Characterisation of a phospholipase Cδ from *Schizosaccharomyces pombe*

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We recently described the use of a PCR-based approach to identify the gene encoding a phospholipase C from the fission yeast *Schizosaccharomyces pombe* [1]. The putative Plc1 protein has 899 amino acids and is structurally most similar to the δ class of PLC isozymes. To further investigate the role of this enzyme, we have used a one step gene replacement strategy (Figure 1) to generate yeast strains that lack the *plc1* gene. Initial experiments demonstrate that strains lacking *plc1* are temperature sensitive (Figure 2).

![Figure 1 Disruption of the *plc1* gene.](image)

A restriction map of the *plc1* locus is shown with the extent and direction of the open reading frame (ORF) indicated. The structure of the linear fragment used to disrupt the *plc1* gene in *vivo* is shown below the map. Genomic DNA was digested with EcoRV, separated by gel electrophoresis and probed with the fragment shown above the map. Lane 1 contains genomic DNA from a wild type diploid strain (*plc1*/*plc1*), lane 2 contains DNA from a heterozygous diploid (*plc1*/*plc1::ura4) in which one of the wild type *plc1* alleles has been disrupted with the *ura4* gene. Lanes 3 to 6 contain DNA from the haploid strains produced following sporulation of the heterozygous diploid.

![Figure 2 Strains lacking *plc1* are temperature sensitive.](image)

The four haploid strains produced by sporulation of the heterozygous diploid strain shown in Figure 1 were plated on yeast extract containing 2% glucose and incubated at the temperatures indicated.

In addition to their temperature sensitivity, strains lacking *plc1* are unable to grow on minimal medium (not shown). The growth defect of this mutant could not be overcome by the addition of either 0.5 M KCl or 1.2 M sorbitol to the medium. It is clear that, under the appropriate conditions, *plc1* is not essential for yeast growth and we are currently trying to more closely define the role of this enzyme. The amenability of the yeast to biochemical, genetical and morphological analyses provides a powerful system in which to perform these studies.

This work was supported by the Cancer Research Campaign and the Biotechnology and Biological Sciences Research Council. JD is a Lister Institute Research Fellow.