

climate reconstruction from borehole data. Results indicate that uncertainties in the SAT time series and thermal parameterization of the ground are reduced by an order of magnitude, while uncertainties in the geotherm are magnified by an order of magnitude in the solution-space.

### **Biology** **with Microbiology and Molecular Biology**

THE EFFECTS OF BACTERIOPHAGE INFECTION ON *BACILLUS ANTHRACIS* DELTA STERNE AND *BACILLUS THURINGIENSIS*. Catherine A. Johnson & Lynn O. Lewis, Department of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401. *Bacillus anthracis* and *Bacillus thuringiensis* are soil dwelling bacteria that are capable of forming endospores. These bacteria go through the processes of sporulation and germination to form the endospores and vegetative cells. Both species of bacillus can be found in various soil samples, along with species of bacteriophages that target each bacterium. The goal of this research was to isolate one temperate bacteriophage for *Bacillus anthracis* Delta Sterne (Delta Sterne) and one temperate bacteriophage for *Bacillus thuringiensis* Al Hakam (Al Hakam) and then determine the effects the bacteriophages have on the sporulation and germination processes for Delta Sterne. A temperate bacteriophage was isolated for each bacillus species, with the one that infects Delta Sterne being called Texas 1 4CI. It was able to cross infect Al Hakam, but the Al Hakam temperate phage was unable to cross infect Delta Sterne. There were no apparent morphological differences between colonies of infected Delta Sterne and uninfected Delta Sterne through sporulation and germination. However, it appears that with successive sporulation and germination, the infectivity of the bacteriophage for Delta Sterne increases from 1 plaque at  $10^{-3}$  dilution, to 32 plaques at  $10^{-3}$  after a second sporulation and germination. (Supported by UMW Undergraduate Student Research Grant.)

CHARACTERIZATION OF MYCOBACTERIOPHAGE VENKMAN. Thien T. Phan & Lynn O. Lewis, Department of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401. Bacteriophage are abundant in the environment and have co-evolved with the bacteria they target. Through soil collected, we were able to have a better understanding of how mycobacteriophage interact with their host, *Mycobacterium smegmatis*. One bacteriophage (Venkman) was chosen for genomic sequencing. Restriction enzyme digests were performed to compare the phage's DNA to other known bacteriophage DNA. Electron microscopy was performed to examine the morphology of the Mycobacteriophage. By using different bioinformatics programs, we determined that the Mycobacteriophage Venkman is in the F cluster and within the F1 subcluster. The genome size is approximately 50 kbp – to 60 kbp, but is unfinished at this time. Further investigations are still being performed on the genome of Mycobacteriophage Venkman. (Supported by UMW Undergraduate Student Research Grant.)

POST TRANSLATIONAL MODIFICATION OF A BACTERIAL PROTEIN IN HUMAN CELLS. Moushimi Amaya, Ancha Baranova & Monique van Hoek, Department of Systems Biology, George Mason University, 10900 University Blvd., Manassas, VA 20110. This study is an on-going investigation into the post translational modification, the prenylation, of a *Francisella tularensis* protein in human cells. Prenylation is a lipid modification, whereby either a farnesyl or geranylgeranyl group is added to a nascent protein harboring a C-terminal CAAX motif, which results in subsequent targeting of the mature protein to the cell membrane. *F.tularensis* is a gram negative bacterium that causes the disease tularemia and can potentially be used a bioweapon due to its low infectivity dose (10-15 organisms) upon inhalation. Bioinformatical analysis using the Prenylation Prediction Suite program revealed a single *F.tularensis* protein that could potentially undergo prenylation. The proposed prenylated protein is 13kDa in size and is of unknown function. Human alveolar lung epithelial cells (A549) are the model system chosen to test for prenylation of the *Francisella* protein. Preliminary Western blot experiments have confirmed that the protein of interest is expressed in A549 cells. We aim to determine if prenylation does indeed occur in A549 cells. We further aim to investigate, by use of inhibitors, which type of prenylation is undertaken: farnesylation or geranylgeranylation. We also aim to determine the localization of the potentially prenylated *Francisella* protein in A549 cells by employing confocal microscopy analysis.

FUNCTIONAL GEMOMIC ANALYSES REVEAL COMPLEX TRANSCRIPTIONAL REGULATORY NETWORKS MEDIATING DENDRITIC ARCHITECTURE. Eswar Prasad R. Iyer<sup>1,2</sup>, Srividya Chandramouli Iyer<sup>1,2</sup>, Ramakrishna Meduri<sup>1,2</sup>, Dennis Wang<sup>1,2,3</sup>, and Daniel N. Cox<sup>1,2†</sup> School of Systems Biology, George Mason University, Manassas, VA 20120, USA,<sup>2</sup>Krasnow Institute for Advanced Study, George Mason University, Fairfax, VA 22030, USA, <sup>3</sup>Present Address: Yale University, New Haven, CT 06520, USA Elucidating the molecular mechanisms controlling dendrite development is key to understanding the pivotal role these structures play in influencing synaptic integration and neural function. Despite significant advances in this field, genetic pleiotropy remains a significant impediment to investigating such complex developmental processes. To circumvent this problem, we have applied class specific neuron transcriptional expression profiling coupled to an *in vivo* RNAi functional validation screen in order to dissect the molecular bases of *Drosophila* class IV dendritic arborization (da) neuron dendritogenesis. Microarray analyses reveal transcriptional regulation as one highly enriched biological and functional category with 420 transcription factors significantly expressed in class IV neurons. Among these, we identify roles for 268 genes in mediating a broad spectrum of functions including dendritic field coverage, branching, routing, and tiling. Collectively, our analyses provide a more comprehensive framework of the role complex transcriptional networks play in directing distinct aspects of class specific dendrite morphogenesis.

CUT MEDIATED TRANSCRIPTIONAL REGULATION OF THE COPII SECRETORY PATHWAY DIRECTS CLASS SPECIFIC DENDRITE MORPHOGENESIS IN *DROSOPHILA*. Srividya C. Iyer, Eswar P.R. Iyer,

Ramakrishna Meduri, Madhu Karamsetty, & Daniel N. Cox, School of System Biology, Krasnow Institute for Advanced Study, George Mason University, Fairfax VA 22030. Elucidating the molecular mechanisms controlling dendrite development is key understanding how neuronal morphologies arise and how they function in achieving synaptic integration and neuronal function. Recent studies demonstrate select secretory pathway genes act in preferentially affecting dendritic growth. Phenotypic analyses of *sec31* mutants reveal a reduction in dendritic branching implicating the COPII secretory pathway in regulating dendritic complexity. Furthermore, gain-of-function (GOF) analyses indicate *sec31* differentially effects dendritic complexity in distinct da neuron subclasses. Microarray analyses, quantitative RT-PCR and immunohistochemistry experiments reveal that overexpression of the homeodomain transcription factor Cut upregulated expression levels of the COPII-mediated secretory pathway genes as well as another key transcription factor, CrebA. Moreover, simultaneous expression of Cut coupled with RNAi knockdown of *CrebA* suppressed the Cut GOF phenotype indicating that CrebA functions as a downstream effector of Cut mediated transcriptional regulation in da neurons. Consistent with this regulatory relationship, overexpression of CrebA in da neurons likewise leads to higher expression levels of components of ER-to-Golgi transport. Collectively, these findings provide novel insight into the role of transcriptional regulation of the COPII-mediated secretory pathway in mediating class specific dendrite morphogenesis.

NEUROPEPTIDE AF-INDUCED ANOREXIA IS ASSOCIATED WITH CHANGES IN HYPOTHALAMIC CHEMISTRY IN SPRAGUE-DAWLEY RATS. Brandon A. Newmyer and Mark A. Cline. Dept of Biol, Radford University, Radford VA 24142. We recently demonstrated that NPAF's anorectic effect is associated with changes in hypothalamic chemistry in nuclei associated with satiety perception in Cobb-500 chicks. In order to elucidate whether this effect was conserved through divergent evolution, NPAF was centrally administered to Sprague-Dawley rats and food intake as well as NPAF-associated changes in brain chemistry were observed. NPAF reduced food intake in rats at similar doses and magnitudes as it did in chicks and also affected hypothalamic chemistry. Similar to chicks, central NPAF was associated with increased neuronal activation in the magnocellular region of the paraventricular nucleus. These data support that NPAF's anorectic effect is conserved in a mammalian model and thus may be a logical target utilize in the treatment of human eating disorders.

EFFECTS OF COMBINED VITAMIN C & E TREATMENT ON PLAQUE FORMATION IN ALZHEIMER'S DISEASE. Anum K. Shaikh & Deborah A. O'Dell, Dept. of Biol., University of Mary Washington, Fredericksburg VA 22401. Alzheimer's disease (AD) is characterized by the inflammation and  $\beta$ -amyloid plaques in the brain. The improper cleavage of amyloid precursor protein (APP) which leads to  $\beta$  amyloid plaques may result from inflammation. We studied the effects of these Vitamins E and C on inflammation and plaque formation in a transgenic mouse model of AD. Mice were divided into three experimental groups that received Vitamin C, Vitamin E or both Vitamin C and E for a period of 34 weeks beginning at 14 weeks of age. Levels of tumor necrosis factor alpha (TNF- $\alpha$ ) and

$\beta$ -amyloid in homogenized brain preparations were measured using a direct ELISA method. The number and size of the plaques in sections of formalin fixed and paraffin embedded cerebral cortex was compared between the groups. The levels of  $\beta$ -amyloid were marginally significantly lower in the combined Vitamin C and E treatment group compared to the control ( $p=0.056$ ). Vitamin E treatment alone did not lower  $\beta$ -amyloid relative to the control. Vitamin treatments also show reduced levels of inflammation as measured by levels of TNF- $\alpha$  compared to the control with Vitamin C and Vitamin C&E showing the lowest levels. There was no significant difference noted in comparison of the number and size of plaques between all the groups. These results show that Vitamin C and E may afford the greatest protective effects against AD. (Supported by: Chi Beta Phi Honorary Society, The Virginia Academy of Science, Univ. of Mary Washington Undergrad Research Funds).

A SYSTEM GENOMICS APPROACH FINDS CANDIDATE GENES FOR NON-INSULIN DEPENDENT DIABETES MELLITUS. Lataisia Jones & Glenn C. Harris, Dept of Biology, Virginia State University, VA 23806. Non-insulin dependent diabetes mellitus (NIDDM) is one of the most significant chronic human diseases, affecting over 20 million people in the United States (7% of the population). NIDDM is associated with obesity and characterized primarily by insulin resistance and impaired insulin production. Evidence has been building that NIDDM is a multifactorial complex disease, with many different genes and gene-gene and gene-environment interactions potentially contributing to the physiological symptoms. Here we demonstrate a systems genomics approach to finding likely candidates for NIDDM. A review of linkage analysis studies found a region on human chromosome 01 significantly associated with NIDDM symptoms. Syntenic regions in rats and mice were identified and subjected to a single nucleotide polymorphism (SNiP) analysis. Only one gene was found in the mouse and rat syntenic regions that exhibited functional SNiPs in symptomatic models. A functional SNiP was defined as producing an amino acid change in the resulting protein. The gene, *Tchhl1*, has not been previously implicated as a candidate gene for NIDDM or obesity. Future projects are described that will attempt to characterize the function of *Tchhl1* in NIDDM phenotypes.

CHROMATIN STATE MAPPING IN NEUROBLASTOMA CELLS IDENTIFIES GENES POTENTIALLY IMPORTANT FOR MAINTAINING PLURIPOTENCY. Shaili Shah & Melissa A. Henriksen, Dept. of Biol., University of Virginia, Charlottesville VA 22903. Neuroblastoma is a common childhood cancer that arises from the neural crest. The clinical diversity seen in neuroblastoma is due to its cellular heterogeneity. The Intermediate (I-type), highly malignant, cell differentiates into both a non-malignant (S-type) cell and a less malignant Neuroblastic (N-type) cell. The multipotent I-type cell has been identified as a cancer stem cell due to its ability to self-renew and differentiate. Since epigenetic mechanisms underlie the phenotypic differences among these three cell types, chromatin state mapping was used to identify potential bivalent genes involved in cancer stem cell multipotency and differentiation. Of the 6 genes identified, one gene, *MSX2*, was examined further due to its known role in triggering apoptosis during neural crest development. Since this gene was not expressed in I-type cells, the potentially inappropriate silencing of

this gene was reversed by over-expression in I-type cells. Ongoing experiments focus on using inducible lentiviral based methods to infect the cancer stem cell and quantify cell death in the cancer stem cell in comparison to the other, differentiated, neuroblastoma cells. These preliminary over-expression experiments suggest that MSX2 may also trigger apoptosis of the cancer stem cell. The aberrant chromatin pattern at MSX2's promoter may cause inappropriate gene silencing, and future experiments will determine how crucial this gene is for maintenance of the neuroblastoma cancer stem cell. Understanding the epigenetic mechanisms that govern genes implicated in cancer stem cell maintenance may offer novel therapeutic targets for cancer treatment.

MELANOMA-ASSOCIATED SUPPRESSION OF DENDRITIC CELL MATURATION/ACTIVATION DEPENDS ON TUMORIGENICITY. Kristian M. Hargadon, Osric A. Forrest, & Pranay R. Reddy, Dept. of Biol., Hampden-Sydney College, Hampden-Sydney VA 23943. The accumulation of data demonstrating immunity to cancer over the last two decades has sparked significant interest in the field of tumor immunology. However, while the existence of anti-tumor immunity is promising, tumor cells have frequently been reported to induce anti-tumor immune dysfunction. Despite the overwhelming significance of this problem, the basis for this immune dysfunction is often poorly understood. Dendritic cells play critical roles in both innate and adaptive immunity, and their numerous functions are tightly linked to their maturation and activation status. Here, we characterize the murine dendritic cell line DC2.4 as a model for studying dendritic cell maturation and activation, and we evaluate the influence of melanoma tumor cells on these processes. Exposure of DC2.4 cells to the Toll-like receptor ligand lipopolysaccharide induces both maturation and activation of these cells, characterized by upregulation of costimulatory molecule expression and proinflammatory cytokine/chemokine production. This maturation and activation is suppressed by soluble factors derived from both the highly tumorigenic B16-F1 and the poorly tumorigenic D5.1G4 murine melanoma cell lines. Interestingly, the extent of DC2.4 immunosuppression by these melanomas correlates with their tumorigenicity, suggesting a vital role for dendritic cell/tumor cell interactions in the regulation of anti-tumor immunity and tumor outgrowth.

PATTERNS OF TRANSIENCY, SEX BIAS, AND BODY WEIGHT IN OPEN-HABITAT RODENT POPULATIONS. Stephen E. Rice & Robert K. Rose. Department of Biological Sciences, Old Dominion University, Norfolk, Virginia 23529-0266. As the most numerous and diverse order of the Class Mammalia, rodents often have served as a common basis for study and modeling biological concepts. Dating from the writings of Robert Collett in the late 19th Century, rodents are believed to live their lives within a small area, with juvenile males the most likely to disperse. To test these assumptions we examined long-term capture-mark-release data from grid populations with attention to the weights at first capture and the transiency/residency patterns of herbivorous rodents studied in old-fields in Virginia, Illinois, and Kansas. Although few studies report the proportions or sexes of juveniles among first-captured animals, many studies have indicated that nearly half of tagged rodents are never seen again. In order to

evaluate the evidence that different species of rodents are philopatric, we examined the sex ratios and body weights of newly tagged old-field rodents and their patterns of transience or residency. Transience was associated with season for Virginia and Kansas cotton rats. Both populations had significant proportions of adults and displayed an association of population groups. Animals in breeding condition were male-biased for Virginia and Kansas. The breeding individuals in Virginia were independent of residency status, whereas Kansas breeders were associated with a high level of transience, 59.33%. Transience was found to be significant for the Virginia population, 54.41%, but not for the Kansas population, 51.00%. No difference in body weight was found between residents/transients for either population. Seasonal sex biases were found in both populations.

THE LIFE HISTORY FEATURES AND ECOLOGY OF *TENODERA SINENSIS* AND *T. ANGUSTIPENNIS* MANTISES IN EASTERN VIRGINIA. Cory A. Gall & Robert K. Rose, Department of Biology, Old Dominion University, Norfolk, VA 23529. Praying Mantises, *Tenodera sinensis* and *T. angustipennis*, are generalist predators located at the top of the arthropod food chain. These species inhabit eastern North America, and are common among old-field vegetation. Virginia is considered the southern limit of their natural range, with few field studies of these two mantises coexisting. My study was conducted on the Wildlife Refuge located on Virginia's Eastern Shore and is focused on their life history, movement, and species interaction in this region. Previous studies have shown that *T. sinensis* hatches earlier in the spring, giving it the ability to become larger in size than *T. angustipennis*. My initial results support these findings. In addition, I investigated the species ratio and sex ratio for both species throughout their lifecycle. I found that *T. sinensis* dominated for the majority of the year, but at the end of fall the species ratio was nearly 50:50. Past research has indicated *T. sinensis* and *T. angustipennis* hatch with 50:50 sex ratios; however, male presence are found to be greater until sexual maturity is reached, then females become more numerous. However, my results illustrate that *T. sinensis* and *T. angustipennis* sex ratios fluctuated throughout the life cycle, with *T. sinensis* and *T. angustipennis* females are dominating at different times in their growth season. My study also supports past research that females are more stationary to measured plots, while males often moved off measured plots.

A SMALL MAMMAL COMMUNITY IN A CHANGING LANDSCAPE IN SOUTHEASTERN VIRGINIA, 2005-2011. Jana Eggleston & Robert K. Rose. Department of Biological Sciences, Old Dominion University, Norfolk, VA 23508. In 2005 we began a monitoring program of the small mammal community on an old field site in southeastern Virginia. This site is a part of the Nature Conservancy Stewardship of lands adjacent to the Great Dismal Swamp National Wildlife Refuge, and a part of a greater wetland habitat restoration plan. An assortment of trees was planted on the site, drainage was altered, and vegetation succession was allowed to progress. We hypothesized this small mammal community would change from herbivorous old field species to those of forested wetlands, and that the numerically dominant species would change as succession progressed. Our study site was an 8 x 8 grid, with 12.5m intervals, and with two modified Fitch traps per station. We trapped for three days each month, averaging 4600 trap nights per year. Results to

date show the general decline of all old field species, the virtual disappearance of *Mus musculus* and *Oryzomys palustris*, stable densities of *Reithrodontomys humulis*, a shift in dominance from *Microtus pennsylvanicus* to *Sigmodon hispidus*, and most recently, the arrival of *Peromyscus leucopus*, a forest species.

THE EFFECTS OF VISUAL AND CHEMICAL CUES ON MALE MATE CHOICE IN EASTERN MOSQUITOFISH (*GAMBUSIA HOLBROOKI*). Nouman J. Rana & Lisa Horth, Dept. of Biol. Old Dominion Univ., Norfolk, VA 23529. Previous studies have illustrated that pheromones released by gravid females western mosquitofish (*Gambusia affinis*) increased male sexual activity during mating selection. However, little is known about the activities during mating selection in eastern mosquitofish (*Gambusia holbrooki*). In this study, we conducted experiments to determine (1) whether pheromones from gravid female mosquitofish induce different male activities as compared to non-gravid females and (2) if male mosquitofish prefer both a chemical and visual cues during mate selection. Our preliminary results indicate that pheromones released by gravid females are different from non-gravid females. In addition, gravid females induce a more active response from males during mate selection than non-gravid females. Also, males prefer to have both visual and chemical cues during the mate selection process. These results suggest that males are more likely to pick a female that they can visually see and one that has recently given birth as compared to one that cannot be seen and has not recently given birth.

EXPLORING THE ROLE OF THE C-TERMINAL REGION OF APQ12 IN THE REGULATION OF CELL CYCLE PROGRESSION AND CELL SEPARATION. James R. Oliver & Michael J. Wolyniak, Department of Biology, Hampden-Sydney College, Hampden-Sydney, VA 23943. The *Saccharomyces cerevisiae* peripheral nuclear membrane protein Apq12 is believed to play a critical role in the regulation of membrane fluidity and, ultimately, the way in which cells divide and separate. To further investigate the proposed role of Apq12 in these processes, we performed a series of site-directed mutagenesis experiments that attempted to delete the transmembrane domains of the protein as well as generate C-terminal truncations via nonsense point mutations. This allowed us to investigate if there was a specific domain of the Apq12 sequence that is necessary for its putative role in the regulation the cell cycle processes and cell separation. Our findings indicated that while Apq12 is, as a whole, a dispensable protein with respect to cell viability, the C-terminal region of the protein may provide a fine-tuned regulation of cell morphology, with different domains of this region acting in an antagonistic relationship to determine cell shape. Since we also found that cells deleted for Apq12 were slower in overall growth rates, our findings suggest that the C-terminal region of Apq12 may contribute to the efficiency of yeast cell cycle progression through the regulation of cell shape during the cell division process.

ISOLATION AND CHARACTERIZATION OF A NEW *CAENORHABDITIS* SPECIES TO BE USED IN EVOLUTIONARY AND DEVELOPMENTAL STUDIES. Borwyn A. Wang, Mejgan Mukhtarzada, Erin Haynes & Theresa M. Grana, Department of Biological Sciences, University of Mary Washington,

Fredericksburg VA 22401. The worm community of researchers is actively seeking a sister species for comparative genomics analysis for the identification of a *C. elegans* sister species. The purpose of this project was to isolate, identify, and characterize several new *Caenorhabditis* species that are phylogenetic relatives of the model organism *Caenorhabditis elegans*. Soil samples were collected in Fredericksburg, VA as well as in King George County in which nematodes were isolated and stocks were established on agar plates. Isolated nematodes were tested for model organism characteristics such as the ability to survive freezing, starvation, and male/female/hermaphrodite classifications. Nomarski microscopy was used to film mouth parts, pharyngeal bulbs, vulvas, and tails to further compare each species. Additional studies include filming embryo development and sequencing a part of the rDNA encoding the 18s ribosomal subunit to construct phylogenetic trees. This study was supported by UMW Undergraduate Research Grants and a VAS undergraduate grant.

EFFECT OF CpG OLIGONUCLEOTIDE AND LL-37 ON PROSTATE CANCER CELL GROWTH AND INVASION. April Lao<sup>1</sup>, Angela Gupta<sup>1</sup>, Maria Craig<sup>2</sup>, & Paul Deeble<sup>1</sup>, Departments of <sup>1</sup>Biology and <sup>2</sup>Chemistry and Physics, Mary Baldwin College, Staunton, VA 24401. CpG oligonucleotides, short pieces of DNA with characteristics of bacterial DNA, have recently been considered as an alternative to chemotherapy. Some studies found CpGs to stimulate anti-tumor immune responses with none of the side effects associated with traditional chemotherapy. However, CpGs effects are weak, and previous results are contradictory, with anti-tumor effects seen in ovarian cancer in mice but increased invasion and decreased growth in PC-3, LNCaP, and DU-145 prostate cancer cell lines. LL-37 is an antimicrobial DNA-binding peptide that increases the body's immune response to CpG. LL-37 is found throughout the body and in higher amounts in breast, lung, and prostate cancer tumors. The combination of CpG and LL-37 has recently been shown to enhance the anti-tumor effects seen with CpG in ovarian cancer in mice. In this project, the effect of CpG in combination with LL-37 on prostate cancer cell growth and invasion was investigated. The addition of LL-37 was hypothesized to enhance the effects seen with CpG (i.e. decreased growth and increased invasion). Experiments utilized androgen-independent PC-3 cells and androgen-responsive LNCaP cells. Results indicate the combination of CpG and LL-37 increases growth in PC-3 cells and increases invasion in LNCaP cells. These results could indicate that CpG oligonucleotides in combination with LL-37 should not be used as a therapy against prostate cancer.

NEUROENDOCRINE DIFFERENTIATION AND ITS EFFECTS ON CHEMORESISTANCE IN PROSTATE CANCER CELLS. Alex Kelly, Sophia Stone, Caitlin Combs, & Paul Deeble, Department of Biology, Mary Baldwin College, Staunton, VA, 24401. Neuroendocrine (NE) differentiation, induced by forskolin (Fsk) and 3-isobutyl-1-methylxanthine (IBMX), was analyzed in a prostate cancer progression model using androgen-responsive LNCaP and androgen-independent PC-3 prostate cancer cells. NE differentiation was quantified by measuring neuritic branch points and mean process length, and LNCaP cells were



found to exhibit a higher level of NE characteristics when compared to PC-3 cells. Cell viability in response to various chemotherapeutics, including Docetaxel and Rapamycin, was measured in undifferentiated and NE differentiated cancer cell lines using an MTT assay. NE differentiation was found to protect LNCaP cells from cell death induced by chemotherapeutic agents.

EFFECTS OF PHENOLIC ACIDS ON OVIPOSITIONAL SELECTION IN THE CABBAGE WHITE BUTTERFLY, *PIERIS RAPAE*. Jessica L. Bray, Mary E. Lehman & Amanda J. Lentz-Ronning, Department of Biological and Environmental Sciences, Longwood University, Farmville VA 23909. *Pieris rapae* oviposit on plants that are most suitable for larval growth and survival. The presence of a variety of plant secondary metabolites may be one factor in determining ovipositional choices. Phenolic acids are secondary metabolites that are widespread among plants, but their effects on *Pieris rapae* have not been well characterized. Wisconsin Fast Plants were treated with four different phenolic acids (1.0 mM concentrations) to see if they would deter or stimulate oviposition. Female butterflies were provided a choice of whether to lay eggs on the control plants sprayed with deionized water or the plants sprayed with a phenolic acid. *p*-coumaric acid significantly stimulated oviposition, whereas salicylic acid and protocatechuic acid had no significant effect. With the removal of one extreme outlier from the data set, the stimulatory effect of ferulic acid was also significant. Ferulic acid and *p*-coumaric acid are very similar in chemical structure and preliminary experiments suggest that *p*-coumaric acid increases larval growth.

EFFECTS OF HOST PLANT ALLELOCHEMICAL AND NUTRIENT STATUS ON THE CABBAGE WHITE BUTTERFLY, *PIERIS RAPAE*. Kristen, S. Walker, Mary E. Lehman & Amanda J. Lentz-Ronning, Department of Biological and Environmental Sciences, Longwood University, Farmville VA 23909. *Pieris rapae* is known to detect the chemical content of plants that would be best suited for offspring development. Plant allelochemical and nutritional status were manipulated to determine the main and interactive effects on ovipositional choices of *Pieris rapae*. Wisconsin Fast Plants were supplied with Hoagland's nutrient solution at concentrations of 1/2X (control) or 1/8X (reduced nutrient treatment). Plants were sprayed with deionized water (control) or 1.0 mM *p*-coumaric acid (PCO; allelochemical treatment). The effects of PCO and nutrient concentration were first assessed individually and then in an interaction experiment. Females and plants were placed in light boxes and eggs were counted after a 24- hour period. A significantly higher number of eggs were deposited on the 1/2X control plants (mean  $\pm$  SE:  $21.7 \pm 5.5$ ) compared to the 1/8X reduced nutrient treatment ( $9.5 \pm 2.8$ ). Females oviposited significantly more eggs on the PCO-treated plants ( $35.3 \pm 3.7$ ) than on the DI water controls ( $25.2 \pm 4.4$ ). In the interaction experiment, only the main effect of nutrient concentration was significant. PCO's lack of significance and the lack of a significant interaction may be due to the low number of replicates (N=6). Overall, results suggest that female *Pieris rapae* can distinguish the nutrient status of plants and that this is a stronger determinate of ovipositional choice than host plant allelochemicals.

THE ROLE OF INTERFERON GAMMA IN EXPRESSION OF KLF4 IN LUNG FIBROBLASTS. H. Rushdi<sup>1</sup>, E. Quraishi<sup>1</sup>, S Qureshi<sup>1</sup>, J. Forrest<sup>1</sup>, S.D. Nathan & G Grant<sup>1</sup>, <sup>1</sup>SSB GMU, Manassas VA, <sup>2</sup>IHVI Inova Fairfax, Falls Church, VA. Krüppel like 4 Factor (KLF4) is a member of the zinc finger transcription factor family which is involved in cells' transcriptional response to multiple critical stimuli, such as DNA damage, growth stimulation and differentiation. KLF4 activity is cell-type dependent acting as both an inducer and repressor of gene expression. It is predominantly associated with post-mitotic terminally differentiated/ing cells, playing a major role in cell homeostasis by induction of p21 and inhibition of p53. However KLF4 can also act as a tumor promoter in cells that have bypassed the p21 checkpoint. Idiopathic pulmonary fibrosis (IPF) is a terminal lung disease propagated by the fibroblasts. IPF fibroblasts over-express KLF4 *in vivo*. Therefore we investigated the potential role of KLF4 overexpression in IPF by inducing its expression with Interferon gamma (INF $\gamma$ ). IPF and normal fibroblasts were seeded at  $2.5 \times 10^5$  cells per 60 mm dish in 10% DMEM at 37°C in a 5% CO<sub>2</sub> incubator. Cells were serum starved for 16 hours to synchronize prior to addition of INF $\gamma$  at 0, 200 and 400 Units per ml for 24 hours. RNA was extracted (RNeasy Kit Qiagen) and cDNA generated (iscript BioRad) from 1 $\mu$ g of total RNA. Real Time Quantitative PCR was carried out for KLF4 induction using ribosomal 18S as a normalization control. INF $\gamma$  exposure of IPF and normal fibroblasts resulted in increased expression of KLF4 protein which may influence the proliferative and survival behavior of these fibroblasts in the IPF lung.

INVESTIGATIONS OF THE EFFECT OF THYMOSIN-BETA4 ON LUNG FIBROBLAST SURVIVAL. S Qureshi<sup>1</sup>, J. Forrest<sup>1</sup>, H. Rushdi<sup>1</sup>, E. Quraishi<sup>1</sup>, S.D. Nathan<sup>2</sup> & G Grant<sup>1</sup>. <sup>1</sup>SSB, GMU, Manassas VA. <sup>2</sup>IHVI Inova Fairfax, Falls Church VA. Idiopathic Pulmonary Fibrosis (IPF) is a fatal lung disease, with no therapy and no cure. IPF is propagated by over abundant pulmonary fibroblasts which deposit excessive extracellular matrix (ECM) causing damage to the alveoli. The excessive ECM interferes with gaseous exchange and ultimately results in organ failure. Thymosin Beta 4 (T $\beta$ 4) is a tiny cellular protein with hormone like properties. Initially identified as a cytoskeletal protein, it has been recently identified with wound healing, cell migration, angiogenesis, and anti-apoptosis. T $\beta$ 4 is upregulated in IPF cells compared to normal lung fibroblasts. In this experiment we investigated the effect of T $\beta$ 4 to prevent hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) induced apoptosis in primary IPF and normal fibroblasts. IPF and normal fibroblasts were exposed to lethal doses of H<sub>2</sub>O<sub>2</sub> in the presence and absence of T $\beta$ 4 for 24 hours in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum in a 96 well format. After 24 hours of exposure cell survival was determined by the Acid Phosphatase and MTT cell proliferation assay. T $\beta$ 4 exposure increased cell survival in both normal and IPF cells. Results indicate that T $\beta$ 4 has the potential to play a significant role in preventing apoptosis in IPF lung fibroblasts. This cytoprotective property of T $\beta$ 4 may contribute to the overall abundance of fibroblasts in the IPF lung, a hallmark and contributing factor of this disease. Further investigations into the action of this protein may lead to therapeutic and biomarker discoveries which may aid in preventing and treating IPF.

THE *DROSOPHILA* SPECTRAPLAKIN SHORT STOP DIFFERENTIALLY REGULATES CLASS SPECIFIC DENDRITE MORPHOGENESIS. James Boddu, Sarah A. Trunnell, & Daniel N. Cox, School of System Biology, Krasnow Institute for Advanced Study, George Mason University, Fairfax VA 22030. Disorders of the nervous system can often be attributed to developmental abnormalities occurring during neurogenesis, which affect the morphology, and ultimately functionality of neurons. The *Drosophila* peripheral nervous system (PNS) provides an excellent model system in which to elucidate the molecular mechanisms governing dendrite morphogenesis. Spectraplakins are an evolutionarily conserved family of cytoskeletal cross-linking proteins that link the actin and microtubule cytoskeletons. Cytoskeletal structure and organization are key mediators of neuronal shape, and by extension neuronal function. To address the role of Spectraplakins in dendrite morphogenesis, we focused on the sole known Spectraplakins gene in *Drosophila* referred to as *short stop* (*shot*). Here we demonstrate that *shot* is present in all classes of dendritic arborization (da) neurons where it exerts differential effects on class specific dendrite morphogenesis. Comparative morphological analyses reveal *shot* is required to restrict dendritic complexity among the simpler class I and II da neurons, whereas *shot* is required to promote dendritic complexity among the more complex class III and IV da neurons. These results suggest dendrite morphogenesis is subject to context-dependent regulation mediated via *shot*.

INVESTIGATING THE ROLE OF RNAi REGULATION IN CLASS-SPECIFIC DENDRITE MORPHOGENESIS. Myurajan Rubaharan, Eswar P.R. Iyer, & Daniel N. Cox, School of System Biology, Krasnow Institute for Advanced Study, George Mason University, Fairfax VA 22030. Dendrites function as the primary sites of synaptic and/or sensory input and integration in the developing nervous system. The initiation and maintenance of dendritic arbors determine both the number and type of inputs they receive and thus is a critical determinant in establishing functional neural networks. Despite their functional importance, the molecular mechanisms governing cell-type specific dendrite morphogenesis remain largely unknown. One emerging mechanism involves the regulation of differential gene expression in neuronal subclasses via the evolutionarily highly conserved RNA interference (RNAi) pathway. The *Drosophila melanogaster*, peripheral nervous system, an excellent genetic and morphological model system for studying class specific dendrite morphogenesis, was used to perform loss-of-function (LOF) and gain-of-function (GOF) phenotypic studies to understand the role(s) of ten key genes essential for mediating RNAi in the fruitfly. These studies provide novel mechanistic insight into the role RNAi pathway plays in controlling class-specific da neuron dendrite morphogenesis. The broader implications of these studies are related to the relative contribution of this conserved pathway in controlling gene expression at the class-specific neuronal level in mammalian systems and how this regulation ultimately contributes to acquisition of distinct neuronal morphologies that underlie the establishment of complex neural networks.

FORAGING AT THE SNAIL DINER: THE EFFECTS OF PARASITE INFECTION ON SNAIL FORAGING BEHAVIOR. Sabrina Brooks<sup>1</sup>, Jeremy M. Wojdak<sup>2</sup> & Lisa K. Belden<sup>1</sup>, <sup>1</sup>Virginia Tech, Department of Biological Sciences, Blacksburg, VA 24060, <sup>2</sup>Radford University, Department of Biology, Radford, VA 24142. Snails are common hosts of parasitic flatworms (trematodes). Snails typically feed at dawn and dusk, and during this time they are especially vulnerable to predators. Parasite infections can alter this feeding pattern, which can increase the probability of infection or transfer of the parasite to the next host. We examined how infection by *Echinostoma trivolvis* can affect the foraging behavior of *Helisoma trivolvis* snails. In separate trials, we examined foraging behavior in (1) snails infected as first intermediate hosts and (2) snails either exposed or not exposed to cercariae (second intermediate host infection). We used two assays to assess foraging behavior. First, we examined how the frequency of snails on the tiles changed over twelve hours and the rate at which the snails in the different infection groups reached the tiles. The second assay compared the amount of lettuce consumed within 25 hours among snails in the different infection groups. We did not see a difference in proportion of snails on the resource tiles between cercariae exposed and non-exposed snails. However, for the first intermediate host snails, the proportion on the tiles was higher after 120 minutes and remained higher until the end of the trial. For the lettuce trials, there was no difference in the amount of lettuce consumed among the three categories of snails. While there were some differences in foraging behavior between first and second intermediate host snails in our first assay, we did not see dramatic difference in foraging based on infection status in *Helisoma* snails.

SUSCEPTIBILITY OF *PSEUDOMONAS AERUGINOSA* BIOFILM TO ALPHA-HELICAL PEPTIDES: D-ENANTIOMER OF LL-37. Scott N. Dean, Barney M. Bishop & Monique L. van Hoek, Department of Systems Biology, George Mason University, 10900 University Blvd., Manassas, VA 20110. *Pseudomonas aeruginosa* is a highly versatile opportunistic pathogen and its ability to produce biofilms is a direct impediment to the healing of wounds and recovery from infection. Interest in anti-microbial peptides has grown due to their potential therapeutic applications and their possible use against antibiotic resistant bacteria. We tested the human AMP, LL-37, and the effect of a protease-resistant LL-37 peptide mimetic, the peptide enantiomer D-LL-37, for anti-microbial and anti-biofilm activity against *P. aeruginosa*. The CD spectra of D- and L-LL-37, and the trypsin resistance of D-LL-37 was confirmed. The helical cathelicidin from the cobra *Naja atra* (NA-CATH), and synthetic peptide variations (ATRA-1, ATRA-2, NA-CATH:ATRA1-ATRA1) were also tested. Although the cobra cathelicidin and related peptides had strong anti-microbial activity, they did not inhibit *Pseudomonas* biofilm formation. D-LL-37 inhibited attachment, promoted *Pseudomonas* motility, and decreased biofilm formation by altering the rate of twitching and downregulating the expression of the biofilm related genes, *rhlA* and *rhlB*. D-LL-37 protected *Galleria mellonella* *in vivo* against *Pseudomonas* infection, while NA-CATH:ATRA1-ATRA1 peptide did not. This study shows D-LL-37 is able to promote bacterial twitching motility and inhibit biofilm formation, and protect

against infection, and suggest that this peptide may be a critical component for the development of new treatments for *P. aeruginosa* infection.

CHARACTERIZATION OF DOWNSTREAM EFFECTORS MEDIATING CUT TRANSCRIPTIONAL REGULATION OF CLASS-SPECIFIC DENDRITE MORPHOGENESIS. Luis Sullivan, Eswar P.R. Iyer, Madhu Karamsetty, & Daniel N. Cox, School of System Biology, Krasnow Institute for Advanced Study, George Mason University, Fairfax VA 22030. Neuronal form dictates function and in a circuitry as complex as the human brain the post-synaptic properties of the neuron are established in large part by dendritic morphology. Transcriptional regulation has emerged as a pivotal mediator of class specific dendrite morphogenesis; however, the downstream effectors of these transcription factors remain largely unknown as are the cellular events that direct morphological change. Recent studies have implicated the *Drosophila* homeodomain transcription factor Cut and its vertebrate homolog in mediating dendrite morphogenesis in the peripheral and central nervous systems. To characterize putative transcriptional targets of Cut regulation, a genetic suppressor screen has been performed in which Cut overexpression has been coupled with target gene-specific *in vivo* RNAi knockdown. Preliminary analyses have identified >400 genes that represent potential direct targets of Cut regulation in *Drosophila* dendritic arborization (da) neurons. Here we report the discovery of target genes that either suppress or enhance Cut-mediated effects on da neuron dendritic morphology. The molecules uncovered in our screen cover a broad range of biological functions. Collectively, these analyses reveal novel transcriptionally regulated pathways and cell biological processes essential to the specification of class specific dendritic morphologies.

THE DETECTION OF *MYCOPLASMA* SPECIES CONTAMINATION IN CULTURED HUMAN CELLS. Amy Yu, Pranvera Ikononi, Nadine Kabbani, Ancha Baranova & Aybike Biredinc, College of Science, George Mason University, Fairfax, VA. *Mycoplasma* can contaminate eukaryotic cell cultures. It can alter reproducibility of experiments or completely destroy the cell line. In most of the cell culture labs, only one or a few species of *Mycoplasma* are systematically monitored. The novel Universal Mycoplasma Detection Kit from American Type Culture Collection (ATCC) provides a way to detect over 60 species of *Mycoplasma*. AIM: To perform field test of the Universal Mycoplasma Detection kit using continuous cell cultures. Methods: The kit contains reagent for a PCR reaction using universal primers that are specific to the 16S rRNA coding region in the mycoplasma genome, allowing to specifically amplify *Mycoplasma* DNA. If a cell culture is contaminated, after gel electrophoresis, the presence of a 464-bp amplicon is detected. In our experiment, 8 PCR reactions were performed for each cell culture, including sample A, B and C provided by the kit as reference examples of different levels of mycoplasma contamination; two test samples; positive control; purified *Mycoplasma* DNA, positive control with lysate to confirm that inhibition of PCR by the cell lysate did not occur; and negative control. Results: Cell culture samples from Dr. Kabbani's and Dr. Van Hoek's lab were negative for *Mycoplasma*. Sample B, Sample C, positive control and positive control with lysate all showed a band at 464-bp. Sample A, all test samples and negative control showed no bands. This confirms the absence

of *Mycoplasma* in the cell cultures and proves that ATCC kit is reliable, easy to use and can be recommended for introduction to the market.

ADAR FACILITATED RNA EDITING IN HUMAN PLASMACYTOID DENDRITIC CELLS (PDC). A. Sharma<sup>1</sup>, K. Doyle<sup>1</sup>, M. Connors<sup>4</sup>, A. Patamawenu<sup>4</sup>, P. Gillevet<sup>3</sup>, A. Birerdinc<sup>1,2</sup>, & A. Baranova<sup>1,2</sup>, <sup>1</sup> School of Systems Biology, George Mason University, Fairfax VA 22030, <sup>2</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA 22042, <sup>3</sup> Microbiome Analysis Center, George Mason University, Manassas, VA 20100, <sup>4</sup>National Institute of Health, Bethesda, MD 20892. Adenosine (A) to Inosine (I) RNA editing is facilitated by enzymes known as ADAR (Adenosine Deaminase that Act on RNA). ADARs specifically recognize double stranded RNA structures or RNA duplex structures as their substrates. Inosine is translated as Guanosine, since most enzymes recognize Inosine as Guanosine. Examples of physiological ADAR editing are edits to neuronal Glutamate and Serotonin receptor transcripts. Here we set to find out whether ADAR-editing in human PDCs (Plasmacytoid Dendritic Cell) is limited to TLR7, or whether it covers other known ADAR targets, including other TLR receptors, FLNA, IGFBP7, KCNA1, GABRA3, and CYFIP2. Site specific primers around previously known edited sites were designed using NCBI primer blast and then tested on cDNA derived from universal RNA and adipose tissue. cDNAs from purified PDC cells will be used as templates for PCR amplification, tagged, purified and subjected to Multitagged (MTPS) pyrosequencing on Roche GS-FLX instrument. If successful, these experiments will be the very first demonstration that RNA editing activity is present in PDCs and acts on physiologically important gene targets.

USP22 ACTIVITY REQUIRED FOR EFFICIENT mRNA 3' PROCESSING OF STAT1 REGULATED GENES. Edmond Chipumuro and Melissa A Henriksen Department of Biology, University of Virginia, Charlottesville VA 22904. Histone modifications represent a major mechanism of eukaryotic transcriptional regulation at multiple levels. There is increasing experimental evidence that histone H2B monoubiquitination (ubH2B) and its deubiquitination are both involved in gene activation. Recent findings have suggested that, the ubiquitin specific peptidase 22 (USP22) regulates c-MYC and p21 expression and is required for appropriate cell cycle progression. However, a mechanistic understanding of USP22 function in gene expression remains elusive. Here, we utilized the rapid and transient STAT1 activation of the *IRF1* gene to characterize roles of USP22. RNAi mediated depletion of USP22 significantly increased *IRF1*-associated levels of ubH2B and down regulates *IRF1* expression. Importantly, USP22 knockdown consistently resulted in inefficient mRNA 3' processing of the *IRF1* gene and led to increased read through RNA polymerase II. Furthermore, depletion of USP22 resulted in lower occupancy of RNA Pol II phosphorylated at serine 2 (CTDS2) and cleavage and polyadenylation specificity factor 73 (CPSF 73) on the *IRF1* gene body and its read through. Taken together, our results suggest that, USP22 activity is required during *IRF1* transcription to regulate co-transcriptional processing of the mRNA.

DEVELOPMENT OF A SIMPLIFIED METHOD FOR DETECTING NUCLEAR IMPORT. Adil Quraish & Stephen Gallik, Ph. D., University of Mary Washington. Nuclear import is an important cellular process through which proteins enter the nucleus through the nuclear pore complex. It is commonly detected and studied using sophisticated protocols too time-consuming, complicated and expensive to be routinely used in the undergraduate teaching or undergraduate research laboratory. The specific objective of the study reported here is to develop a simplified method for the detection of nuclear import that can be more easily used in an undergraduate setting. The simplified approach taken here involves homogenization of rat liver, incubation of the homogenates with commercially-available fluorescently-labeled proteins, isolation of the rat liver nuclei from the homogenates using a standard Optiprep density gradient centrifugation techniques, followed by the fluorescent-microscopic inspection of the isolated nuclei. Results showed nuclei isolated from homogenates incubated with fluorescently-labeled proteins conjugated to a 13-amino acid nuclear localization signal (NLS) fluoresced, showing nuclear import of the fluorescent protein, while nuclei isolated from homogenates incubated with fluorescently-labeled proteins lacking an NLS did not fluoresce.

THE EFFECT OF VEGF ON ADIPOSE STEM CELL MIRGRATION. Laila Almahdali & Kathryn E. Loesser-Casey, Dept. of Biol., University of Mary Washington, Fredericksburg VA 22407. Stem cell research is one of the cutting edge ways scientists and clinicians hope to treat human diseases in the future, but stem cells are difficult to obtain in sufficient quantities. In order for stem cells to be of any use following a myocardial infarct or similar injury, they must be able to migrate to the place of damage. It has been found that growth factors such as Vascular Endothelial Growth Factor (VEGF) can assist in the migration pathway by stimulating endothelial growth, migration and blood vessel formation. It is also found the VEGF is secreted by adipose stem cells, leading to the possibility that VEGF may be necessary for stem cells release or migration. The goal of this experiment was to determine if treatment with 50ng/ml VEGF increased migration and whether the age of the stem cells affected migration. Cells were collected from both young (10 to 12 week) and old (over 30 week) CF-1 mice and used for migration assays using Boyden blind-well chambers. The number of cells that had migrated towards media with or without VEGF was counted and data evaluated using a one-sample T-test. Cells isolated from the younger mice appeared to show an increase in cell migration towards the VEGF-containing media; however statistics indicates no significant change in cell number over the control. When cells from adult mice were tested we saw a decrease in cell migration, but no statistics can be performed on this data because only one assay was performed due to very slow cell growth. We plan to repeat these experiments, changing the cell number used for each migration and increasing the concentration of VEGF to get more consistent results.