Genetically Screening for Gain-of-Function Mutations in Telomerase RNA

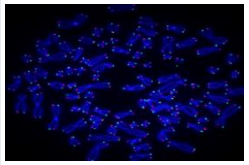
Jose Childers and Dr. Melissa Mefford
Morehead State University, College of Science, Department of Biology and Chemistry
Morehead, KY 40351

ABSTRACT

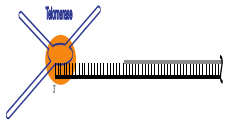
Telomeres are DNA structures located at the ends of linear chromosomes. They function, in part, to allow DNA ends to be copied before cells divide. Since the standard DNA replication machinery can't fully copy telomeres, most eukaryotic cells require the vital ribonucleoprotein enzyme telomerase. Telomerase is composed of a TERT (telomerase reverse transcriptase protein) and telomerase RNA. As we age, telomeres shorten since most human cells do not express telomerase. If telomeres grow too short, it triggers a cell-cycle arrest known as senescence, can ultimately result in cell death. On the other hand, >85% of human cancers show over-expression of telomerase, which is required for the uncontrollable cell division that is a hallmark of this disease. Interestingly, despite the importance of telomerase RNA, its structure and function is not well understood. To shed light on the correlation between telomere length and telomerase RNA structure, we devised a genetic screening strategy using the yeast *Saccharomyces cerevisiae* to identify novel gain-of-function (GOF) mutations in telomerase RNA. First, we transform a library of random telomerase RNA mutant plasmids into yeast. Then, we select for yeast that appear to have longer telomeres using a selectable marker in a sub-telomeric region. Finally, we rescue the plasmids and send them for sequencing to determine the mutations. To date, I have screened ~1000 colonies and identified ~11 putative GOF alleles. I am currently working to identify the mutations present in the putative GOF alleles. Identification of more active versions of telomerase RNA could ultimately lead to an understanding of enzyme function that lengthens telomeres and decreases the rate of aging.

Background

Telomeres are repetitive DNA sequences at the end of chromosomes.(In pink)



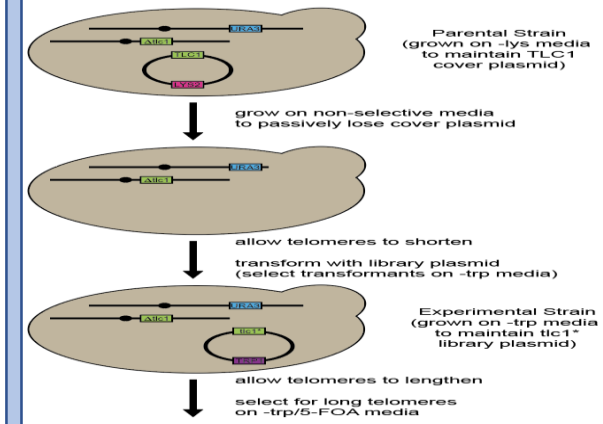
Telomerase is the ribonucleoprotein enzyme that helps maintain telomere length. It is composed of TERT and telomerase.



*We are interested on the changes that telomerase RNA has on enzyme functions

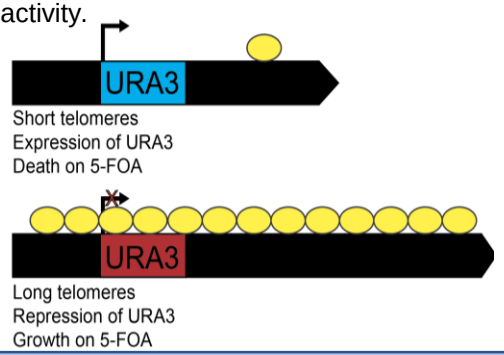
Plasmids, Strain and Yeast Plasmid Shuffle

We start with TLC1 on a LYS2 cover plasmid, then grow the yeast on a non-selective media to lose the LYS2 cover plasmid. Then we transform the TLC1 mutant library plasmid with a TRP1 selectable marker.



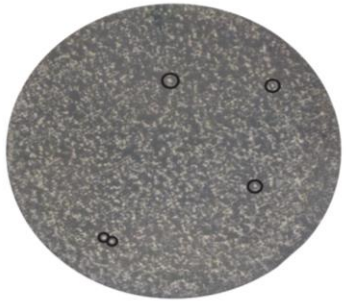
Telomere Position Effect (TPE)

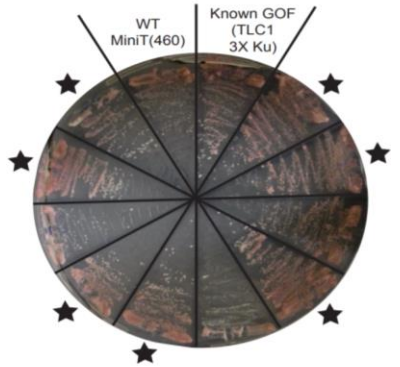
Silencing of genes near the telomeres indicates longer telomeres, thus an increase in telomerase activity.



Replica Plate

The circled colonies indicate possible GOF mutants due to their increased size.





Streaking Plate

This shows that the colonies that grew on the replica plate are indeed GOF mutants due to growth on 5-FOA.

Preliminary Conclusion

Using the genetic screening strategy, I have:

- Screened ~ 1,000 colonies
- Identified 11 putative GOF mutants
- We composed the first Novel of Genetic Screen of GOF mutations

Current Work

- Rescuing GOF plasmids from yeast
- Sequencing telomerase RNA gene

Long- Term Goals

Identification of more active versions of telomerase RNA could ultimately lead to an understanding of enzyme function that lengthens telomeres and decreases the rate of aging.

Hypothesized function	Experimental Approach
Increased enzyme activity	*In vitro* telomerase assay
Change in RNA structure	Compensatory analysis
Increased RNA abundance	qRT-PCR
Increased repeat addition processivity	Two template assay
Increased protein binding	CARRY-Y2H
Increased telomere recruitment	CHIP, Est2-Cdc13 fusion

Acknowledgements

This work was supported by a New Faculty Start-up Award and Investigator Development Award to Dr. Melissa Mefford from KY INBRE (NIGMS 8P20GM103436), as well as Start-up Funds from Morehead State University. Thank you to the Cech and Zappulla Labs for providing yeast strains and plasmids.