

**DEPARTMENT OF
BIOCHEMISTRY
&
MOLECULAR
BIOLOGY**

UNDERGRADUATE POSTER SESSION

**Friday, April 24, 2015
11:30a - 12:30p**

The Origins Of Life

Andrew Baker and Tyler Rhinesmith

Mentor: Dr. Robert Root-Bernstein, Department of Physiology

Abstract: In 1953 Stanley Miller demonstrated that an apparatus simulating the atmospheric conditions of the primordial earth could synthesize important amino acids (Miller 1953). The apparatus included a reducing gas mixture, a reservoir of deionized water, a heat source, and a high-voltage electric spark. Others have expanded on his work, synthesizing sugars, nucleotides, and lipids by varying the gas mixtures, temperatures, and energy sources used (Fitz. et al 2007). We have refined Miller's methods further, adding simulated seawater, longer run times, and more precise control over atmospheric conditions. Biomolecules were identified via GC-MS and UHPLC-MS/MS. Analysis revealed simple sugars, amino acids, fatty acids, nitriles, and alcohols. In addition, enzyme and immunoassays demonstrated the presence of ATP and cAMP. Previously, each could only be separately synthesized. To our knowledge, this is the first report of many diverse biomolecules being synthesized in one mixture. Close proximity of relevant chemicals is imperative for abiogenesis; therefore, demonstrating their co-synthesis from the same starting materials is significant.

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## Mutagenesis of the *E. coli* RNAP in the N-Terminal Hinge of the Bridge Helix

\*Hailey Caudill

**Mentor: Dr. Zachary Burton, Department of Biochemistry and Molecular Biology**

**Abstract:** RNA polymerase (RNAP) is an enzyme involved in gene expression and transcription of many organisms. *Escherichia coli* RNAP is comprised of four important subunits,  $\alpha$ ,  $\beta$ ,  $\beta'$ , and  $\omega$  as well as an associated transcription factor,  $\sigma$ . The bridge helix domain, an alpha helix found between the two large  $\beta$  and  $\beta'$  subunits, has been observed to coordinate the movement of other domains, acting as a hinge. Mutations in the *E. coli* hinge sequence, GARKGL, directly affect cellular processes such as elongation and fidelity as the bridge helix is involved intimately with RNAP function. Site-directed mutations, specifically in the N-terminal hinge, have the potential to impact the flexibility of the hinge, which may alter various RNAP activities. For the purpose of this study the pVS10 vector which encodes the closely related *E. coli* RNAP was constructed due to the immense complexity of the RNAP structure. Various mutations, specifically focusing on mutations of the basic lysine residue, will be amplified via polymerase chain reaction as chosen by their ability to alter the flexibility of the hinge. Protein purification can be achieved through Ni-NTA column chromatography followed by heparin column chromatography, in which the final product is dialyzed. Fidelity and transcriptional elongation rates will then be assayed to examine the effects involved in the variation of hinge flexibility. Manipulation of specific hinge residues may alter various cellular functions or prevent gene expression entirely as the bacterial bridge helix plays a central role in RNAP activity.



\*1st place Undergraduate Research and Arts Forum winner

# Repurposing an Aminomutase from Taxus Plants: Stereo- and Regioselective Amination of Cinnamate Epoxides Produces Ring-opened erythro-Phenylserines

Olivia Goethe

**Mentors:** Dr. Dilini Ratnayake (Dept of Chemistry) and Dr. Kevin Walker (Dept of Chemistry and Dept of Biochemistry and Molecular Biology)

**Abstract:** The antineoplastic, natural product Paclitaxel has nineteen chiral centers. The stereochemistry of the phenylisoserine side chain of Paclitaxel accounts for two stereocenters that are important for its efficacy. The side chain is derived from R- $\beta$ -phenylalanine, the stereoselective product of an aminomutase (*TcPAM*) that isomerizes an S- $\alpha$ -phenylalanine substrate. Earlier studies showed that *TcPAM* functions as a transaminase and ring-opens cinnamate epoxide to an *erythro*-phenylserine. Additionally, *meta*-substituted cinnamate epoxides with bromo-, chloro-, and nitro-substituents were tested as substrates for *TcPAM* using styrylalanine as the amino group source in the transamination reaction. LC-MS revealed masses that were predicted for aminated, ring-opened products and showed that the biosynthetic product of the cinnamic epoxide was *erythro*-phenylserine. To solve the absolute stereochemistry of the biosynthetic phenylserine, we used the stereospecific L-threonine aldolase (from *Escherichia coli*) to assess the D- or L- stereoisomerism of the *erythro*-products. The aldolase cleaves the C $\alpha$ -C $\beta$  bond of a [2S, 3S] stereoisomer, producing benzaldehyde and glycine. GC-MS helped discern which molecules are present before and after incubation with the aldolase and if benzaldehyde is formed. An authentic standard of a DL-racemate of *erythro*-phenylserine will be derivatized with a chiral auxiliary before and after treatment with the aldolase. The derivatized stereoisomer with a reduced peak intensity will help assign the *erythro*-L-isomer on GC-MS. The biosynthetic sample eluting at the same retention time and showing fragment ions identical to that of the L-stereoisomer in the standard would support the absolute stereochemistry of the *erythro*-phenylserine product as [2S, 3S]

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Developing a Quantitative Western Blot Procedure for Chromatin-Associated Proteins

Tyler Miksanek

Mentor: Dr. Monique Floer, Department of Biochemistry and Molecular Biology

Abstract: Cells selectively regulate DNA's transcription through chromatin packing. Chromatin is comprised of histones, which are proteins that form complexes called nucleosomes within the cell. The fractional nucleosome occupancy at a specific genomic location helps determine gene expression along that segment of DNA. The SWI/SNF complex is a nucleosome remodeling complex. One model of SWI/SNF function holds that the SWI/SNF complex lowers nucleosome occupancy by weakening interactions between histones and DNA, allowing for transcription factor binding and eventual protein expression. This epigenetic control is exceptionally important in the immune response, as many macrophage promoters are blocked by nucleosomes unless SWI/SNF is present to reduce nucleosome occupancy.

Our lab's research project is focused on understanding the role and mechanism of SWI/SNF function in macrophage development and activation, using murine bone marrow derived macrophages. Our lab measures variations in nucleosome occupancy quantitatively at specific pro-inflammatory genes after lentiviral shRNA knockdown of SWI/SNF subunits. However, to quantify the ultimate effect of SWI/SNF knockdown, our lab must be able to reliably quantify variations in SWI/SNF subunits in the entire chromatin fraction. This chromatin fractionalization assay would complement our nucleosome occupancy assay and allow for quantification of global changes in histone binding under various conditions.

Starch and sucrose partitioning in plants able to transport G6P into the chloroplast.

Comparing Acylsucrose Acyltransferase-2 (ASAT2) Activity in Wild Tomato Species

Abigail Miller

Mentors: Dr. Robert Last (Dept of Biochemistry and Molecular Biology & Dept of Plant Biology) and Dr. Pengxiang Fan (Dept of Biochemistry and Molecular Biology)

Abstract: Glandular trichomes in tomato plants secrete acyl sugars, which play a role in plant herbivore defense. These modified sugars are made in a biosynthetic pathway catalyzed by four BAHD acyltransferase in the cultivated tomato *Solanum lycopersicum*. ASAT2, which catalyzes the second step of the pathway, adds a C12 or iC5 acyl chain to the R3 position of monoacyl sucrose to make diacyl sucrose; however, it has low activity to add iC5 at this position. The main focus for this project was to study the variations of ASAT2 in wild tomato species. In this research, ASAT2 alleles were cloned from various accessions. The proteins were expressed, and enzyme activities were tested. Interestingly, it was found that ASAT2 alleles from multiple accessions have a high activity for iC5-CoA as a substrate, unlike *S. lycopersicum*. Through alignment of protein sequences, four amino acid differences were found to correlate with iC5-CoA substrate preference of ASAT2. Through protein homology modeling, one out of the four amino acids: Phe408 in M82 and Val408 in *S. chilense* were found close to the predicted binding site of the enzyme, and through site-directed mutagenesis it was found that changing Phe to Val showed an increase in enzyme activity for M82 allele to use iC5-CoA as a substrate. The finding that one amino acid difference can change the acyl-donor substrate specificity of acyltransferase will help in the understanding and manipulation of acyl sucrose biosynthesis in the future.

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## Long Distance Lipid Signaling In Plants

Michael O'Keefe

**Mentor: Dr. Susanne Hoffmann-Benning, Department of Biochemistry and Molecular Biology**

**Abstract:** The plant phloem acts as a highway system in plants, facilitating transport of essential nutrients, photoassimilates, and other molecules. These compounds are transported as energy carriers or building blocks but can also serve as signaling molecules. The latter are essential for the plant to respond to environmental changes. One group of components that has not been studied closely is lipids and lipid binding proteins. They may function in a novel long distance signaling path through the phloem and elicit adaptive responses to stress conditions (such as drought) by changing the development of the plant. We are investigating the structure; function and localization of PLAFP (Phloem Lipid-Associated Binding Protein) to better understand this signaling mechanism and its effect on plant development. PLAFP is localized in the periphery of cells which, combined with its small size, suggests ability to move through plasmodesmata and into the phloem. PLAFP-overexpression affects root length. PLAFP transcription as well as Phosphatidic acid (PA) levels increase in response to osmotic stress and ABA. This suggests that PLAFP and PA act together in long distance (drought-) stress signaling through the phloem. I have developed models suggesting PLAFP binds PA, possibly along the whole length of the lipid, pointing to removal of PA from the membrane as a signaling molecule.

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# Starch and sucrose partitioning in plants able to transport G6P into the chloroplast

Alyssa Schreur

**Mentors: Dr. Thomas Sharkey and Dr. Sean Weise, Department of Biochemistry and Molecular Biology**

**Abstract:** During the day, triose phosphates are exported from the chloroplast to be used in sucrose synthesis and hexose phosphates are used within the chloroplast for starch synthesis. In  $C_3$  plants hexose phosphates, intermediates of both starch and sucrose synthesis, are not normally transported across the chloroplast membrane. However, in  $C_3$  Arabidopsis there are two glucose 6-phosphate/phosphate anti-transporters in chloroplast membrane. We have over expressed GTP2, which is not normally expressed in green leaves. It was shown that GTP2 over-expressing plants have higher starch accumulation in comparison to controls. From this, we hypothesized that G6P is being imported from the cytosol, bypassing regulation by PGI to increase the amount of starch synthesized. Disruptions of the transport of intermediates of the syntheses of starch and sucrose also changed starch and sucrose partitioning. In GTP2 over-expressing plants, the percentage of assimilated carbon partitioned to starch was higher than empty vector plants. This [came](#) at the expense of the ionic fraction of metabolites. The changes in partitioning demonstrate that expression of GTP2 needs to be regulated since constant expression of GTP2 compromises normal carbon partitioning.

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Association Between the Dash Diet and Blood Pressure in Women

*Diana Xu

Mentors: Dr. Jenifer Fenton (Dept of Food Science and Human Nutrition) and Dr. Claudia Holzman (Dept of Epidemiology and Biostatistics)

Abstract: The Dietary Approaches to Stop Hypertension (DASH) diet lowers blood pressure (BP) in some populations. However, the effect of the DASH diet on blood pressure in middle-age women is poorly studied. To determine if the DASH diet was associated with BP, anthropometric and Block food frequency data collected at follow-up were analyzed from a subset of women (25–50 yrs, N=677) who participated in the Pregnancy Outcomes and Community Health (POUCH) Study (1998–2004). The DASH diet score was the sum of eight component scores calculated using a modified Fung's DASH diet index. The index is based on rankings for intakes of fruits, vegetables, whole grains, nuts/seeds/legumes, sodium, sweets, meats/poultry/fish and total dairy products. The total DASH score was divided into quartiles for analysis (lowest quartile = poorest adherence). Weighted regression models were run to estimate the means of systolic BP (SBP) and diastolic BP (DBP) for quartile 1 vs. 2-4 of DASH scores. Adjusted models controlled for maternal age, race and season at enrollment. Women in the lowest quartile had a higher mean blood pressure compared to women in quartiles 2-4: SBP (119.2 vs 115.8 mmHg, $p=0.03$) and DBP (78.7 vs. 76.3 mmHg, $p=0.04$). When stratified by BMI, the DASH diet and BP association was primarily found in non-obese women. The DASH diet adherence was associated with lower blood pressure in some, but not all POUCH study women. These findings motivate us to further explore the effect modification by women's BMI.



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