# The roles of Ric1, a protein involved in Golgi transport, and Ost4, an ER membrane protein, in telomere defective strains

Kaiqiang Li*, Marta Markiewicz, David Lydall
*130062444, BSc Biomedical Sciences, k.li12@newcastle.ac.uk

## Aim

- To determine whether Ric1 and/or Ost4 affect telomere function.

## Introduction

Telomeres are the nucleoprotein structures found at the end of eukaryotic chromosomes, which are important in maintaining genome integrity and preventing age-related diseases or cancer. Multiple telomere capping proteins work together to prevent telomere being recognized as double strand breaks (DSBs) and processed by repair machinery. In this study, we focused on budding yeast capping proteins Cdc13 and Yku70. By inactivating these two capping proteins, telomeres are perceived as DSBs and induce the cell cycle checkpoint. As a result, these telomere uncapped strains are temperature-sensitive. Using these strains allow us to study the role of other proteins when telomeres are uncapped.

This project investigate the roles of Ric1 (Ribosomal Control) and Ost4 (OligoSaccharylTransferase) in telomere function. Previous Quantitative Fitness Analysis (QFA) results from the lab suggested both *ric1Δ* and *ost4Δ* are suppressors of *cdc13-1* mutant temperature sensitivity (ts) and enhancers of *yku70Δ* mutant ts [1]. Protein Ric1 is located in Golgi membrane, and is required for efficient fusion between endosome-derived vesicles with Golgi membrane during retrograde transport [2]. Ost4 is a subunit of the oligosaccharyltransferase (OSTase) found on the ER membrane, which is essential in N-linked glycosylation [3].

## Methods

- *RIC1* and *OST4* were deleted respectively (*ric1Δ::KANMX*, resistant to antibiotic G418, and *ost4Δ::HPHMX*, resistant to antibiotic hygromycin).
- Deletion was performed in *cdc13-1/CDC13, yku70Δ/YKU70* and WT/WT diploids respectively, using lithium-acetate (LiAc) transformation.
- Gene knock out was confirmed with PCR (Fig.1). Diploid yeast was then sporulated and dissected to select single and double haploid mutants.

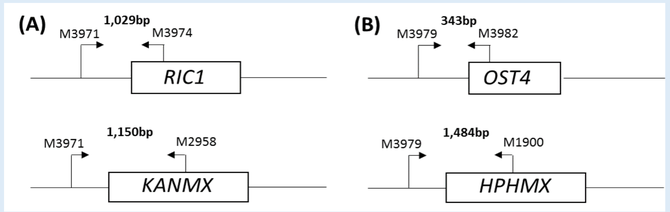


**Figure 1**.
**(A)** Model of *RIC1* and *ric1Δ ::KANMX* detection primers. **(B)** model of *OST4* and *ost4Δ::HPHMX* detection primers. Models not to scale.

- Spot tests in various temperatures were performed with selected haploid strains to determine how these two proteins affect the temperature fitness of the telomere uncapped strains.

## Results- Ric1

- *ric1Δ* suppress the *cdc13-1* mutant ts (Fig. 2A).
- *ric1Δ* enhance the *yku70Δ* mutant ts (Fig. 2B).
- *ric1Δ* single mutant is temperature-sensitive, inviable at 36°C (Fig. 2B).

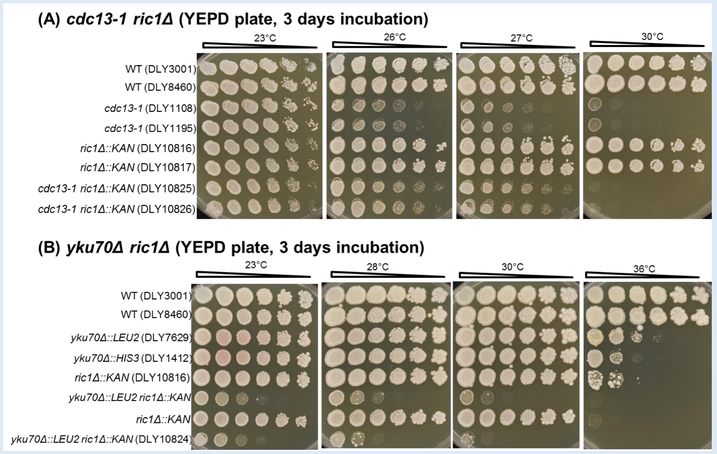


**Figure 2.** Ric1 affect the fitness of telomeres uncapped strains.

## Results- Ost4

- *ost4Δ* suppress the *cdc13-1* mutant ts (Fig. 3A).
- *ost4Δ* has no effect on the *yku70Δ* mutant ts (Fig. 3B).
- *ost4Δ* single mutant is temperature-sensitive, sick at 23°C (Fig. 3A&B)
- Ost4 is required for optimum growth of yeast on –LEU plates (Fig. 3C).

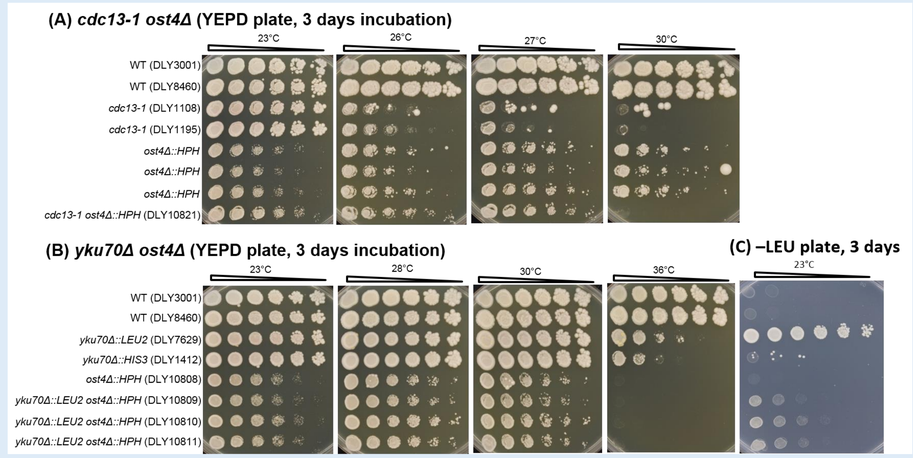


**Figure 3.** Ost4 affect the fitness of *cdc13-1*, but not *yku70Δ,* and is required for optimum growth on Leucine absent agar plates.

## Conclusion and future studies

- Growth assay verified the QFA genome-wide results, suggesting both protein Ric1 and Ost4 affect telomere function.
- *ost4Δ* cover the effect of *yku70Δ* on temperature fitness.
- Ost4 is essential for optimum growth of budding yeast on –LEU plates.
- Future work should focus on understanding the molecular basis of found genetic interactions, which include experiments like in-gel assay to test the ssDNA accumulation and Southern blot to measure telomere length.

## Acknowledgements

I would like to thank Prof. David Lydall and Marta Markiewicz for the project supervision and support. I also thank the Genetics Society in founding this project.

References:
1. Addinall *et al*., (2011) 'Quantitative Fitness Analysis Shows That NMD Proteins and Many Other Protein Complexes Suppress or Enhance Distinct Telomere Cap Defects', *PLoS Genet*, 7(4), p. e1001362.
2. Mizuta *et al*., (1997) 'RIC1, a novel gene required for ribosome synthesis in Saccharomyces cerevisiae', *Gene*, 187(2), pp. 171-178.
3. Chi *et al*., (1996) 'The OST4 gene of Saccharomyces cerevisiae encodes an unusually small protein required for normal levels of oligosaccharyltransferase activity', *J Biol Chem*, 271(6), pp. 3132-40.