Creating a Circular Chromosome in the Budding Yeast *Saccharomyces cerevisiae*

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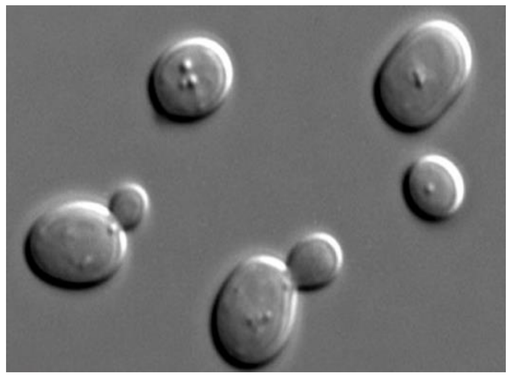
Introduction

At the ends of linear chromosomes there are unique regions of repeated nucleotide sequences called telomeres. In each round of DNA replication, the termini of telomeres may shorten. To maintain telomeres an enzyme called telomerase adds nucleotides at the ends of the chromosomes. The function of telomerase in telomere maintenance has important implications for human health. Lack of telomerase function and telomere shortening plays a role in aging, while unregulated increased telomerase activity occurs in most human cancers. Given these significant effects on organisms, we will experimentally explore how linear chromosomes, telomeres, and telomerase evolved in the simple model organism *Saccharomyces cerevisiae*. To accomplish this, I will genetically engineer the linear chromosome XI of *Saccharomyces cerevisiae* into a circularized version that lacks telomeres.

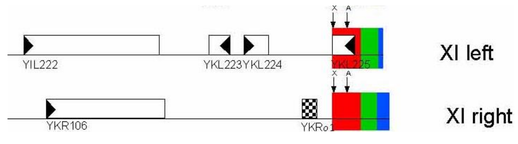


Individual cells of *S. cerevisiae*

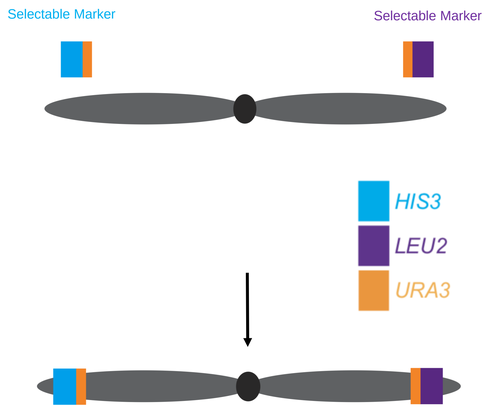
Methodology

DNA cassettes will be constructed that have homology to the sub-telomeric regions of chromosome XI. In one cassette, a selectable auxotrophic marker, *HIS3*, and half of the *URA3* gene will be added; the cassette for the other end will contain the complimentary half of *URA3* and the other auxotrophic marker, *LEU2*. These cassettes will be transformed into yeast, where they can integrate into the sub-telomeric regions of Chromosome XI. To select for integration, strains of yeast will be grown on media lacking histidine and leucine. Yeast strains that grow on this media will then be transferred onto a media lacking uracil, which will select for cells that underwent recombination to create an intact *URA3* gene. The intact gene will create a circular chromosome.

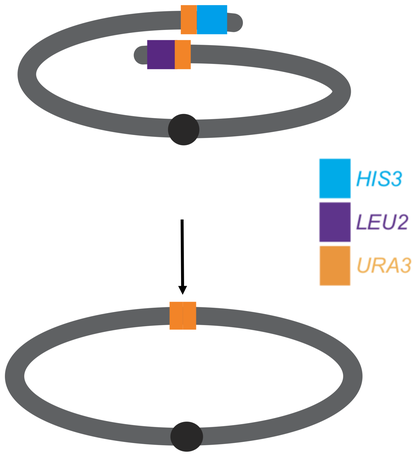
The viability and fitness of strains with a circular chromosome will then be characterized.



The Ends of Chromosome XI:
The DNA cassette is inserted between the start of the telomeric sequence (red) and the terminal-most gene (YKL 224 and YKR 106)
Image Credit: https://www2.le.ac.uk



DNA cassettes containing selectable markers are inserted into the ends of Chromosome XI



***URA3* can recombine and circularize the chromosome.**
Yeast strains with circular chromosomes will be selected in media lacking uracil.

Expected Results & Future Plans

- Strains with a circular chromosome XI are expected to have normal fitness as compared to strains with only linear chromosomes.
- The overall fecundity of strains with a circular chromosome XI is expected to be reduced due to difficulties with crossing over in meiosis.
- If the circular chromosomes are viable, then a strain will be genetically engineered to have all 16 of its chromosomes circularized in the near future.

Key Literature

Klar, A. J., Stratnern, J. N., Hicks, J. B., & Prudente, D. (1983). Efficient Production of a Ring Derivative of Chromosome III by the Mating-Type Switching Mechanism in Saccharomyces cerevisiae. *Molecular and Cellular Biology, 3*(5), 803-810.

Naito, T., Matsuura, A., & Ishikawa, F. (1998). Circular Chromosome Formation in a Fission Yeast Mutant Defective in two ATM Homologues. *Nature Genetics, 20*, 203-206.

Acknowledgements

Thank you to the KBRIN New Faculty Start Up Award to Dr. Melissa Mefford for funding.
(NIGMS P20GM103436)