EVOLVING A MORE ACTIVE TELOMERASE ENZYME IN *SACCHAROMYCES CEREVISIAE*

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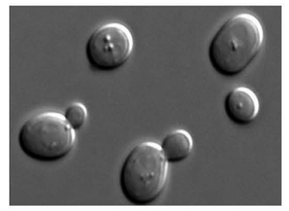
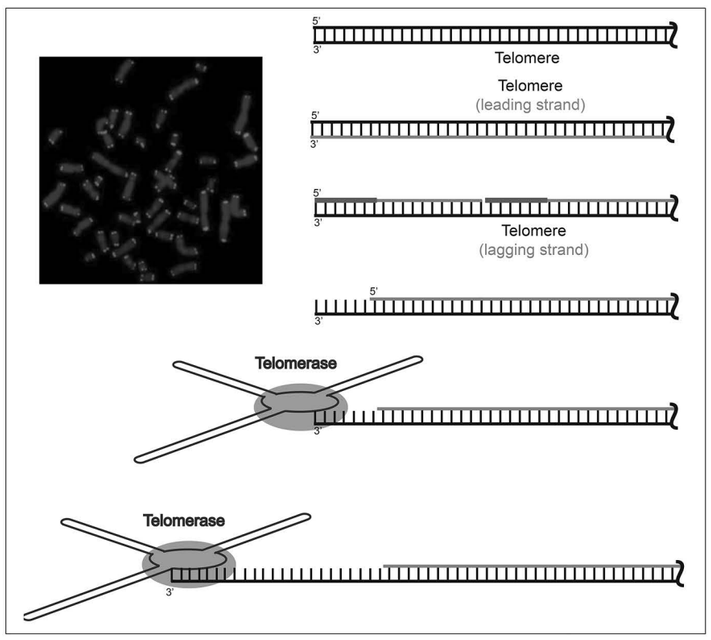


ABSTRACT

Telomeres are repetitive DNA sequences found at the ends of linear chromosomes in eukaryotic organisms ranging from yeast to humans. Though telomeres act to protect the end, they cannot be fully copied by the DNA replication machinery. This “end replication problem” is overcome by a telomere-lengthening enzyme called telomerase. Telomerase is minimally composed of a non-coding telomerase RNA and a reverse transcriptase protein. Without telomerase, telomeres shorten over time, eventually causing cells to senesce and contributing to cellular aging. On the other hand, more than 85% of human cancers overexpress telomerase to support the uncontrolled cell division characteristic of this disease. In order to better understand how the telomerase enzyme works, we are screening a library of telomerase RNA mutations for gain-of-function alleles that increase the activity of telomerase. This screen utilizes a counter-selectable marker located in the telomeric region that exhibits the telomere position effect (TPE). With TPE, longer telomeres increase silencing of genes near telomeres allowing us to select yeast that have more active telomerase. Our results will shed light on how the structure of telomerase RNA contributes to enzyme function. Ultimately, being able to create more active telomerase that lengthens telomeres could be used to slow aging or treat diseases of pre-mature aging.

WHAT ARE TELOMERES?

Telomeres are found on the ends of linear eukaryotic chromosomes, from organisms including budding yeast to humans. Telomeres are specialized repeats of DNA that protect these ends of chromosomes by capping the ends like the aglets on shoelaces. Over time, telomeres shorten, contributing to aging due to the ”end replication problem.”

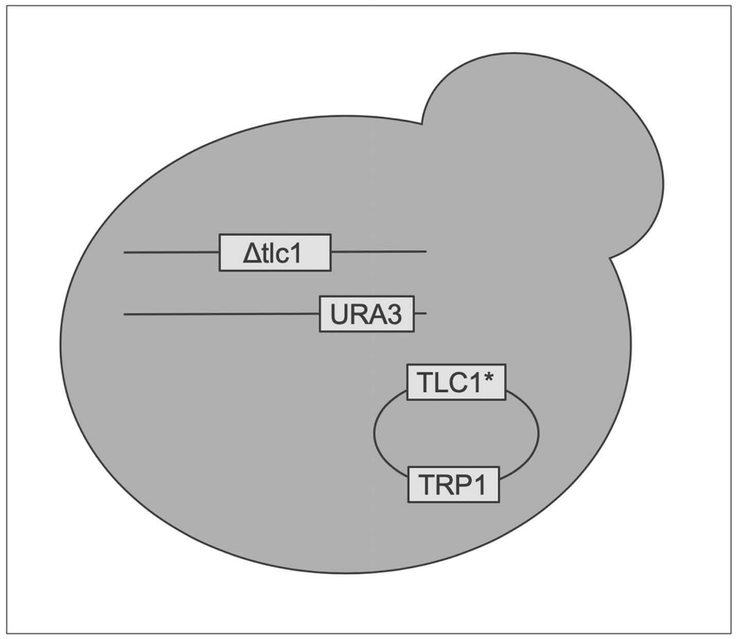


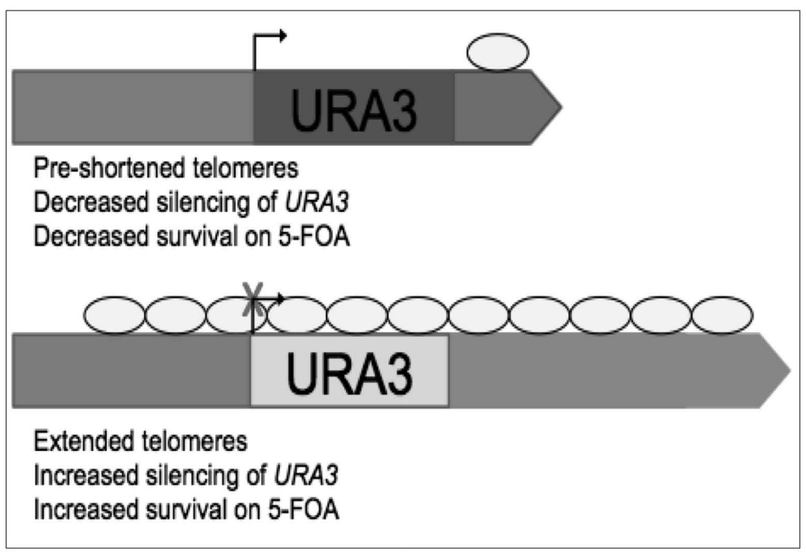
WHAT IS TELOMERASE?

In most eukaryotic organisms, the “end replication problem” is overcome by the enzyme telomerase. Telomerase is made up of a special non-coding telomerase RNA and a reverse transcriptase protein. In the absence of telomerase, telomeres gradually shorten, which will cause senescence. Telomerase has also been linked with human cancers. Over 85% of cancers exhibit an overexpression of telomerase, which supports cancer’s hallmark uncontrolled division of cells.

SCREENING PROCESS FOR GAIN-OF-FUNCTION MUTATIONS

We are interested in variants of telomerase with more active function. First, we will create a library of telomerase RNA mutants using error-prone PCR. The products that are gained from the PCR reaction will then be placed into a plasmid. We will then transform a strain of the budding yeast species *S. cerevisiae* with the telomeres RNA mutant library. To identify telomerase RNA mutants that are more active, we will use the telomere position effect (TPE).



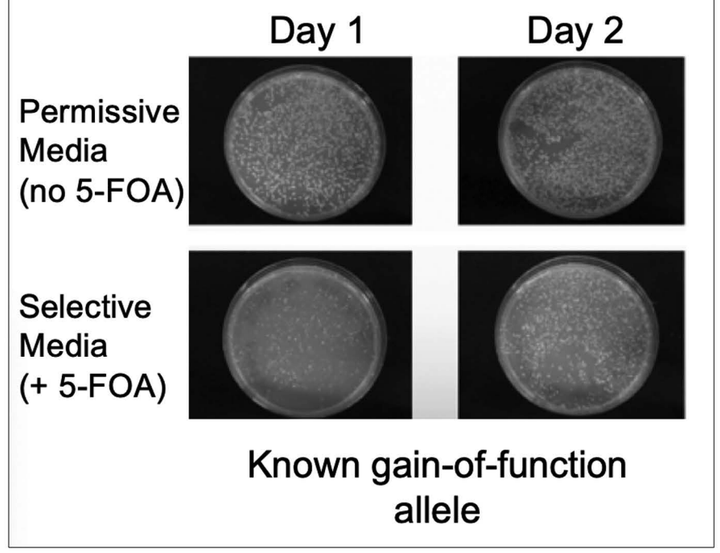


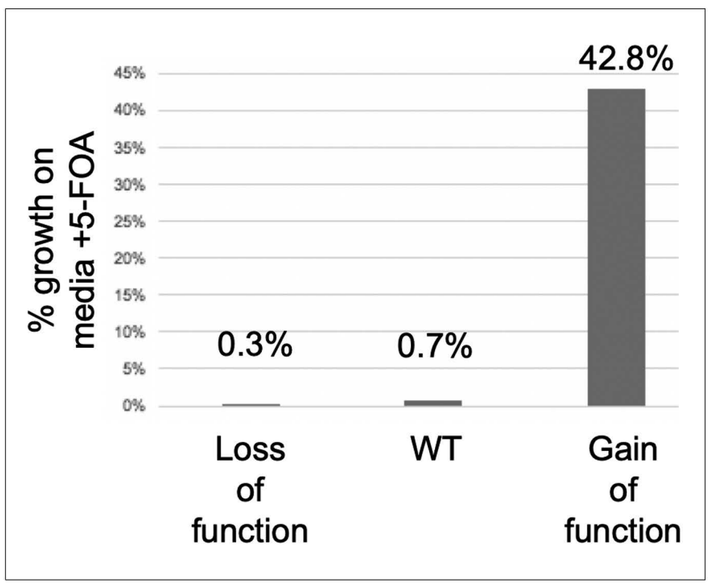
TELOMERE POSITION EFFECT (TPE)

Telomere position effect is a silencing mechanism used by eukaryotes including *S. cerevisiae.* TPE involves formation of highly condensed heterochromatin. This heterochromatin begins at the telomere and spreads inward. Interestingly, the degree of spreading is related to telomere length. Longer telomeres exhibit greater spreading of silencing. We will use the increased silencing at longer telomeres to select for more active telomerase.

PROOF OF PRINCIPLE EXPERIMENT

To test if this novel approach will work, a previous researcher tested a known gain-of-function mutation. After two days of growth on selective media, the gain-of-function allele showed 40-fold increase in survival. These data suggest our approach will successfully select for gain-of-function variants.





FUTURE IMPLICATIONS

The direct goals of this project are to better understand yeast telomerase structure and function. Long-term impacts could lead to creation of more active human telomerase, which could be used to slow aging or treat diseased of pre-mature aging.

ACKNOWLEDGEMENTS

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