

**DEPARTMENT OF  
BIOCHEMISTRY  
&  
MOLECULAR BIOLOGY**

***UNDERGRADUATE POSTER  
SESSION***

**Tuesday, April 21, 2009  
3:30pm – 5:00pm**

## **“EXPRESSION, PURIFICATION, AND ACTIVITY STUDIES OF ACTIVATION-INDUCED DEAMINASE (AID) PROTEIN”**

Paul Harris, under the direction of Dr. Dennis Arvidson, Department of Microbiology and Molecular Genetics

### **Abstract:**

Activation-Induced Deaminase (AID) protein functions in class switch recombination and somatic hypermutation in mammalian cells. The AID protein deaminates cytidine sites in single stranded DNA. We designed and constructed an expression plasmid that encodes AID as a recombinant fusion protein. Our construct includes an N-terminal maltose binding protein as a solubility and expression tag followed by a hexa-histidine tag to allow affinity purification linked via a TEV cleavage site to AID. This construct will allow us to express the fusion protein at adequate levels, to purify the fusion protein using NiNTA affinity chromatography, to cleave the fusion protein with TEV protease releasing native AID, and to purify the native AID away from both the his-tagged TEV protease as well as the cleaved N-terminal tag using a second NiNTA affinity chromatography step. In previous work with recombinant AID protein, it was found that amino acids remaining after tag cleavage altered AID function. As our new construct will allow us to produce AID protein with no non-native amino acids we expect that it will have full wild type function.

## “THE ROLE OF PHOTOPERIOD IN COLD ACCLIMATION”

Cynthia Collings, under the direction of  
Dr. Michael F. Thomashow, Plant Research Laboratory

### **Abstract:**

Plants sense and adapt to environmental changes, such as light quality and duration, or changes in temperature. Plants are subject to numerous stresses in their environment, such as freezing stress. Many plants, such as *Arabidopsis*, are able to increase their freezing tolerance in response to a period of low, non freezing temperature. Many plants undergo metabolic and physiological changes at low, nonfreezing temperatures in a process known as cold acclimation, which increases their ability to survive freezing temperatures. Plants can acclimate to the cold by altering their metabolome and transcriptome. Several transcription factors induced upon low-temperature exposure, such as CBF1-3(C-Repeat Binding Factor), ZAT10, and ZAT12 contribute to freezing tolerance. Seasonal changes, such as decrease in temperature and day length in the fall, enable some plants to establish freezing tolerance. It has been shown that plants grown under short day (8h light/16h dark) are more freezing tolerant those grown under long day (16h light/8h dark); however, the molecular mechanism is not well understood. To determine whether different photoperiods alter the expression of cold-induced genes during cold acclimation, the transcript levels and kinetics of some early cold-induced transcription factors of *Arabidopsis thaliana* grown in two different photoperiods will be examined.

## **“Elucidate the Role of a Novel DnaJ-Like Protein in Fatty Acid Metabolism Within the Chloroplast”**

Ardian Coku, under the direction of  
Dr. Robert Last, Departments of Biochemistry/Molecular Biology and  
Plant Biology

### **Abstract:**

DnaJ-like proteins are a part of the HSP40 family, which function as molecular chaperones involved in protein folding. As part of the Chloroplast 2010 project to identify the function of roughly 4,400 genes predicted to be targeted to the plastid, an *Arabidopsis thaliana* T-DNA mutant of a gene, At1g08640, which encodes for a DnaJ-like protein, was discovered to exhibit an unusual fatty acid profile. The mutant was identified by fatty acid methyl ester analysis with the use of GC-FID and showed a consistent increase in 16:0, 16:1 $\Delta$ 7, and 18:1 $\Delta$ 9 and a decrease in 16:3. The subcellular localization of DnaJ was determined by generating a DnaJ:GFP fusion protein and analyzing via confocal microscopy. The results, along with a chloroplast import assay, confirmed that the protein is embedded within the chloroplast. Expression and purification of the J-domain:6XHis of Ni affinity column eluted fractions yielded the presence of several other proteins whose identity was determined via Mass-spectrometry and was found to include DnaK and proteins involved in lipid metabolism. An *in vivo* activity assay involving transformation of the J-domain into a DnaJ deficient strain of *E. coli* resulted in restoration of its ability to withstand heat shock. Moreover, complementation of the *Arabidopsis* DnaJ mutants resulted in recovery of the wild-type phenotype. Furthermore, *in vivo* lipid labeling experiments with [1-<sup>14</sup>C] sodium acetate will be conducted to determine whether DnaJ is involved in lipid trafficking.

## “PROBING ENZYME/SUBSTRATE INTERACTION OF AN INTRAMEMBRANE-CLEAVING PROTEASE”

Paul Luethy, under the direction of Dr. Lee Kroos, Department of Biochemistry and  
Molecular Biology

### **Abstract:**

SpoIVFB mediates regulated intramembrane proteolysis in the soil bacterium *Bacillus subtilis*. When starved, this bacterium undergoes spore development directed by several transcription factors. Pro- $\sigma^K$  is one of these factors, and requires that SpoIVFB cleave it to  $\sigma^K$  before gene expression can begin. SpoIVFB is a model protein for the intramembrane-cleaving proteases, whose active sites are buried within membranes, where they cleave transmembrane segments of their substrates. Studying the interaction between SpoIVFB and Pro- $\sigma^K$  will reveal how this unusual type of protease functions. Through site-directed mutagenesis, an active, cysteine-less version of the protein was created by mutating either one or three of SpoIVFB's five cysteine residues to leucine, while the others were changed to serine. Through further mutagenesis, a single cysteine can now be placed in SpoIVFB at residues believed to be close to Pro- $\sigma^K$  during cleavage. A single cysteine can also be placed into Pro- $\sigma^K$ , allowing us to study enzyme/substrate interactions through cross-linking experiments.

**“A STABILIZED SPLICE VARIANT OF THE JASMONATE ZIM-DOMAIN PROTEIN JAZ10  
REPRESSES JASMONATE SIGNALLING IN ARABIDOPSIS”**

Thom Cooke, under the direction of Dr. Gregg Howe, Departments of Biochemistry/Molecular  
Biology and Plant Research

**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 JA-dependent 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.

## **“COMPARISON OF MESOPHYLL AND BUNDLE SHEATH CHLOROPLAST ENVELOPES REVEALS NOVEL TRANSMEMBRANE PROTEINS WITH DIFFERENTIAL EXPRESSION”**

Evan Pratt, Samuel Saitie, and Urs Benning under the direction of Dr. Susanne Hoffmann-Benning, Department of Biochemistry and Molecular Biology

### **Abstract:**

The chloroplast is the site for a number of biosynthetic functions essential to plants, including photosynthesis. Chloroplasts of the C<sub>4</sub> plant *Zea mays* differentiate into bundle sheath (BS) and mesophyll (MS) types. C<sub>4</sub> plants have a higher CO<sub>2</sub> fixation rates than most C<sub>3</sub> plants. PEPC in the MS fixes CO<sub>2</sub> into oxaloacetate which is converted to malate and transported to the BS. There it is released and enters the Calvin cycle. The accumulation depends on higher fluxes of metabolic intermediates and thus a number of chloroplast envelope proteins, which transport those intermediates across the chloroplast envelope membranes. Only a few of these transport proteins have been studied at a molecular level. We hypothesized that comparative proteomics of *Zea mays* will reveal transport proteins that are differentially expressed in the BS and MS cells. Our lab has characterized over 200 proteins from maize chloroplast envelopes. 70% of these proteins contain transmembrane regions and 45% are known chloroplast envelope proteins. 25% are of unknown function. YFP labelling has confirmed the chloroplast localization of eight of these proteins. A semi-quantitative technique based on the peptide counts of the proteome of *Zea mays* MS and BS chloroplast envelopes has revealed 23 differentially expressed hypothetical proteins. We are using RT-PCR to confirm the differential expression between mesophyll and BS cells as well as during different developmental stages and in different plant organs. In parallel, we are analyzing T-DNA knockout mutants and the effect of the lack of the putative transporters on photosynthesis and plant development.

**“COMPARATIVE TOXICOGENOMICS OF TCDD AND TCDF-ELICITED EFFECTS IN MICE:  
EROD ANALYSIS AND EVALUATION OF TCDF TOXIC EQUIVALENCY FACTOR”**

Ashley R Burg, Lyle D Burgoon, and Alhaji N’jai under the direction of  
Dr. Tim R Zacharewski, Department of Biochemistry & Molecular Biology, National Food Safety  
& Toxicology Center and the Center for Integrative Toxicology,

**Abstract**

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a halogenated coplanar molecule, is known to be the most toxic man-made compound. TCDD enters the environment as an industrial byproduct and due to its high lipophilic properties and resistance to degradation it bioaccumulates within mammalian systems, particularly in the liver. Toxic effects elicited by dioxin and dioxin-like compounds are mediated via the Aryl Hydrocarbon Receptor, including induction of Cyp1a1, a prototypical marker of dioxin exposure. To better assess toxicity, a Toxic Equivalency Factor (TEF) concept has been adopted, where toxic effects of a dioxin-like compounds are contrasted with TCDD (TEF=1.0). In this study, a comparative hepatotoxic evaluation following exposure to 2,3,7,8-tetrachlorodibenzofuran (TCDF, TEF = 0.1) was examined in immature, ovariectomized C57BL/6 mice, and compared to TCDD. In a dose-response study, mice were orally gavaged with either TCDD (0.03 µg/kg to 30 µg/kg), TCDF (0.3 µg/kg to 300 µg/kg) or sesame oil vehicle. Mice were sacrificed 72 hrs post-treatment, and hepatic microsomal fractions were extracted. Kinetic analysis of ethoxyresorufin-O-deethylation (EROD) activity were assessed to determine the relative potency of TCDF. Enzyme activities were calculated for each treatment at each dose, and the dose-response was modeled to determine the ED<sub>50</sub> of each compound. ED<sub>50</sub>s of 1.47 and 40.5 for TCDD and TCDF, respectively, were calculated with a TCDF Relative Effect Potency value of 0.036. The results suggest that the World Health Organization’s established TEF of 0.1 may be an overestimate of the hepatotoxicity of TCDF. However, more comprehensive analyses including dose-response studies at different time points are required.

**“DNA BINDING AND DISSOCIATION CONSTANT OF ALKYLATION RESPONSE PROTEIN  
AIDB IN *ESCHERICHIA COLI*”**

Michael Howard, under the direction of Dr. Robert Hausinger, and Dr. Scott Mulrooney,  
Departments of Biochemistry/Molecular Biology & Microbiology/Molecular Genetics

**Abstract**

Exposure of *Escherichia coli* to chemical 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 a crystalline structure similar to the flavin- containing acyl-CoA dehydrogenases. Though the molecular mechanism by which AidB reduces the effects of alkylating damage is unknown, two theories exist. The Presence of a redox-active flavin prosthetic group implicates its use in the direct repair of alkylated DNA. Alternatively, the crystalline structure implicates a role of AidB in protecting DNA by binding and destroying alkylating agents that have yet to contact their DNA target. In this study, the protein was purified to homogeneity and its DNA binding properties were examined. By using electrophoretic mobility shift assays (EMSA) AidB was shown to have an approximate  $K_d$  of 30  $\mu$ M.

## **“IDENTIFICATION OF RESIDUES ESSENTIAL FOR DNA LYASE ACTIVITY SPECIFIC TO ABASIC SITES IN HUMAN ABH1 ”**

Megan Andrzejak, under the direction of Dr. Robert Hausinger, Departments of Biochemistry/Molecular Biology & Microbiology and Molecular Genetics

### **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. ABH1, closest in sequence to AlkB, also catalyzes the unanticipated cleavage of DNA containing abasic sites. The goal of the experiments described here was to identify critical residues of the protein that are responsible for binding to abasic sites in DNA and lyase activity. A series of mutant forms of ABH1 was created in which carefully selected amino acid residues were altered. These mutations were chosen by comparison of the sequences of AlkB and ABH1 from diverse organisms, using conserved portions of the sequences and emphasizing Lys side chains that are often critical for lyases. Site-directed mutagenesis using specific primers was carried out in order to incorporate the desired mutations into the sequence. The variant forms of ABH1 were over-expressed in E. coli BL21(DE3)-RIPL cells, cell extracts were obtained, and the proteins were purified by using a Ni-NTA Sepharose column. The purified proteins were subjected to DNA binding and lyase activity assays to determine whether the mutations affected the binding and/or cleavage of DNA containing abasic sites. The seven mutations initially chosen for study exhibited little or no affect on binding or activity; however, the results of additional mutations will be described.

## **“TEMPLATED NUCLEOSIDE TRIPHOSPHATE LOADING TO DOWNSTREAM SITES BY HUMAN RNA POLYMERASE II”**

Anthony Nazione, under the direction of Dr. Zachary Burton,  
Department of Biochemistry and Molecular Biology

### **Abstract**

As human RNA polymerase II slows elongation in response to a low concentration of an upcoming templated nucleoside triphosphate (NTP) substrate, upstream NTPs induce sequestration of otherwise limiting downstream NTPs. Higher concentrations of an upstream NTP enhance downstream NTP commitment and limiting concentrations of an upstream NTP appear to cause reduced downstream NTP commitment resulting in reversal of phosphodiester bond synthesis. Because of the concentration dependence, upstream NTPs appear to act both at the active site and downstream of the active site. At the active site, NTP concentration is likely to be irrelevant, because of stable NTP-Mg<sup>2+</sup> commitment in the active site. This result indicates that multiple NTPs (i+1 (active site), i+2, i+3) must be on template simultaneously. The apparent  $K_d$  for GTP binding to the active site at the A43 position (43 nucleotide RNA ending in 3'-AMP) is 16.1 mM, but through multiple bonds the reaction runs significantly faster at 2500 mM than at 50 mM, indicating that NTPs may have roles outside of the active site. These results are consistent with the NTP-driven translocation model for NTP loading by multi-subunit RNA polymerases and appear inconsistent with NTP loading primarily or solely through the secondary pore.

## “CRYSTALLOGRAPHY STUDY OF THE COP9 SIGNALOSOME SUBUNIT 8 (CSN8)”

Lauren Grenzicki, under the direction of Dr. R. William Henry,  
Department of Biochemistry and Molecular Biology

### Abstract

The COP9 signalosome (CSN) complex is composed of eight subunits and was originally discovered in *Arabidopsis* during studies of photomorphogenesis. Further studies determined that CSN is conserved across multiple eukaryotic species. In some cases, CSN contains isopeptidase activity in an ubiquitin-like pathway involving neddylation rather than ubiquitination. Mutations in *Drosophila* are lethal early in development, suggesting that the deneddylation of certain target proteins by CSN is essential to embryogenesis. Indeed, one of the subunits, CSN8, is essential to oogenesis and adult development of *Drosophila*. In both mouse and *Drosophila* cells, the lack of CSN8 blocks re-entry of the cell into the cell cycle from the G0 phase, providing a plausible pathway for disrupted developmental programs that rely on cell cycle control. Studies in adult mice also indicate that deletion of CSN8 negatively impacts the development of the CSN complex, reducing T-lymphocyte proliferation and survival. To elucidate the function of CSN8, a crystallographic study will be pursued to determine the subunit's atomic structure. A purification protocol is being developed to obtain a high yield of protein. Though earlier studies have yielded structures of a few truncated CSN subunits, pursuing studies of the full length protein will provide a better idea of the possible function of CSN8 in the CSN isopeptidase activity and its influence on the cell cycle.

**“REGULATION OF C-SIGNAL-DEPENDENT PROMOTER 4403 IN  
*MYXOCOCCUS XANTHUS*”**

Jun-Seok Lee, under the direction of Dr. Lee Kroos, Department of Biochemistry and  
Molecular Biology

**Abstract**

*Myxococcus xanthus* is a social bacterium that can glide on solid surfaces and prey on other bacteria in topsoil. Upon starvation, *M. xanthus* forms multicellular fruiting bodies, each containing approximately  $10^5$  cells. This developmental process provides a model to study cell behavior in biofilms, including how cell-cell signaling regulates gene expression. C-signaling is essential for fruiting body development, but the mechanism remains a mystery. C-signaling appears to involve cell-cell contact, which is unusual for bacteria (most signals are diffusible). To understand how C-signaling regulates gene expression, the promoter regions of several C-signal-dependent genes have been characterized, including one called 4403. Since two transcription factors (MrpC2 and FruA) have been shown to bind cooperatively to the promoter regions of 3 other C-signal-dependent genes, electrophoretic mobility shift assays (EMSAs) were used to test for binding to the 4403 promoter region. With 4403 DNA from -80 to -16 (relative to the transcriptional start site), MrpC2 produced 2 abundant shifted complexes, FruA produced 1 complex, and the combination of proteins showed enhanced formation of shifted complexes, indicative of cooperative binding. EMSAs with shorter or mutant DNA fragments support a model in which MrpC2 binds to at least 2 sites to repress promoter activity, and C-signal-dependent activation of FruA allows it to displace MrpC2 from the downstream site to activate transcription. This model proposes a novel regulatory mechanism for the 4403 gene, whose expression depends absolutely on C-signaling, as compared with other genes that have been studied, whose expression depends only in part on C-signaling.