PCR Detection of a Deletion of the *CDC7* Gene in Yeast

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ABSTRACT

The *CDC7* gene of *Saccharomyces cerevisiae*, or Baker's yeast, plays an important role in the regulation of DNA replication during the mitotic cell cycle. However, its role in meiosis is uncertain. Although *cdc7*Δ strains die in the absence of DNA replication, a suppressor called *bob1* allows the deletion mutant to grow mitotically. This same suppresser, however, has been shown to be unable to rescue *cdc7*Δ's meiotic phenotype. This research describes the development of a PCR protocol to analyze the proper construction of haploid *cdc7*Δ strains being constructed in the laboratory. These strains will be use to construct a diploid strain to examine the meiotic phenotype of the *cdc7*Δ mutation.

INTRODUCTION

Cell division cycle, or *cdc*, genes encode essential proteins that are responsible for controlling the critical sequence of cell cycle events. These cell cycle events regulate growth and division of individual cells, and alteration of this regulation may result in cancerous tumors as the cells grow and divide at an accelerated rate. *CDC7* in *Saccharomyces cerevisiae*, or Baker's yeast, encodes a protein kinase required for initiation of DNA replication between the G1 and S phases of the mitotic cell cycle (Sclafani and Jackson, 1994). However, the role of *CDC7* in meiosis remains unclear. Recently, a human homolog of yeast *CDC7* was identified and shown to be abnormally overexpressed in some tumor cells (Hess, *et al.*, 1998). Determining the mechanisms of cell cycle control and cell commitment to DNA replication is important for determining the etiology of a number of diseases, including cancer, in which the regulation is altered.

Typically, temperature sensitive *cdc7* mutations prevent yeast from growing mitotically and also prevent the proper completion of meiosis to form four haploid spores (Sclafani and Jackson, 1994). Recently, a mutation called *bob1* was found to allow a *cdc7* mutant to grow, suppressing the altered phenotype of both a *cdc7* temperature sensitive mutation and a deletion of the *CDC7* gene in the mitotic cell cycle. That same *bob1* mutation, however, appeared unable to suppress the meiotic defect of these *cdc7* mutations (Hardy, *et al.*, 1997). If true, then a *cdc7*Δ *bob1* strain would be able to grow mitotically due to the suppression of *cdc7*'s growth defect by *bob1*, but would not be able to complete meiosis, allowing the meiotic phenotype to be examined. An understanding of the role of *CDC7* in yeast meiosis may provide a better understanding of the human version of this gene (Hess, et al., 1998) and its putative role in human meiosis.

The primary goal of this research project was to construct the strains required to test a *cdc7*Δ *bob1* diploid for its meiotic phenotype. Because the deletion allele is 1.3kb smaller than the wild-type *CDC7* allele, a PCR protocol was used to screen the strains for the presence of the *cdc7*Δ allele.

METHODS AND MATERIALS

Strain Construction

Both "a" and "α" haploid *CDC7 bob1* strains were used to construct "a" and "α" *cdc7*Δ *bob1* double mutants using standard two-step gene replacement (Rothstein, 1991). Briefly, a plasmid containing a deletion of 1.3kb of the *CDC7* gene (including part of the promoter and the entire reading frame) was cut with the restriction enzyme *Mlu*I (Promega) and transformed into the *bob1* haploid strains using lithium acetate (Figure 1). Two individual colonies of each of the a and α transformants were grown as large patches on YPD medium which were then replica plated to plates containing the negative selection chemical 5-FOA (Angus Buffers and Biochemicals). The resulting popouts were tested by PCR to determine whether they contained the *cdc7*Δmutation or the wild-type *CDC7* allele.

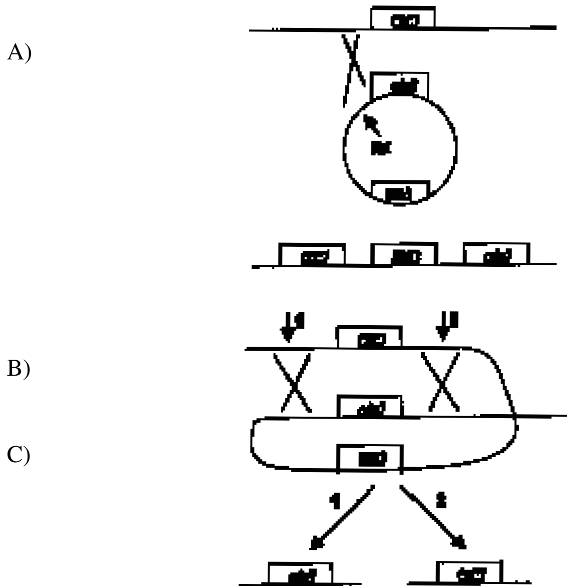

Figure 1. Construction of a yeast strain containing the *cdc7*Δ mutation using two-step gene replacement. A) A plasmid containing the *cdc7*Δ construct (cdc7) and the selectable marker *URA3* was cut with the restriction endonuclease MluI and transformed into a wild-type *CDC7* strain. B) This plasmid spontaneously integrated into the yeast chromosome by homologous recombination, resulting in a strain that had both the wild-type CDC7 and *cdc7*Δ mutant alleles at the chromosomal locus with the intervening *URA3* gene used for selection of the transformants on media lacking uracil. C) After transfer to nonselectable YPD medium, spontaneous intrachromosomal recombination resulted in the loss of the *URA3* gene and one of the two *CDC7* alleles. Only if the crossover occurred on the left side of *CDC7* (#1) did the "popout" contain the desired *cdc7*Δ construct.

PCR Analysis

PCR primers (CTTAAACATTTGTCCCCGG, TCCTGTTTGCAACATGCCC) were constructed that flank both the wild-type and *cdc7*Δ alleles, resulting in PCR products that were 1.9kb and 0.6kb, respectively. The PCR Core System II kit (Promega) was used to perform the reactions. Conditions for optimizing the protocol were obtained using plasmid DNAs that contained either the wild-type *CDC7* gene (pAG4) or the deletion (pAG5). The optimized conditions consisted of 7' at 95ºC, followed by 30 cycles of 1' at 94ºC, 1' at 56ºC, and 1' at 72ºC. To analyze the popouts, about 0.25ul of freshly grown yeast was added to a 20ul PCR reaction mix and run using the same conditions that were used for the plasmid DNA.

RESULTS AND DISCUSSION

Two haploid *CDC7 bob1* strains, an "a" and an "α", were used to construct *cdc7*Δ *bob1* double mutants by two-step gene replacement (Rothstein, 1991). A plasmid containing a 1.3kb deletion of the *CDC7* gene was cut with the restriction enzyme *Mlu*I to linearize it (Figure 1A). This linear DNA, which contained the selectable marker *URA3*, was transformed into a *bob1* mutant strain. These transformants contained the wild-type *CDC7* gene, the *cdc7*Δ mutation, and the *URA3* gene that allowed them to grow on media lacking uracil (Figure 1B). Two individual transformants of each of the a and α strains were grown as large patches on non-selective YPD medium to allow intrachromosomal recombination to occur, resulting in loss of the *URA3* gene and one of the two copies of the *CDC7* genes (Figure 1C). These YPD plates were replica plated to plates containing 5-FOA, allowing growth of these Ura- strains. The "popouts" contained either the wild-type *CDC7* gene as they did initially (Figure 1C, option #2), or they now contained the desired *cdc7*Δ allele (Figure 1C, option #1).

Because the *cdc7*Δ mutation is shorter than the wild-type *CDC7* gene by 1.3 kb, PCR was used to distinguish between the two types of popouts. The conditions for the PCR reactions were initially determined by performing PCR on plasmid DNAs that either contained the wild-type *CDC7* gene (pAG4) or the *cdc7*Δ mutation (pAG5). The results of the successful PCR analysis of the plasmid DNAs is shown in Figure 2.

1 2 3 4

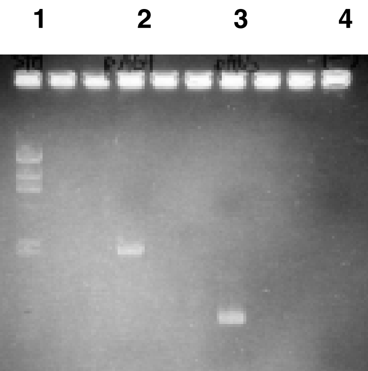

Figure 2. PCR analysis of plasmid DNAs using primers that flank the *CDC7* gene. Lane 1 contains the λ *Hind*III DNA molecular weight standard. Lane 2 contains the 1.9kb PCR product (upper arrow) from the plasmid pAG4 that contains the wild-type *CDC7* gene. Lane 3 contains the 0.6kb PCR product (lower arrow) from the plasmid pAG5 that contains the *cdc7*Δ construct. Lane 4 contains the result from a PCR reaction performed with no DNA added to the tube (negative control). As expected, the PCR product from pAG5 traveled farther through the gel than the PCR product from pAG4, and no band was observed in the negative control lane.

Once the PCR was working using plasmid DNA, the same conditions were used to analyze yeast chromosomal DNA from the popout strains grown on the 5-FOA plates. Using this technique, both an "a" and an "α" haploid were obtained that contained the *cdc7*Δ allele (Figure 3.)

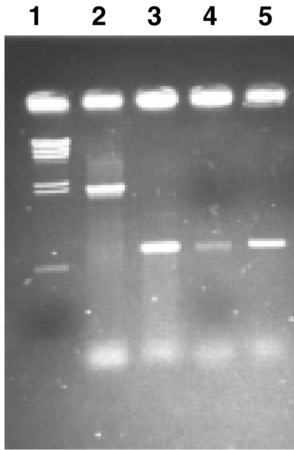

Figure 3. PCR analysis of haploid yeast popouts. Lane 1 contains the λ *Hind*III DNA molecular weight standard. Lane 2 contains the 1.9kb PCR product (upper arrow) from the plasmid pAG4 that contains the wild-type *CDC7* gene. Lane 3 contains the 0.6kb PCR product (lower arrow) from the plasmid pAG5 that contains the *cdc7*Δ construct. Lanes 4 and 5 contain the results from PCR reactions performed on an "a" and an "a" popout, respectively. Both of these haploid strains contain the *cdc7*Δ allele.

Now that the two haploid *cdc7*Δ *bob1* strains have been constructed, they will be crossed together to produce a diploid strain that is capable of undergoing meiosis. That strain, which can grow mitotically because of the suppression of the *cdc7*Δ mitotic phenotype by the *bob1* mutation, will be induced to undergo meiosis so that the meiotic phenotype can be determined. This will be the first careful analysis of the meiotic phenotype of a deletion of the *CDC7* gene and will serve an important role in the understanding of the requirement for *CDC7* in yeast meiosis.

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