

**DEPARTMENT OF
BIOCHEMISTRY
&
MOLECULAR
BIOLOGY**

UNDERGRADUATE POSTER SESSION

**Monday, April 11, 2011
2:00-4:00pm**

Lisa Pinkava
Under the Direction of Dr. Michael Thomashow
Plant Research Laboratory

Title: *Potential involvement of posttranslational and posttranscriptional regulation of CBF in cold acclimation*

Abstract:

Freezing tolerance in many plants is shown to increase after exposure to low non-freezing temperatures, a process called cold acclimation. Our lab showed that CBF 1, 2 and 3 transcription factors play a crucial role in cold acclimation of Arabidopsis. In response to cold, CBF transcripts levels rapidly peak after 2h in the cold while their target genes peak 12-24 hours later. Even though CBF transcripts quickly diminish after their peak, their target genes transcript levels remain relatively high even 7 days later. Since CBF proteins control the target gene expression, this result suggests the possibility of posttranslational regulation of CBF proteins in the cold, a process which is not understood well in Arabidopsis. To understand potential roles of posttranslational regulation, transgenic plants with the CBF genes fused to a MYC or GUS tag under control of its endogenous promoter were generated. Interestingly, the CBF2-GUS line had high levels of transcripts at warm temperatures whereas the CBF2-MYC line behaves similarly to that of native CBF2 transcripts levels. Since both transgenes have the same promoter, posttranslational regulation of CBF mRNA may play a role in CBF cold induction. Preliminary data has shown under cold treatment CBF2-GUS protein level peaks at 24h, while CBF2-GUS transcript levels peak at 3h. This result also suggests posttranslational regulation of CBF mRNA in the cold, which could be appropriately tested by CBF-MYC protein expression since CBF-MYC mRNA behaves more like native CBF. Finding an effective MYC antibody for CBF-MYC protein experiments are still under way.

Matthew Faber
Under the Direction of Dr. Julia Busik
Department of Physiology

Title: *ASMASE Activity As A Possible Indicator Of Cellular Memory In HRVE Cells*

Abstract:

Diabetic retinopathy is a complication associated with diabetes, a disease that afflicts approximately 7 % of the population of the United States. Diabetic retinopathy leads to visual impairment and blindness. Vascular dysfunction is central in the pathology of diabetic retinopathy. Human retinal vascular endothelial (hRVE) cells composing the vasculature of the retina are the focus of this study. An increase in pro-inflammatory cytokine levels is associated with an increased rate of endothelial cell death. Previous studies have linked increased expression of proinflammatory cytokines with increased acid sphingomyelinase (ASMase) activity in hRVEs. ASMase is an enzyme responsible for the conversion of sphingomyelin to ceramide. Both of these lipids play a role in membrane dynamics and cytokine signaling. ASMase activity was compared in non-diabetic vs diabetic donors. Treatment of hRVE cells with insulin increased the ASMase activity in the cells from diabetic and non-diabetic donors; treatment with IGF-1 decreased the ASMase activity in non-diabetic donor cells and increased ASMase activity in diabetic donor cells. RT-PCR results indicated no significant changes in mRNA expression between diabetic and non-diabetic donor cells indicating that differences exist outside the level of gene transcription. Increased basal level ASMase activity persisted in cells from diabetic donors even after being cultured in medium containing normal glucose levels for numerous passages. These findings indicate that diabetes induces epigenetic changes in hRVE cells that impose a cellular “memory”. Our studies indicate that pharmacologically reducing ASMase activity in hRVE cells may improve the outcomes of diabetic retinopathy.

Kimberly Aung
Under the Direction of Dr. Thomas D. Sharkey
Department of Biochemistry and Molecular Biology

Title: *Altering the timing of leaf starch accumulation in Arabidopsis*

Abstract:

Currently, grain starch is the main source of bioethanol production in the United States. However, using grain starch for ethanol competes with food production. Ethanol production from leaf starch has potential to overcome this issue. Leaf starch is synthesized during the day and broken down during the night. It functions as a carbon and energy source at night when photosynthesis is not occurring. Two enzymes are required for starch breakdown: Glucan, water dikinase (GWD) and Phosphoglucan phosphatase coded for by the *SEX4* gene. We designed RNAi constructs against the gene for GWD or *SEX4* in Arabidopsis. These constructs were placed behind an alcohol-inducible promoter. This allowed the timing of starch accumulation to be controlled. Upon alcohol induction, transcription of GWD or *SEX4* was suppressed by 50%. Levels of starch accumulation in transgenic lines were seven times greater than that of wild type while maintaining similar growth rates and total biomass.

Jasmine Lucas
Under the Direction of Dr. Donna Koslowsky
Department of Microbiology and Molecular Genetics

Title: *Effects of poly-U tail on gRNA/mRNA hybridization*

Abstract:

Trypanosomes are protozoan eukaryotic parasites that cause African Sleeping Sickness, Chagas disease and Leishmaniasis; all of these parasitic diseases can be fatal. Together, these parasites affect approximately 550 million people in the developing world. Trypanosomes are able to regulate their energy metabolism using a unique RNA editing mechanism that may be a good target for drug development. Editing of mitochondrial mRNA in Trypanosomes is done through uridylyte insertion or deletion and up to 50% of an mRNA can be created post transcriptionally. The insertion and deletion is done by a complex of proteins called the editosome and is directed by small RNAs called guide RNAs (gRNA). One mRNA is edited by multiple gRNAs in a sequential fashion that suggests extreme evolutionary pressure for high efficiency and accuracy for successful editing. All known gRNAs in Trypanosomes have a polyuridylyte tail. This study investigates how the polyuridylyte tails of gRNAs contributes to the editing process. Using electrophoretic mobility shift assays (EMSA), we determined that the presences of a poly-U tails significantly increased the affinity between a gRNA and its target mRNA. To understand the affects of the U-tail on the kinetics of the bimolecular interaction, we used competitive EMSA to determine the mRNA/gRNA dissociation rate. Using the formula $K_d = k_{off}/k_{on}$ we can calculate the on rate to determine how the U-tail contributes.

Anna Hardy
Under the Direction of Dr. Thomas D. Sharkey
Department of Biochemistry and Molecular Biology

Title: *Expression of sugar phosphate transporters in starch metabolism mutants*

Abstract:

Transport of sugar phosphates across the chloroplast envelope is vital for sucrose synthesis in the cytosol and for providing intermediates for metabolic pathways, such as the MEP pathway and the shikimic acid pathway inside the chloroplast. Sugar phosphate transport into amyloplasts is necessary for storage starch synthesis. The transcript levels of three sugar phosphate transporters: triose phosphate transporter (TPT), xylulose phosphate transporter (XPT), and glucose phosphate transporter, in three different *Arabidopsis* starch metabolism mutants were quantified. The starchless mutant increased expression 32.0-fold in the glucose phosphate transporter versus the wild type, but expression of XPT and TPT were unchanged. In the triose phosphate transporter KO, glucose phosphate transporter expression decreased 3.2-fold and XPT expression slightly increased. Expression of all three phosphate transporters in the starch phosphorylase mutant did not change. Based on this data, we hypothesized that transitory starch levels may be involved in the regulation of glucose phosphate transporter expression.

Tiara Ahmad
Under the Direction of Dr. Katherine Osteryoung
Department of Plant Biology

Title: *Mapping of chloroplast altered number (can) mutations*

Abstract:

Chloroplasts are plant-specific organelles that mainly perform photosynthesis. Similar to their prokaryotic ancestors, chloroplasts replicate by binary fission, involving many proteins of both prokaryotic and eukaryotic origin. Although many chloroplast division proteins have been characterized, some factors remain unknown. In a screen of ethyl methanesulfonate (EMS)- mutagenized *Arabidopsis Col-0* plants, 13 viable, fertile mutants were found to have aberrant chloroplast morphology, termed chloroplast altered number (*can*). Compared to wild-type, mesophyll cells of *can8*, *can12*, and *can13* mutants have fewer chloroplasts of various sizes. In this study, the causative mutations in these plants are determined using two methods. In the first method, mutants are crossed to wild-type *Ler-0* plants, and the F₂ generations of each cross serve as mapping populations. The chromosomal location of each mutation is determined by analyzing linkage to SSLP genetic markers distinguishing the *Col-0* and *Ler-0* accessions. Rough mapping data indicate *can8* is located on the right arm of chromosome five while *can12* is on the left arm of chromosome one. Fine mapping of these mutations is in progress and will be followed by DNA sequencing as well as complementation analyses. The second method is to test for allelism. The *can* mutants are crossed to known chloroplast division mutants with similar phenotypes. This study will reveal the mutations responsible for *can8*, *can12*, and *can13*, specifying amino acid residues essential for correct functioning of their corresponding proteins in chloroplast division, and may reveal new genes involved in the division process.