Investigation of helicases, exonucleases, and TERRA non-coding RNAs in the maintenance of telomeres in *Saccharomyces cerevisiae*

Taizina Momtareen and Jennifer E.G. Gallagher
Department of Biology, Eberly College of Arts and Sciences, West Virginia University

Abstract

The telomeres of *S. cerevisiae* are coated with a variety of proteins that function in replication, recombination, and providing structural stability to the chromosomal ends. Among these proteins are the helicases Sgs1 and Y'-Help1. Sgs1, the yeast homolog of the bacterial RecQ helicases and the human WRN protein, is important in the maintenance of yeast telomeres. Sgs1 carries out C-strand resection of the 5' telomeric end, producing a G-rich overhang (G-tail) at the 3' end. It works along with the exonucleases Dna2, Exo1, and MRX complex. Close to the telomeres are the subtelomeric regions consisting of X and Y' elements. The YRF1 gene in the Y' elements encode a helicase known as Y'-Help1; however, this helicase is not very well-characterized. Another element of the yeast telomere that demands comprehensive research is the Telomeric Repeat containing RNA (TERRA), that is transcribed directly from the telomeres. Although TERRA RNA has been implicated in several telomere maintenance processes, its function is yet to be discovered. In the absence of telomerase, some *tlc1*Δ cells (Type I and Type II survivors) can utilize recombination-based ALT pathways to extend telomeres. The copy number of Y' elements as well as the levels of Sgs1, Y'-Help1, and TERRA are upregulated in these telomerase mutant cells. Because Y'-Help1 is overexpressed in type I cells and Sgs1 in type II, my hypothesis is that these two helicases carry out similar functions in their respective survivor pathways. Moreover, the presence of subtelomeric sequences in TERRA transcripts, it's implicated role in ALT, and its ability to form DNA: RNA duplexes that are preferentially unwinded by helicases like Sgs1, leads to the proposition that TERRA functions in telomere length maintenance along with Sgs1 and Y'-Help1. The goal is to determine the roles of these three components in the telomere biology.

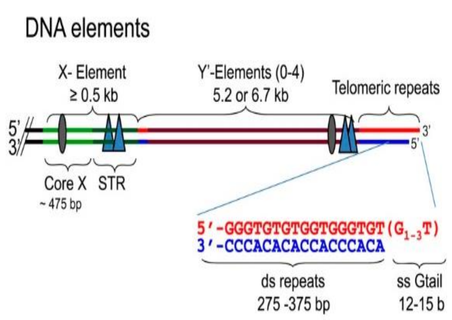
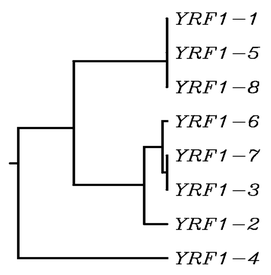


Fig 1: DNA components of a *S. cerevisiae* telomere. (A) X and Y' elements constitute the subtelomeric region upstream of telomere

Subtelomeres have repeat elements X and Y'

- The Y' elements contain the YRF-1 gene that has 8 paralogs, YRF1-1 to YRF1-7. These have high sequence homology and are located in different locations of different chromosomes.
- Y' elements are not essential
- YRF-1 encodes the Y'Help1 helicase which is expressed in telomerase mutant cells (Yamada et. al, 1996)
- Type I survivors: high copy number of Y' subunits;
- Type II survivors: amplified TG_{1-3} repeats.
- Both types require Rad 52 and Pol 32. The telomeres of Type I survivors acquire extra copies of Y' elements by recombination (Chen et. al., 2001).
- The copy number increase possibly prevents chromosomal loss and cell death

Gene	CHROM.	Length (bp)	Orientation
YRF1-1	IV	5391	R
YRF1-2	V	5046	R
YRF1-3	VII	5728	R
YRF1-4	XII	4149	R
YRF1-5	XII	5391	R
YRF1-6	XIV	5728	R
YRF1-7	XVI	5728	L
YRF1-8	XV	5391	L



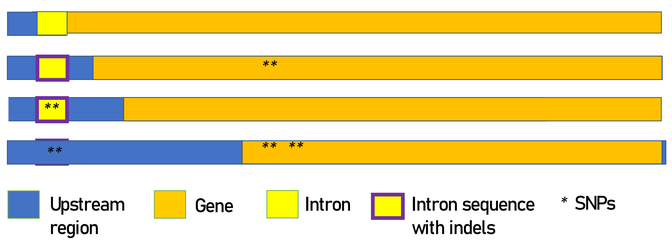


Fig2: Quantitative liquid growth assay of mCherry tagged strains from SWAT genomic overexpression collection and W303 strains with YRF1-4 cloned into the expression vector pESC-URA.

Do Y'-Help1 and Sgs1 play similar roles in recombination?

YRF1-4 was cloned into pESC-URA expression vector and transformed into W303 and W303 *sgs1Δ*. Overexpression was induced under the *GAL* promoter. Because DNA damage and its repair pathways can induce HR, quantitative liquid growth assays were carried out to observe the effect of various DNA damaging agents on these strains and BY4741 strains overexpressing *YRF1-2* from the genome.

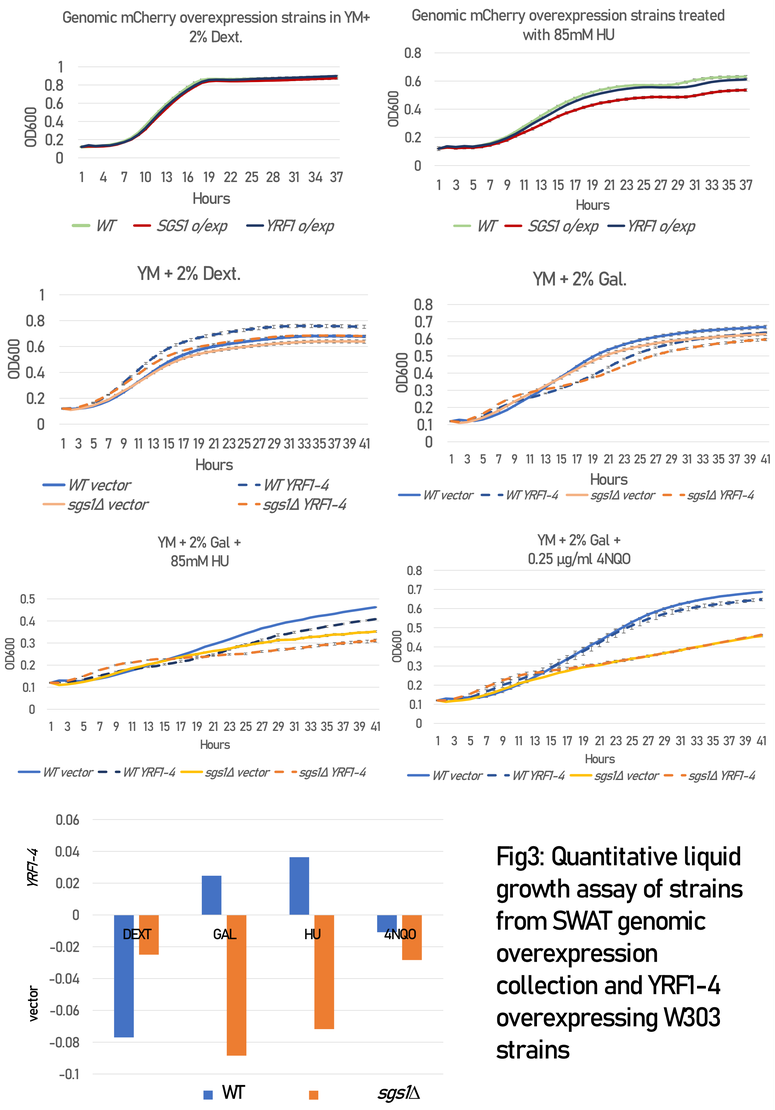


Fig3: Quantitative liquid growth assay of strains from SWAT genomic overexpression collection and YRF1-4 overexpressing W303 strains

1. Y' amplification presumably occurs via recombination to compensate telomere loss
2. Amplified Y' elements produce Y'-Help1 in *Δtlc1* cells, indicating a role in recombination to facilitate Y' amplification
3. Y'-Help1 shares high homology with RecG protein, a helicase having similar function as RecQ helicases

Sgs1: the yeast homolog of the bacterial RecQ helicase and the human WRN protein. Mutation of Sgs1 causes the Werner's syndrome. It is involved in both DSB repair mechanism and telomere lengthening. In telomeres, Sgs1 resection causes G-tail formation which can either bind telomerase or invade strands for homologous recombination (HR). Sgs1 mutants show:

- Slow growth
- Increased mitotic and missegregation
- higher sensitivity to DNA-damaging agents

Conclusion

- Overexpression of *YRF1-4* did not rescue growth.
- The overexpression strains grow very well in dextrose, however in galactose media, once overexpression begins and *YRF1-4* starts to accumulate, growth is arrested.
- Growth arrest in both the WT and *sgs1*Δ mutants; stronger effect in drug treated mutants
- YRF1-4 either not expressed, or not rescuing growth

Future plans

- Western blot and qRT PCR in survivor cells
- Clone the rest of the paralogs into survivor cells
- CRISPR the 8 paralogs from the genome
- Determine if the non-coding RNA TERRA interacts with *YRF* genes
- Determine if the R-loops caused by TERRA can inhibit Y' amplification
- Determine if Sgs1 and Y'-Help1 can unwind DNA:RNA hybrids caused by TERRA

Acknowledgement

Thanks to the Schuldiner Lab for the mCherry-tagged overexpression library used in this project. Thanks to David Lydall and lab for providing W303 *cdc13-1* strains