

2009 Research Presentations

Department of Biology

Urban Ecology in Miniature: Spatial Analysis of Antibiotic Resistance in *Enterobacter* spp. Isolates Gathered from Soils of Lancaster City, Pennsylvania

Ryan McDonald, Dr. David Bowne and Dr. Debra Wohl

The increase in microbial antibiotic resistance is a major public health concern but is also an interesting ecological phenomenon. Antibiotic resistance in the environment may result from ecological interactions among soil microbes, from anthropogenic application of antibiotics, or from co-selection for resistance to heavy metals (e.g. lead, copper, mercury). The relevant importance of each factor is currently unknown. If resistance is related to soil contamination, then urban areas may show high levels of antibiotic resistance. We examined the spatial heterogeneity of antibiotic resistance in an urban environment and are relating observed levels of resistance to soil metal concentrations. We gathered isolates of *Enterobacter* spp. from 26 sites in the city of Lancaster and analyzed them for resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. Of the 26 sites, 18 sites yielded *Enterobacter* spp. with 70% or more of the isolates resistant to each antibiotic. Doubling these antibiotic concentrations dropped the average to 47%. In addition to this overall high level of resistance, 4 sites also showed antibiotic resistance in excess of 90% to all three antibiotics at the highest concentrations. In ongoing studies, we are examining soil lead, copper, and mercury content in order to determine whether presence of metals is correlated with drug resistant bacteria. Our analysis contributes to our understanding of urban ecology, both in how human health is impacted by the environment and on the ecological and evolutionary consequences of human activity.

Determining the function of Atu2115 in *Agrobacterium tumefaciens*

Jason Matakas and Dr. Debra Wohl

Agrobacterium tumefaciens (*A. tumefaciens*), causal agent of Crown Gall disease in plants, is a soil bacterium that is well known for its Ti plasmid. This plasmid affords it the ability to transfer and integrate specific genes into a host plant cell genome, and it is this ability that has been exploited commercially as a biotechnological tool. My goal for this project was to obtain further knowledge of the genomics of this bacterium through the use of knockout mutants, as well as explore certain cellular functions. The gene I targeted, Atu2115, is thought to code for a pyrophosphate-dependant fructose-6-P 1 phosphotransferase. This gene product may serve the same function as phosphofructokinase, which is 'missing' bioinformatically from the genome of *A. tumefaciens*. Using recombinant DNA cloning, I created an Atu2115 disruption mutant, and assessed the function of the gene to determine if my hypothesis is supported. Once certain about the successful transformation, the mutant was characterized. While additional analyses are still needed, observed differences in growth rates between the wild type and mutant do support my hypothesis that Atu2115 functions as a phosphofructokinase. These findings contribute to the field of bioinformatics through a better understanding of the function of the Atu2115 gene.

The Effects of Chemical and Physical Properties of Soil on Earthworm Abundance

**Derek Faust, Kurt Deschner,
Dr. David Bowne and Dr. Kristi Kneas**

The interactions between the environment and the ability of an organism to function in that environment may have repercussions not only on the population of that organism, but also the ecosystem as a whole. Earthworms aerate soil, can speed the release of nutrients as they decompose organic matter, and furthermore enhance water infiltration and solute transport. As a result, the growth rate and productivity of farmers' crops is significantly improved. In this study, we investigated the effects of physical and chemical properties of the soil—temperature, elevation, moisture, and the amounts of organic material, magnesium, calcium, phosphorus, and carbonates—on the abundance of earthworms present in the soil. There were 21 randomly selected sample sites from a soy bean field on the property of Elizabethtown College in Elizabethtown, Pennsylvania. The earthworms were extracted from the soil using a hot mustard solution. The carbonate contents and exchangeable calcium and magnesium concentrations were determined titrimetrically. Moisture was measured gravimetrically, and organic material was measured gravimetrically through combustion. Phosphorus was measured by UV-Visible spectrometry. Based on a multiple linear regression, the elevation, moisture, and amount of carbonates in the form of calcium carbonate were found to be statistically significant in affecting the number of earthworms ($F_{1, 14} = 6.533$, $p = 0.006$, $R^2 = 0.601$). Our results provide guidance on how farmers can manage their soil to promote earthworm abundance and thus the positive ecosystem services that earthworms provide.

Effects of Gradient Background Noise on fMRI Study of Sound-Color Synesthesia

**Janet Richards, Jason Matakas, Jeffrey Mastrangelo,
Dr. Catherine Lemley and Dr. Khristy Thompson**

Synesthesia occurs when one sense reliably activates another. Sound-color synesthetes perceive distinct colored shapes (photisms) upon hearing sounds. This fMRI study is investigating the neural mechanisms of TH and DR, two sound-color synesthetes. No previous studies on sound-color synesthesia reported triggering of photisms from the background noise of the MRI. However, both synesthetes reported a distinct photism for the noise the fMRI generated. To determine the location of the participants' color centers, a real-color paradigm was given in which blocks of colorful patterns were presented and baselines were determined by presenting patterns with no color. To determine the activation that occurs when synesthesia is experienced, the participants were given experimental blocks of audio clips. The baseline was silence and the participants were instructed to count backwards by threes so they would not attend to the fMRI noise while in another run they did not count. TH reported that during the counting task there was a decrease in his colored photisms while DR reported that the background noise continued to produce consistent photisms. In the sound-color paradigm, TH showed activation in his color center, angular gyrus, and superior parietal lobe with reduced activity while counting. For DR, the sound-color paradigm showed some activation in the angular gyrus during the counting task. DR's baseline was more difficult to obtain since the backward counting task did not reduce the colored photisms elicited by the fMRI noise. This reveals that there are differences in the sensitivity to sounds that cause photisms in sound-color synesthesia.

Neural plasticity of grapheme-color synesthesia: fMRI correlates of recently learned symbols

**Janet Richards, Jason Matakas, Jeffrey Mastrangelo,
Dr. Catherine Lemley and Dr. Christy Thompson**

Synesthesia is a condition in which one perceptual modality involuntarily activates another. For grapheme-color synesthetes, familiar graphemes (letters, numbers) produce a color percept. Marks and Odgaard (2005) suggested that learning should be considered in theories of synesthesia since many 'cultural artifacts' elicit synesthetic experiences. We used fMRI to investigate whether recent learning of symbols could elicit color center activation or other brain activations in an adult grapheme-color synesthete. During Phase 1, D.R., a grapheme-color synesthete, was presented 5 Graphemes that show color activation, 10 unknown Chinese characters that do not produce synesthesia, and 5 Nonsense symbols that do not produce synesthesia. After Phase 1, she learned the English meaning of 5 of the original 10 Chinese characters. Phase 2 was run as Phase 1. Phase 1 revealed activation in the color center (V4/V8), dorsolateral prefrontal cortex, mid cingulate, temporal pole and superior parietal cortex for the Grapheme condition. The Chinese and Nonsense characters showed activation in the visual association structures, but not V4/V8. Phase 2 showed no significant recruitment of V4/V8, but D.R. reported experiencing color perceptions. Activations were observed in the bilateral superior parietal lobe and right temporal pole-regions which were also activated in the grapheme-color condition. Our findings demonstrate the neural substrate corresponding to new synesthetic color experiences in grapheme-color synesthesia. Because of the highly associative nature of this experimental learning paradigm, associative cortices of the parietal and temporal lobes may be particularly important. Future studies should examine the time course of neural change underlying such learning activities.

PVF genes and Colony Growth in the Invertebrate Podocoryna carnea

Lindsey Evans and Dr. Diane Bridge

VEGF and PDGF proteins play critical roles in human blood vessel growth and remodeling. The marine invertebrate *Podocoryna carnea* forms a simple network of fluid-bearing tubes as it grows. Network growth responds to flow rate and nutrient levels in some of the same ways that growing blood vessels do. If *Podocoryna* growth involves molecules related to those important in vertebrate blood vessel growth, *Podocoryna* could serve as a simple, experimentally tractable model for research on the circulatory system. To characterize the function of PDGF/VEGF (PVF) proteins in *Podocoryna* growth, we are working to produce genetically modified *Podocoryna* in which any tissue expressing a Pvf gene also expresses a fluorescent marker protein. To do this, we have cloned portions of two *Podocoryna* Pvf genes. We are using PCR walking techniques to clone sequence 5' of the coding regions of these genes. Expression constructs including these sequences and an EGFP gene will be used to produce transgenic *Podocoryna*.

Screening of an Arrayed Human Genomic Library in the PAC Shuttle Vector pJCPAC-MAM2

**Stephanie Bireley, Matthew Kochuba, Michael Wagner
and Dr. Jonathon Coren**

Human genomic libraries constructed in the PAC shuttle vector pJCPAC-Mam2 allow scientists to recover ample quantities of DNA from any clone in bacteria, and then introduce the PAC clone into a variety of human cell lines for functional genomic studies. We have constructed an arrayed 115,000 member library housed in twelve hundred 96 well plates. The library was then pooled in a columns and rows fashion. Each column (12 clones) was pooled into a single well of a second-generation plate (100 total). Next, entire second-generation plates were pooled. Two additional rounds of pooling resulted in the production of 19 cryovials containing ~6,000 PAC clones/vial. PCR screening is currently underway with oligonucleotides representing several genes (p53, DNA ligase, AP endonuclease, and HSA) in order to determine library coverage.

Stress and Aging in a Basal Metazoan: FOXO Regulation in Hydra vulgaris

Rebecca Holler, Alex Theofiles and Dr. Diane Bridge

FOXO transcription factors have been implicated in the regulation of longevity and cellular stress responses in bilaterians, including vertebrates, *Drosophila*, and *C. elegans*. Existing data suggest that aging does not occur in the cnidarian *Hydra vulgaris*. To investigate the role of a Hydra FOXO gene, we have generated transgenic *Hydra* which express a FOXO:EGFP fusion protein. FOXO activity is regulated by post-transcriptional modification, with active FOXO localized to the nucleus. We found that heat shock significantly increased localization of the fusion protein to cell nuclei. Our results also show that inhibition of Akt activity increased nuclear localization of the fusion protein, while inhibition of JNK activity decreased it. We did not find that dietary restriction has an effect on FOXO:EGFP localization in *Hydra*. Our results provide evidence for a role for the *Hydra* FOXO gene in responses to heat shock. They raise the possibility that FOXOs may be involved in responses of other cnidarians, including corals, to heat stress.

T Antigen and the Transactivation of the Ribosomal and Cyclin A Promoters

Stacey Lehman and Dr. Jane Cavender

Large tumor antigen, a viral oncoprotein, is a permissive transactivating factor in infected cells. In the monkey system, prior research has shown that T antigen mutants that cannot transactivate the ribosomal promoter also cannot transactivate the cyclin A promoter. One possible explanation is that these mutants lack the ability to bind TATA binding protein (TBP), a transcription factor held in common between both promoters. Studies using GST-fusions have previously demonstrated that T antigen has the ability to bind TBP in vitro. However, results from these current co-immunoprecipitation experiments indicate that both wild-type and mutant T antigens do not bind TBP in vivo, although wild-type T antigen retains its ability to bind p53 and p53 retains its ability to bind TBP. Thus, it appears that another mechanism is responsible for the mutants' inability to transactivate the ribosomal and cyclin A promoters. Other possibilities are that phosphorylation activity of the cyclin A-cdk 2 complex is required for RNA polymerase I transcription or that it is an indirect effect of T antigen's ability to upregulate the cell cycle.

Corticosterone Decreases Tumor Growth and T Cell Numbers in Balb/c Mice

Lisa Sether and Dr. Jodi Yorty

Psychological and physical stressors decrease the body's ability to fight infection. Evidence is now mounting for a connection between stress, the immune system, and cancer. This project seeks to develop an in vivo mouse model to assess the effects of stress hormones (corticosterone) on the immune response to tumors. Subcutaneous injection of 1×10^7 tumor cells (MKSA or MethA) into Balb/c mice elicited tumor growth at approximately one week, increasing the total number of T cells in the spleen. CD4+ and CD8+ T cells were present in tumors, regardless of tumor cell type. However, there was little evidence of cytokine production by T cells present in MKSA tumors. Administration of 1×10^{-6} M CORT in the drinking water decreased the total T cell number in the spleen and markedly slowed tumor development. Future research will investigate whether CORT treatment causes regression of established tumors. Additionally, we hope to further explore the role of CD4+ and CD8+ T cells recruited to the tumor site. Combined, these studies explore the relationships that exist between the immune system, tumor development, and stress hormones.

Manganese Uptake and Distribution in the Brain after MeBr Induced Lesions in the Olfactory Epithelium

Sandip Savaliya, Dr. Ramon M. Molina, Dr. Joseph D. Brain, Dr. James E. Schwob and Dr. Khristy Thompson

Manganese (Mn) is an essential nutrient with potential neurotoxic effects when chronically inhaled resulting in Manganism, a Parkinson's like disorder. Mn deposited in the nose can be transported to the brain by olfactory neurons through retrograde axonal transport, bypassing the blood-brain-barrier. The role of the olfactory epithelial cells in Mn transport from the nasal cavity is not well understood. Olfactory epithelial cells can fully regenerate after injury in a time-dependent and cell-specific manner. We utilized the methyl bromide (MeBr) model to ablate the olfactory epithelium of rats. In this model, supporting cells reappear within a week, and complete neurogenesis in 6-8 weeks. $^{54}\text{MnCl}_2$ was administered by intranasal instillation at 1.5, 3.5, 7, 21 and 56 days post-MeBr treatment. ^{54}Mn concentration in blood and the brain distribution were determined 1 week post-instillation. Blood ^{54}Mn returned to control levels by 7 days, but brain ^{54}Mn reached control levels at 21 and 56 days post-MeBr treatment. Our data show that an intact olfactory epithelium is necessary for Mn transport to the brain and blood where (1) supporting cells are necessary for Mn transport to the blood and (2) intact axonal projections are required for direct Mn transport from the nasal cavity to the brain.

Characterization of Dysfunctional DMT1 in the Belgrade Rat

Michael Nelson, Dr. Jane Cavender and Dr. Khristy Thompson

Iron is essential for growth and development; however, high levels of iron can result in toxicity and deficiency can result in cognitive delays. The iron transporter, DMT1 (Divalent Metal Transporter-1), plays a major role in dietary non-heme iron uptake and systemic iron acquisition post-intestinal-absorption. DMT1 expression is up-regulated by iron deficiency. The anemic Belgrade rat contains a glycine-to-arginine substitution (G185R) that encodes a DMT1 protein with little or no activity in iron uptake assay. These rats manifest microcytic anemia and require iron supplementation to survive. Past research has shown that the Belgrade rat is able to acquire iron from the diet, most likely from the functional transferrin receptor, and they load iron in the liver; yet, they remain anemic. We hypothesized that the mutation in the DMT1 transporter would not adversely affect the transport of iron into red blood cells by the transferrin receptor. However, the iron would be trapped in the RBC lysosomes unable to be moved into the cytosol by the mutant DMT1 protein. Iron staining of RBC from homozygous and heterozygous Belgrade and normal Fisher 344 rats has confirmed that the Belgrade rats do contain iron in the RBC. Currently research is being conducted to determine specific levels of iron within the serum and RBCs through atomic absorption. Additionally, altered production of spectrin and elastin in RBCs and other tissues, respectively, is currently being assessed.

Corticosterone Suppresses Tumor Cell Growth in Vitro

Catherine O'Connor and Dr. Jodi Yorty

Corticosteroids are stress hormones that regulate a wide range of processes in the body including the stress response, immune response and electrolyte levels. One corticosteroid, corticosterone (CORT), has been shown to halt the cell cycle prior to DNA synthesis. This study was designed to explore whether CORT exhibits the same inhibitory effect on tumor cells that, unlike normal cells, exhibit uncontrollable cell proliferation. The MSKA-TIA murine tumor cell line was infected with Simian virus 40 (Sv40) which caused transformation due to expression of the oncogene, T-antigen. To examine the effects of CORT on the MSKA-TIA tumor cells in vitro, CORT was introduced into the culture media. Treating the MSKA-TIA cells with CORT resulted in a dose-dependent down-regulation in proliferation over three days. Somewhat surprisingly, the removal of CORT from the culture media did not restore cell growth. The glucocorticoid antagonist, RU486, was used to confirm that the CORT was acting through its specific receptor and not by way of an indirect mechanism. Future experiments will look to confirm that CORT causes G1 arrest as indicated by a decrease in key cell cycle regulators. There will also be exploration in whether CORT affects expression of the T-antigen protein. To date, it has been established that stress and stress hormones in vitro are toxic to a growing tumor.

The Uptake of Manganese by Olfactory Epithelium in Iron-Deficient Rats

Jessalyn Donnelly, Ashley Bryner, Stephanie Usef, Thomas Reinmiller and Dr. Khristy Thompson

Environmental exposure to toxic levels of manganese can result in a parkinsonian syndrome in humans as a result of manganese deposition in brain regions involved in motor control. Neurobehavioral abnormalities also result from manganese exposure. The route of entry to the affected regions of the brain is from inhalation of manganese rather than ingestion. The olfactory system forms a direct interface between the air and brain, bypassing the blood-brain barrier, where the primary olfactory neurons provide a pathway to the brain for manganese. The proposed study will contribute understanding of potential physiological risks of metal-induced neurotoxicity and, specifically, the interactions between iron deficiency and manganese neurotoxicity. Proteins involved in the regulation of iron homeostasis also transport manganese. The hypothesis of this proposal is that absorption of inhaled manganese is upregulated upon iron-deficiency such that neurological complications of poor iron status are compounded by an increased vulnerability to the toxic effects of manganese exposure. Previously, iron regulated proteins in olfactory manganese transport were tested by studying olfactory uptake and brain distribution of manganese in iron-deficient rats and control rats using MRI. Behavioral and motor tests were performed as an indicator of manganese neurotoxicity. Histopathology and neurochemical analysis will be used to evaluate whether increased manganese accumulation associated with toxicity occurs in regions associated with manganese. This study is working to establish the parameters to study neurobehavioral endpoints of manganese toxicity in the rat and if the hypothesis is correct, may highlight a critical influence of iron deficiency on the metal's neurotoxicity.