DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

UNDERGRADUATE POSTER SESSION

Friday, April 19, 2013

11:30am - 12:30pm

Biochemistry Building

Identification and Implications of Active Line-1 Elements in Canines

Hillary Cullison

Under the Direction of Dr. Patrick Venta, Canine Genetics,
Department of Veterinarian Medicine

Long interspersed nuclear elements (LINEs) are non-long terminal repeat retrotransposons that have risen to such a high copy number in mammalian DNA as to be considered ubiquitous. The purpose of this research is to identify those LINEs that are active in the canine genome. This is of interest for numerous reasons including understanding of cellular response to retrovirus exposure, the role of LINEs in various regulatory elements, and their use as a tool to identify evolutionary histories. The most highly studied of these elements is LINE-1 (L1), for which genomic signatures allow the detection of both actively transposing LINEs and inactive fixed LINEs. In order to identify potentially active LINEs, the UCSC Genome Browser was used to search canine chromosomes 1-3 for full length LINEs (~6000 bp). These LINEs were then translated in silico by use of the Expasy translate tool and examined for complete open reading frames for the two proteins encoded by active LINEs. Thirty potentially active LINEs were identified. These elements were then compared to a generated consensus sequence to approximate the number of mutations accrued in each element as an indication of relative age. Of the 30 candidates initially identified, the three youngest, and presumably most active, elements are now available to undergo isolation, amplification, and testing in cell culture in order to determine activity.

Purification of Methylated Histone H3 by PIMAX System

Wei-Yu Liu

Under the Direction of Dr. Min Hao Kuo, Department of Biochemistry & Molecular Biology

The purification using PIMAX system is efficient and accurate. In our case, we use the cloned plasmid expressing two proteins. One contains 6x-his tag, leucine zipper Fos and SumoN, and enzyme methyl transferase Set7/9 and G9A. The other one includes leucine zipper Jun and SumoC, and the substrate histone H3. We take the advantage of leucine zipper and got the two proteins together to allow enzyme modifying the substrate. The following step is to use Ni-NTA to co-purify the complex of these two proteins and use Sumo Protease which specifically cuts the peptide bond between SumoC to release methylated H3.

Discovery of 3KPZS Antagonists for Sea Lamprey Control

Santosh Kumar Gunturu

Under the Direction of Dr. Leslie Kuhn, Department of Biochemistry & Molecular Biology

Male sea lampreys release a mating pheromone, 7?,12?,24-trihydroxy-3-one-5?-cholan-24-sulfate (also known as 3-ketopetromyzonol sulfate or 3kPZS) which attracts females to spawning grounds. 3kPZS binds to the receptor SLOR1 on the surface of sensory neurons in the olfactory organ, causing a behavioral response, and is detected in femtomolar concentration. SLOR1 was best modeled using human beta1-adrenergic receptor. This structural template allows reliable modeling of the 3D structure of SLOR1. By computational screening of a database of more than 8 million drug-like organic molecules represented by 240 million low-energy conformations, we aim to find a molecule that binds to SLOR1 and blocks its detection of 3kPZS. We are selecting molecular analogs for electro-olfactogram assays based on having the same steroid ring as 3kPZS and matching its important 3-keto and sulfate oxo groups, while also incorporating different side groups. Molecules with different molecular scaffolds that mimic the shape and electrostatics of 3kPZS are also being selected, as assessed by ROCS software and our own scripts. An additional approach we are using for screening is the SLIDE (Screening for Ligands by Induced-fit Docking, Efficiently) docking software developed by Dr. Kuhn's lab, to assess the quality of interactions for each candidate with SLOR1.

Mixed-Lineage Kinases in Glioma Viability

Ashley Sample

Under the direction of
Dr. Kathleen Gallo, Physiology

Glioblastomas are highly malignant and highly lethal cancers of the central nervous system. The mixed lineage kinases are a family of serine/threonine kinases that can activate multiple MAPK pathways, including JNK. Our lab has shown a critical role for mixed lineage kinase 3-JNK signaling pathway in breast cancer migration and invasion. My current studies demonstrate that treatment with an MLK inhibitor markedly decreases viability of multiple human glioma cell lines. The mechanism through which this occurs is currently being examined. Experiments include determining the impact of an MLK inhibitor on cell cycle and MAPK signaling pathways in glioma cell lines. In addition we are testing the utility of combination therapies with an MLK inhibitor and chemotherapeutic drugs commonly used to treat glioma.

**1st Place Award Winner -
University Undergraduate
Research and Arts Forum**

Analysis of Metal-Binding Sites in Urease Accessory Protein by Combination of Metal-Catalyzed Oxidation and Mass Spectrometry

Adib Abdullah

Under the Direction of Dr. Robert Hausinger

Departments of Biochemistry and Molecular Biology and Microbiology and Molecular Genetics

The metalloenzyme urease catalyzes the hydrolysis of urea into ammonia and carbonic acid, a reaction that provides cells with a useful nitrogen source or used to increase virulence (e.g., in *Helicobacter pylori* colonization of the stomach). The model urease system from *Klebsiella aerogenes* consists of UreA, UreB, and UreC subunits and uses a di-nickel active site for catalysis. The accessory proteins UreD, UreF, UreG, and the nickel metallochaperone UreE are required to sequester and shuttle nickel ions to the active site *in vivo*. Previous *in vitro* studies have shown that all of the accessory proteins except UreF bind nickel ions, but the residues required for nickel binding are only known for UreE. To investigate the metal-binding sites in selected protein species, we performed *in vitro* metal-catalyzed oxidation (MCO). MCO generates short-lived reactive oxygen species (ROS) that can locally react with nearby residues and result in oxidative modification or cleavage at residues close to the metal-binding sites. Samples were subjected to proteolytic digestion and the peptides analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) to identify the oxidized regions. Our results show that nickel or iron catalyzed oxidation successfully oxidizes a tryptic peptide fragment on UreE that contains the known metal binding residues. Ongoing studies are using pepsin and chymotrypsin digests or tandem MS approaches to identify the exact oxidized residues in other accessory proteins. Site directed mutagenesis of the suspected metal-binding residues will be used to validate the assignments by testing for loss of metal binding.

Contractility in Uterus and Cervix

Emma Darios

Under the direction of Dr. Stephanie Watts

Department of Pharmacology and Toxicology

Both the uterus and cervix express smooth muscle that is pharmacologically sensitive to contractile agonists. Due to the difference in function of these two tissues, we hypothesized that their basic contractile mechanisms would be different. Strips and rings of virgin Sprague Dawley uterus and cervix tissues, respectively, were mounted in isolated tissue baths for measurement of isometric contraction. We studied two agonists, which activate heptahelical G protein linked receptors, oxytocin (OT) and the muscarlinic cholinergic agonist carbamylcholine (carbachol). The contractile mechanisms investigated have been validated in vascular smooth muscle. The Erk MAPK pathway was inhibited by PD098059 (1 μM), but did not modify contraction (potency or efficacy) in either tissue to either agonist (table). Similarly, the phosphoinositide-3-kinase inhibitor LY294002 (10 μM) and reactive oxygen species inhibitor 4-hydroxy Tempo (1 mM) were without effect on agonist-induced contraction in the uterus. 4-hydroxytempo reduced OT-induced contraction 59% (vs. vehicle max) in the cervix. By contrast, the L-type voltage dependent calcium channel antagonist Nifedipine (1 μM) inhibited maximal contraction to OT in the uterus 32% and cervix 20%. A similar inhibition was observed with carbachol as the agonist in the uterus (26%) and cervix (33%). Notably, the Rho Kinase inhibitor Y-27632 (10 μM) was ineffective in reducing carbachol-induced contraction in the uterus and cervix, but significantly reduced contraction to OT in the uterus (39%) and cervix (47%). These findings illustrate that reproductive smooth muscle mechanisms are not identical to those in vascular muscle and contractile agonists do not activate the same pathways. There are subtle differences in mechanisms activated within the uterus and cervix. Understanding these mechanisms is an important step to understanding how cervix and uterus function might be independently influenced.

Characterization of the Re-emerging Epizootic Epitheliotropic Disease Virus of Lake Trout in the Great Lakes

Ashley Bourke

Under the Direction of Dr. Mohamed Faisal, Departments of Fisheries and Wildlife, and Pathobiology and Diagnostic Investigation

In the mid-1980s, more than 15 million mortalities of hatchery-reared lake trout (*Salvelinus namaycush*) populations occurred in seven hatcheries in three states (lakes Michigan and Superior watersheds) due to a deadly herpesvirus known as Epizootic Epitheliotropic Disease Virus (EEDV). Despite stringent control measures, a major EEDV outbreak killed over one hundred thousand lake trout in Michigan's Marquette State Fish Hatchery in Fall 2012. By using realtime PCR to detect and quantify virus titers in both freshly collected and archived tissues from various salmonid species, this study aims to determine the susceptibility and carrier status of species in order to understand how to prevent the spread of EEDV. In addition to identifying the host range and disease course, the tissue tropism of EEDV will be determined by testing gill, kidney, spleen, heart, and mucous samples. Characterization of EEDV is extremely important for the improved design of control strategies since developing captive lake trout brood stocks are critical for the restoration of the severely depleted lake trout populations in the Great Lakes.

The Effects of Lineage-Determining Transcription Factor PU.1 on Chromatin Architecture

Brandon Wilkinson

Under the Direction of Dr. Monique Floer, Department of Biochemistry & Molecular Biology

How lineage-specific transcription factors (TFs) regulate cell-type specific gene expression has remained elusive. We have investigated the role of PU.1, a TF required for macrophage identity, in determining chromatin architecture at three pro-inflammatory genes in mouse macrophages. Previous studies showed that PU.1 is bound to putative enhancers and promoters of these genes in macrophages, and it has been suggested that PU.1 binding may alter chromatin architecture at these regions (Ghisletti, S. et al. 2010 Immunity 32, 317, Heinz, S. et al. 2010 Mol. Cell 38, 576). We have used a quantitative nucleosome occupancy assay (Bryant, G. et al. 2008 PLoS Biology 6, 2928) to determine changes in nucleosome binding at these genes upon PU.1 binding using an inducible PU.1 system. Our results show that PU.1 binding to a putative enhancer 10kb upstream of IL1A leads to relative depletion of nucleosomes from the enhancer and our preliminary data suggest that this may be mediated by PU.1-mediated recruitment of the nucleosome remodeler PBAF. We hypothesize that cell-type specific chromatin architecture at enhancers facilitates rapid induction of the associated genes in response to an appropriate signal.

1st Place Award Winner - University Undergraduate Research and Arts Forum

C-Terminal Domain of Retinoblastoma Protein Governs PRB Stability and Its Role in Apoptosis

Raj Lingnurkar

Under the Direction Drs. R William Henry and Satyaki Sengupta,
Department of Biochemistry & Molecular Biology

Mutations in the Retinoblastoma gene are present in one third of all human cancers. A noted tumor suppressor, Retinoblastoma protein (pRB) binds transcriptional activator E2F1, repressing transcription of genes critical to cell cycle progression. Consistent with its tumor suppressor activity, pRB also governs apoptosis in proliferating cells in response to DNA damage. Tethering of pRB to pro-apoptotic gene promoters is mediated through its C-terminus region, which contains critical intermolecular contacts with E2F1. Intriguingly, retinoblastoma patients harbor a premature stop codon in the RB gene, resulting in a truncated pRB lacking the entire C-terminus. We hypothesize that these mutants are debilitated for inducing apoptosis in the face of genotoxic stress, and thus offer cancer cells a distinct survival advantage. To test this, mutations were introduced into the RB gene to mimic this clinically observed genotype, and mutant proteins are being assayed for their ability to induce apoptosis in cancer cells. The pRB C-terminus region also shows structural homology to the C-terminal region of other RB family proteins, p107 and p130. Based on our recent work, we hypothesize that this region is also linked to the targeted destruction of pRB, contributing to the etiology of cancer progression. Currently, mutant forms of human pRB bearing defined C-terminal deletions are being tested for stability in cancer cells. Findings from these studies would uncover regulatory pathways impingent on pRB, and provide us insight to therapeutic strategies for cancers involving loss of pRB function.

Conservative Binding Function of TGD2 Protein throughout Chloroplast Organisms

Peter Hsueh

Under the Direction of Dr. Christoph Benning, Biochemistry and Molecular Biology

The transportation of lipid building blocks from the endoplasmic reticulum into the chloroplast envelope membranes is essential for chloroplast thylakoid membrane biosynthesis. Previous studies in Arabidopsis showed the trigalactosyldiacylglycerol (TGD 1, 2 and 3), protein complex to form ABC transporter in the inner envelope membrane of chloroplast. TGD2 is the substrate binding protein and it is anchored with an N-terminal membrane-spanning domain in the inner envelope membrane;its soluble C-terminus appears to bind phosphatidic acid (PtdOH) specifically. Here, we takes an evolutionary approach by studying TGD2 homologs from green algae, Chlamydomonas reinhardti (Cr), and cyanobacteria, Syncocystis sp. 6803 (Sy) which two organisms do not have lipid transport from ER to the chloroplast. To investigate the PtdOH binding in detail, the soluble C-terminal regions of each protein were fused with Discosoma sp. Red fluorescent protein (DsRed).

The fusion protein, DsRed-CrTGD2 and DsRed-SyTGD2, are greatly improved in solubility and easier to detect during protein production in Escherichia coli. Their lipid binding activities are investigated in vitro using liposome association assays. Results show that both proteins have similar PtdOH binding activity to the Arabidopsis TGD2.

We hypothesize that although similar protein activities appear in DsRed-CrTGD2 and DsRed-SyTGD2, We hypothesize that TGD2 function conserved throughout chloroplast organisms and crucial for cyanobacteria and algae biosynthesis.

Using Lipopolysaccharide Truncation Mutants To Investigate Surface Characteristics And Metal Reduction in Geobacter Sulfurreducens

Michael Paxhia

Under the Direction of Dr. Gemma Reguera, Department of Microbiology and Molecular Genetics

Lipopolysaccharide (LPS) is a major component of the outer membrane of gram-negative bacteria. Its general structure of a Lipid A base, phosphorylated core and polysaccharide O-antigen protects cells from harm, retains their cell envelope integrity, and allows attachment to different surfaces. In pathogenic bacteria, LPS is well characterized as a means to attach to host cellular membranes. However, LPS is also present in non-pathogenic bacteria. The metal-reducing bacterium Geobacter sulfurreducens has a unique form of LPS lacking the O-antigen and is believed to improve binding of metals through hydrophobic interactions. We created several truncated LPS mutants and characterized their hydrophobic properties and ability to reduce both soluble and insoluble electron acceptors to determine the role of LPS in the reduction of metals, such as iron and uranium. In addition, we investigated the role of LPS in uranium immobilization and reduction, examining the preservation of cell integrity throughout the process. This characterization will broaden our understanding of metal eduction in G. sulfurreducens and lead to the development of biomimetic devices for in-situ remediation of uranium-contaminated environments.