# Rif2 Protein, Product of Subfunctionalization or Neofunctionalization

Shawn Gibson, Theresa Rubi, Laura Rusche

State University of New York at Buffalo, Department of Biological Sciences, Amherst, NY 14260

## Abstract

The telomere is a region of repetitive DNA that conserves the integrity of chromosomal ends. It is essential to understand the telomere because it dictates the cell's viability. Rap1, a regulatory protein involved in telomere length, recruits Rif2 proteins. The Rif2 protein in *Saccharomyces cerevisiae* protects telomeres by blocking the kinase Tel1, thereby inhibiting telomere elongation. Comparative genome analyses reveal that Rif2 proteins descended from the replication protein Orc4. We wanted to understand how a telomere-binding protein evolved from a replication protein. Specifically, we wanted to distinguish whether the Rif2 protein is a product of subfunctionalization or neofunctionalization. To do so, we used Chromatin Immunoprecipation (ChIP) to determine whether the non-duplicated Orc4 protein from *Kluyveromyces lactis* is associated with telomeres. Our results show that KlOrc4 does not associate with telomeres. Therefore, the Rif2 protein may have evolved this trait after duplication and therefore may be a product of neofunctionalization.

## Background

- In the yeast species *Saccharomyces cerevisiae*, the Rif2 protein protects telomere ends.
- Sequence studies[2] shown that Rif2 proteins evolved from Orc4, which is an initiator of DNA Replication.
- We want to determine the divergence of Orc4 and Rif2 in the yeast species *K.lactis.*

**Differences between Subfunctionalization and Neofunctionalization**

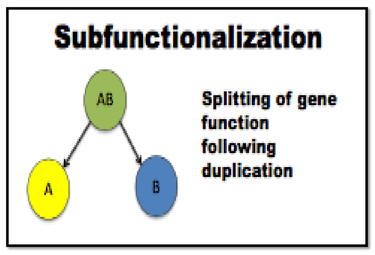


*Fig.1: Subfunctionalization Divergence[3]*

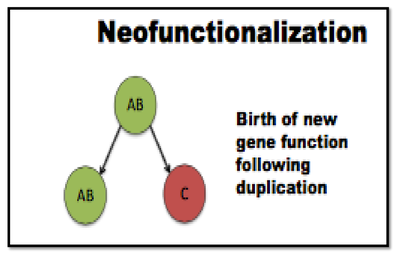


*Fig.2: Neofunctionalization Divergence[4]*

- If Orc4 shows a large enrichment at the telomeres, then Rif2 is a product of **subfunctionalization**
- Alternatively, if Orc4 does not show a large enrichment at the telomeres, then Rif2 is a product of **neofunctionalization.**

**Correlation to this project**

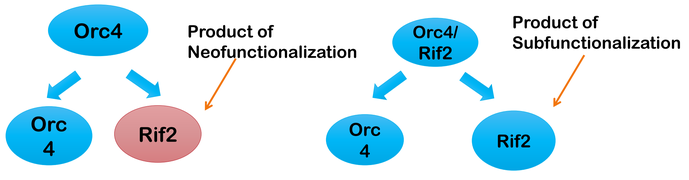


*Fig.3: Rif2 as Neofunctionalization*

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*Fig.4: Rif2 as Subfunctionalization*

## Hypothesis

We hypothesize that the Rif2 protein is an example of subfunctionalization in the non-duplicated yeast species *Kluyveromyces lactis.* Neofunctionalization is rare simply because few mutations confer new properties on a protein.

## Methods and Materials

We used Chromatin Immunoprecipation (ChIP) to discover whether or not Orc4 is located at the telomeres. We then analyzed our ChIP samples using qPCR (Quantitative PCR) . The following is a overview of the ChIP phases, pictures taken in the laboratory and the steps leading up to our analysis.

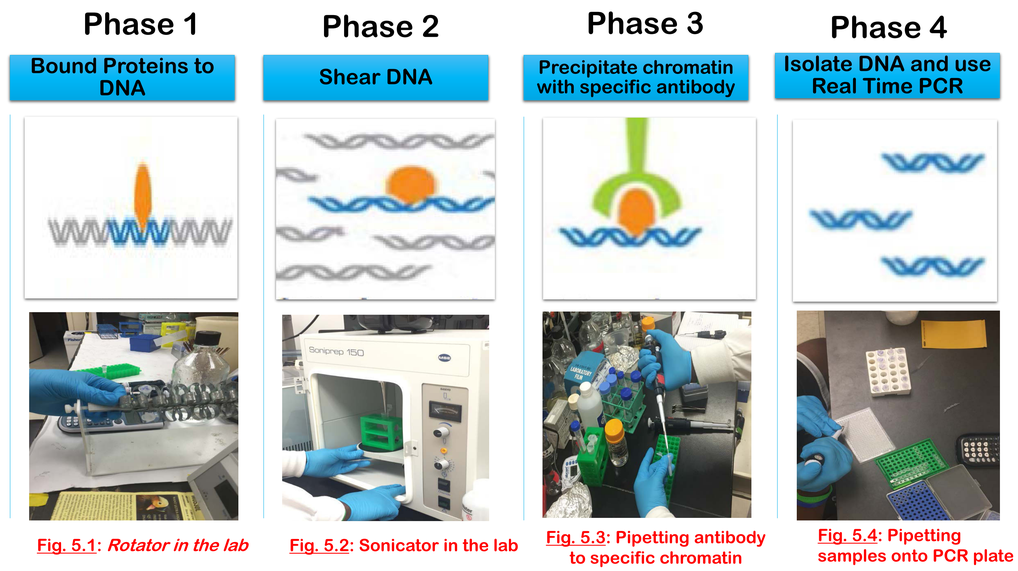



Fig. 5.1: *Rotator in the lab*

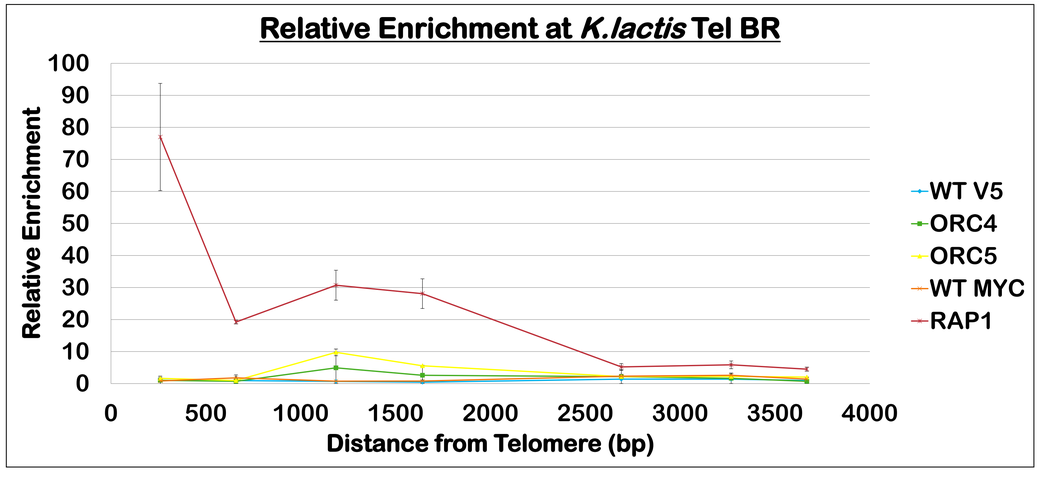
Fig. 5.2: Sonicator in the lab

Fig. 5.3: Pipetting antibody to specific chromatin

Fig. 5.4: Pipetting samples onto PCR plate

*Fig. 5: Chromatin Immunoprecipation Phases[4].* ChIP is a procedure used to determine whether a protein binds to a specific DNA sequence

## Results



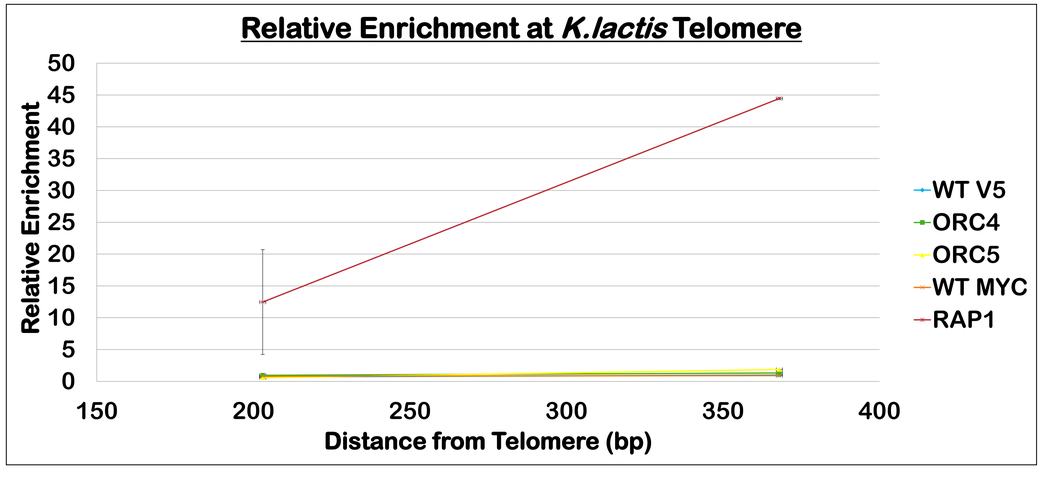




*Fig. 6 and 7: ChIP Analysis of Orc4 and Rap1 at telomeres:* ***Y- axis error bars are present for a clarified analysis.***

## Results (cont'd)

- Orc4 appears to have very little enrichment near the telomere. This is shown in both Figures 6 and 7.
- A total of nine primers were used in this experiment. Seven of the nine primers were found on one telomere, while the remaining two primers were found on another telomere.
- The relationship between ORC4 and Rap1 is important to emphasize on because according to Figures 6 and 7 we don't observe a similar pattern amongst the two proteins.
- With that being said, the data presented here suggests Rif2 function as a product of neofunctionalization.

## Conclusion

- Based on our results, we can conclude that our hypothesis was incorrect. The ChIP analysis in the results section support the idea that the Orc4 protein does not share any telomere-like function. By not observing an ample amount of Orc4 DNA enrichment near the telomere, these results are indicative to the fact that the Rif2 protein is an example of neofunctionalization and that the Rif2 protein may have evolved this trait after duplication.
- Understanding how new protein function evolves is the reasoning behind this research project. We want to understand how new complexities evolve. If it turned that Rif2 was a product of subfunctionalization, then Orc4 would play an important role in telomeres in many organisms. This project could broaden knowledge related to telomere biology. We want to understand telomeres work because they dictate the cell's lifespan.

## Refrences

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5. Rada-Iglesias, A. *et al.* Binding sites for metabolic disease related transcription factors inferred at base pair resolution by chromatin immunoprecipitation and genomic microarrays. *Hum. Mol. Genet.* **14**, 3435–3447

Additional questions? Contact Shawn Gibson or Theresa Rubi at
**E-mail: shawngib@buffalo.edu or jtheresa@buffalo.edu**