DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

UNDERGRADUATE POSTER SESSION

Wednesday, April 16, 2008
3:00pm – 5:00pm

“Deciphering the Role of a Novel DnaJ-Like Protein in Fatty Acid Metabolism”

Ardian Coku, under the direction of Drs. Robert Last and Imad Ajjawi,
Department of Biochemistry and Molecular Biology

Abstract:

DnaJ-like proteins are apart 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 (FAME) analysis using gas chromatography. The results of several screenings showed a consistent increase in 16:0, 16:lΔ7, and 18:1Δ9 in addition to a decrease in 16:3. Extracting RNA from the mutants and performing reverse transcriptase PCR revealed that the mutants produced no transcript for At1g08640. The wild-type cDNA for At1g08640 was extracted from a gel, purified, and then with the use of gateway cloning was ultimately transformed into *Agrobacterium tumefaciens*. This was done for the purpose of over expressing the gene in wild-type plants and to complement mutant plants. To confirm results from proteomic studies, that the protein is located in the chloroplast, the protein will be fused with GFP for subcellular localization using fluorescent microscopy. Further experiments will be conducted involving inserting a polyhistidine-tag to the C-Terminus of the protein in order to purify the protein and thus determine how and with what proteins the DnaJ-like protein interacts with.

“Characterization of Proteins from Corn Coleoptile Epdidermis involved in Auxin-Induced Growth”

Tiffany J. Dickerson, under the direction of Dr. Susanne Hoffmann-Benning,
Department of Biochemistry and Molecular Biology

Abstract:

Rapidly growing corn coleoptiles display a phenomenon called “tissue tension”. When they are cut longitudinally, they curve outwards with the epidermis on the concave side of the section (Kutschera et al., 1987). Tissue tension has been interpreted as the manifestation of two conflicting forces in the rapidly growing coleoptile: the epidermis is under tension because it is growth limiting, while the inner tissue does not limit growth and is under compression (Kutschera, 1989). During examination of the cell ultrastructure of rapidly growing plants, osmiophilic particles (OPs) had been observed in several plant species (Kutschera et al., 1987; Kutschera and Kende, 1988; Hoffmann-Benning et al., 1994; Funke and Edelmann, 2000). These particles can be between 80-300nm in diameter. Electron microscopy and labeling experiments had shown that they are closely associated with the outer epidermis of growing tissues, are going through the secretory pathway, and are, at least in part, proteinaceous (Hoffmann-Benning et al., 1994). From the location and time of appearance of the OPs we can assume that they are related to either cell-wall or cuticle biosynthesis. However, so far they have eluded identification. We decided to use a proteomics approach to try to identify proteins by comparing the protein profile of slow versus rapidly growing coleoptile and coleoptile epidermis.
In our experiments we were able to identify over 80 proteins that appear to be induced in rapidly growing coleoptile epidermis. Half of those are related to protein synthesis/maintenance and 11% are potentially associated with the cell wall, cuticle, or lipid metabolism. We are analyzing the expression and distribution of those proteins plus an additional three hypothetical proteins with unknown function.

“Regulation of Oil Biosynthesis in Algae ”

Marie Fedewa, under the direction of Dr. Christoph Benning, Department of Biochemistry and Molecular Biology

Abstract:

The widely recognized need for the development of biomass-based domestic production systems for high energy liquid transportation fuels is addressed by exploring oil (triacylglycerol) biosynthesis in microalgae. Initial efforts are focused on the unicellular model green alga *Chlamydomonas reinhardtii* with its abundance of genetic and genomic resources. Like many algae, Chlamydomonas accumulates triacylglycerols under conditions of nutrient limitation. The identification of microalgal genes encoding the enzymes and regulatory factors required for the induction of triacylglycerol biosynthesis will be essential for the engineering of triacylglycerol biosynthesis in algae. Genetic mutant screens are currently conducted for loss of triacylglycerol accumulation under induced conditions, and for gain of triacylglycerol biosynthesis under non-induced conditions. Efforts are under way to biochemically and physiologically characterize these mutants. The affected genes are currently being identified and characterized based on a gene tagging approach. In addition, a high-throughput cDNA pyrosequencing experiment has been conducted under induced and non-induced conditions to generate a deep set of expressed sequence tags for comparative transcriptional profiling. The newly identified genes and the functional genomic information will provide novel targets for future engineering approaches towards optimizing microalgal oil production strains.

“Molecular Systematics of the Butterfly Genus *Speyeria*”

Edita Klimyte, under the direction of Dr. Barry Williams,
Department of Zoology

Abstract:

Elucidating the evolutionary history of organisms is central to many aspects of biology. However, for both analytical and biological reasons, evolutionary tree construction can be quite problematic. The goal of this study is to generate a robust phylogenetic hypothesis for a relatively unknown, but highly complex genus of butterflies, *Speyeria*. *Speyeria* is an exemplar group of organisms to study, because there are roughly 14 species and 120 subspecies found throughout much of North America; however, these estimates taxonomic delineations and patterns are only based on field observations of highly labile morphologic characters. Until now, there was no formal phylogenetic tree proposed for this group even though they are some of the most photogenic and charismatic butterflies in the world. Also, they have a complex biographic distribution across North America, with partially or completely overlapping distributions for closely related taxa, several disjunctive species and subspecies, and several highly endangered species. Further, many of these are notoriously difficult to distinguish, likely due to a very recent evolutionary origin for all of the taxa from a recent common ancestor. This rapid, but complex pattern of diversification is likely to result in an equally complex phylogeny history. In order to accomplish this task, we have sequenced multiple genes from all of the species and several of the subspecies of the genus; the genes included in this study consist of cytochrome oxidase subunit I, elongation factor-1 alpha, wingless, and triosephosphate isomerase. Then, multiple analyses were performed to obtain the most statistically-supported phylogenetic tree.

“*Engineering a Cystein-Less SpoIVFB*”

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-σk is one of these factors, and requires that SpoIVFB process it to σk before gene expression can begin. To determine accessibility of SpoIVFB to cysteine-modifying reagents, we are trying to construct an active, cysteine-less version of the protein. Previous work showed that of SpoIVFB's 5 cysteine residues, two could be changed to serine without loss of activity, but the other three residues, when changed to serine, inactivated the enzyme.

If these three residues were instead mutated to alanine, could a functional cysteine-less SpoIVFB be created?

“Analyzing the Evolutionary Rates of Intracellular and Extracellular Proteins”

Josh Mackaluso, under the direction of Dr. Maria Zavodszky, Department of Biochemistry and Molecular Biology

Abstract:

Comparing the evolutionary rates of intracellular and extracellular proteins of various species is a useful approach to understanding the effects of mutations at the molecular level. While many mutations result in no significant observable changes, some mutations can lead to the development of drug resistance in bacteria or the development of harmful diseases in humans. The accumulation of mutations can result in proteins that have entirely new functions. In order to better understand how species evolve, more knowledge regarding the evolution of proteins is necessary. Not all proteins evolve at the same rate. Proteins with many interacting partners or those that have critical functions for life have been shown to be more conserved than others. It is believed that the cellular location of a protein affects its evolution, with proteins residing in the extracellular space showing higher rates of evolution than intracellular proteins due to higher exposure to environmental stress. In order to test this hypothesis, a dataset has been constructed of human protein structures with known cellular location. Each protein within the set has been paired to its orthologous mouse protein. As a first approximation, the rate of evolution is calculated as the percent of amino acids that differ between the human and mouse proteins. Further analysis is expected to reveal whether surface residue mutations contribute differently to the rate of evolution than the mutations of buried ones.

"*GUN201* encodes a TPR protein of unknown function and defines a new plastid-to-nucleus signaling pathway"

Andrea Stavoe, under the direction of Dr. Robert Larkin, Department of Biochemistry and Molecular Biology and Plant Research

Abstract:

Plastid signals help coordinate the expression of nuclear genes that encode proteins active in photosynthesis with the functional state of the chloroplast and help coordinate the expression of the nuclear and chloroplast genomes. Because we know very little about plastid-to-nucleus signaling mechanisms, we cannot speculate on what types of molecules this form of signaling might depend. Therefore, we are taking a forward genetic approach to fill the many gaps in our knowledge of this form of interorganellar communication. We have isolated new *genomes uncoupled* (*gun*) mutants in *Arabidopsis thaliana* that appear defective in plastid-to-nucleus signaling because the expression of genes that encode proteins active in photosynthesis is uncoupled from chloroplast function in these mutants. Here we describe the isolation and characterization of *genomes uncoupled 201* (*gun201*) and the positional cloning of a *gun201* nonsense allele. In addition to the *gun* phenotype, *gun201* also exhibits a far-red block of greening phenotype. The far-red block of greening refers to the inability of seedlings irradiated with dim far-red light (720-780 nm) to green when subsequently irradiated with white light. In contrast to wild type, *gun201* does not exhibit a far-red block of greening. We mapped *gun201* to a 70 kb interval on chromosome 1. We sequenced ten genes in this interval and found that one of these genes contains a premature stop codon in *gun201*. We conclude that this gene is likely *GUN201*. *GUN201* encodes a 200 kDa protein of unknown function that contains three tetratricopeptide repeat (TPR) domains. Our current understanding and future plans for GUN201 will be presented.

“Effects on RcaE on Cell Morphology in *Fremyella diplosiphon*”

Michaela TerAvest, under the direction of Dr. Beronda Montgomery-Kaguri,
Department of Biochemistry and Molecular Biology

Abstract:

RcaE is an important light-sensing protein found in the cyanobacteria *Fremyella diplosiphon*. It is involved in regulating the relative concentrations of the phycobiliproteins phycocyanin and phycoerythrin *in vivo* in response to red and green light, respectively. These phycobiliproteins harvest light of different wavelengths with different efficiencies. RcaE regulates the relative amounts of the phycobiliproteins to maximize light absorption for photosynthesis. In prior studies that characterized the physiological roles of RcaE in *F. diplosiphon*, another interesting effect was noticed; mutating RcaE caused changes in cell morphology. Wild-type *F. diplosiphon* cells are rod shaped, while RcaE-deficient cells are circular. This cell-shape phenotype could be complemented by expressing a wild-type copy of *rcaE* in the RcaE-deficient strain. These results indicate that RcaE may also have some role in regulating cell shape and perhaps cell wall structure. To examine whether the cell wall of RcaE-deficient cells is impacted and thereby resulting in the observed morphological changes, the lysozyme sensitivity of varying strains of *F. diplosiphon* were compared. Lysozyme sensitivity was tested by assaying for the release of phycobiliproteins from treated cells. Relative concentrations of extracted phycobiliproteins were quantified for wild-type, RcaE-deficient and complemented strains. To assess the impact of RcaE activity on cell shape and cell wall structure, quantified values of released phycobiliproteins were correlated with both cell shape and the presence or absence of functional RcaE in tested strains.

“Structural Models of the Active Site of Acetyl-coenzyme A Synthase”

Jason Thomas, under the direction of
Dr. Eric Hegg, Department of Biochemistry and Molecular Biology

Abstract:

The unusual bifunctional enzyme CODH/ACS catalyzes the biosynthesis of acetyl-CoA. In the first step, CO2 is reduced to CO by CODH, and ACS then combines the CO with -CH3 and coenzyme A to generate acetyl-CoA. The active site of the ACS enzyme is comprised of a unique Ni-Ni bimetallic site. One of the Ni ions is in a square-planar environment and is coordinated to two thiolates and two deprotonated amides in a Cys-Gly-Cys motif. The second Ni ion, however, is coordinated to three Cys thiolates and is considerably more comformationally flexible. To address the many mechanistic questions related to ACS, we began synthesizing small metal complexes that serve as well-defined mimics of the active site. In order to model the unusual

Ni(C·G·C) site of ACS and probe the reactivity available to it, we designed an asymmetric ligand and made its corresponding Ni-complex. The Ni-complex crystallizes as the trinuclear complex. The mononuclear complex is synthesized by breaking down the trinuclear complex with excess base and Ni-salt. The model complexes were characterized by various spectral techniques.

“Examining the Affect of Mixed Lineage Kinase-3 in Mammary Epithelial Cell Architecture”

Kelly Watson, under the direction of
Dr. Kathleen Gallo, Depts. of Biochemistry and Molecular Biology & Physiology

Abstract:

Protein phosphorylation is an important cellular process that controls many vital physiological processes, including cell proliferation and programmed cell death. Regulation of protein phosphorylation ensures proper cell cycle progression. Since protein kinases catalyze protein phosphorylation, they are also subjected to regulation which is critical for cell survival. Cancer has been connected to the improper regulation of protein kinases. Dr. Kathleen Gallo’s lab is primarily concerned with how protein kinases are regulated during both normal and cancerous cell cycles.

The mixed-lineage kinases (MLKs) are a family of serine/threonine kinases that function as mitogen-activated (or responding to extra cellular stimuli) protein kinase kinase kinases (MKKKs) to activate the JNK pathway. A member of the MLK subfamily, MLK3, plays a crucial role in the propagation of a stimulus from the environment, such as growth factors, stress, and cytokines, to elicit a biological response through phosphorylation of downstream kinases and transcription factors. Protein levels of MLK3 were found to be much higher in breast cancer cells than in human cell lines derived from other tissues. Consequently, MLK3 regulation is a good model for studying the mixed lineage family of protein kinases.

Induced MLK3 expression in MCF10A cells has shown an enhanced proliferation effect when grown in Matrigel (extracellular matrix proteins). The 3D culture system mimics various qualities of normal glandular epithelium, such as polarization, luminal space and controlled growth. It is a useful way of studying cancerous phenotypes *in vitro*. Expressing mutant forms of MLK3 in MCF10A cells in 3D culture might lead to additional understanding of the function and regulation of the MLK family. My research is concerned with validating whether kinase activity is required for an enhanced proliferation effect, and if constitutive activity of MLK3 can increase the proliferation phenotype, leading to invasion of the extracellular matrix.

“Chloroplast envelope transporter in mesophyll and bundle sheath of maize”

Alex Mikelonis, under the direction of Dr. Susanne Hoffmann-Benning,
Department of Biochemistry and Molecular Biology

Abstract:

During harsh conditions when CO_2 levels become limited plants that utilize a C4 photosynthetic pathway fix carbon with better efficiency than those plants that utilize the C3 mechanism. Research in the photosynthetic pathway of C4 maize plants, has led to the isolation of unidentified proteins that may be a part of a transport mechanism that prevents C4 plants from having excess photorespiration. Recent research of the membrane protein Mep3 has led to conformations that the protein is localized in the chloroplast. Other research involving maize includes the extraction of protoplasts from the mesophyll and bundle sheath cells in order to obtain a pure DNA from the different chloroplasts.

“The role of Jumonji-domain containing proteins in transcription and chromatin modification”

Jacqueline Lapp, under the direction of Dr. Steven van Nocker,
Department of Horticulture

Abstract:

In eukaryotes, DNA is packaged into chromatin, composed mostly of histone proteins. Histones can be post-translationally modified at their N-terminal tail by methylation, acetylation, or phosphorylation of specific amino acid residues, and these modifications help to regulate the transcriptional activity of nearby genes. JumonjiC-domain containing proteins (Jumonji proteins), such as human UTX1 and JMJD3, have recently been found to enzymatically remove methyl marks from histones, and thus act as potentially important regulators of transcription. However, higher eukaryotes contain numerous additional Jumonji proteins, and the activity and biological function of most of these remains unknown.
To further investigate the role of Jumonji proteins in growth, development, and chromatin modification, we used artificial microRNA (amiRNA) technology to disrupt each of the 21 Jumonji genes in transgenic Arabidopsis. We are analyzing the resulting transgenic lines for defects in histone methylation patterns and development.