

Telomere binding protein Rif2 as a product of subfunctionalization or neofunctionalization following the whole genome duplication in yeast species

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Abstract

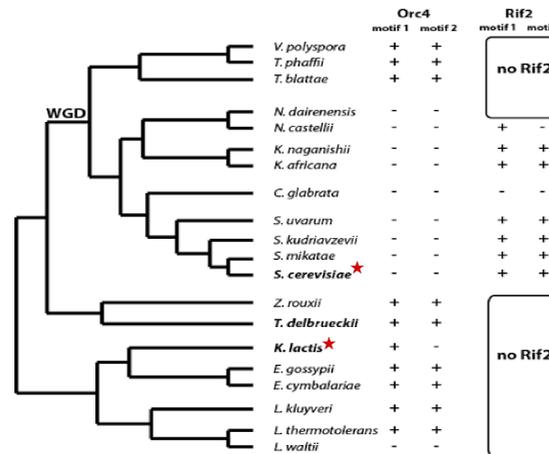
In the yeast species *Saccharomyces cerevisiae*, the Rif2 protein serves to protect telomere ends. Rif2 associates with Rap1, which binds directly to telomeres, to inhibit telomere lengthening by telomerase. Sequence studies have shown that Rif2 proteins descended from replication protein Orc4 as a result of a gene duplication. This raises the question as to how a telomere protein evolved from a replication protein. Our hypothesis is that Rif2 is a product of subfunctionalization, meaning that the duplicated Orc4 resulted in two genes, each retaining a different portion of the original function. To test this hypothesis, Orc4 telomeric association was assessed through chromatin immunoprecipitation (ChIP) in the pre-duplicated species *Kluyveromyces lactis*. Our results show that Orc4 does not associate with telomeres, and thus does not share the Rif2-like function. This observation suggests that the function of Rif2 evolved after the duplication event, making it a product of neofunctionalization.

Methods

- Orc4 and Rif2 are paralogs
- No Rif2 observed in pre-duplicated yeast species
- K. lactis* chosen as model organism

Purpose

Determine if Orc4 in pre-duplicated yeast species has similar function to Rif2 in duplicated species by observing association of Orc4 with Rap1 at the telomeres.



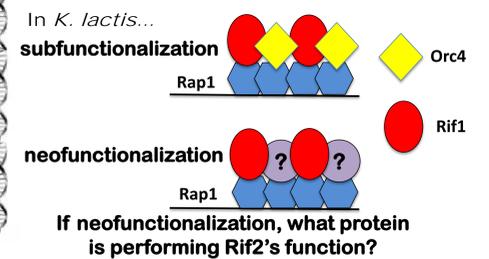
Chromatin Immunoprecipitation

- Cross-link bound proteins to DNA
- Isolate chromatin and shear DNA
- Precipitate chromatin with protein-specific antibody
- Isolate target DNA and quantify by PCR

	Tagged	Transformed
Subtelomere indicators		
ORC4::V5	?	✓
RAP1::MYC	✓	✓
RIF1::V5	?	?
Heterochromatin indicators		
ORC1::V5	✓	✓
SIR4::V5	✓	✓
Origin indicators		
ORC3::V5	✓	?
ORC5::V5	✓	✓
CDC6::MYC	✓	?

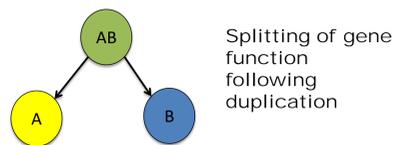
Red - what we did
Black - what was already prepared

Both Orc5 and Rap1 successfully tagged and transformed

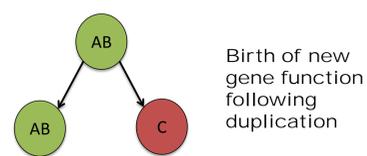


Background

Subfunctionalization

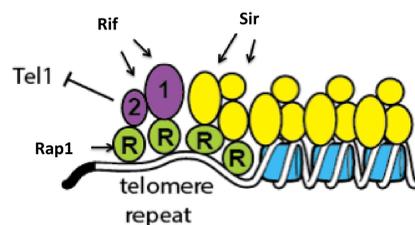


Neofunctionalization



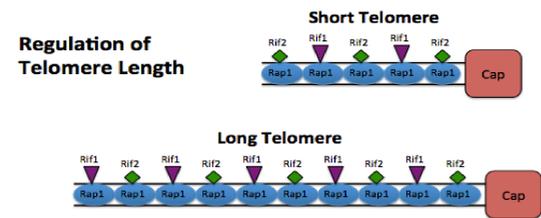
The Telosome

In *S. cerevisiae*...

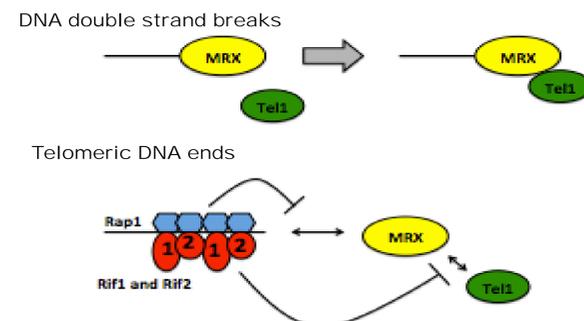


- Rap1 binding to telomeric repeats
- Rif1 and Rif2 binding to Rap1
- Rif2 blocking Tel1 (kinase) association to telomeric ends
- Tel1 recruits telomerase

Regulation of Telomere Length



- Longer telomeres have more Rap1 binding sites
- The tendency to increase in telomere length is inversely proportional to the amount of telomeric repeats



- MRX complex recruits Tel1 at double strand breaks
- Rap1 inhibits MRX binding to DNA ends
- Rif2 distinguishes telomeres from DNA breaks by inhibiting the binding of Tel1

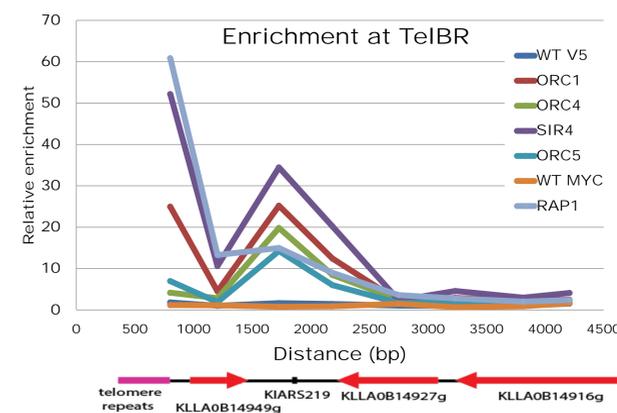
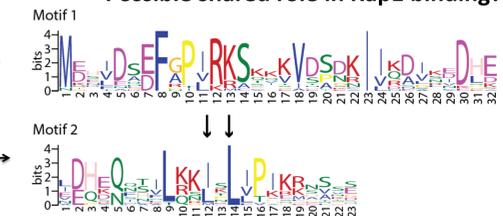
Question

Was the Rif2 function of binding to Rap1 at the telomeres formed as a production of neofunctionalization or subfunctionalization?

Results

Sequence alignment reveals Rif2 and Orc4 share 2 motifs

Possible shared role in Rap1 binding?



Conclusion

- Though they may share a possible Rap1 binding motif, no Orc4 association is observed at the *K. lactis* telomere
- Replication proteins are indeed enriching at known origin
- Rif2 function a production of neofunctionalization

Future Direction

- Perform ChIP with Rif1 at *K. lactis* Tel BR
- ChIP all proteins at other *K. lactis* telomere ends
- Co-IP of Orc4 and Rap1 in *K. lactis*
- Mutate Orc4 and observe effect on binding efficiency
- Observe interactions in *T. delbrueckii*

Bibliography:

Bonetti et al., PLoS Genetics 6(5):e1000966, 2005
Hirano et al., Cell 33, 312-322, 2009
Martina et al., Mol Cell Bio 1604-1617, 2012
Shi et al., Cell 153, 1340-1353, 2013