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Online Proceedings

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Modulation of Cell Behavior and its Components

Session Moderator: Valerie Daggett, Bioengineering

JHN 175

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Measuring the Replicative Lifespan of Yeast

Blaise John (Blaise) Pascual, Senior, Microbiology
Hieu Trung (Hieu) Nguyen, Senior, Microbiology
Diego Molina Ochoa, Junior, Biology (Physiology)
UW Honors Program
Mentor: Matt Kaeberlein, Pathology
Mentor: Brian Wasko, Department of Pathology

The biological basis behind aging in eukaryotic species is defined by a wide range of factors, from diet to environmental conditions and metabolic predisposition. The genetic factors associated with aging are of particular interest because they are highly conserved among all eukaryotes. Determining longevity genes that are broadly conserved among eukaryotic species is a critical step in understanding aging, and is one of the primary goals of this project. The Kaeberlein lab has analyzed the replicative lifespan (RLS) of more than 4700 single-gene deletion mutants of *Saccharomyces cerevisiae*, a eukaryotic model organism. RLS experiments measure the number of daughter cells produced by a mother cell prior to senescence, and is thought to provide a model of aging in mitotically active cells. Mother and daughter cells are visibly differentiated under a light microscope and physically separated using a fiber-optic needle. The number of daughter cells dissected are quantitated and recorded. The total number of cell divisions are then summed for statistical analysis. The result of the investigation yielded 238 gene deletion mutants that exhibited a statistically significant lifespan extension. Longevity genes were found to be clustered within specific parts of an organism's biology. Ribosomal genes and genes related to metabolic responses to nutrients were particularly significant. One particularly significant gene is the LOS1 gene, which functions as a tRNA exporter from the nucleus. Deletion of the LOS1 gene resulted in a dramatic enhancement of yeast RLS, opening the possibilities of lifespan research regarding the subcellular localization of tRNA. Also significant was the discovery that a majority of the longevity genes were concentrated among highly evolutionarily conserved parts of the genome. Ultimately, the success of the yeast RLS project in identifying novel longevity genes and establishing a database of conserved longevity genes in eukaryotes points to the efficacy of such methodology.

Does rDNA Copy Number Influence Genome-Wide Replication Initiation?

Mackenzie Erin (Mackenzie) Croy, Senior, Biology (Molecular, Cellular & Developmental)
Madison Amber (Madison) Miller, Senior, Biochemistry
Sarah Ariana (Sarah) Johnson, Senior, Biology (Molecular, Cellular & Developmental)
UW Honors Program
Mentor: Bonita Brewer, Genome Sciences

Successful genome replication is essential for cell division and has been correlated with longevity in the model organism *Saccharomyces cerevisiae*. DNA replication is initiated at specific sites on chromosomes called origins and requires the recruitment of several low abundance replication factors. The highly repetitive ribosomal DNA (rDNA) locus, known to encode the RNA components of ribosomes in both yeast and humans, has recently been suggested to be influential in genome replication and stability. For instance, the genome of the budding yeast *S. cerevisiae* contains 450 origins, 150 of which reside at the rDNA locus and could therefore create competition with origins located in the rest of the genome. We hypothesize that the preponderance of rDNA origins compete for essential replication factors and that reduction of rDNA origin competition, achieved by reducing rDNA copy number or compromising the rDNA origin sequence, would promote non-rDNA genome replication. We wanted to examine the role of rDNA competition by using four yeast strains that have either high or low rDNA copy number and either a normal or compromised rDNA origin sequence. We measured the ability of these four strains to maintain a non-essential plasmid containing a non-rDNA origin of replication as an indicator of genome replication. We expect that if rDNA origins compete with other origins, including those on the plasmid, DNA replication will be unable to initiate at the plasmid ori-

gin. Therefore, we predict that strains with high rDNA copy number arrays and strong rDNA origins will lose the plasmid at a greater rate than strains with low rDNA copy number and weak rDNA origins. Cells with such intense competition for replication factors could consequently struggle to complete chromosome replication, potentially leading to decreased cellular health and lifespan in yeast, which could be extended to our understanding of human cellular health.

Does Single-Stranded DNA Accumulation Cause Death of Ligase-Deficient Yeast Cells?

Sarah Ariana (Sarah) Johnson, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar, UW Honors Program
Mentor: M.K. Raghuraman, Genome Sciences
Mentor: Bonita Brewer, Genome Sciences

DNA replication is a crucial step in the duplication of all cells, requiring the cooperative function of multiple enzymes. In one of the final steps of chromosomal DNA replication, short pieces of DNA, Okazaki fragments, are joined by the essential enzyme DNA ligase. Mutations in human DNA ligase have been linked to genome instability. To study the consequences of ligase deficiency, my lab uses a temperature-sensitive mutation in the *CDC9* gene that encodes DNA ligase in the budding yeast *Saccharomyces cerevisiae*. When *cdc9^{t}s* mutant yeast cells replicate at restrictive temperatures, they lose viability while simultaneously and unexpectedly accumulating loose pieces of single-stranded DNA (ssDNA). The current model of DNA replication cannot explain this accumulation, as newly formed ssDNA fragments should be base-paired to the template strand. We hypothesize that the absence of ligase causes this accumulation of ssDNA, which in turn causes cells to lose viability. I am currently testing this hypothesis in *S. cerevisiae* cells that have the *cdc9^{t}s* mutation as well as a mutation in either *EXO1* or *RAD27*, two genes that code for exonucleases. These exonucleases process Okazaki fragments before ligation, and preliminary evidence suggests that the *rad27* mutation decreases viability, while the *exo1* mutation improves viability. By growing mutant cells at restrictive temperatures and visualizing ssDNA quantity at multiple time points while determining viability, I am correlating these two phenotypes. If ssDNA causes loss of viability, *cdc9^{t}s rad27* double-mutant cells should have more accumulated ssDNA, correlating to decreased viability, while *cdc9^{t}s exo1* double-mutants should have less accumulated ssDNA. These experiments could provide novel insights into a universal process—DNA replication—and clarify the consequences of defects in that process. Additionally, because ssDNA accumulation has been linked to autoimmune disorders in humans, understanding the causes and consequences of ssDNA accumulation may have implications for human health.

Drugs that Inhibit Sirtuin 2 and Activate Gene Expression

Angela Yunji (Angela) Park, Junior, Pre Public Health
Mentor: Antonio Bedalov, Medicine/Biochemistry
Mentor: Sarwat Chowdhury, Clinical Sciences Division

Sirtuins are histone deacetylases- enzymes that remove acetyl and various acyl groups from a lysine amino acid side chains on histone and other client proteins. Sirtuins are NAD+ dependent, meaning that they function in the presence of the oxidized form of the coenzyme NAD. When an acyl group is removed, the resultant positively charged protein has a stronger affinity for the negatively charged DNA phosphate backbone. As a result, DNA wraps more tightly around the histone and transcription is repressed due to closed chromatin. Along with chromatin modification, sirtuins modulate various cellular processes through deacetylation of other clients. There are seven types of sirtuins, of which Sirtuin 2 (SIRT2) is the main focus of my research. The goal is to identify compounds that inhibit SIRT2 activity, while have minimum inhibition to structurally similar to SIRT1. Compounds are initially screened at 50 μM to test for high inhibition of SIRT2 and low inhibition of SIRT1. Compounds that inhibit SIRT2 activity by more than 90% are then evaluated for a dose response curve in order to determine the IC50 of each compound, or the concentration at which the enzymatic activity is reduced to 50%. The last stage of testing in my research is to test the compounds on various mammalian cancer cell lines. We found that Burkitt's lymphoma derived cell lines are most sensitive to SIRT2 inhibition, giving IC50 values in the $<$10 μM range. My research will contribute to the ongoing search for Sirtuin2-inhibiting compounds for the treatment of B-cell malignancies.

Immunostaining Histone acetyltransferase p300 (also known as p300) and cAMP response element-binding protein (CBP)

Noah Schlenk, Junior, BBMB (Biochemistry, Biophysics, Molecular Biology), Whitman College
Mentor: Paul Yancey, Biology, Whitman College
Mentor: Samuel LaBarge, Orthopaedic Surgery, University of California, San Diego
Mentor: Simon Schenk, Orthopaedic Surgery, University of California, San Diego

My presentation focuses on research I conducted using immunohistochemistry to identify proteins in insulin stimulated pathways. My research contributed to the lab's ability to analyze p300 and CBP proteins for their use of acetylation. p300 and CBP often are talked about hand in hand as two proteins that function together to induce and carry out acetylation of other proteins. Generally, you can think of them as a messenger, carrying the information from one source to the next in a long line of messangers. Phosphorylation has long been seen as the primary driving force of downstream signal-

ing. Our lab examined the importance and underestimation of acetylation through these two proteins. Initially, the lab used Western Blotting to look for protein presence, but the results we obtained were faint and difficult to analyze. I was assigned the task of trouble-shooting a protocol for the staining of these two proteins. I sliced tissue, plated it, stained it, and calculated the changes between knockout- and wild-type tissue. I was ultimately successful in identifying p300 but statistically unsuccessful in identifying CBP due to a technical error. My research showed a much more reliable way of identifying at least 1 of the proteins rather than Western Blotting techniques. This has a broader implication that there will possibly be more accurate or reliable research done on these p300 and CBP proteins, and acetylation as a primer for downstream pathway messaging done in the future.

Auto-Inhibition of SspH1, a Bacterial E3 Ubiquitin Ligase

Thomas Paul (Thomas) Schweppe, Senior, Biochemistry, English
Mentor: Peter Brzovic, Biochemistry

With the rise of antibiotic resistant bacteria, understanding how pathogenic bacteria invade and survive within our bodies is becoming more important as we search for novel ways to combat bacterial infection. Some pathogens, such as *Salmonella typhimurium*, inject host cells with proteins that suppress the host cell's ability to respond to bacterial invasion. One such protein, SspH1, is an E3 ubiquitin ligase. E3 ubiquitin ligases attach ubiquitin, an important regulatory protein used to mark other proteins for degradation, to a substrate. Ubiquitin, the biochemical machinery that activates ubiquitin, and the pathways that utilize ubiquitin as a signaling molecule, are not found in bacteria, only in eukaryotes. However, bacteria have evolved proteins, such as SspH1, that hijack a host cell's biochemical machinery in order to target host proteins for degradation. SspH1 consists of two domains: a catalytic E3 domain that transfers ubiquitin to substrate, and a substrate recognizing Leucine-Rich-Repeat (LRR) domain. I have shown through biochemical assays that the LRR domain inhibits the catalytic activity of the E3 domain. I have also shown that this inhibition is relieved in the presence of substrate. Through the use of 2D NMR and further biochemical assays, I hope to characterize the binding between the LRR and E3 domain in order to further the understanding of how SspH1 is inhibited. Understanding the mechanism of SspH1 auto-inhibition can increase our knowledge of the mechanisms and activities of related bacterial effector proteins, furthering our understanding of how pathogenic bacteria evade our immune system, and potentially lead to the development of novel treatments that can combat bacterial infection.

Characterizing the Effect of Zinc Binding on the Structure and Chaperone Activity of a Small Heat Shock Protein

Bobby Shih, Senior, Chemistry, Biochemistry
UW Honors Program
Mentor: Rachel Klevit, Biochemistry
Mentor: Amanda Clouser, Biochemistry

Proteins serve very specific and powerful functions in the cell by maintaining a specific shape through chemical interactions in the protein sequence. Under stress conditions however, these chemical interactions holding the protein together may break apart, leading to the protein's loss of function and exposure of hydrophobic patches that can interact with other proteins to form toxic aggregates. Heat shock proteins (sHSPs) act as safeguards under conditions of stress and protein misfolding as molecular chaperones that interact with exposed hydrophobic patches to stabilize unfolded proteins. The importance of sHSPs in the cell is exemplified through various disease associated mutations of sHSPs. sHSPs are a large and diverse family of proteins distinctive in their ability to form multimers of various sizes, which correlates with varying ability to prevent aggregation. However, little is known about how different structural elements of sHSPs influence function. The Klevit lab aims to better understand the molecular interactions of human sHSPs with themselves, how these sHSPs are activated in cellular conditions, and how they recognize and bind unfolded protein. My work focuses on the characterization of the structure and function of the human sHSP, HSPB8. Specifically, I am studying the effects of HSPB8 binding to zinc, the cellular levels of which vary in response to stress and regulate the function of many other proteins. By comparing zinc bound and non-zinc bound protein samples using various biophysical techniques such as NMR, circular dichroism and fluorescence, I aim to elucidate the effects of zinc binding on structural and functional elements of HSPB8.

Determining the Contributions of Genetic Modifiers to Complex Cell Aggregation Traits in *Saccharomyces cerevisiae*

Kolena Dang, Senior, Biochemistry
Mary Gates Scholar
Mentor: Maitreya Dunham, Genome Sciences
Mentor: Elyse Hope, Genome Sciences

Model yeast *Saccharomyces cerevisiae* normally grows in the lab as isolated cells but can evolve the ability to clump, protecting the cells from stress factors including antimicrobial treatments. Clumping can occur either due to a failure of cell separation during division or through physical cell adhesion ("flocculation"). Understanding the genetics behind these traits could give us insight into how microbes develop antimicrobial resistance. Previously, we evolved *S. cerevisiae* from a non-clumping ancestor strain for 300 generations in

miniature chemostats, multiplexed culture devices that maintain a constant environment. Using a combination of whole genome sequencing and bulk segregant analysis, a pooling and sequencing approach, we identified causal mutations in 23 independent, clumping clones from the evolution experiments. We identified two strains with cell separation defects which had mutations in both budding gene *BEM2* and cell separation gene *ACE2*. In the remaining clones, the majority of the causal mutations affected the regulation of known flocculation gene *FLO1*. Despite this shared mutation, a number of these clones exhibited varying flocculation phenotypes. We hypothesized that other "modifier" mutations might also be contributing to the complexity of the phenotype. For one strain with unusual cell morphology, targeted sequencing of candidate modifier genes revealed that the modifier mutation is either in the *IRA1* or *HSL7* gene, which are genetically linked. The goal of our current experiments is to determine the contributions of *BEM2* and *ACE2* to the separation defect phenotype and of *HSL7* and *IRA1* to the flocculation phenotype. We are using a method called complementation testing, in which the wild-type gene is added to the mutated strain using molecular cloning techniques to see if the mutant phenotype is repaired. This will enable us to identify which genes are contributing to the complexity of these traits and facilitate a better understanding of their underlying mechanisms.