common complementary and alternative medications (CAM) used by cancer patients, both in the treatment of cancer and the management of cancer symptoms. However, information concerning the safety and efficacy of many CAM treatments has not been established. *Rosmarinus officinalis*, commonly known as rosemary, has been implicated as a possible cancer chemo preventive agent as well as a treatment due to its inherent antioxidant activity. It has been demonstrated in several cancer lines that rosemary extracts (RE) have a significant anti-proliferative effect through modifications on the cell cycle. RE’s have not been tested on Ewing’s sarcoma (ES) cell lines. This study aimed to evaluate the proliferative activity of RE’s on an ES cell line. Also, one of RE’s main active components, rosmarinic acid (RA) was evaluated. Both of these analyses used the MTT viability assay. Contrary to the effects on other cell lines, this study indicated a significant increase in cell proliferation. The Folin-Ciocalteu assay was used to determine the approximate phenol concentration within an RE. RE was applied to the system at concentrations of 30 µg/ml, 15 µg/ml, 7.5 µg/ml, and 3.5 µg/ml, an increase in cell viability by 119%, 140%, 157%, and 289%, respectively, was demonstrated. When the RA was applied to the system at concentrations of 30 µg/ml, 15 µg/ml, 7.5 µg/ml, and 3.5 µg/ml, an increase in cell viability by 120%, 175%, 246%, and 310% was recorded. These results suggest that RA is the major active component of the extract.

EFFECT OF SOCIAL STATUS ON BEHAVIOR AND NEUROENDOCRINE SYSTEMS IN MALE MICE. Meagan T. Darling¹, Hanna M. Belski¹, David H. Stahlman², & R. Parrish Waters¹, ¹Department of Biology and ²Department of Psychological Sciences, University of Mary Washington, Fredericksburg VA 22401. Mice are social animals that form hierarchies, in which higher-ranking (dominant) animals display aggressive behavior toward lower-ranking (subordinate) animals. These social interactions are intensely stressful.
and have profound effects on the physiological and behavioral state of both the dominant and subordinate individual. The effects of this social stress often resemble the symptoms of human stress-related pathology, including depression, PTSD, and addiction. Multiple laboratory paradigms utilize social stress in mice to model these disorders. However, many of these paradigms fall short of ethological and ecological validity: they use short-term and/or heavily weighted interactions between animals, which do not match the true nature of mouse social interactions, and are therefore less applicable to human pathology. Our model attempts to address this deficit. We assess the position of mice in dominance hierarchies and the stability of these hierarchies to analyze their effects on physiology and behavior. Our preliminary results suggest that aggressive home cage behaviors correlate to dominance status, that social cages are dynamic, and that social status impacts monoamine expression in the amygdala. This study was funded in part by the Irene Piscopo Rodgers ’59 and James D. Rodgers Student Research Fellowship II and the UMW Summer Science Institute.

CHIMERIC PD1-EXPRESSING T CELLS AS A POTENTIAL TREATMENT FOR MULTIPLE TYPES OF CANCER. Kelsey Deal, Geoffrey Parriott, & Amorette Barber, Dept. of Biol. and Env. Sci., Longwood Univ., Farmville VA 23901. CD8 T cells are one of the immune system’s best defenses against tumors. However, some tumors accumulate enough mutations to evade T-cell detection. On the other hand, most tumors express ligands for the Programmed Death receptor (PD1). Since PD1 ligands are expressed on most tumor types, and not many other cells, they are ideal targets for potential tumor therapies. One such therapy is the development of chimeric antigen receptors (CAR). CARs are modified receptors that use genetic engineering to replace the normal signaling domain with a different one. In our experiments we used a CAR with the PD1 receptor as the tumor-targeting domain attached to CD3 zeta activation and Dap10 costimulatory domains, called chimeric-PD1 receptor (chPD1). Previously, chPD1 expressing T cells were shown to effectively treat murine models of lymphoma and melanoma. In this study, we tested the anti-tumor efficacy of chPD1 T cells against murine kidney, pancreatic, liver, and colon cancers. Flow cytometry and RTPCR were used to determine that all tumor cells tested expressed the PD1 ligands. Then we measured tumor cytotoxicity of the chPD1 T cells compared to wild type T cells. For all tumor types tested, chPD1 T cells significantly increased killing of the tumor cells. Therefore, chPD1 T cells could be a novel therapeutic strategy to treat multiple types of cancer.
INFLUENCE OF ENVIRONMENTAL TEMPERATURE ON METABOLIC RATE IN AQUATIC ECTOTHERMS. J. C. Doran1, J. M. Doran1, S. Henkanaththegedara2 & P. A. P. deHart1, 1Dept. of Biol., Virginia Military Institute, Lexington VA 24450 and 2Biol. & Environ. Sci., Longwood University, Farmville VA 23909. This research aimed to quantify the effects of environmental temperature on metabolic rate in aquatic ectotherms through the use of a model ectotherm, the crayfish. With rising global temp., environments are expected to rise in average temp., including aquatic environments. Due to the essential roles that aquatic ectotherms carry out in their ecosystems, it is crucial to discover how these organisms will be affected by environmental temp. changes. It is hypothesized that crayfish inhabiting warmer environments will reflect higher stress levels, indicated through increased metabolic rates. Crayfish of genus *Cambarus* were collected in streams within the Lexington, VA area. Three sets of ten crayfish were maintained for an average of 15 days in three separate aquaria held at 17, 21, and 25°C. After maintaining the crayfish, each organism was prepped and subsampled for stable isotope analysis (SIA). By observing metabolic rates from isotopic signatures, insight on the stress levels of each crayfish could be assumed. Values of δ13C and δ15N did not reflect the hypothesis. Both 17 and 25°C treatment groups reflected statistically more enriched signatures relative to the 21°C treatment in regards to δ15N. This would suggest that any deviation from an intermediate temp. would increase stress levels of ectotherms. However, these overall signatures are more likely to have occurred due to the short exposure time within treatment aquaria. Longer exposure will allow for the effects of varying environmental temp. to be reflected within the tissues of crayfish.

HABITAT MEDIATED DIFFERENCES IN THE TROPHIC NICHE OF ARACHNIDS AS CLARIFIED BY STABLE ISOTOPE ANALYSIS. J. M. Doran1, O. Howell2, J. C. Doran1, L.E. Hurd2, & P.A.P. deHart1, 1Department of Biology, Virginia Military Institute, Lexington, VA 24450 2Biological Department, Washington & Lee University, Lexington, VA 24450. Our research aimed to determine the consequences that differing habitats has on arachnids’ eating behaviors. In order to accomplish this, we utilized stable isotope analysis to quantify trophic levels on which each are operating. The objective for this project was to collect arachnids of varying species, including the harvestman of Order *Opiliones* and the orbweaver of Order *Araneae*, from within the Lexington, VA area from two different habitats; a woody habitat and a field habitat. Once collected, samples were prepared for stable isotope analysis (SIA) and taken to a stable isotope facility, for isotope analyses. Data was then be analyzed, examining trends in the ratios of heavy to light carbon and nitrogen isotopes across
treatment groups. Values of $\delta^{15}N$ and $\delta^{13}C$ indicated that field harvestmen operate at one trophic level higher than their counterparts in the forest habitat. However, the same was not witnessed for orbweavers, as both were operating on relatively equal trophic levels. Arachnids are a generalist predator that can influence species diversity, how they interact trophically is extremely important for the ecosystem as a whole. Determining how the habitat influences this trophic interaction has not been studied notably in the past. How and what each species eats in the two different habitats can reveal valuable information about how the environment influences each arachnid.

**INHIBITORY RECEPTORS PD-1 AND CTLA-4 DIFFERENTIALLY REGULATE NFkB ACTIVATION IN NKG2D- AND CD28-COSTIMULATED CD8 T CELLS.** Zachary Fasana & Amorette Barber, Dept. of Biol. and Env. Sci., Longwood Univ., Farmville VA 23901. Effector CD8 T cells are important for successful antitumor immune responses. The balance of activating and inhibitory receptors affects the potency of the CD8 T cell response by activating kinases and/or phosphatases. The optimal combination of receptors that induces effective antitumor immunity is still unknown. CD8 T cell activity is enhanced by activation of costimulatory receptors, including CD28 and NKG2D. Inhibitory PD1 and CTLA4 receptors are highly expressed by T cells and their ligands are expressed in the tumor microenvironment. While these activating and inhibitory receptors are all likely stimulated on T cells in the tumor, it is currently unknown how the CD28 and NKG2D receptor signaling is altered by concurrent activation of inhibitory receptors PD1 and CTLA4. Therefore, this study compared gene expression of NFkB pathway members involved in antitumor responses. Specifically, murine effector CD8 T cells were costimulated by activating NKG2D or CD28 receptors in combination with inhibitory PD1 or CTLA4 receptors and an RT-PCR array was used to measure expression of over 80 genes in the NFkB pathway. Compared to CD28 costimulation, CD8 T cells stimulated by NKG2D and CTLA4 or PD1 had fewer changes in gene expression. Thus, NKG2D is likely the more effective antitumor costimulatory receptor due to its resistance to be inhibited by PD1 and CTLA4 receptors.

**OPTIMIZATION OF ASSAY FOR IDENTIFICATION OF RNA-RNA INTERACTION.** A. Filatova, A. Baranova and M. Yu Skoblov, Centre for Medical Genetics, Federal Agency for Scientific Organizations, Moscow, 115478, Russian Federation, School of Systems Biology, George Mason University, Manassas, VA, 20110, USA and Moscow Institute of Physics and Technology, Moscow, 117303, Russian Federation. RNA-RNA interactions play an important
role in regulation of gene expression and maintenance of cell homeostasis. RNA-RNA duplex formation is a trigger for many cellular mechanisms which alter target RNA structure, half-life time, translational efficiency, and ability to form complexes with other targets. According to recent reports, the human genome contains more than 15,000 long non-coding RNAs (ncRNAs) genes. Many of these play an important role in diverse biological processes. ncRNA functions are often associated with their ability to form complexes with other RNA-molecules, both coding and non-coding. Thus, analysis of RNA partners of previously unexplored ncRNA allows us to investigate its possible function. One of the approaches to identify RNA-RNA interactions is RNA-RNA pull-down with short biotin-labeled ssDNA-oligonucleotides complementary to target RNA. This study is devoted to determine the most appropriate conditions for effective and specific detection of RNA partners. We made RNA-RNA pull-down experiments for high-level expressed and low-level expressed RNAs with different amount of probes on different hybridization conditions. We measured enrichment of target RNA, its partner, and reference RNA in pull-down samples relative to input samples by qPCR. Additionally, we evaluated the specificity of different protocols using non-specific probe controls.

CHARACTERIZATION OF CELL-TYPE SPECIFIC RESPONSES TO MISFOLDED PROTEIN STRESS IN C. ELEGANS. Claire Gormley, Kristen Hoffman, Rana Ihsan & Tim Bloss, Dept. of Biol. James Madison University, Harrisonburg, VA 22807. Cells experiencing misfolded protein stress can become debilitated and die, contributing to the onset of disease. Different cell types display varying sensitivities to this stress, with neurons being particularly susceptible to death. When a cell experiences misfolded protein stress in the endoplasmic reticulum (ER), the unfolded protein response (UPR) initiates cell-saving mechanisms that mitigate stress and, if the stress cannot be resolved, triggers cell death by apoptosis. The nascent polypeptide-associated complex (NAC) is a heterodimeric chaperone that mediates proper protein folding and localization during translation, and also triggers the UPR when the ER experiences misfolded protein stress. The role of the NAC in relation to the UPR is not well understood, nor is it known if this role is different in different cell types; our goal is to characterize the relationship of the NAC with the UPR in different cell types in the model organism C. elegans. We are characterizing how neurons respond to misfolded protein stress in the absence of the NAC by depleting the NAC via RNA interference and quantifying the number of neurons observed in the ventral nerve cord.
Generally, depletion of the NAC decreases the number of ventral nerve cord neurons while also leading to the mis-localization of the neurons that remain. In addition, we are characterizing the effects of depletion of the NAC in hypodermal cells, which are relatively more resistant to stress-induced death. Through these experiments, we hope to better understand how different cell types handle misfolded protein stress, and why some cell types are more likely to die in response to this stress while others live.

TAU AND BETA-AMYLOID INTERACTIONS IN ALZHEIMER'S DISEASE. Tyrrell C. Graham & Deborah A. O'Dell. Dept. Biol. Sci., Univ. Mary Washington, Fredericksburg, VA 22401 Characteristics of Alzheimer’s disease include abnormal Tau protein phosphorylation and the presence of beta-amyloid plaques. The stimulus for changing Tau phosphorylation is unknown. The effect of the presence of beta-amyloid aggregates on the phosphorylation state of Tau was examined. We exposing cultured human cortical neurons to beta-amyloid aggregates for 48 hours and looked for the presence of p-231 Tau using immunohistochemistry. Microscopic examination of cells showed that cultured cells exposed to beta-amyloid aggregates had increased levels of p231-Tau compared to control cells. This suggests that the presence of beta-amyloid plaques stimulates the changes in the phosphorylation of Tau. This work was sponsored by an Undergraduate Research Grant to GH from UMW.

COMPARATIVE GENOMICS OF STREPTOCOCCUS PARAUBERIS IN FISH AND CATTLE. Ashley N. Haines1, Elvira N. Besong1, Kimaya R. Council1, Onaysha A. Lambert1, Miranda Ryan2, Dillion B. Matthews2 & David T. Gauthier2, 1Department of Biology, Norfolk State University, Norfolk, VA 23504 and 2Dept. of Biological Sciences, Old Dominion University, Norfolk, VA 23529. Streptococcus parauberis is a gram-positive bacterium that infects fish and cattle, causing streptococcosis and mastitis respectively. We have sequenced, assembled and annotated 14 genomes, eight isolated from fish and six from non-piscine sources. In this study we investigated the selective pressures on the genomic resources of these isolates. We hypothesized that genes would be under different selective pressures, depending upon the host or environment in which they live. To demonstrate this, we created a phylogenetic tree of all available isolates based upon single nucleotide polymorphisms (SNPs). We then assessed whether the dN/dS ratio differed between branches on this tree and identified eight specific genes under positive selection. We also identified 45 genes unique to isolates infecting Chesapeake Bay striped bass (Morone saxatilis) and two genes not found in Chesapeake Bay...
isolates. Identifying these genetic differences will help us understand how these bacteria adapt to different niches and become pathogens of new hosts.

RESPONSE OF IMMUNE CELLS TO NANODIAMONDS IN VITRO. Maisoun E. Bani Hani & Christopher Osgood, Dept. of Biol., Old Dominion University, Norfolk VA 23529. Nanodiamonds (NDs) are gaining more attraction for both imaging and theranostic applications. In addition to their unique physical and chemical properties, NDs are described as highly biocompatible with very low cytotoxicity in cultured cells, nematodes and mice. The intrinsic fluorescence of ND due to crystal defects in their lattice, usually known as nitrogen vacancies, made it possible to track ND both in vivo and in vitro. In order to achieve the safe use and application of NDs in biomedicine, it is essential to understand the effects of this material on the immune system at the cellular level. Our goal was to investigate the impact of ND on the immune system by studying their effects on innate immune cells viability, proliferation and function in vitro. Measuring the absorbance and the fluorescence from cells incubated with different concentrations of ND, the uptake by a mouse macrophage cell line increased with increasing the time of incubation and the concentration of NDs. Incubation of mouse cells with up to 100 µg/ml ND did not significantly reduce cell viability as assessed by MTS assay. In order to investigate the ability of ND to induce an immune response from mouse macrophages, we performed RT-qPCR for different immune markers and found increased expression of these genes which indicates an induced response in these cells. We also looked at the ability of these cells to respond to LPS after treatment with ND but did not find a significant difference. Our future work will be to perform a microarray assay to further investigate immune signaling pathways activated by NDs.

EFFECTS OF ANTIGEN-SPECIFIC AVIAN IGY ON PATHOGEN SHEDDING AND MURINE ANTIBODY RESPONSE IN CITROBACTER RODENTIUM INFECTION OF A MURINE MODEL. Nicole Hawkins, Robert Welch, & Randall Hubbard, Department of Biology and Chemistry, Liberty University, Lynchburg VA 24502. Objective: Assessment of whether administration of antigen-specific IgY would neutralize the pathogen C. rodentium in mice. Method: The following were performed: a 21-day infection of CF1 mice with DBS 101 (n=26) and a 10-day infection of C57BL/6 mice (n=6) with DBS 100. ELISA of serum and serial dilutions of pathogen load in stool were used to compare the responses of two treatment groups and a non-treated group to infection. Results: In both trials mice demonstrated lack of susceptibility to infection (50% of n=26 mice with stool assessed grew no pathogen). ELISA showed a decreased C. rodentium-specific IgG serum response level in treated CF1 mice but no similar
decrease in treated C57BL/6 mice. Serial dilutions of stool showed no significant advantage of treated groups versus non-treated in pathogen load reduction. **Conclusion:** A stronger pathogen and more susceptible model are needed to better assess therapeutic potential of IgY as well as additional trials.

**EFFECTS OF SB100 ON RAGE RECEPTOR CONCENTRATION IN GLIOBLASTOMA CELLS.** Brittany Hylander, Mary-Alison Lane & Deborah O’Dell, Dept. of Bio. Sci. Univ. Mary Washington, Fredericksburg, VA 22401 We studied the relationship between S100B protein and the RAGE receptor. It is known that at nanomolar concentrations, S100B produces neurotrophic effects on surrounding cells while micromolar levels lead S100B to induce neurotoxic effects. We hypothesized that if cells were exposed to a constant high level of S100B, this would lead to a change in RAGE expression. To test this hypothesis we examined the levels of RAGE protein in cultured glioblastoma cells that were exposed to high and low concentrations of S100B. After being exposed, some cells were homogenized while others were fixed. Homogenized cells were used to determine the concentration of RAGE receptors in the cells through an ELISA test using an antibody agonist of RAGE. The cells that were fixed were mounted onto slides and photographed to determine if there was an increase in RAGE receptors due to S100B concentration increase. The results of this experiment showed that levels of S100B do not seem to affect the amount of RAGE receptor, at least as measured by immunochemistry in glioblastoma cells. This work was supported by an Undergraduate Research Grant to BH and MAL from the University of Mary Washington.

**EFFECTS OF ANTHOCYANINS ON ALCOHOL-INDUCED HEPATIC DAMAGE IN FETAL MICE.** Chris D. Miller, Justin D. Paul & Roman J. Miller, Dept of Biol., Eastern Mennonite Univ., Harrisonburg VA 22802. Fetal Alcohol Syndrome (FAS) occurs when a mother chronically consumes alcohol during pregnancy. While the detrimental effects of FAS are well known, antioxidants, may have the potential to negate some alcohol-induced damage. To investigate this potential, three groups of mice were utilized: control group (CO) injected with saline, binge alcohol group (BA) injected with alcohol, and alcohol/anthocyanin group (AA) injected with both alcohol and anthocyanins. At 14 days gestation, fetuses were removed from pregnant mothers to determine gross level body weight, crown-rump length (CRL), total fetal area, and liver area parameters. For gross level parameters, BA fetuses were significantly smaller than CO and AA fetuses, based on ANOVA testing (p < 0.05). In contrast CO versus AA fetuses did not significantly differ. When liver area was compared to total body area for each group, all of the groups were significantly
different from each other. On a cellular level based on microscopic observation and stereological determinations (liver nuclei, cytoplasm, and vessel/sinusoid volume density parameters), no statistically significant differences were found between the three groups. When liver function was biochemically assessed for total protein production and acid phosphatase activity, no statistically significant differences emerged between the groups. In summary, the data indicate that alcohol damage has an impact on a gross level, but not on liver cellular/biochemical level parameters. The addition of anthocyanins indicated that antioxidants negate some alcohol damage that was seen on gross level parameters. (Research sponsored in part by EMU’s Daniel B. Suter Endowment in Biology.)

STUDYING REGULATION OF HUMAN GENES AFAP1-AS1, RIC8A, S100A13. I. A. Krivosheeva¹, J. V. Vyakhireva¹, A. V. Baranova¹², M. Yu Skoblov¹³, ¹Centre for Medical Genetics, Federal Agency for Scientific Organizations, Moscow, 115478, Russian Federation, ²School of Systems Biology, George Mason University, Manassas, VA, 20110, USA and ³Moscow Institute of Physics and Technology, Moscow, 117303, Russian Federation. Regulation of gene expression is a necessary process for homeostasis of cells and whole organisms. It can be implemented by various mechanisms; e.g. sense-antisense interaction or modification of mRNA half-life time with different pathways such as miRNA-mediated or STAU-mediated decay. Here we study 3 genes which have natural antisense transcripts. RIC8A encodes guanine-exchange factor while S100A13 plays an important role in cell cycle regulation. AFAP1-AS1 is a NAT for the AFAP1 gene, which encodes proteins of unknown function that are associated with actin filaments of the cell. As a model to study regulation, we chose cell line HEK293N for all genes and K562 as a cell line with high level of AFAP1-AS1. We designed siRNA to these genes and made a knockdown. Knockdown of RIC8A decreased the level of its antisense partner, SIRT3, approximately 25 times relative to control. Knockdown of S100A13 didn’t show any significant change in the level of CHTOP (NAT of S100A13). AFAP1-AS1 knockdown in K562 cells showed significant decrease of both partners. For overexpression of AFAP1-AS1 we created a vector based on pEYFP. It included regions of AFAP1-AS1 second exon with 2 overlapping regions with AFAP1. This construction was transfected to HEK293N. We showed significant overexpression, but no change in AFAP1 level. We also suggested that some mRNAs can interact with AFAP1-AS1 through Alu-repeat. This interaction may lead to degradation of both molecules by Stau-mediated decay (SMD). These partners must have an Alu-repeat in 3'-UTR, express in HEK293N and K562, and have an
alignment length of 130 bp. qPCR after AFAP1-AS1 knockdown showed significant increased level of 2 partners – PPIA and PAICS.

GENETICALLY ENGINEERING A PLASMID EXPRESSION VECTOR FOR NUCLEAR LOCALIZATION STUDIES, PART 1: ENGINEERING A 3GFP-3NLS PLASMID. Kristina Krumpos & Stephen Gallik, Dept. of Biol. Sci., University of Mary Washington, Fredericksburg, VA 22401. The long-term goal of this research project is to create a plasmid expression vector that can be used by future students to study the nuclear localization of proteins and the nuclear localization signal (NLS). Due to its relatively high molecular weight and its natural fluorescence, a fusion protein consisting of 3 copies of green fluorescent protein (GFP) linked to a single NLS is an ideal reporter protein for such studies. The specific objective of this study is to modify a commercially-available plasmid (pCMV/myc/nuc/GFP, ThermoFisher) that contains 1 copy of a GFP open reading frame (ORF) linked to 3 tandemly-arranged copies of an NLS, to create a new plasmid containing 3 copies of a GFP ORF linked to the 3 copies of the NLSs (3GFP_3NLS). Once created, future studies would remove 2 of the NLSs to create the desired final 3GFP_1NLS plasmid. To create the 3GFP_3NLS plasmid, a 48 bp linker (spacer) was first inserted upstream of the parent GFP ORF. A second copy of the GFP ORF was then added upstream of the linker, creating an intermediate 2GFP_3NLS plasmid. A 24 bp linker (spacer) was then added upstream of the second GFP ORF, followed by a third copy the GFP ORF, added upstream of the 24 bp linker, thus creating the 3GFP_3NLS plasmid. Single pass DNA sequencing (ACGT, Inc) confirmed the successful insertions of the linkers and the GFP ORFs. The protein expressed by the 3GFP_3NLS plasmid is expected to accumulate in the nucleus via the classic nuclear import process, to be confirmed in transfected HEK cells with fluorescence microscopy.

MOLECULAR MODELS OF AGING: COMPARATIVE ANALYSIS OF GENE SIGNATURES IN REPLICATIVE SENESCENCE AND STRESS INDUCED PREMATURE SENESCENCE. Kamil C. Kural & Ancha V. Baranova, School of Systems Biology, George Mason University, Manassas VA 20110. Senescence is defined as terminal phase of human cell populations. It can happen naturally termed as replicative senescence or form as a consequence of external challenges such as oxidative stress, radiation, activated oncoproteins and others termed as stress induced senescence. It is widely believed that the mechanisms at work for entering senescence provides a protection against cancer formation and suppresses tumor formation. Therefore, further identification is necessary to solve the mysteries behind
senescence. The goal is to find the differentially expressed genes in replicative and stress induced senescence in human fibroblast cells using geneXplain bioinformatics software platform. By doing so, we are hoping to highlight the important mechanisms underlying the causes between these two phenomena and identify the elements responsible for the process. Our approach was to classify the differentially expressed genes by their functions to predict the involvement of these genes in various cell cycle processes and different pathways involved in senescence. The list of genes which are exclusively up and down regulated in stress induced senescence and replicative senescence are compared at first step. These unique genes between two types of senescence are then used to perform promoter analysis to get potential transcription factor (TF) binding sites in combination with upstream analysis to predict master regulators that control the activity of these transcription factors.

THE EFFECT OF TEMPERATURE SENSATION ON ACUTE TOLERANCE TO ALCOHOL IN C. ELEGANS. Maroua Lahrach, Laura Mathies & Jill Bettinger, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Alcohol is a widely used drug, and 17 million Americans have an alcohol use disorder, a pathological pattern of alcohol use. The acute mechanisms that contribute to alcohol’s effects on human behavior are uncertain. We use Caenorhabditis elegans to understand the effects of alcohol as well as the development of acute functional tolerance (AFT) because C. elegans and mammals experience similar effects of alcohol at equivalent tissue concentrations, suggesting that there are similar drug targets in these animals. We tested how temperature sensitivity affects the worm’s initial sensitivity and AFT levels. We observed that wild-type animals demonstrate different levels of AFT if they are reared at different cultivation temperatures and tested at 20°C. At 15°C, animals show no significant AFT, while at 20°C, high AFT levels are exhibited. We hypothesized that animals reared at 15°C may have experienced heat shock while being tested at 20°C, and this disrupted AFT. To test this, we examined temperature response of hsf-1 animals. hsf-1 is a heat shock transcription factor that is used as one method of temperature sensation, and hsf-1 mutant animals are defective in temperature sensation. Results demonstrate that both initial sensitivity and AFT is temperature dependent, the wild types that were observed at both 20°C and 15°C differed in their adaptability to alcohol. Animal’s reared at 15°C were unable to develop AFT compared to those reared at 20°C. Although we cannot conclude that temperature sensitivity contributes to different AFT levels, we can predict that alcohol may
have an effect on many lurking variables including temperature change. More tests should be conducted to gain a better understanding on the significance gene hsf-1 has on the development of AFT; Absence of this gene might lead to improvement in AFT levels and also providing evidence that sensation of temperature is one of many factors that affects AFT.

MOTIF ENRICHMENT ANALYSIS FOR DIFFERENTIALLY EXPRESSED GENES IN ALZHEIMER’S DISEASE IDENTIFIES SREBF2 AS POSSIBLE TRANSCRIPTIONAL REGULATOR. John T. Lawson, Rachel C. Bordelon, Bria E. Johnston & Gary D. Isaacs, Department of Biology and Chemistry, Liberty University, Lynchburg VA 24515. Alzheimer’s disease (AD) is a complex neurodegenerative disorder that affects over five million Americans every year. A previous study generated a list of genes found to increase or decrease in expression in hippocampus from an AD mouse model compared to normal. It is possible that differential expression in AD could be due to altered functionality or availability of important transcription factors (TFs). To identify TFs that may regulate the genes differentially expressed in AD, proximal promoters (-1900 to +100 relative to the transcription start site of the most 5' promoter of each gene) were analyzed using the Analysis of Motif Enrichment program, which is part of the MEME Suite toolset, for relative enrichment of certain TF motifs compared to the promoters of random, unchanging hippocampal genes. Seven statistically significant (alpha = 0.05) TF motifs were found to be enriched in the upregulated gene list, including the motif for SREBF2, which was associated with 148 of the upregulated genes. Two of these upregulated genes, Gfap and Trem2, were selected for further study because both show significant change in expression in AD compared to normal, have been linked in literature with AD pathology, and have never before been shown to be regulated by SREBF2. Electrophoretic mobility shift assays (EMSAs) can be used to validate these putative DNA-TF binding interactions in vitro, potentially helping to elucidate mechanisms behind some of the differential expression in AD.

THE EFFECTS OF THE TREATMENT OF VARIOUS LIPID SPECIES ON MITOCHONDRIAL AND CELLULAR FUNCTIONS OF HEPATOCYTES. Peter M. Masschelin1,3, Katie Gwilliam2,3, Aybike Birerdinc1,3, Rohini Mehta3 & Ancha Baranova1,3, 1School of Systems Biology, George Mason University; 2 College of Science, George Mason University; 3 Center for Integrated Research, Inova Health Systems, Falls Church VA. Non-Alcoholic Fatty Liver Disease (NAFLD) is a chronic, progressive liver disease characterized by fatty infiltration of the hepatocytes and inflammation. Elucidation of the molecular mechanisms behind this disease can help our understanding
of how the disease progresses and potential treatments. Treatment of hepatocellular carcinoma cells, HepG2, with different lipid species serves as a model system to understand molecular pathways involved in lipid accumulation in hepatocytes. Mitochondria plays central role in energy homeostasis. Thus, mitochondrial dysfunction will be measured at several levels along with assessment of intracellular lipid accumulation, cellular apoptosis and proliferation, epigenetic modifications and change in mitochondrial mass. The goal is to understand mitochondrial dynamics upon exposure to a lipid and glucose rich environment to help determine the cause of NAFLD.

THE INVOLVEMENT OF A DROSOPHILA MITOCHONDRIAL FISSION GENE IN OXIDATIVE STRESS RESISTANCE. Grayson Mast, Charise Garber, and Jeff Copeland, Department of Biology, Eastern Mennonite University, Harrisonburg, VA. While oxidative damage is known to play an important role in the aging process, the molecular mechanisms are still poorly known. To better understand the cellular response to oxidative stress, we screened the X chromosome in Drosophila melanogaster to find mutants resistant to elevated oxygen levels. A loss-of-function mutant in the mitochondrial fission gene CG7772 showed increased resistance to hyperoxia, but not to paraquat, another reactive oxygen species generator, nor starvation. CG7772 mutants also had a normal life span. Since mitochondrial fusion and fission is essential for spermatogenesis, and CG7772 is highly expressed in Drosophila testes, CG7772 mutants showed defects in male fertility.

CHARACTERIZATION BY DELETION OF GENES RELATED TO CARBON METABOLISM IN CRYPTOCOCCUS NEOFORMANS. Kirk Nickish1, Joshua Herts1, John R. Perfect2, & Michael S. Price1,2, 1Dept. of Biology and Chemistry, Liberty University and 2Dept. of Medicine, Duke University Medical Center. The mechanism by which C. neoformans recovers glucose metabolism is currently unknown. The goal of the presented project is to identify genes that show increased activity in the rescue mutant compared to the pyk1 mutant. Three genes in C. neoformans putatively related to recovery of glucose metabolism in pyk1 mutants will be knocked out and characterized. The characterization process will include testing for various auxotrophies and ability to grow on different carbon sources. Several markers of virulence in C. neoformans, such as melanin production, will be evaluated in the new strain. Two of the knockout constructs have been produced successfully. Both biolistics and electroporation have been used to insert the chimeric DNA into the cells. Negative controls have exhibited growth after treatment by electroporation, although neither
the positive nor experimental samples have produced viable colonies. This failure indicates that the procedure reported by Lin X. et al. may be incomplete, as the cells have been shown to be viable after treatment and the pJAF1 plasmid includes the selectable marker.

EXAMINING THE EFFECTS OF ALDOSTERONE ON THE EXPRESSION OF THE LINCRNA GAS5 IN IMCD3 AND MPKCCD CELLS. B. Nolan¹, M. L. Gumz² & D. L. Zies¹, ¹Department of Biological Sciences, University of Mary Washington and ²Department of Medicine, Division of Nephrology, Hypertension & Renal Transplantation, University of Florida College of Medicine. Hypertension is the number one risk factor for premature death worldwide, and yet many of the cases have no obvious underlying cause. Understanding the normal regulation of blood pressure may help determine these underlying causes and reduce hypertension rates and deaths. Aldosterone is a steroid hormone that binds to the mineralocorticoid receptor (MR) and acts as a primary blood pressure regulator. This research will explore the relationship between the long non-coding RNA growth arrested specific 5 (Gas5) gene and aldosterone. It was recently shown that Gas5 acts as a decoy for the glucocorticoid receptor and reduces its ability to activate target gene transcription in the presence of its steroid hormone, cortisol. Additional data suggests that Gas5 may do the same for aldosterone and MR. Preliminary data from our collaborator, Dr. Michelle Gumz, shows that aldosterone treatment of kidney cell lines increases the expression of Gas5 at 4 hours after treatment, but not at 24 hours. We hypothesize that aldosterone first acts to increase the expression of Gas5, but as Gas5 accumulates it will act as a decoy for aldosterone bound MR and thereby reduce its own expression. To test this hypothesis, mouse inner medullary collecting duct (IMCD3) cells and minipig kidney cortical collecting duct (mpkCCD) cells were grown in conditions that mimic their physiological state. The cells were treated with Aldosterone for 0, 4, 8, and 24 hours. After treatment total RNA was isolated and converted into cDNA. Quantitative real-time PCR (qRTPCR) was performed to measure the expression of Gas5, Sgk1, and actin. Our initial results suggest that aldosterone does not regulate Gas5 in IMCD3 cells at any time point but does have an effect on Gas5 expression in mpkCCD cells. Current work is aimed at replicating experiments to generate data necessary to determine statistical significance. Progress on these experiments will be reported. Further study will be necessary to understand the relevance of differences in gene regulation between these two kidney cell lines.
INTIMIN IS LIKELY USED TO CAUSE DISEASE DURING COMPETITION WITH COMMENSAAL *ESCHERICHIA COLI*. Dominique J. Richburg, Dept. of Biol. and Chem., Liberty University., Lynchburg VA 24515. The intimin gene in the Locus of Enterocyte Effacement (LEE) pathogenicity island is the primary attachment mechanism in *Citrobacter rodentium*. Intimin is a bacterial adhesin that attaches to the epithelial wall of the intestine. An intimin deletion mutant bacteria is used to study colonization and pathogenesis in the mouse gastrointestinal tract. Additionally, *Citrobacter rodentium* is an attaching/effacing pathogen, and a useful murine model in understanding Enterohemorrhagic *Escherichia coli* (EHEC) infection in humans. *E. coli* and *C. rodentium* cause gastroenteritis in humans and mice, respectively. *C. rodentium* is a murine pathogen commonly used to model gastrointestinal disease. Results have shown intimin and *C. rodentium* useful in causing disease during competition with commensal *E. coli*. By studying the mechanisms and genes involved in pathogenic adhesion in *C. rodentium*, it will be easier to find out a cure or treatment for illness cause by the before mentioned *E. coli* strains such as Crohn’s disease, ulcerative colitis and colonic tumorigenesis. (supported by: The Virginia Academy of Science).

THE ROLE OF ATRX DURING DEVELOPMENT IN *XENOPUS LAEVIS*. N.D. Taliaferro, S.E. Wahl & A.J.G. Dickinson, Dept. of Biology, Virginia Commonwealth University, Richmond VA 23284. Alpha thalassemia syndrome X-linked (ATR-X) is a rare recessive disease affecting multiple organ systems of the body. Symptoms include developmental delay, gastrointestinal defects, and alpha thalassemia. Facial deformities are common and can include microcephaly, a protruding tongue with drool, and improper positioning of the teeth of the upper jaw in relation to those of the lower jaw. This disease is caused by a defect of the ATRX gene, which lies on the X-chromosome. ATRX is a nuclear protein that localizes to nuclear compartments called PML bodies and pericentromeric heterochromatin, where it interacts with a component of heterochromatin, HP1. It is suggested, ATRX helps regulate the activity of other genes through a process known as chromatin remodeling, as well as regulate HB1 and HB2, which are necessary for hemoglobin production. To determine the role of ATRX in orofacial development, we examined expression of ATRX during development in the African clawed frog (*Xenopus laevis*). PCR, using primers we designed, was used to characterize ATRX mRNA. A commercially available ATRX antibody was used to investigate protein expression during development. Results demonstrate that ATRX is expressed at the mRNA level during
development, ATRX expression changes as development progresses, and ATRX protein expression increases during development. Additional time points and samples are needed to further characterize the expression of ATRX at the mRNA and protein levels.

IDENTIFYING PHENOTYPES IN OVEREXPRESSION OF PUTATIVE KINASES IN CRYPTOCOCCUS NEOFORMANS. Nicolas Terreri & Joshua Sellwood, Dept. of Biology and Chemistry, Liberty University, 1971 University Blvd, Lynchburg VA, 24502. Cryptococcus neoformans persists in the central nervous system via the utilization of carbon sources mainly from sugars like glucose. In a prior study assessing the role of pyruvate kinase in the central nervous system persistence by C. neoformans, we observed the delayed appearance of pyk1Δ colonies on glucose-containing medium. When the C. neoformans grew on glucose containing media, another possible kinase which activates only when the pyk1Δ suppressor mutant was deleted, was brought into question as the possible substitute for the pyk1 gene. The colonies appeared as one of three different morphotypes: filamentous, pseudohyphal, or yeast. Increased filamentation and haploid fruiting has been observed with overexpression of STE12 in C. neoformans. This observation leads us to believe that this gene coding for the kinase is located in the MAT locus of chromosome 5 in the C. neoformans Serotype A strain, H99. Due to the filamentous nature of many of the pyk1Δ suppressor mutants, We hypothesize that a partial genome duplication may be responsible for suppression of the pyk1Δ mutant glucose utilization phenotype, and that the likely area of duplication centers around the MAT locus on Chromosome 5. A possible genetic basis for the expression of a different kinase’s growth phenotypes will be assessed using novel molecular approaches. Different putative genes, found using various microarray data, will be amplified and transformed into Cryptococcus neoformans pyk1Δ gene mutants. Overexpression of the putative rescue genes in C. neoformans may show the same phenotypes of the original pyk1Δ mutant phenotypes of delayed growth on glucose containing media and a complete loss of virulence in the host.

EARLY NEUTROPHIL RECRUITMENT BY CHEMOKINE-RELEASING NANOPARTICLES IMPROVES BACTERIAL CLEARANCE AND SURVIVAL OF ANTHRAX-CHALLENGED MICE. Allison L. Teunis1,2, Taissia G. Popova1 & Serguei G. Popov2, 1Center for Applied Proteomics and Molecular Medicine, Department of Molecular Biology, School of Systems Biology, George Mason University, 2National Center for Biodefense and Infectious Diseases, School of Systems Biology, George Mason University. Nanomaterials capable of directing immune cell recruitment hold promise as novel tools for basic research and therapeutic applications. In this study,
hydrogel nanoparticles chemically coupled with Cibacron Blue affinity bait were used as carriers for the directed release of chemokines, human CXCL8 and mouse CCL3, to enhance migration of neutrophils and to improve outcome of anthrax infection in a mouse model. Mice were given a prophylactic dose of nanoparticles and challenged into footpads a few hours later with Bacillus anthracis Sterne 34F2 spores. Released chemokines induced a massive influx of neutrophils to the site of spore inoculation and regional lymph nodes resulting in reduced bacterial burden, decreased inflammatory response and up to 70% survival of mice over 13 days (p<0.0001). All untreated mice died within 4 days with a strong inflammation of footpads. Stimulation of neutrophil chemotaxis with nanoparticle-released chemokines may be considered as a novel strategy to treat anthrax. This work was supported by the grant 1R21AI117425-01 from the National Institutes of Health, USA.

GENETICALLY ENGINEERING A PLASMID EXPRESSION VECTOR FOR NUCLEAR LOCALIZATION STUDIES, PART 2: ENGINEERING A 2 GFP-0NLS AND A 3GFP-0NLS PLASMID. Rachel Thomas & Stephen Gallik, Dept. of Biol. Sci., University of Mary Washington, Fredericksburg, VA 22401. The long-term goal of this research project is to create a plasmid expression vector that can be used by future students to study the nuclear localization of proteins and the nuclear localization signal (NLS). Due to its relatively high molecular weight and its natural fluorescence, a fusion protein consisting of 3 copies of green fluorescent protein (GFP) linked to a single NLS is an ideal reporter protein for such studies. In part 1 of this project, plasmids containing 2 and 3 copies of a GFP open reading frame (ORF) linked to 3 tandemly-arranged copies of an NLS (2GFP_3NLS and 3GFP_3NLS, respectively) were created from a commercial plasmid. The specific objective of part 2 of this project is to remove the 3 NLSs from the 2GFP_3NLS and 3GFP_3NLS plasmids, to create plasmids containing 2 GFP ORFs and 3 GFP ORFS, respectively, but lacking the NLSs (2GFP_0NLS and 3GFP_0NLS). Once created, a future study would insert a single NLS to create a 2GFP_1NLS plasmid and the desired final 3GFP_1NLS plasmid. To create the 2GFP_0NLS and 3GFP_0NLS plasmids, a NotI cut site was, in each case, inserted downstream of the 3 NLSs, sandwiching the NLSs between the new NotI cut site and an original NotI cut site located just upstream of the 3 NLSs. The 3 NLSs were then removed with NotI restriction digestion followed by ligation. DNA sequencing confirmed the successful creation of the 2GFP_0NLS, but not the 3GFP_0NLS. Slow diffusion of the fusion protein expressed by the 2GFP_0NLS plasmid in transfected HEK cells will be demonstrated by fluorescence microscopy.