What happens to offspring that inherit abnormal length telomeres from their parents?

Sarah Mearns, 160288159, BSc Biomedical Sciences with Professional Placement, S.Mearns@newcastle.ac.uk, Supervisor: Professor David Lydall

Introduction

Human and yeast cells, have structures called telomeres capping their chromosomes (Figure 1). *Saccharomyces cerevisiae* (budding yeast), commonly used for baking and brewing was used as a simple model in this experiment. Yeast have 16 chromosomes and thus 32 telomeres.

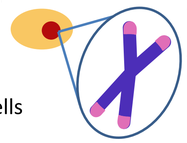


Figure 1: *Yeast cell with nucleus (red), showing a chromosome (purple) with telomeres (pink) capping the ends*

Yeast are fast growing, making them great to study telomere length inheritance. Two haploid cells in yeast (similar to sperm and egg cells in humans) mate to form a diploid cell (progeny).

For human females approximately 31 cell divisions occur between fertilisation of the egg and production of eggs. In males the equivalent number is estimated at 400 for a 30 year olds sperm. Divisions are higher as sperm are produced constantly in adulthood[1] (Figure 2). By calculating the number of cell divisions in yeast, it will allow comparison with human telomere length inheritance.

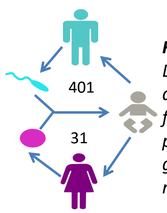


Figure 2: *Diagram of cell divisions between fertilisation and production of gametes in the next generation*

The aim was to see what happens to telomere length in budding yeast diploids that inherit abnormal telomeres from their parents.

Methods

- Mate haploid yeast parental strains with different length telomeres (normal - **N**, long – **L** and short - **S**), with another 3 (**N**, **L** and **S**) creating 9 diploids, by mixing the parent strains.
- Streak (spread) some of each diploid onto plates, to grow single colonies.
- Select 2 of these colonies (A and B) , each a clone.
- Patch (to keep some yeast for later DNA extraction) and streak (to passage) A and B every 3.5 days.
- Grow some of each patch in media overnight.
- Extract DNA, cut with an enzyme (Xho1), measure telomere length via a Southern Blot using a Y'-TG probe that binds to the telomeres, then strip blots and use a chromosome VI probe. (Figure 3)

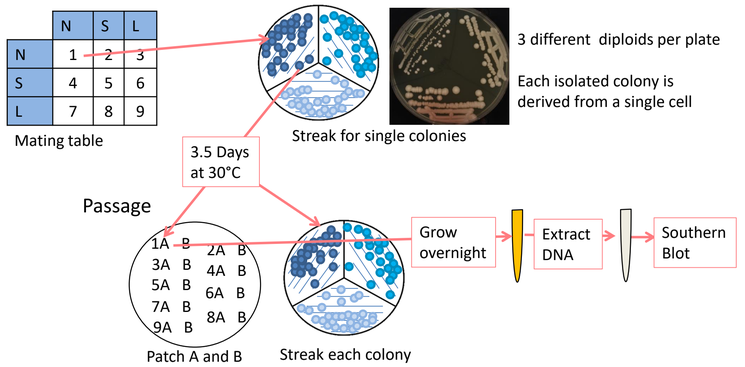
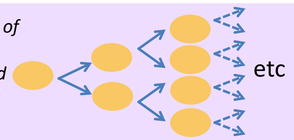


Figure 3: *Schematic diagram of experimental methods: crossing yeast strains, patching and streaking each cross, then growth, DNA extraction and Southern blot*

- Passage/ subculture- a culture made by transferring microorganisms from a previous culture to a fresh medium[2] .
- Count cells in single colony and also cells before and after growth overnight using a microscope, to calculate the number of cell divisions (Figure 4).

Figure 4: *Diagram of cells dividing (exponentially) and equation produced from this pattern*



$$\text{Final cell number} = \text{Original cell number} \times 2^{\text{divisions}}$$

Rearrange

$$\text{Divisions} = \log_2\left(\frac{\text{Final number}}{\text{Original}}\right)$$

- The yeast parental strains used had different mutations affecting telomeric proteins (Figure 5).
  - Short: *mre*11Δ or *yku*70Δ
  - Long: *rif*1Δ *or rif2*Δ

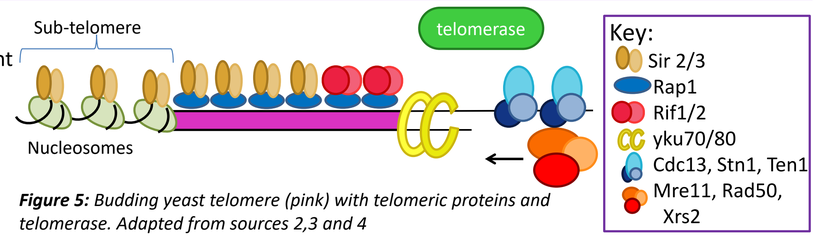


Figure 5: *Budding yeast telomere (pink) with telomeric proteins and telomerase. Adapted from sources 2,3 and 4*

Results

Normal (N) and short (S) length telomere diploids (*mre*11Δ or *yku*70Δ), aren't quite normal length in P1 but quickly normalise by P5.

Long telomere diploids of all combinations begin normal length or longer in P1, these can then increase further (L/L Fig 6a), become shorter (L/S) or remain long (L/L Fig 6b). But in all cases they remained normal length or longer in P9.

Most cases in the Chr VI blots, where there are two bands, the longer one shortens and the shorter one lengthens or remains the same. The exception is L/L A where both lengthen (Fig 6a)

*Rif*2Δ (N/L) mutants normalise in telomere length quickly. The longer band is fainter in P1 and lost completely by P5.

Molecular weight ladders are for size reference, the further down the band, the smaller it is in size. The DIG ladder is digoxigenin labelled.

Cell Divisions

- 22 to 26 divisions between passages, depending on colony size. A mean of 24.6.
- 2 to 3 divisions during growth of patch.
- 3 to 4 divisions during growth in media.
- Totalling 30 to 32 divisions.

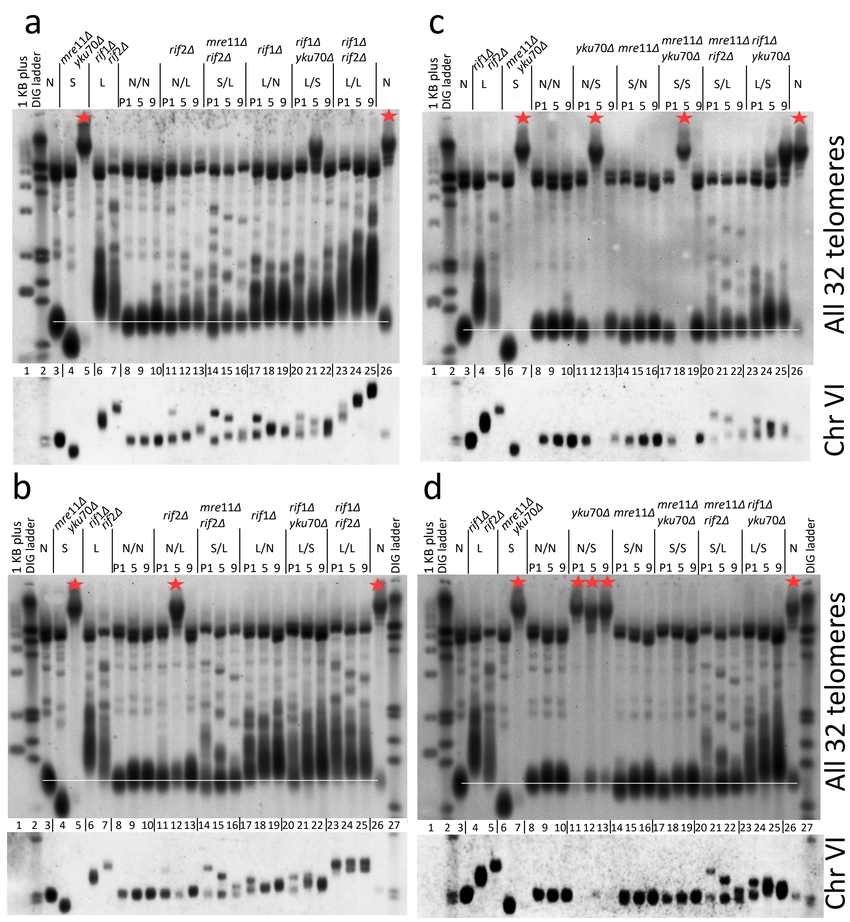


Figure 6: *Southern blots of parents normal (N), short (S) and long (L) length telomeres and crosses Passage 1, 5 and 9. Clones A (top blots) and B (bottom blots). Y'-TG probe for larger blots, same blots then stripped and reprobed with chromosome (Chr) VI probe, shown as the smaller blots.* Some DNA did not fully cut with the enzyme (Xho1), indicated by ★

Discussion

- Each passage is comparable with a single generation in human females (32 divisions in oocytes)[1].
- Differences between clones A and B suggest other mutations may have spontaneously developed affecting their telomeres.
- Differences seen between mutations for the same telomere length, suggest the impact on telomere length depends on the specific mutation. The *mre*11Δ mutation for example results in shorter telomeres than the *yku*70Δ mutation, even though both mutations create short telomeres.

Conclusion

- Telomere length inheritance in budding yeast is complex and many patterns can be seen.
- In humans the patterns are likely to be at least as complex.

Acknowledgements

Thank you to Professor Lydall for letting me undertake this project in his lab, and for all his help. Thank you to Marta Markiewicz-Potoczny for her advice and to her and Carolin for the probes. Also many thanks to the Wellcome Trust for funding this project.

References: **1** Drost JB, Lee WR. Biological basis of germline mutation: Comparisons of spontaneous germline mutation rates among drosophila, mouse, and human. Environmental and Molecular Mutagenesis. 1995;25:48-64. **2** Farflex Partner Medical Dictionary. Subcultural capital. 2012 [cited 23/08/18] . Available from: https://medical-dictionary.thefreedictionary.com/Subcultural+capital **3** Di Domenico EG, Romano E, Del Porto P, Ascenzioni F. Multifunctional role of ATM/Tel1 kinase in genome stability: from the DNA damage response to telomere maintenance. Biomed Research International. 2014;2014:787404. **4** Auriche C, Di Domenico EG, Ascenzioni F. Budding yeast with human telomeres: A puzzling structure. Biochimie. 2008;90:108-115. **5** Lydall D. Taming the tiger by the tail: modulation of DNA damage responses by telomeres. The EMBO Journal. 2009;28:2174.