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The *Saccharomyces cerevisiae SRK1* Gene, a Suppressor of *bcy1* and *ins1*, May Be Involved in Protein Phosphatase Function

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The *Saccharomyces cerevisiae SRK1* gene, when expressed on a low-copy shuttle vector, partially suppresses the phenotype associated with elevated levels of cyclic AMP-dependent protein kinase activity and suppresses the temperature-sensitive cell cycle arrest of the *ins1* mutant. *SRK1* is located on chromosome IV, 3 centimorgans from *gcn2*. A mutant carrying a deletion mutation in *srk1* is viable. *SRK1* encodes a 140-kDa protein with homology to the dis3$^+$ protein from *Schizosaccharomyces pombe*. The ability of *SRK1* to alleviate partially the defects caused by high levels of cyclic AMP-dependent protein kinase and the similarity of its encoded protein to dis3$^+$ suggest that *SRK1* may have a role in protein phosphatase function.

***SRK1* suppresses traits associated with hyperactivity of cAMP-dependent protein kinase.** *Saccharomyces cerevisiae* strains with mutations in the low-K_m cyclic AMP (cAMP) phosphodiesterase (*pde2/sra5*) gene fail to properly regulate cAMP levels in the presence of exogenous cAMP and lose viability as a consequence (27). This property was used to clone the *PDE2* gene from a yeast genomic library constructed in the yeast shuttle vector YCp50 (provided by M. Rose, Princeton University) (27). In addition to plasmids containing the bona fide *PDE2* gene, we recovered another plasmid, pW31 (Fig. 1), which protected the *pde2* strain from loss of viability in the presence of exogenous cAMP but failed to influence the low glycogen levels associated with *pde2*. To determine whether pW31 could complement other mutations in the cAMP-dependent protein kinase pathway, we transformed yeast strains (2) which contain a *HIS3* disruption in the gene encoding the regulatory subunit of cAMP-dependent protein kinase (*bcy1/sra1*) (6, 25) with pW31 and tested Ura$^+$ transformants for traits associated with *bcy1*. *bcy1*::*HIS3* transformants containing pW31 were more resistant to a 52°C heat shock than were those *bcy1*::*HIS3* transformants containing only the YCp50 vector (Fig. 2B) and were also able to grow on maltose (Fig. 2C)- or ethanol (Fig. 2D)-containing medium. pW31 did not fully complement *bcy1*; the pW31 transformants of EG286-1-10C were still sensitive to nitrogen starvation (Fig. 2E), although some *bcy1* strains that we tested did show partial resistance to starvation. *bcy1* strains containing the *SRK1* plasmids were unable to grow on acetate- or glycerol-containing medium (data not shown), and glycogen levels remained low, as judged by the inability of iodine vapors to stain the transformants (Fig. 2F). When present on a 2μm multicopy vector, the genomic DNA present in pW31 did not complement *bcy1* to any greater extent than on YCp50 (data not shown). The gene present in pW31 was named *SRK1* because it suppressed mutations in the regulatory subunit of cAMP-dependent protein kinase.

The *SRK1* gene was independently identified as a suppressor of the *ins1* mutation in strain SC3B (*MATα leu2 ura3-52 ade2-101 his3 ins1*). This mutation causes a temperature-sensitive defect in normal progression through the mitotic cell cycle. Temperature-arrested cells carrying the *ins1* mutation do not initiate DNA synthesis and contain either an unduplicated or an unseparated spindle-pole-body. *ins1* was mapped to chromosome IV by a chromosome loss technique

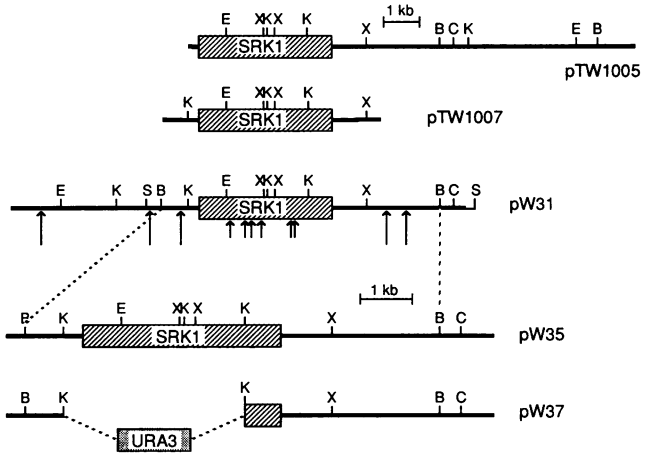

FIG. 1. *SRK1* gene structure. The genomic DNAs found in pTW1005, pTW1007, and pW31 are depicted. Thick lines represent insert sequences, and the thin line represents vector sequence. The striped box represents the *SRK1* open reading frame, with the start codon at the left end. Short vertical arrows below pW31 denote Tn*5* insertions (22) that abolish *SRK1* activity (suppression of *bcy1*::*HIS3*); long vertical arrows denote Tn*5* insertions that do not affect *SRK1* activity. pW35 was constructed by inserting the 9.3-kb *Sal*I fragment of pW31 into the *Sal*I site of YEp24. pW37 was derived from pW35 by replacement of the 1.2- and 2.3-kb *Kpn*I fragments of pW35 with the *URA3* gene. The *URA3* gene was derived from the plasmid pWJ-120, which was kindly provided by John Hill. Abbreviations: B, *Bgl*II; C, *Cla*I; E, *Eco*RI; K, *Kpn*I; S, *Sal*I; X, *Xba*I.

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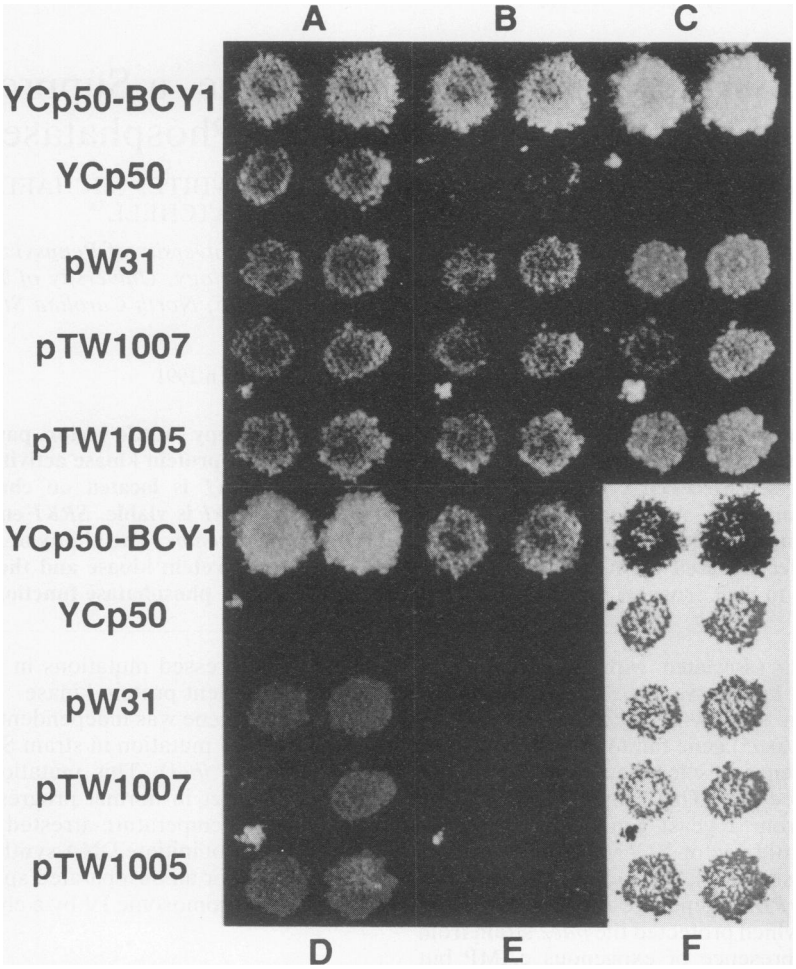

FIG. 2. Suppression of *bcy1*::*HIS3* by *SRK1*. Strain EG286-1-10C (*MATα his3 leu2 ura3 bcy1*::*HIS3*) was transformed (12) with either YCp50-BCY1, YCp50, pW31, PTW1007, or pTW1005, and two representative transformants that contained each plasmid were plated onto synthetic medium lacking uracil (sc-ura) (20), allowed to grow overnight at 30°C, and replica plated to different plates to screen for the *bcy1* phenotype. Plates were photographed after an additional 48 h. (A) sc-ura (30°C). (B) sc-ura (52°C for 4 min and then shifted to 30°C). (C) YEP (1% yeast extract, 2% Bacto-Peptone 2% agar) plus 2% maltose (37°C). (D) YEP plus 2% ethanol (30°C). (E) Synthetic medium lacking ammonium sulfate (5) for 24 h (30°C), followed by replica plating to sc-ura (30°C). (F) sc-ura (30°C), stained with iodine vapor (5). Note that one of the pTW1007 transformants does not grow well on maltose or ethanol, although it remains heat shock resistant. We have observed this with 10% of the pTW1007 and pTW1005 transformants but have no explanation for the variability.

(26), but it complemented other cell cycle mutations located on chromosome IV (*cdc2*, *cdc7 cdc9*, *cdc36*, and *cdc39*) that have functions known to be involved in the process of DNA replication or cell division (4). In attempts to clone the *ins1* gene, two plasmids, pTW1005 and pTW1007, whose genomic inserts overlap the insert in pW31 (Fig. 1), were recovered from a YCp50 yeast genomic library (provided by J. L. Campbell, California Institute of Technology). pW31 complemented the temperature sensitivity of *ins1*, and pTW1005 and pTW1007 also suppressed *bcy1* (Fig. 2). Unlike the limited suppression of *bcy1*, the *SRK1* plasmids allowed *ins1* strains to grow nearly as well as wild-type strains (to 39°C). *SRK1* and *ins1* are not linked, however. The temperature sensitivity and the Ura$^+$ phenotype of a *URA3*-tagged *srk1* allele (see below) segregated independently in a cross (5 parental ditype:4 nonparental ditype:17 tetratype) between strains EG262-18B (*MATa ura3 leu2 ins1*) and RW240-7-6B (*MATα ura3 leu2 Δtrp1 lys2 srk1*::*URA3*). As for *bcy1*, *SRK1* plasmids act as suppressors of *ins1*.

***SRK1* maps near *gcn2* on chromosome IV.** The genomic yeast DNA present in pW31 was genetically mapped to test the possibility that *SRK1* is tightly linked to a previously identified gene. We first localized *SRK1* to chromosome IV

TABLE 1. Mapping data for *srk1*[a]

Gene pair	No. of:			Map distance (centimorgans)
	Parental ditypes	Nonparental ditypes	Tetratypes	
srk1-trp4	24	2	51	40.9
srk1-gcn2	73	0	4	2.6
gcn2-trp4	23	3	51	44.8

[a] Data are from a cross between JG94 (*MATα leu2 ura3 his4 srk1*::*URA3*) and JG93 (*MATa ura3 trp4 leu2 gcn2*::*LEU2*). The *gcn2*::*LEU2* allele was derived from strain H752 (*MATa ura3-52 leu2-3,112 gcn2*::*LEU2*), kindly provided by Alan Hinnebusch.

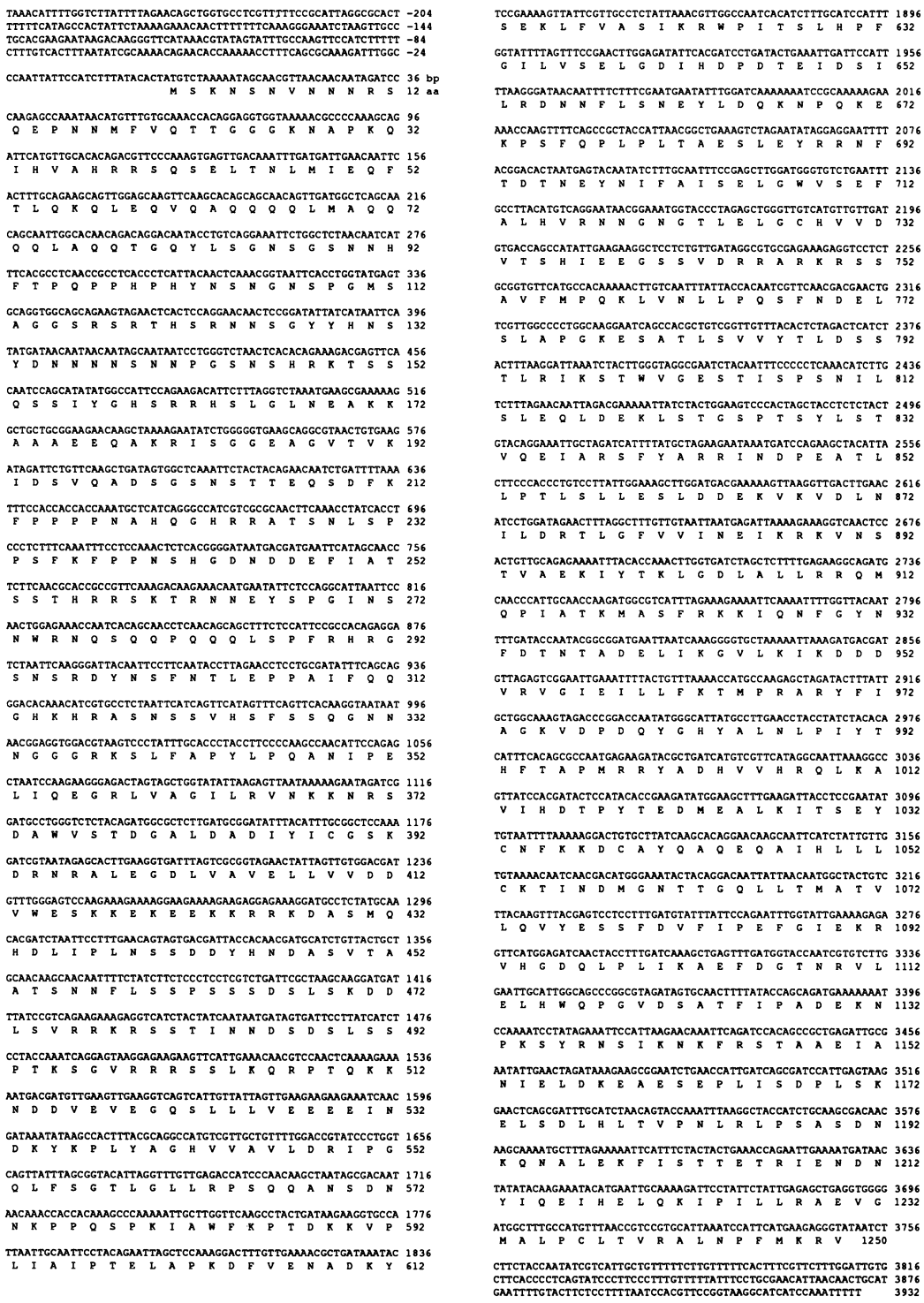

FIG. 3. Nucleotide sequence of *SRK1* from pW31 and pTW1007 and predicted amino acid sequence of the encoded product. The sequence of the entire open reading frame and the sequence 5′ to the initiation codon were determined on both strands. The numbers to the right of each line refer to the nucleotide or amino acid (aa) residue starting from the initiation codon. In-frame stop codons begin 153 and 264 nucleotides 5′ to the initiation codon. The 1,250-codon open reading frame could encode a 139,944-Da protein.

by hybridizing the 7.9-kb *Bgl*II fragment of pW31, which had been labeled with ^{32}P by nick translation, to *S. cerevisiae* chromosomes separated by orthogonal-field-alternation gel electrophoresis (7). Tetrad analysis between strains that contained *URA3* integrated into the *SRK1* locus (*srk1*::*URA3*; see below) and markers on chromosome IV revealed that *SRK1* mapped to a novel locus between *gcn2* and *trp4* on the right arm of chromosome IV (Table 1).

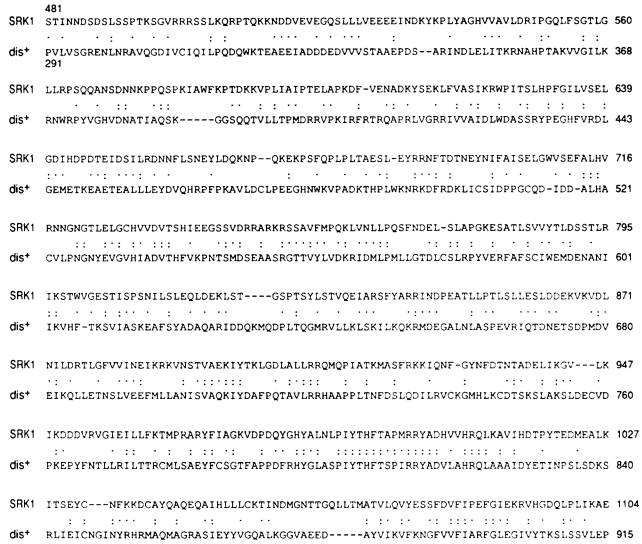

FIG. 4. Alignment of the amino acid sequences of the SRK1 and dis3$^+$ proteins. Identical and conserved amino acids are denoted with two dots and one dot, respectively, between the two sequences. Conservative amino acid changes are defined by the following groups of amino acids: (L, I, V, M), (A, G, P, S, T), (Q, D, E, N), (R, K, H), and (F, Y, W).

***SRK1* encodes a protein with homology to the dis3$^+$ protein of *S. pombe*.** The sequences in pW31 that allowed *bcy1* strains to grow on ethanol as a carbon source were defined by Tn*5* mutagenesis (22) as diagrammed in Fig. 1. The DNA sequence of this region (Fig. 3) revealed a single uninterrupted reading frame of 1,250 amino acid codons encoding a predicted protein of 139,944 Da. The *SRK1* genes from pTW1005 and pTW1007 were sequenced independently and were found to have a DNA sequence identical to that from pW31. A comparison of the *SRK1* coding sequence with a protein data base provided by Mark Goebl (University of Indiana Medical School) revealed that *SRK1* is identical to *SSD1*, which was isolated as a suppressor of the protein phosphatase sit4 (23), and is similar over a region of 600 amino acids to the dis3$^+$ gene product of *Schizosaccharomyces pombe* (14). More than 21% of the amino acids in the region of similarity between SRK1 and dis3$^+$ (Fig. 4) are identical. dis3$^+$ is thought to be involved in protein phosphatase function (see below).

The *SRK1* gene is not essential. To test the consequences of a null mutation in *srk1*, we replaced the 1.2- and 2.3-kb *Kpn*I fragments of pW35 with *URA3* to create the *srk1*::*URA3* deletion plasmid pW37 (Fig. 1). All but the 142 C-terminal codons were removed in *srk1*::*URA3*. A diploid strain homozygous for *ura3* (RW233) (*MAT*a/*MAT*α *ura3*/*ura3 leu2*/*leu2 ras2*::*LEU2*/*RAS2* Δ*trp1*/*TRP1 lys2*/*LYS2 his4*/*his4*) was transformed with the 5.8-kb *Bgl*II fragment of pW37, and stable Ura$^+$ transformants were screened by Southern analysis (16) for the replacement of the *SRK1* locus with the *srk1*::*URA3* deletion. One such transformant was sporulated, and the resulting tetrads were dissected. Four viable spore clones germinated from each tetrad, and no observable phenotype was found to segregate with *srk1*::*URA3* in our genetic background. We compared *SRK1* and *srk1*::*URA3* strains for glycogen content, temperature sensitivity, staining with phloxine B (which can detect even slight variations in viability) (5), and enhancement or suppression of the ability of strains carrying *ras2*::*LEU2* to grow on nonfermentable carbon sources (5) but found no reproducible differences. The failure to observe a distinct phenotype for the *srk1*::*URA3* deletion would be expected if our strains contained a *SRK1* homolog. However, we have not consistently detected cross-hybridizing bands in our Southern blots that would suggest the presence of such a homolog.

***SRK1* may have a role in protein phosphatase function.** The ability of *SRK1* to partially suppress the phenotypes associated with hyperactive cAMP-dependent protein kinase suggests that *SRK1* may act either to negatively regulate protein kinase activity or to moderate some of the effects of hyperphosphorylation. We favor the hypothesis that *SRK1* has an as yet undefined role in protein phosphatase function for several reasons. *SRK1* was independently identified as a suppressor of the *sit4* protein phosphatase mutant. Null mutations in the *sit4* protein phosphatase are lethal in some backgrounds but viable in others (23). Sutton et al. (23) have discovered that this discrepancy is due to allelic differences in *SRK1*/*SSD1*. The suppression we observe could also be due to allelic differences between the *SRK1* gene in the backgrounds we used and the cloned *SRK1* genes in pW31, pTW1005, and pTW1007, although we have not ruled out the possibility that the *SRK1* plasmids act by a dosage mechanism. Temperature-sensitive *sit4* mutants arrest in G_1, prior to DNA synthesis (23), like *ins1* mutants (4). *sit4* and *ins1* are not allelic, however, as the temperature sensitivity of *ins1* is not suppressed by a plasmid containing *SIT4* (17). The *SRK1*/*SSD1* gene therefore suppresses the mutant phenotypes of three genes, *bcy1*, *sit4*, and *ins1*.

The similarity between the SRK1 and dis3$^+$ protein sequences also suggests a role for SRK1 in protein phosphatase function. Cold-sensitive *dis3* mutants have phenotypes nearly identical to those of cold-sensitive *dis2* mutants (18); *dis2* encodes a type 1 protein phosphatase (19). *dis2* and *dis3* mutants both arrest at the same point in mitosis, and are both caffeine sensitive (18). *dis2 dis3* double mutants are lethal, suggesting that both genes may act in the same pathway (14). Unlike protein kinases, which belong to a single protein family (11), protein phosphatases belong to a number of unrelated protein families. Protein phosphatase types 1, 2A, and 2B belong to a family (10, 13) that is distinct from that of protein phosphatase type 2C (24) and from the tyrosine phosphatase family (8). It is possible that the dis3$^+$/SRK1/SSD1 protein family defines another unrelated group of protein phosphatases. Alternatively, SRK1/SSD1 could act to modulate the activity of one of the previously identified protein phosphatases. Proteins with high homology to mammalian type I and type 2A phosphatases have been identified in both *S. pombe* and *S. cerevisiae* (1, 3, 15, 19, 21, 23). These proteins are known to exist as complexes with inhibitory, modulatory, or targeting subunits (9). SRK1/SSD1 may play such a role.

Nucleotide sequence accession number. The nucleotide sequence accession number of *SRK1* in GenBank is M63004.

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