



Lincoln's Inn Fields

Cell Cycle

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The major objective of the Cell Cycle laboratory is to understand the regulation of the cell cycle and cell shape, both of which are important for the development of cancer. To study these problems we use the fission yeast *Schizosaccharomyces pombe*, which is a single celled eukaryotic organism and typically has many of the features found in higher eukaryotes. It has, for example, large centromeres and origins of replication, introns and an interphase microtubular cytoskeleton. We have used a set of genome wide gene deletions in fission yeast to identify new cell cycle and cell morphology genes and to develop whole genome approaches to studying these and related problems.

Genome analyses in fission

We are part of an international consortium to generate and analyse a genome wide set of gene deletions. As part of the validation of this set of deletions strains and to also develop methodologies for screening fission yeast mutants we have examined a subset of 2650 viable haploid deletion mutants in collaboration with a number of other laboratories.

This collection has been used to investigate the role of the DNA glycosylase MutY, which is highly conserved and found in both

eukaryotes and prokaryotes. MutY has previously been shown to be involved in the repair of oxidative damage. We have shown that myh1 (MutY in fission yeast) also genetically interacts with a number of DNA repair genes particularly Rad1 and Rad9, which are involved in the DNA damage checkpoint. Double mutants of rad1 myh1 and rad9 myh1 have increased UV sensitivity. This work extended the known range of DNA damaging agents that require MutY for DNA repair (Jansson *et al.*, 2008).

The deletion collection has also been used to screen for deletion mutants showing defective growth in different conditions. In one study the role of oxidative stress in fission yeast was investigated and it was found that a number of deletion mutants with defective mitochondrial function led to increased oxidative stress and a reduced life span. There were 51 deletion mutants sensitive to growth on both respiratory proficient medium and hydrogen peroxide containing fermentable media and 19 of these were defective in mitochondrial function. These mutants exhibit elevated levels of reactive oxygen species (ROS), which has been linked to reduced life span in other organisms and may explain why these fission yeast cells have reduced life span (Zuin *et al.*, 2008). In a second study a screen for cadmium sensitive mutants identified 237 genes, including genes involved in sulphur metabolism, inorganic stress and the cell cycle. As well as identifying genes with previously known function we also identified genes of unknown function. These genes will help elucidate the process of cadmium tolerance in fission yeast and will also help to reduce the number of unknown genes which is currently about 18% of the total 4914 protein coding genes in fission yeast (Kennedy *et al.*, 2008).

In budding yeast synthetic lethal interaction networks identified using synthetic gene arrays (SGA) have been used to define interactions that regulate essential gene functions. However the extent to which these interactions have been conserved in other organisms is not known. Double mutants of 222 genes including ones involved in DNA replication, DNA damage repair,

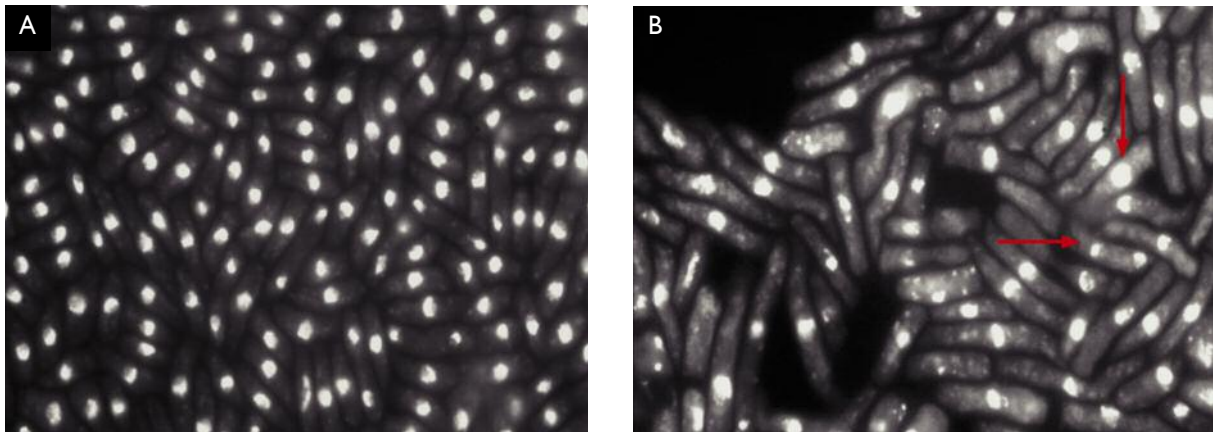


Figure 1. Wild type fission yeast cells and deletion mutant cells grown in rich medium and stained with DAPI to show the nucleus (A) Wild type cells and (B) deletion mutant of *SPAC17A3.05c* which encodes a DNAJ protein and was not previously identified as a cell cycle gene. This mutant displays a number of nuclear defects including enlarged nuclei and mis-segregated nuclei (see red arrows). Both A and B are the same magnification.

chromatin remodelling and intracellular transport and 2650 deletion mutants have been constructed to identify interacting genes. These data were then compared with interaction studies from a literature screen and with data from budding yeast. We found that ~29% of interactions are conserved between the two organisms (Dixon *et al.*, 2008). A second, similar approach identified conserved networks using an epistasis mapping (Emap), which also analyses double mutant phenotypes. These data were then compared with data from an Emap of budding yeast. We found that functional modules are conserved between the two yeasts but that the wiring between them may be different between the two organisms (Roguev *et al.*, 2008).

Cell cycle

The cell cycle in fission yeast is typically eukaryotic and has distinct G₁, S, G₂ and M phases. The genome is around 14Mb and is replicated bi-directionally from origins of replication dispersed throughout the 3 linear chromosomes. Previous studies have estimated that it takes around 20 minutes to replicate the whole genome and it has also been shown that although there are around 900 origins of replication only about one third are used in any one S phase. We have used a

stochastic hybrid modelling approach based on genome wide data of origin of replication efficiency and position to study genome replication. We observed that because of the stochasticity of origin firing there are regions of the genome that would not be replicated within the estimated length of S phase using conventional methods. Modelling showed that increased fork rate or increasing the number of origins did not reduce S phase to the reported length. We propose that in fission yeast the length of S phase is longer than previously reported and extends into the 'G₂' phase of the cell cycle (Lygeros *et al.*, 2008).

We have also carried out a genome-wide screen for new cell cycle mutants to identify a near complete set of genes required for the cell cycle (Figure 1). We are using these to carry out a haploinsufficiency screen for cells that delay entry into mitosis and to create temperature sensitive mutants for further study of new cell cycle genes that are conserved in humans and not previously characterised in any other organism. These genes will be important for identifying new human genes important for cell cycle progression.

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