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# A putative protein kinase gene (*kin1*$^+$) is important for growth polarity in *Schizosaccharomyces pombe*

(oligonucleotide probes/conserved sequence/*KIN* homolog)

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ABSTRACT **Mixed synthetic oligonucleotides encoding a sequence conserved among tyrosine-specific protein kinases were used to probe the genome of the fission yeast *Schizosaccharomyces pombe*. A single gene (*kin1*$^+$) was isolated that encodes a putative protein kinase closely related to the *KIN1*- and *KIN2*-encoded serine/threonine-specific protein kinases of *Saccharomyces cerevisiae*. *kin1*$^+$ is transcribed into a 3.5-kilobase mRNA that contains an uninterrupted open reading frame encoding a polypeptide of 98 kDa. In contrast to results obtained with *kin* mutants of *S. cerevisiae*, disruption of the *Sc. pombe kin1*$^+$ gene resulted in recessive morphological and growth defects. *kin1*-disrupted cells grew slowly on enriched medium and grew as spheres, in contrast to wild-type *Sc. pombe* cells, which grow as rods. Relative to *kin1*$^+$ cells, *kin1*-disrupted cells were differentially sensitive to lysis by treatment with α- and β-glucanases, suggesting an alteration in either the composition or the organization of their cell walls.**

Protein kinases comprise a diverse group of regulatory proteins widely suspected to be involved in growth control and malignant transformation in eukaryotes (for reviews, see refs. 1–3). These enzymes exert their regulatory effects by phosphorylating other proteins. Identification of important substrates of protein kinases is the key to understanding the physiological significance of protein phosphorylation. To understand the roles of protein kinases in normal and malignant cells, it will be useful to take advantage of the existence of these enzymes in species that lend themselves to rigorous genetic analysis. Both the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe* are exceptionally amenable to such investigation.

Nearly 100 unique protein kinases have been described to date. These have been classified into two subfamilies based on substrate specificity. Some specifically phosphorylate tyrosine residues, whereas others modify serine and threonine residues (4). Members of these subfamilies can be distinguished by characteristic structural features that are highly conserved within each subfamily. More than 30 protein kinase genes have been isolated from budding and fission yeast, but all have been shown to be, or are predicted from their amino acid sequences to be, serine/threonine-specific enzymes. Although tyrosine-specific protein kinase genes have yet to be found in the genomes of yeast, biochemical evidence suggests that both *S. cerevisiae* and *Sc. pombe* possess enzymes that phosphorylate their substrates on tyrosine residues (5, 6). Previously we reported the isolation of two putative protein kinase genes from *S. cerevisiae* (designated *KIN1* and *KIN2*) by using mixed oligonucleotide probes encoding sequences conserved among tyrosine-specific protein kinases (7). These genes have been shown to encode protein kinases specific for serine and threonine residues (8). Using the same oligonucleotide probes, we have isolated a gene from *Sc. pombe* that is closely related to the *S. cerevisiae KIN* genes. Here we describe the molecular and genetic characterization of this gene.†

## MATERIALS AND METHODS

**Materials.** The *Sc. pombe* genomic DNA libraries in shuttle vectors pDB248 (9) and pFL20 (10) were the gift of David Beach (Cold Spring Harbor Laboratories), as was the *Sc. pombe ura4*$^+$ gene cloned into pUC19 (pUC19-SU4). NovoZym 234 (α-glucanase) was from Novo BioLabs (Danbury, CT). Zymolyase-20T (β-glucanase) was from ICN. Oligonucleotide pools have been described (7).

**Strains, Media, and Transformations.** The stable diploid *Sc. pombe* strain used in this study was DLP100 (*h*$^{+N}$ *ura4-294 leu1-32 ade6-704*/*h*$^{+N}$ *ura4-294 leu1-32 ade6-704*) provided by Paul Nurse (University of Oxford). The diploid strain that was heterozygous for the *kin1* disruption (*kin1*$^+$/*kin1*::*LEU2*; DLP101) was derived from strain DLP100. Haploid segregants of this heterozygous diploid were DLP102 (*h*$^{+N}$ *kin1*::*LEU2*) and DLP103 (*h*$^{+N}$ *kin1*$^+$). Wild-type strain 972 *h*$^{-s}$ was the gift of David Beach. Yeast cells were grown in YEA medium (0.5% yeast extract/75 μg of adenine per ml) supplemented with 3% glucose. Synthetic minimal medium (PMA; ref. 11) supplemented with the appropriate nutrients was used to select for plasmid maintenance, gene replacement, and identification of mating-type convertants with iodine vapors (12). Transformation of *Sc. pombe* was by the procedure of Beach *et al.* (9).

Bacterial strains HB101 (13) and JM101 (14) were used for the propagation of plasmids and phage, respectively. *Escherichia coli* cells were cultured in Luria broth and transformed or infected with phage by standard methods (15).

**Nucleic Acid Preparation and Hybridization.** Genomic *Sc. pombe* DNA was isolated by the method of Beach *et al.* (9). Poly(A)$^+$ RNA was isolated from strain 972 *h*$^{-s}$ during proliferation and after 12 hr in stationary phase and prepared for hybridization as described by Jensen *et al.* (16). Hybridization analyses with end-labeled oligonucleotides (see Fig. 1), strand-specific probes, and nick-translated DNA fragments were carried out as described (7). The plasmid libraries were screened by the method of Grunstein and Hogness (17). Plasmid DNA was prepared from *E. coli* by the alkali lysis method (18).

DNA sequence analysis was conducted by the dideoxynucleotide chain-termination method (19) after subcloning of restriction endonuclease fragments into M13mp18 and M13mp19 (20). The sequence of the 3.8 kilobases (kb) of

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M36060).

DNA spanning the three *Hin*dIII sites of the *kin1*$^+$ locus (see Fig. 2) was determined for both strands.

***kin1*$^+$ Gene Replacement.** A mutant allele of *kin1*$^+$ was constructed by the method of Rothstein (21). The 2.2-kb *Xho* I/*Sal* I fragment of the *S. cerevisiae LEU2* gene (22) was inserted into the *Sal* I site of the 3.8-kb *Pst* I/*Pvu* II fragment of *kin1*$^+$ (Fig. 2) cloned into pUC18. This construction was digested with *Hin*dIII to liberate a 4.0-kb fragment containing the 5′ end of the *kin1*$^+$ gene disrupted by the selectable marker. The *Hin*dIII fragment was used to transform the stable diploid strain by selection for leucine prototrophy. Stable Leu$^+$ transformants were identified by replicate plating from YEA medium to PMA supplemented with uracil. Restriction and hybridization analysis of genomic DNA from such transformants was done to identify those possessing single gene replacements at the *kin1*$^+$ locus.

## RESULTS

Protein kinases possess a 30-kDa domain responsible for their catalytic activity. Some sequences within the so-called kinase domain are universally conserved among all members of the family; others are conserved among only those members whose kinase activity is specific for tyrosine residues (4).

In an effort to isolate genes encoding tyrosine-specific protein kinases from the genome of *Sc. pombe*, mixed oligonucleotide pools encoding an amino acid sequence highly conserved among this subfamily of enzymes were used as hybridization probes. Considering the complexity of the fission yeast genome and the requisite degeneracy of the probes, the optimal length of these oligonucleotides was calculated to be 17 bases. The probability of random occurrences of these sequences within the *Sc. pombe* genome was low enough so as not to be prohibitive. The oligonucleotide pools used encoded the conserved six-amino acid sequence Asp-Val-Trp-Ser-Phe-Gly (DVWSFG) (7).

**Isolation of the *Sc. pombe kin1*$^+$ Gene.** The oligonucleotide pools shown in Fig. 1 were used to probe genomic *Sc. pombe* DNA at high stringency. These pools include all of the sequences that encode the amino acid sequence DVWSFG. Since there are six serine codons, it was necessary to synthesize two oligonucleotide pools. Genomic DNA fragments were resolved by agarose gel electrophoresis after digestion with either *Eco*RI or *Hin*dIII and transferred to nitrocellulose filters for hybridization. The fission yeast genome contained one sequence that hybridized to members of pool 1 and two sequences that hybridized to members of pool 2 (Fig. 1).

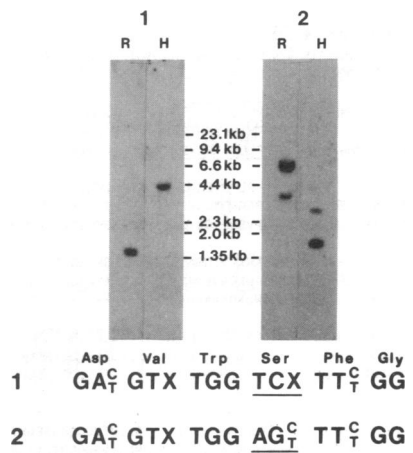


FIG. 1. Hybridization of mixed oligonucleotide probes with genomic *Sc. pombe* DNA. DNA was digested with *Eco*RI (R) or *Hin*dIII (H), electrophoresed through a 1% agarose gel (5 μg per lane), and transferred to nitrocellulose. Pools of 17-mers encoding the six-amino acid sequence DVWSFG were end-labeled and used as probes. Since there are six serine codons, it was necessary to synthesize two pools differing only at the serine codons. Pool 1 contained a mixture of 64 probes, whereas pool 2 contained 32 probes.

Both oligonucleotide pools were used to screen a library of *Sc. pombe* genomic DNA in the shuttle vector pDB248 (9). Signals were detected from 2 of ≈5000 colonies (corresponding to 5 genomic equivalents) probed with pool 1, and from 6 among the same population probed with pool 2. Plasmid DNA from each of these clones was isolated and subjected to restriction and hybridization analysis (data not shown). Both clones isolated using pool 1 possessed restriction fragments that corresponded to the genomic DNA fragment detected by that probe. Among the 6 clones isolated with pool 2, 2 corresponded to the genomic 3.8-kb *Eco*RI and 2.7-kb *Hin*dIII fragments, and 4 corresponded to the 6.0-kb *Eco*RI and 1.8-kb *Hin*dIII fragments.

The sequence that hybridized to a member of pool 1 resided on a 250-base-pair (bp) *Eco*RI/*Hin*dIII fragment, which was subcloned into M13 and sequenced. This fragment contained the following sequence: 5′-GACGTTTGGTCGTTTGG-3′, which is identical to one member of the probe pool. Since the reading frame defined by this sequence was closed three amino acids C terminal to the region homologous to the probe, and the subclone possessed no additional sequence similarity to protein kinase genes, no further work was done with this cloned sequence. The sequences responsible for hybridization with members of pool 2 resided on a 450-bp *Hin*dIII/*Bgl* II fragment and a 450-bp *Bgl* II fragment within the 2.7-kb and the 1.8-kb *Hin*dIII fragments, respectively. The *Hin*dIII/*Bgl* II fragment contained the following sequence: 5′-GACGTTTG-GAGTTTCTT-3′, which differs from the closest member of the probe pool at two 3′ thymine residues (underlined). The reading frame defined by this sequence was closed one amino acid C terminal to the region homologous to the probe, and no additional sequence similarity to protein kinase genes was identified within this subclone.

The *Bgl* II fragment that hybridized with a member of pool 2 contained the following sequence: 5′-GACGTTTG-GAGTTTTGG-3′, which is identical to one member of the probe pool. The reading frame defined by this sequence was open throughout the subclone. In addition, this reading frame possessed other hallmark sequences indicative of protein kinases. Complete DNA sequence analysis of this locus revealed that it encodes a 2.7-kb uninterrupted open reading frame corresponding to a polypeptide with a predicted size of 891 amino acids (98 kDa). This value assumes the use of the 5′-most methionine codon in the open reading frame. No consensus sequences for intron splicing were identified within the predicted coding region or in the 500 bp 5′ to the predicted initiation site.

The amino acid sequence of this putative protein kinase was compared with sequences in the GenBank and EMBL protein data bases. Although sequence similarity was identified between the predicted *Sc. pombe* protein and many protein kinases, this protein was most closely related to the *KIN1* and *KIN2* protein kinases of *S. cerevisiae* (7) and was therefore designated *kin1*$^+$. A restriction map of *kin1*$^+$ is shown in Fig. 2. Probes derived from the *kin1*$^+$ coding sequence failed to detect additional members of this gene

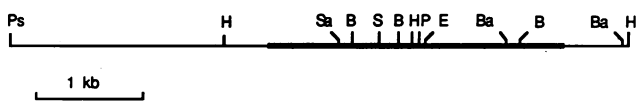


FIG. 2. Restriction map of the *kin1*$^+$ gene. Only selected sites are shown. The direction of transcription is from left to right as drawn, with the thick line representing the coding sequence. Ps, *Pst* I; H, *Hin*dIII; Sa, *Sal* I; B, *Bgl* I; S, *Sph* I; P, *Pvu* II; E; *Eco*RI; Ba, *Bam*HI.

family within the *Sc. pombe* genome under hybridization conditions of reduced stringency (data not shown).

The putative kinase domain of *kin1*$^+$ (encoded by residues 132–386) was most highly conserved with the *S. cerevisiae* KIN1 and KIN2 proteins at 62% and 63%, respectively (Fig. 3). Like the *S. cerevisiae KIN* genes, *kin1*$^+$ possessed some structural features that are distinctive of serine/threonine protein kinases (e.g., amino acid sequence SLYFAAPE at positions 303–310), with the only sequence characteristic of tyrosine-specific kinases being the target of the oligonucleotide probe. Outside of the kinase domain, sequence similarity was patchy, with some regions highly conserved and others diverged when compared to the *S. cerevisiae* KIN proteins.

**Expression of *kin1*$^+$.** A nick-translated probe derived from the coding sequence of the *kin1*$^+$ gene (Fig. 2) was hybridized with *Sc. pombe* poly(A)$^+$ RNA. Fig. 4 shows that *kin1*$^+$ is transcribed into a 3.5-kb mRNA in proliferating h$^{-s}$ cells. The steady-state level of kin1$^+$ mRNA was similar to that of the ura4$^+$ message in logarithmically growing cells. The kin1$^+$ mRNA level was not reduced in cells that had arrested growth in the $G_0$ phase of the cell cycle, suggesting either that the message is unusually stable or that expression of this gene continues into the stationary phase. Strand-specific probes derived from the *kin1*$^+$ gene confirmed that the direction of transcription corresponds to that specified by the open reading frame (data not shown).

***kin1*$^+$ Is Important for *Sc. pombe* Cell Growth.** To determine the phenotypic effect of a loss of *kin1*$^+$ function, a disruption mutant of *kin1*$^+$ was constructed. The *S. cerevisiae LEU2* gene, which rescues *leu1* mutants of *Sc. pombe*, was inserted into the unique *Sal* I site within *kin1*$^+$ (Fig. 2). The *Sal* I site separates the predicted ATP-binding site from the remainder of the catalytic domain. The disrupted allele (*kin1*::*LEU2*) was used to transform a stable diploid strain (DLP100; $h^{+N}/h^{+N}$) with multiple auxotrophic markers by selecting for leucine prototrophy. Eight stable Leu$^+$ transformants were tested for replacement of a wild-type allele of *kin1*$^+$ with the disrupted allele by restriction and hybridization analysis

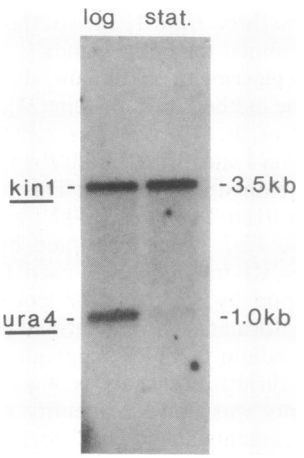


FIG. 4. mRNA of *kin1*$^+$. Poly(A)$^+$ RNA, prepared from strain 972 $h^{-s}$ growing logarithmically (log) in PMA medium (left lane) or arrested for 12 hr in stationary phase (stat) (right lane), was electrophoresed (5 $\mu$g per lane) through a 6% formaldehyde/1.2% agarose gel and transferred to nitrocellulose. A nick-translated probe for the *kin1*$^+$ mRNA was derived from the 700-bp *Sal* I/*Hin*dIII fragment (see Fig. 2). A probe prepared from the 1.8-kb *Sph* I fragment bearing the *Sc. pombe ura4*$^+$ gene (from pUC19-SU4) was included at the same concentration and specific activity as a quantitation control. Markers were the 0.24- to 9.5-kb RNA ladder from Bethesda Research Laboratories.

(data not shown). Two of these transformants gave the hybridization pattern expected for *kin1*$^+$ heterozygous diploids (*kin1*::*LEU2*/*kin1*$^+$; DLP101). Rare sporulation-proficient convertants ($h^{90}/h^{+N}$; ref. 23) of these diploids were identified by staining the ascospores with iodine vapors (12). After sporulation, tetrads were dissected, and haploid spores were allowed to germinate on YEA glucose medium. All four spores from each tetrad gave rise to colonies, but the colonies were not uniform in size. All the tetrads examined

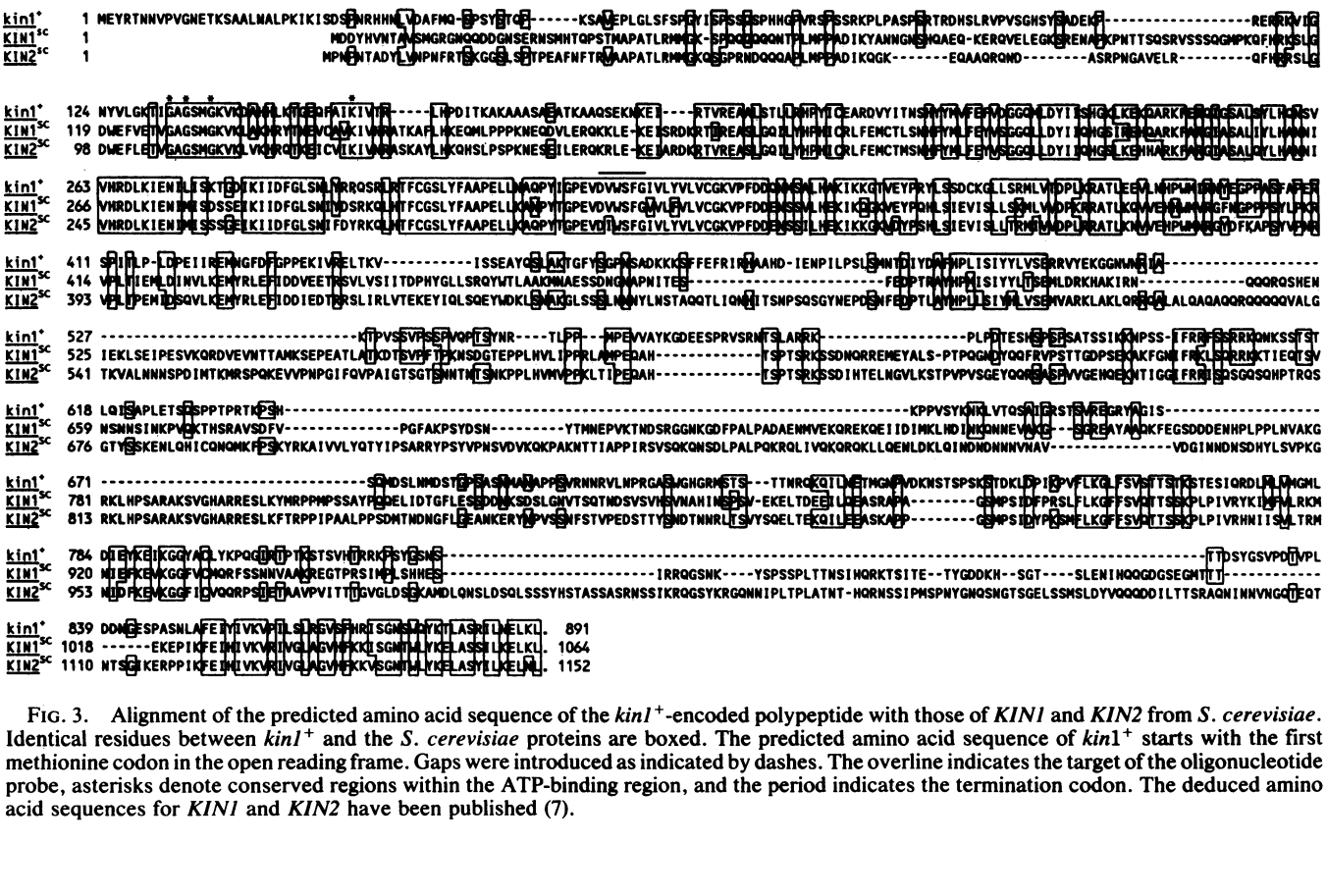

FIG. 3. Alignment of the predicted amino acid sequence of the *kin1*$^+$-encoded polypeptide with those of *KIN1* and *KIN2* from *S. cerevisiae*. Identical residues between *kin1*$^+$ and the *S. cerevisiae* proteins are boxed. The predicted amino acid sequence of *kin*1$^+$ starts with the first methionine codon in the open reading frame. Gaps were introduced as indicated by dashes. The overline indicates the target of the oligonucleotide probe, asterisks denote conserved regions within the ATP-binding region, and the period indicates the termination codon. The deduced amino acid sequences for *KIN1* and *KIN2* have been published (7).

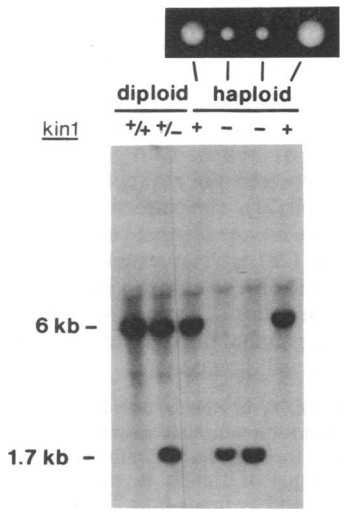


FIG. 5. Disruption of the *kin1*$^+$ gene. Genomic DNA was isolated from a *kin1*$^+$ diploid strain (DLP100), a diploid heterozygous for the *kin1* disruption (*kin1*$^+$/*kin1*::*LEU2*; DLP101) and the haploid segregants of a representative tetrad from DLP101 (*Upper*). DNA (5 μg) was digested with *Eco*RI and prepared for hybridization with the nick-translated 700-bp *Sal* I/*Hin*dIII fragment from *kin1*$^+$. The hybridizing 1.7-kb fragment is derived from the disrupted allele and is due to cleavage at the single *Eco*RI site within the *LEU2* gene.

gave two large colonies and two small colonies. The small colonies were Leu$^+$ and possessed the disrupted allele of *kin1*$^+$ (Fig. 5). The doubling time of the *kin1*::*LEU2* haploid strain at 30°C (DLP102) in YEA glucose medium was 200 min, as compared with 120 min for the isogenic *kin1*$^+$ strain (DLP103). A genomic copy of the *kin1*$^+$ gene cloned into the plasmid vector pFL20 (carries *URA3*) was capable of complementing the slow-growth defect associated with the *kin1*::*LEU2* disruption.

Microscopic examination of cells containing the *kin1*::*LEU2* disruption revealed morphological defects associated with the loss of *kin1*$^+$ function. Cells grown at 23°C separated poorly after septation, resulting in pairs of cells attached by remnants of their septa (Fig. 6*B*). Also found within this population of cells were filamentous structures like the one shown in Fig. 6*C*. These structures are morphologically similar to *Sc. pombe* cells that carry mutations in β-tubulin (24). However, unlike the β-tubulin mutants, which display a variety of cytokinetic abnormalities (24), *kin1*::*LEU2* cells carry out apparently unimpaired cytokinesis. The *kin1*::*LEU2* cells were uniformly uninucleate, with nuclei centrally located, as judged by 4′,6-diamidino-2-phenylindole staining, and did not show differential sensitivity to the microtubule antagonist nocodazole (data not shown).

*kin1*::*LEU2* cells grown at 30°C displayed a growth polarity defect. Wild-type *Sc. pombe* cells are rod shaped (Fig. 6 *A* and *D*), grow exclusively from their ends, and divide medially. In contrast, *kin1*::*LEU2* cells grew as spheres while continuing to divide medially. These spherical cells grew stuck together in clumps of up to 100 cells (Fig. 6*E*). Cells separated by sonication or by treatment with α-glucanase retained high viability (>80%), indicating that cell division proceeds to completion in the *kin1*::*LEU2* mutant.

A temperature-shift experiment was conducted to characterize further the phenotypic differences observed at low and high temperatures. Cells grown on YEA glucose at 23°C were transferred to 30°C for 6 hr. Many of the cells were observed to have an "ice-cream cone" shape, with a spherical end and a rod-shaped end (Fig. 6*F*). It is likely that the rod-shaped ends represent a structure that was established at 23°C and the spherical ends result from new growth at the higher temperature. Temperature shifts for periods of <2 hr did not induce morphological changes.

***kin1*::*LEU2* Cells Have Altered Cell Walls.** Structures similar to those seen in our temperature-shift experiments can be induced by treatment of growing *Sc. pombe* cells with aculeacin A (25). This antifungal antibiotic is a specific inhibitor of β-glucan synthase, the enzyme responsible for synthesis of one of the two major cell wall components in yeast (26). Inhibition of β-glucan synthase by aculeacin A results in a weakened cell wall composed mainly of α-glucans. Because the integrity of the cell wall is compromised by this agent, cells assume a spherical shape (26). To test the possibility that the composition of the cell wall is altered in *kin1*::*LEU2* mutant cells, cultures were subjected to lysis by treatment with specific glucanases. Fig. 7 shows that *kin1*::*LEU2* cells were more sensitive than wild-type cells to lysis by treatment with β-glucanase. In contrast, wild-type cells were more sensitive than the *kin1*::*LEU2* cells to lysis by α-glucanase. These results suggest that either the relative

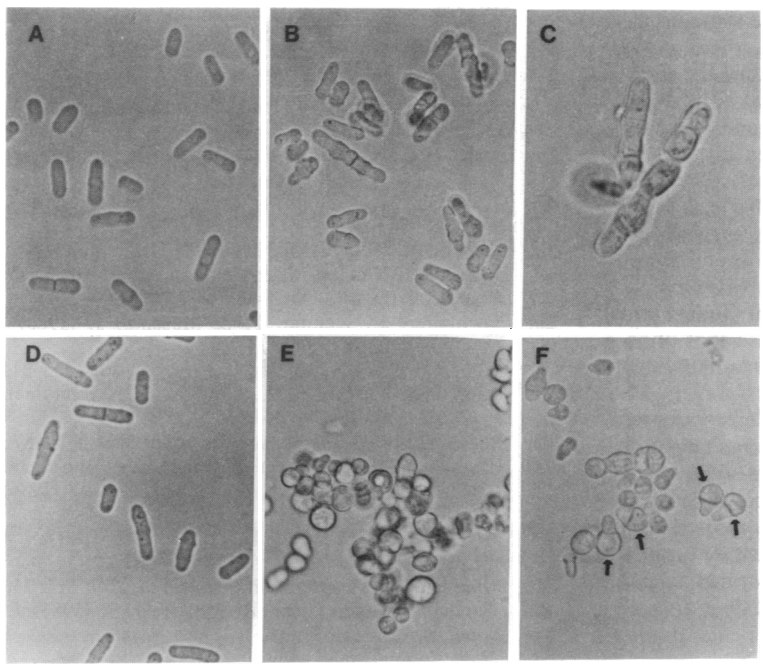


FIG. 6. Morphological defects associated with disruption of the *kin1*$^+$ gene. (*A*) Haploid strain DLP103 (*kin1*$^+$) grown at 23°C. (*B*) Haploid strain DLP102 (*kin1*::*LEU2*) at 23°C. (*C*) Filamentous structure observed in cultures of strain DLP102 at 23°C shown at additional magnification (×2). (*D*) DLP103 at 30°C. (*E*) DLP102 at 30°C. (*F*) A temperature-shift experiment in which strain DLP102, which had been cultured at 23°C, was transferred to 30°C for 6 hr. Cells were grown in YEA glucose medium and photographed using a ×40 objective mounted on a Zeiss photomicroscope III.

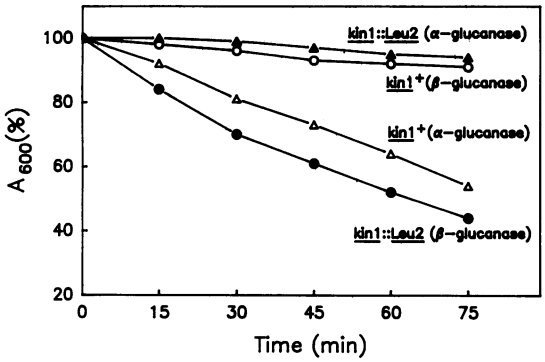


FIG. 7. Sensitivity of *kin1*$^+$ and *kin1*::*LEU2* cells to lysis by glucanases. Cultures of DLP103 (*kin1*$^+$) and DLP102 (*kin1*::*LEU2*) were grown to midlogarithmic phase ($A_{600}$, ≈0.7) in YEA glucose medium at 30°C, washed, and resuspended in aqueous solutions of either 100 μg of Zymolyase-20T per ml (β-glucanase) or 25 μg of NovoZym 234 per ml (α-glucanase). Cell suspensions were maintained at 30°C with vigorous shaking and were monitored for cell lysis by $A_{600}$ measurements. ▲, DLP102 with α-glucanase; △, DLP103 with α-glucanase; ●, DLP102 with β-glucanase; ○, DLP103 with β-glucanase.

amounts of α- and β-glucans, or their organization within the cell wall, may be altered in the *kin1*::*LEU2* mutant.

## DISCUSSION

***kin1*$^+$ Encodes a Homolog of the *S. cerevisiae KIN* Genes.** We used mixed oligonucleotide pools encoding a region conserved among tyrosine-specific protein kinases to probe the *Sc. pombe* genome. These probes allowed the isolation of a single gene, designated *kin1*$^+$, which is predicted to encode a protein with a high level of amino acid sequence similarity to the *S. cerevisiae KIN1* and *KIN2* protein kinase genes (7). The topography of the predicted *kin1*$^+$-encoded protein is similar to that of *KIN1* and *KIN2*, with the catalytic domain near the N terminus of a relatively large protein (98 kDa). Through its predicted catalytic domain, *kin1*$^+$ shares 62% and 63% amino acid identity with *KIN1* and *KIN2*, respectively. Outside the catalytic domain, sequence similarity is patchy but still evident through most of the protein. The C termini of all three proteins are particularly well conserved, perhaps indicating a site for effector interaction. *kin1*$^+$ is not appreciably more closely related to one of the *S. cerevisiae KIN* genes than to the other. It is noteworthy that *kin1*$^+$ does not appear to be structurally redundant in *Sc. pombe* as it is in *S. cerevisiae*.

*KIN1* and *KIN2* were isolated previously by using the same oligonucleotide probes used in the present study (7). These budding yeast genes have been shown to encode glycosylated, serine/threonine-specific protein kinases (8), diminishing the likelihood that *Sc. pombe kin1*$^+$ encodes a tyrosine-specific protein kinase. As was found of the KIN1 and KIN2 proteins, the *kin1*$^+$ catalytic domain possesses several features that are characteristic of serine/threonine-specific protein kinases, but only one that is distinctive of tyrosine-specific kinases. Although it is clear from recent studies that *Sc. pombe* possesses at least one protein kinase that phosphorylates its target at a tyrosine residue (6), it is unlikely that *kin1*$^+$ encodes this tyrosine kinase.

***kin1*$^+$ Is Important for Cell Growth Polarity.** Cells disrupted in the *kin1*$^+$ gene display multiple growth defects. This is in contrast to results obtained with deletion mutants of *KIN1* and *KIN2* in *S. cerevisiae*, for which no phenotypic defect has been identified (7). *kin1*-defective cells grow at approximately half the rate of *kin1*$^+$ cells on rich medium. When cultured at 23°C, these mutant cells are morphologically deformed and are apparently stuck together in pairs by remnants of their division septa. At the normal growth temperature for *Sc. pombe* (30°C), *kin1*-defective cells grow as spheres rather than as rods. These spheres grow in clumps of up to 100 cells, the result of what may be a more severe manifestation of the phenotype observed at the lower temperature. *kin1*-defective cells also appear to have an altered cell wall composition. Because wild-type *Sc. pombe* cells grow exclusively from the ends of the cell, deposition of new cell wall material is focused to the tips of the cell (27). The pattern of movement of the actin cytoskeleton in *Sc. pombe* suggests an association with newly created cell wall (28). It is possible that a *kin1*$^+$-encoded protein kinase controls the cytoskeletal structures thought to be responsible for focusing growth to the cell tips. Alternatively, *kin1*$^+$ may regulate the biosynthetic pathways involved in generating cell wall components.

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1. Cohen, P. (1982) *Nature (London)* **296**, 613–620.
2. Hunter, T. & Cooper, J. A. (1985) *Annu. Rev. Biochem.* **54**, 897–930.
3. Bishop, J. M. & Varmus, H. (1985) in *RNA Tumor Viruses*, eds. Weiss, R., Teich, N., Varmus, H. & Coffin, J. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 999–1108.
4. Hanks, S. K., Quinn, A. M. & Hunter, T. (1988) *Science* **241**, 442–452.
5. Schieven, G., Thorner, J. & Martin, G. S. (1986) *Science* **231**, 390–393.
6. Gould, K. & Nurse, P. (1989) *Nature (London)* **342**, 39–45.
7. Levin, D. E., Hammond, C. I., Ralston, R. O. & Bishop, J. M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6035–6039.
8. Hammond, C., Tibbetts, M. & Donovan, M. (1988) *J. Cell Biol.* **107**, 278 (abstr.).
9. Beach, D., Piper, M. & Nurse, P. (1982) *Nature (London)* **290**, 140–142.
10. Losson, R. & Lacroute, F. (1983) *Cell* **32**, 371–377.
11. Nurse, P. (1975) *Nature (London)* **256**, 547–551.
12. Gutz, H., Heslot, H., Leupold, U. & Loprieno, N. (1974) in *Handbook of Genetics*, ed. King, R. C. (Plenum, New York), Vol. 1, pp. 395–446.
13. Boyer, H. & Roilland-Dussoix, D. (1969) *J. Mol. Biol.* **41**, 459–472.
14. Messing, J., Crea, R. & Seeburg, P. H. (1981) *Nucleic Acids Res.* **9**, 309–321.
15. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
16. Jensen, R., Sprague, G. F. & Herskowitz, I. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3035–3039.
17. Grunstein, M. & Hogness, D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965.
18. Benton, W. D. & Davis, R. W. (1977) *Science* **196**, 180–183.
19. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **83**, 5463–5467.
20. Norrander, J., Kempe, T. & Messing, J. (1983) *Gene* **26**, 101–106.
21. Rothstein, R. (1983) *Methods Enzymol.* **101**, 202–211.
22. Andreadis, A., Hsu, Y.-P., Kohlhaw, G. & Schimmel, P. (1984) *J. Biol. Chem.* **259**, 8059–8062.
23. Beach, D. H. (1983) *Nature (London)* **305**, 682–687.
24. Hiraoka, Y., Toda, T. & Yanagida, M. (1984) *Cell* **39**, 349–358.
25. Miyata, M., Miyata, H. & Johnson, B. F. (1986) *J. Gen. Microbiol.* **132**, 883–891.
26. Miyata, M., Kanbe, T. & Tanaka, K. (1985) *J. Gen. Microbiol.* **131**, 611–621.
27. Mitchison, J. M. & Nurse, P. (1985) *J. Cell Sci.* **75**, 357–376.
28. Marks, J., Hagan, I. M. & Hyams, J. S. (1986) *J. Cell Sci. Suppl.* **5**, 229–241.