Telomerase Activity Enhancement in *Saccharomyces cerevisiae*

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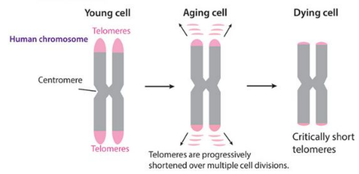
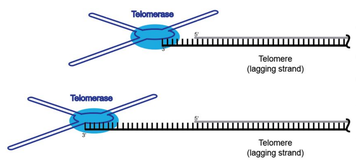
Abstract

Telomeres are located at the ends of eukaryotic linear chromosomes and are composed of repeated nucleotide sequences. One of their main functions is to protect chromosomal ends from being damaged. Telomeres cannot be completely copied during DNA replication so they gradually shorten during each replication cycle in what is known as the “end replication problem”. To counteract this problem, the RNA dependent enzyme complex telomerase works to extend telomeres and help protect the ends of chromosomes. Telomeres and the telomerase enzyme are heavily involved in the aging process and cancer progression. Telomeres gradually shorten with age and eventually become so short that cells begin to senese and undergo apoptosis. Most cancers avoid senescence and apoptosis by activating telomerase at an excessive rate to reduce telomere shortening. The structure and function of telomerase RNA is not well understood. Most past research has focused on identifying loss-of-function mutations rather than identifying gain-of-function mutations. We set out to identify gain-of-function mutations to help us learn more about telomere length and telomerase and how they relate to aging and cancer. We screened nearly 10,000 colonies and identified 32 possible gain-of-function candidates that we are currently in the process of verifying.We used *Saccharomyces cerevisiae* as the model organism in our gain-of-function genetic screen to identify mutations that lengthen telomeres by increasing telomerase activity. The gain-of-function mutants identified through our screen will further enhance our understanding of how significant increasing telomerase activity could be to human health as a whole.

Background

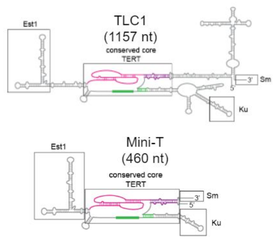


Telomeres are located at the ends of chromosomes to protect them; however, they shorten as we age since they aren’t conserved during DNA replication. The shortening of telomeres through many cycles of DNA replication is known as the “end replication problem”.



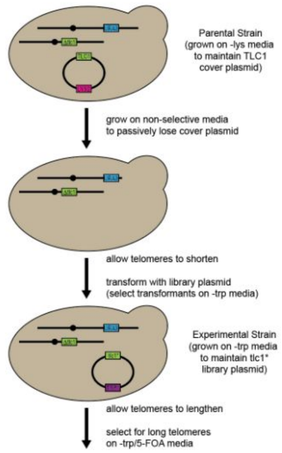
The telomerase enzyme complex counteracts the end replication problem by preserving the length of telomeres.

In our lab we use Mini-T(460) mutant plasmids, a shortened version of TLC1, to screen for gain of function mutations. While Mini-T (460) is less than half the size of TLC1, it still contains all the necessary components needed to maintain stable telomeres and appropriate biological function

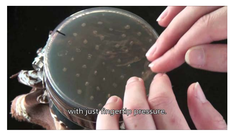


Genetic Screen for Gain of Function Alleles

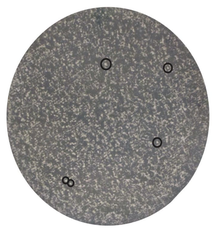
Step 1: Transform yeast with mutant library plasmid



Step 2: Grow transformed yeast on selective media for several generations using replica plating to allow changes in telomere length to occur

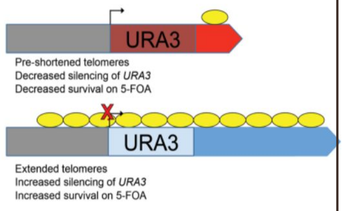


Step 3: Replica Plate onto Media Containing 5-FOA to select for yeast with lengthened telomeres and identify potential gain of function mutations



Potential gain of function candidates are selected by choosing larger colonies after growth on -trpFOA plates.

The URA3 gene can be used to select for longer telomeres and gain of function mutations using the telomere positioning effect (TPE)



Step 4: Rescue gain of function mutant plasmids from yeast and sequence the plasmids to determine positions of possible gain of function mutations

```
MPN86       CTTATCGTTAACTCTGGAAAAAGGAACATGAGTATATATAGAAATGGTTCATTCTAGTTT  420
MINI-T(460) CTTATCGTTAACTCTGGAAAAAGGAACATGAGTATATATAGAAATGGTTTATTCTAGTTT  307
            ************************************************** T297C

MPN86       TTTCCGTTTTTTCTGTAGATTTTTGCCTTTAAAAGAATAAATCCCACTACAAAAAGGTAA  480
MINI-T(460) TTTCCGTTTTTTCAGTAGATTTTTGCCTTTAAAAGAATAAATCCCACTACAAAAAGGTAA  367
            ********** A321T ************************************************
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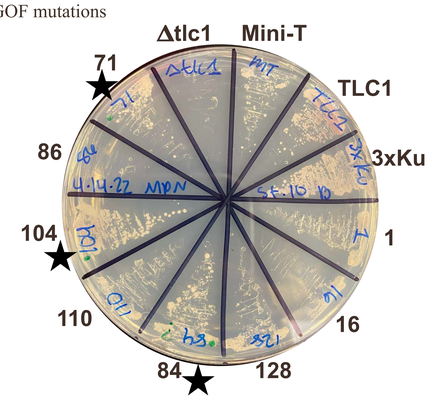
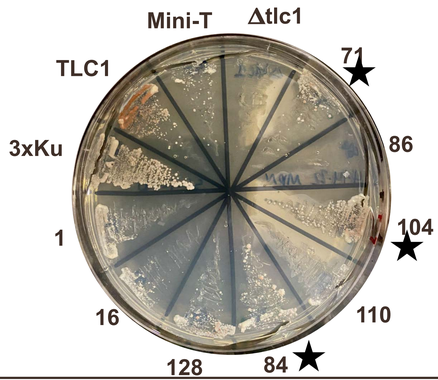
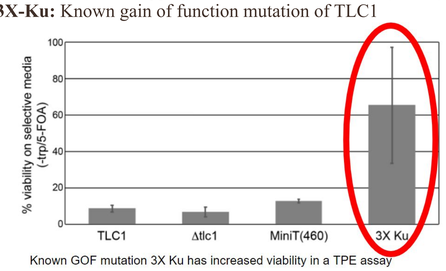
Step 5: Retransform plasmids into fresh yeast and repeat growth on 5-FOA to confirm GOF mutations



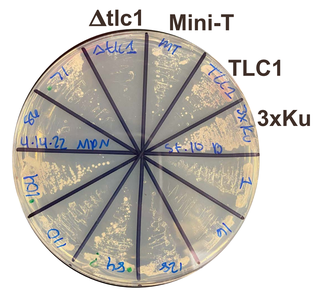


Controls Used During Growth on 5-FOA

1. **TLC1:**Full length wild type yeast strain (1157 nt)
2. **Δtlc1:**Complete Loss of Function mutation that eventually undergo senescence
3. **Mini-T(460):** Wild type yeast strain used in our lab (460 nt)
4. **3X-Ku:** Known gain of function mutation of TLC1



Known GOF mutation 3X Ku has increased viability in a TPE assay



Results

- Nearly 10,000 individual yeast colonies screened
- 32 possible gain of function candidates identified and sequenced
- Currently confirming gain of function candidates by transforming into fresh competent yeast cells

Mutant	GOF phenotype	Weak phenotype	Mutations
51	3 plates	1 plate	Weak sequence
68	3 plates	1 plate	G49A, A163T, A275G, T391A, T416C
71	3 plates	1 plate	T221A, T233G, T252C, G254T
84	2 plates	2 plates	T45A
104	4 plates	0 plates	Weak sequence
119	2 plates	2 plates	C36A, A87G, A121C,A124C,A133C
121	3 plates	1 plate	Weak sequence
128	3 plates	1 plate	A76T, C228T, G387A
160	3 plates	1 plate	Weak sequence

Each mutant was struck in quadruplicate to have 4 colonies to compare when confirming gain of function phenotype

Future Plans

- Continue sequencing and verifying gain of function candidates
- Identify causative gain of function mutations from gain of function candidates with multiple mutations
- Map gain of function alleles onto telomerase RNA secondary structure model to look for trends
- Determine the mechanism behind gain of function mutations

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Since some gain of function candidates have more than one mutation, we need to determine with specific mutations are truly gain of function

Acknowledgments

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