Research Internship Program

Abstract Booklet
Summer 2006
**BIOLOGICAL SCIENCES**

**Expression of draper homolog, jedi, found in peripheral glia of zebrafish.**

Julie Dunlap, Sarah Kucenas, and Bruce Appel.

Regulation of the time and place of gene expression during development are fundamental properties of gene function. In fruit flies the *draper* gene promotes glial cell engulfment of severed axons. Because gene functions are conserved between species, we hypothesize that the draper homolog *jedi* is expressed similarly in peripheral glia of vertebrates. We began to test this hypothesis by identifying the zebrafish *jedi* gene and examining its expression by in situ RNA hybridization. We found that *jedi* is expressed in a pattern consistent with the distribution of peripheral glia. Understanding the details of gene expression in model organisms like zebrafish will bring us closer to understanding the basis of human developmental defects and their potential cures.

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**Synthetic genetic analysis of DRS2 with adaptor proteins AP-1 and GGA.**

Court Reese, Ke Liu, and Todd R. Graham.

Transport of proteins and lipids between organelles of secretory and endocytic pathways are carried out by transport vesicles. Among different carrier vesicles, clathrin-coated vesicles are the most prominent. Adaptors as are key factors in clathrin-coated vesicle formation, coupling clathrin to cargo and/or the lipid bilayer. Both AP-1 and Gga adaptors participate in clathrin-mediated protein transport between the trans-Golgi network and endosomes, although results from genetic studies in yeast and mammalian cells suggest Gga and AP-1 function in distinct pathways. Drs2, an integral membrane protein that function as a flippase, is involved in the formation of clathrin-coated vesicles from the late Golgi. To assess whether Drs2 preferentially acts in conjunction with AP-1 or Gga adaptors, a synthetic genetic analysis was used. Synthetic growth defects were observed when gga1Δgga2Δ but not apl4Δ was introduced together with deletion of DRS2 gene, suggesting that Drs2 function in the same pathways AP-1 but not Gga.
Determining the three dimensional structure and assembly and disassembly of potato virus X through protein expression and purification, x-ray crystallography, and fiber diffraction.

Nishi Shah, Ian McCullough, Michele McDonald, and Gerald Stubbs.

X-ray crystallography is used to determine the three dimensional structure of a protein that crystallizes into a homogenous lattice. The potato virus X coat protein will not form a crystal lattice; therefore, we have little idea about the coat protein structure of the virus. We have mutated the carboxylate groups in the amino acid sequence, which are responsible for virus disassembly within a host cell. We have also expressed the mutated coat protein in E. coli and isolate the protein through different purification methods. All these techniques will eventually help us find out the three dimensional structure of pieces of the coat protein, which will be used with the fiber diffraction pattern of PVX to reveal the three dimensional structure of the entire virus. This will also help to disclose the information on the disassembly of the virus. Potentially, this information can help in human virology, biotechnology, and agricultural studies.
BIOMEDICAL ENGINEERING

Dynamic Flow Hybridization of Microarrays.

Mike Czubakowski, Michael Poku, Chidinma Iwueke, and Frederick R. Haselton.

Microarrays provide a genetic snapshot of cell behavior. However, the hybridization of microarray slides can often be time consuming, taking upwards of 24 hours. Hybridizing in a flow is one potential method to speed up this process. We compared the effects of steady flow hybridization to no flow and examined the delivery of Panomer 9 target solution to genes arrayed on 37,440 spots on a human DNA microarray. We found that no flow may provide a more intense hybridization but dynamic flow provides more uniformity.

Nanoengineering of stent surfaces using layer-by-layer assembly.

Monica Guan, Tom Soike, and Prasad Shastri.

Millions of stents are implanted annually. Stents help regulate blood flow and reduce plaque buildup. This study uses layer by layer assembly techniques to attach negatively charged nanoparticles to a charged surface. The procedure was tested on 316L stainless steel samples. The data suggests that a 0.4% NP solution with five or more layers at 10-20 minutes of dipping time would yield the most amount of nanoparticles adhering to the surface of the stainless steel sheets. In the future we hope to replace the current nanoparticles with gold and gadolinium particles to improve imaging properties of stents.
**CANCER BIOLOGY**

**Detecting tumor-associated MMP activity using proteolytic beacons.**

Sarah M. McIntyre and Lynn Matrisian.

Increased expression and activity of matrix metalloproteinase (MMP) have been linked to the growth and metastasis of tumors. Therefore, development of MMP specific detecting devices would be useful for further advancement in cancer detection and treatment technology. In this study, novel polymer-based fluorogenic substrates, which serve as selective proteolytic beacons (PBs) for MMPs, were prepared, purified, and tested for cleavage by MMPs. These PBs contain both an internal reference and an optical sensor, which fluoresces when the peptide chain is cleaved by a specific MMP. This allows the MMP activity to be detected in a fluorescence assay. PBs were prepared with either visible fluorophores (fluorescein, green, and tetramethyl-rhodamine, red) or near infrared (NIR) dyes (Alexa fluor 700 and 750). DQgelatin, a non-specific detector, was first assayed with various MMPs as a baseline. Then, the MMP activity with the PBs was measured in vitro to characterize the specificity and efficacy of each beacon. Studies of these conditions showed increased fluorescence of the MMPs with four new beacon preparations, indicating successful synthesis. Reagent 2 was shown to have similar activity with MMPs 2, 7, and 9. Reagents 3 and 8 were shown to be selective for MMP2 and MMP9 with approximately 10 fold lower activity with MMP7. The NIR PB (reagent 7) was shown to have increased fluorescence of the sensor after treatment with MMP2. To study MMPs produced by polyoma virus middle-T derived (PyVT) breast cancer cells, a 3D culture model was developed using PyVT cells from culture seeded onto a gelfoam collagen sponge (in order to mimic the natural 3D in vivo environment) and were then tested for MMP activity using DQgelatin. Cells incubated with DQgelatin showed variable fluorescence in the presence or absence of the MMP inhibitor GM6001. The conditioned media harvested from the cells after 24 hours exhibited minimal MMP or proteinase activities with DQgelatin. In conclusion, new optical PBs were shown by fluorescence proteinase assays to be selective for the gelatinases MMP2 and MMP9. Detection of proteinase activities associated with the breast cancer cells in 3D culture will require additional studies such as increasing incubation time and/or increased cell number.
The Notch signaling pathway is important in development found throughout the entire body and used for all cell differentiation and specification decisions. Examining when and where this pathway is affecting the liver is key to exploring and analyzing liver development and regeneration. In order to analyze this pathway’s effect we need to employ a Cre excision line specific for the liver, due to complicating secondary lethal effects. In order to do this competently, we must first address which Cre mouse lines and which reporting alleles act as the most effective tools when used in conjunction in the liver. We used both the Albumin::Cre coupled with ZEG or ROSA26R reporting alleles. While in theory ZEG would appear to be the more effective reporter, we have found that in practice the ROSA26R reporter is more successful. Using the ROSA26R we have also been able to observe that the Albumin::Cre excision line is activated earlier in liver development than has previously been reported. Furthermore, when compared to another Cre excision line, Sonic Hedgehog, the Albumin::Cre line remains the most effective and specific in the liver. Our findings suggest that Albumin::Cre when in conjunct with ROSA26R is the most useful tool in studying genetic development throughout the liver.
Using MBP-MS2 (fusion protein) to determine what proteins bind the Arp2 complex.


RNA splicing is an important mechanism in all eukaryotic cells. In our lab, we are using the fission yeast *Schizosaccharomyces pombe* to purify the splicing machinery called the spliceosome. Our goal is to purify the spliceosome bound to a single RNA species. In order to purify this splicing machinery, we are using a series of mutants in *arp2*+, which is a spliced gene containing a single intron. In addition, these *arp2*+ constructs also contain sequences, which encodes a hairpin loop that is recognized by an RNA binding protein. I used Northern analysis to make sure these constructs express *arp2*+ properly. Western Blotting was done to make sure Arp2 is properly produced. Further evidence for the interactions between the spliceosome and apr2 RNA will be studied by purifying bound proteins using the MBP-MS2 fusion protein produced in *E.coli*. 
**CHEMISTRY**

**Practical approach towards protein biomimics: Synthesis of low temperature crosslinker for intramolecular chain collapse.**

Nevena Kozekova, Sharon Hamilton, Bill Evans, and Eva Harth.

A new low temperature crosslinker synthesis yielding benzocyclobutenol is presented. The crosslinker was obtained through three reactions. First, 2-Aminobenzoic acid was activated using the diazotation method with iso-amyl nitrite yielding benzenediazonium-2-carboxylate. In the second reaction, the benzenediazonium-2-carboxylate was heated with excess of vinyl acetate in dichloroethane and directly obtained benzocyclobutenyl acetate. In the last reaction, a hydrolysis of the acetate with 5% aqueous sodium carbonate in ethanol yielded benzocyclobutenol crosslink precursor. Benzocyclobutenol was chosen as a linker due to the expected low temperature at which it will crosslink. In future research, the crosslinker will be attached to a linear polymer which will be collapsed to form a nanoparticle tailored for a drug delivery system.

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**Investigation of Peptide Protein Interfaces.**

Saransh Midha, Andrew Morin, and Jens Meiler.

In this study, we used the PDB files to trace the specific combination of peptide/protein to observe the Peptide composition. The PDB Data provided with us the binding modes used by nature and through the PDB file, we selected the specific proteins which did not contain any non-specific amino acids in the HEATM. In those proteins, we searched for AA Composition, binding mode, Peptide length, H-Bond donors, H-bond acceptors, Water-Mediated Bonds, and Bond-Configuration. After obtaining the data, we created graphs to analyze the information and we discovered that Proline was the most common amino acid, the binding mode was largely groove, many H-bond acceptors existed than H-bond donors, and the bond configuration for most proteins was Side-chain/Side-Chain. The data was critical in the evaluation of these nature proteins to determine their features.
Earth and Environmental Sciences

Dating monazites from a fault zone in southern Appalachian Mountains.

Jason Cox, Scott Crombie, Yan Lao, and John C. Ayers.

In the Unaka Mountains, located in eastern Tennessee and western North Carolina, the Devil’s Fork fault zone was formed. I took two samples that were collected by Dr. Ayers and a few of his graduate students, and started the mineral separation process. This process takes about four to five days per sample. One sample (UK-5) was collected from inside the fault zone and another sample (UK-6) was collected outside the fault zone. Collecting a sample from each location will allow us to date approximately when the fault was formed. We can do this by analyzing each grain of monazite found within the sample with a sophisticated machine called the Laser Ablation ICP-MS. What the machine does is shoot a very focused laser on to each grain of monazite and analyzes the concentration of Th (Thorium) and Pb (Lead). The Th to Pb ratio is found by using the Th-Pb isotopic system. The lower the Th-Pb ratio is, the older the monazite is, which means the fault is older.
**Medicine**

**Defining the cellular location of PAH associated BMPR2 mutant proteins in NMuMG epithelial cells.**

Apoorwa Thati, Tom Blackwell, and Kirk Lane.

Pulmonary Artery Hypertension (PAH) is often a genetic disorder which causes an overgrowth of vascular cells in small arteries of the lung. Mutations in the gene coding for Bone Morphogenic Protein Receptor Type II (BMPR2) are causative for PAH. By expressing a epitope-tagged mutant BMPR2 in to our NMuMG (Normal Murine Mammary Gland) epithelial cells, we will be able to determine if altered receptors move to the cell membrane. Extracellular Receptor Kinase (ERK) signaling controls the growth of cells in vessels. Initiation of the ERK signal occurs at the cell surface. We propose that mutant BMPR2 receptors alter the ERK response and that this occurs at the cell surface. These studies will demonstrate the necessary first step, the presence of PAH associated BMPR2 proteins in the cell membrane.

**The Effect of Resveratrol on Hypertonicity induced Apoptosis of cultured Inner Medullary Collecting Duct [IMCD-3] Cell.**

Gerald Wakefield, Wenjuan He, Chuan-ming Hao, Reena Rao, Reyadh Redha, Betsy Srichai, and Matthew Breyer.

Two separate laboratory experiments were carried out to examine the effects of resveratrol on hypertonicity induced apoptosis of cultured mouse renal IMCD-3 cells and to explore the potential role of resveratrol in reducing renal damage in chronic kidney disease. Using various concentrations of resveratrol [0-50μM], we observed in vitro that cell viability was positively affected by increased concentration of resveratrol. Cells treated with 50μM of resveratrol without hypertonicity showed the greatest viability, while cells treated with 0.5μM resveratrol with hypertonicity exhibited the least viability. It appears that resveratrol may be exerting some protective effect on the viability of IMCD-3 cells at higher concentrations, but it is difficult to ascribe the toxic effects of resveratrol at lower concentration.
Molecular Physiology and Biophysics

To determine which part of the glucose processing is possibly responsible for diabetes.

Cheng Zhang, Stacie Braswell, Tammy Santomango, and Owen McGuinness.

The liver and muscle cells are the major sites of glucose metabolism in the body. In diabetes these cells fail to appropriately regulate their uptake and/or production of glucose. To be metabolized glucose must be transported in and then phosphorylated. In each cell there are channel forming molecules called GLUT’s to transport glucose and an enzyme called hexokinase, which phosphorylates glucose. These molecules come in multiple types (i.e. isoforms) that are expressed in differing amounts in each tissue. We set up an assay to measure their content by western blot and/or activity in the tissue. Fructose and xylitol are known to activate liver glucose uptake in the acute setting. However the increase is not sustained when they are given chronically. We wanted to determine if decreases in glucokinase (the isoform of hexokinase present in the liver) activity antagonized the effects of these molecules. We observed that glucokinase activity decreased by 40% with fructose infusion and further decreased when both fructose and xylitol were infused (2.2±0.2, 1.5±0.2 and 0.9±0.2 mU/g liver; control, fructose, and fructose +xylitol, respectively). The analysis of GLUT’s using western analysis was not complete, however tissue differences in the content of the different isoforms was detected. We will continue to work on the subject of glucose management by the liver and muscle, and attempt to determine how the liver and muscle regulate the metabolism of glucose.
PATHOLOGY

Immonoassay determination of metallothionein concentrations in brains of control and N,N-diethyldithiocarbamate exposed rats.

Elyse Sadeghi, Olga Viquez, and William M. Valentine.

Human exposure to dithiocarbamates such as N, N-diethyldithiocarbamate (DEDC) can result in neurotoxicity due to their ability to redistribute copper across lipid membranes. Whereas severe myelin lesions characteristic of DEDC exposure have been observed in the peripheral nervous system of experimental animals, similar lesions have not been recognized in the brain and spinal cord. Metallothionein (MT) is a protein believed to play an important role in the detoxification of copper. We hypothesized that the decreased sensitivity to DEDC exposure demonstrated by the CNS is due to the presence of astrocytes in the brain that are capable of producing increased levels of metallothionein. We used immunoblotting techniques to determine the concentrations of metallothionein in control and DEDC exposed rat brain proteins. According to our results, there was no significant difference between metallothionein concentrations of the control and exposed groups. This suggests that up-regulation of metallothionein is not responsible for the decreased sensitivity of the brain to DEDC exposure.
**Pediatrics**

Screening ornithine transcarbamylase to detect polymorphisms in urea cycle disorder patients.


The purpose of our experiment is to target polymorphisms in the ornithine transcarbamylase (OTC) gene that cause Urea Cycle Disorders. We discovered 26 possible polymorphisms spanning 7 exons of the OTC gene. We are currently in the process of sequencing the DNA to determine what these polymorphisms are and how they affect the Urea Cycle. Thus far, 5 polymorphisms have been found. With this knowledge it may be possible to modify the Urea Cycle with reactants and/or products that it fails to code for in order to complete the cycle and dispose of excess ammonia.

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Loss of Raf increases inflammation during recovery following colitis-induced injury.

Thuy Dang, Karen Edelblum, and Brent Polk.

A single epithelial layer serves an important barrier function in the intestine. Intestinal epithelial cell survival is regulated by pro- and anti-apoptotic signaling pathways. In the healthy intestine, the cytokine, TNF, promotes cell survival; however, Inflammatory bowel disease (IBD) is characterized by increased TNF production during inflammation. The involvement of several regulatory molecules including KSR activation of Raf, serine/threonine kinase may be required for TNF-induced cell survival. Western blot analysis demonstrated that TNF promotes anti-apoptotic signaling in Rafflx intestinal epithelial cells. To further address the role of Raf in the intestine, PCR was performed to genotype Raf intestine-specific knockout mice. Western blot analysis confirmed that loss of Raf expression was restricted to the intestinal epithelium. Using immunohistochemistry to stain for neutrophils in colon sections of wild type and Raf intestine-specific knockout mice, we have found that loss of Raf results in increased inflammation during recovery from colitis-induced injury. Determining the role of Raf in inflammation and in the regulation of intestinal cell survival may lead to novel therapeutic approaches for the treatment of IBD.
We screened DNA samples of 47 patients with urea cycle disorders for polymorphisms in the N-acetyl-glutamate synthase (NAGS) gene. NAGS produces Carbamyl Phosphate Synthase I (CPSI), the enzyme involved in the first and rate-limiting step of the urea cycle, and, therefore, plays a critical role in maintaining proper urea cycle function [2]. Currently, we have screened 6 of the 7 exons in the NAGS gene and have found 4 polymorphisms. We are in the process of screening the remaining exon, exon 1.
Expression of myocyte enhancing factor MEF2 in rat brain.

Molly Robert, M. Diana Neely, and Ariel Y. Deutch.

Spines, dendritic protrusions where synapses are formed have been shown to be depleted in the striatum of Parkinsons Disease patients and the prefrontal cortex of Schizophrenia patients. The mechanisms responsible for these long lasting spine changes are not known, but likely involve changes in gene expression. Myocyte enhancing factor, MEF2, a transcription factor has recently been demonstrated to play a role in spine and synapse formation and maintenance. However, not much is known about the expression and regulation of this protein in the brain. Here, we describe expression of MEF2A and MEF2D in the cortex, striatum and septal nuclei of the rat brain. In the cortex, both MEF2A and MEF2D were expressed in neurons but not glia, and were more prominent in the superficial layers. In the striatum MEF2A and 2D were expressed mostly in neurons, but also observed in some non-neuronal cells. The immunoreactive signal was strongest in the medial parts of the striatum. In the dorso-lateral and ventro-lateral septal nuclei, the two isoforms showed a very different distribution. While MEF2D was exclusively nuclear, we observed a dense fiber-like staining for MEF2A. We show here that these fibers do not colocalize with the dopaminergic innervation of these areas. High magnification images reveal a punctate staining over the soma and fibers. The identity of these puncta will be analyzed in the future.
**Physics and Astronomy**

**Optical properties of europium sulfide nanocrystals.**

Justine Hart, Marcela Redígolo, Sameer Mahajan, Dustin Kavich, Suseela Somarajan, Heungman Park, Will Brown, Saad Hasan, Alhaji Cherif, and James Dickerson.

The purpose of our study is to explore the optical properties of Europium Sulfide nanocrystals. Our method of studying the nanocrystals involves electrophoretic deposition, or EPD. For our study, we manufactured glass electrodes and used evaporation to coat them in chromium and gold, cleaned them using deionized water, isopropyl alcohol, and acetone, and coated them with a thin film of Europium Sulfide nanocrystals using EPD. After EPD was performed, we measured their photoluminescence to confirm that the films were Europium Sulfide. We also studied the films using optical and scanning electron microscopy to determine the optical properties. The EuS films that were made were compared to films made with other materials, such as Europium Oxide. We were successful in making films from Europium Sulfide, and now these films are undergoing further testing of their optical and magnetic properties. Europium Sulfide bulk material is useful in the manufacture of television and computer screens because of its optical properties, and by using Europium Sulfide nanocrystals, we hope to refine and expand these uses.

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**Comparison of collateral damage resulting from free electron laser ablation of cornea at 2.77 and 6.45 µm.**

Lauren Hughes and Yaowu Xiao, and M. Shane Hutson.

The goal of tissue ablation with the free electron laser is to eliminate the problem area, such as brain tumors or corneas, at a more effective rate as that of a usual knife or other lasers. The problem with this is that it is very hard to find a direct wavelength that produces as little collateral damage as possible. The goal of our research is to find this ideal wavelength that produces as little collateral damage as possible, so that it may be reproduced in lasers at as little cost as possible (A free-electron laser usually costs one million dollars, and then another million dollars per year to maintain, therefore making it difficult to supply these in medical centers).