DNA Repair at chromosome ends

Temperature sensitivity assessment at telomere ends of *Saccharomyces cerevisiae* cdc13-1 double mutant strains to study genetic interactions between DNA repair genes

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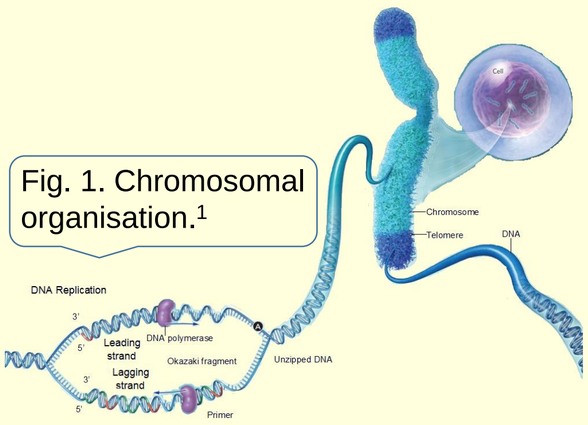

Fig. 1. Chromosomal organisation.[1]

Fig. 2. Cdc13 caps protect exposed chromosome ends. Loss of Cdc13 would stimulate DNA damage response.

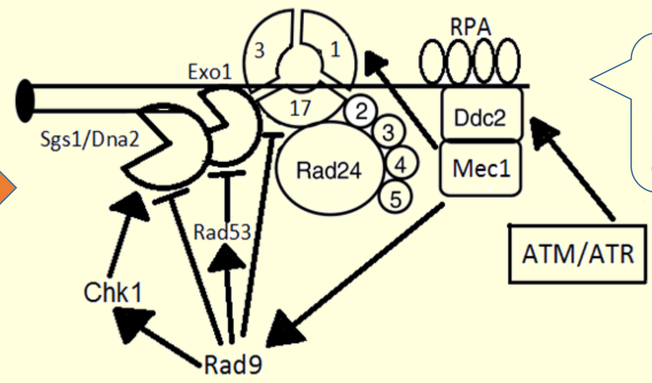

Fig. 3. DNA repair protein interactions at Cdc13 deficient yeast cells.[2]

Background

Cancer is a genetic disease that involves uncontrolled cell division. Genetic studies aimed at understanding cell division processes would therefore attempt to understand cancer progression mechanisms better.

Cdc13 capping protein at yeast chromosome ends used as tool for genetics study. The temperature sensitive cdc13-1 strain could be manipulated, such that growth of cdc13-1 strains at non-permissive temperature of 30ºC will have non-functional Cdc13 whereas growth of cdc13-1 strains at permissive temperature of 20ºC will have functional Cdc13.

This way, we can study effect of cdc13-1 yfgΔ (i.e. variable gene of interest) strains, thereby inferring possible functions of the YFG gene and its interactions with other gene products.

Aims

Identify DNA repair proteins involved in repair of Cdc13 deficient yeast cells

Conclusions

- Rad24, Exo1, Ddc1, Rad17, and Chk1 proteins found be involved in DNA repair of Cdc13 deficient yeast cells
- Experimental results replicate previous results

1. Methodology: Synthetic Genetic Array

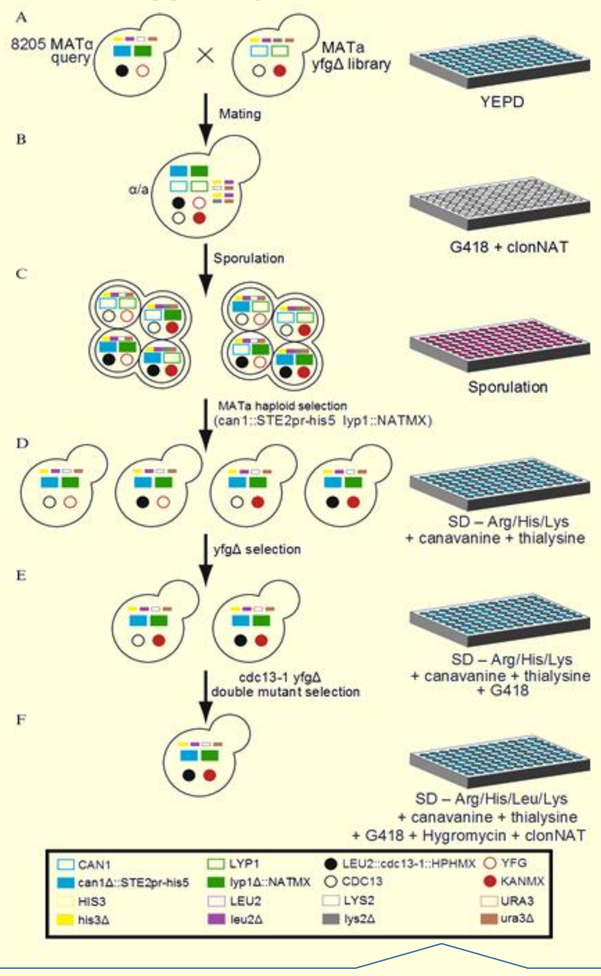

Fig. 4. Synthetic Genetic Array (SGA) produces deletion mutants using automated robots. In this experiment, array of cdc13-1 yfgΔ double mutants are produced as shown to select for only the haploids of desired genotype.[3]

2. Sample SGA Images

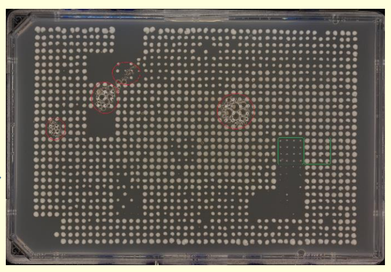

Fig. 5. Sample raw SGA. Red circles show regions of air bubbles. The 2 green boxes each show an array of 16 cell culture replicates of a single cdc13-1 yfgΔ double mutant strain.

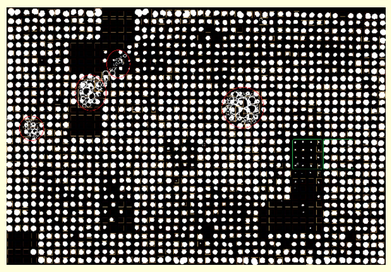

Fig. 6. Gridded image of Fig.5 analysed by Gitter software application.

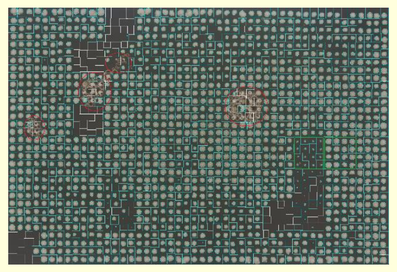

Fig. 7. Gridded image of Fig.5 analysed by IRIS software application.

3. Software analysis

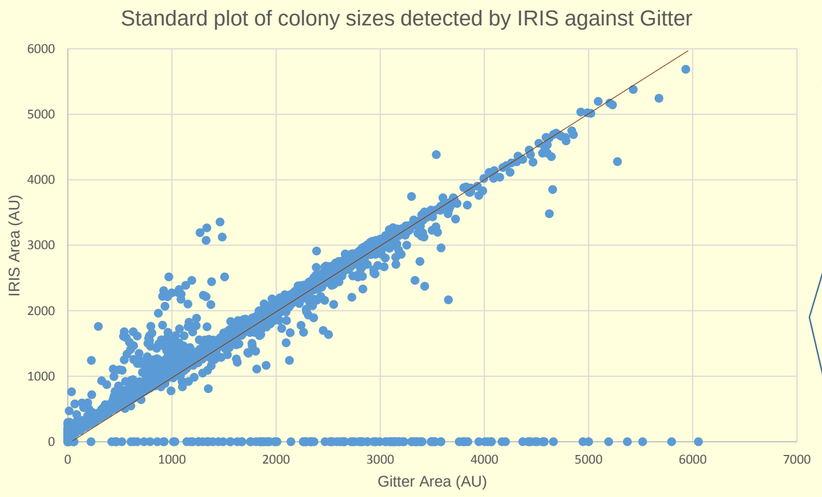

Fig. 8. Software analysis between IRIS and Gitter application performed to identify the better application:

1. Scatter plot of all the data points plotted.
2. Outliers at the axes are then individually inspected.
3. Outliers at the Gitter axis are due to quantification of air bubbles (as shown within red circles in fig. 5-7), whereas IRIS completely disregards such colonies.

Therefore, IRIS is deemed to be the better software application and would be used for subsequent data analysis and interpretation.

4. Quality control

Standard plot of mean colony sizes of 8205 strain yfgΔ deletion control strains at 20ºC vs 30ºC

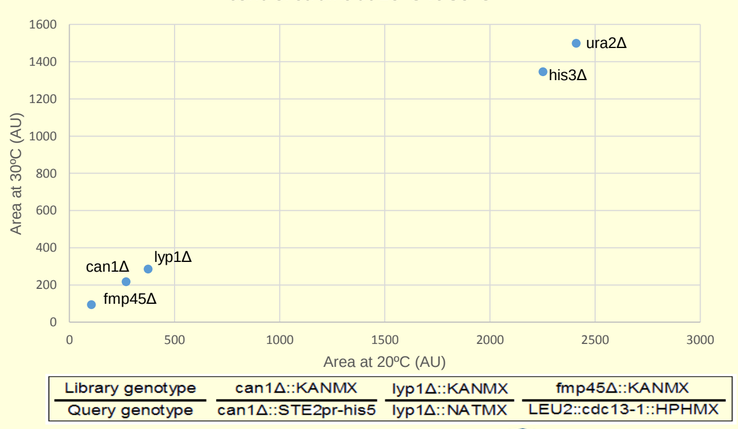

Fig. 9. Growth plot of selected test markers to assess accuracy of results. In this experiment, growth rates observed in fig.9 correspond to expected growth rates:

- low rates for cdc13-1 fmp45Δ, can1Δ, lyp1Δ
- high rates for cdc13-1 his3Δ and ura2Δ

Therefore, experimental data reliable.

5. Colony growth analysis

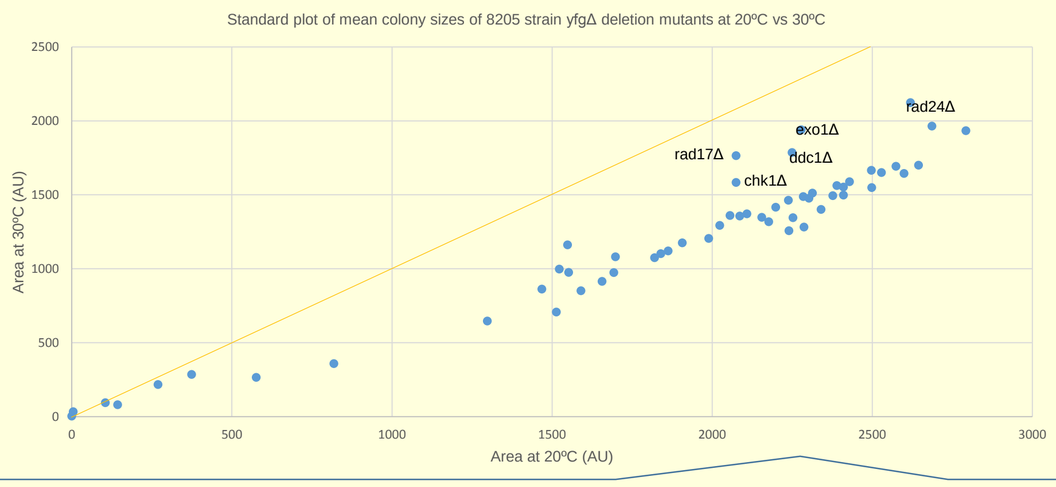

Fig. 10. Plot of mean colony sizes of all deletion mutant strains. cdc13-1 rad24Δ, exo1Δ, ddc1Δ, rad17Δ, and chk1Δ double mutants have higher than expected growth rates.

- ➢ Rad24, Exo1, Ddc1, Rad17, and Chk1 proteins found to suppress Cdc13 deficient cell growth
- ➢ Additional experiments needed to elucidate genetic interactions

1. Adapted from Leighton S. Telomere Basics. *The Scientist*. [online]. 2012. Available at: https://www.the-scientist.com/infographics/telomere-basics-41041 [Accessed 3 Aug. 2018].
2. Adapted from Morin I, Ngo HP, Greenall A, Zubko MK, Morrice N, Lydall D. Checkpoint-dependent phosphorylation of Exo1 modulates the DNA damage response. The EMBO journal. 2008 Sep 17;27(18):2400-10.
3. Adapted from Baryshnikova A, Costanzo M, Dixon S, Vizeacoumar FJ, Myers CL, Andrews B, Boone C. Synthetic genetic array (SGA) analysis in Saccharomyces cerevisiae and Schizosaccharomyces pombe. InMethods in enzymology 2010 Jan 1 (Vol. 470, pp. 145-179). Academic Press.