Investigating Rif1 in DNA damage response pathways in yeast

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Introduction

Organisms such as yeast have evolved many DNA damage response (DDR) pathways to counteract environmental and replication stresses on DNA. One of these pathways involves the formation of chromatin; these are highly organised structures of DNA, tightly packaged into repeating units called nucleosomes. Rif1 is a protein that is known to be involved in the DDR via other pathways, and is implicated in chromatin formation.

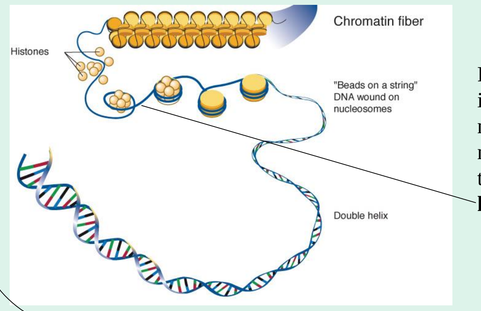

Figure 1 [1]: DNA is packaged into nucleosomes; these nucleosomes are in turn made up of **histones**.

Aims

In this experiment, I aimed to knock out 2 genes involved in chromatin production: ASF1 and RTT106, and observe any effects on chromatin (by measuring expression of histone genes), or Rif1 phosphorylation.

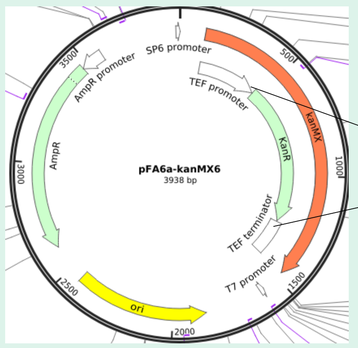

Figure 2 [2]: This is the plasmid I used to knock out the genes; by designing primers which match defined sequences at the **promoter** and **terminator** regions, the cassette of genes from the plasmid can be inserted and can function in place of the host genes by PCR (which amplifies the plasmid). .

Method

Designed primers to knock the genes of interest out and replace them with the plasmid cassette, containing an antibacterial resistance gene, for positive selection.

Transformed yeast strains with the plasmid for each respective gene knockout (culture on selective antibacterial plates which should kill all colonies that have not incorporated the plasmid)

Test integration of plasmids by selecting colonies for colony PCR, and running them on an agarose gel for analysing fragment length (to eliminate false positives)

Measure histone gene expression in positive colonies by real-time PCR (quantitative)

Measure levels of Rif1 phosphorylation in positive colonies by Western Blot.

Results

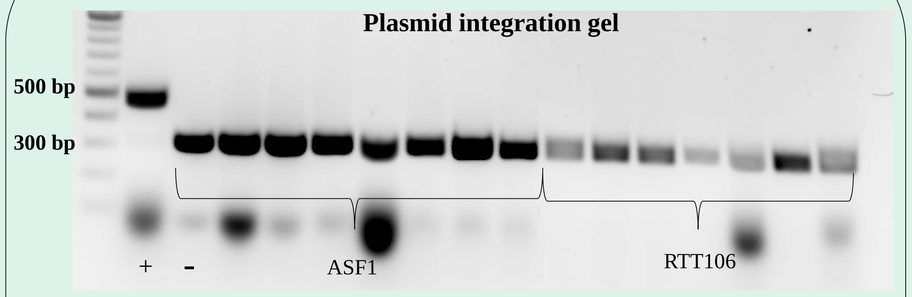

Figure 3: Gel image of colonies grown on selective plates. If the yeast had taken up the plasmid, a 500 base pair fragment was expected, untransformed cells produce a 300 bp fragment. As the gel shows, all tested colonies produced 300 bp fragments; they are all false positives. These can be compared with the negative control strain (untransformed yeast, ("-" in the figure)) and the positive control strain (a strain from the lab confirmed to have been transformed ("+" in the figure)).

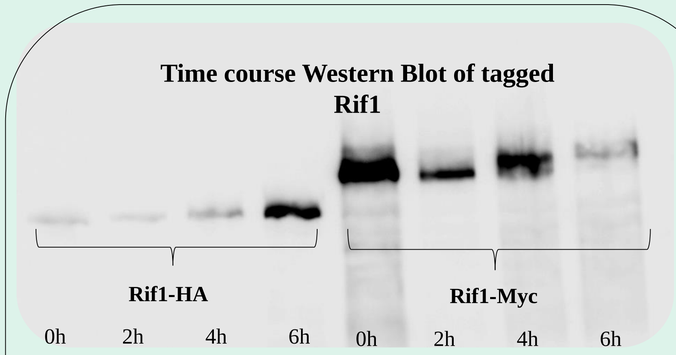

Figure 4: Western Blot of two yeast strains containing Rif1 tagged with two different antibodies. The tags do not interfere with Rif1 function and do not affect the results. Since the chromatin could not be disrupted (with the gene knockouts), DNA damage was caused in the cultures by placing them at a high temperature. The rise in bands over time is indicative of an increasing population of phosphorylated Rif1 (which has a higher molecular weight due to the addition of the phosphate group).

Discussion

Unfortunately, we were not able to successfully transform the yeast with the plasmid cassette. The gel image shown in Figure 3 is one of many trials after changing various conditions in the experiment. We concluded that it was not possible to knock these genes out in the haploid yeast strain used in this experiment; to investigate this further, the experiment could be repeated using a diploid yeast strain (containing an extra copy of the gene to potentially rescue the cell).

The Western Blot shows increasing phosphorylation in response to increasing DNA damage; this reflects the prevailing literature wherein Rif1 phosphorylation is required for its localisation to damaged DNA in the DNA damage response [3].

References

1. Image from: www.unlockinglifescode.org/sites/default/files/chromatin_lg.jpg
2. Image from: www.addgene.org/39296. Last accessed 29/09/17
3. Chapman JR, Barral P, Vannier J-B, Borel V, Steger M, Tomas-Loba A, et al. RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. Mol Cell 2013 Mar;49(5):858–71.