The effect of Replication Protein A phosphorylation on telomere shortening in *Saccharomyces cerevisiae* using a PCR amplification-based protocol

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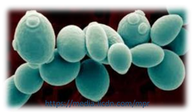
Abstract

Replication Protein A (RPA) is a highly conserved, heterotrimeric, single-stranded DNA binding protein that is essential for many DNA maintenance pathways such as DNA replication, DNA damage repair, cell cycle regulation, and telomere maintenance. RPA is phosphorylated in a cell-cycle dependent manner and in response to DNA damage, suggesting that phosphorylation may play a vital role in regulating its function. We hypothesized that phosphorylation may regulate RPA functions in telomere synthesis, and our lab has previously shown that mutations in RPA that effect phosphorylation cause changes in telomere length in the baker's yeast *Saccharomyces cerevisiae* (*S. cerevisiae*). However, the mechanism for RPA phosphorylation's regulation of telomere synthesis is unclear. As a result, we have been measuring telomere length of various *S. cerevisiae* mutants, including those with phosphorylated and dephosphorylated states of RPA. After isolating genomic DNA from *S. cerevisiae,* telomere lengths were amplified using Endpoint Polymerase Chain Reaction (PCR) and measured through gel electrophoresis. We are currently analyzing the telomere lengths of *S. cerevisiae* mutants and seeing positive and negative effects on telomere length caused by the addition of different mutations. We have confirmed our Endpoint PCR protocol using a control and have been able to verify our protocol in terms of amplifying telomere lengths. By comparing telomere lengths of RPA phosphorylated mutants to known telomere synthesis mutants, we will have a better understanding of the role RPA phosphorylation plays in regulating telomere synthesis. Since the abnormal regulation of telomere synthesis is required for cancer cell formation and cancer progression, our research may provide insights into novel treatment targets for cancer.

Saccharomyces cerevisiae

- Also known as baker's yeast
- Ideal organism for studying and understanding RPA and telomere synthesis
  - RPA that is homologous to humans, making it a model organism
- Entire yeast genome is readily accessible
- Yeast telomeres: ~300 bp of simple repeats
  - $G_{2\text{-}3}/TG_{1\text{-}3}$



Telomeres

- These are simple, non-coding G- rich repeating DNA sequences found at the ends of Chromosomes
- Protect against
  - Non-homologous end joining
  - Folding over → caps
  - Loss of important genes on chromosomes (End Replication Problem)

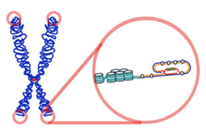


Figure 1: End folding of telomere to protect against non-homologous end-joining

https://upload.wikimedia.org/wikipedia/commons/6/6a/Telomere.png

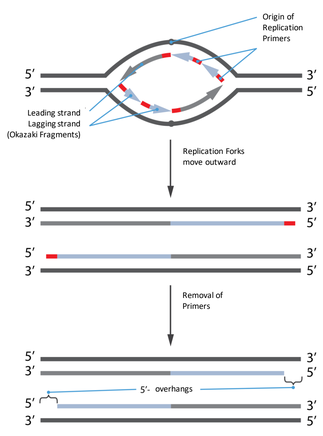


Image Modified From https://commons.wikimedia.org/wiki/File%3ATelomere_shortening.svg

Figure 2: The end replication problem created due to removal of primers during replication. This shows why telomeres are necessary for linear chromosomes

How is this problem solved?

- Once the RNA primer is removed, there is an overhang, and if not fixed, chromosomes will continue to shorten significantly with each round of cell division
- In actively proliferating cells, the telomere synthesis pathway is activated, and telomerase adds repeats to the ends of telomeres
- Telomere replication is both cell cycle and developmentally regulated, and its control is likely to be complex.
- Telomere loss causes the kinds of chromosomal changes associated with cancer, and telomere synthesis is abnormally regulated in cancer cells.

Replication Protein A (RPA)

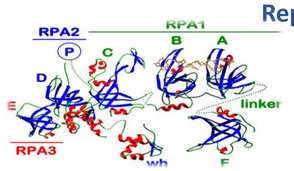
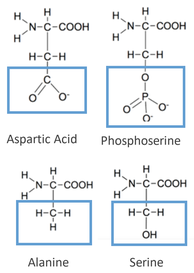


Figure 3: Replication Protein A
Haring, S.J., Mason, A.C., Binz, S.K. and Wold, M.S. 2008



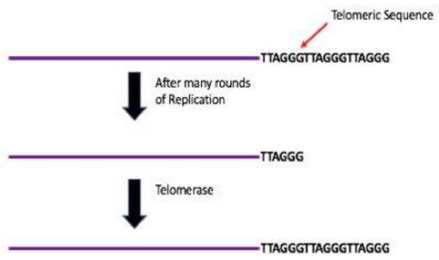
- Highly conserved, heterotrimeric single stranded DNA binding protein involved in:
  - DNA replication, cell cycle regulation, and DNA repair
  - Involved in telomere maintenance
- 3 subunits
- Focus on 2nd subunit
- Phosphorylation affects function

Phosphorylation of RPA *in vitro*

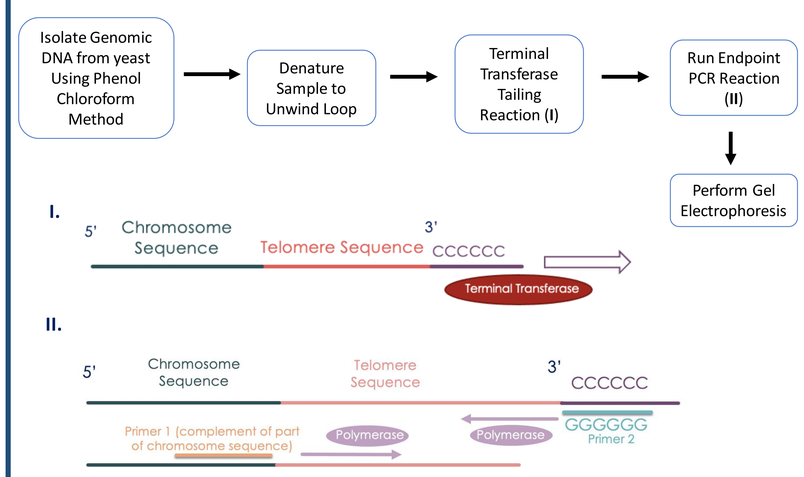
- Phosphomimetics
- *Rfa2-asp*
  - Acts phosphorylated
- *Rfa2-ala*
  - Acts dephosphorylated

Telomerase

- RNA-dependent DNA polymerase
- Reverse transcriptase
- Lengthens telomeres
  - Enzyme binds to a special RNA molecule that contains a sequence complementary to the telomeric repeat, then extends the overhanging strand of the telomere DNA using complementary RNA as a template
  - Next, a normal DNA polymerase and RNA primer come in and fill in the rest
- Active in cancer cells



Methods



Results

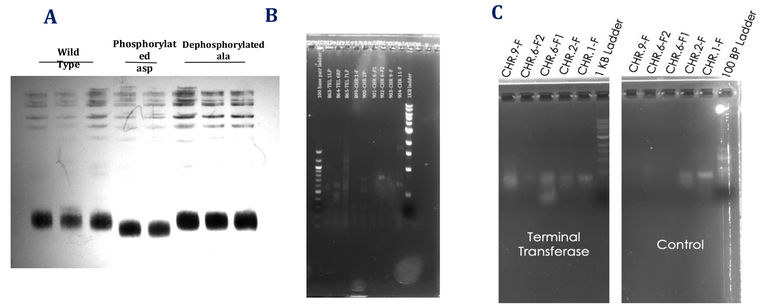


Figure 4: A) Southern blot analysis run on WT and phosphorylated RPA as well as unphosphorylated RPA strain showing phosphorylated strains have shorter telomeres B) Terminal Transferase Method for AWY 28 shows telomeres about 300-700 BP C) Terminal Transferase method with control for AWY 28 shows telomeres around 500 BP for Terminal Transferase method and mostly no bands in control (non-tailed DNA)

Strain name:

- AWY 28: JKM139 Mat a (WT)
- AWY 29: JKM179 Mat alpha (WT)
- AWY 31: JKM139 *ku70::KAN* Mat a
- AWY 32: JKM179 *ku70::KAN* Mat alpha

- Southern Blot confirms that wild type telomeres and dephosphorylated RPA show longer telomeres than phosphorylated RPA strains, which have shorter telomeres

Analysis of Results

- Previous Southern blot analyses confirmed that *rfa2-asp* telomeres were shorter than *rfa2-ala*
- PCR method confirmed using control (non-tailed DNA)
- RPA phosphorylation plays an important role in regulating telomere synthesis
- Through further examination of additional telomere mutants, we will be able to give more compelling insights into regulation of telomeres in both yeast and humans which can be used to study how we can regulate telomeres of cancerous cells

Future Direction

- Continue PCR method on strains involving phosphorylated RPA, and dephosphorylated RPA
- Perform another more precise tailing method (RNA Ligase method) and compare to current methods
- Confirm more results with southern blot method.
- Introduce potential pathway for RPA involvement in Telomere synthesis pathway

References

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