

A yeast gene coding for a putative protein kinase homologous to *cdc25* suppressing protein kinase

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A yeast gene termed *YKR* coding for a putative protein kinase was isolated by using the cloned cDNA for rabbit protein kinase C as a hybridization probe. The encoded protein (*YKR*), composed of 380 amino acid residues, shows extensive sequence homology to serine/threonine-specific protein kinases from various species in the approx. 320 C-terminal amino acid residues, strongly suggesting that *YKR* is endowed with a protein kinase activity. The observed homologies to the *cdc25* suppressing protein kinase from yeast, the catalytic subunit of mammalian cAMP-dependent protein kinase, and mammalian protein kinase C were 76, 48 and 37%, respectively. Gene replacement experiments showed that *YKR* itself is not essential for cell proliferation.

Protein kinase; cyclic AMP-dependent protein kinase; Protein kinase C; *cdc25* suppressor gene; (*Saccharomyces cerevisiae*)

1. INTRODUCTION

Phosphorylation of proteins is extremely important in the response of cells to a variety of internal and external signals [1–3]. In yeast, many regulatory genes encode protein kinases [4–8]. For example, cell division cycle genes *CDC28* [4] and *CDC7* [5] have been shown to encode protein kinases. The suppressor gene for *cdc25* [6] also encodes a protein kinase.

In order to verify the existence of a yeast homologue of mammalian protein kinase C (PKC), which is implicated in the signalling system mediated by inositol phospholipid hydrolysis [3],

we screened yeast genomic libraries using fragments of cDNA for rabbit PKC [9] as hybridization probes. Isolation and characterization of one of the most homologous sequences to rabbit PKC cDNA revealed a novel yeast gene *YKR* encoding a putative protein kinase. This protein shows 76% amino acid identity with the kinase region of *cdc25* suppressing protein kinase, PK25.

2. EXPERIMENTAL

Genomic DNA of *Saccharomyces cerevisiae* was prepared from the strain X2180-1A as described [10]. Two sets of yeast genomic libraries were constructed from the complete *EcoRI* or *HindIII* digest of the genomic DNA using the vectors λ gt10 and pUC8, respectively. Screening was carried out using the rabbit PKC cDNA fragments as hybridization probes as described [11] with the following modifications. For low-stringency hybridization conditions, the filters were hybridized in the standard solution at 57°C and washed in $6 \times$ SSC/0.1% SDS at the same temperature.

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The rabbit PKC cDNA fragments used as probes were PKC β 11-C3 (nucleotide nos 1017–1954, R33) and PKC α -C3 (1260–1977, R812) [9] (for nomenclature of PKC see [12]). Suitable overlapping DNA fragments from the cloned genomic DNA were subcloned into pUC or BKS (Stratagene Cloning Systems) plasmids and the DNA sequencing was performed by primed DNA synthesis on the denatured DNA template [13] in the presence of dideoxy nucleotide triphosphates [14]. One-directional serial deletions were carried out by *E. coli* exonuclease III and mung bean nuclease to accomplish overlapping sequencing of fragments subcloned into the BKS plasmid. The entire protein coding region was sequenced with both strands. For gene disruption experiments, the *Hind*III fragment containing *URA3* was inserted into the *Hind*III site at position 367 of the plasmid Y6 (cf. fig.2), and the resultant 3.3 kb *Eco*RI fragment was used for the transfection as described [15].

3. RESULTS

3.1. Identification and isolation of PKC-related sequences in *S. cerevisiae*

In an attempt to identify a yeast homologue of mammalian PKC, we carried out Southern hybridization analysis of the yeast genomic DNA using rabbit cDNA corresponding to the kinase domain of PKC as a probe. As shown in fig.1a, the rabbit cDNA probe detected multiple hybridization bands under low-stringency hybridization conditions, indicating the presence of PKC-related sequences in the yeast genome. Thus we screened a yeast genomic library and isolated a clone, Y6, containing a 2.2 kb *Eco*RI fragment. Southern

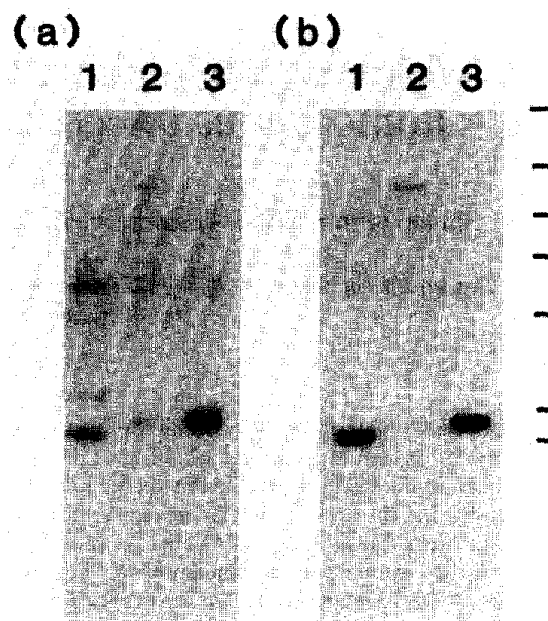


Fig.1. PKC-related sequences in the yeast genome. (a) Yeast genomic DNA was digested with *Hind*III (1), *Bam*HI (2) or *Eco*RI (3) and the blotted filter was hybridized with the rabbit cDNA for PKC under low-stringency hybridization conditions. (b) The same filter was washed and then hybridized with the cloned 2.2 kb *Eco*RI insert of plasmid Y6 under high-stringency hybridization conditions. Size markers are *Hind*III digests of λ DNA.

hybridization analysis of yeast DNA using the cloned *Eco*RI fragment as a probe under high-stringency hybridization conditions (fig.1b) showed that the cloned DNA contains sequences that give strong signals upon hybridization with mammalian PKC cDNA fragments under low-stringency conditions.

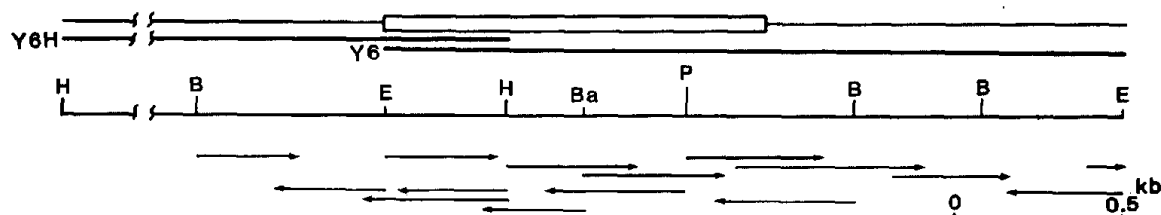


Fig.2. Restriction map and sequencing strategy of a yeast genomic DNA fragment containing the *YKR* gene. The open reading frame encoding *YKR* is boxed. The two overlapping genomic clones Y6 and HY6 are shown by thick lines. Arrows indicate the direction and length of individual sequence determinations. Restriction enzymes used were *Hind*III (H), *Bgl*II (B), *Eco*RI (E), *Pst*I (P) and *Bam*HI (Ba).

[illegible]

Fig. 3. Nucleotide and deduced amino acid sequences of the *YKR* gene. The in-phase termination codon, TATA-like sequences and AATAAA sequence are underlined.

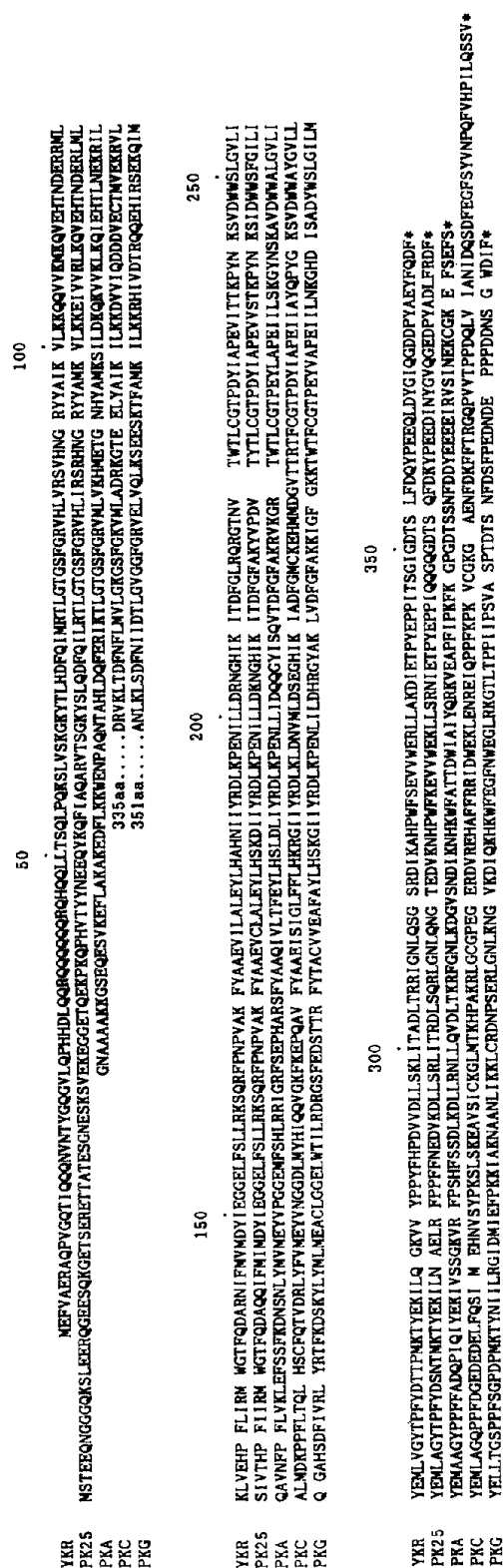


Fig. 4. Sequence alignment of YKR with homologous protein kinases. The complete sequence of YKR is aligned with those of *cdc25* suppressor protein kinase (PK25) and the catalytic subunit of bovine cAMP-dependent protein kinase (PKA), as well as those of the kinase domains of rabbit protein kinase C α (PKC) (amino acids 336–672) and bovine cGMP-dependent protein kinase (PKG) (amino acids 352–670) [20].

3.2. Nucleotide sequence of *YKR*

The nucleotide sequence of the 2.2 kb *EcoRI* fragment was determined. In addition, the overlapping 10 kb *HindIII* fragment was also cloned and the sequence of a portion (1 kb) was determined (figs 2,3). There is a long open reading frame which encodes a protein of 380 amino acid residues. This open reading frame is preceded by an in-phase termination codon at -96 and TATA-like sequences, AAATATA (-67) and ATAAT-TAA (-145), and followed by a sequence AATAAA (1642). The homology of this gene (*YKR*) to the rabbit PKC cDNA fragment, used as the hybridization probes, was found within this open reading frame. A sequence of 363 bp (corresponding to 121 amino acids) at nucleotide positions 436-798 (amino acids 146-266) shows about 60% sequence identity to the corresponding region of rabbit PKC cDNA (α and β) at the nucleotide level (a 9 bp insertion was introduced into *YKR* at position 663).

3.3. A putative protein kinase *YKR*

The open reading frame predicts a protein (*YKR*) of 380 amino acid residues of M_r 44269. The sequence contains all the conserved characteristics of serine/threonine-specific protein kinases: a sequence for an ATP-binding site, GXGXXG.....AXKXL starting at G-77, and a sequence for a catalytic site, RDL...DFG...APE starting at R-192. Thus, it is most likely that *YKR* possesses protein kinase activity. The sequence of *YKR* can be aligned with those of other protein kinases as shown in fig.4. The highest homology was to PK25 [6] as shown in table 1. Mammalian protein kinases, such as PKA [16] and PKC [9], also showed high degrees of sequence homology to *YKR*. As is the case with other protein kinases, such as PK25 and PKA, *YKR* possesses an N-terminal extension of approx. 60 amino acid residues in addition to the kinase region. The N-terminal extension is highly rich in Gln. A Gln-rich sequence is also found in a homeo-box-containing protein, *en*, from *Drosophila melanogaster* [17].

3.4. Gene disruption experiments

In order to examine whether the *YKR* gene is essential for cell growth, gene disruption experiments were carried out. A 1.1 kb *URA3* fragment was inserted into the *HindIII* site of *YKR*

(fig.5a). The *HindIII* site exists at amino acid position 123 and the insertion of the *URA3* DNA separates the ATP-binding site from the catalytic site, most likely resulting in the loss of protein

Table 1

Percentage amino acid sequence homologies between protein kinases^a

	PK25	PKA	PKC	PKG
<i>YKR</i>	76	48	37	39
PK25		48	36	39
PKA			37	39
PKC				41

^a Only the kinase domains corresponding to amino acid nos 63-380 of *YKR* (see fig.4) were considered

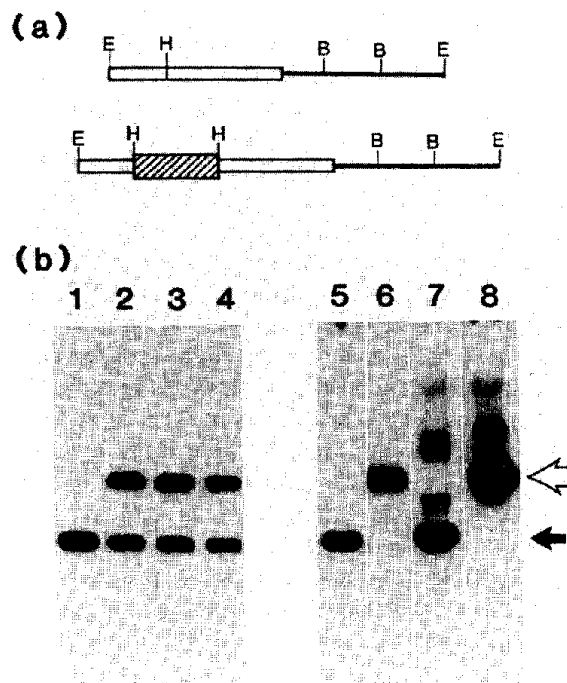


Fig.5. (a) Restriction map of the disrupted *YKR* gene (see also fig.2). Open boxes indicate the protein coding regions. The hatched box indicates the *URA3* sequence. (b) Southern hybridization analysis of the *YKR* gene in the segregated spores. Genomic DNAs were digested with *Bgl*II and probed with the *EcoRI*-*Pst*I fragment (see fig.1). Lanes: 1, wild-type diploid YK21; 2-4, *URA*⁺ transformants; nos 3, 4, 2, respectively; 5-8, four spore colonies from no.2. White and black arrows indicate the positions of the normal and the disrupted *YKR* gene, respectively.

kinase activity. One of the chromosomal genes of diploid cells was replaced by the disrupted gene and used for tetrad analysis. Tetrads derived from 6 transformants all showed 2:2 segregation for the uracil auxotrophy. Southern hybridization analysis of chromosomal DNA from diploid transformants and haploid segregants of a tetrad is shown in fig.5b. Both the haploid mutants and diploid cells homozygous with respect to the mutated gene grew normally, and homozygous diploid cells showed normal sporulation (not shown). These data indicate that the *YKR* gene itself is not essential for cell growth.

4. DISCUSSION

We have isolated a yeast gene (*YKR*) encoding a novel putative protein kinase (YKR) using rabbit PKC cDNA fragments as hybridization probes. The predicted amino acid sequence of the encoded protein YKR shows higher sequence homology to mammalian PKA (48%) than to mammalian PKC (37%). Computer analysis of the nucleotide sequences for *YKR* and rabbit PKC cDNA showed that the rabbit cDNA probe recognizes the sequence corresponding to the catalytic site of YKR where amino acid sequences IIYRDLKPEN-ILLDRNGHIKITDFG and CGTPDYIAPE are located. These sequences are highly conserved between YKR and PKC, whereas the sequences around the ATP-binding site and the latter half of the kinase domain are somewhat more divergent. This explains why we could isolate such a distantly related sequence. Multiple bands detected in the genomic Southern experiments (fig.1a) using the rabbit cDNA fragments for PKC as probes may indicate the existence of additional genes encoding protein kinases related to PKC in the yeast genome.

YKR shows the highest sequence homology to PK25. Although they differ significantly in their N-terminal sequences, the following kinase regions are highly homologous (76%), indicating that these two proteins are closely related and distinct from the other protein kinases in the yeast such as *CDC28* [4], *CDC7* [5] and *STE7* [7]. It is tempting to speculate that these two kinases have analogous functions in the cellular protein kinase cascades. Disruption of the *YKR* gene did not cause any apparent effect on cell growth and spore formation.

One possible explanation is that the function of *YKR* was substituted for by homologous genes, such as the *cdc25* suppressor.

High sequence homology between yeast PK25/YKR and mammalian PKA (48%) suggests their close evolutionary relationship. Thus, YKR, as well as PK25 [6], is a candidate for the yeast version of PKA [18]. From the analysis of *cyr2* mutants [19], *CYR2* is suggested to encode PKA [18], although the *CYR2* gene itself has not been analysed. As shown by the gene disruption experiments, *YKR* as well as *cdc25* suppressor gene [6] is different from *CYR2*. The biochemical relationships between PKA and YKR (or PK25) remain to be established. During the preparation of this manuscript, we learned that Toda et al. identified three yeast genes which encode proteins related to mammalian PKA.

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