Online Proceedings

SESSION 2B

GENETIC EVOLUTION ACROSS THE LIFESPAN

Session Moderator: Joachim Voss, Biobehavioral Nursing & Health Systems
228 MGH
3:30 PM to 5:00 PM
* Note: Titles in order of presentation.

Do Centromere-Like Regions (CLRs) Regulate DNA Replication Timing?
Seungbeen (Steven) Lee, Senior, Biochemistry
Mary Gates Scholar, UW Honors Program
Mentor: Bonita Brewer, Genome Sciences

Chromosomal DNA synthesis begins at origins of replication, with some origins activating earlier than others during S-phase. Neither the mechanism by which origin activation time is determined nor the biological significance of this temporal program is fully understood. Interestingly centromeres, sites where kinetochore complexes form to correctly separate chromosomes during mitosis, replicate early. In the budding yeast *Saccharomyces cerevisiae*, centromeres actively promote their own early replication by advancing the activation time of their neighboring origins. We previously showed, in cells lacking the mitotic checkpoint, an artificially delayed, late replicating centromere causes a dramatic increase in chromosome instability. In this study, I am investigating the function of centromere-like regions (CLRs) in *S. cerevisiae*. These sequences can bind the centromeric histone (Cse4), especially when *CSE4* is overexpressed. Under this condition, additional kinetochore proteins can build up and CLRs take on some of the properties of functional centromeres. Because the kinetochore protein Ctf19 recruits a replication initiation factor, I hypothesize that the centromere-mimicking DNA sequences are also capable of recruiting the kinetochore protein associated with early replication to advance the initiation time of nearby origins. To test this possibility, I will examine the effects of the CLR on replication origin activation using a plasmid that contains two identical origins. I will clone a CLR next to one of the origins and determine whether this origin is now the earlier activated origin on the plasmid when *CSE4* is overexpressed. The CLR I will use resides in an early replicating part of the yeast genome. In a complementary experiment I will delete the genomic copy of this CLR and measure its effect on firing time of the adjacent origins. The results of this study will provide us deeper insights on how cells orchestrate when and where in their genome to start duplication.

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A Screen for Genetic Regulators of rDNA Copy Number
Xiaobin (Summer) Wang, Senior, Biology (Molecular, Cellular & Developmental)
UW Honors Program
Mentor: Bonita Brewer, Genome Sciences
Mentor: Elizabeth Kwan, Genome Sciences

Eukaryotic genomes contain many copies of ribosomal DNA (rDNA) encoding the RNA components of ribosomes, ranging from 150 tandem repeated copies in the budding yeast *Saccharomyces cerevisiae* to approximately 700 copies in diploid human cells. Recent work in the Brewer/Raghuraman and Bedalov labs used *S. cerevisiae* to identify connections between rDNA copy number and cellular processes such as genome replication and replicative aging. Based on their findings, I hypothesize that a cell with too many rDNA copies may have difficulty with genome-wide DNA replication, while a cell with too few copies will not be able to satisfy ribosome demand from protein synthesis. What dictates the size of rDNA region remains unknown, but we hope to learn about the relationship between the size of rDNA region and DNA replication by identifying its genetic regulators. A preliminary survey found single gene deletion strains with altered numbers of rDNA repeats, supporting the idea that rDNA copy number is under genetic control. Encouraged by this result, I screened 400 mutants from *S. cerevisiae* deletion collection for rDNA copy number. I also examined the putative link between rDNA size and longevity: half of the mutants chosen in this screen were reported to have longer

replicative lifespan and the other half were randomly selected. I found that the average rDNA copy number is not significantly different between the two groups, suggesting that the relationship between the size of rDNA region and replicative lifespan is not direct. I did observe a notable enrichment for mutants with altered rDNA size in genes that have mitochondrial function, defects in cell cycle progression, and abnormal cytoskeleton phenotypes. By continuous investigation and exploring the molecular mechanisms that govern rDNA copy number, I hope to gain a deeper understanding of how the rDNA region interacts with genome-wide DNA replication.