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A Synthetic Lethal Screen Identifies SLK1, a Novel Protein Kinase Homolog Implicated in Yeast Cell Morphogenesis and Cell Growth

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The *Saccharomyces cerevisiae* SPA2 protein localizes at sites involved in polarized cell growth in budding cells and mating cells. *spa2* mutants have defects in projection formation during mating but are healthy during vegetative growth. A synthetic lethal screen was devised to identify mutants that require the *SPA2* gene for vegetative growth. One mutant, called *slk1-1* (for synthetic lethal kinase), has been characterized extensively. The *SLK1* gene has been cloned, and sequence analysis predicts that the SLK1 protein is 1,478 amino acid residues in length. Approximately 300 amino acids at the carboxy terminus exhibit sequence similarity with the catalytic domains of protein kinases. Disruption mutations have been constructed in the *SLK1* gene. *slk1* null mutants cannot grow at 37°C, but many cells can grow at 30, 24, and 17°C. Dead *slk1* mutant cells usually have aberrant cell morphologies, and many cells are very small, approximately one-half the diameter of wild-type cells. Surviving *slk1* cells also exhibit morphogenic defects; these cells are impaired in their ability to form projections upon exposure to mating pheromones. During vegetative growth, a higher fraction of *slk1* cells are unbudded compared with wild-type cells, and under nutrient limiting conditions, *slk1* cells exhibit defects in cell cycle arrest. The different *slk1* mutant defects are partially rescued by an extra copy of the *SSD1/SRK1* gene. *SSD1/SRK1* has been independently isolated as a suppressor of mutations in genes involved in growth control, *sit4*, *pde2*, *bcy1*, and *ins1* (A. Sutton, D. Immanuel, and K. T. Arnat, Mol. Cell. Biol. 11:2133–2148, 1991; R. B. Wilson, A. A. Brenner, T. B. White, M. J. Engler, J. P. Gaughran, and K. Tatchell, Mol. Cell. Biol. 11:3369–3373, 1991). These data suggest that *SLK1* plays a role in both cell morphogenesis and the control of cell growth. We speculate that *SLK1* may be a regulatory link for these two cellular processes.

Cell polarity is essential for cell morphogenesis in *Saccharomyces cerevisiae*. During vegetative growth, yeast cells grow and divide by budding, a process in which new cell growth begins at one edge of the cell. Bud formation begins in late G_1 at a specific site (22), and bud growth continues until cytokinesis (13). Prior to mating, yeast cells also undergo polarized cell growth (16). When haploid cells of one mating type are mixed with cells of the other mating type, they arrest growth in late G_1 and form a projection toward their mating partner. The pear-shaped cells, called shmoos, fuse via their projections.

Bud formation in mitotic cells and shmoo formation in mating cells each require an integrated series of cellular activities. Secretion and cell wall deposition, cytoskeletal reorganization, and growth are all required to produce a new bud or projection (reviewed in references 17 and 35). These activities must be orchestrated both temporally and spatially, and transcriptional as well as posttranscriptional regulatory mechanisms are expected to be important for regulating the different processes involved in polarized cell growth in yeast.

A variety of components that participate in different processes involved in bud formation and shmoo formation have been identified (1–3, 5, 13, 15, 18, 23, 26, 30, 32, 37, 38). One of these components is the SPA2 protein, which colocalizes with sites of polarized cell growth (23, 52, 53). The SPA2 protein localizes as a patch in unbudded cells, and as the bud emerges and enlarges, the SPA2 protein is present at the bud tip. In large budded cells, the SPA2 protein becomes delocalized, and at cytokinesis it appears at the neck. In haploid cells which have been exposed to mating pheromone, SPA2 localizes at the tip of the shmoo. *spa2* mutants have polarized growth defects in shmoo formation; in the presence of mating pheromone, these cells usually remain round or ovoid. Consequently, *spa2* mutants do not mate well. Thus, *SPA2* plays an important role in cell polarity and morphogenesis in mating. Although the SPA2 protein appears to be abundant at sites of polarized cell growth in vegetatively growing cells (23, 52), the defects found in *spa2* mutants are relatively minor. *spa2* mutants grow at the same rates as do wild-type cells, but they are marginally rounder, have altered budding patterns, and display weak defects in cytokinesis.

The preparation for bud formation begins well in advance of any morphological change. The SPA2 protein, actin spots, and many of the putative neck filament proteins (the CDC3, CDC10, CDC11, and CDC12 proteins [21, 24, 33, 41]) arrive at the incipient bud site during the beginning or middle of G_1 (21, 33, 53). The decision to initiate the formation of a bud does not occur until late G_1 (after Start), after these components have already begun to assemble at the bud site. Therefore, it seems likely that cell cycle control signals that trigger bud emergence might act upon components that have already begun preparation for budding.

In an attempt to identify additional genes important for cell morphogenesis in *S. cerevisiae*, we searched for mutants that could not grow in the absence of the *SPA2* gene (i.e., a synthetic lethal screen). We speculated that this screen would identify gene products that interact with the *SPA2* protein or function in a pathway parallel to that of *SPA2*. Using this screen, we have identified mutants that require

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the *SPA2* gene for growth. One of these, *slk1*, has been extensively characterized. *SLK1* encodes a protein kinase homolog that is implicated in both morphogenesis and cell growth.

MATERIALS AND METHODS

Yeast strains and general methods. Congenic S288C strains were used for all experiments except the caffeine sensitivity assays, in which strains of different backgrounds were employed. All strains are listed in Table 1 except for the chromosomal mapping strains YNN295 and YPH149, which are described by Vollrath et al. (58). Growth media and genetic manipulations were as described by Sherman et al. (50). Yeast media limited for adenine (*i*ade) contained 9 to 18 μg of adenine per ml. 5-Fluoro-orotic acid (5-FOA) plates contained standard media supplemented with 1 μg of 5-FOA per ml (11). Cloning procedures were as described by Sambrook et al. (46). Strain Y779 is a 5-FOA revertant of Y761.

Isolation of mutants that require *SPA2* for growth. Strain Y574 (*spa2-Δ1::TRP1 ade2-101* + pSPA2/TRP1/SUP4/CEN) was grown to early log phase (10^7 cells/ml) in minimal medium. One milliliter of this culture was diluted with 14 ml of YPD in a 90-mm petri dish. The dish was mutagenized with UV, and aliquots were removed after 50, 60, and 70 s. These conditions resulted in approximately 25, 45, and 60% killing, respectively. The irradiated cells were maintained in three separate pools. Cells were allowed to recover in the dark at 24°C for 23.5 h and then were plated on synthetic complete medium containing *i*ade at a cell density of approximately 370 cells per plate. Colonies were grown at 24°C for 5 days to allow the development of red color (29).

Of 46,000 colonies screened, 150 homogeneous white colonies were identified and patched onto glycerol plates. The 78 mutants that grew on medium containing glycerol were transformed with p182, a YEp24 plasmid containing a wild-type copy of the *SPA2* gene and *URA3* but lacking the *SUP4* gene. Transformants were selected on synthetic complete plates containing *i*ade but lacking uracil. Six mutants that formed sectored colonies in the presence of p182 were identified. Transformants of these mutants were retested by plating cells onto *i*ade plates containing 5-FOA. All yielded homogeneous white colonies.

Genetic analysis of mutants. The six mutants were each mated to strain Y554 (a *spa2 ade2* strain containing the *SPA2/SUP4* plasmid). The resulting diploids in each case formed colonies with many red and pink sectors; the pink sectors are due to the presence of one copy of *SUP4*, and red sectors do not contain the *SUP4* plasmid. Thus, because the heterozygous diploids can lose the *SPA2* plasmid, the mutations are recessive.

To determine whether the SPA2-dependent phenotype was due to the presence of a single mutation, heterozygous *slk* diploids were grown in minimal medium containing *i*ade to maintain the centromeric plasmid and then sporulated. Tetrads were dissected onto YPD plates and scored for their sectoring phenotypes. Four of the mutants, including *slk1-1*, segregated two sectoring colonies and two nonsectoring colonies when all four segregants had a *SPA2* plasmid present (number of tetrads with this phenotype, ≥8). Many tetrads produced only two progeny that contained plasmids, presumably because one copy of the plasmid was lost during mitotic growth. In these tetrads ($N \geq 20$) the sum of the presumed mutants (dead progeny plus nonsectoring colonies) always equaled two, and the number of wild-type cells (red colonies plus sectoring colonies) was two. Thus, we conclude that the *SPA2*-dependent mutations segregated 2:2 for these four strains. Complementation analysis revealed that all four mutations reside in different complementation groups. Two other strains had substantially higher proportions of wild-type cells, presumably because these strains had more than one mutation.

Cloning of genes that rescue lethality. *slk1-1* mutant strains Y753 and Y754 were transformed with two different yeast genomic DNA libraries in centromeric vectors: a library in YCp50 (*URA3*) (43) and a library in a *LEU2*-YCp50 derivative (28a). Both libraries were derived from S288C DNA. A total of 12,000 transformants were selected on appropriate plates containing synthetic complete medium and *i*ade. Of 12,000 transformants, 14 sectoring colonies were identified. The genomic library plasmids were prepared from red colonies and rescued in *Escherichia coli*; retransformation into *slk1-1 spa2* yeast strains revealed that 8 of the 14 plasmids allowed red sectoring in all transformed colonies and therefore complemented the *slk1-1 spa2* mutation(s). Presumably the other six plasmids were from transformants that had acquired chromosomal mutations. The eight plasmids were rechecked for the ability to cause sectoring by plating transformants onto plates containing *i*ade and 5-FOA; all eight produced homogeneous white colonies. pSLK1, which was isolated from the *LEU2* library, contained a 10.5-kb DNA insert and is depicted as the first complementing plasmid in Fig. 2. pSSL1, which was isolated from the *URA3* library, contained a 15-kb DNA insert and is depicted as the first complementing plasmid in Fig. 10.

Complementation analysis. To identify the minimum complementing region in pSLK1 and pSSL1, two strategies were used. First, deletions were generated in the library clones by digesting with restriction enzymes (see appropriate figures) and religating the vector piece so that one or more restriction fragments containing yeast genomic DNA were removed. For fragments that did not have complementary ends, it was necessary to make the ends flush with the large fragment of DNA polymerase I and deoxynucleoside triphosphates (dNTPs) prior to ligation. The second approach involved direct subcloning of pSLK1 and pSSL1 restriction fragments into the yeast shuttle vectors pRS315 (a *LEU2* vector) and pRS316 (a *URA3* vector), respectively (51). Complementation was assayed by the appearance of sectoring in the *SPA2/SUP4* plasmid-containing strains.

Genetic mapping of pSLK1 sequences to the *SLK1* locus. To determine whether the *SLK1* gene corresponded to the wild-type locus of *slk1-1*, the chromosomal locus of *SLK1* was marked with *URA3*. Strain Y554 was transformed with a Bluescript SK^+ plasmid carrying the *slk1-Δ1::URA3* allele (see below); this plasmid was linearized with *Bgl*II (see Fig. 2) prior to transformation. Three different Ura^+ transformants in which the plasmid had integrated at the *SLK1* locus (Y759a, -b, and -c) were crossed to strain Y753 (*slk1-1 spa2*). The *SLK1::URA3/slk1-1* strains were grown nonselectively (resulting in the loss of the *SPA2* plasmid) and sporulated. Thirty-eight tetrads segregated two Ura^+ progeny that produced red colonies and two progeny which failed to produce colonies. Three tetrads produced one red Ura^+ colony and three progeny that failed to produced colonies.

DNA sequencing and analysis. Sequencing was carried out by the double-stranded sequencing procedure described in the Pharmacia T7 polymerase sequencing kit (47). Sequencing reactions were performed on fragments cloned into pRS315, pRS316, and Bluescript SK^+ (Stratagene). Random deletions were generated by the Erase-a-Base system

TABLE 1. Strains

Strains	Genotype
S288C congenic strains	
Y270 (YNN318)	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200*
	MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200
Y431	*MAT***a** *ura3-52 lys2-801 ade2-101 trpl-Δ1 leu2-Δ98*
Y554	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 spa2-Δ1::TRP1* + *pSPA2/TRP1/SUP4/CEN*
Y574	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ98 spa2-Δ1::TRP1* + *pSPA2/TRP1/SUP4/CEN*
Y545	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 spa2-Δ1::TRP1*
Y601	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 spa2-Δ3::URA3*
Y753	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ98 spa2-Δ1::TRP1 slk1-1* + *pSPA2/TRP1/SUP4/CEN*
Y754	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ98 spa2-Δ1::TRP1 slk1-1* + *pSPA2/TRP1/SUP4/CEN*
Y758 (a–f)	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 slk1-Δ2::URA3*
	MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 SLK1
Y759 (a–c)	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 spa2-Δ1::TRP1 SLK1::slk1-Δ2::URA3*
Y760 (a–c)	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 slk1-Δ2::URA3*
Y761 (a and b)	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 slk1-Δ2::URA3*
Y762	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200*
Y763	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200*
Y764	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ98 HIS3 spa2-Δ1::TRP1*
	MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 LEU2 his3-Δ200 spa2-Δ1::TRP1
Y765 (a–c)	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ98 HIS3 spa2-Δ1::TRP1 slk1-Δ1::URA3*
	MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 LEU2 his3-Δ200 spa2-Δ1::TRP1 SLK1
Y766	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 ssd1-Δ1::URA3*
Y767	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 ssd1-Δ1::URA3*
Y768	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 ssd1-Δ1::URA3*
	MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 ssd1-Δ1::URA3
Y769	*MAT***a***ura3-52lys2-801ade2-101trp1-Δ1his3-Δ200ssd1-Δ1::URA3spa2-Δ1::TRP1*
Y770	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 ssd1-Δ1::URA3 spa2-Δ1::TRP1*
Y778	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ98 his3-Δ200 slk1-Δ2::URA3*
Y779	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 slk1-Δ2::ura3*
Y782	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 slk1-Δ1::TRP1*
Y784	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ98 HIS3 slk1-Δ2::URA3*
	MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 LEU2 his3-Δ200 slk1-Δ2::URA3
C276 congenic strains from J. Pringle	
Y580	*MAT***a**
	MATα
Y582	*MAT***a** *cdc42-1*
	MATα cdc42-1
Y583	*MAT***a** *cdc43-2*
	MATα cdc43-2
Y729	*MATα ura3 bem1-Δ::URA3*
Y730	*MATα ura3*
A364A congenic strains from L. Hartwell	
Y145	*MAT***a** *his7 ura1*
Y171	*MATα ura3-52 ade2-101 trp1-289 leu2-3,112 can1 sap3 his7*
Y172	*MAT***a** *ura3-52 ade2-101 trp1-289 leu2-3,112 can1 sap3 his7*
Y524	*MAT***a** *ura1 ade1 ade2 gal1 lys2 tyr1 his7 cdc3-1*
Y526	*MAT***a** *ura1 ade1 ade2 gal1 lys2 tyr1 his7 cdc10-1*
Y528	*MAT***a** *ura1 ade1 ade2 gal1 lys2 tyr1 his7 cdc11-1*
Y529	*MAT***a** *cdc12-1*
Y538	*MAT***a** *ura1 cdc35-1*
Y588	*MAT***a** *cdc24-2 ura1 ade2 ade1 tyr1 gal1 lys2 his7*
	MATα cdc24-2 his7 hom3 can1
Congenic strains from K. Tatchell	
Y488	*MAT***a** *leu2 ura3-52 his4*
Y489	*MAT***a** *leu2 ura3-52 his4 sra1-20::URA3*
Y490	*MAT***a** *leu2 ura3-52 his4 ras2-530::LEU2*
Y495	*MAT***a** *leu2 ura3-52 his4*
	MATα leu2 ura3-52 his4
Y493	*MAT***a** *leu2 ura3-52 his4 cdc25-1*
	MATα leu2 ura3-52 his4 cdc25-1
Y494	*MAT***a** *leu2 ura3-52 his4 cdc25-5*
	MATα leu2 ura3-52 his4 cdc25-5
Congenic strains from J. Szostak	
Y516	*MATα leu2 ura3 his3*
Y517	*MATα leu2 ura3 his3 ard1-Δ::URA3*
Y518	*MAT***a** *leu2 ura3 his3 ard1-Δ::URA3*

(Promega Biotec) (28). A 5.2-kb region containing the *SLK1* gene was sequenced. A 4.3-kb fragment containing the complementing region of pSSL1 was also sequenced. The sequences of both strands of DNA were determined. Because the *SSL1* sequence proved nearly identical to the recently published sequences of *SSD1/SRK1* (54, 62), only the nucleotide differences are reported. Initial comparisons of the predicted protein sequences and DNA sequences with those in the NBRF and GenBank data bases was performed, using the FASTA and tFASTA programs (40). Later comparisons of the predicted protein sequences were generously performed by Mark Goebl.

Chromosome mapping. Chromosome size DNAs were prepared from yeast strains Y231 (YNN231) and Y424 (YP149) and separated in a pulsed-field gel by the method of Chu et al. (14). Gel blots were prepared and probed with hexamer primer-labeled DNA from the insert of pSLK1 (20).

***SLK1* gene disruptions.** To generate disruptions of *SLK1*, the 5.7-kb *Pvu*II fragment of pSLK1, which contains most of the *SLK1* open reading frame (ORF), was subcloned into Bluescript SK$^+$ (Stratagene). *slk1-Δ1::URA3* was generated by digesting this plasmid with *Nde*I and *Sna*BI and ligating a 1.1-kb *Hin*dIII fragment containing the *URA3* gene to the DNA fragment containing the vector plus *SLK1* flanking regions. Prior to the ligation, the ends of both fragments were made flush by treatment with the large fragment of DNA polymerase I and dNTPs. The *slk1-Δ1::TRP1* mutation is identical to *slk1-Δ1::URA3* except that a 0.8-kb *Eco*RI-*Bgl*II fragment containing the *TRP1* gene was inserted into the SLK1 gene at the *Nde*I-*Sna*BI sites. In this construct, the *TRP1* gene is oriented in the same direction as SLK1.

A more complete deletion of the *SLK1* gene, *slk1-Δ2::URA3*, was also generated. The *slk1-Δ1::URA3* plasmid was digested with *Sma*I (which cleaves at the end of the *Hin*dIII fragment), and the 280-bp upstream fragment of *SLK1* was replaced with a 1.3-kb *Pvu*II fragment from pSLK1 which contains a fragment further upstream (see Fig. 2).

Linearized DNA containing the mutant alleles was generated by digestion of the appropriate plasmids with *Cla*I and *Not*I and used to transform diploid yeast cells by electroporation (8, 44). Strain Y270 was used for disruption in a wild-type strain, and Y764 was used for disruption in a *spa2* background. Gel blot analysis of genomic DNA from the yeast transformants and segregants was used to verify correct substitution at the genomic locus.

Although the *slk1* deletion mutations extend 36 nucleotides past the 3′ end of the SLK1 ORF, it is unlikely that these mutations affect the downstream gene for two reasons. First, the ORF that lies 3′ to *SLK1* is at least 130 bp away and is oriented so that the direction of transcription converges with that of *SLK1*. Thus, it is unlikely that promoter sequences for an adjacent gene are affected by the deletion mutation. Second, an *SLK1* DNA fragment extending only 240 bp past the termination codon complements an *slk1* deletion mutation.

SSD1/SRK1* gene disruption.** A disruption mutation in the *SSD1/SRK1* gene was created by first cloning a *Pvu*II fragment containing the full-length gene into Bluescript SK$^+$. This plasmid was then cleaved with *Bsa*BI and *Hpa*I, and the ends of the vector were filled in as described above. A 1.1-kb *URA3*-containing *Hin*dIII fragment, with filled-in ends, was ligated into the *SSD1/SRK1* plasmid. Linearized DNA containing *ssd1-Δ1::URA3* was generated by digestion with *Cla*I and *Not*I and used to transform yeast strain Y270. Haploid *ssd1-Δ1::URA3* segregants were crossed to Y545, and the phenotypes of the *ssd1-Δ1::URA3 spa2-Δ1::TRP1* segregants were analyzed. Diploid *ssd1-Δ1/ssd1-Δ1* cells were generated by crossing two haploid *ssd1-Δ1::URA3* segregants of opposite mating type and isolating zygotes by micromanipulation. The *MATa**/*MAT*α genotype was confirmed by checking for an inability to mate. Gel blot analysis of genomic DNA of the yeast transformants and segregants was used to verify correct substitution at the genomic locus.

Growth measurements. Exponentially growing *SLK1* and *slk1* cells were diluted to an optical density at 600 nm (OD_{600}) of ~0.03 and incubated at 30°C. After 2 hours, OD_{600} measurements were made five times over the course of 5.5 h.

Morphology of mutant segregants. Heterozygous *slk1-Δ2/SLK1* diploids (Y758) were sporulated and dissected. An estimate of the number of *slk1-Δ2::URA3* segregants which were unbudded, budded, and aberrant or shmoolike was determined by using the dissecting microscope (160×) after micromanipulation. For Fig. 6, representative cells were transferred from the dissecting plate onto a coverslip covered with a thin layer of YPD in 1.5% agar. The coverslips were placed agar side up on glass slides and covered with a second, larger coverslip. The second coverslip was sealed to the slide with nail polish, and cells were examined under a 100× oil immersion objective by differential interference contrast microscopy (magnification of 1,250×). Viable *slk1* cells were examined directly by differential interference contrast microscopy.

Pheromone-treated cells were prepared as described by Gehrung and Snyder (23). *MAT***a** yeast cells were grown at 30°C to an OD_{600} of 0.3. Strains Y760a, Y762, Y782 containing pSSL1, Y778 containing pSLK1 or constructs 1 to 5 (Fig. 2B), and control strains containing plasmid vectors were tested. α-Factor was added to a final concentration of 4 μg/ml, and after 50 min a second equivalent aliquot of α-factor was added. Incubation was continued for a total of 100, 120, or 150 min. Aliquots were fixed at room temperature by the addition of formaldehyde to 3.7% and viewed with differential interference contrast microscopy.

Immunofluorescence with anti-SPA2 antibodies was performed as previously described (23, 53).

Assays for entry into stationary phase. Stationary-phase assays were performed according to Toda et al. (55, 56). Yeast cells (Y760, Y761, Y762, Y779 containing pSSL1, Y778 containing pSLK1 or constructs 1 to 5 [see Fig. 2B], and control strains containing plasmid vectors) were grown on YPD plates (or synthetic complete plates lacking appropriate amino acids or nutrients if plasmid selection was necessary) for 2 days at 30°C. Heat shock sensitivity was tested by replica plating cells to YPD plates warmed to 55°C, incubating the plates at 55°C for 1 h, then transferring the plates to 30°C to allow growth. Cells were tested for sensitivity to nutrient starvation by replica plating to minimal medium lacking nitrogen, 2% agar, or yeast minimal medium plates lacking glucose and incubated at 30°C for 7 days. After incubation on starvation media, the cells were replica plated back onto YPD and incubated at 30°C for 24 h.

Homozygous *slk1/slk1* diploids (Y784) and wild-type controls (Y270) were tested for sporulation ability by standard methods (50).

Glycogen accumulation was assayed by growing cells (Y60, Y761, and Y762) on YPD supplemented with adenine (60 μg/ml) for 1 week at 24°C. Adenine supplementation was necessary to prevent the accumulation of the red pigment which occurs in our *ade2* strains on YPD plates. Strains were inverted over iodine crystals for ~10 min, until wild-type strains had stained brown (61).

Nucleotide sequence accession number. The sequence of

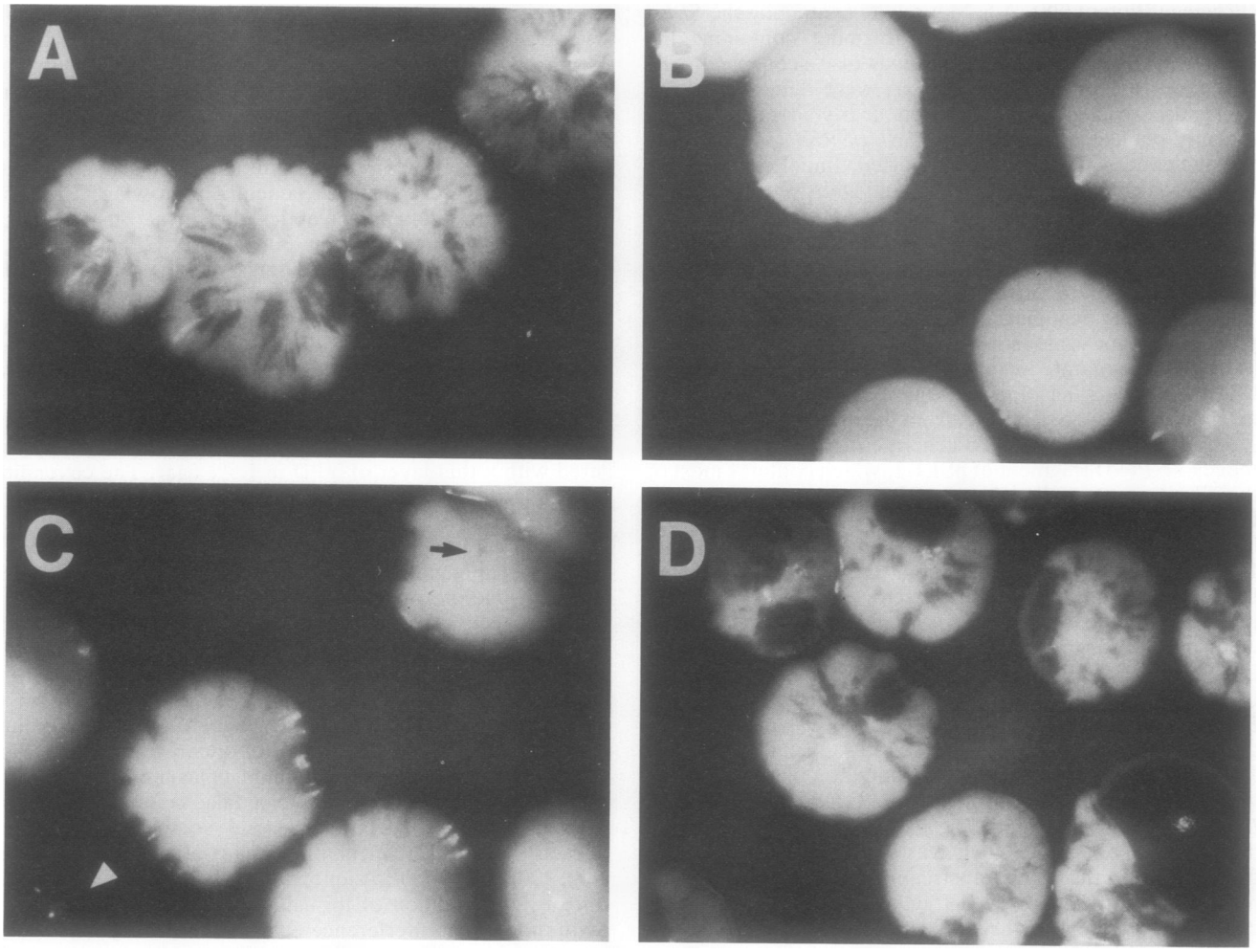

FIG. 1. Synthetic lethal screen: colonies of strains containing the *SPA2/SUP4* plasmid. (A) Starting strain Y574 (*spa2-Δ1*). (B) Synthetic lethal mutant Y753 (*slk1-1 spa2-Δ1*). (C) Synthetic lethal mutant Y753 containing the suppressor gene, *SSL1*, on a centromeric plasmid (*slk1-1 spa2-Δ1* + pSSL1). Small red colonies (arrowhead) and small red sectors (arrow) are present when the *SPA2/SUP4* plasmid is lost. (D) Synthetic lethal mutant Y753 containing the *SLK1* gene on a centromeric plasmid (*slk1-1 spa2-Δ1* + pSLK1).

the *SKL1* gene can be obtained under GenBank/EMBL accession number M84389.

RESULTS

Identification of mutants that require *SPA2* for growth by using a color colony synthetic lethal screen. The SPA2 protein plays an important role in mating cells of *S. cerevisiae*: it localizes to the tip of the projection, and *spa2* mutant cells usually fail to form projections (23). In vegetatively growing cells, the SPA2 protein is abundant and localizes to sites of yeast cell growth; however, *spa2* mutants have only minor growth defects (see introduction). It therefore seemed plausible that some gene or pathway exists in yeast cells that partially overlaps with *SPA2* function.

In an attempt to identify genes involved in pathways that overlap with that of the *SPA2* gene, and to identify genes that might be involved in the same pathway as is *SPA2*, we devised a synthetic lethal screen in which mutants that require the *SPA2* gene for growth were sought. This screen makes use of a simple *ade2/SUP4* colony color assay. A haploid yeast strain was constructed that contains a deletion of the *SPA2* gene, *spa2-Δ1*, and *ade2-101*, an *ade2* ochre mutation. These cells turn red in medium containing limited adenine because of the accumulation of a red pigment in the *ade2* mutant strains (29). A centromeric plasmid containing *SPA2* and *SUP4*, an ochre-suppressing tRNA gene, was introduced into these strains. In the presence of *SUP4*, the haploid *ade2-101* mutant cells are white (48). When *spa2 ade2* cells containing this plasmid are grown on nonselective plates, the small circular plasmid is normally lost at a high frequency (2 to 4% loss per cell division), and thus the cells form white colonies with many red sectors (29, 48) (Fig. 1A). Plasmid loss occurs because the *SPA2* gene is not essential in the *spa2* mutant cells. To search for mutants that require the *SPA2* gene for growth, this strain was mutagenized with UV, and potential mutants that require the *SPA2* gene for growth were identified as homogeneous white colonies; i.e., they were dependent on the *SPA2* gene for viability and consequently could not grow in the absence of the plasmid (see Materials and Methods). One example is shown in Fig. 1B. White colonies can also arise from other sources: petite strains (i.e., those that have lost mitochondrial function), various other *ade* mutants (e.g., *ade8*) that act upstream of

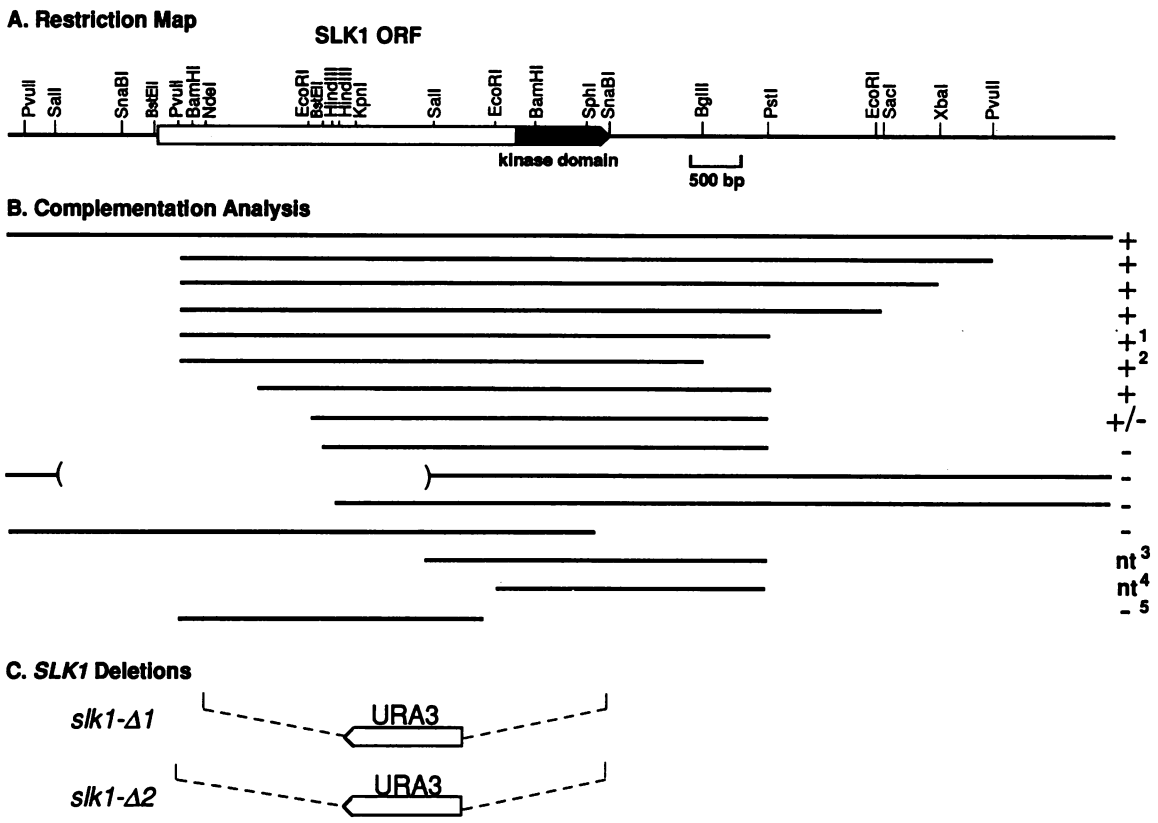

FIG. 2. (A) Restriction map of the *SLK1* region. The map is incomplete outside the coding region. (B) Complementation analysis of *SLK1* clones. Various subclones on centromeric plasmids were tested for complementation in a *slk1-1 spa2-Δ1* strain (Y754). +, complementation of the *slk1-1* nonsectoring phenotype; −, failure to complement *slk1-1*; nt, not tested. Clones 1 to 5 were also tested for complementation of the 37°C growth defect, caffeine sensitivity, shmoo formation defect, and cell cycle arrest defects of *slk1-Δ2* (Y778). (C) *slk1* deletion mutations. The *URA3* gene was substituted for a large fragment of *SLK1* as described in Materials and Methods to produce *slk1-Δ1::URA3* and *slk1-Δ2::URA3*.

ADE2, and other types of mutants that might require the plasmid (e.g., ochre mutants). To rule out other types of mutants that might form white colonies yet not require the *SPA2* gene for growth, potential mutants were transformed with a second plasmid that contains a *SPA2* gene and *URA3* but lacks *SUP4*. Six *SPA2*-dependent mutants were identified that lost the original *SPA2/SUP4* plasmid in the presence of another copy of *SPA2* and formed sectored colonies. A strain containing one mutation, called *slk1-1*, that is recessive and segregates as a single locus has been characterized extensively. *slk1-1* mutant cells grow normally in the presence of the *SPA2* plasmid but are inviable in its absence.

Cloning the *SLK1* gene by complementation: isolation of *SLK1* and a second suppressing gene, *SSL1*. The *SLK1* gene was cloned by complementation of the nonsectoring phenotype (see Materials and Methods for details). Eight plasmids that complemented the *slk1-1 spa2* mutation(s) were identified. Restriction mapping analysis revealed that six of the eight plasmids contained the *SPA2* gene; the other two plasmids each contained unique DNA, as judged by restriction mapping and DNA sequence analysis (see below). One of these plasmids, pSLK1, fully restores the sectoring level to that of *SLK1* strains (Fig. 1D). Red colonies that have lost the *SPA2* plasmid are the same size as wild-type strains. The other plasmid, pSSL1 (for suppressor of *spa2* synthetic lethality), only partially suppresses the *slk1-1* mutation; in colonies that contain the *SPA2* plasmid, only tiny red sectors are seen; without the *SPA2* plasmid, very small red colonies are produced (Fig. 1C).

Plasmid pSLK1 was shown to encode the *SLK1* gene by integration of a *URA3* gene at the locus encoded by the pSLK1 insert (see Materials and Methods). Sporulation of *SLK1::URA3/slk1-1 spa2/spa2* diploids revealed that *URA3* segregated away from the *slk1-1* locus in each of 41 tetrads (see Materials and Methods). Thus, we conclude that pSLK1 encodes the *SLK1* gene.

DNA sequence analysis reveals that *SLK1* encodes a protein kinase homolog. To learn more about the function of *SLK1*, the *slk1-1*-complementing region was identified (Fig. 2), and the nucleotide sequence of a 5.2-kb DNA segment containing this region was determined (Fig. 3) (47). A single long ORF that is predicted to encode a protein 1,478 amino acids in length was identified. Complementation of the *slk1-1* mutation is due to the ORF and not to adjacent sequences, because deletions extending into the *SLK1* ORF from either direction abolish *slk1* complementation. Interestingly, however, the amino-terminal coding sequences of *SLK1* are not necessary for complementation. A DNA fragment which lacks 311 codons at the 5′ end of the ORF still fully complements the *slk1-1* mutation (Fig. 2). We speculate that the truncated gene is expressed by a cryptic promoter and ATG in the yeast shuttle vector or by promoter and/or ATG sequences encoded in the *SLK1* ORF. The truncated fragments work in either orientation in the vector; thus, if vector

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-505 ACATTTTAAACATGACCGATGTAACAACTTTAGGATCTAGAAACTACAAGCTAGTTGGTCATCCAATCAATACTTCTCTGTATACTTTAAAAGCAACCGTTGTTGGGAAAACTAAAAGG
-386 GCTTTCCAAACTAGGGAGGTAACGAAGAGAAGAAATAGACGGGTCCGTCATGCAAAGAGTAAAGGTGATTTGACAATCTTAAGAATATCAGAACTGAGTATGAACTGAATTTTAGGGAT
-267 ATGTACATATACGCATACGTATAAAATCATGTAAATAACAATAAAATTTGACACACTTTCTTCGCCCCAATGGCCATTCAATTCCAGACCGACGCGTCTTCTAAGTTATAATGCTATGA
-148 AATTTCAACAAGTTAATTAAAAGTAAGCATAATAATAAAATCAGGTAATGGATTCACCAAAAAAAAAAATAAAAAAACAAGGGCAGAGGGGTGACCATTAGTACGAAACACTAAATATA
-29  GTATTAAAATAGTTCAACTCCACCTCCAA

1    ATG CCC TTT TTG AGG AAA ATA GCG GGG ACA GCA CAT ACA CAT TCT AGG TCT GAT TCG AAC TCA TCT GTG AAA TTC GGC CAT CAG CCG ACT
1    M  P  F  L  R  K  I  A  G  T  A  H  T  H  S  R  S  D  S  N  S  S  V  K  F  G  H  Q  P  T

91   AGT TCG GTA GCA TCA ACC AAA AGT TCA AGC AAA AGC CCT CGT GCA ACA TCT CGC AAA AGC ATT TAT GAT GAT ATT AGA AGC CAA TTT CCC
31   S  S  V  A  S  T  K  S  S  S  K  S  P  R  A  T  S  R  K  S  I  Y  D  D  I  R  S  Q  F  P

181  AAC CTA ACC CCC AAC TCT ACC TCT TCT CAG TTT TAC GAA AGC ACG CCA GTT ATC GAA CAA TCC TTT AAT TGG ACG ACA GAT GAC CAC ATC
61   N  L  T  P  N  S  T  S  S  Q  F  Y  E  S  T  P  V  I  E  Q  S  F  N  W  T  T  D  D  H  I

271  TCA GCT GGA ACG CTT GAA AAC CCA ACG AGC TTT ACA AAC AGT TCT TAT AAA AAT GAC AAT GGA CCT AGT AGC CTC TCT GAT TCG AGG AAA
91   S  A  G  T  L  E  N  P  T  S  F  T  N  S  S  Y  K  N  D  N  G  P  S  S  L  S  D  S  R  K

361  TCC TCC GGT GGC AAT AGC GTA AAT AGT TTG TCC TTT GAC AAG CTA ATT CTA TCG TGG GAT CCT ACA GAC CCT GAT GAA TGG ACA ATG CAT
121  S  S  G  G  N  S  V  N  S  L  S  F  D  K  L  I  L  S  W  D  P  T  D  P  D  E  W  T  M  H

451  CGC GTC ACC TCA TGG TTT AAA TTT CAT GAT TTT CCA GAA TCC TGG ATA TTG TTT TTC AAA AAG CAT CAA TTG TTT GGT CAC AGA TTT ATA
151  R  V  T  S  W  F  K  F  H  D  F  P  E  S  W  I  L  F  F  K  K  H  Q  L  F  G  H  R  F  I

541  AAG TTG CTT GCA TAT GAT AAT TTC GCT GTT TAT GAA AAG TAT TTG CCG CAG ACT AAA ACT GCT TCA TAT ACC AGG TTT CAG CAG TTA TTG
181  K  L  L  A  Y  D  N  F  A  V  Y  E  K  Y  L  P  Q  T  K  T  A  S  Y  T  R  F  Q  Q  L  L

631  AAA AAA ACA ATG ACC AAG AAC GTA ACA AAT AGC CAT ATT CGT CAG AAG AGC GCT AGC AAA CTT AAA AGT TCC AGG TCT TCC AGC GAA TCG
211  K  K  T  M  T  K  N  V  T  N  S  H  I  R  Q  K  S  A  S  K  L  K  S  S  R  S  S  S  E  S

721  ATC AAA TCA AAA TTA AAA AAT AGT AAA TCG CAA GAG GAT ATT TCA AAT TCT AGA TCA ACG TCA GAA TCT GCA TTG AGC CCA ACA AAA TCG
241  I  K  S  K  L  K  N  S  K  S  Q  E  D  I  S  N  S  R  S  T  S  E  S  A  L  S  P  T  K  S

811  GGC CCT TCC AAG ACC GAT GAA AAG AAT TTT TTA CAT TCT ACT TCA ACA CAC CAA AAA ACC AAA AGC GCA AGT TCA CTA TAC AGA AGA AGT
271  G  P  S  K  T  D  E  K  N  F  L  H  S  T  S  T  H  Q  K  T  K  S  A  S  S  L  Y  R  R  S

901  TTT ATA TCC CTA AGA GGC TCA TCA TCG AGC AAT GCT TCC TCA GCA AAA TCA CCT TCA AAC ATC AAG TTA AGT ATA CCG GCT CGG CCG CAC
301  F  I  S  L  R  G  S  S  S  S  N  A  S  S  A  K  S  P  S  N  I  K  L  S  I  P  A  R  P  H

991  TCA ATT ATT GAA TCT AAC AGT ACA CTT ACC AAA TCG GCG AGC CCA CCT GCA TCT CCT TCG TAT CCT AGC ATA TTT AGA AGA CAT CAC AAA
331  S  I  I  E  S  N  S  T  L  T  K  S  A  S  P  P  A  S  P  S  Y  P  S  I  F  R  R  H  H  K

1081 AGT AGT TCA TCT GAG TCG TCA TTA TTA AAT TCC CTT TTT GGT AGT GGA ATA GGC GAG GAA GCT CCA ACA AAG CCT AAT CCA CAA GGT CAT
361  S  S  S  S  E  S  S  L  L  N  S  L  F  G  S  G  I  G  E  E  A  P  T  K  P  N  P  Q  G  H

1171 AGT CTG TCT AGT GAA AAT TTA GCT AAA GGA AAA TCT AAA CAC TAT GAA ACT AAT GTG TCT TCA CCT TTA AAA CAA TCT TCA CTA CCC ACT
391  S  L  S  S  E  N  L  A  K  G  K  S  K  H  Y  E  T  N  V  S  S  P  L  K  Q  S  S  L  P  T

1261 TCG GAT GAT AAA GGT AAT TTA TGG AAT AAA TTC AAA AGA AAG AGC CAA ATA GGG GTT CCT AGC CCA AAT ACG GTA GCT TAT GTA ACG TCT
421  S  D  D  K  G  N  L  W  N  K  F  K  R  K  S  Q  I  G  V  P  S  P  N  T  V  A  Y  V  T  S

1351 CAA GAA ACT CCA TCC TTA AAA TCG AAT TCG AGT ACT GCT ACC TTA ACC GTA CAA ACG GCA GAT GTA AAT ATA CCA TCT CCA TCT TCA TCA
451  Q  E  T  P  S  L  K  S  N  S  S  T  A  T  L  T  V  Q  T  A  D  V  N  I  P  S  P  S  S  S

1441 CCA CCG CCA ATA CCC AAA ACT GCA AAC AGA AGT TTG GAG GTC ATC AGC ACA GAA GAT ACA CCT AAA ATT TCT TCA ACC ACG GCG TCT TTT
481  P  P  P  I  P  K  T  A  N  R  S  L  E  V  I  S  T  E  D  T  P  K  I  S  S  T  T  A  S  F

1531 AAA GAA ACG TAT CCT GAT TGT ATT AAT CCA GAC AAG ACA GTT CCA GTG CCG GTA AAT AAT CAA AAG TAT AGT GTA AAG AAC TTT TTA CTG
511  K  E  T  Y  P  D  C  I  N  P  D  K  T  V  P  V  P  V  N  N  Q  K  Y  S  V  K  N  F  L  L

1621 GAC CAA AAA TTT TAT CCT CTG AAG AAA ACA GGG TTA AAT GAT AGT GAG AAT AAA TAT ATT CTG GTT ACC AAA GAT AAT GTT AGT TTT GTT
541  D  Q  K  F  Y  P  L  K  K  T  G  L  N  D  S  E  N  K  Y  I  L  V  T  K  D  N  V  S  F  V

1711 CCG CTA AAC TTA AAA AGT GTA GCA AAA TTA TCC AGT TTC AAA GAA TCT GCT CTC ACA AAA TTG GGA ATC AAT CAC AAA AAT GTC ACT TTC
571  P  L  N  L  K  S  V  A  K  L  S  S  F  K  E  S  A  L  T  K  L  G  I  N  H  K  N  V  T  F

1801 CAT ATG ACA GAC TTT GAT TGC GAT ATT GGT GCT GCA ATT CCA GAT GAT ACT TTG GAA TTT TTG AAA AAA AGC TTG TTT TTG AAC ACT TCT
601  H  M  T  D  F  D  C  D  I  G  A  A  I  P  D  D  T  L  E  F  L  K  K  S  L  F  L  N  T  S

1891 GGA AAA ATT TAT ATC AAA GAC CAA ATG AAG CTT CAA CAA AAA CCG AAA CCT GCT CCT CTC ACC TCA GAA AAC AAT GTT CCT TTA AAA TCG
631  G  K  I  Y  I  K  D  Q  M  K  L  Q  Q  K  P  K  P  A  P  L  T  S  E  N  N  V  P  L  K  S

1981 GTG AAA AGT AAG AGT TCA ATG AGG TCC GGA ACA AGC AGT CTG ATA GCA TCG ACA GAT GAT GTT TCC ATT GTC ACT TCG TCT TCT GAC ATA
661  V  K  S  K  S  S  M  R  S  G  T  S  S  L  I  A  S  T  D  D  V  S  I  V  T  S  S  S  D  I

2071 ACA TCA TTT GAT GAA CAT GCA TCA GGA AGT GGG CGC AGG TAC CCC CAA ACC CCG AGT TAT TAC TAT GAC AGA GTT TCC AAT ACT AAT CCA
691  T  S  F  D  E  H  A  S  G  S  G  R  R  Y  P  Q  T  P  S  Y  Y  Y  D  R  V  S  N  T  N  P

2161 ACT GAA GAA TTG AAT TAT TGG AAT ATT AAA GAA GTT CTT TCT CAT GAG GAA AAT GCA CCA AAA ATG GTT TTT AAA ACA AGT CCA AAA TTA
721  T  E  E  L  N  Y  W  N  I  K  E  V  L  S  H  E  E  N  A  P  K  M  V  F  K  T  S  P  K  L
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FIG. 3. Sequence of the *SLK1* region. The nucleotide sequence is depicted above the predicted SLK1 protein sequence. Numbers indicate the positions of nucleic acid and amino acid residues.

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2251 GAA CTC AAC CTA CCA GAT AAA GGA AGT AAA TTA AAT ATT CCT ACC CCC ATA ACA GAA AAT GAA AGC AAG AGT AGT TTT CAA GTG CTA AGA
751  E   L   N   L   P   D   K   G   S   K   L   N   I   P   T   P   I   T   E   N   E   S   K   S   S   F   Q   V   L   R

2341 AAA GAT GAG GGG ACT GAA ATT GAT TTC AAT CAT CGT AGG GAA TCG CCT TAT ACA AAA CCA GAA CTG GCA CCA AAA AGA GAA GCT CCC AAG
781  K   D   E   G   T   E   I   D   F   N   H   R   R   E   S   P   Y   T   K   P   E   L   A   P   K   R   E   A   P   K

2431 CCT CCC GCA AAT ACT TCT CCT CAG AGG ACC TTA TCA ACT TCT AAA CAG AAT AAA CCG ATC CGC CTA GTG AGG GCA AGT ACA AAA ATT TCG
811  P   P   A   N   T   S   P   Q   R   T   L   S   T   S   K   Q   N   K   P   I   R   L   V   R   A   S   T   K   I   S

2521 AGA AGC AAA AGA TCG AAA CCA TTG CCG CCA CAA TTA TTA TCA TCT CCT ATA GAA GCT AGC AGC TCG TCT CCT GAT TCG CTT ACT TCC TCA
841  R   S   K   R   S   K   P   L   P   P   Q   L   L   S   S   P   I   E   A   S   S   S   S   P   D   S   L   T   S   S

2611 TAT ACT CCT GCT TCG ACT CAT GTT TTG ATA CCG CAA CCT TAT AAG GGT GCA AAC GAT GTT ATG CGT AGG TTG AAA ACA GAC CAG GAC TCG
871  Y   T   P   A   S   T   H   V   L   I   P   Q   P   Y   K   G   A   N   D   V   M   R   R   L   K   T   D   Q   D   S

2701 ACG AGT ACT TCC CCA TCT TTG AAA ATG AAA CAG AAA GTG AAT CGC TCA AAT TCA ACT GTA TCG ACT TCA AAT TCA ATT TTC TAT TCT CCT
901  T   S   T   S   P   S   L   K   M   K   Q   K   V   N   R   S   N   S   T   V   S   T   S   N   S   I   F   Y   S   P

2791 TCA CCA TTG TTA AAA AGA GGT AAC TCA AAA AGA GTT GTT TCG TCG ACA TCT GCG GCC GAT ATA TTT GAA GAG AAT GAC ATA ACA TTC GCG
931  S   P   L   L   K   R   G   N   S   K   R   V   V   S   S   T   S   A   A   D   I   F   E   E   N   D   I   T   F   A

2881 GAT GCT CCG CCG ATG TTT GAC AGC GAT GAT AGT GAT GAC GAT TCT AGT TCA TCC GAT GAC ATT ATC TGG TCC AAG AAA AAA ACA GCT CCT
961  D   A   P   P   M   F   D   S   D   S   D   D   D   S   S   S   S   D   D   I   I   W   S   K   K   K   T   A   P

2971 GAG ACT AAT AAT GAA AAC AAA AAG GAT GAG AAA AGC GAT AAC AGT TCT ACG CAT TCT GAC GAA ATA TTC TAT GAT TCT CAA ACG CAG GAC
991  E   T   N   N   E   N   K   K   D   E   K   S   D   N   S   S   T   H   S   D   E   I   F   Y   D   S   Q   T   Q   D

3061 AAA ATG GAG AGA AAG ATG ACC TTT AGA CCA TCT CCG GAG GTC GTT TAT CAA AAT TTA GAG AAA TTC TTC CCA AGG GCT AAC TTA GAT AAG
1021 K   M   E   R   K   M   T   F   R   P   S   P   E   V   V   Y   Q   N   L   E   K   F   F   P   R   A   N   L   D   K

3151 CCA ATC ACT GAA GGA ATA GCT TCA CCA ACA TCT CCG AAA TCC TTA GAC AGC CTA CTT TCA CCA AAG AAT GTG GCT TCA TCG AGA ACT GAG
1051 P   I   T   E   G   I   A   S   P   T   S   P   K   S   L   D   S   L   L   S   P   K   N   V   A   S   S   R   T   E

3241 CCA AGC ACT CCT TCC CGT CCC GTC CCT CCT GAT AGC TCA TAC GAG TTC ATA CAG GAT GGA CTT AAC GGT AAA AAT AAA CCA TTG AAT CAA
1081 P   S   T   P   S   R   P   V   P   P   D   S   S   Y   E   F   I   Q   D   G   L   N   G   K   N   K   P   L   N   Q

3331 GCT AAG ACA CCT AAA AGA ACA AAA ACC ATA AGA ACC ATT GCA CAT GAA GCT AGT TTA GCA AGA AAA AAC TCT GTA AAA CTA AAA AGA CAG
1111 A   K   T   P   K   R   T   K   T   I   R   T   I   A   H   E   A   S   L   A   R   K   N   S   V   K   L   K   R   Q

3421 AAC ACC AAA ATG TGG GGT ACA AGA ATG GTC GAA GTG ACC GAA AAC CAT ATG GTG TCA ATT AAT AAA GCC AAA AAT TCG AAA GGT GAG TAT
1141 N   T   K   M   W   G   T   R   M   V   E   V   T   E   N   H   M   V   S   I   N   K   A   K   N   S   K   G   E   Y

3511 AAG GAA TTC GCC TGG ATG AAG GGT GAA ATG ATA GGG AAG GGA TCT TTC GGT GCT GTT TAT TTA TGT TTA AAC GTT ACT ACA GGT GAG ATG
1171 K   E   F   A   W   M   K   G   E   M   I   G   K   G   S   F   G   A   V   Y   L   C   L   N   V   T   T   G   E   M

3601 ATG GCC GTT AAG CAG GTT GAG GTC CCC AAG TAT AGC TCA CAA AAT GAA GCC ATT CTA AGT ACC GTG GAA GCA TTA AGA TCT GAA GTG TCC
1201 M   A   V   K   Q   V   E   V   P   K   Y   S   S   Q   N   E   A   I   L   S   T   V   E   A   L   R   S   E   V   S

3691 ACG TTA AAA GAT TTA GAT CAT CTT AAT ATT GTT CAA TAC TTA GGT TTT GAG AAT AAA AAC AAT ATT TAC AGT TTG TTT TTA GAA TAT GTT
1231 T   L   K   D   L   D   H   L   N   I   V   Q   Y   L   G   F   E   N   K   N   N   I   Y   S   L   F   L   E   Y   V

3781 GCT GGT GGC TCC GTG GGA TCC TTG ATT AGA ATG TAT GGA AGA TTC GAT GAA CCG TTG ATC AAA CAT TTA ACA ACA CAA GTA TTA AAA GGA
1261 A   G   G   S   V   G   S   L   I   R   M   Y   G   R   F   D   E   P   L   I   K   H   L   T   T   Q   V   L   K   G

3871 TTG GCA TAC CTA CAC TCG AAA GGT ATT CTC CAC AGG GAT ATG AAG GCA GAC AAC TTA CTT TTG GAT CAA GAT GGT ATC TGC AAA ATC AGT
1291 L   A   Y   L   H   S   K   G   I   L   H   R   D   M   K   A   D   N   L   L   L   D   Q   D   G   I   C   K   I   S

3961 GAC TTC GGA ATT TCA AGA AAA TCA AAG GAC ATA TAC TCT AAT TCG GAT ATG ACC ATG CGA GGA ACA GTC TTC TGG ATG GCT CCT GAA ATG
1321 D   F   G   I   S   R   K   S   K   D   I   Y   S   N   S   D   M   T   M   R   G   T   V   F   W   M   A   P   E   M

4051 GTT GAT ACA AAG CAA GGC TAC AGT GCA AAA GTT GAT ATA TGG TCT CTG GGA TGC ATC GTT CTG GAA ATG TTT GCT GGT AAG CGC CCG TGG
1351 V   D   T   K   Q   G   Y   S   A   K   V   D   I   W   S   L   G   C   I   V   L   E   M   F   A   G   K   R   P   W

4141 TCC AAC TTA GAA GTC GTC GCA GCC ATG TTC AAA ATT GGA AAG TCA AAA TCG GCA CCA CCA ATT CCT GAG GAC ACT TTA CCA TTG ATA TCG
1381 S   N   L   E   V   V   A   A   M   F   K   I   G   K   S   K   S   A   P   P   I   P   E   D   T   L   P   L   I   S

4231 CAA ATC GGA CGA AAT TTT CTG GAC GCA TGC TTC GAG ATA AAT CCA GAG AAA AGG CCA ACC GCT AAC GAG CTT CTT TCT CAT CCT TTT AGT
1411 Q   I   G   R   N   F   L   D   A   C   F   E   I   N   P   E   K   R   P   T   A   N   E   L   L   S   H   P   F   S

4321 GAA GTA AAT GAA ACA TTC AAT TTC AAA TCT ACC AGA CTC GCG AAG TTT ATA AAG TCA AAT GAT AAG TTA AAC TCT TCA AAA TTA AGG ATA
1441 E   V   N   E   T   F   N   F   K   S   T   R   L   A   K   F   I   K   S   N   D   K   L   N   S   S   K   L   R   I

4411 ACC TCT CAG GAG AAT AAA ACT GAA TAG
1471 T   S   Q   E   N   K   T   E   Trm

4438 CTATTAGGATCGATCTATACTTAAGATATTTATGCATACGTAATAATATAGAACAATAAACATAGATGACGGAATGACGGAATGACTGAAAATTAAGAACAAAGAAAATTAAAAAATTT
4557 CTGCTGATACTTATTCAGACGAAAAATGGTGCACAATAAGGGTGTCAAAACGACGATTCGACCCTTCAAATATTACAGTCCTCACCTCTTCGCTGAAGGGCTCACTTTCATCAGGAAGGT
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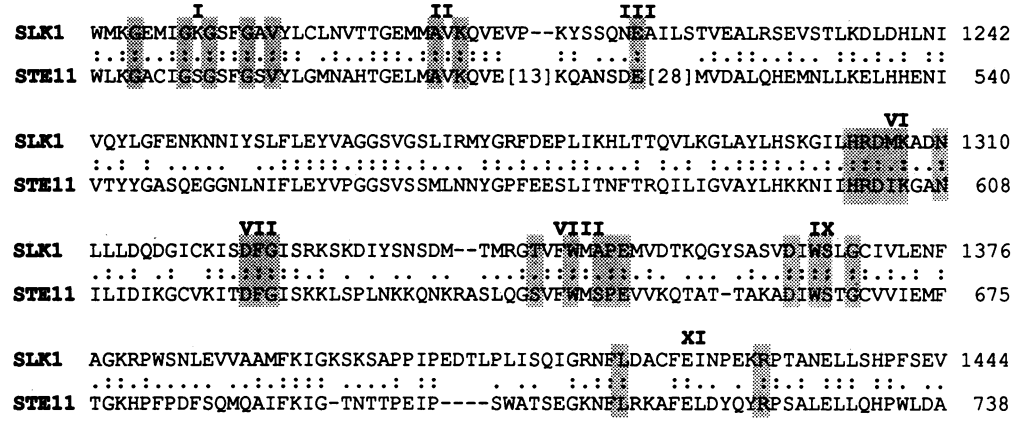

FIG. 4. Similarity between the predicted protein sequences of *SLK1* and *STE11*. The shaded boxes indicate the residues that are highly conserved among most Ser/Thr protein kinases (from reference 25). The roman numerals above them indicate the domain to which they correspond. :, identical residue; ., similar residue.

sequences do contribute to expression of the *SLK1* fragment, there must two different regions that are capable of performing this function.

The predicted protein sequence of *SLK1* was compared with those in GenBank, using the tFASTA program (40). A 300-amino-acid region at the carboxy terminus was found to have significant sequence similarity to that of protein kinases, particularly Ser/Thr kinases. Comparisons by Mark Goebl revealed that this region is most similar to the *STE11* gene of *S. cerevisiae* (41.4% identity over 244 amino acids) and to the *byr2* gene of *Schizosaccharomyces pombe* (36.9% identity over 339 amino acids) (42, 59) (Fig. 4 shows comparison with *STE11*). Both *ste11* and *byr2* exhibit defects in mating, and *STE11* has been shown to be important for cell cycle arrest at G_1. Sequence similarity of the predicted SLK1 protein sequence to the protein kinase C family was also noted (see Discussion). SLK1 contains the 11 domains conserved among all protein kinases (25) (Fig. 4).

The predicted SLK1 kinase sequence contains a number of potential phosphorylation sites for other protein kinases, including cyclic AMP (cAMP)-dependent protein kinase (RRES; amino acid residues 792 to 795 [31]), protein kinase C (RGNSKR; residues 936 to 941 [31]), and casein kinase II (SDDSDDDSSSSDD; residues 968 to 980 [57]). There are also 10 potential phosphorylation sites for cdc2/CDC28 kinase (49) and numerous potential sites for S6 kinase and calmodulin-dependent protein kinase (31). It is therefore possible that the SLK1 protein is itself regulated by phosphorylation.

***SLK1* is important for cell growth at 30°C and essential for cell growth at 37°C.** To learn more about the role of *SLK1* in vivo, deletion mutations were constructed in this gene (Fig. 2). *slk1-Δ1::URA3* contains a substitution of a 3,923-bp region of *SLK1* for a DNA fragment containing the *URA3* gene. This deletion leaves 185 codons of amino-terminal coding sequence upstream of the mutation and extends 36 nucleotides past the termination codon. *slk1-Δ2::URA3* is similar to *slk1-Δ1::URA3* except that only 90 codons remain upstream of the *URA3* fragment.

The *slk1-Δ2::URA3* mutation was introduced into wild-type diploid yeast strains. Six heterozygous diploid transformants were sporulated, and progeny from individual tetrads were dissected onto plates and analyzed. The segregants from each of the transformants yielded identical results, which are described collectively below. The viability of the *slk1-Δ2* segregants differed depending on the temperature at which the dissection plates were incubated (Fig. 5). When incubated at 37°C, 38 of 39 tetrads yielded two progeny that formed colonies and two that did not. The remaining tetrad produced progeny that formed one colony and three that did not. All colonies were Ura^-, indicating that they did not contain the *slk1-Δ2::URA3* mutation. Thus, the *SLK1* gene is necessary for cell growth at 37°C.

When tetrads from heterozygous diploids were incubated at 30, 24, or 17°C, different results were obtained. At 30°C, 30% of the predicted *slk1-Δ2* mutants formed very small colonies (n = 164), and 70% failed to form colonies. At 24 and 17°C, the viability of the *slk1-Δ2* strains was improved; 41 and 50%, respectively, formed colonies (n = 22 and 20, respectively). Thus, the *SLK1* gene is not essential for growth at lower temperatures but is important for cell growth and viability.

Analysis of *slk1-Δ2* mutant cells that grow at the permis-

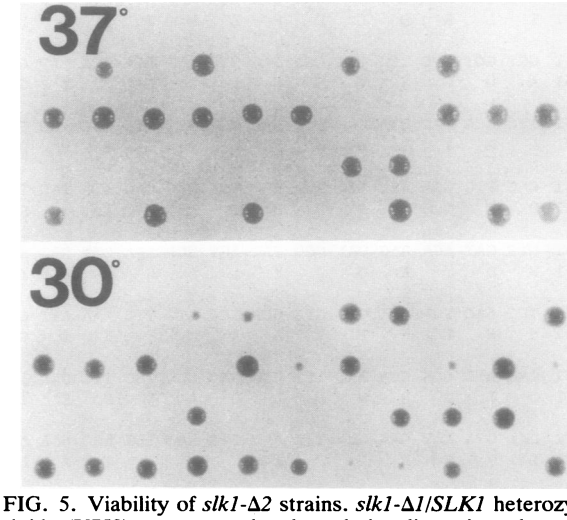

FIG. 5. Viability of *slk1-Δ2* strains. *slk1-Δ1/SLK1* heterozygous diploids (Y758) were sporulated, and the dissection plates were incubated at either 37, 30, 24, or 17°C (not shown for the latter two temperatures). *slk1-Δ2* segregants fail to form colonies at 37°C, and many fail to form colonies at the lower temperatures. The *slk1-Δ2* (Ura^+) colonies that do form at the lower temperature are significantly smaller than the Ura^- wild-type colonies.

TABLE 2. Growth of cells at the permissive temperature[a]

Strain	Doubling time (min)	% Unbudded	R	n
SLK1(Y762)	114.6	40.3	0.7	300
SLK1 (Y763)	112.2	NT	NT	NT
slk1-Δ2 (Y760a)	149.4	64.3	1.8	322
slk1-Δ2 (Y760b)	157.2	65.5	1.9	261
slk1-Δ2 (Y760c)	122.4	NT	NT	NT

[a] Y760a, Y760b, and Y760c refer to independent *slk1* isolates. nt, not tested; R, ratio of unbudded to budded; *n*, number of cells counted.

sive temperature revealed that they grow at 72 to 93% of the rate of wild-type cells (Table 2). *slk1-Δ2* isolates failed to form colonies at 37°C, as expected. To determine the arrest phenotype of *slk1-Δ2* mutants, cells were grown at the permissive temperature and shifted to the restrictive temperature of 38°C. The OD_{600} of the culture increased normally for 2.5 h, after which it remained constant. Microscopic analysis revealed that *slk1* mutant cells arrested at all stages of the cell cycle, indicating that *slk1* is not a cell division cycle mutant. Many of the cells incubated at the restrictive temperature appeared more transparent than the wild-type cells, suggestive of cell death.

The *slk1-Δ1::URA3* mutation was introduced into *spa2-Δ1/spa2-Δ1* diploid strains. Heterozygous *slk1-Δ1/SLK1* strains were sporulated. Analysis of 39 tetrads revealed that all segregated two progeny that produced Ura^- colonies and two that failed to produce colonies. Thus, the *slk1-Δ1::URA3* null mutation, like *slk1-1*, is lethal in a *spa2-Δ1* background.

***SLK1* is important for cell morphogenesis in vegetatively growing yeast.** Tetrad analysis revealed that most *slk1-Δ2* mutants failed to form colonies in a wild-type background, even at lower temperatures. Inspection of the cells that did not produce colonies revealed that at 37°C the cells had divided a total of 1 to 14 times (average number of total cell divisions = 5) prior to cell death. At 30°C, this number increased to an average of 10 cell divisions prior to death. Thus, in each case, the cells had germinated but were not able to sustain cell growth. At 30 and 37°C, 15 to 27% of the mutant cells were extremely small, less than one-half the size of wild-type cells (Fig. 6B). Greater than 90% of the cells which were of wild-type size or larger were morphologically abnormal. Representative cells are shown in Fig. 6. The cells either appeared shmoolike or had abnormal buds or irregular cell shapes. Some cells had intermediate bud/shmoo morphologies, such that the constriction at the neck was larger than expected for vegetative cells (Fig. 6C). Some cells had more than one projection or bud (not shown). It is possible that some of the small cells also had abnormal shapes, but this could not be determined easily because of their small size. Morphologically abnormal cells are not typical of dead cells. Similar examination of *nuf1* mutants (which, like *slk1* mutants, die several cell divisions after dissection of heterozygous diploids [35a]) and *cik1* cells (which are dead at 37°C [38b]) did not reveal any morphological abnormalities. Thus, *SLK1* may play a role in cell morphogenesis (see Discussion).

Microscopic analysis of the *slk1* mutant cells that formed colonies at the lower temperatures revealed that at 24 to 30°C, most of the cells appear normal. However, approximately 65% of *slk1-Δ2* cells in exponential growth at 30°C are unbudded, compared with 40% unbudded for wild-type cell populations (Table 2). Approximately 10% of *slk1-Δ2* mutant cells are very small, about one-half the diameter of a wild-type cell, and another fraction (5%) have abnormal morphologies similar to those described above. These phenotypes are consistent with a defect in both control of cell growth and morphogenesis.

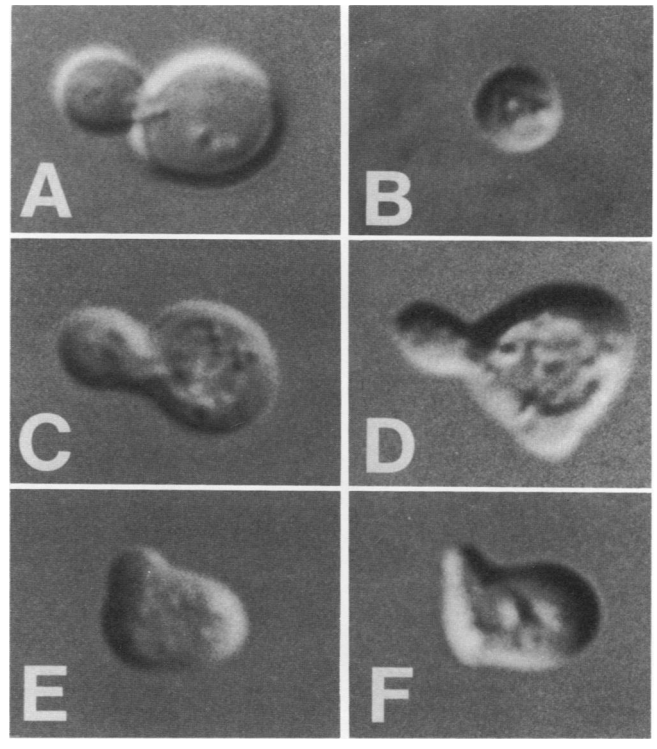

FIG. 6. Differential interference contrast microscopy showing that *slk1* progeny often have abnormal morphologies. (A) Wild-type cell. (B to F) *slk1-Δ2* mutants from progeny that failed to form colonies. Most large cells exhibit abnormal cell shapes. Some defects are subtle, such as the one shown in panel C, where the neck does not appear to have a normal constriction. (E and F) Cells that appear shmoolike (except that the projections do not usually emerge from the end of the cell as in wild-type cells) are often observed. The cells were photographed on a thin layer of agar; hence, the images are not as sharp as when agar is not used.

Defects in morphogenesis might reflect a loss of internal polarity of components involved in bud formation. To test this possibility, the distribution of the SPA2 protein was examined in *slk1-Δ2* mutant cells (Y760b and -c) grown at 30°C by indirect immunofluorescence with anti-SPA2 antibodies. The SPA2 protein localized to the bud tip in budded cells and to a small patch on the edge of unbudded cells, just as is observed for wild-type cells (data not shown). Thus, polarity of the SPA2 protein is not disrupted in *slk1* mutant strains.

***slk1-Δ* mutants are defective in pheromone-induced morphogenesis.** *spa2* mutants are defective in pheromone-induced morphogenesis. To test whether *SLK1* is important for this process, exponentially growing wild-type and *slk1-Δ2* MATa cells were treated with α-factor. After incubation at 30°C for 100, 120, and 150 min, the ability to form projections was examined by differential interference contrast microscopy. As shown in Fig. 7, when wild-type cells are exposed to α-factor for 100 min, they arrest as unbudded cells with long projections. *slk1-Δ2* mutants also arrest cell division (Table 3); however, the projections are very short.

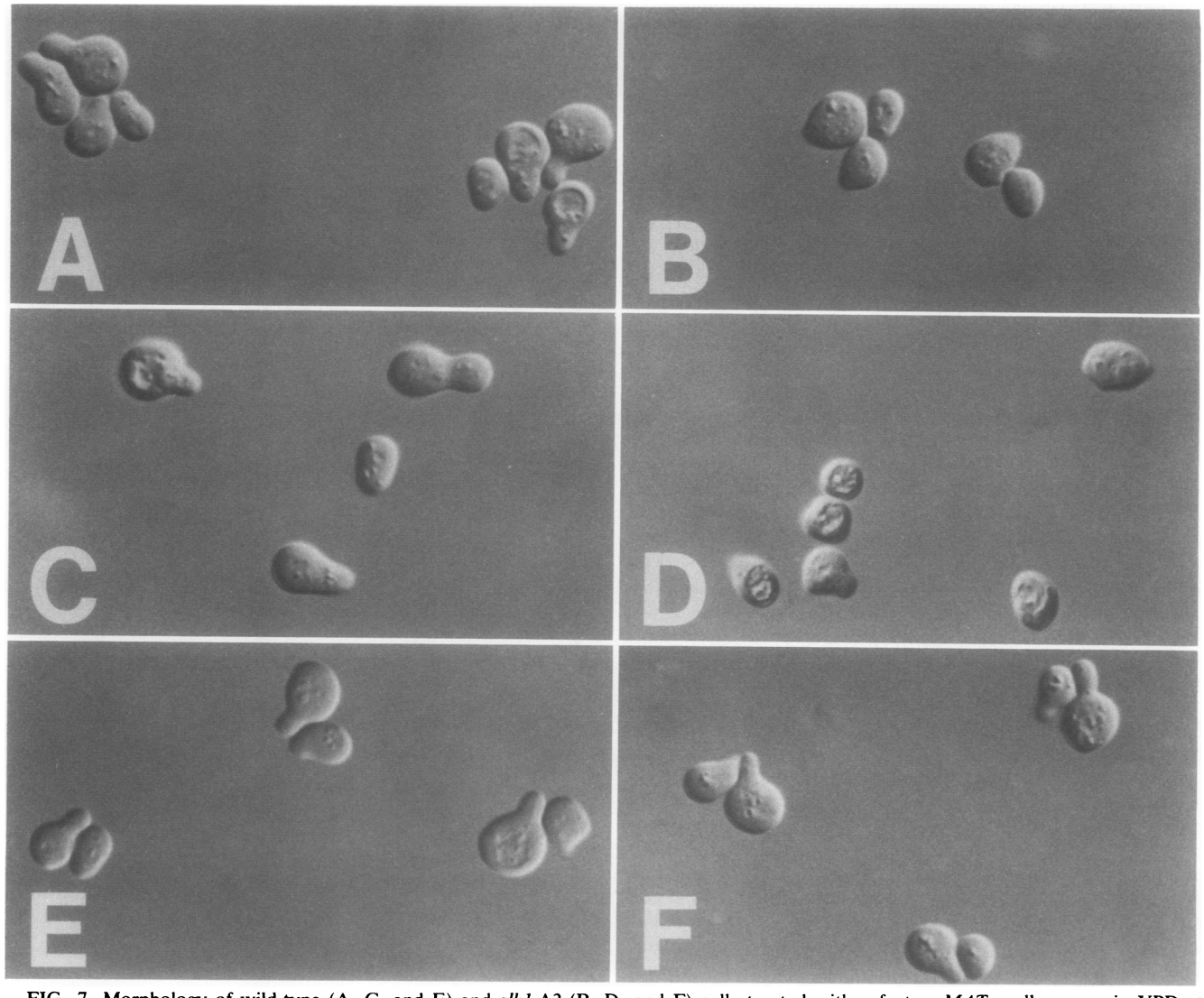

FIG. 7. Morphology of wild-type (A, C, and E) and *slk1*-Δ*2* (B, D, and F) cells treated with α-factor. *MAT***a** cells grown in YPD at 30°C were treated with α-factor for 100 min, and cells were viewed by differential interference contrast microscopy. (A and B) Cells grown at 30°C; (C and D) cells grown at 30°C and shifted to the restrictive temperature simultaneously with the addition of α-factor; (E and D) same as panels C and D except that 1 M sorbitol was present in the growth medium at both the permissive and restrictive temperatures.

TABLE 3. Shmoo formation[a]

Strain	Temp (°C)	% Unbudded	% Shmoos (P ≥ 1.5)	No. of cells tested
SLK1 (Y762)	30	100	73	700
slk1-Δ*2* (Y760a)	30	98	6.0	718
SLK1 (Y762)	38	100	44	713
slk1-Δ*2* (Y760a)	38	90	1.0	723
slk1-Δ*2* (Y760a)[b]	38	99	1.0	710
SLK1 (Y762)	38 + sorbitol	100	60	767
slk1-Δ*2* (Y760a)	38 + sorbitol	98	18	736

[a] Quantitation was determined for cells incubated in α-factor for 120 min.
[b] Cells were treated with α-factor at 30°C for 50 min and then shifted to 38°C. In the other experiments, α-factor was added simultaneously with the shift to 38°C.

The relative projection length for individual cells was quantitated by dividing the sum of the projection length and radius of each cell by the radius of the main portion of the cell (P value; Fig. 8). Those cells that have P values greater than 1.5 generally have a projection length greater than one-half the radius of the cell. For *slk1*-Δ*2* mutants, only 6% of the population have P values greater than or equal to 1.5, compared with 73% of wild-type cells after 120 min of exposure to α-factor (Table 3). Thus, *slk1*-Δ*2* mutants arrest cell division but are defective in pheromone-induced morphogenesis.

Wild-type and *slk1*-Δ*2* mutant cells were also exposed to α-factor for 150 min. Although the projections in *slk1*-Δ*2* mutant cells were slightly longer than those observed in cells treated for only 100 or 120 min, they were still considerably shorter than the projections of wild-type cells at either 110, 120, or 150 min (data not shown). The shmoo defect in *slk1*-Δ*2* cells is not due to a general growth deficiency,

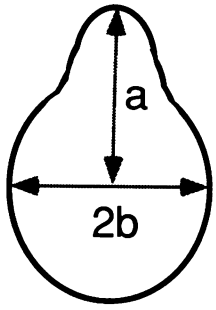

FIG. 8. Determination of P values to quantitate relative lengths of projections. P equals a/b.

because the OD of the culture continued to increase and was greater than 90% of the wild-type levels over the course of this experiment (up to 150 min tested). Thus, cell growth still occurs, but it is no longer directional.

We also tested whether *slk1*-Δ2 mutant cells can form projections at the restrictive temperature. Wild-type and *slk1*-Δ2 mutant cells were grown at the permissive temperature and shifted to 38°C simultaneously with the addition of α-factor. After incubation for 100, 120, and 150 min, *slk1*-Δ2 mutants remained round with small projections, as observed for cells grown at 30°C (for 120 min, 1% cells have a P value of ≥1.5; Table 3). In contrast, wild-type cells usually formed normal projections, although they were generally slightly shorter than for cells grown at 30°C. As above, the defect observed at 120 min is unlikely to be due to a general growth deficiency, because after a shift of *slk1*-Δ2 cells to the restrictive temperature and addition of α-factor, the culture still increases normally in OD for up to 150 min.

Lee and Levin (33a) have independently identified the *SLK1* gene and found that the growth defects of *slk1* cells grown at 37°C can be suppressed by the addition of sorbitol to the medium (see Discussion). We therefore tested whether the presence of sorbitol also suppressed the shmoo formation defects of cells incubated at the restrictive temperature. Wild-type and *slk1*-Δ2 mutant cells were grown in the presence and absence of 1.0 M sorbitol and shifted to 38°C simultaneously with the addition of α-factor. The presence of sorbitol partially suppressed the projection formation defect of *slk1*-Δ2 mutant cells relative to wild-type cells (Fig. 7). Many cells had long projections similar to those of wild-type cells, although the fraction of such cells was still less than that of wild-type cells (Table 3).

In summary, *SLK1* is implicated in cell morphogenesis during vegetative growth and shmoo formation.

***slk1* mutants exhibit defects in entry into meiosis and stationary phase.** Cells defective in cell cycle arrest such as those carrying *bcy1*, which encodes the regulatory subunit of cAMP-dependent protein kinase, and *ard1*, which encodes a subunit of an amino-terminal acetyltransferase (36), fail to enter meiosis or stationary phase (55, 61). Under nutrient-limiting conditions, the cells remain budded and are sensitive to heat shock, and prolonged incubation results in a loss of cell viability.

slk1-Δ cells exhibit a variety of phenotypes indicative of a defect in exit from the vegetative growth cycle. Upon transfer to sporulation medium, *slk1*-Δ cells fail to undergo meiosis and do not form tetrads. Instead, the cells remain budded. In addition, *slk1*-Δ2 mutants exhibit defects in entry into stationary phase, as assayed by four criteria. First, when a culture of wild-type cells is grown in rich YPD medium to stationary phase for 10 days, 0.5% of the cells remain budded. In contrast, approximately 30% of *slk1* mutant strains remain budded under similar conditions. Second, when wild-type cells are transferred to plates lacking either nitrogen, glucose, or all nutrients, they remain viable. When *slk1* mutant cells are similarly starved, they rapidly lose viability (results of a nitrogen starvation assay are shown in Fig. 9; other data are not shown but were similar to those in Fig. 9). Third, when patches of *slk1* cells are exposed to a heat shock at 55°C, the cells rapidly lose viability, whereas wild-type cells remain viable. Finally, when wild-type cells are grown for 1 week on YPD plates, they accumulate glycogen, whereas *slk1* cells do not (data not shown). These different defects can be complemented by the presence of a plasmid containing *SLK1* or partially suppressed by the *SSL1* plasmid (see below). All of these defects, a high fraction of budded cells in saturated cultures, loss of viability upon nutrient starvation, sensitivity to heat shock, and failure to accumulate glycogen, are suggestive of an inability to respond properly to nutrient-limiting conditions and exit the cell cycle.

The entire *SLK1* gene is not necessary for *SLK1* functions. During the course of this work, we noticed that truncations in the amino-terminal coding region of *SLK1* still complemented many of the *slk1*-Δ2 defects. The truncations are simple deletions of the coding sequence; presumably, vector sequences are responsible for expression of the *slk1* truncation alleles (constructs 1 to 4; Fig. 2B). The growth defect of *slk1*-Δ2 mutants at 37°C was complemented by plasmids containing several amino-terminal deletions (constructs 1 to

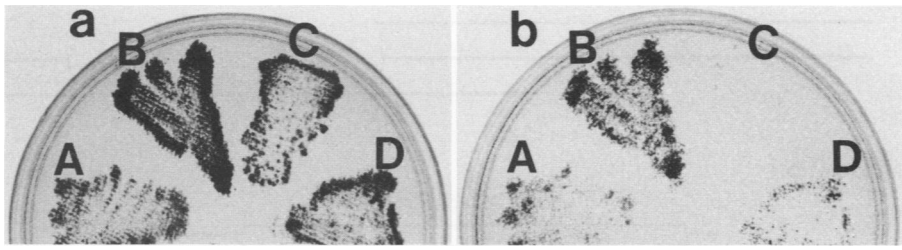

FIG. 9. Sensitivity of *slk1*-Δ2 mutants to nitrogen starvation. Cells were replica plated to media lacking nitrogen and to control YPD plates. Cells on media lacking nitrogen were incubated at 30°C for 7 days and then replica plated back to YPD and incubated at 30°C for 24 h (b). The YPD control plates were incubated at 30°C for 24 h (a). (C) *slk1*-Δ2 cells (Y778) fail to survive transient starvation for nitrogen. This defect can be complemented by (B) pSLK1 and partially suppressed by (D) a plasmid carrying the *SSD1/SRK1* gene (pSSL1 in strain Y779) and by (A) a plasmid carrying an *SLK1* truncation which lacks the amino-terminal 1,133-amino-acid coding sequence of *SLK1* (construct 4; Fig. 2B). Yeast patches appear dark because plates were photographed with transillumination.

3), including a plasmid lacking the 893 codons predicted to encode the amino terminus of the SLK1 protein (construct 3). The shmoo formation defect could be complemented with a plasmid lacking the amino-terminal 90 codons of *SLK1* (constructs 1 and 2) but not by other deletions (constructs 3 or 4). The heat shock sensitivity, inability to survive nitrogen starvation, and failure to grow in the presence of 8 mM caffeine could be complemented with *SLK1*-containing plasmids lacking up to 1,133 codons encoding the amino-terminal domain, leaving the kinase domain (constructs 1 to 4). A plasmid that lacks the kinase domain (construct 5) failed to complement any of the growth, cell cycle exit, or shmoo formation defects, indicating that this domain is necessary for *SLK1* function. Thus, the entire *SLK1* ORF is not necessary for complementing the different *slk1-Δ2* phenotypes in yeast cells, and in some cases only a small region containing the catalytic domain is necessary. These results indicate that different functional domains reside in the SLK1 protein or that different levels of *SLK1* activity are needed for each of these processes and growth conditions.

Chromosomal mapping of *SLK1*. The chromosomal location of the *SLK1* gene was determined by pulsed-field gel electrophoresis. Gel blots of chromosome-size yeast DNA separated in a pulsed-field gel (14) were probed with a ^{32}P-labeled DNA fragment derived from pSLK1. Hybridization corresponding to chromosome X was observed (data not shown).

***SSL1* encodes a recently identified gene called *SSD1/SRK1* that is involved in protein phosphatase function.** pSSL1 weakly suppressed the defects of *slk1-1 spa2-Δ1* synthetic lethality. pSSL1 also partially suppressed the various *slk1-Δ* mutant defects, including the inability to grow at 37°C, the failure to enter stationary phase (heat shock sensitivity, and viability loss when transferred to medium lacking nitrogen or all nutrients), the shmoo formation defect, and the inhibition of growth by caffeine (see below; rescue of the viability loss on media lacking nitrogen is shown in Fig. 9; rescue of the caffeine sensitivity defect is reported in Table 4).

To understand its interaction with *spa2* and *slk1*, we analyzed the *SSL1* gene. The minimum complementing region of pSSL1 was narrowed to 4.7 kb (Fig. 10). A 4,304-bp region was sequenced and found to contain a single large ORF, 3,750 nucleotides in length, which is capable of encoding a protein of 1,250 amino acids. Mark Goebl compared the predicted sequence with those in his data base. Except for two nucleotide differences, *SSL1* was identical to two recently reported sequences, *SSD1* and *SRK1* (54, 62). *SSD1/SRK1* has been identified as a suppressor of both cAMP pathway mutants and mutants defective in the G_1/S transition (see Discussion). Its product is implicated in protein phosphatase function.

The nucleotide and predicted amino acid differences between *SSL1* and *SSD1/SRK1* are shown in Fig. 11. It is unlikely that these differences are due to sequencing errors; our sequence is unambiguous at these positions, and the sequence of *SSD1/SRK1* was independently determined by two different groups. Thus, they reflect either natural polymorphisms or changes that occurred during cloning or propagation events. Our clone and those encoding *SSD1* and *SRK1* are all derived from the same genomic DNA library. The *SSL1* gene will subsequently be referred to as *SSD1/SRK1*.

There are two interesting features about the predicted SSD1/SRK1 protein sequence. First, glutamine- and asparagine-rich regions are found in the amino-terminal portion of the protein. A polyglutamine-rich region is located at residues 60 to 81 (13 of 22 residues are glutamine), and another short glutamine-rich region (6 of 8, residues are glutamine) extends from position 277 to 284. An asparagine-rich region lies at position 135 to 141 (6 of 7 residues are asparagine). Second, two potential phosphorylation sites for cAMP-dependent protein kinase are present in the sequence at

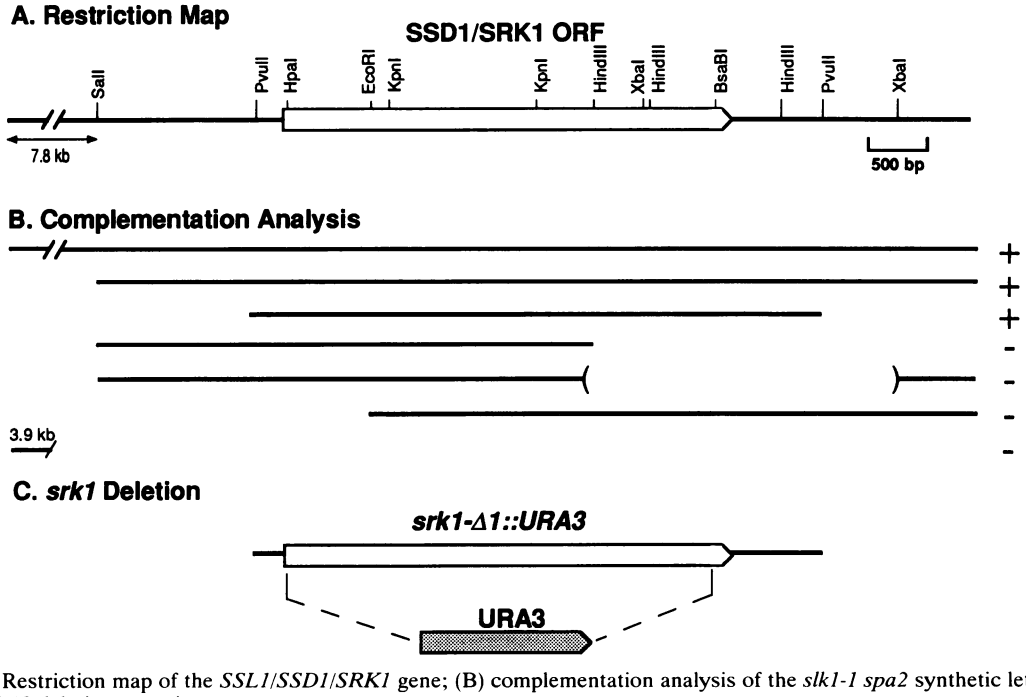

FIG. 10. (A) Restriction map of the *SSL1/SSD1/SRK1* gene; (B) complementation analysis of the *slk1-1 spa2* synthetic lethal phenotype; (C) *ssd1-Δ1::URA3* deletion mutation.

TABLE 4. Caffeine sensitivity at 30°C[a]

Mutant	Strain(s)	Growth in caffeine at concn (mM) of:						
		1	3	5	8	11	13	15
SLK1/SLK1 (S288C)	Y270	+	+	+	+	+	+	±
SLK1	Y762, Y763	+	+	+	+	+	+	±
slk1-Δ1	Y782	+	−	−	−	−	−	−
slk1-Δ2	Y760, Y761, Y779	+	−	−	−	−	−	−
slk1-Δ + pSSL1	Y779[b], Y782[b]	+	+	−	−	−	−	−
slk1-1	Y753	+	+	+	+	+	+	±
ssd1-Δ1	Y766, Y767	+	+	+	+	+	+	±
ssd1-Δ1/ssd1-Δ1	Y768	+	+	+	+	±	−	−
ssd1-Δ1 spa2-Δ3	Y769, Y770	+	+	+	+	+	+	±
spa2-Δ3::URA3	Y601	+	+	+	+	+	+	±
spa2-Δ1/spa2-Δ1	Y764	+	+	+	+	+	+	±
bcy1	Y489	+	+	−	−	−	−	−
ard1	Y518	+	+	+	−	−	−	−
bem1	Y729	+	+	+	−	−	−	−
Other[c]	See strain list	+	+	+	+	+	+	+[d]

[a] Strains were patched on YPD plates containing different concentrations of caffeine and scored for the ability to grow. +, normal growth; ±, impaired growth; −, no growth.

[b] Containing plasmid pSSL1.

[c] This category includes the mutants cited in the text and the isogenic wild-type strains for each mutant (genotypes and specific strain numbers are provided in Table 1).

[d] ± or + for S288C strains or other strain backgrounds, respectively.

positions 161 to 165 (RRHSL) and 499 to 503 (RRRSS). Thus, the SLK1 and SSD1/SRK1 proteins are both potentially regulated by the cAMP-dependent protein kinase.

To determine the role of *SSD1/SRK1* in our S288C yeast strains, a deletion mutation was constructed. *ssd1-Δ1::URA3* contains a substitution of a DNA fragment containing the *URA3* gene for a 3,510-bp internal fragment of *SSD1/SRK1*; only 7 codons lie upstream of the *URA3* insertion, and 72 codons lie downstream of the insertion. This mutation was transformed into diploid yeast strains, and the strains were sporulated. Four viable spores were obtained; these segregated 2 Ura^+:2 Ura^-. The *ssd1-Δ1::URA3* strain was crossed to a strain containing the *spa2-Δ1::TRP1* mutation. *ssd1-Δ1::URA3 spa2-Δ1::TRP1* segregants were observed at the expected frequency. No growth defects of *ssd1-Δ1::URA3* and *ssd1-Δ1::URA3 spa2-Δ1::TRP1* mutant strains were observed when cells were incubated on plates at either 37, 30, or 17°C. *ssd1-Δ1/ssd1-Δ1* strains also undergo meiosis well and form tetrads with four viable spores. Thus, the *SSD1* gene is not essential for mitosis or meiosis, nor is it essential for growth in a *spa2* mutant background (however, see Discussion).

Caffeine inhibits growth of *slk1-Δ2* and *ssd1-Δ1* mutants. Sutton et al. (54) reported that strains containing a deletion of *SSD1/SRK1* do not grow in the presence of caffeine, an inhibitor of cAMP phosphodiesterases (7, 39). We therefore tested whether *slk1-Δ2*, *ssd1-Δ1*, and *ssd1-Δ1 spa2-Δ1* cells would grow in the presence of 1, 3, 5, 8, 11, 13, and 15 mM caffeine at 30°C. We also tested a variety of other mutants involved in growth control (*bcy1/sra1-20*, *cdc25-1*, *cdc25-5*, *cdc35-1*, *ras2-530*, and *ard1-Δ*), budding (*spa2-Δ1*, *cdc24-1*, *cdc42-1*, *cdc43-1*, and *bem1-Δ*), and cytokinesis (*cdc3-1*, *cdc10-1*, *cdc11-1*, and *cdc12-1*) and one mutant defective in chromosome segregation, *cik1-Δ* (38b). The *bcy1*, *cdc25-1*, *cdc25-5*, *cdc35-1*, *ras2-530*, and *ard1-Δ* mutants exhibit defects in the cAMP pathway and/or growth control (12, 55, 61). *cdc24-2*, *cdc42-1*, and *cdc43-2* mutants are temperature sensitive; they fail to form buds at the restrictive temperature and arrest as large unbudded multinucleate cells (4, 41). *bem1* (for bud emergence) also is defective in bud formation and does not form colonies at 39°C (10). The *cdc3*, *cdc10*, *cdc11*, and *cdc12* mutants are also temperature sensitive for growth; at the restrictive temperature, these cells fail to undergo cytokinesis and form long chains of connected cells (41). *cik1-Δ* is also temperature sensitive for growth. A temperature of 30°C is semipermissive for growth of most of these mutants. Wild-type strains congenic with the various mutants were also tested.

slk1-Δ2, *bcy1*, *ard1*, and *bem1-Δ* mutants do not grow in the presence of 3, 5, 8, and 8 mM caffeine, respectively (Table 4). *ssd1-Δ1/ssd1-Δ1* diploid cells do not grow in the presence of 13 mM caffeine, whereas wild-type cells are unaffected by this concentration. This inhibition of growth in the presence of caffeine is observed only in *ssd1-Δ1/ssd1-Δ1* diploids; no inhibition was observed in haploid strains. None of the other mutants tested are more sensitive than wild-type cells at any of the concentrations tested (1, 3, 5, 8, 11, 13, and 15 mM). In summary, *slk1-Δ2* (and *bem1-Δ*) mutants, like *bcy1* and *ard1* strains, are more sensitive to caffeine than are the other mutant strains.

	Promoter	ORF
Position (aa)		186 to 188
(bp)	-133 to -126	556 to 564
		E **A** G
SSL1	TAA**G**ACAA	GAA **G**CA GGC
		E **T** G
SSD1/SRK1	TAA**A**ACAA	GAA **A**CA GGC

FIG. 11. Nucleotide differences between the *SSL1* gene sequenced in this study and the *SSD1/SRK1* gene sequence reported by others (54, 62). Differences are indicated in bold type. Nucleotide positions are relative to the first nucleotide of the initiator ATG codon. aa, amino acids.

DISCUSSION

Role of *SLK1* in yeast cells. This report describes the use of a simple synthetic lethal screen to search for mutants that require the *SPA2* gene for growth. By using this screen, a mutant called *slk1-1* was identified. The *SLK1* gene was cloned, and sequence analysis predicts that it encodes a protein kinase.

The various phenotypes of *slk1-Δ* mutants (abnormal cell morphology, high fraction of unbudded cells, small cell size, and cell cycle arrest defects) suggest that *SLK1* may directly or indirectly play a role in both morphogenesis and growth control. Analysis of *slk1-Δ2* cells that fail to form colonies from the dissection plates indicates that the mutants usually divide several times before cell death. These cells often display aberrant morphologies with one or more projections or budlike structures emanating from the cell. In those cells that survive at the lower temperatures, a small percentage (5%) of cells are also morphologically abnormal. Many of the cells are unbudded and a small fraction are very small, suggestive of a growth defect during G_1.

Consistent with the hypothesis that *SLK1* plays a role in morphogenesis, *slk1* mutants exhibit a severe defect in projection formation. The predicted SLK1 protein sequence is most similar to the predicted protein sequences of *STE11* of *S. cerevisiae* and *byr2* of *S. pombe*. Both the *STE11* and *byr2* genes are important in mating (42, 59). The *STE11* gene has been shown to be important for cell cycle arrest in late G_1 during mating. *SLK1* is also involved in the mating

response, but it appears to be more important for projection formation than for cell cycle control because *slk1* mutants still arrest as unbudded cells when exposed to mating pheromone. In this respect, *slk1* mutants are similar to *spa2* mutants, which also arrest properly upon exposure to mating pheromones and are defective in projection formation (23).

slk1 phenotypes are similar to those of *bcy1* and *ard1* mutants, which appear to be defective in cell cycle arrest (55, 61). *slk1*-Δ*2* cells strains fail to undergo meiosis and fail to enter stationary phase, as assayed by the presence of budded cells, heat shock sensitivity, and loss of viability under nutrient-limiting conditions. *slk1*-Δ*2* mutants fail to grow in the presence of low concentrations of caffeine, similar to findings for *bcy1* and *ard1* strains. One of the effects of caffeine is to inhibit cAMP phosphodiesterases (7, 39); mutants that are induced in the cAMP response such as *bcy1* mutants might be expected to be sensitive to this drug. In *S. cerevisiae*, caffeine affects other cellular process such as DNA repair (63), so the sensitivity of *slk1* and *ssd1//srk1* mutants to caffeine is not direct evidence of participation of *SLK1* and *SSD1/SRK1* in growth control processes. Nevertheless, the caffeine sensitivity and the aforementioned phenotypes are certainly consistent with a role in growth control.

SLK1 interacts genetically with other genes involved in morphogenesis and growth control. *slk1* mutants are lethal in combination with *spa2*, and both *slk1*-Δ*2* and *slk1-1 spa2* mutants are partially suppressed by the presence of an extra copy of *SSD1/SRK1*. *SSD1/SRK1* has been implicated in both growth control and morphogenesis (54, 62). *ssd1* mutants exhibit weak defects in the G_1/S transition (54), and *SSD1/SRK1*, when present on a centromeric plasmid, can suppress mutations in *PDE2* (cAMP phosphodiesterase), *BCY1*, and temperature-sensitive *ins1* mutations (62). The adenylate cyclase/cAMP protein kinase pathway is implicated in growth control in yeast cells, and *ins1* mutants arrest at late G_1, a critical cell cycle control point (62). In wild-type strains, *SSD1/SRK1* also suppresses the lethality of *sit4* mutants. *SIT4* encodes a putative phosphatase implicated in the G_1/S transition (54). *SSD1/SRK1* may also affect cell morphogenesis. In the presence of the *ssd1* mutation, *cdc28* cells fail to retain their elongated shape and instead form large round cells (54). Thus, *SSD1/SRK1* itself is implicated in both growth control and morphogenesis.

The SSD1/SRK1 predicted protein sequence is similar to the sequence of the dis3 protein of *S. pombe*, a protein involved in protein phosphatase function (62), and contains two potential sites of phosphorylation by cAMP-dependent protein kinase. Thus, it is possible that *SSD1/SRK1* is involved in phosphatase function and the regulation of growth control and is itself potentially regulated by the cAMP pathway. Alternatively, *SSD1/SRK1* may simply act as a general suppressor of many mutations. As a protein involved in protein phosphatase function, general suppression might be achieved by modulation of many cellular activities which are controlled by phosphorylation/dephosphorylation events.

There are several possible explanations for why *slk1*-Δ mutants may have defects in both morphogenic and cell growth processes. First, *SLK1* may participate in two separate pathways, one involving growth control and another involving budding and morphogenesis. Since protein kinases can have many substrates, this possibility is certainly plausible.

Dephosphorylation has been implicated in two aspects of morphogenesis in yeast cells: cell surface growth and cytokinesis. Yeast strains with mutations in *CDC55* (which encodes a protein homologous to the β subunit of type 2A phosphatases) exhibit defects in both of these processes (27). Presumably phosphorylation is also important for certain morphogenic events, and *SLK1* may play an role in some of these processes.

A second possible manner in which *SLK1* participates in both growth control and cell morphogenesis is by playing an important role in a step that cell growth and morphogenesis have in common (e.g., cell wall synthesis or cytoskeletal rearrangements) or in a regulatory mechanism that simultaneously monitors both of these processes. Bud formation normally occurs only after cells have reached a critical size and have been exposed to sufficient nutrients, parameters that involve monitoring of growth conditions (6, 60). However, a cell prepares for budding by depositing cytoskeletal and other components at the incipient bud site well before bud emergence (21, 33, 53). Hence, the decision at Start to initiate bud emergence must be superimposed on processes involved in the preparation for bud formation. Controls may exist that simultaneously regulate both of these processes (35, 52). Defects in such a regulatory system might be expected to lead to an enrichment of unbudded cells and cause morphogenic abnormalities.

These different possibilities, which are not mutually exclusive, cannot yet be distinguished. Nevertheless, *SLK1* is a possible regulatory component that directly or indirectly links both cell morphogenesis and cell growth.

Independently of our work, Lee and Levin (33a) identified the *SLK1* gene, which they call *BCK1*, as a dominant suppressor of a strain carrying a deletion of protein kinase C. They report that *slk1/bck1* mutants lose intracellular components upon prolonged incubation at 37°C. This effect can be rescued by osmotic stabilizers (e.g., 1 M sorbitol). Loss of such components may be indicative of a direct role of *SLK1/BCK1* in cell integrity (e.g., cell wall synthesis or cytoskeleton assembly). Consistent with this hypothesis, cytoskeletal defects in erythrocytes lead to abnormal cell shape and lysis; these defects can be rescued by the addition of osmotic stabilizers to the medium (19, 34). In yeast cells, temperature-sensitive *cdc24* mutants fail to form buds at the restrictive temperature; this defect can be rescued by muticopy suppressors only in the presence of sorbitol (9), consistent with the observation that sorbitol can help rescue morphogenic defects. It is also possible that the effect of sorbitol is indirect; for example, sorbitol slows cell growth (unpublished observations) and may provide cells with an opportunity to repair defective processes. Further experiments will be necessary to distinguish the different possibilities.

Synthetic lethal screens. The synthetic lethal screen described in this report was designed to search for mutants that require the *SPA2* gene for growth. Advantages of using a colony color assay for this type of screen are its simplicity and extreme sensitivity. This latter feature is particularly useful. For example, in the course of cloning a gene by screening for plasmids which restore the sectoring phenotype, additional genes which partially suppress the mutant phenotype can be identified (such as for the case of *SSD1/SRK1*).

Furthermore, mutants that have very low viability can still be isolated. For example, we have identified one mutant that forms very small colonies in the absence of the *SPA2* plasmid after 2 weeks of growth (data not shown). This assay is potentially advantageous if two redundant genes exist that are necessary for creating a synthetic lethal phenotype with

a third gene. Mutations in one gene might reduce, but not eliminate, sectoring and be detected by this method.

One disadvantage of the *SUP4/ade2* approach is that metabolic mutants and ochre mutations will also be identified in the initial screening. These mutations contribute to the initial background, although they are subsequently removed when a second plasmid containing the gene of interest is transformed into the potential mutants. New vectors that contain the *ADE2* gene instead of a tRNA suppressor have been constructed (38a). These vectors will avoid ochre mutations that are not of interest.

One general concern about synthetic lethal screens is that combining two mutations which individually are deleterious to the health of a cell might result in lethality. This is not the case for *spa2* or *slk1-1* mutants. Aside from relatively minor defects, *spa2* mutant cells have normal doubling times and appear quite healthy during vegetative growth. *slk1-1* mutants in the presence of the *SPA2* gene are also quite healthy; no observable defects in colony size or growth rate are apparent. Thus, since both the *spa2* and *slk1-1* strains appear healthy, it is unlikely that general growth defects contributed by both cause lethality. Rather, we expect that the synthetic lethality is due to defects in the same cellular processes. Consistent with this interpretation, we have found that *spa2-Δ2* is synthetically lethal when placed in combination with *bem1-Δ* (data not shown). The *BEM1* gene is a nonessential gene important for budding (10), and its gene product has a subcellular localization similar to that of the SPA2 protein (15a).

Another concern about synthetic lethal screens is that lethality may be due to a protein acquiring a new function. This is of particular concern for mutations involving protein kinases, which might act on new substrates. This new activity may cause lethality in the artificial background (in our case, *spa2*), but the original activity may be irrelevant to the biological process of interest. Although *slk1-1* mutants are recessive, this concern cannot be completely eliminated because gain-of-function mutations in the presence of a wild-type gene can sometimes be recessive. *slk1-Δ* (i.e., null) mutations, similar to *slk1-1* mutations, are also synthetically lethal in the presence of the *spa2* mutation; thus both mutations probably result in a decrease of *SLK1* activity. In the absence of *spa2*, *slk1-1* mutant strains have no major growth defect, whereas *slk1-Δ2* strains grow slowly; hence, *slk1-1* is probably not a null mutant and is only partially reduced in *SLK1* function.

Synthetic lethality has now demonstrated genetic interactions among genes involved in a common pathway. Mutations in genes involved in late steps of the secretory pathway cause synthetic lethality when combined with one another but not when combined with mutations in the early steps (45). The promising results of this screen and an independent screen of Bender and Pringle (10) indicate that this approach may be generally useful for identifying genes involved in common cellular processes.

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REFERENCES

1. **Adams, A., and J. Pringle.** 1984. Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenic-mutant *Saccharomyces cerevisiae*. J. Cell Biol. **98**:934–945.
2. **Adams, A. E. M., and D. Botstein.** 1989. Dominant suppressors of yeast actin mutations that are reciprocally suppressed. Genetics **121**:675–683.
3. **Adams, A. E. M., D. Botstein, and D. G. Drubin.** 1989. A yeast actin-binding protein is encoded by *SAC6*, a gene found by suppression of an actin mutation. Science **243**:231–233.
4. **Adams, A. E. M., D. I. Johnson, R. M. Longnecker, B. F. Sloat, and J. R. Pringle.** 1990. *CDC42* and *CDC43*, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. J. Cell Biol. **111**:131–142.
5. **Baba, M., N. Baba, Y. Ohsumi, K. Kanaya, and M. Osumi.** 1989. Three-dimensional analysis of morphogenesis induced by mating pheromone α factor in *Saccharomyces cerevisiae*. J. Cell Sci. **94**:207–216.
6. **Bartlett, R., and P. Nurse.** 1990. Yeast as a model system for understanding the control of DNA replication in eukaryotes. BioEssays **12**:457–463.
7. **Beach, D. H., L. Rodgers, and J. Gould.** 1985. RAN1+ controls the transition from mitotic division to meiosis in fission yeast. Curr. Genet. **10**:297–311.
8. **Becker, D. M., and L. Guarente.** 1991. High efficiency transformation of yeast by electroporation. Methods Enzymol. **194**:182–187.
9. **Bender, A., and J. R. Pringle.** 1989. Multicopy suppression of the *cdc24* budding defect in yeast by *CDC42* and three newly identified genes including the *ras*-related gene *RSR1*. Proc. Natl. Acad. Sci. USA **86**:9976–9980.
10. **Bender, A., and J. R. Pringle.** 1991. Use of a screen for synthetic lethal and multicopy suppressor mutants to identify two new genes involved in morphogenesis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11**:1295–1305.
11. **Boeke, J., J. Trueheart, G. Natsoulis, and G. R. Fink.** 1987. 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. **154**:164–175.
12. **Broek, D., T. Toda, T. Michaeli, L. Levin, C. Birchmeier, M. Zoller, S. Powers, and M. Wigler.** 1987. The *S. cerevisiae CDC25* gene product regulates the RAS/adenylate cyclase pathway. Cell **48**:789–799.
13. **Byers, B.** 1981. Cytology of the yeast life cycle, p. 97–142. *In* J. N. Strathern, E. Jones, and J. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance, vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Chu, G., D. Vollrath, and R. W. Davis.** 1986. Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science **234**:1582–1585.
15. **Cleves, A. E., P. J. Novick, and V. A. Bankaitis.** 1989. Mutations in the *SAC1* gene suppress defects in yeast Golgi and yeast actin function. J. Cell Biol. **109**:2939–2950.

15a. **Corrado, K., and J. Pringle.** Unpublished data.

16. **Cross, F., L. H. Hartwell, C. Jackson, and J. B. Konopka.** 1988. Conjugation in *Saccharomyces cerevisiae*. Annu. Rev. Cell Biol. **4**:429–457.
17. **Drubin, D.** 1991. Development of cell polarity in budding yeast. Cell **65**:1093–1096.
18. **Drubin, D. G., K. G. Miller, and D. Botstein.** 1988. Yeast actin-binding proteins: evidence for a role in morphogenesis. J. Cell Biol. **107**:2551–2561.
19. **Eber, S. W., R. Armbrust, and W. Schroter.** 1990. Variable clinical severity of hereditary spherocytosis-relation to erythrocytic spectrin concentration, osmotic fragility, and autohemolysis. J. Pediatr. **117**:409–419.
20. **Feinberg, A. P., and Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
21. **Ford, S., and J. Pringle.** 1991. Cellular morphogenesis in the *Saccharomyces cerevisiae* cell cycle: localization of the CDC11

gene product and the timing of events at the budding site. Dev. Genet. **12**:281–292.
22. **Freifelder, D.** 1960. Bud position in *Saccharomyces cerevisiae*. J. Bacteriol. **124**:511–523.
23. **Gehrung, S., and M. Snyder.** 1990. The *SPA2* gene of *Saccharomyces cerevisiae* is important for pheromone-induced morphogenesis and efficient mating. J. Cell Biol. **111**:1451–1464.
24. **Haarer, B., and J. R. Pringle.** 1987. Immunofluorescence localization of the *Saccharomyces cerevisiae* CDC12 gene product to the vicinity of the 10 nm filaments in the mother-bud neck. Mol. Cell. Biol. **7**:3678–3687.
25. **Hanks, S. K., and A. M. Quinn.** 1991. Protein kinase catalytic domain sequence database: identification of conserved features of primary structure and classification of family members. Methods Enzymol. **200**:38–81.
26. **Hasek, J., I. Rupes, J. Svobodova, and E. Streiblova.** 1987. Tubulin and actin topology during zygote formation of *Saccharomyces cerevisiae*. J. Gen. Microbiol. **133**:3355–3363.
27. **Healy, A. M., S. Zolnierowicz, A. E. Stapleton, M. Goebl, A. A. DePaoli-Roach, and J. R. Pringle.** 1991. *CDC55*, a *Saccharomyces cerevisiae* gene involved in cellular morphogenesis: identification, characterization, and homology to the B subunit of mammalian type 2A protein phosphatase. Mol. Cell. Biol. **11**:5767–5780.
28. **Henikoff, S.** 1987. Unidirectional digestion with exonuclease III in DNA sequence analysis. Methods Enzymol. **155**:156–165.
28a. **Hieter, P.** Unpublished data.
29. **Hieter, P., C. Mann, M. Snyder, and R. W. Davis.** 1985. Mitotic chromosome stability of yeast chromosomes: a colony color assay that measures nondisjunction and chromosome loss. Cell **40**:381–392.
30. **Johnston, G. C., J. A. Prendergast, and R. A. Singer.** 1991. The *Saccharomyces cerevisiae MYO2* gene encodes an essential myosin for vectorial transport of vesicles. J. Cell Biol. **113**:539–551.
31. **Kemp, B. E., and R. B. Pearson.** 1990. Protein kinase recognition sequence motifs. Trends Biochem. Sci. **15**:342–346.
32. **Kilmartin, J. V., and A. E. M. Adams.** 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*. J. Cell Biol. **98**:922–933.
33. **Kim, H. B., B. K. Haarer, and J. R. Pringle.** 1991. Cellular morphogenesis in the *Saccharomyces cerevisiae* cell cycle: localization of the CDC3 gene product and the timing of events at the budding site. J. Cell Biol. **112**:535–544.
33a. **Lee, K., and D. Levin.** Personal communication.
34. **Liu, S. C., L. H. Derick, P. Agre, and J. Palek.** 1990. Alteration of the erythrocyte membrane skeletal ultrastructure in hereditary spherocytosis, hereditary elliptocytosis, and pyropoikilocytosis. Blood **76**:198–205.
35. **Madden, K., C. Costigan, and M. Snyder.** 1992. Cell polarity and morphogenesis in *Saccharomyces cerevisiae*. Trends Cell Biol. **2**:22–29.
35a. **Mirzayan, C., C. Copeland, and M. Snyder.** J. Cell Biol., in press.
36. **Mullen, J. R., P. S. Kayne, R. P. Moerschell, S. Tsunasawa, M. Gribskov, M. Colavito-Shepanski, M. Grunstein, F. Sherman, and R. Sternglanz.** 1989. Identification and characterization of genes and mutants for an N-terminal acetyltransferase from yeast. EMBO J. **8**:2067–2075.
37. **Novick, P., and D. Botstein.** 1985. Phenotypic analysis of temperature-sensitive yeast actin mutants. Cell **40**:405–416.
38. **Novick, P., B. C. Osmond, and D. Botstein.** 1989. Suppressors of yeast actin mutations. Genetics **121**:659–674.
38a. **Padmanabha, R., and M. Snyder.** Unpublished data.
38b. **Page, B. D., and M. Snyder.** Unpublished data.
39. **Parsons, W. J., V. Ramkumar, and G. L. Stiles.** 1988. Isobutylmethylxanthine stimulates adenylate cyclase by blocking the inhibitory regulatory protein, G_i. Mol. Pharmacol. **34**:37–41.
40. **Pearson, W. R.** 1990. Rapid and sensitive sequence comparison with FASTP and FASTA. **183**:63–98.
41. **Pringle, J. R., and L. H. Hartwell.** 1981. The *Saccharomyces cerevisiae* cell cycle, p. 97–142. *In* J. N. Strathern, E. Jones, and J. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
42. **Rhodes, N., L. Connell, and B. Errede.** 1990. STE11 is a protein kinase required for cell-type-specific transcription and signal transduction in yeast. Genes Dev. **4**:1862–1874.
43. **Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink.** 1987. A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene **50**:237–243.
44. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
45. **Salminen, A., and P. Novick.** 1987. A ras-like protein is required for a post-Golgi event in yeast secretion. Cell **49**:527–538.
46. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
47. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
48. **Shaw, K., and M. Olson.** 1984. Effects of altered 5′ flanking sequences on the in vivo expression of a *Saccharomyces cerevisiae* $tRNA^{Tyr}$ gene. Mol. Cell. Biol. **4**:657–665.
49. **Shenoy, S., J. K. Choi, S. Bagrodia, T. D. Copeland, J. L. Maller, and D. Shalloway.** 1989. Purified maturation promoting factor phosphorylates $pp60^{c\text{-}src}$ at the sites phosphorylated during fibroblast mitosis. Cell **57**:763–774.
50. **Sherman, F., G. Fink, and J. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
51. **Sikorski, R., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122**:19–27.
52. **Snyder, M.** 1989. The SPA2 protein of yeast localizes to sites of cell growth. J. Cell Biol. **108**:1419–1429.
53. **Snyder, M., S. Gehrung, and B. D. Page.** 1991. Studies concerning the temporal and genetic control of cell polarity in *Saccharomyces cerevisiae*. J. Cell Biol. **114**:515–532.
54. **Sutton, A., D. Immanuel, and K. T. Arndt.** 1991. The SIT4 protein phosphatase functions in late G_1 for progression into S phase. Mol. Cell. Biol. **11**:2133–2148.
55. **Toda, T., S. Cameron, P. Sass, M. Zoller, J. D. Scott, B. McMullen, M. Hurwitz, E. G. Krebs, and M. Wigler.** 1987. Cloning and characterization of *BCY1*, a locus encoding a regulatory subunit of the cyclic AMP-dependent protein kinase in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:1371–1377.
56. **Toda, T., I. Uno, T. Ishikawa, S. Powers, T. Kataoka, D. Broek, S. Cameron, J. Broach, K. Matsumoto, and M. Wigler.** 1985. In yeast, RAS proteins are controlling elements of adenylate cyclase. Cell **40**:27–36.
57. **Tuazon, P. T., and J. A. Traugh.** 1989. Casein kinase I and II-multipotential serine protein kinases: structure, function and regulation, p. 123–164. *In* Advances in second-messenger and phosphoprotein research. Raven Press, New York.
58. **Vollrath, D., R. W. Davis, C. Connelly, and P. Hieter.** 1988. Physical mapping of large DNA by chromosome fragmentation. Proc. Natl. Acad. Sci. USA **85**:6027–6031.
59. **Wang, Y., H.-P. Xu, M. Riggs, L. Rogers, and M. Wigler.** 1991. *byr2*, a *Schizosaccharomyces pombe* gene encoding a protein kinase capable of partial suppression of the *ras1* mutant phenotype. Mol. Cell. Biol. **11**:3554–3563.
60. **Wheals, A.** 1987. Biology of the cell cycle in yeasts. Yeasts **1**:283–377.
61. **Whiteway, M., and J. W. Szostak.** 1985. The ARD1 gene of yeast functions in the switch between the mitotic cell cycle and alternate developmental pathways. Cell **43**:483–492.
62. **Wilson, R. B., A. A. Brenner, T. B. White, M. J. Engler, J. P. Gaughran, and K. Tatchell.** 1991. The *Saccharomyces cerevisiae SRK1* gene, a suppressor of *bcy1* and *ins1*, may be involved in protein phosphatase function. Mol. Cell. Biol. **11**:3369–3373.
63. **Zaborowska, D., and J. Zuk.** 1990. The effect of DNA replication on mutation of *Saccharomyces cerevisiae* CDC8 gene. Curr. Genet. **17**:275–280.