DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

UNDERGRADUATE POSTER SESSION

Abstract Poster Presentations

Thursday, April 1, 2004
Atrium, Biomedical & Physical Sciences Building
3:00 – 5:00 p.m.

Posters by:

Nathan Lord
"Cloning the GCN5-encoding cDNA from tomato"
Mentor: Dr. Steven Triezenberg, Department of Biochemistry & Molecular Biology

Michelle Manente
"Characterization of *Pseudomonas syringae* HrpA via protein-protein cross linking: An assay for self-assembly"
Mentor: Dr. Dennis Arvidson, Department of Microbiology & Molecular Genetics

Sze-Ling Ng
"Identification of proteins that bind specifically phosphorylated CTD of RNA Pol II using a tethered catalysis/yeast two-hybrid system"
Mentor: Dr. Min-Hao Kuo, Department of Biochemistry & Molecular Biology

David Taggart
"Protein expression and deletion mutagenesis of hepatitis delta antigen "
Mentor: Dr. Zachary Burton, Department of Biochemistry & Molecular Biology

Daniel Wood
"Effects of DNA in GTP hydrolysis activity of the *Neisseria gonorrhoeae* signal recognition particle protein PilA"
Mentor: Dr. Cindy Arvidson, Department of Microbiology & Molecular Genetics

Katie Zobeck
"Regulation of translocation by transcription factor IIF"
Mentor: Dr. Zachary Burton, Department of Biochemistry & Molecular Biology

Cloning the GCN5-Encoding cDNA from Tomato

Nathan Lord, under the direction of Dr. Steve Triezenberg, Department of Biochemistry and Molecular Biology

The eukaryotic protein Gcn5 is a transcriptional coactivator with the enzymatic function of a histone acetyltransferase (HAT). GCN5 homologs have been identified in yeast, fruit flies, mice and humans, but few have been identified in plant species. Our laboratory has characterized the GCN5 gene and protein from the plant Arabidopsis thaliana, an organism useful for molecular genetics experiments but less than optimal for biochemical research. The purpose of this study is to clone the GCN5 gene from Lycopersicon esculentum (tomato), a plant that offers advantages in biochemical study of protein complexes involved in transcriptional regulation. A cDNA encoding the complete leGCN5 open reading frame and putative 5′ and 3′ untranslated regions, approximately 2.0 kb, was cloned using a combination of phage library screening and 5′-RACE (rapid amplification of cDNA ends). Northern blot analysis of tomato total RNA indicated a GCN5 primary transcript of approximately 2.1 kb. The sequence of the leGCN5 cDNA clone revealed regions of conservation, such as the HAT catalytic domain and the bromodomain, and other features that exhibit greater diversity, such as the N-terminal region of Gcn5. This work provides a foundation for future biochemical efforts to identify components of the coactivator protein complex(es) containing Gcn5, and thereby to define one mechanistic aspect of transcriptional activation in plants.

Characterization of *Pseudomonas syringae* HrpA via protein-protein crosslinking: An assay for self-assembly

Michelle Manente under the direction of Dr. Dennis N. Arvidson, Department of Microbiology and Molecular Genetics

Pseudomonas syringae is a plant pathogenic bacterium that delivers effector virulence proteins to the extracellular milieu or directly into the host cell by way of the type III secretion system. The *hrp* gene cluster encodes HrpA, the structural subunit of the Hrp-pilus. The Hrp pilus functions as the type III secretion conduit and is required for virulence. HrpA can self-assemble in solution to form a pilus-like structure. We have identified negative mutations of the HrpA protein that inhibit formation of the functional Hrp pilus and the secretion of effector proteins. Current research goals are to express truncated wild-type and mutant HrpA proteins in *Escherichia coli*, purify them by liquid chromatography, and assay the oligomeric state of each variant with protein-protein crosslinking in conjunction with SDS PAGE and western blotting for detection. Non-assembling mutants were identified and are being subjected to crystallization trials. We hypothesize that mutants of HrpA that no longer self-assemble are more likely to crystallize.

Identification of proteins that bind specifically phosphorylated CTD of RNA Pol II using a tethered catalysis/yeast two-hybrid system

Sze-Ling Ng, under the direction of Dr. Min-Hao Kuo, Department of Biochemistry and Molecular Biology

Many protein-protein interactions are facilitated or inhibited by post-translational modifications. These modifications are frequently critical for the functions, stability, structures, and localization of the underlying proteins. While research into protein-protein interactions is quite prevalent, the studies on those that are regulated by post-translational modifications are still in their infancy, mainly due to the lack of a suitable experimental approach.

The tethered catalysis/yeast two-hybrid system was designed to study this gap in the proteomic puzzle. This system is an extension of the yeast-two hybrid system (Y2H), where the incorporation of the tethered catalysis allows for the study of interactions induced by post-translational modifications. For example, one such modification, the phosphorylation of the carboxyl terminal domain (CTD) of RNA Pol II contributes to its selective recruitment of different proteins and plays a critical role in regulation of transcription.

We hypothesize that the use of our tethered catalysis/yeast two-hybrid system for the identification of phospho-CTD-interacting proteins will help to further elucidate the molecular mechanisms as to how CTD phosphorylation exerts its function in regulating transcription, and even its links to certain human diseases.

Bait constructs were created by physically fusing CTD to its modifying enzyme, Kin28, whereby constitutive modification of the bait protein is achieved. An E54Q mutant was also created to test for phospho-CTD specific interactions. Our Y2H screen yielded several adenine plus candidates. The first five were re-tested against various control baits and four showed phospho-CTD specific interactions. Sequencing results revealed two of the candidates to be pyruvate kinase, another to be UXT and the fourth to be OVCA2. The relationship of these proteins have yet to be uncovered. The other candidates are in the process of being screened.

Protein expression and deletion mutagenesis of hepatitis delta antigen

David Taggart, under the direction of Dr. Zachary Burton, Department of Biochemistry and Molecular Biology

Hepatitis Delta Antigen (HDAg) is a viral protein required for the replication of the Hepatitis Delta Virus, a satellite virus of Hepatitis B. HDAg exists in two forms, HDAg-S (195 amino acids long) and HDAg-L (214 amino acids long) that stem from the editing of a common mRNA. HDAg-S binds RNA polymerase II (RNAPII), stimulates RNAPII transcription, and promotes RNAPII elongation, however, the mechanism for such stimulation is not yet known. Our lab has studied HDAg-S in millisecond phase kinetic studies of RNAPII to determine how HDAg-S stimulates elongation. HDAg-S has well-characterized interactions with RNA and many defined functional domains including: a coiled-coil domain that participates in protein oligomerization, a helix-turn-helix domain that participates in RNA binding and a C-terminal region that binds RNAPII. In order to determine the relationship of RNA binding by HDAg-S to the stimulation of RNAPII transcription, deletion mutants of HDAg-S were created. Deletion of increasing portions of the RNA binding domain while leaving the RNAPII binding region intact had little effect upon the stimulation of transcriptional elongation of RNAPII by HDAg-S. However, deletion of the HDAg-S region involved in RNAPII binding while leaving the RNA binding domain intact eliminated elongation stimulation. We conclude that RNA interaction has little or no importance for stimulation of RNAPII elongation by HDAg-S.

Effects of DNA in GTP hydrolysis activity of the *Neisseria gonorrhoeae* signal recognition particle protein PilA

Daniel Wood, under the direction of Dr. Cindy Arvidson, Department of Microbiology and Molecular Genetics

Gonorrhea, which is caused by *Neisseria gonorrhoeae*, is the second most prevalent sexually transmitted disease in the United States. *Neisseria gonorrhoeae* uses a protein targeting system called the Signal Recognition Particle to export up to 20% of synthesized proteins out of the cytoplasm. It has been previously shown that PilA is the receptor for the gonococcal Signal Recognition Particle and has an intrinsic GTPase activity that is essential for proper functioning. Also, the pilin expression locus *pilE* has been shown to stimulate the GTPase activity of PilA. The objective of this study was to determine if the DNA promoter sequences of other proteins associated with the SRP would also cause a stimulation of the PilA GTPase activity. Initially the promoter regions of *zipA*, *mtrC*, and *mexB* were studied because the proteins for which they encode are known to utilize the SRP. It was shown that *zipA* and *mexB* stimulate GTP hydrolysis of PilA to a similar degree that *pilE* does, and that *mtrC* stimulates to a slightly lesser degree. Additional studies were performed on 12 promoter regions that were identified in a previous search that attempted to identify gonococcal promoters that were bound by PilA. Thus far, however, these regions have demonstrated nominal GTPase stimulation. The fact that DNA promoter sequences other than *pilE* causes PilA to increase its' GTPase activity lends more support to the notion that DNA plays a major role in targeting proteins through the SRP.

Regulation of translocation by transcription factor IIF

Katie Zobeck, under the direction of Dr. Zachary Burton, Department of Biochemistry and Molecular Biology

Transcription factor IIF (TFIIF) is involved in both initiation and elongating by RNA polymerase II (RNAPII). TFIIF is composed of two subunits, RNAPII associating protein 74 (RAP74) and RAP30. Our lab has previously shown that mutations in the alpha1 helix of RAP74 cause a serious defect in elongation. It has also been found that TFIIF and Stimulatory factor II (SII) can cooperate to suppress transcriptional pausing by increasing the rate for the elongation complex to escape from the highly paused state. Mutations in RAP74 that limit transcription stimulation allow for pause suppression in the presence of SII. Wild type RAP74 stimulates elongation by supporting the post-translocated elongation complex, while a mutant RAP74(1-158) deleted for the alpha1 helix re-distributes the elongation complex into the pre-translocated state. In the presence of SII the RAP74(1-158) mutant causes RNAPII to advance slowly but efficiently, further demonstrating that TFIIF supports translocation and pause suppression.