DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

UNDERGRADUATE POSTER SESSION

Wednesday, April 14, 2010
3:30pm – 5:00pm

Dennis Miner
Under the Direction of Dr. Robert Last and Dr. Anthony Schilmiller
Department of Biochemistry and Molecular Biology

Title: Identification of a Sesquiterpene Synthase in Tomato Type VI Leaf Trichomes

Abstract:
Trichomes are hair-like appendages that protrude from the epidermis of many plant species and are involved in the biosynthesis of specialized metabolites. These metabolites can serve to protect plants from insect infestation, but also contribute to the scent of flowers that can attract pollinators. Tomato trichomes produce significant amounts of specialized metabolites of the terpene class including three sesquiterpenes: germacrene C, β-caryophyllene, and α-humulene. GC-MS analysis of type VI leaf trichomes compared to stem showed significant amounts of β-caryophyllene and α-humulene produced in leaf type VI glands, but not in trichomes from the stem. The amount of δ-elemene (the thermal degradation product of germacrene C from the inlet injection of the GC) remained the same in glands from both tissues. Searching the trichome EST database for sesquiterpene synthases revealed two highly expressed genes. The first, SSTLE1, encodes a protein that was previously shown to catalyze production of germacrene C in vitro, and was originally suggested to also produce β-caryophyllene and α-humulene. Another contig, that we named CAHS (for β-caryophyllene/α-humulene synthase), was found that is 91% identical at the nucleotide level to SSTLE1. EST counts for CAHS from leaf vs. stem trichomes showed an expression pattern matching that of β-caryophyllene and α-humulene production. From these observations, we hypothesized that CAHS is responsible for β-caryophyllene and α-humulene production in leaf. Activity assays using recombinant CAHS expressed in E. coli demonstrated that this enzyme produces β-caryophyllene and α-humulene using farnesyl diphosphate as substrate.

Michael Howard
Under the Direction of Dr. Robert Hausinger
Departments of Biochemistry and Molecular Biology, Microbiology and Molecular Genetics

Title: Computational modeling and molecular docking of ligands to the putative active site of AIDB an alkylation response protein of *Escherichia Coli*

Abstract:
Exposure of *Escherichia coli* to alkylating agents activates expression of three DNA repair proteins Ada, AlkA, and AlkB along with AidB. Previous studies have shown that AidB binds to double-stranded DNA, contains a redox active flavin adenine dinucleotide (FAD) prosthetic group, and exhibits a crystalline structure similar to the flavin-containing acyl-CoA dehydrogenases (ACADs). The molecular mechanism by which AidB reduces the effects of alkylation damage is unknown, but direct enzymatic repair of alkylated DNA, protection of DNA from the reagents, and destruction of alkylating agents have been proposed. In this study, the accessibility of the putative FAD active site was examined by computational modeling using two different ligand docking programs: Dock Blaster and SLIDE (Screening for Ligands by Induced-fit Docking). Both programs suggest that the FAD active site could bind molecules resembling free pyrimidines and purines, thus supporting a direct enzymatic role in DNA repair.

Catherine Nezich
Under the Direction of Dr. Christina Chan
Departments of Biochemistry and Molecular Biology, Chemical Engineering and Material Science, and Computer Science and Engineering

Title: Effects of palmitic acid on the Unfolded Protein Response in HepG2 cells

Abstract:
The Unfolded Protein Response (UPR) is a set of signal transduction pathways initiated in the endoplasmic reticulum (ER stress) and used by eukaryotic cells to adapt to perturbations in protein folding. It is thought to be activated in diseases like Alzheimer?s Disease, cancer, and diabetes. Past research shows that ER stress can be induced by saturated free fatty acids such as palmitate (PA), modulating calcium homeostasis. Our lab has demonstrated that PA inhibits the phosphorylation of PKR and other cytosolic kinases by binding to the conserved ATP binding site. The purpose of this study is to determine whether PA also binds directly to UPR transmembrane kinases, PERK and IRE1, to activate its signaling. Human hepatoblastoma (HepG2) cells were treated with various PA concentrations (0-0.4 mM) for 0-48 hr and analyzed by western blot for IRE1, PERK, and BiP (UPR misfolded protein sensor protein). Preliminary results show that palmitate may induce the expression of BiP and phosphorylated PERK and IRE1. However, the effect of PA on the transmembrane kinases is opposite to what was observed with PKR, suggesting that PA may have different interactions with cytosolic versus transmembrane kinases. These results also raise the possibility that PA may have implications on the efficacy of current drug therapies that target kinases. We are currently working to determine the effects of PA on the protein expression levels of several downstream signaling molecules in the UPR, as well as isolating and purifying IRE1 for PA- and ATP- binding studies, as previously performed with PKR.

Bongjun Son
Under the Direction of Dr. Lee Kroos
Department of Biochemistry and Molecular Biology

Title: Regulation of C-signal dependent promoter W4406 during development of *Myxococcus xanthus*

Abstract:
Myxococcus xanthus is a soil bacterium that provides a model for investigating cell-cell signaling and gene regulation. Upon starvation, *M. xanthus* forms multicellular fruiting bodies and expresses genes that lead to sporulation. C-signaling, which depends on contact between cells and communicates positional information, is essential for this developmental process. Previous studies have shown the importance of C-signaling in regulation of gene expression, and the W4406 promoter region is under C-signal control. Three *cis*-regulatory elements have been identified upstream, downstream, and proximal to the W4406 promoter. The upstream element has a negative effect, whereas the downstream element has a positive effect on promoter activity. Electrophoretic mobility shift assays (EMSAs) show cooperative binding of two proteins, MrpC2 and FruA, to each of the three sites. MrpC2 is a transcription factor induced by starvation that also plays a second role in regulating programmed cell death during the developmental process. FruA is involved in the response to C-signal. Using 5’ and 3’ deletions, and multiple-base-pair changes, one end of the upstream element has been localized between -120 and -110, and one end of the downstream element has been localized between +93 and +116, relative to the transcriptional start site. In each case, a FruA binding site appears to define the end of the element, and binding of FruA to that site is important for regulatory function of the element.

Megan Andrzejak
Under the Direction of Dr. Robert Hausinger and Tina Mueller
Departments of Biochemistry and Molecular Biology and Microbiology and Molecular Genetics

Title: Identification of Residues Essential for Abasic Site-Specific DNA Lyase Activity of Human ABH1

Abstract:
Humans possess eight homologues, known as ABH1-ABH8, of the *Escherichia coli* DNA repair enzyme AlkB that catalyzes the oxidative demethylation of alkylated DNA and RNA. In addition to its demethylation activity, ABH1, closest in sequence to AlkB, also catalyzes the unanticipated cleavage of DNA at abasic sites (AP) according to a b-elimination mechanism. The goal of the experiments described here was to identify critical residues of the protein that are responsible for this lyase activity. This class of enzyme typically uses a lysine side chain to catalyze the reaction, so all 22 lysines were mutated (24 mutations in total) by site-directed mutagenesis. The variant forms of ABH1 were over-expressed in *E. coli* BL21(DE3) cells, cell-free extracts were obtained, and the His-tagged proteins were purified by using a Ni-NTA Sepharose column. AP-lyase activity assays demonstrated that all of the purified proteins retained the ability to cleave AP-containing DNA, providing no significant insight as to which lysine is responsible for the DNA-cleaving activity. This result indicates that ABH1 possesses an ?opportunistic lysine? which takes over once the original lysine has been mutated as has been seen in certain other AP-lyases. An additional approach to identify the region containing the critical lysine involved construction of N- and C-terminally truncated proteins in maltose binding protein fusion variants. The mutants were enriched with an amylose resin; however, again, all truncation variants were active indicating that the two lysines responsible for the DNA-cleavage are not located in the same region of the protein.

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Brittnie DeVries
Under the Direction of Dr. Robert Hausinger
Department of Biochemistry and Molecular Biology

Title: Investigations into the nickel-dependent stabilization of a UreG-UreE complex via site-directed mutagenesis

Abstract:
Urease, a nickel-containing enzyme, requires a series of four accessory proteins for activation: UreD, UreE, UreF, and UreG. The goal of these experiments was to characterize the interaction of UreG with urease (UreABC) and the other accessory proteins. In order to characterize this interaction, highly conserved UreG residues were selected for site-directed mutagenesis. These mutations were created in *Strep*-tagged UreG ($UreG_{Str}$), which binds tightly to *Strep*-Tactin resin, thus allowing for pull-down assays to reveal associated proteins. Two different types of pull-down assays were performed. First pull-downs were performed using *Escherichia coli* DH5-α cells that over-expressed the *Klebsiella aerogenes* urease cluster modified to encode $UreG_{Str}$ or its variants, and grown with and without nickel ions. Cell-free extracts were obtained and pull-down assays revealed the presence of the UreABCDF complex, except in the case of D80A $UreG_{Str}$ where the interaction was interrupted for all components except UreE. These assays also indicated that nickel stabilized the interaction between $UreG_{Str}$ and UreE, but not with urease, UreD, or UreF. A second set of pull-down assays used purified $UreG_{Str}$ or its variants plus isolated UreE, and the effect of varied nickel ion concentration was again examined. The interaction between $UreG_{Str}$ and UreE was stabilized when nickel was present, and none of the site-directed mutations disrupted this interaction.

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Thomas Cooke
Under the direction of Drs. Gregg Howe,
Hoo Sun Chung and Leron Katsir
Departments of Biochemistry/Molecular Biology and Plant Research

Title: A STABILIZED SPLICE VARIANT OF THE JASMONATE ZIM DOMAIN PROTEIN JAZ10 REPRESSES JASMONATE SIGNALLING IN ARABIDOPSIS

Abstract:
Jasmonate (JA) is a lipid derived hormone that regulates various aspects of plant growth and defense. JASMONATE ZIM domain (JAZ) proteins act as repressors of JA signaling. Perception of bioactive JAs by the F box protein CORONATINE INSENSITIVE1 (COI1) causes degradation of JAZs via the ubiquitin proteasome pathway, which in turn activates the expression of genes involved in plant growth, development, and defense. JAZ proteins contain two highly conserved sequence regions: the Jas domain that interacts with COI1 to destabilize the repressor, and the ZIM domain, recently shown by yeast two hybrid assay to mediate homo and heteromeric interactions between JAZ proteins through its conserved TIFY motif (TIFF/YXG). Here, we use an in vitro pulldown assay to show that an alternatively spliced form of JAZ10 (JAZ10.4) that lacks a Jas domain is unable to interact with COI1 in a JAdependent manner, and is therefore resistant to JA induced degradation. We also use a pulldown assay to show that JAZ10.4 can form homodimers, and that this interaction requires an intact TIFY motif. Our findings support the hypothesis that JAZ10.4 functions to attenuate signal output in the presence of JA, and that the dominant negative action of this splice variant involves protein protein interaction through the ZIM/TIFY domain.