Telomere and telomerase evolution in *Saccharomyces cerevisiae*

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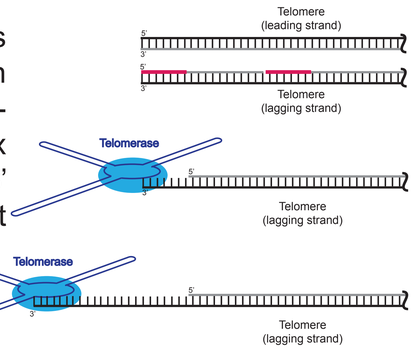
Abstract

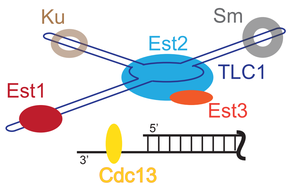
Telomeres, composed of repetitive DNA sequences at the termini of linear chromosomes, serve as protective caps. However, telomeres cannot be fully replicated without the ribonucleoprotein enzyme telomerase. Understanding the balance between telomere length and telomerase activity has important implications for human health concerns: aging and cancer. Telomeres shorten with age, acting as a "biological clock" limiting the rounds of division a cell can undergo. To circumvent this proliferative limit, >85% of cancers aberrantly up-regulate telomerase expression. The goal of our work is to understand the evolution of telomeres and telomerase, and how these contribute to the health and lifespan of an organism.

To address these questions, two projects are underway. First, we are screening for gain-of-function mutations in telomerase RNA to identify novel alleles that lengthen telomeres. These mutants will shed light on how the RNA component of telomerase contributes to enzyme action. We are currently optimizing our selection strategy and creating a library of mutations. Second, we are genetically engineering yeast to circularize each of their 16 linear chromosomes, allowing experimental investigation of the advantages and disadvantages of circular chromosomes in a eukaryotic organism. We have built DNA cassettes with selectable markers and inserted these into the ends of four different chromosomes. We are currently selecting for recombination events that generate circular chromosomes. These novel yeast strains will build a foundation for creating an innovative eukaryotic organism with all linear chromosomes circularized, allowing experimental exploration of telomere and telomerase evolution.

Background

Lagging strand telomeres are not fully replicated. In most eukaryotes, the ribo-nucleoprotein 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).

Acknowledgements

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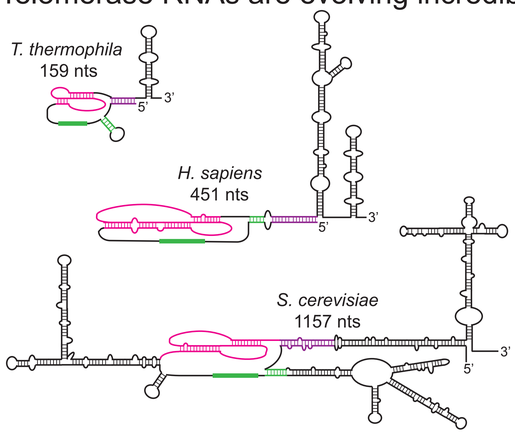
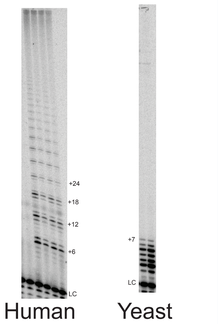
Screening for gain-of-function mutations in telomerase RNA

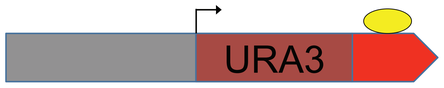
Telomerase RNAs are evolving incredibly rapidly



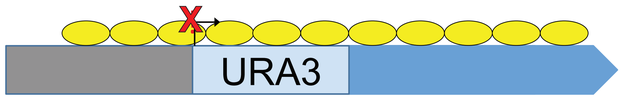


S. cerevisiae has relatively weak *in vitro* telomerase activity

Approach: use the telomere position effect (TPE) to select for lengthened telomeres



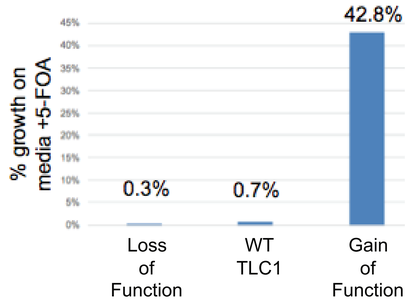
Pre-shortened telomeres
Decreased silencing of *URA3*
Decreased survival on 5-FOA



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

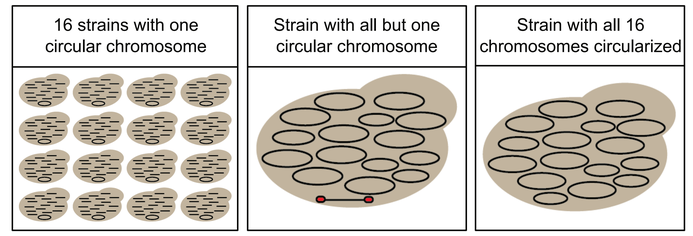
Preliminary Data: a known gain-of-function TLC1 allele shows >40-fold increase in growth

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.

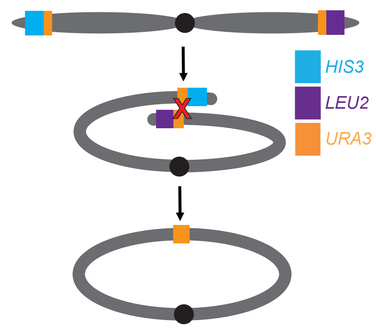


Circularizing linear chromosomes

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