Circularization of linear chromosomes and telomerase RNA gain-of-function mutations in *Saccharomyces cerevisiae*

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Abstract
Telomeres are repeated DNA sequences located at the ends of linear eukaryotic chromosomes. While they act as protective caps, they cannot be fully copied by the DNA replication machinery. To overcome this end-replication problem, most eukaryotic organisms express the ribonucleoprotein enzyme complex telomerase. I will present preliminary results investigating basic, broad questions of telomere and telomerase evolution in the budding yeast, *Saccharomyces cerevisiae*. First, we are genetically engineering strains with circularized versions of each of the 16 chromosomes to assess the viability and potential fitness effects on the organism. Second, we are screening for gain-of-function mutations in telomerase RNA to gain further insights into how this component contributes to enzyme function.

Background
Lagging strand telomeres are not fully replicated. In most eukaryotes, the ribonucleoprotein complex telomerase extends the 3’ overhang to counteract telomere shortening. Telomerase minimally requires an RNA (TLC1) to provide a template and a reverse transcriptase (TERT, Est2p).

Screening for gain-of-function mutations in telomerase RNA
Telomerase RNAs are evolving incredibly rapidly

Approach: use the telomere position effect (TPE) to select for lengthened telomeres
Pre-shortened telomeres
Decreased silencing of URA3
Decreased survival on 5-FOA

Extended telomeres
Increased silencing of URA3
Increased survival on 5-FOA

Preliminary Data: a known gain-of-function TLC1 allele shows >40-fold increase in growth
Yeast containing either WT TLC1, Dtlc1, or a triple-Ku gain-of-function allele were grown in strain TCy43. Equal ODs of cells were plated on both rich media and counter-selective media containing 5-FOA. The proportion of cells that were able to survive on media containing 5-FOA is shown.

Circularizing linear chromosomes

Long-term goals

Overview of genetic engineering approach
DNA cassettes containing a selectable marker (HIS3 or LEU2) and half of the URA3 selectable marker will be inserted into the left and right arms of a chromosome. Genetic recombination between the URA3 regions will create a circular chromosome that reconstitutes the URA3 gene function, allowing for selection of circularized chromosomes.

Design of cassettes to circularize Ch. III

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