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M P Ward and S Garrett

Suppression of a Yeast Cyclic AMP-Dependent Protein Kinase Defect by Overexpression of SOK1, a Yeast Gene Exhibiting Sequence Similarity to a Developmentally Regulated Mouse Gene

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Received 3 March 1994/Returned for modification 6 April 1994/Accepted 25 May 1994

Saccharomyces cerevisiae cyclic AMP-dependent protein kinase (A kinase) activity is essential for growth and cell cycle progression. Dependence on A kinase function can be partially relieved by the inactivation of a second kinase encoded by the gene YAK1. We have isolated two new genes, SOK1 and SOK2 (suppressor of kinase), as gene dosage suppressors of the conditional growth defect of several temperature-sensitive A kinase mutants. Overexpression of SOK1, like lesions in YAK1, also restores growth to a strain (tpk1 tpk2 tpk3) lacking all A kinase activity. The SOK1 gene is not essential, but a sok1::HIS3 disruption abrogates suppression of an A kinase defect by yak1. These results suggest that Yak1 and Sok1 define a linear pathway that is partially redundant with that of the A kinase. Activation of SOK1, by SOK1 overexpression, is also by inactivation of the negative regulator Yak1, renders a cell independent of A kinase function. The implications of such a model are particularly intriguing in light of the nuclear localization pattern of the overexpressed Sok1 protein and the primary sequence homology between SOK1 and a recently described, developmentally regulated mouse gene.

In the yeast Saccharomyces cerevisiae, cyclic AMP (cAMP)-dependent protein kinase (A kinase) activity is essential for growth and cell cycle progression. Cells deficient in this activity stop growing and arrest in G1 in a manner similar to that observed for wild-type cells deprived of nutrients (16, 20, 21). In contrast, mutations yielding elevated A kinase activity prevent cells from arresting in G1 following nutrient starvation or heat shock (4). Such mutations also cause sporulation deficiency, loss of carbohydrate reserves, and sensitivity to various forms of stress, including heat shock and nutrient starvation. Together, these phenotypes have been taken as evidence that the yeast A kinase pathway plays a central role in mediating the growth and cell cycle arrest of starved cells (2). Low-level activity promotes exit from the mitotic cycle and entry into Go, whereas high-level activity precludes access to Go.

Yeast A kinase activity is regulated by a complex signal transduction pathway that includes the yeast homologs of the mammalian ras products. S. cerevisiae contains two RAS genes (RAS1 and RAS2) which encode functionally redundant, membrane-associated proteins that bind and hydrolyze GTP (32). In their active, GTP-bound state, yeast Ras proteins activate adenylate cyclase, which is encoded by a single gene, CYRI (18). Like its mammalian counterpart, yeast A kinase is a heterotrimeric protein consisting of two catalytic subunits and two regulatory subunits. The yeast catalytic subunits are encoded by three functionally redundant genes, TPKI, TPK2, and TPK3 (31), whereas the regulatory subunit is specified by a single gene, BCY1 (4). Binding of cAMP to Bcy1 results in its dissociation from the catalytic subunits and in stimulation of A kinase activity.

The growth arrest and cell cycle arrest of conditional Ras and A kinase mutants are consistent with the notion that A kinase phosphorylation regulates many cellular processes. Targets of the yeast A kinase have been described and include proteins involved in processes such as carbohydrate storage and metabolism, phospholipid metabolism, and transcriptional regulation, as well as functions involved in the synthesis and breakdown of cAMP (for reviews, see references 1 and 2). Nevertheless, it remains unclear whether A kinase phosphorylation of these known targets can influence whether the cell exits the cell cycle or continues proliferation (3).

Previous attempts to identify downstream effectors of the Ras/A kinase pathway have exploited classical and gene dosage (high-copy-number) suppressor analyses (4, 9, 13, 24, 30). Two genes exhibiting significant homology to known protein kinase genes were identified in separate selections for suppressors of conditional defects in the A kinase pathway. Null mutations of one gene, YAK1, allowed strains completely deficient in A kinase activity to grow; tpk1 tpk2 tpk3 YAK1+ strains are inviable, but tpk1 tpk2 tpk3 yak1 strains grow (13). These and other results led us to propose that Yak1 served as a negative regulator of cell growth, in a pathway parallel to that of the A kinase, with overlapping but antagonistic effects (14). The second gene exhibiting protein kinase gene homology, SCH9, was isolated as a high-copy-number suppressor of a temperature-sensitive cdc25(Ts) mutation. Although the mechanism by which Sch9 overproduction alleviates the A kinase defect is not known, the reciprocal suppression of null mutations in both pathways (a tpk strain grows in the presence of a SCH9 high-copy-number plasmid, and the slow-growth defect caused by an sch9 disruption is alleviated by high-level expression of A kinase activity) is consistent with a model in which Sch9 and the A kinase have partially overlapping functions. An alternate model, based in part on the significant sequence similarity between the two kinases, suggests that hyperactivation of either kinase results in the abnormal phosphorylation of essential kinase substrates of the opposite pathway (15, 30).

Since previous suppressor selections were conducted with mutations in RAS (5, 8, 13) and CDC25 (30), we elected to isolate temperature-sensitive tpk2 mutants [tpk1 tpk2(Ts) tpk3 mutants] and use them to isolate second-site suppressors. We
reasoned that by doing so, and thereby eliminating the majority of suppressors already isolated (CDC25, RAS1 and RAS2, and the three TPK genes, etc.), we would identify rare or weak suppressors that might originally have been overlooked. This communication describes the isolation and characterization of several independent, temperature-sensitive mutations in the TPK2 gene, as well as the isolation of two gene dosage suppressors of the resulting conditional growth defect. Characterization of one of the high-copy-number suppressors has identified a novel gene, SOK1, that exhibits sequence similarity to a developmentally regulated mouse gene. Tests of epistasis are consistent with a model in which activation of Sok1, by Sok1 overexpression or by inactivation of YAK1, results in suppression of the A kinase defect.

**MATERIALS AND METHODS**

**Media and growth conditions.** Media used, including yeast rich and minimal media as well as bacterial media, were prepared as described previously (7, 13). Yeast cells were heat shocked by replicating patches to prewarmed agar and placing the agar plates in a shallow water bath set to 55°C. After 10 min at the elevated temperature, the plates were incubated at 23 or 30°C for several days. Physiological characterization of the temperature-sensitive A kinase mutants for glycerol catabolism and growth arrest was carried out by essentially the same methods described previously (13). Gene dosage suppressors of the A kinase conditional mutants were isolated by transforming two temperature-sensitive tpk2 (Ts) strains, MWY63 and MWY65, to adenine prototrophy and temperature resistance (34.5 and 36°C, respectively) with a high-copy-number plasmid library based on the Ade8 2μm vector YEpADE8 (14).

**Strains and plasmids.** Yeast strains are listed, with references where applicable, in Table 1. Bacterial strains CM1066 [Δ(lac)X74 galU galK strA hsdR rfbC930 leuB6 pyrF::Trp5] and DH5α [F′ [proAB lacI Q12 recA1 thi-1 endA1 hsdR17 (λmcr B) supE44 thi-1 recA1 gyrA relA1 lacZΔM15] have been described previously (6, 35). The high-copy-number yeast vector YEpADE8 was constructed by Toda and Cameron and has been described previously (14). To circumvent the repeated isolation of plasmids containing TPK1, TPK2, and TPK3, a high-copy-number library was constructed by digesting chromosomal DNA of a tpk-deficient strain (tpk1::URA3 tpk2::HIS3 tpk3::TRP1 yak1::LEU2) (SGP406) by partial completion with Sau3A and ligating size-selected fragments into the single BamHI site of YEpADE8. Eight separate pools of transconjugants (>8,000 colonies per pool) were collected and tested for the fraction of plasmids with inserts as well as for insert size. Physical analysis of plasmids isolated from random bacterial colonies showed that >80% (10 of 12) contained inserts, with an average insert size of 14 kb. The bacterial vector pBSK+ has been described previously (Stratagene Product Catalogue). Plasmids pMW25 and pMW42 are class I suppressors, whereas plasmid pMW43 is a class II suppressor.

**DNA manipulations.** Plasmid DNA was prepared from *Escherichia coli* by the alkaline lysis method (19). All enzymes were used according to the specifications of their suppliers (New England Biolabs or Bethesda Research Laboratories), and cloning techniques were as described previously (19).

The HIS3 disruption of Sok1 was constructed by digesting plasmid pMW22 (YEpADE8 containing a 6-kb BglII Sok1 fragment at the BamHI site; see Fig. 3, plasmid C) with NcoI, filling in the ends with Klenow fragment, ligating on BamHI linkers, and then inserting the 1.7-kb BamHI fragment of HIS3. The resulting plasmid pHis3 plasmid was designated pMW26. The yak1::HIS3 and yak1::ADE8 disruptions have been described previously (13).

The sequence of Sok1 was determined by a modification of the method of Sanger et al. (28), using double-stranded plasmid DNA containing random deletions of Sok1. Random deletions of Sok1 in pBSK+ were constructed by digesting pMP11 and pMPP13 with *KpnI* and *XhoI* and then sequentially digesting with exonuclease III and S1 nuclease according to the instructions of the supplier (Promega).

The Sok1 gene was epotope tagged by converting the single *Hind* III site (bp 312 in Fig. 4) within the 5′ end of the coding region to a NotI site and inserting a 112-bp NotI DNA fragment (GTEP) containing three repeats of a 27-codon sequence specifying the hemagglutinin (HA) peptide, YPYDVPDYA (11, 26a). The NotI site was created by cutting Sok1 with HindIII, filling in the 5′ overhang with Klenow fragment, and inserting NotI linkers (8-mers from New England Biolabs). One of the clones containing the correct linkers was digested with *NotI*, dephosphorylated with calf intestinal phosphatase, and then ligated with the GTEP fragment. Plasmids containing a single insert were tested by digestion with several restriction

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**Table 1. Yeast strains used in this study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S7-7A × S7-5A</td>
<td>MATα/A+ tpk2::URA3/TPK1 tpk2::HIS3/TPK2 tpk3::TRP1/TPK3 ura3-52/ura3-52 leu2-3,112/leu2-3,112 his3/his3 trp1/adel ade8/ade8</td>
<td>31</td>
</tr>
<tr>
<td>SGY356</td>
<td>MATα tpk1::URA3/TPK3 tpk2::URA3/TPK2 tpk3::TRP1/TPK3 bpcl::LEU2/ura3-52 his3 leu2-3,112 trp1 ade8</td>
<td>This study</td>
</tr>
<tr>
<td>SGP3</td>
<td>MATα ras1::HIS3 RAS2 his3 ura3-52 leu2-3,112 trp1 ade8</td>
<td>This study</td>
</tr>
<tr>
<td>SGP34</td>
<td>MATα ras1::HIS3 ras2-34-URA3(Ts) his3 ura3-52 leu2-3,112 trp1 ade8</td>
<td>This study</td>
</tr>
<tr>
<td>MWY63</td>
<td>SSGY356 tpk2-63(Ts)</td>
<td>This study</td>
</tr>
<tr>
<td>MWY65</td>
<td>SSGY356 tpk2-65(Ts)</td>
<td>This study</td>
</tr>
<tr>
<td>MWY123</td>
<td>MWY63 yak1::HIS3</td>
<td>This study</td>
</tr>
<tr>
<td>MWY131</td>
<td>MWY65 yak1::HIS3</td>
<td>This study</td>
</tr>
<tr>
<td>SGP398</td>
<td>MWY63 tpk3::ADE8</td>
<td>This study</td>
</tr>
<tr>
<td>1029</td>
<td>MATα/MATα ura3/ura3 leu2-3,112/leu2-3,112 his3/his3 lys2/lys2 trp1/TPR1 ade2/ade2</td>
<td>13</td>
</tr>
<tr>
<td>MWY264</td>
<td>1029 sok1::HIS3/SOK1</td>
<td>This study</td>
</tr>
<tr>
<td>MWY285</td>
<td>MWY63 sok1::HIS3</td>
<td>This study</td>
</tr>
<tr>
<td>MWY273</td>
<td>MWY63 yak1::ADE8</td>
<td>This study</td>
</tr>
<tr>
<td>MWY313</td>
<td>MWY63 sok1::HIS3 yak1::ADE8</td>
<td>This study</td>
</tr>
<tr>
<td>SGP406</td>
<td>MATα leu2-3,112 trpl ura3-52 his3 tpk1::URA3 tpk2::HIS3 tpk3::TRP1 yak1::LEU2</td>
<td>13</td>
</tr>
<tr>
<td>RS13.58A-1</td>
<td>MATα tpk1Δts tpk2::HIS3 tpk3::TRP1 bpcl::LEU2/ura3-52 his3 leu2-3,112 trp1 ade8</td>
<td>3</td>
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</tbody>
</table>

* WI is the notation used by Cameron et al. (3) for the wimp alleles of TPK1, TPK2, and TPK3.

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enzymes to monitor the orientation of the insert (there is a single BamHI site positioned asymmetrically within the GTEP fragment). Finally, the single Ssr1 fragment from one of the derivatives containing the GTEP insert in the correct orientation (as well as a clone containing the same fragment in the opposite orientation) was inserted into the unique Ssr1 site of the original vector (YEpADE8).

**Immunofluorescence of Sok1-HA.** Strains were stained for indirect immunofluorescence essentially as described previously (27). The primary antibody was anti-HA monoclonal antibody 12CA5 (the kind gift of Ken Ferguson and Mike Wigler) and was diluted 1:1,000 for staining. The secondary antibody was fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin G from Boehringer Mannheim.

**RESULTS**

**Isolation and characterization of A kinase temperature-sensitive mutations.** To isolate temperature-sensitive A kinase mutations, we took advantage of the stress-sensitive phenotype of strains with elevated A kinase activity (4). Strains lacking the regulatory subunit of the A kinase and containing one or more of the three, redundant catalytic subunit genes fail to survive regimens such as brief exposure to high temperatures (10 min, 55°C) and nutrient deprivation. This stress sensitivity was previously exploited to isolate partial-loss-of-function mutations in the A kinase catalytic subunit genes (3). Although the mutations isolated by Cameron et al. (3) were not analyzed for conditional activity, we reasoned that a subset of them might result in the complete loss of A kinase function at an elevated temperature. Strains containing such mutations would be resistant to heat shock and viable at the permissive temperature (i.e., 23°C) and would arrest in G1 on a shift to the nonpermissive temperature. A similar scheme was used by others to identify conditional alleles of RAS2 (26).

Stress-resistant revertants of a *tpk1 TPK2 tpk3 bcy1* strain were isolated by exposing cells to 55°C for 10 min and then incubating the cells at 23°C for 3 days. To ensure that all of the revertants were independent, only one heat shock-resistant colony from each patch was saved (see Materials and Methods). Stress-resistant revertants were then retested for their ability to survive exposure to extreme heat as well as for their viability at 36°C (Fig. 1). Of a total of 150 heat shock-resistant survivors, 30 (20%) exhibited a temperature-sensitive growth defect.

At least three of the stress-resistant revertants contain temperature-sensitive mutations in *TPK2* as judged by several criteria (Table 2). First, the temperature-sensitive growth defect of each strain was recessive and was complemented by a low-copy-number plasmid carrying *TPK1* or *TPK3*. Second, the temperature-sensitive mutations were shown to be tightly linked to *TPK2* by mating each of the revertants to a *TPK1 tpk2::HIS3 tpk3::TRP1 Bcy1* strain and scoring tetrads. Only His+ Ura+ strains were temperature sensitive for growth (of 50 tetrads), suggesting that the conditional mutation was tightly linked to *TPK2* and was masked by the presence of a wild-type *TPK1* allele. Third, the temperature-sensitive growth defect was suppressed by the disruption of a gene, *YAK1*, identified previously as a recessive suppressor of conditional A kinase activity (13). Finally, all of the mutants accumulated at least moderate levels of glycogen, in contrast to the stress-sensitive *TPK2 bcy1* parent, which was unable to accumulate measurable glycogen under any condition. In *S. cerevisiae*, glycogen synthesis and degradation have been shown to be

**FIG. 1.** Characterization of conditional A kinase mutants. Growth was tested by replicating patches to agar that was either incubated at 23°C, heat shocked (HS) for 10 min and returned to 23°C, or incubated at 36°C. Strains: *TPK2*, SGY356 (*TPK2 bcy1::LEU2*); *tpk1*, RS13.58A-1 (*tpk1* bcy1::LEU2); *tpk2-63*, MWY63 [*tpk2-63(Ts) bcy1::LEU2*]; *tpk2-65*, MWY65 [*tpk2-65(Ts) bcy1::LEU2*].

**TABLE 2.** Characterization of *tpk2(Ts)* mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Growth at:</th>
<th>HS*</th>
<th>Iodine*</th>
<th>Complementation</th>
<th>TPK2 linkage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>23°C</td>
<td>34.5°C</td>
<td>36°C</td>
<td></td>
<td>TPK1</td>
</tr>
<tr>
<td>SGY356</td>
<td><em>TPK2</em></td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>Y</td>
</tr>
<tr>
<td>MWY63</td>
<td><em>tpk2-63(Ts)</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>B</td>
</tr>
<tr>
<td>MWY65</td>
<td><em>tpk2-65(Ts)</em></td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>B</td>
</tr>
<tr>
<td>MWY123</td>
<td><em>tpk2-63(Ts) yak1::HIS3</em></td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MWY131</td>
<td><em>tpk2-65(Ts) yak1::HIS3</em></td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

a +, growth; +/-, slow growth; -, no growth. ND, not done; Y, yellow (no glycogen accumulation); B, brown (glycogen accumulation).

b Growth at 23°C after exposure to 55°C for 10 min.

c Color of colonies after exposure to iodine vapors.

**FIG. 2.** High-copy-number suppressors of conditional A kinase mutants. Patches of strain MWY63 [*tpk2-63(Ts) ade8*] containing the indicated high-copy-number plasmids were replicated to minimal medium (lacking adenine) and incubated at 23 and 34.5°C for several days. Plasmids were YEp (YEpADE8), 35S (YEpADE8-SPK1) (pMW25), 42S (YEpADE8-SPK1) (pMW42), and 76S (YEpADE8-SPK1) (pMW45).
regulated by A kinase phosphorylation, with glycogen levels exhibiting an inverse relation to cellular A kinase activity (4, 12). By these criteria, the conditional growth defects of at least three of the heat shock-resistant revertants were due to a temperature-sensitive mutation in TPK2 (Table 2). Eight other conditional mutants contained recessive, temperature-sensitive mutations in TPK2 as judged by their inability to complement one of the tpk2(Ts) mutants (tpk2-63 mutant) of the opposite mating type.

Isolation of gene dosage suppressors of the conditional A kinase mutations. The technical difficulties of characterizing and cloning dominant suppressors have been partially circumvented by the recent exploitation of gene dosage, or high-copy-number, suppressors. Gene dosage suppressors of the conditional A kinase mutations were isolated by transforming two of the temperature-sensitive A kinase mutants (tpk2-63 and tpk2-65 mutants) with a high-copy-number plasmid library and incubating the transformants at the nonpermissive temperature for several days. Since A kinase phosphorylation may affect many essential processes, we determined the lowest temperature at which each of the strains could be incubated without exhibiting any growth. By doing so, we hoped to make the phosphorylation of a single essential substrate limiting. In addition, the plasmid library from which the high-copy-number suppressors were isolated was generated from a tpk1 tpk2 tpk3 yakl strain (see Materials and Methods). The use of this library eliminated the reisolation of the three TPK genes, any one of which was able to complement the conditional A kinase mutation and alleviate the growth defect.

To confirm that the temperature-resistant growth of the transformants was plasmid dependent, colonies that grew at the nonpermissive temperature were allowed to lose the plasmid (by growth in nonselective medium at 23°C for several days) and then retested for conditional growth. By this criterion, 30 colonies (of a total of 55,000 Ade⁺ transformants) exhibited plasmid-dependent, temperature-resistant growth. Plasmid DNA from each of the 30 colonies was rescued in E. coli and used to retransform the original temperature-sensitive yeast strain (MWY63 or MWY65) from which each was derived. In this second screen, plasmid DNA from 8 of the 30 temperature-resistant colonies was able to retransform the conditional A kinase mutants to temperature resistance (Fig. 2). At least 6 of the remaining 22 colonies may have contained a mixture of high-copy-number plasmids, since temperature resistance was restored to greater than one-eighth of the yeast transformants when plasmid DNA from a pooled bacterial transformation, rather than a single colony, was used.

The gene dosage suppressors define two new genes, SOK1 and SOK2. Since the plasmid library was constructed from a strain lacking the three A kinase catalytic genes, we could rule out the possibility that any of the eight gene dosage suppressors contained a functional TPK gene. To determine the identities of the inserts contained within the eight high-copy-number plasmids, each plasmid was subjected to restriction fragment analysis. Plasmids with nonidentical but overlapping inserts were grouped into two classes: class I and class II were defined by five and three plasmids, respectively. Consistent with this physical assignment, class II plasmids suppressed the temperature-sensitive growth defect more weakly than the five class I suppressors (see below). Despite this quantitative

FIG. 3. Cloning and restriction map of SOK1. Patches of strain MWY63 (tpk2-63 ade8) containing the indicated plasmids were replicated to minimal medium (lacking adenine) and incubated for several days at 23 and 34.5°C. The yeast DNA fragments of some of the plasmids are shown at the bottom along with the results of the test of suppression. Plasmids not shown are A (YEpADE8) and B (pMW25, the original SOK1 clone).
The nucleotide sequence extends 2,500 bp from the leftmost Sal site (the sequence does not include the Sal site) to an asterisk. Fig. 4. Nucleotide and predicted amino acid sequences of SOKI. The nucleotide sequence extends 2,500 bp from the leftmost Sal site (the sequence does not include the Sal site) to an asterisk. Fig. 4. Nucleotide and predicted amino acid sequences of SOKI. The nucleotide sequence extends 2,500 bp from the leftmost Sal site (the sequence does not include the Sal site) to an asterisk. Fig. 4. Nucleotide and predicted amino acid sequences of SOKI. The nucleotide sequence extends 2,500 bp from the leftmost Sal site (the sequence does not include the Sal site) to an asterisk.
tion sites were conspicuously missing from the predicted protein.

An epitope-tagged Sok1 protein is localized to the nucleus. Since the primary sequence of the Sok1 protein did not suggest an obvious function or activity, we determined its cellular localization by indirect immunofluorescence (27). The Sok1 protein was epitope tagged by inserting a 115-bp oligonucleotide specifying three repeats of the 9-amino-acid HA epitope into the single HindIII site within the 5' end of the SOK1 coding region (Fig. 4; see Materials and Methods). Insertion of the oligonucleotide in frame and in the correct orientation did not perturb the suppressor activity of the altered SOK1 gene (data not shown), so we reasoned that the tagged polypeptide would be properly localized. The epitope-tagged Sok1 protein was localized to the nuclei of cells containing the high-copy-number Yep4ADE8-SOK1-GTEP plasmid by using the anti-HA monoclonal antibody 12CA5 and a fluorescein isothiocyanate-conjugated goat antimouse antibody (Fig. 6). In contrast, no nuclear staining was observed with strains bearing various control plasmids, including the YepADE8 vector and a SOK1 derivative containing the epitope in the inverse orientation (data not shown). Consistent with this observation, it is possible to identify at least one potential nuclear localization signal within the Sok1 coding region (Fig. 4). The mouse testis-specific protein also contains a sequence that might serve as a nuclear localization signal, although neither sequence falls within the regions shared by the two proteins. Finally, we have confirmed by Western blot (immunoblot) analysis (data not shown) that strains bearing the SOK1-GTEP plasmid, but not those bearing the SOK1-PETG construct, specify a protein that, at 65 kDa, is consistent with the size predicted for the epitope-tagged SOK1 gene product (565 plus 27 amino acids).

SOK1 overexpression suppresses total loss of A kinase function. To determine the relation of the SOK1 and SOK2 products to the Ras/A kinase pathway, we tested the abilities of the corresponding plasmids to suppress other mutations of the pathway. A representative high-copy-number plasmid from each class was transformed into temperature-sensitive cdc25-5(Ts) and ras1 ras2(Ts) strains, and Ade+ transformants were tested for growth at several temperatures. As shown in Table 3, the SOK1 plasmid was able to partially suppress the growth defects caused by both mutations. By contrast, the high-copy-number SOK2 plasmid suppressed neither mutation, consistent with the observation that SOK2 was a relatively weak suppressor of the tpk2(Ts) defect.

Disruption of yak1 or overexpression of SCH9 restored growth to a strain lacking all three A kinase catalytic genes, as well as to strains lacking cdc35 and both ras genes (13, 30). Accordingly, we tested whether either high-copy-number suppressor isolated in these studies could do the same. The heterozygous tpk diploid S7-7A × S7-5A was transformed to Ade+ with a plasmid bearing either SOK1 or SOK2, sporulated, and picked to rich medium. Haploid spores containing disruptions of TPK1, TPK2, and TPK3 germinated only when they contained the high-copy-number plasmid containing SOK1 (Table 3). Interestingly, the pattern of suppression by SOK1 overexpression was similar to that conferred by inactivation of YAK1, as judged by the slow growth (Table 3) (13) and hyperaccumulation of glycogen. Thus, neither SOK1 overexpression nor Yak1 inactivation is capable of completely relieving the A kinase growth dependence of a cell. In contrast, colonies lacking all three catalytic subunit genes were never recovered from the diploid containing the SOK2 plasmid, despite the high transmission frequency of the SOK2 plasmid during sporulation.

Overexpression of SOK1 has no effect on glycogen accumulation or sensitivity to stress. Several distinct phenotypes have been associated with strains containing diminished or elevated levels of A kinase. For example, activation of the A kinase pathway results in cells that are exquisitely sensitive to various forms of stress as well as in a failure to accumulate storage

<table>
<thead>
<tr>
<th>Mutant genotype</th>
<th>Growth† with the following suppressor:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>yak1</td>
</tr>
<tr>
<td>ras1 ras2-34(Ts)</td>
<td>+</td>
</tr>
<tr>
<td>cdc25-5(Ts)</td>
<td>+</td>
</tr>
<tr>
<td>tpk1 tpk2-63(Ts)tpk3</td>
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<tr>
<td>tpk1 tpk2-63(Ts)tpk3</td>
<td>+</td>
</tr>
</tbody>
</table>

* See Table 2, footnote a.
¹ HC, high copy number.

FIG. 5. Amino acid sequence homology between the SOK1 product and a testis-specific mouse protein. Sequence similarity of Sok1 (residues 111 to 237) and the protein encoded by pBS13 is displayed. Amino acid identity is denoted by bars, and conservative changes are denoted by colons.

FIG. 6. Cellular localization of the Sok1 protein. The localization of an epitope-tagged derivative of the Sok1 protein was visualized by indirect immunofluorescence with fluorescein isothiocyanate-conjugated secondary antibody (a) and contrasted with the localization of cellular DNA as judged by DAPI (4',6-diamidino-2-phenylindole) staining (b). Cells contained an epitope-tagged derivative of the original high-copy-number SOK1 suppressor (see Materials and Methods).
carbohydrates such as glycogen. Strains that are compromised for A kinase activity are abnormally resistant to stress and accumulate elevated levels of glycogen. To examine the effect of SOK1 overexpression on these phenotypes, we determined the heat shock and starvation sensitivities of a wild-type strain containing the high-copy number SOK1 plasmid or the YEplcADE8 vector. The same plasmid-containing strains were inverted over iodine to gauge the effect of SOK1 overexpression on glycogen accumulation. By these criteria the SOK1-overexpressing strain was identical to its isogenic YEplcADE8 control and was in stark contrast to a congenic bcy1 strain, which was exquisite sensitive to all forms of stress and failed to accumulate glycogen even on prolonged incubation (data not shown). Disruption of the SOK1 gene also had no appreciable effect on stress sensitivity or the capacity to accumulate glycogen.

**Disruption of SOK1 prevents suppression of the A kinase defect by loss of Yak1 function.** To determine the role of the SOK1 gene product in cell growth and division, a sok1::HI53 disruption was placed in the chromosome. The 4.7-kb fragment of plasmid pMW26 (YEplcADE8-sok1::HI53) was used to transform diploid strain 1029 (SOK1/SOK1 his3/his3) to His+, and two transformants were subjected to tetrad analysis. Both transformants contained a single disrupted copy and a wild-type copy of SOK1 as determined by DNA-DNA hybridization (data not shown). All tetrads of both strains (17 of 17) had four equal-sized colonies, with the His+ marker segregating 2:2 (data not shown). Thus, the SOK1 gene is not essential for growth. Although we cannot rule out the possibility that SOK1 is a member of a family of genes with related functions, we have been unable to detect another yeast gene exhibiting significant structural similarity as judged by low-stringency hybridization (data not shown).

Tests of epistasis placed the SOK1 suppressor downstream, or on a pathway parallel with that of, the A kinase gene (Table 3). Two other genes thought to encode growth regulators related to the A kinase pathway include SCH9 and YAK1, identified as gene dosage and loss-of-function suppressors of mutations in the A kinase, respectively. We have previously shown that suppression by SOK1 is independent of the Sch9 kinase (15). To determine the relation between Sok1 and Yak1, the sok1::HI53 disruption was introduced into isogenic tcp2(Ts) YAK1 and tcp2(Ts) yak1 strains, which were then tested for growth at the permissive and nonpermissive temperatures. While the loss of SOK1 function had no apparent effect on the growth of the Yak1/proficient strain [Fig. 7; compare the growth of the tcp2(Ts) YAK1 Sok1 strain with that of the tcp2(Ts) YAK1 sok1 mutant], its inactivation totally blocked growth of the tcp2(Ts) yak1 strain at elevated temperatures. Thus, suppression of the A kinase defect by the inactivation of Yak1 required a functional SOK1 gene product.

**DISCUSSION**

We have identified a new gene, SOK1, whose overexpression alleviates the growth defect of yeast strains lacking A kinase activity. Although the mechanism of this suppression is not known, our results are consistent with a model in which Sok1 identifies a downstream component of the Yak1 kinase pathway (Fig. 8). In that scenario, the Yak1-Sok1 pathway would stimulate a set of essential cellular processes under A kinase control. Activation of Sok1, by SOK1 overexpression or by inactivation of the negative regulator Yak1, would render each of the processes independent of A kinase activity. Given the nuclear localization pattern of Sok1 on overexpression, it is tempting to speculate that at least one process made independent of A kinase activity might include the general or specific activation of transcription. These results are particularly intriguing in light of the structural similarity between the predicted product of SOK1 and the product of a recently described, developmentally regulated mouse gene (21).

The most important result of these studies is that overexpression of SOK1 can suppress the growth defect of a strain lacking all three TPK genes. This marks SOK1 as the second gene whose overexpression is capable of bypassing the need for A kinase for growth. The first such gene, SCH9, encodes a protein kinase exhibiting significant homology to the yeast A kinase catalytic subunits (30). The structural similarity between Sch9 and the A kinase, along with physiological and genetic studies (15), is at least consistent with the notion that suppression of a tpk strain by SCH9 overexpression occurs as a result of the overlapping specificities of the two kinases. The SOK1 gene, in contrast, corresponds to a protein that bears no relation to known protein kinases (Fig. 4). Thus, the SOK1 high-copy-number suppressor appears to act by a mechanism that is different from that of SCH9.

One intriguing possibility is that SOK1 encodes an A kinase substrate involved in cell growth and division. Its dependence on A kinase phosphorylation might, therefore, be abrogated by an increase in SOK1 abundance. Arguing against this proposal, however, is the fact that deletion of the SOK1 gene does not result in a noticeable growth defect, contrary to the expectation for an effector protein in an essential pathway. This result could be reconciled if SOK1 was a member of a duplicated protein family.
gene family. However, SOK1 and SOK2 have each been isolated multiple times, and genetic and physical analyses suggest that the two genes are not functionally related (for example, a sok1 sok2 TPK strain exhibits no obvious growth defects [33a]).

An alternate hypothesis is that the Sok1 protein participates in a pathway that is partially redundant with that of the A kinase (Fig. 8). A kinase-dependent processes would be regulated independently by Sok1, such that a decrease in A kinase activity (with a concurrent diminution in some metabolic process) could be compensated for by an increase in stimulations by Sok1. In such a model, the phenotypic effect of loss of either pathway would depend on the relative contribution of each to the overall function of the process. Judging by the apparent wild-type growth of the sok1 null mutant (Fig. 7), it seems likely that under normal conditions, the contribution by the Sok1 pathway is small. On activation, the contribution by Sok1 presumably increases to levels sufficient to relieve the A kinase requirement.

Our model also posits that Sok1 activity is normally repressed by the Yak1 kinase. Such a model explains our earlier observation (13) that Yak1 activity is antagonistic to growth of an A kinase-deficient mutant, and it predicts that the growth of a tpk(Ts) yak1 strain would be abrogated by the loss of Sok1 function. In other words, relief from Yak1 repression would result in A kinase independence only if a functional Sok1 protein was present. As predicted, the growth of a tpk(Ts) yak1 strain is made conditional by the disruption of SOK1. Thus, our results are consistent with a model in which the defect in yeast A kinase can be alleviated by activation of Sok1 function, either by an increase in SOK1 expression or by a decrease in Yak1 kinase activity. The model shown in Fig. 8 is consistent with the possibility, but does not require, that the interaction between Yak1 and Sok1 is direct, such that Sok1 is inactivated by a Yak1-specific phosphorylation event.

The lack of identity between Sok1 and other protein kinases does not eliminate the possibility that SOK1 overexpression activates a kinase that shares overlapping specificity with the A kinase. For example, if Sok1 stimulated SCH9 transcription, overexpression of either SOK1 or SCH9 might result in suppression of the A kinase defect. However, several results argue against Sok1 regulation of the Sch9 kinase. First, the synthetic lethality of a tpk(Ts) sch9 strain can be overcome by SOK1 overexpression or disruption of yak1 (15). Thus, the SOK1 and yak1 suppressors must alleviate the A kinase defect by an SCH9-independent mechanism, placing Sok1 function distal to Sch9 function. Second, cells lacking Sch9 activity grow extremely slowly (15, 30), whereas sok1 deletion mutants are unaffected in growth. Thus, the two functions appear unrelated. It remains possible that Sok1 might alleviate the A kinase defect through the activation of another A kinase homolog; however, such a kinase has been notably absent from the suppressors identified to date (5, 9, 13, 30, 33a).

The structural similarity between Sok1 and a testis-specific mouse transcript thought to play a role in sperm development implicates a shared determinant or functional domain of these two proteins in the regulation of a variety of important growth and developmental processes. Unfortunately, the primary structures of the two proteins provide few clues to their specific biochemical functions or the nature of the processes they regulate. Potential sites of N glycosylation and membrane attachment (21) are not conserved between the two proteins, and several putative sites of protein kinase C phosphorylation that are present in the mouse pBs13 product are displaced in Sok1. Moreover, the region most conserved between the two proteins, a central core of 143 amino acids, does not appear to reveal a consensus sequence that is shared with any other proteins in the available data banks. In this context, it will be interesting to determine if the mouse protein, like Sok1, resides in the nucleus. Localization of the mouse protein to the nucleus would be consistent with the two proteins sharing some function, such as the regulation of transcription. Finally, Sok1 appears to be devoid of any potential A kinase phosphorylation sites. This would seem to conform well to the notion that Sok1 activates an A kinase-regulated process by a mechanism that is independent of A kinase function. Since A kinase plays a critical role in complex developmental pathways of other organisms (10, 23, 29), it is reasonable to imagine that A kinase-regulated circuits in S. cerevisiae might be equally complex.

In contrast with the broad pattern of suppression exhibited by overexpression of SOK1, the SOK2 high-copy-number suppressors were able to alleviate the growth defects caused by only a restricted set of conditional defects in the A kinase pathway. While SOK2 reversed the temperature-sensitive defect caused by mutations in the A kinase catalytic subunit gene TPK2, several conditional lesions in upstream elements of the Ras/A kinase pathway [ras2(Ts) and cdc25(Ts)] were unaffected. Although the number of alleles tested is too few to make a strong conclusion, it is tempting to speculate that this result may point to the mechanism by which SOK2 overexpression alleviates the conditional growth of the tpk2(Ts) strains. One can imagine, for example, that overproduction of Sok2 might alleviate a conformational defect of the free, but altered, tpk2(Ts) product but have a negligible effect on the activity of a wild-type A kinase catalytic subunit guestimated by Bcy1 [as would be the case in a ras2(Ts) or cdc25(Ts) background]. Since preliminary sequence and genetic analyses have determined that SOK1 and SOK2 do not have overlapping functions, it will be interesting to determine the role of SOK2 in A kinase-dependent growth control and division.

Finally, it seems likely that the temperature-sensitive A kinase mutants isolated in this study will continue to contribute to our understanding of the growth and cell cycle control processes regulated by the yeast A kinase. The two high-copy-number suppressors described here may reflect only a subset of the genes that can be altered to alleviate the A kinase defect. For example, we recently have shown that at least one dominant suppressor of the tpk2-63(Ts) allele is unlinked to SOK1 and may define yet another function of the A kinase pathway (33a). In addition, analyses of the physiological and morphological properties of conditional mutants with defects in each step of the pathway [i.e., cdc25(Ts), ras1 ral2(Ts), cys1(Ts), and now tpk1 tpk2(Ts) tpk3 mutants] are certain to contribute to our understanding of A kinase regulation (1, 25) and may help define the A kinase-independent function of the yeast Ras protein (1, 22, 33, 34).

ACKNOWLEDGMENTS

This work was supported by grant GM44666 from the National Institutes of Health. M.P.W. was supported by NIH training grant 5P32GM07184, and S.G. is a Junior Faculty Scholar of the American Cancer Society (JFRA 395).

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