

The *KNS1* gene of *Saccharomyces cerevisiae* encodes a nonessential protein kinase homologue that is distantly related to members of the *CDC28/cdc2* gene family

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Summary. A novel protein kinase homologue (*KNS1*) has been identified in *Saccharomyces cerevisiae*. *KNS1* contains an open reading frame of 720 codons. The carboxy-terminal portion of the predicted protein sequence is similar to that of many other protein kinases, exhibiting 36% identity to the *cdc2* gene product of *Schizosaccharomyces pombe* and 34% identity to the *CDC28* gene product of *S. cerevisiae*. Deletion mutations were constructed in the *KNS1* gene. *kns1* mutants grow at the same rate as wild-type cells using several different carbon sources. They mate at normal efficiencies, and they sporulate successfully. No defects were found in entry into or exit from stationary phase. Thus, the *KNS1* gene is not essential for cell growth and a variety of other cellular processes in yeast.

Key words: Protein kinase – Yeast – *CDC28* – Cell cycle

Introduction

Protein phosphorylation has been implicated in a wide variety of cellular functions as the mechanism by which both enzymatic and non-enzymatic activities of proteins are controlled (Krebs 1986). Events influenced by protein phosphorylation include enzyme regulation, assembly of cytoskeletal components, and cell cycle control. The *cdc2* gene of *Schizosaccharomyces pombe* and its closely related homologue in *Saccharomyces cerevisiae*, *CDC28*, encode protein kinases that are necessary for cell cycle control at G1/S and G2/M transitions (Wheals 1987). Several additional homologues of *CDC28* have been identified in *S. cerevisiae*. These include *KIN28*, an essential gene in yeast (Simon et al. 1986), *KSS1* and *FUS3*, two genes involved in growth control (Courchesne et al. 1989; Elion et al. 1990), and *PHO85*, a gene involved in negative regulation of the *PHO* genes (Toh-e et al. 1988). More distantly related members of this fami-

ly include casein kinase II the catalytic subunits of which are encoded by two genes, *CKA1* and *CKA2* (Chen et al. 1988; Padmanabha et al. 1990). All of these proteins are thought to phosphorylate serine and threonine residues and in most cases this has been directly demonstrated.

It is presently unclear just how large the *CDC28* gene family is, and whether or not most members of this family participate in cell cycle regulatory functions is also not known. Described below is a novel protein kinase homologue which was identified while studying the organization of the *SPA2* region on chromosome XII of *S. cerevisiae* (Snyder 1989). This putative kinase gene is a distantly related member of the *CDC28* gene family.

Materials and methods

General methods. Yeast growth media and genetic manipulations were as described in Sherman et al. (1986). All bacterial media and manipulations were as described in Maniatis et al. (1982). Yeast strains used are listed in Table 1.

DNA sequence analysis. Individual subclones in either M13mp18 or mp19 (Yanisch-Perron et al. 1985) were sequenced by the dideoxy chain-termination method (Sanger et al. 1977) using the Sequenase kit (United States Biochemical) according to the recommendations of the manufacturer.

Gene disruption. A *XhoI-SpeI* DNA fragment containing a full-length *KNS1* gene was cloned into pUC18. The resulting plasmid was designated p217. *kns1-Δ1* was constructed by partially digesting this plasmid with *StyI* and *XbaI*, the ends were made flush by treatment with the large fragment of DNA polymerase I plus dNTPs, and ligated to a 1.1 kb *HindIII* fragment containing the yeast *URA3* gene. The ends of the *URA3* DNA fragment had previously been made flush by treatment with the large fragment of DNA polymerase I. Similarly, *kns1-Δ2* was

Table 1. Yeast strains

Strains		genotypes						
Y264	α	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>his3-Δ200</i>	<i>trp1-901</i>		
Y428	<i>a</i>	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>trp1-901</i>	<i>leu2-Δ98</i>		
Y270	<i>a</i>	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>his3-Δ200</i>	<i>trp1-901</i>		
Y650	α	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>his3-Δ200</i>	<i>trp1-901</i>		
	<i>a</i>	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>his3-Δ200</i>	<i>trp1-901</i>	<i>kns1-Δ1::URA3</i>	
Y651	α	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>his3-Δ200</i>	<i>trp1-901</i>	<i>KNS1</i>	
	<i>a</i>	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>his3-Δ200</i>	<i>trp1-901</i>	<i>kns1-Δ2::URA3</i>	
Y652	α	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>his3-Δ200</i>	<i>trp1-901</i>	<i>KNS1</i>	
	<i>a</i>	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>his3-Δ200</i>	<i>trp1-901</i>	<i>kns1-Δ3::URA3</i>	
Y657	α	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>his3-Δ200</i>	<i>trp1-901</i>	<i>KNS1</i>	
	<i>a</i>	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>his3-Δ200</i>	<i>trp1-901</i>	<i>kns1-Δ1::URA3</i>	
Y659	<i>a</i>	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>HIS3</i>	<i>trp1-901</i>	<i>leu2-Δ98</i>	<i>KNS1</i>
Y660	α	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>his3-Δ200</i>	<i>trp1-901</i>	<i>LEU2</i>	<i>kns1-Δ1::URA3</i>
	<i>a</i>	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>trp1-901</i>	<i>leu2-Δ98</i>	<i>his3-Δ200</i>	<i>kns1-Δ1::URA3</i>
Y661	α	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>trp1-901</i>	<i>leu2-Δ98</i>	<i>his3-Δ200</i>	<i>kns1-Δ1::URA3</i>
Y662	α	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>trp1-901</i>	<i>leu2-Δ98</i>	<i>his3-Δ200</i>	<i>kns1-Δ1::URA3</i>
Y663	<i>a</i>	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>trp1-901</i>	<i>leu2-Δ98</i>	<i>his3-Δ200</i>	<i>kns1-Δ1::URA3</i>
Y664	<i>a</i>	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>trp1-901</i>	<i>leu2-Δ98</i>	<i>his3-Δ200</i>	<i>kns1-Δ1::URA3</i>
Y665	α	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>trp1-901</i>	<i>leu2-Δ98</i>	<i>his3-Δ200</i>	<i>kns1-Δ1::URA3</i>
	<i>a</i>	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>trp1-901</i>	<i>leu2-Δ98</i>	<i>his3-Δ200</i>	<i>kns1-Δ1::URA3</i>
	α	<i>ura3-52</i>	<i>lys2-801</i>	<i>ade2-101</i>	<i>trp1-901</i>	<i>leu2-Δ98</i>	<i>his3-Δ200</i>	<i>kns1-Δ1::URA3</i>

All strains are congenic and in an S288C background. Y664 and Y665 are derived from different isolates

constructed by replacing the *StyI* fragment with the *URA3* DNA fragment, and in *kns1- Δ 3* the internal *XbaI* fragment was replaced with the *URA3* gene. The orientation of the *URA3* fragment in all the constructs was determined by restriction map analysis. Linearized DNA fragments containing the mutations were generated by digestion with *EcoRI* and *HindIII* and transformed into diploid yeast (Y270) (Ito et al. 1983; Rothstein 1983). The heterozygous diploids were then sporulated and the asci dissected (Sherman et al. 1986). Disruption of the *KNS1* genomic locus was confirmed by gel blot analysis of genomic DNA as described by Maniatis et al. (1982).

Phenotype studies. The growth rates of *KNS1* and *kns1- Δ 1* strains were determined in YPD rich media. The doubling time was determined by measuring the OD₆₀₀ of exponentially growing cultures every 4–5 h starting at OD₆₀₀ = 0.2, for a total of 24 h. Mating efficiencies were determined by mixing equal numbers (2.5×10^6 cells; 1 ml total volume) of exponentially growing *MATa* and *MAT α* cells and allowing them to stand upright in an 18 mm tube without agitation at 30° C. After 5 h, the cultures were vortexed vigorously and serial dilutions of the cell suspensions plated on selective media to determine the number of diploids. For each mating experi-

ment between 150 and 280 diploids were counted. Sporulation efficiencies were determined essentially as described by Sherman et al. (1986). Stationary phase cells were washed in 1% potassium acetate and then incubated in 1% potassium acetate at 30° C for 3 days. The percentage of asci present was then determined by examining appropriate dilutions under a microscope.

The induction of thermotolerance in the different strains was determined in the following manner. Exponentially growing cells were diluted into fresh YPD media to OD₆₀₀ = 0.5 and then grown at 30° C. Aliquots were periodically removed and appropriate dilutions plated on YPD rich media either with or without a heat treatment. The portions that were heat-treated were transferred to glass 18 mm test-tubes and incubated at 53.5° C for 5 min. The cells were then cooled rapidly on ice and plated. Between 500 and 1500 cells were counted at different incubation times and the percentage survival determined.

RNA blot analysis. Total RNA was prepared by K. Voelkel-Meiman from vegetatively growing diploid yeast cells by the method of Schmitt et al. (1990). Between 7 and 15 μ g of the RNA was then subjected to electrophoresis in a 1.5% agarose gel in the presence of formal-

dehyde (Maniatis et al. 1982), transferred to a nylon membrane according to the recommendations of the manufacturer (Zeta-probe, Bio-Lab Laboratories), and probed with a 1.5 kb *Bgl*III *KNS1* DNA fragment labelled with [α - 32 P]ATP (Feinberg and Vogelstein 1983). After autoradiography, the probe was removed from the filter by incubation in 0.015 M NaCl, 0.0015 M sodium citrate at 95° C, and the blot was rehybridized with a 0.5 kb yeast actin probe (a gift from S. Roeder, Yale University). The actin probe hybridized with a 1.5 kb yeast RNA at less than one-third the intensity of the *KNS1* probe.

KNS1-lacZ fusions. In order to determine whether the kinase protein was expressed, fusions between *KNS1* and the *lacZ* gene of *Escherichia coli* were constructed. A 1.5 kb *Bgl*III fragment which contained 341 nucleotides located upstream of the putative initiator methionine and extended into the kinase domain of *KNS1*, was inserted into the *Bam*HI site of plasmid B5 (a gift from S. Roeder, Yale University; plasmid B5 contains a truncated *lacZ* gene inserted into YCp50). In the proper orientation, this produces an in-frame fusion between *KNS1* and *lacZ*. Recombinants containing the *Bgl*III *KNS1* fragment inserted in both orientations were isolated and transformed into yeast.

β -Galactosidase assays were performed as follows. Patches of yeast cells containing the plasmids were allowed to grow overnight at 30° C, after which a piece of Whatman 3 mm filter paper was placed over the patches until the paper was completely moist. The filter paper was removed with the yeast cells adhering to it; the paper was then exposed to chloroform vapours for 5 min to lyse the cells. The paper was then placed on a petri dish containing the assay medium [0.7% agar, 0.1 M sodium phosphate, pH 7.0, 1 mM MgSO₄, 120 μ g/ml 5-bromo-4-chloro-3-indolyl- β -D-galactoside (X-Gal)], and incubated at 30° C for 20 h until color development was complete. The assay was performed in duplicate.

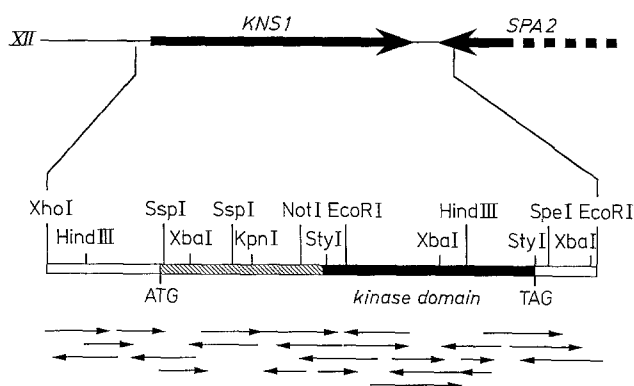


Fig. 1. Restriction map of *KNS1* region and sequencing strategy. Large arrows indicate the direction of transcription of the *KNS1* and *SPA2* genes respectively. Arrows beneath the map indicate the direction and extent of DNA sequencing as determined by the dideoxy chain-termination method (Sanger et al. 1977), using M13 vectors (Yanisch-Perron et al. 1985)

Results

A protein kinase homologue lies 3' to the SPA2 gene of yeast

During the sequencing of the *SPA2* gene of *S. cerevisiae*, our analysis extended into the 3' flanking region (Figs. 1 and 2). An open reading frame was identified which could potentially code for a protein the sequence of which shows significant similarity to those of a number of protein kinases. This gene was named *KNS1* for kinase next to *SPA2*. The *KNS1* open reading frame is 2208 bp in length and lies 339 bp away from the *SPA2* open reading frame. There are two methionine codons located at the beginning of the *KNS1* ORF, one at nucleotide 655 and the other at 703. It is likely that the second methionine is the one used for translation initiation since sequences near the second methionine more closely resemble the consensus sequences surrounding the initiator methionine of other eukaryotic genes (Kozak 1983). If this is the case, then the *KNS1* translation product is 720 amino acids long. It is unlikely that the *KNS1* gene is spliced within the coding region, as this sequence lacks the TACTAAC sequence found close to the 3' end of all yeast introns (Teem et al. 1984). The predicted amino acid sequence of the *KNS1* gene was compared with those in the Genbank data base using the FASTA programs (Pearson and Lipman 1988); the last 420 amino acids of the putative *KNS1* gene product have significant amino acid sequence similarity to many protein kinases. A more detailed comparison of the *KNS1* sequence with the data of Hanks et al. (1988) confirmed that the protein encoded by *KNS1* contained every invariant and semi-variant residue present in all known protein kinases (Figs. 3A and 4). These conserved residues span ten domains (Hanks et al. 1988).

The KNS1 protein kinase is most similar to the CDC28/cdc2 kinase family

The sequences of a large number of protein kinases have been grouped into families based on their sequence similarities (Hanks et al. 1988). The closest matches of the *KNS1* predicted sequence were with the *CDC28* and *FUS3* genes of *S. cerevisiae*, and *cdc2* gene of *S. pombe* (Fig. 4). The *KNS1* protein shares a number of residues characteristic of the members of this family (indicated by asterisks in Fig. 4). The number of identical residues shared with the *CDC2*, *CDC28*, and *FUS3* proteins is 36%, 34%, and 32%, respectively over a ca. 350 amino acid stretch. The number of similar residues between the *KNS1* protein and these three proteins is 60%–70%. The *KNS1* protein, like other members of the *cdc2/CDC28* family, contains a large domain X in its carboxy-terminus (see Figs. 3 and 4).

Although the *KNS1* protein sequence is most similar to that of members of the *CDC28* family, there are several noteworthy differences. First, the *KNS1* protein lacks the conserved tyrosine residue at position 308; a tyrosine residue at this position in the *cdc2* protein of *S. pombe*

1 C T G G A G G C T G T G C G G G C C A G T G C C C G T T T C T T T T T T T C T A G G G G C G G C A A G G G G C C A A A G G G G T C A G C G T G A T T G G G C G G T T T T C C A C A A A G C T T T A G T C T C T C C T C A C
116 G C C T T G G C T T A G T G A G T G C C G G A G T T A A A G T T G C G A G G A A C A C A C A G C G A A G G G A A A A A G C T T T G G C G T G C T T A A C G A T A T G C C T T A T G C C C G G T T G C A A T G C T G G T T A G T
231 T T C G A T T A C G A T T T C G A T T T G A A T C T T G C C A C A T C C C A C C C T T T T T T A T C T G A G A T C G T G C G T T G G A T T T T A T T A T G A A G T C C T A T A T T A G A A T A A A A G T
346 T A G T A T T T A C T T C C C A G A T C T A C C G G A T T T A C A A A G T A T T G T T A C C T C A C C A A G T G A A A G G A A C A G T G C A G T A A C T C C T C G T G C T T A C G C T A A C T A A A T A G A T A T T A
461 T T C A C G A A A T A C T G C C A G A T A G C A A G C A A C C C A A A A A A A A A A A A A A A A G A A A A A A A A A A A G A A A A A A A A A A A G A G T G A A A G A A C A T A G T A T T T A C C A A G T C G
576 A A G A T T T A G C A C A C A A A A G A C T A A T C T A C A C G C A T A C T G C T C T T C A T T A T T G C T T A T C T T C C G C G T A C C T T A G T T A T G T C A C A G A A T A T T C A A A T T G G C A C T A G A A A A C G T
691 T C A A G A G C A A A T A T G A A A A T A A T S C C A T A C T A C G G C C C T G C T A A C A A C A C A G T C T A A C A A G A A C C C A A A A A C C C A A A A T T T T T G G A T A A T T T C G A A G A A
1 M N N S T T T T G P A N N T S S N K T F L D N F E E
778 A C G C G C A C A A A C A A G C T G C T G C G A G A T G T T T G C T C G G C A A A A T T C T T T C T A A C A G A C A A T C T G A G G A A C A G N C T A G A C C T G A A T
26 T R T N K L L D E M F A R Q N S F L T D N L R N S L D L N
865 C A A G C A G A C A A T C C G C T C C G T C C T C G G C A A C A C C A A C A G C A G T T G T T C T T G A C A A T G A A A A T G C C A T A G A A C T A G A C G A G G A G C C G
55 Q A D N P L R P R Q H Q H Q L F L D N E N A I E L D E E P
952 C G T A T C A T T A A C A C C A C A A T C A A T A A C A G T A A T A A T C A T A A C A G T A G T C G A G T G C A G A G G A T G C T G A C G A T G A C A T C A T T T T A T C
84 R I I N T T I N N S N N H N S S R V D E D A D D I I F I
1039 A A A G A G C A G C C A A T T C A G T T T T C G T C A C C T T T G A T T C T G C C T A G T A G C A G C A G T A T C A A T A A C A A C A A T A A T A T T G T T A C T T C A A A T
113 K E Q P I Q F S S P L I L P S S S S I N N N N N I V T S N
1126 A A T C C A G G G T G C G G T A C C G C A G C T A C A G C A A T A G T A C G T A T A T C A C G A C A C C A A A G A A A T T C A A G A A G C A A A G A C C A T C T C A C T G
142 N P G C G T A A A T S N S T Y I T T P K K F K K Q R T I S L
1213 C C T C A G T T G C C T C T A T C G A A G C T G A G C T A C C A A T C G A A C T A T T T A A T G T T C C T G A T C A A A C C A A C G C T A T C G T G C C A A G A G T A C G
171 P Q L P L S K L S Y Q S N Y F N V P D Q T N A I V P R V T
1300 C A A A C T G A A A A T G A G C T G T T G C A T C T A C G G G T C G T G T G C A A A G A C T C T G A G G G C A A C A A G G C A G T G A A T C T C A C G A T T G C T C A C
200 Q T E N E L L H L T G S C A K T L E G N K A V N L T I A H
1387 A G C A C T T C T C C C T T T T C C A A C C C A C C A G C A A A T A G C T C C C T G C C T C A A T C C A A C C T C A A G A A A C A A A T T G G T T C T T C A C T A C G G
229 S T S P F S N P P A Q I A S L P Q S N L K K Q I G S S L R
1474 A A A T T T A C A T C A A A C G G C T C T C A G A A A G C G A T C A T C C A A T A A A T C A A A T T T C A A A A C G G A C A A A G A C G G T C A C T A C G T T A C C A G
258 K F T S N G S S E S A S S N K S N F K T D K D G H Y V Y Q
1561 G A A A A C G A C A T A T T T G C C A G T G C C G G C G C G T T T G T T G T G A A G G A C C T A T T G G C C A A G G C A C G T T T G G T A A A G T A C T T A A A T G C A T C
287 E N D I F G S G G R F V V K D L L G Q G T F G K V L K C I
1648 G A T A A C A A G T A T G A A C C T A A T T A C G T G G T G T A A A G T G A T A A G G C T G T A G A T A T A G A G A A G C C C A A A C A G A A C T A A G A
316 D N K Y E P N Y V A V K V I R A V D R Y R E A A K T E L R
1735 A T T C T A C A G A C T A T C C T G A A T A A T G A C C C T C A A G G T C A G T T C C A G T G C C T C T T G C T A A G G A G T G C T T C G A T T A C A A A A A T C A C A T T
345 I L Q T I L N N D P Q G Q F Q C L L L R E C F D Y K N H I
1822 T G T T T G T G A C A G A T C T A T A C G G C A G G T C C A T T A C G A T T T A T G T G C T C C A A C G G C A T T G C A G G T T C C C G G C T C T A T A T C A G G C
374 C L V T D L Y G R S I Y D F M C S N G I A G S P A L I S G
1909 C A T T G C A G A C A G C T A A T A G A T C T G T G C T T T G C A C G A T T T G G C A T A A T A C A C C A G G A T T T G A A C C A G A A A T A T C C T G A T T
403 H C R Q L I R S V C F L H D L G I I H T D L K P E N I L I
1996 T G T G A C G A A A C C A T A T T G C T C A A A A T G C C T T T G A A A A C C G T A C A G T C G C T A A G C A A G A G A C G C C G T G A A G C C A G T A A G G G A A G
432 C D E T H I A Q K L P L K T V Q S L S K R R R E A S K G K
2083 C G C A A A A T C T T G A A A A T C C A G A A T C A A A A T C A T T G A T T T C G G T A G C G C A A T T T C A T T A C G A A T A T C A T C C T C C T G T A A T A T C C
461 R K I L K N P E I K I I D F G S A I F H Y E Y H P P V I S
2170 A C T C G T C A C T A T A G A G C C C G G A A T T G T C T T G G C T T G G C T G G T G C G T T C C C T G C G A C A T T T G G T C C A T C G G T G T C T A G T A
490 T R H Y R A P E I V L G L G W S F P C D I W S I A C V L V
2257 G A A C T G T T A T T G G C G A A T C T C T T A C C C A T T C A T G A A A A T C T A G A A C A T A T G C T A T G A T G A A C G A A T C A A T G G A A C C C T T C
519 E L V I G E S L Y P I H E N L E H M A M M Q R I N G T P F
2344 C C C A C A G A C A T T A T T G A T A A G A T G T T T A C A A A T C T A A A C A T A A A T T G G G C A A C T C T C C A T C A G A C C T A A A T C A A C G G T G A T A A G
548 P T D I I D K M F Y K S K H K L G N S P S D L N S T V I K
2431 C A T T T C G A C A G A A A A C T T T A A G C T T A C A A T G G C C T G A A A A A T A A A C G C G G A G A T A C C A T A A C C A A A G A A G T C A A T G A A A A G G
577 H F D R K T L S L Q W P E K N K R G D T I T T E K S M K R
2518 G T C T T G C A G T C A T G T G A T C G C T T A G A T A T T A C A T C A A A G G T C T T G A A A C A G G A T T A C G G C G A T A G C T T A A G C A T C A A T T G G A A T
606 V L Q S C D R L D I Y I S K V L K Q D Y G D S L S I N W N
2605 C T A C C G C C G G A G A A A A C T G G T C T T T G A T A A T T C G A A A T T G C A T G G A A A A G A C A A C A T T C C T C T T C C T C A A C T A C T G A T
635 L P P E K N W S L I N S K L A W K R Q T H S S S S S S T T D
2692 G A A C T C G A T A A G G A A A C T T T C T T G T T T G G T A C T G G T T C A T C A T C T A G G A A A A T G T T T G A G T T C G A C C C A A A A A A G G A T T
664 E L D K E T F L F W Y W F I D L L R K M F E F D P T K R I
2779 A C C G C A A A G G A C G C T C T G A C C A T G A A T G G T T T A A T C T G G C A T A C T G A T G A T G G T A T T G C A A C T T A T A A T A A C C A A G G A T A G
693 T A K D A L D H E W F N L G I L D D G I A T Y N N T Q G Trm
2866 T G G G T G T T C T G T T C T A C T T T T C T T C T T T T T T T T C A C A T C T T T A C T A G T T C C G T T T T T T T T T T A A C T C T T T T T C A T T T C C G G T T T T A A A T A T T T C T T A A T
2981 T T C T A T A T T C A A T A A A A T T C T T C C T A C A T A T A T A T C T T C A T A T T C A A C A C A T T A T A G T A T T T T A C T A A T T T T T G T T G T T T A A T A T A C T A T A A T A T A T T A T T A C T G C T
3096 T T A A C T A C T A T A T T A T T A T A T A T A T A T A T A T A T A T G C A T A C A T A A T A C A T T C T A T T G A T T G C T T T G C T T C C T T T C T T C C T C T A G A

Fig. 2. DNA sequence of the *KNS1* region. The translation of the *KNS1* open reading frame starting at the second methionine is shown beneath the DNA sequence. The first and second methionine residues are indicated in italics. Numbers adjacent to the nucle-

otide sequence refer to the nucleotide position; numbers in *italics* indicate the respective amino acid residue positions downstream of the second methionine

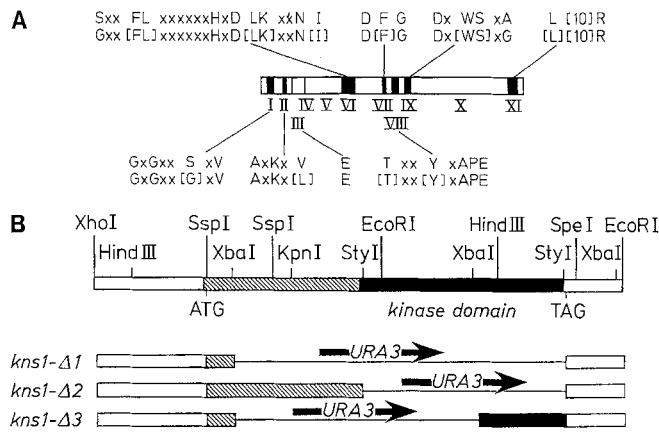


Fig. 3. A Amino acid residues in the predicted KNS1 protein (top line) that are present in other protein kinases (bottom line). Regions conserved in all known kinases are indicated by the filled segments. X=any residue. Residues in the lower line are in all protein kinases; residues in brackets are usually found in other protein kinases, but other residues are sometimes found. **B** *kns1* deletions in which the *URA3* gene has been substituted for portions of the *KNS1* gene. *kns1-Δ1* and *kns1-Δ3* begin at codon 51. *kns1-Δ2* begins at codon 305, which encodes the first conserved residue of the kinase domain indicated by the filled segment; the remaining fragment of KNS1 is indicated by shading. *kns1-Δ1* and *kns1-Δ2* deletions end at exactly the last codon of the *KNS1* open reading frame

has been shown to be phosphorylated in vivo (Gould and Nurse 1989). The KNS1 protein also contains 305 amino acids upstream of the putative kinase domain; an upstream domain of comparable size is not present in other members of the CDC28 family. Although we do not yet know its function, this upstream region may be involved in the regulation of kinase activity, via interaction with substrates and/or interaction with as yet undiscovered subunits. This region also contains a possible nuclear localization sequence, KKFKKQR, beginning at residue 176 (see Kalderon et al. 1984 for examples of other nuclear localization sequences), suggesting that perhaps the KNS1 protein functions within the nucleus.

Throughout the KNS1 protein sequence there are several homopolymer tracts of amino acids, for example, at positions 142 (SSSS), 147 (NNNNN), and 671 (SSSSS) based on numbering relative to the first methionine of the *KNS1* ORF (Fig. 2). The latter polyserine stretch is part of a potential recognition sequence for casein kinase II (SSSSSTTDE) (Tuazon and Traugh 1989). This raises the possibility that the *KNS1* protein itself may be regulated by phosphorylation.

The KNS1 protein is not essential for cell viability in yeast

To help determine the role of the *KNS1* protein in yeast, three deletion mutations were constructed within the *KNS1* region, and these are shown in Fig. 3B. *kns1-Δ1* is a substitution of *URA3* for a large region of *KNS1*; only 51 codons of *KNS1* coding sequence lie upstream of the *URA3* gene, assuming the second methionine co-

don is the initiator methionine. The *kns1-Δ1* deletion ends exactly at the last codon of the open reading frame. *kns1-Δ2* contains a precise substitution of the *URA3* gene for the 1274 bp coding segment of the kinase domain; this deletion begins at the codon for the first conserved residue of the kinase domain (residue 305) and also ends exactly at the last codon of the open reading frame. A third deletion, *kns1-Δ3*, removes an intermediate amount of DNA relative to *kns1-Δ1* and *kns1-Δ2*.

Each of the different *kns1* alleles were introduced into diploid yeast strains, and the resulting heterozygotes were sporulated. For all three alleles, four viable spores were obtained, and the markers segregated 2 Ura⁺: Ura⁻, indicating that the *KNS1* gene is not essential for cell viability. Proper substitution at the *KNS1* locus was determined by gel blot analysis of genomic DNA (not shown). *kns1-1* mutants grow at the same rate as wild-type cells in rich media (YPD) at 30° C and at 11° C (see Table 2 for 30° C results), and have the same proportion of unbudded, small budded, and large budded cells. *kns1* cells also form normal-sized colonies when incubated at either 18°, 30° or 37° C, or when incubated on plates containing either galactose or glycerol as their primary carbon source. *kns1* cells mate at approximately the same efficiency as wild-type cells (Table 3), and *kns1/kns1* diploids successfully undergo meiosis to form four-spored tetrads at equivalent levels to wild-type cells (Table 4).

Since *CDC28* homologues participate in control steps of the cell cycle we also examined entry into and exit from stationary phase. Wild-type yeast cells acquire thermal tolerance upon entry into stationary phase (see Snyder 1989). When cultures of yeast cells were incubated for up to 2 days, *kns1* mutant cells acquired thermal tolerance at a rate equivalent to that of wild-type cells, indicating that entry into stationary phase was normal (Fig. 5). Exit from stationary phase into a proliferative cycle was also tested. Incubation of wild-type cells in the presence of glucose caused a rapid loss of cell viability; the loss of cell viability is suggested to be caused by initiation of cell growth in media that cannot sustain growth (Granot and Snyder 1991). Glucose-induced loss of cell viability occurred in *kns1* mutant cells at a rate similar or faster to that in wild-type cells (not shown), indicating that exit from stationary phase was normal in *kns1* mutant cells. Thus, *kns1* cells appeared normal compared to wild-type cells in all aspects examined.

The KNS1 protein is expressed in vegetatively growing yeast cells

The lack of any observable defect in *kns1* cells might be due to failure of the *KNS1* gene to produce a transcript or protein in yeast. To determine whether the *KNS1* gene is expressed, two types of experiments were performed, RNA blot analysis and *KNS1::lacZ* fusions. Total RNA from vegetatively growing yeast cells was prepared and probed with a *KNS1* probe (see the Materials and methods). As shown in Fig. 6, a 2.5 kb tran-

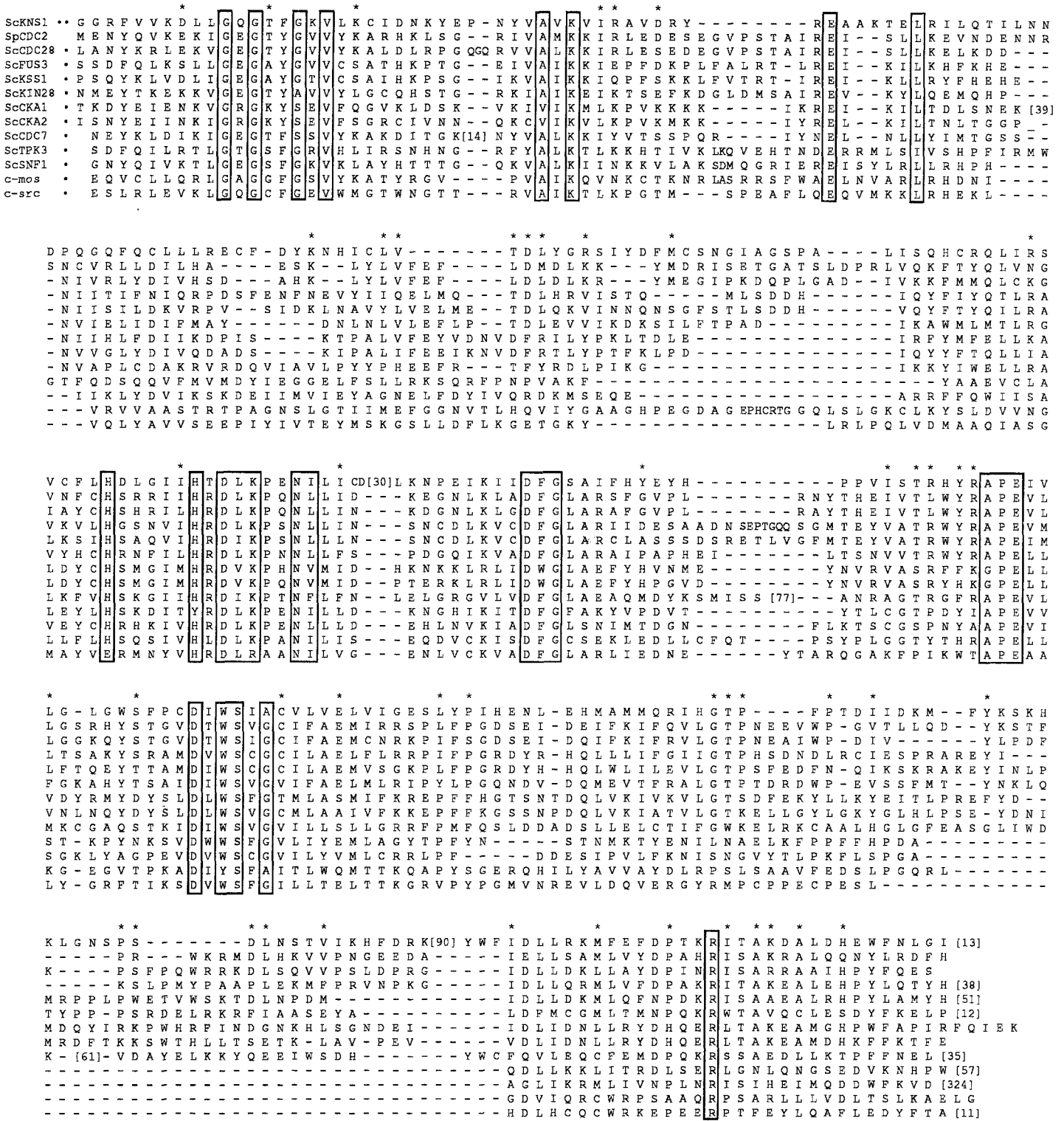


Fig. 4. Comparison of the putative *KNS1* protein sequence with that of other protein kinases. Residues often found in members of the *cdc2/CDC28* family and not often present in other protein kinases are indicated by an asterisk. Residues common to all family members are enclosed in boxes. For comparison *CDC7*, *TPK3*, *SNF1*, *c-mos*, and *c-src* protein sequences are included; these are not members of the *cdc2/CDC28* family. The number of amino acid residues upstream of the depicted kinase domains are 291 (*KNS1*); 0, (*cdc2*); 4 (*CDC28*); 9 (*FUS3*); 9 (*KSS1*); 4 (*KIN28*);

36 (*CKA1*); 46 (*CKA2*); 5 (*CDC7*); 85 (*TPK3*); 52 (*SNF1*); 57 (*c-mos*); and 267 (*c-src*). The protein sequences were obtained from Hindley and Phear 1984 (*cdc2*); Lorincz and Reed 1984 (*CDC28*); Courchesne et al. 1989 (*KSS1*); Elion et al. 1990 (*FUS3*); Simon et al. 1986 (*KIN28*); Chen et al. 1988 (*CKA1*); Padmanabha et al. 1990 (*CKA2*); Patterson et al. 1986 (*CDC7*); Toda et al. 1987 (*TPK3*); Celnza and Carlson 1986 (*SNF1*); Watson et al. 1982 (*c-mos*); Anderson et al. 1985 (*c-src*)

Table 2. Growth rates

Strain	Doubling time at 30° C
<i>MATα KNS1</i> (Y428)	110
<i>MATα KNS1</i> (Y264)	120
<i>MATα kns1-Δ1</i> (Y660)	126
<i>MATα kns1-Δ1</i> (Y661)	115

KNS1 and *kns1- Δ 1* yeast strains were grown in rich media. The doubling time was determined by measuring the OD₆₀₀ of exponentially growing cultures every 4–5 h starting at OD=0.2 for a total of 24 h

Table 3. Mating efficiencies

<i>MATα</i>	<i>MATα</i>	Mating efficiency
<i>KNS1</i> (Y428)	<i>KNS1</i> (Y264)	1.0
<i>KNS1</i> (Y428)	<i>kns1-Δ1</i> (Y661)	0.753
<i>kns1-Δ1</i> (Y660)	<i>KNS1</i> (Y264)	0.825
<i>kns1-Δ1</i> (Y660)	<i>kns1-Δ1</i> (Y661)	0.514
<i>kns1-Δ1</i> (Y663)	<i>kns1-Δ1</i> (Y662)	0.921

MAT α and *MAT α* cells growing exponentially were incubated to OD₆₀₀=0.5 and mating efficiency were determined as described in the Materials and methods. For each mating between 150 and 280 diploid colonies were counted

Table 4. Sporulation efficiencies

Diploids	No. of cells counted	No. of spores	% Ascii
<i>KNS1/KNS1</i> (Y270)	187	43	22.9
<i>kns1-Δ1/kns1-Δ1</i> (Y664)	149	34	22.8
<i>kns1-Δ1/kns1-Δ1</i> (Y665)	153	28	18.3

Diploid yeast cells were sporulated in 30° C in 1% potassium acetate, and the % asci was determined after 3 days

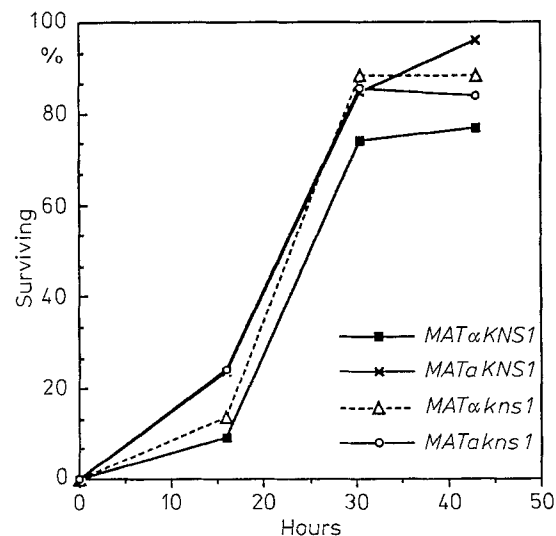


Fig. 5. Test for entry into stationary phase of wild-type cells and of *kns1* mutant cells of the following strains was performed as described in the Materials and methods: *MAT α KNS1* (Y264), *MAT α KNS1* (Y428), *MAT α kns1* (Y661), and *MAT α kns1* (Y662)

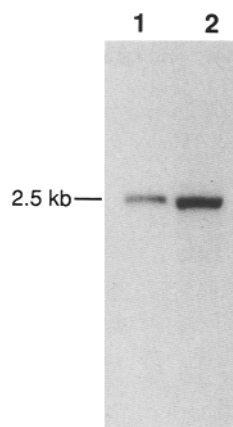


Fig. 6. RNA blot analysis of *KNS1*. Lane 1, 7 μ g of total yeast RNA; lane 2, 15 μ g of total yeast RNA. The size of the hybridizing band was estimated based on the mobility of the ribosomal RNA bands

script capable of encoding the entire *KNS1* protein was present. The *KNS1* transcript appears to be more abundant than yeast actin RNA (not shown).

In order to determine whether this *KNS1* transcript was capable of being translated, the *lacZ* gene of *E. coli* was fused in frame to *KNS1* coding sequences (see the Materials and methods), transformed into yeast, and assayed for the presence of β -galactosidase. A low level of β -galactosidase activity was observed; this level was at least 50-fold lower than that of the *SPA2:: β -gal* fusion protein (Snyder 1989; C. Costigan and M. Snyder, unpublished results). Control strains with *lacZ* present in the opposite orientation relative to *KNS1* failed to produce any detectable β -galactosidase. The low levels of enzyme activity detected in β -galactosidase assays can be explained in several ways: (i) the *KNS1* transcript may be poorly translated, (ii) the presence of the *KNS1* protein may be only transient during the yeast cell cycle, (iii) the *KNS1* protein or the *KNS1:: β -gal* fusion protein might be particularly sensitive to proteolysis, or (iv) the β -galactosidase activity of this particular fusion construct may itself be intrinsically low. In any event, the *KNS1* gene appears to be expressed as RNA and occurs, at least at a low level, as protein in yeast.

The presence of the KNS1 gene on a high copy number plasmid in yeast does not suppress the cdc28-1 mutation of yeast

The *KNS1* gene is distantly related to the *CDC28* gene. To determine whether the *KNS1* protein might contain overlapping functions with that of the *CDC28* protein, we tested whether the *KNS1* gene when present on a high copy number plasmid, YEp24, might suppress the *cdc28-1* mutation of *S. cerevisiae*. Wild-type and *cdc28* mutant cells containing either a *KNS1*/YEp24 high copy number plasmid or YEp24 alone exhibited no difference in colony size when cells were grown at either the restric-

tive temperature (37° C) or at semipermissive temperatures (30° and 32° C). Thus, although we have not determined whether the *KNS1*/YEp24 plasmid overproduces *KNS1* RNA or protein, the *KNS1*/YEp24 plasmid does not suppress the *cdc28-1* mutation.

Discussion

A novel kinase homologue, *KNS1*, which is a distant member of the *CDC28/cdc2* gene family has been identified. The *KNS1* protein differs from proteins encoded by other members of the *CDC28/cdc2* gene family in that it contains a 30 kDa segment upstream of the kinase domain, which may regulate the *KNS1* kinase activity. An attached regulatory domain might make the *KNS1* kinase less versatile than other members of the *cdc2/CDC28* family, because the other members of this family can potentially interact with a number of regulatory domains. The *SPA2/KNS1* region lies on the left arm of chromosome XII, 21 cM from the centromere. The *KNS1* gene contains a *NotI* site in its coding region (Fig. 1), which should allow its precise positioning on a physical map, once it becomes available.

A large deletion of the *KNS1* gene was constructed. *kns1* mutant cells appear similar to wild-type cells in all aspects that were examined. The *KNS1* gene produces a 2.5 kb transcript and *KNS1::lacZ* fusions produce β -galactosidase activity indicating that the *KNS1* gene is expressed in yeast. Either we have not found the appropriate conditions under which *KNS1* plays an important function in yeast, or there is another gene or set of genes that has an overlapping function with *KNS1*. There are other examples of dispensable genes in yeast where a second or third homologue exists with a closely related function [examples include cAMP-dependent protein kinase genes *TPK1*, *TPK2*, *TPK3* (Toda et al. 1988); *RAS1*, *RAS2* (DeFeo Jones et al. 1983); histone genes *H2A*, *H2B*, *H3*, *H4* (Hereford et al. 1979; Smith and Murray 1983); and casein kinase II genes *CKA1*, *CKA2* (Padmanabha and Glover 1987)]. Low stringency hybridizations with *KNS1* probes failed to reveal any related homologue, indicating that if *KNS1* homologues exist, they must be divergent in sequence.

Our serendipitous finding of the *KNS1* gene probably reflects the fact that there are many protein kinase genes in yeast. So far, at least 24 yeast protein kinase genes or homologues have been identified. Approximately 520 sequences yeast genes which are expected to be transcribed by RNA polymerase II are present in the GenBank data base (Release 63); thus kinase homologues comprise about 4%–5% of the total, a number slightly higher than that predicted by Hunter (1987). If 4%–5% represents the frequency of protein kinases in yeast, then we predict that the yeast genome contains approximately 400–500 protein kinases among its 10000 genes. We recognize that there are a number of factors that may bias this estimate.

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