DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

UNDERGRADUATE POSTER SESSION

Friday, April 20, 2012
12:00 – 1:30pm

Characterization of the RNA-binding Protein PPR27 from *Trypanosoma Brucei* using Fluorescence Polarization Anisotropy

David Dickson
Under the direction of Dr. Charles Hoogstraten, Biochemistry and Molecular Biology, and Dr. Fadhiru Kamba, Cell and Molecular Biology

Pentatricopeptide repeat protein 27 (PPR 27) is but one of a large family of highly conserved proteins found in *Trypanosoma Brucei*, the parasite responsible for Human African Trypanosomiasis, commonly known as African sleeping sickness. There have only been six of these PPRs found in humans, while over 35 have been discovered in *T. Brucei*. Their nonessential role in our bodies makes them an excellent potential drug target to effectively eliminate the parasite while leaving the human host unharmed. Upon full characterization of the protein and its possible function, efforts can be made to discover how to stop it. Previous data suggested a role for PPR proteins in binding unique single-stranded RNA sequences within trypanosomal mitochondria. Our current focus is using fluorescence polarization anisotropy and extensive user-defined curve fitting and data analysis to investigate the possible protein ligand binding interactions for our protein construct. We tested our protein against several homopolymeric fluorescently labeled nucleotide oligomers to obtain the dissociation constant (K_d) for the interaction. Previous work in the Hoogstraten lab has demonstrated preferential binding to G-rich RNA sequences of approximately 9 bases in length. Current work entails testing several other possibilities such as G-rich DNA, both single and double stranded, as well as double stranded RNA. Our goal is to conclusively determine the validity of our hypothesis that PPR 27 prefers G-rich, single-stranded RNA over all other ligands. Future work will likely entail sequence specific binding as well as in depth structural analysis including X-ray crystallography and 2D NMR.

Two-level developmental gene regulatory model to incorporate cis-regulatory and temporal information.

Irina Pushel
Under the direction of Dr. David Arnosti, Biochemistry and Molecular Biology

High-throughput genome sequencing and transcriptome analysis have provided researchers with a quantitative basis for detailed modeling of gene expression using a wide variety of mathematical models. Modeling of eukaryotic gene regulation has focused on either time-dependent interactions of gene networks or equilibrium approaches that can explore the cis-regulatory grammar of transcriptional enhancers. These models rely on systems of differential equations or thermodynamic descriptions, which can be used either to understand dynamics of a system, or DNA-level regulatory processes. To combine the strengths of each of these approaches, we have developed a novel model that joins these methods to provide a dynamical description of gene regulatory systems, using detailed DNA-based information, as well as spatial transcription factor concentration data. Our ‘two-layer’ modeling approach uses a thermodynamic model as the synthesis term in our differential equation, the term that represents the rate of mRNA production. The differential equation incorporates all other terms, representing decay and diffusion of mRNA, thus the model does not lose its effectiveness in predicting emerging spatial expression patterns over time. By incorporating data on DNA sequence, we are able to successfully model context-specific features of enhancers, as well as replicate the dynamic expression of a simple Drosophila gene regulatory circuit that drives development in the dorsal-ventral axis of the blastoderm embryo, involving Dorsal, Twist, Snail, and rhomboid. Where protein and cis-regulatory information is available, our two-layer model provides a powerful method to recapitulate and predict dynamic aspects of eukaryotic transcriptional systems that will greatly improve understanding of gene regulation at a global level.

Thermodynamic Regulation of Non-Photochemical Quenching

Matthew Smith
Under the direction of Dr. David Kramer, Plant Biology

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 single 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 measuring chlorophyll florescence yields 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 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 Dewar flask that was allowed to slowly heat upon depletion of liquid nitrogen. This apparatus was attached to a spectrofluorometer via a fiber optics light guide, with illumination from a filtered light emitting diode. Preliminary results show clear differences in fluorescence temperature-dependence between the light- and dark-adapted wild type and NPQ mutants, consistent with the proposed thermally activated intermediate. Based on these promising results, we are constructing a new instrument to measure these spectra under precise temperature regulation.

New aspects of phloem-mediated long-distance lipid-signaling in plants

Urs F. Benning and Banita Tamot
Under the direction of Dr. Susanne Hoffmann-Benning,
Biochemistry and Molecular Biology

Plants cannot move to escape adverse conditions. As a consequence, they evolved mechanisms to detect changes in their environment, communicate these to different organs, and adjust development accordingly. One of these adaptations, the phloem, serves as a major trafficking pathway for assimilates, viruses, RNA, plant hormones, metabolites, and proteins with functions ranging from synthesis to metabolism to signaling. The study of signaling compounds within the phloem is essential for our understanding of plant communication of environmental cues. Determining the nature of signals and the mechanisms of their transport will lead to a more complete understanding of plant development and plant responses to stress.

Our analysis of *Arabidopsis* phloem exudates revealed several lipid-binding proteins as well as lipids. Lipid transport in the phloem has been given little attention until now. In other aqueous systems like the human blood lipids are often transported while bound to proteins. In some cases, they serve as messengers and modulate transcription factor activity. We have shown that one phloem lipid-binding protein (PLAFP) binds one of the phloem lipids, phosphatidic acid (PA). PA is known to play a role in intracellular signaling. We will present data showing the effect of PLAFP on development, phloem lipid profile; lipid-binding properties, expression in response to various stresses, and localization within the plant and on a cellular level.

Our data suggest that PLAFP, and with it bound PA, play a role in long-distance developmental signaling.

Cell Expansion Growth in the Epidermial layer of a cell

Paula Boakye
Under the direction of Dr. Susanne Hoffmann-Benning,
Biochemistry and Molecular Biology

Both, the plant cell wall as well as the overlaying cuticle play a vital role in plant growth and development and in the interaction of plants with their environment. Their composition controls the extent and direction of cell expansion. Many lines of evidence show that it is the epidermis which promotes and restricts cell expansion growth. We used auxin to induce rapid cell elongation in isolated coleoptile sections. In order to better understand changes in the epidermal cell layer during expansion growth, we used a proteomics approach to compare epidermal proteins from rapidly (IAA-treated) and slow-growing corn coleoptiles. Of the 86 proteins identified by LC-ESI- MS/MS 15 were predicted to be related to the secretory pathway or to cell wall or cutin biosynthesis. We compared the expression of two hypothetical proteins (HP3 and HP4) to that of several known cell wall biosynthesis proteins. Real-time PCR confirmed that upon auxin treatment HP3 and HP4 are induced 2.5 and 1.8 times, respectively. Expression of both genes is correlated with expansion growth in coleoptiles and leaves. However, while HP3 expression appears to be predominant in the epidermis, HP4 is equally expressed in inner tissues. HP3 is a 22 kDa protein with a esterase-lipase domain and similarity to dienelactone hydrolases and endo-1,3-1,4-beta-D-glucanases and may play an important role in the biosynthesis/ extension of mixed-linked beta glucans in the cell wall. HP4 belongs to a group of proteins with a DUF538 domain. GFP labeling suggests localization of HP4 in the nucleus and at the periphery of the cell, suggesting HP4 maybe function as messenger in auxin signaling. A comparison of gene expression in response to auxin treatment shows an increase in HP4 expression prior to that of other tested genes. Data supporting our findings will be presented.

Chlamydomonas shunts electrons into triacylglycerols to avoid damage to photosynthetic electron transport chain components during nutrient stress

Cassandra Johnny
Under the direction of Drs. Christoph Benning and Xiaobo Li,
Biochemistry and Molecular Biology

Following nitrogen (N) deprivation, microalgae such as *Chlamydomonas reinhardtii* produce triacylglycerols (TAGs). In a previous mutant screen to identify genes central to TAG metabolism, a Chlamydomonas mutant, designated E12, was found to be deficient in TAG biosynthesis. Mechanistic studies on the low TAG phenotype were performed to determine its biochemical defect, likely the loss of a polar lipid lipase. Besides that, this low-TAG mutant also provided an excellent opportunity to study the significance of TAG accumulation in microalgae. Following N deprivation, E12 exhibited chlorosis (bleaching) and a major loss of viability. This phenotype was reverted by inhibiting the photosynthetic electron transport chain with the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea. This suggests that in Chlamydomonas, TAG is synthesized to consume the NADPH generated by the photosynthetic electron transport chain (PETC) to relieve overreduction , which can lead to the production of detrimental reactive oxygen species. If this model is correct, we expect that a second (suppressor) mutation, which can enhance TAG biosynthesis or block the PETC, will suppress the chlorosis phenotype. Mutants were generated in the E12 background and screened for the reversion of chlorosis and 25 suppressor mutants were obtained. Analysis of TAG content, photosynthetic defects, and identification of mutant loci is underway.

THE CHARACTERIZATION OF TRANSACTIVATIONAL PROPERTIES OF ATWRI1

Michael Grix
Under the direction of Drs. Christoph Benning and Que Kong,
Biochemistry and Molecular Biology

The Arabidopsis thaliana WRINKLED1 (AtWRI1) protein is a key regulator in controlling the accumulation of storage compounds during seed filling. AtWRI1 is an excellent candidate gene for biofuels production by increasing the energy density of biomass through accumulation of triacylglycerols (TAGs) not only in reproductive tissues (seeds), also in vegetative tissue (leaves, roots). AtWRI1 belongs to the AP2/ERF family of transcription factors (TFs) and over expression of AtWRI1 results in up-regulation of a set of genes involved in fatty acids (FA) synthesis, indicating AtWRI1 is a transcriptional activator during seed storage compounds accumulation. However, which region in AtWRI responsible for its transactivational properties is still unclear. The transactivational activity determines the rate of downstream gene expression triggered by the TFs. Understanding of their transactivational properties will facilitate our understandings of TFs functions and provide us a clue for engineering TFs to improve their activities. My research aims to characterize the transactivation domain and its properties of AtWRI1. Different regions of AtWRI1protein were fused to a GAL4 DNA binding domain and the fusion proteins were tested for their transactivational activities in the yeast monohybrid system. So far, we found the activation domain of AtWRI1 is located at the C-terminal part of AtWRI1 (amino acid 306 to 430). Further investigation of which amino acid residue(s) or peptide are essential for its transactivational properties is underway.

Exploration of the Alternative Localization of an [FeFe]-hydrogenase in *Volvox carteri*

Robin Green
Under the direction of Dr. Eric Hegg, Biochemistry and Molecular Biology

[FeFe]-hydrogenases are unique metalloenzymes capable of reducing protons in solution to hydrogen gas. Multiple species of biological organisms, in particular green algae, can utilize these enzymes as a way to mitigate high reducing potential within the cell by providing alternative means to channel electron flow. There is currently great interest in using green algae as biological hydrogen factories. Their ability to couple energy from photosynthesis to hydrogen production using [FeFe]-hydrogenases makes them attractive candidates for alternative fuel source development. My research involves the investigation of alternative localization of *Volvox carteri*, a green algae that has recently been demonstrated to possess hydrogen metabolism. Canonical [FeFe]-hydrogenases are localized to the chloroplasts, where they can shuttle electrons generated by photosynthesis to generate hydrogen gas. Current bioinformatics data collected from my lab suggests that an active [FeFe]-hydrogenase, VcHydA1 may be targeted elsewhere. Our lab has speculated that the hydrogenase in question may be localized to the mitochondria based on additional bioinformatics analysis and similarity between the two organelles *in vivo*. Current efforts are being directed to elucidate the possible alternative location of VcHydA1. Using a devised methodology of differential centrifugation, hydrogen evolution experiments, immunoblot detection, and various marker assays *V.carteri* cells have been assayed and screened for hydrogenase activity within the mitochondria. This project has the potential yield new insight into how biological organisms utilize [FeFe]-hydrogenases, possibly diversify the prospect of industrial application. Current data will be reported and future directions for this project will be outlined.

Deep Sequence Profiling of the gRNA Transcriptome in Trypanosoma brucei

Jasmine Lucas
Under the direction of Dr. Donna Koslowsky,
Microbiology and Molecular Genetics

Trypanosoma brucei are parasitic protozoans that are responsible for African Sleeping Sickness. Trypanosomes extensively edit 12 of their mitochondrial mRNA transcripts dependent upontheir life phase. Guide RNAs (gRNA) are the driving force behind this unique RNA editing in Trypanosomes, directing the insertion and deletion of uridylates in the pre-edited mRNAtranscripts. Despite their critical role in the editing process, gRNAs have not been completely characterized due to the complexity of the genome. The genome consists of both 22kb maxicircles which encode the mRNAs and approximately 10,000 minicircles, each encodingthree to four gRNAs. Previously, gRNAs have been identified using classical sequencing methods. Despite numerous attempts, however, large numbers of the gRNAs needed for the extensive editing required were still unidentified. In this project, we used deep sequencing (Illumina) to characterize the full gRNA transcriptome in the insect stage of the life cycle. Guide RNAs were isolated based on transcript size from three Trypanosoma brucei cell lines. Initial analyses of the read data have identified most of the gRNAs required for RNA editing. In addition, analyses of transcript abundance indicate vastly different expression levels for the different gRNAs.

Mutagenesis of Bacterial RNAP

Fadi Assaf, Raj Lingnurkar.
Under the Direction of Dr. Zachary Burton,
Biochemistry and Molecular Biology

Bacterial RNA polymerases (RNAPs) have the subunit composition **$\alpha_2\beta\beta'\omega\sigma$.** The elongating form of the RNAP releases the **σ** subunit. Molecular dynamics simulation of *Thermus thermophilus* (Tt) RNAP with closed and open trigger loop conformations has led to a model for the RNAP elongation mechanism. The trigger loop closes on the nucleotide triphosphate (NTP) substrate during catalysis and opens during translocation, which is the movement of nucleic acids through RNAP. The model involves two GXP hinges (designated H1 and H2) located within the helices at the base of the trigger loop, **β'** 1230-GEPGTQ-1235 and 1255-GLP-1257. On the nearby bridge helix (**β'** 1067 to 1104), two clustered glycine hinges (designated H3 and H4) are hypothesized within the segment **β'** 1076-GARKGG-1081. In addition to these hinges, "switch" residues are identified that may alter their atomic contacts to support catalysis (a closed trigger loop TEC (ternary elongation complex)) or translocation (an open trigger loop TEC). Because of their proposed roles in elongation, hinge and switch residues of RNAP on the β and β' subunits are targets for in vitro mutagenesis. Because of the complexity of the RNAP structure, a polygenic vector encoding the closely related *Escherichia coli* (Ec) RNAP was constructed to co-express **α, β, β'** and **ω** subunits. Mutant RNAPs, which carry a histidine_6-tag, are purified by Ni^{2+} chromatography followed by heparin chromatography. RNAPs are assayed for transcriptional elongation rates and fidelity in vitro. A number of combined substitutions are under construction to test for possible synergistic effects that might indicate coupling of hinges and switches in transcription.

Improving Alkaline Peroxide Pretreatment of Biomass Using Metal Catalysts

Alex Toulokhonov
Under the direction of Drs. Eric Hegg & Vaidyanathan Mathrubootham

Biofuel production from plant biomass requires pretreatment procedures to remove lignin, a polymer, which surrounds cellulose and hemicellulose. Alkaline hydrogen peroxide (AHP) delignification is a widely used method for totally chlorine-free (TCF) delignification of lignocelluloses (bleaching) because it utilizes and releases relatively benign reactants and byproducts.
However, certain challenges hinder the process from reaching its full potential and achieving practical reaction rates. These challenges include great quantities of required base, carbohydrate degradation, and non-productive side reactions that consume large amounts of hydrogen peroxide. In order to lower the pH and hydrogen peroxide loading required for efficient delignification of biomass, we are developing homogenous metal based catalysts for the activation of hydrogen peroxide. The hypothesis is that metal based catalysts would activate H2O2 and form more selective reactive intermediates. In these catalysts, the metal acts either as a Lewis acid and forms a metal peroxide species that can act as a potent nucleophile at a significantly lower pH than standard alkaline peroxide conditions or the metal can undergo redox changes and form reactive metal oxo species. Catalysts were tested for their ability to activate H2O2 in the presence of AHP under different reaction conditions and preliminary results indicate slight improvement of the process by some catalysts at high pH conditions and high enzyme loading. Stability studies under various conditions suggest that the ligand backbone needs to be modified to make the catalysts more water soluble and more resistant to high pH and H2O2.