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Online Proceedings

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BIOLOGICAL STRUCTURE AND FUNCTION

Session Moderator: Matt Kaeberlein, Pathology

JHN 022

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Early Fatty Acid Vesicles Interact Selectively with Amino Acids: The Origins of Life

Sean Christopher Dickson, Junior, Chemistry
Mentor: Sarah L. Keller, Chemistry
Mentor: Roy Black, Bioengineering and Chemistry

Modern cells discriminate among the amino acids chosen to be included in protein synthesis: some, like leucine, serine, glycine, and alanine appear in most proteins across most cells, whereas other amino acids, like (g)-aminobutyric acid and aminoisobutyric acid do not appear. This raises the question of how selectivity among amino acids arose – does the selectivity rely on modern protein machinery or could it have arisen in the first protocells of the Early Earth? For example, could certain amino acids have, by some mechanism, associated more strongly with protocell membranes, increasing their chances of being integrated into the first peptide chains? To test this hypothesis, our group assembles rudimentary protocells from molecules that would have been present on the early-Earth: decanoic acid (a fatty acid), sodium monophosphate, salt, and water. Vesicles of the decanoic acid spontaneously form. We then add different amino acids to the solutions and measure their turbidity to determine whether each amino acid causes the number of lamellae in the vesicles to increase or decrease. Increased lamellarity correlates with a sturdier vesicle. If certain amino acids increase lamellarity of protocells, that could serve as a method of selection for certain amino acids rather than others. Our results are that particular amino acids (most notably serine, glycine, and alanine) do in fact increase the lamellarity of fatty acid vesicles significantly, whereas other, less common, amino acids do not. We are currently exploring the plausibility of a mechanism for this occurrence involving ease of rotation around the alpha carbon of the amino acids, and we are investigating other ways in which interactions between amino acids and fatty acid membranes might be manifested, for example by a shift in the solution's critical vesicle concentration. Our results will fit into the overarching goal of understanding peptide formation and protocell stability in order to gain insight into the origins of life on Earth.

Mechanisms of Inhibition of Glycoprotein from Bacterial Adhesin, FimH

Chantalle Sasha Bell, Senior, Biochemistry
Mentor: Wendy Thomas, Bioengineering
Mentor: Laura Carlucci, bioengineering

Due to antibacterial resistance and the high recurrence of urinary tract infections (UTIs), studies have shifted to focus on anti-adhesive therapies as alternative to antibiotics. Often treated with antibiotics, UTIs are caused by uropathogenic Escherichia coli (UPEC). The bacterial adhesin, FimH, found on the terminal end of fimbria, hair like structures expressed on the perimeter of UPEC, is the main etiological factor of UTI prevalence and recurrence. FimH increases the virulence factor of E. coli by mediating the initial binding of the bacteria to glycosylated cells in the urinary tract. FimH has two domains. The lectin domain (LD) recognizes and binds the terminal mannose on glycosylated cells lining the urinary tract, whereas the pilin domain acts as an anchor to the fimbria. Previous studies have shown that α-methyl-mannose (αMM) competitively inhibits glycoproteins, such as horseradish peroxidase (HRP), from the FimH active site. We hypothesize that αMM can non-competitively inhibit HRP through a novel mechanism of inhibition. To determine the mechanism of inhibition of HRP in the presence of αMM, we are using Enzyme Linked Immunosorbent Assays to measure the dissociation of HRP in the presence and absence of αMM, after the FimH-HRP complex has formed. We expect to see an increase in the dissociation of HRP in the presence of αMM. HRP in this case, will act as a model to the glycosylated cells lining the urinary tract. This study aims to assist in the design of innovative anti-adhesive therapies that inhibit binding of FimH once bound to glycosylated cells lining the urinary tract.

Extrapolating a Synthetic, Orthogonal Auxin-TIR1 Receptor Pair in *Solanum lycopersicum*

Tonio Francisco (Tonio) Chaparro He.Him, Senior, Environmental Science & Resource Management, Biology (Molecular, Cellular & Developmental)

Levinson Emerging Scholar, Mary Gates Scholar, UW Honors Program

Mentor: Aarthi Putarjunan, Biology

Mentor: Keiko Torii, Biology

Auxins are a class of bioregulatory hormones which impact nearly every aspect of plant growth and development. Indole-3-acetic acid (IAA) and one of its corresponding receptors (TIR1) have been shown to be involved in seed germination, lateral root formation, stem elongation, fruit set and development, along with numerous other developmental processes. In an effort to more effectively study auxin-dependent pathways, Torii et al. developed a synthetic version of the IAA/TIR1 receptor pair which was shown to act independent of the endogenous pair in *Arabidopsis*. Here, I extrapolate our synthetically engineered auxin-receptor pair into a model system with agricultural and environmental implications (e.g. Solanum lycopersicum- tomato) and aim to test whether we are able to 'hijack' endogenous auxin signaling in tomato to precisely modulate auxin-dependent, spatiotemporal developmental outcomes. These findings could shed some light onto the vastly complex, auxin-dependent developmental process in *Solanum lycopersicum* and provide a tool to study auxin's specific role in varying developmental processes such as stomatal formation, leaf morphology, temporal flowering and fruit setting.

Humanizing Yeast TOR: Development of a Strain of Yeast to Capture Human Relevant mTOR Inhibitors to Use in Cancer Treatments

Anh Boi Diep, Senior, Biochemistry

Mentor: Matt Kaeberlein, Pathology

Mentor: Michael Kiflezghi, Pathology, School of Medicine

A person's age is one of the greatest risk factors for many age-related diseases, such as Alzheimer's disease, heart disease, and cancer. Developing interventions that target this common risk factor may allow us to slow down the progression of all age-associated diseases. One promising target for aging research is the mTOR (mechanistic Target of Rapamycin) signaling pathway, which is a master regulator of cell growth that allows the cell to fine tune its response to various environmental conditions. Inhibition of mTOR pathway has been shown to extend lifespan in yeasts, worms, fruit flies, and mice. In addition of being a target in aging research, the mTOR pathway is also a well-known effective target in some human cancers to prevent cancerous cells from dividing uncontrollably. We have previously developed a yeast-based system to identify mTOR inhibitors by comparing the growth of yeast strains that are differentially sensitive to mTOR inhibitors; a wild type strain, a tor1Δ mutant, and an fpr1Δ mutant. However, the system is not able to identify a few known ATP competitive inhibitors that have been shown to inhibit mTOR in human cells. This could be due to dissimilarities between yeast and human mTOR protein kinase domains. To address this issue, we are developing a humanized strain of yeast that should allow for identification of additional mTOR inhibitors that are effective in human cells. I am developing a novel hybrid gene containing the human mTOR kinase domain fused with the N-terminus of the yeast TOR2 gene. This new humanized strain will be utilized in our yeast-based assay with the goal of first detecting mTOR inhibitors known to be effective in human cells but unable to inhibit yeast mTOR. Successful completion of this goal will facilitate high-throughput screening of novel compounds that may have mTOR inhibitory and cancer fighting effects.

Elucidatidating the Binding Interaction of the *LINK-A* lncRNA to PIP_3 Using Nuclear Magnetic Resonance (NMR) Spectroscopy

Angelique Amado, Recent Graduate, Chemistry, University of Washington

Howard Hughes Scholar, UW Post-Baccalaureate Research Education Program

Mentor: Gabriele Varani, Chemistry

Long non-coding (lnc)RNAs have multiple biological functions, including recruitment of kinases to regulate signaling pathways involved in tumorigenesis and other human diseases. Of particular interest is the proposed interaction between *L*ong *I*ntergenic *N*oncoding *RNA* for *K*inase *A*ctivation (LINK-A) and the membrane component phosphatidylinositol-3,4,5-triphosphate (PIP_3). The proposed interaction between PIP_3-LINK-A would be the first example of a direct interaction between a non-coding RNA and phospholipid. I investigated the proposed interaction between LINK-A and PIP_3 using Nuclear Magnetic Resonance (NMR) spectroscopy. Multiple NMR-based experiments were performed to assess the degree of binding on the basis of line-width broadening of NMR spectra. Wild-type and mutated RNA constructs were titrated into a 100 uM solution of PIP_3, but the NMR data showed no evidence of line-width broadening, indicating that no direct interaction occurs between wild-type or mutated RNA constructs. Presumably, the reported cellular interaction is not direct and might require an additional mediating factor. I determined the 3D structure of the LINK-A RNA hairpin, required to recruit PIP_3, using biophysical molecular modeling and NMR data. My results provide a biophysical foundation to elucidate the functional role of LINK-A in PIP_3 recruitment and kinase activation.

Rigid Uso1 Protein Construction and Testing for Tethering Mechanism

Weisha Liu, Senior, Bioengineering
Mentor: Alexey Merz, Biochemistry

Membrane trafficking in the eukaryotic cell is a highly controlled and significant process that is related to various inherited disorders and cancers. Among numerous regulatory proteins, Uso1 protein – an essential, long, coiled-coil protein – plays a key role in the tethering process, capturing and pulling the vesicle toward the target membrane. Despite many years of work, the tethering mechanism has not been fully understood. Although several tethering models have been proposed, none of them were tested with Uso1 protein. Hence, to test these models, we engineered a mechanically deficient version of Uso1 protein, which lacks the critical tethering region for functioning. After the protein characterization, in cell survival tests and in test tube tests in chemically defined fusion system have been performed. The failure of the trafficking event, which would support our hypothesis, along with other functional test data, would provide a promising demonstration for the tethering mechanism of the long coiled-coil tether and the role of Uso1 protein.

Investigating Nuclear Localization Patterns in NUP188 Patients using Live Cell Fluorescence

Natalie J. Weed, Senior, Economics, Neurobiology
Mary Gates Scholar, UW Honors Program
Mentor: Heather Mefford, Pediatrics
Mentor: Alison Muir, Pediatrics

The nucleus is the information center of the cell, acting as a hub for DNA storage, regulation, and replication. Therefore, transport of molecules into and out of the nucleus is vital for proper biological functioning. This transport is tightly regulated via the nuclear membrane, and a collection of proteins called nuclear pore proteins that interact with molecular signals. These signals allow molecules that cannot passively diffuse through the nuclear membrane (typically greater than 60 kD) to be shuttled into or out of the nucleus via a variety of pathways. Like all biological pathways, this process can be disrupted and lead to phenotypic abnormalities. Our lab identified two siblings with biallelic variants in NUP188, a known nuclear pore protein; we identified four additional cases in three families through collaborators. Clinical features include brain abnormalities with thin corpus callosum, progressive microcephaly, severely delayed myelination; congenital cataracts; mild dysmorphic features; and hypoventilation leading to death in infancy. In order to better understand our patients' phenotype, we are investigating nuclear import pathways as a potential mechanism of disease. Using green fluorescent protein (GFP), we can directly visualize protein localization in living cells, without the use of additional stains such as immunohistochemistry. Based on size restriction of the nuclear pore, we used a vector construct with three repeats of GFP and our nuclear localization signal (NLS). Specifically, we are investigating four main NLS pathways: Importin α+β (SV40 NLS), Kap β2 (hnRNP NLS), Importin β (CREB NLS), and no NLS. We have been able to successfully create a viral vector for each NLS. Next steps include optimizing expression in patient cell lines. This approach will allow us to quantify and visualize discrepancies between patient and control localization patterns, leading to better understanding of causes of patient phenotype and potential novel therapies.

Improvement and Validation of Dotted Traction Force Microscopy Platform

Robin Zhexuan Yan, Senior, Mechanical Engineering
Mary Gates Scholar
Mentor: Nathan Sniadecki, Mechanical Engineering
Mentor: Kevin Beussman

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) have great potentials in biomedical research and can be used extensively in drug screening and heart simulations. To understand the cardiomyocytes, we need to perform functional analysis on these muscle cells. Therefore, we need a simple, controllable, yet biocompatible and high throughput tool to measure the cellular traction force. At the Sniadecki Lab, we are developing a new technique to measure the force generation of hiPSC-CM: dotted traction force microscopy platform. To create the platform, fluorescent proteins were first absorbed to a dotted polydimethylsiloxane (PDMS) negative and stamped onto a polyvinyl alcohol film. The film was then transferred to a soft PDMS substrate and subsequently dissolved using phosphate buffered saline solution while the patterned fluorescent proteins stained the substrate. Since the stiffness of the soft PDMS substrate is known, the force generation of the cardiomyocytes can be calculated in real time by optically tracking the deformation of the fluorescent dots. Currently, we are able to manufacture the platform with high fidelity and uniform alignment with a production time of less than 2 hours. Moreover, the cardiomyocytes can fully spread out to their in vivo state on the substrate which ensures the force measurement is valid and accurate. Potentially, this method is not limited to cardiomyocyte research and can be applied to study the interaction between force generation and cell performance of other cells. We are also exploring the possibility of automated manufacture and integration with 96-well to enable mass production.