Telomere binding protein Rif2 as a product of subfunctionalization or neofunctionalization following the whole genome duplication in yeast species
Laura Rusche, William Richardson, Breandan Quinn

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

Results
Sequence alignment reveals Rif2 and Orc4 share 2 motifs
- 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

Conclusion
- 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

Future Direction
- Express Rif2 in H. polymorpha

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

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