UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

SESSION 1K

MOLECULAR BASIS FOR HUMAN DISEASE

Session Moderator: Caroline Harwood, Microbiology
MGH 271
12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Utilizing Budding Yeast to Investigate Human Dwarfism
Mackenzie Croy, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Bonita Brewer, Genome Sciences

Meier-Gorlin syndrome (MGS) is a rare form of proportionate dwarfism that has been linked to mutations in proteins that function during the initiation and elongation steps of DNA replication. Although mutations in DNA replication proteins have been shown to be causative for MGS, it is still not clear how defects during S-phase lead to the phenotypes observed in patients with this condition. Therefore, further research is necessary to better understand the molecular and cellular consequences of cells harboring MGS mutations. Tools for studying DNA replication in humans are limited, however, there are several well-established tools for studying this process in the budding yeast *S. cerevisiae*. Some individuals with MGS have been shown to have mutations in the DNA replication initiation factor Cdc45, which functions during both the initiation and elongation steps of DNA replication. Because a specific MGS mutation in CDC45 is in a residue that is conserved between humans and yeast, we have replaced the wild type yeast gene with an allele that contains the equivalent human mutation. Yeast with the MGS mutation exhibit increased sensitivity to elevated temperatures and a reduced ribosomal DNA (rDNA) copy number. In budding yeast, the rDNA locus contains ˜150 copies of a 9.1 kb repeat which encodes the template needed to make ribosomal RNA—the main structural component of ribosomes. Additionally, each repeat contains an origin of replication. We hypothesize that this loss of rDNA repeats is a consequence of impaired replication initiation at the rDNA locus and will test this using 2-D gel electrophoresis. We anticipate to expand our study outside of the rDNA locus and examine how replication might be perturbed at other locations across the genome. Ultimately, we hope studying MGS in yeast will elucidate our understanding of the pathogenesis of this condition in humans.

SESSION 2S

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.

Does Single-Stranded DNA Accumulation Cause Death of Ligase-Deficient Yeast Cells?
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 $cdc9^{ts}$ 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 $cdc9^{ts}$ 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.

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MODULATION OF CELL BEHAVIOR AND ITS COMPONENTS

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JHN 175

3:30 PM to 5:15 PM

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Does rDNA Copy Number Influence Genome-Wide Replication Initiation?

Mackenzie Croy, Senior, Biology (Molecular, Cellular & Developmental)
Madison Miller, Senior, Biochemistry
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 origin. 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.

POSTER SESSION 3

MGH 206, Easel 167

2:30 PM to 4:00 PM

Exploring a Novel DNA Replication Error as a Source of Gene Amplification in Yeast

Madison Miller, Senior, Biochemistry
Mary Gates Scholar
Mentor: Bonita Brewer, Genome Sciences

In March of 2011 Dr. Bonita Brewer published a paper proposing a model to explain how a particular error in replication can lead to the amplification of chromosomal segments in an inverted orientation, such as those seen in classes of chromosomal rearrangements that arise in human developmental disorders and cancers. She hypothesized that when a replication fork encounters a short inverted repeat, the leading strands could erroneously become ligated to the lagging strand and produce an extrachromosomal palindromic DNA intermediate. Through my research I am investigating if this model can explain the amplification of the ADH4 locus in yeast, what features of the short inverted repeats lead to the inverted junctions, and what environmental or genetic factors influence this mode of gene amplification. Yeast cells that lack a functional copy of ADH1 (a gene that encodes the major form of alcohol dehydrogenase) become sensitive to the drug Antimycin-A. However, these cells can regain Antimycin-A resistance by amplification of ADH4 (a gene that encodes a minor form of alcohol dehydrogenase). Previously it has been found that the extra copies of ADH4 reside on an extrachromosomal palindromic DNA molecule. Through my research I aim to determine if the extrachromosomal amplification of ADH4 is occurring via this new model. To do so, I have created my own adh1???:KanMX strain, confirmed that high copy number of ADH4 is sufficient to confer resistance to Antimycin-A, and will isolate the extrachromosomal fragment via pulse-field gel electrophoresis and characterize its structure in detail. In an extension of this work I will test whether genetic and environmental factors that stress the replication process influence the frequency of this form of gene amplification, possibly providing insights into amplification events that contribute to human developmental disorders and cancers.