Cancer repair mechanisms

Uncovering a relation between RIF1 protein and histone regulation

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Introduction:

Most cancers occur due to DNA damage in the form of double strand breaks. A major factor influencing the repair mechanism is specific histone and non-histone proteins that form chromatin.

Histones are proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes, units of chromatin. There are 5 major families of histones: H1/H5 (linker histones), H2A, H2B, H3, and H4 (core histones) (Fig. 1) [1].

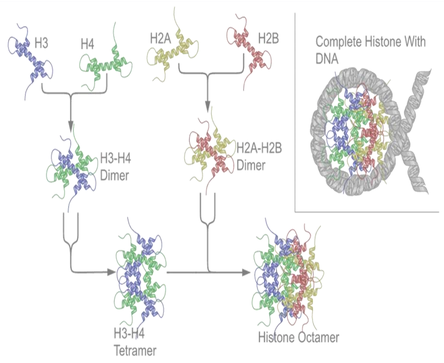

Fig. 1 Schematic representation of the assembly of the core histones into the nucleosome (top-right box).

The process of **DNA double strand break repair**, requires several chromatin alterations to sense damage, recruit factors and determine appropriate pathway for repair, meanwhile conferring accessibility to the repair machinery. [2]

Rif1 (RAP1 Interacting Factor) is a major player in determining whether an error free repair or a different pathway that can lead to loss of DNA information is to be used. Rif1 action is blocked in cell cycle (S phase) to ensure double strand repair occurs via error free (homologous recombination) pathway. [3]

Aims:

- ❑ Disrupt 3 genes of histone interacting proteins:

1) - **HIR1** (inhibits histone gene H2A and H2B transcription)

2) - **RTT109** (histone acetyltransferase (HAT), conferring resistance to DNA damage in S phase)

3) - **CAC1** (subunit of CAF1 -scaffold depositing tetrameric H3/H4 histones onto newly synthesised DNA)

- ❑ Measure the level of histones expressed
- ❑ investigate whether Rif1 is signalled via modifications

Method:

The genes investigated had to be knocked-out from the genome of haploid (genome contains one copy of each gene) S. cerevisiae cells of different strains:

1) **RIF1-Myc cdc13-1** (temperature sensitive)
2) **RIF1-Myc** (Myc tag allows Rif1 to be detected)
3) **rif1Δ** (RIf1 deleted)
4) **WT** (cells with unmodified genome)

Primers were design specifically to have 20 base pair (bp) homology to a plasmid containing a gene cassette conferring resistance to selective medium (e.g. kanamycin) and 40 bp homologies with the gene (Fig. 2).

Using PCR, cassette was multiplicated and the product was used to transform strains of yeast cells, where cassette displaces the gene. PCR technique was used again to test for successful gene knock-outs in colonies using set of designed primers, ready to amplify the region of DNA bound by the primers. The corresponding bands were observed in agarose gels (Fig. 4).

Furthermore, real-time PCR technique was to be used in measuring the levels of histone gene expressed. Finally, investigating Rif for post translational modifications (e.g. phosphorylation) using Western Blot technique.

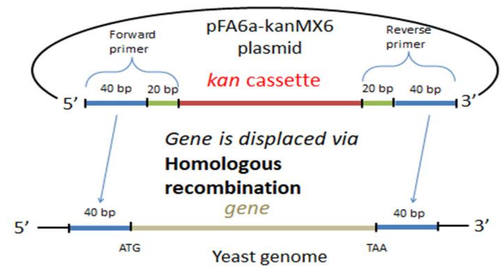

Fig 2. Illustrates how the gene of interest is replaced by PCR amplified *kan* cassette

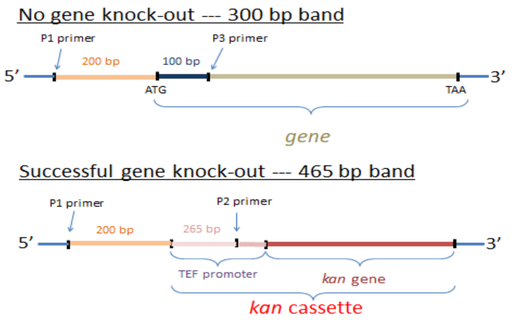

Fig 3. Illustrates how the band size encompassed by the primers allows for identification of un/succesful gene knock-out after transformation

Results:

A number of colonies emerged on kanamycin treated plates were tested (Table 1 – *"on the right"*). None of the genes proved to have been deleted (Fig. 4 a,b). The negative control represents the cell culture untransformed.

Colonies formed after transformation				
Gene	Strain			
	RIF1-Myc cdc13-1	RIF1-Myc	rif1Δ	WT
hir1	0	2	19	0
rtt109	0	4	19	0
cac1	0	19	19	0

Fig 4a. Each band represents 1 colony each formed and tested from the strain *RIF1-Myc.*

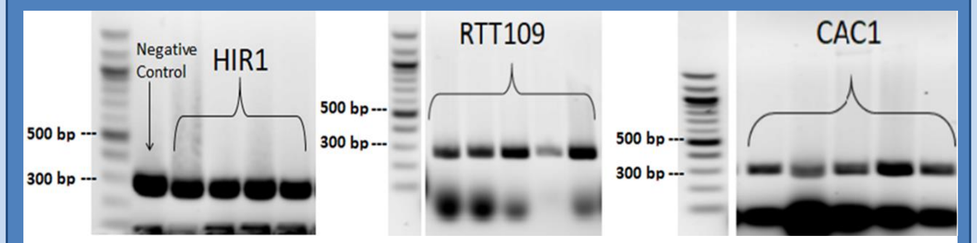

Fig 5b. Each band represents 1 colony each formed and tested from the strain *rif1Δ*.

Conclusion:

Colonies (Table 1) which emerged suggest that *kan* cassette has been introduced in yeast genome after transformation, however cells which actually underwent successful gene knock-outs were unable to survive.

References:

[1] Shapiro JA; Phys Life Rev. 2013 Sep; 10 (3):287-323.
[2] Hunt CR et al; Radiat Res. 2013 Apr; 179 (4):383-92.
[3] J. Ross Chapman; Mol Cell. 2013 Mar 7; 49 (5): 858–871.

Next step:

To research my aims, it would be appropriate to try mate the haploid cells of all strains. After producing gene knockouts in diploid cells, they would undergo sporulation (**yeast** *S. cerevisiae* meiosis packaging haploid nuclei) and selecting the cells with gene deletions, proceed with the investigation.

Acknowledgements:

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