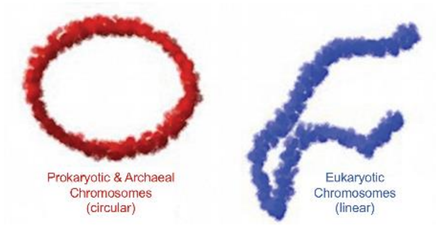
Creating the First Genetically Engineered Eukaryote with Circular Chromosomes

Duncan McGinnis and Dr. Melissa Mefford
Morehead State University, Department of Biology and Chemistry
Morehead, KY 40351

Introduction

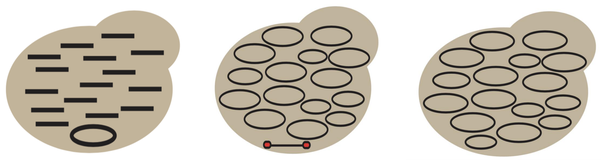
Prokaryotes and eukaryotes differ in their chromosome structure; prokaryotes have a single circular chromosome, while eukaryotes have multiple circular chromosomes.



Linear chromosomes are more complex than circular ones and create problems for eukaryotes. Many of these issues are solved by telomeres—long, repetitive sequences of DNA found on the ends of most eukaryotic chromosomes. They protect the ends from harm and limit how many times a cell can divide. Two of our greatest medical challenges, cancer and aging, are closely related to telomeres; most cancers upregulate telomerase, and aging human cells display shorter telomeres. To investigate the evolution and function of telomeres, we are circularizing chromosome VII in a strain of the single-celled eukaryote *Saccharomyces cerevisiae* (budding yeast). There is precedent set for functional and viable yeast with circular chromosomes; a naturally circularized chromosome III has been found in mutant *S. cerevisiae* (1); natural circularization of all 3 chromosomes has been observed in mutant *Schizosaccharomyces pombe*, another type of yeast (2); and a strain of *S. cerevisiae* has been created with all its chromosomes fused into a single, massive circular chromosome (3).

Objectives

Our research aims to circularize individual chromosomes in *S. cerevisiae* cells and determine their viability and fitness. If these cells are viable, we will circularize additional chromosomes to create strains with all but one and all chromosomes circularized.

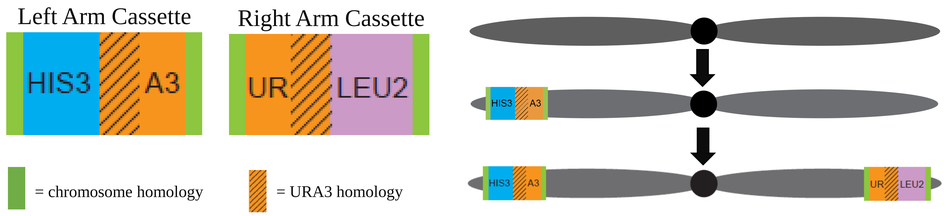


Acknowledgements: This work was supported by KY INBRE IDeA (8P20GM103436) and NIH R15 (1R15GM144954) awards to Dr. Melissa Mefford.

Citations:
1. Klar A et. al. (1982). "Efficient Production of a Ring Derivative of Chromosome III by the Mating-Type Switching Mechanism in *Saccharomyces cerevisiae*". *Molecular and Cellular Biology*, May 1983, p. 803-810.
2. Natio T et. al. (1998). "Circular chromosome formation in a fission yeast mutant defective in two ATM homologues". *Nature Genetics*, 1998, vol. 20 p. 203-206.
3. Shao Y et. al. (2019). "A single circular chromosome yeast". *Cell Research*, 29:87-89.

Methods

A separate DNA cassette must be created and integrated into each arm of chromosome VII. Once each cassette is in place, DNA recombination can occur between the two cassettes, circularizing the chromosome.



Each cassette contains regions of homology with its specific chromosome arm which allow it to be integrated in the correct place. Each cassette also contains a functional marker gene and one half of the *URA3* gene. The *URA3* gene halves have a region of homology with each other.

Each cassette is integrated into its respective chromosome arm. The full selectable marker genes (*HIS3* and *LEU2*) allow for selection of yeast with one or both cassettes inserted.

DNA recombination occurs between the homologous regions of the *URA3* gene, circularizing the chromosome. The cassettes are positioned so that no functional genes will be lost on the new chromosome.

The circularized chromosome now has a functional *URA3* gene. Any colonies with circular chromosomes will survive on a –uracil media.

Current Results

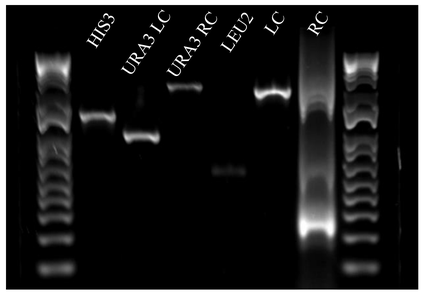
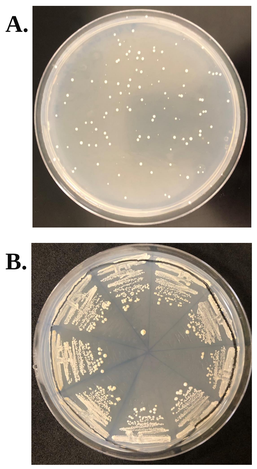


Figure 1. PCR amplification of cassette pieces and whole cassettes. Each cassette is made of two separate halves joined together in a 2nd round of overlap PCR. Products were separated on a 1% agarose gel and are all expected sizes.



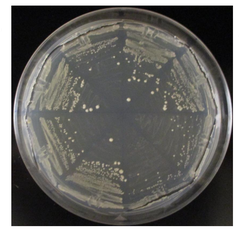


Figure 2. Integration candidate colonies. A. Transformed left cassette integration colonies, growing on a –histidine media. **B.** Replated right cassette integration candidates, growing on a –leucine media. **C.** Replated double integrant candidates growing on –histidine/-leucine

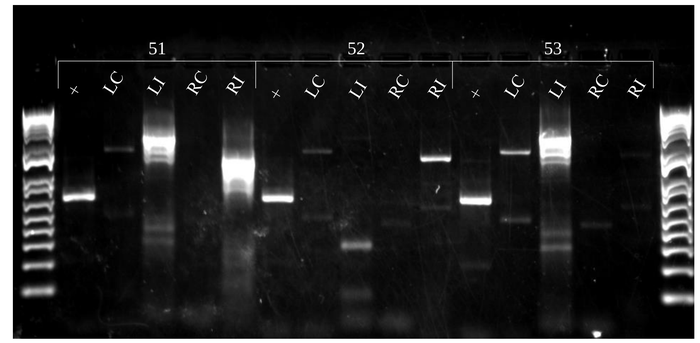
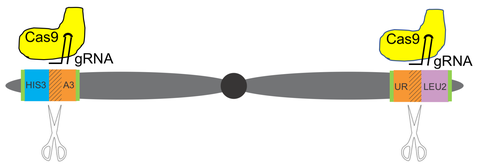


Figure 3. Integration conformation. PCR conformation of left and right cassette presence and integration in colonies 51, 52 and 53. Left to right for each sample: + control, left presence, left integration, right presence, right integration. Products are expected size except all left integration and 53's right integration. 1% agarose gel.

CRISPR Techniques



We are currently using a modified CRISPR plasmid to cause double stranded DNA breaks in the *URA3* regions of the cassettes. With more double stranded breaks, more recombination is likely to take place and more circular chromosome colonies are likely to develop.

Current Progress

So far in our work to circularize chromosome VII in *S. cerevisiae*, we have successfully created both DNA cassettes and integrated them into 3 separate yeast colonies. We are currently troubleshooting the conformation of the colonies left cassette integration PCR and using CRISPR to encourage double strand DNA breaks and recombination between the cassettes. We are plating cells on to –uracil media to select for potential circularized chromosomes.