DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

UNDERGRADUATE POSTER SESSION

Friday, April 11, 2014
11:30a - 12:30p

BIOCHEMICAL CHARACTERIZATION OF ALKBH1 VARIANTS FOCUSING ON PUTATIVE PROTEIN-DNA ADDUCT FORMING RESIDUES

Kristen Clark

Mentors: Drs. Tina Muller and Robert Hausinger, Departments of Biochemistry & Microbiology

Abstract: Alkbh1 is a human homolog of the Escherichia coli AlkB protein that directs DNA repair by removing alkylation damage to DNA bases. While the in vivo role of Alkbh1 is still unknown, the protein was recently shown to possess abasic site lyase activity in vitro and to cleave abasic sites according to a beta-elimination mechanism. Surprisingly,
Alkbh1 forms a covalent adduct to the 5?-DNA product. In this project, different Alkbh1 variants were characterized, focusing on residues that may form the protein-DNA adduct as well as several amino acids which are predicted to bind metal ions. Site-directed mutagenesis was used to make variant proteins which differ in one or more amino acids from the wild type protein, with expression in E. coli and purification by affinity chromatography. Assays were carried out to investigate whether adduct formation and AP lyase activity differed from the wild type enzyme. This approach has begun to provide us with additional knowledge about key amino acids in Alkbh1, with the hope of identifying residues critical to adduct formation. Thus far, a series of variants of putative zinc-finger residues and selected other potential amino acids involved in adduct formation were shown to not affect adduct formation.

~~~~~~~~~~~~~~~~~

The role of carboxylesterases in acyl sugar biosynthesis/degradation in *Solanum*

Karin Hanisch

Mentor: Robert Last, Department of Biochemistry and Molecular Biology

Abstract: Glandular trichomes are hair-like structures found on the surface of tomato plants that secrete a variety of secondary metabolites including acyl sugars, which play a role in insect defense. Previous screening of a set of tomato lines containing regions of the *Solanum pennellii*, LA0716, genome in a *Solanum lycopersicum*, M82, background showed a region on chromosome 5 to be influential in acyl sugar production. Two genes from this region, annotated as carboxylesterases (CXEs), are highly expressed in trichomes. Both CXEs in *S. pennellii*, SpASH1 and SpASH2, appear to encode functional proteins while *S. lycopersicum* has a deletion in the second carboxylesterase, SlASH2, resulting in a non-functional protein. In assays using purified recombinant enzyme, SpASH1, SlASH1 and SpASH2 all demonstrated esterase activity in vitro by removing specific acyl chains from purified tomato acylsucroses. SpASH1 and SlASH1 also showed activity in degrading acylglucoses. A search of the tomato genome for other trichome expressed CXEs identified two genes, 09g075710 and 04g005230. Assays revealed that the lycopersicum and pennellii alleles of the gene 09g075710 encode enzymes that hydrolyze acylsucroses. No activity has been detected with 04g005230 enzyme, however not all possible substrates have yet been tested. The exact biological role of CXEs in acyl sugar biosynthesis *in planta* is not yet known. Future experimentation will utilize transgenic plants to further explore *in vivo* carboxylesterase function.
~~~~~~~~~~~~~~~~~

In vitro selection and enrichment of ssRNA binding targets of the 41 kDa and 61 kDa PPR proteins from Trypanosoma brucei

Michael Lenard

Mentor: Dr. Charles Hoogstraten, Department of Biochemistry and Molecular Biology

Abstract: African trypanosomiasis, or African sleeping sickness is a disease caused by infection from the parasitic protozoan Trypanosoma brucei. This parasite is transmitted by the bite of an infected Tsetse fly. Most cases occur in sub-Saharan Africa. If left untreated, the parasite may cross the blood-brain barrier and cause extensive neurological damage, eventually leading to coma, organ failure and death. Problems arise in treating this disease due to the parasite?s natural defenses, which include a frequently mutating glycoprotein coat which allows it to circumvent the host?s immune system. Current treatment options are inefficacious and some are toxic organoarsenic compounds.

Pentatricopeptide repeat (PPR) proteins are a family of single stranded RNA binding proteins that have greatly expanded genetically in T. brucei compared to typical eukaryotes. PPR proteins are characterized by degenerate 35 amino acid motif repeats. Knocking down PPR-coding genes in T. brucei causes extreme phenotype changes, indicating that these proteins are necessary for the development of the parasite. Because T. brucei contains many more PPR proteins than humans, they may comprise an ideal target for therapy. Therefore, we are determining PPR protein binding target sequences to facilitate the development of novel drugs to be used for treatment of trypanosomiasis.

The structure of PPR proteins suggests that they may be involved in RNA editing. These proteins form a series of ?-helices that interact with single stranded RNA via sequence-specific hydrogen bonding. We hope to find a consensus sequence for the RNA targets of PPR41 and

PPR61 by way of in vitro selection, in which an initial random sequence pool is subjected to iterative enrichment by exerting selection pressure for protein binding over a number of rounds.

~~~~~~~~~~~~~~~~

Production and Aggregation Studies of Phosphorylated Tau Protein Related to Alzheimer's Disease

Wei-Yu Liu

Mentor: Min-Hao Kuo

Abstract: Tauopathies are a class of neurodegenerative diseases featured by intraneuronal deposits of abnormal phosphorylated tau protein. Though the association between phosphorylation of tau and the most well-known neurodegenerative disease, Alzheimer's disease, is not well-established, we only know the component for neurofibrillary tangles is hyperphosphorylated tau and the roles of phosphorylation in pathology and protein aggregation are still intriguing. One of the bottlenecks in the in vitro studies of tau aggregation is to obtain quantitatively phosphorylated tau (p-tau). To this end, we are developing the Zippers-Assisted Catalysis (ZAC) to produce efficiently p-tau. For now, we are processing ligation independent cloning (LIC) in order to insert kinase CDK5, protein folding chaperone FKBP and tau into ZAC vector.
~~~~~~~~~~~~~~~~

Structural Chemistry and Magnetic Properties of Copper Pyromellitate Coordination Polymers Containing Pyridylnicotinamide Ligands

Jessica Mizzi

Mentor: Dr. Robert LaDuca, Lyman Briggs College and Department of Chemistry

Abstract: A series of divalent copper pyromellitate (1,2,4,5-benzenetetracarboxylate, pyro) coordination polymers containing either 3-pyridylnicotinamide (3-pna) or 4-pyridylnicotinamide (4-pna) was hydrothermally prepared and structurally characterized by single-crystal X-ray diffraction. $[Cu_2(pyro)(pyroH_2)(3\text{-}pnaH)_2(H_2O)_2]_n$ (**1**) is a 2-D coordination polymer built from $\{Cu_2O_2(OCO)_2\}$ dimeric units, while $\{[Cu(pyro)(3\text{-}pnaH)_2(H_2O)_2]\bullet 4H_2O\}_n$ (**2**) possesses cationic 1-D chain motifs and unligated $pyroH_2$ dianions. $\{[Cu_2(pyroH_2)_3(4\text{-}pnaH)_2]\bullet 6H_2O\}_n$ (**3**) is also a 1-D coordination polymer, but built from the linkage of $\{Cu_2(pyroH_2)\}$ dimeric units. $\{[Cu_3(pyroH)_2(4\text{-}pna)_2(H_2O)_2]\bullet 2H_2O\}_n$ (**4**) manifests a 3-D coordination polymer network with rare frl topology, containing embedded $\{Cu_3(OCO)_2\}$ linear trimers. Moderately strong antiferromagnetic coupling (J = –76.4(3) cm^{-1}) was observed within the $\{Cu_2O_2(OCO)_2\}$ dimeric units in **1**, while very weak ferromagnetic coupling (J = 0.8(2) cm^{-1}) was observed within the $\{Cu_3(OCO)_2\}$ linear trimers in **4**.

~~~~~~~~~~~~~~~~~

***Irf6* and Receptor Tyrosine Kinase signaling interact in craniofacial development**

Raeuf R. Roushangar

Mentor: Dr. Brian Schutte, Microbiology and Molecular Genetics

Abstract: Mutations in Interferon Regulatory Factor 6 (*Irf6*) lead to Van der Woude Syndrome and Popliteal Pterygium Syndrome, dominantly inherited orofacial clefting. *Irf6* knockout mice ($Irf6^{gt/gt}$) have severe bilateral oral adhesion and a cleft palate. In addition, variants within the *Irf6* locus contribute at least 12% of all isolated orofacial clefting risk. Likewise, variants within Receptor Tyrosine Kinase (RTK) signaling pathway contribute additional risk for isolated orofacial clefting. In the mouse, like *Irf6*, perturbation of *FGF* signaling leads to oral adhesions and palatal clefting. In this study, we ask if *Irf6* genetically interacts with RTK signaling. To answer this question, we over-express *Spry4* in oral epithelium using the *KRT14* promoter ($Tg^{KRT14::Spry4}$). Considering that *Spry4* represses RTK signaling, we predict that over-expressing *Spry4* will lead to oral adhesions. We further predict that *Irf6* and *Spry4* interact in craniofacial development. To examine our predication, we use a mouse model and hypothesize that $Irf6^{gt/+}$;$Tg^{KRT14::Spry4}$ embryos will have more severe oral adhesions than either $Tg^{KRT14::Spry4}$ and $Irf6^{gt/+}$ littermates. Our analysis quantitatively shows that whereas $Irf6^{gt/+}$ embryos develop mandible-maxilla oral adhesions, $Tg^{KRT14::Sprouty4}$ develop palate-tongue oral adhesions. Furthermore, $Irf6^{gt/+}$;$Tg^{KRT14::Spry4}$ embryos have more severe mandible-maxilla and palate-tongue oral adhesions than either singly mutant embryo. Molecularly, we show that $Irf6^{gt/+}$ and $Tg^{KRT14::Spry4}$ effect a common molecular signature for periderm. Significantly, $Irf6^{gt/+}$ and $Tg^{KRT14::Spry4}$ interact in regulating *Grhl3*, a recently discovered human orofacial clefting gene. Together, these data suggest that *Irf6* and *RTK* signaling regulate periderm development in the mouse and contribute significant risk to orofacial clefting in humans.
~~~~~~~~~~~~~~~~~

The mechanism of photo-protection in plants: Involvement of thermally activated states

Matt Smith

Mentor: David M. Kramer: Biochemistry and Molecular Biology/Plant Biology

Abstract: Photosynthesis is a pivotal biological process for all life, and learning how to increase photosynthetic efficiency can benefit society and positively impact all life on Earth. In plants, overexposure to sunlight can reduce photosynthetic efficiency by creating harmful reactive oxygen species, including singlet oxygen and superoxide. Plants have adapted to protect themselves by dissipating excess excitation energy as heat; this process is known as non-photochemical quenching (NPQ). The mechanism of NPQ is under intense debate. Some models posit that NPQ involves exciton transfer to low-energy carotenoid energy levels, while others propose that de-excitation involves electron transfer. NPQ can be quantified by comparing the differing chlorophyll florescence yields in light and dark adapted plants after saturating normal photochemical quenching with light. However, these differences in fluorescence seem to disappear at liquid nitrogen temperatures, 77K. Based on this observation, we hypothesized that NPQ involves a thermally activated intermediate. If so, characterizing this intermediate may allow us to identify the thermodynamic mechanism of NPQ. We are testing this hypothesis by measuring the temperature-dependence of chlorophyll fluorescence emission spectra of wild type and mutant plants with altered NPQ responses. Preliminary work was performed in a temperature regulated cryogenic chamber; this apparatus was attached to a spectrofluorometer via a fiber optics light guide, with illumination from a filtered light emitting diode. Results show clear differences in fluorescence temperature-dependence between the light- and dark- adapted wild type and NPQ deficient mutants, consistent with the proposed thermally activated intermediate. Further data is currently being collected via Time Correlated Single Photon Counter (TCSPC) with hopes of observing correlations between previous experiments and fluorescence decay kinetics in samples. Future studies may pertain to the analysis of the PsbS protein, which is inhibited in the NPQ deficient mutants

~~~~~~~~~~~~~~~~

DIPEPTIDYL PEPTIDASE-IV/CD26 ENZYME ACTIVITY IN HEALTHY AND EQUINE METABOLIC SYNDROME (EMS) EQUIDS

Lara Stephens-Brown

Mentor(s): Patty Sue D Weber, Raymond J Geor, L Jill McCutcheon

Abstract: Equine Metabolic Syndrome (EMS) is a metabolic condition characterized by increased adiposity, insulin resistance, hyperinsulinemia, and laminitis. The dipeptidyl peptidase--IV (DPP-IV) enzyme plays an important role in glucose metabolism in many species. Soluble DPP-IV circulates in the blood and its activity is higher in diabetic humans. Neither DPP-IV concentration nor enzyme activities have been studied in horses previously. The objective of this study was to (1) validate a commercial DPP-IV activity assay in equine serum and (2) compare DPP-IV activity in healthy control and EMS animals. Serum samples (collected after 8 hours of feed withholding [“fasting”]) from 114 Morgan horses and Welsh ponies (n=46 control, n=68 EMS) were used in this study. Assay protocols were adapted from the manufacturer’s recommendations with emphasis on accurate dilution, inhibition, and assay read length. DPP-IV activity control intra-assay and inter-assay coefficients of variation (CV) were 0.8 and 7.1%, respectively, while a pooled equine serum control CVs were 3.6 and 15.1%. DPP-IV activity was higher in EMS equids (22.80 +1.43 RFU/min) than healthy controls (17.56 +1.28 RFU/min; P=0.0013). Significant Spearman correlations (P<0.05) were observed between DPP-IV activity and body condition score (r=0.340), and fasting serum insulin (r=0.243), triglyceride (r=0.516), and non-esterified fatty acid (r=0.398) concentrations. Univariate analysis shows breed is strongly associated with DPP-IV activity. These results indicate that this assay may be used to determine DPP-IV activity in equids. The higher DPP-IV activity in EMS when compared to control equids suggests that DPP-IV may play an important role in glucose/insulin dynamics in EMS.
~~~~~~~~~~~~~~~~