DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

UNDERGRADUATE POSTER SESSION

Abstract Poster Presentations

Wednesday, April 11, 2007
1st floor of the Biochemistry Building
3:00 – 5:00 p.m.

Posters by:

Kimberly Anderson
"Analysis of putative nickel-binding residues in Klebsiella aerogenes UreG"
Mentor: Dr. Robert Hausinger, Department of Microbiology & Molecular Genetics

Jacqueline Brosius
"Analysis of the Csn5 subunit of the COP9 signalosome; a complex required for retinoblastoma protein stability in Drosophila."
Mentor: Dr. David Arnosti, Department of Biochemistry & Molecular Biology

Seth W. Dickey
"Interaction of Galectins and TFII-I: Their Role in pre-mRNA Splicing"
Mentors: Drs. John Wang and Ron Patterson, Depts. of Biochemistry and Molecular Biology and Microbiology and Molecular Genetics

Rebecca L. Kornas
"Preliminary evidence of a novel neutral polysaccharide in human brain that is age and disease regulated"
Mentor: Dr. Birgit Zipser, Department of Physiology

Atsushi Kuno
"Purification of the HU Protein."
Mentor: Dr. Jon Kaguni, Department of Biochemistry & Molecular Biology

"Analysis of putative nickel-binding residues in *Klebsiella aerogenes* UreG"

Kimberly Anderson, under the direction of Dr. Robert Hausinger, Department of Microbiology and Molecular Genetics

Abstract:

Urease is a nickel-containing enzyme that plays an important role in agriculture and as a virulence factor. Enzyme activation occurs through the use of several accessory proteins, one of which is UreG. Sequence alignment between UreG and its hydrogenase-related homolog HypB, along with the recently published crystal structure of HypB, suggested that residues His74, Cys72, Ser111, and Ser115 were of potential importance in coordinating Ni(II) ions.

Previous work added a biotin tag to UreG for purification purposes, mutated the gene encoding UreGb to change His74 to Ala, Asn, and Cys, and showed that when expressed with the other urease genes the UreG His74 variants almost completely eliminated urease activity.

In this study, Cys72, Ser111, and Ser115 were mutated to Ala to determine their importance in urease activation. The variants were tested for their ability to activate urease when expressed with the rest of the operon in *Escherichia coli* (*E. coli*). The variants were also expressed separately for purification, and the purified proteins will be used for equilibrium dialysis to determine their ability to bind Ni(II).

"Analysis of the Csn5 subunit of the COP9 signalosome; a complex required for retinoblastoma protein stability in Drosophila."

Jacqueline Brosius, under the direction of Dr. David Arnosti, Department of Biochemistry and Molecular Biology

Abstract:

In certain organisms, the COP9 complex regulates signaling pathways and protein degradation, and our laboratory has identified a role of this complex in controlling the degradation, and our laboratory has identified a role of this complex in controlling the levels of the Retinoblastoma Tumor Suppressor protein. However, the biochemical function of this complex in Drosophila is unclear. My work consists of analyzing the function of the CSn5 gene, which encodes a subunit of the COP9 complex.

To accomplish this, I PCR amplify two exons of this gene, and engineer in an epitope tag. I then modify these gene segments and return them back into the genome of Drosophila organisms in order to study the modified protein that results, that will lead to clues about this gene's function. This protein will be used for biochemical purification of the entire COP9 complex and in-vitro studies of its action.

"Interaction of Galectins and TFII-I: Their Role in pre-mRNA Splicing"

Seth W. Dickey, under the direction of Ronald J. Patterson and John L. Wang,
Departments of Microbiology and Biochemistry

Abstract:

Galectin-1 (Gal1) and galectin-3 (Gal3) are two members of a family of carbohydrate binding proteins found in nuclei of cells. Using HeLa cell nuclear extracts (NE), depletion-reconstitution experiments documented that they are factors involved in pre-mRNA splicing. An in vitro pull-down experiment using fusion proteins containing glutathione S-transferase (GST) identified the general transcription factor TFII-I as an interacting partner of Gal1 and Gal3

Our studies yielded several key conclusions: (a) Either GST-Gal1 or GST-Gal3 can specifically pull-down TFII-I out of NE. (b) β-galactosides, ligands of galectins, inhibited this interaction whereas non-binding carbohydrates failed to yield the same effect. (c) Site-directed mutants of Gal1, devoid of carbohydrate-binding activity, retained the ability to interact with TFII-I and, unlike the wild-type protein, this interaction was no longer sensitive to saccharide inhibition.

Along with assays of splicing, our results suggest that the interaction of Gal1 and Gal3 with TFII-I was closely correlated with their splicing activity and that saccharide-binding, per se, was not necessary for either of the former two activities. Interestingly, a recent proteomic analysis of spliceosomes identified TFII-I as one of the components.

"Preliminary evidence of a novel neutral polysaccharide in human brain that is age and disease regulated"

Rebecca L. Kornas, under the direction of Dr. Birgit Zipser, Department of Physiology

Abstract:

Alzheimer's disease is a neurodegenerative disease characterized byamyloid plaque and neurofibrillary tangle deposition in brain tissue. Typically Alzheimer's researchers study the amyloid beta peptide or hyperphosphorylated tau that precipitated into these plaques or tangles, as well as negatively charged glycans, polysialic acid and glycosaminoglycans. Investigating the so far ignored neutral glycans, we have discovered a novel type of polysaccharides,that we termed chitinaceous polymers (CP) that, according to our preliminary evidence, are regulated by age and disease.

To elucidate and compare the chemical compositions and structures of CPs fromAlzheimer's disease brains, age-matched controls and 1 yr old brain, we have developed the following purification method: We performed delipidation,hydrazinolysis, ion exchanges and size exclusion chromatography to harvest and purify the neutral polysaccharides from brain tissue. Based on the analysis of Bio-Gel P4 fractions, a high molecular weight fraction of chitinaceous polymers appear to be more highly expressed in AD brain tissue than in control brain tissue.

The prominent HMW fraction from the AD tissue was separated using Bio-Gel P30 fractionation from the low molecular weight (LMW common to all brain tissues. The multiple peaks apparent within the AD HMW fraction infer that it contains several discrete polysaccharides, which we will attempt to fractionate using HPLC. We will elucidate the chemical composition and structure of these AD-specific HMW species and further examine with NMR spectroscopy GC/MSS and MALDI.

“Purification of HU Protein”

Atsushi Kuno, under the direction of Dr. Jon Kaguni, Department of Biochemistry and Molecular Biology

Abstract:

HU protein is a heterodimetric protein which consists of and subunits. The hup and hup genes encode and subunits, respectively. HU protein is a bacterial histonelike protein which is known to be involved in the initiation process of replication. With the help of HU protein and ATP, DnaA protein molecules recognize and successively denature the A=T-rich region of DNA in the origin.

[1] The purpose of this experiment is to confirm the method which Pellegrini et al. [2] have made, and, if possible, to find out the better process of HU purification. HU proteins were amplified through PCR and purified through ion-exchange chromatography. 5 different columns were tested and confirmed that Heparin Sepharose is the optimum column for the HU purification.