Shk1, a homolog of the Saccharomyces cerevisiae Ste20 and mammalian p65PAK protein kinases, is a component of a Ras/Cdc42 signaling module in the fission yeast Schizosaccharomyces pombe

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ABSTRACT We describe a protein kinase, Shk1, from the fission yeast Schizosaccharomyces pombe, which is structurally related to the Saccharomyces cerevisiae Ste20 and mammalian p65PAK protein kinases. We provide genetic evidence for physical and functional interaction between Shk1 and the Cdc42 GTP-binding protein required for normal cell morphology and mating in S. pombe. We further show that expression of the STE20 gene complements the shk1 null mutation and that Shk1 is capable of signaling to the pheromone-responsive mitogen-activated protein kinase cascade in S. cerevisiae. Our results lead us to propose that signaling modules composed of small GTP-binding proteins and protein kinases related to Shk1, Ste20, and p65PAK, are highly conserved in evolution and participate in both cytoskeletal functions and mitogen-activated protein kinase signaling pathways.

ras genes are highly conserved in evolution and encode small GTP-binding proteins that regulate cell growth and differentiation in a broad spectrum of eukaryotic organisms (1). The fission yeast Schizosaccharomyces pombe possesses a single known ras homolog, ras1, the product of which is required for at least two distinct cellular functions. First, Ras1 is required for sexual differentiation—namely, conjugation and sporulation—which is induced by starvation and by peptide mating pheromones that bind to serpentine receptors (2–4). Ras1 functions upstream of a mitogen-activated protein (MAP) kinase (MAPK) module composed of the Byp2 [MAP kinase kinase (MAPKKK)], Byp1 [MAP kinase (MAPKK)], and Spk1 (MAPK) protein kinases (5). Data suggest that Ras1 acts in concert with a G protein α subunit homolog, Gpa1, and that both signal to the MAPK module (6). The yeast two-hybrid system was used to show that Ras1 physically associates with the N-terminal regulatory domain of the Byp2 component of the module (7). Numerous studies have demonstrated a role for Ras proteins in the activation of MAPK cascades induced by receptor tyrosine kinases in mammalian cells (for review, see ref. 8). However, recent studies have suggested that Ras proteins are also key elements of MAPK cascades induced by certain mammalian heterotrimeric G protein-coupled serpentine receptors (for review, see ref. 9), suggesting that signal-transduction pathways analogous to the Ras-dependent peptide mating factor response pathway of S. pombe are conserved in evolution.

A second and distinct function of Ras1 in S. pombe is the control of cell morphology. Wild-type S. pombe cells are rod-like in morphology, whereas ras1null mutants are spherical in shape (2, 3). This aspect of Ras1 function is separable from its functions on the MAPK module, as byr1, byr2, spk1, and gpa1 null mutants are normal in morphology (10–13). We recently reported that a member of the Rho subfamily of Ras-related GTP-binding proteins, Cdc42 (14), acts downstream of Ras1 in the regulation of morphology in S. pombe, and that Ras1 and Cdc42 form a multiprotein complex with two other proteins, Sec1 and Sec2 (15). Interestingly, Ras proteins also participate in the morphologic transformation of mammalian cells. Stimulation of mammalian cells with growth factors or with oncogenic Ras proteins rapidly induces actin reorganization and membrane ruffling (for review, see ref. 16). As for S. pombe, Ras-dependent cytoskeletal control in mammalian cells is regulated by small GTP-binding proteins belonging to the Rho subfamily (17, 18).

In this report, we describe a protein kinase, Shk1, and provide evidence that it mediates functions of the Ras1/Cdc42 signaling complex in S. pombe. Shk1 is highly related in structure to the Ste20 kinase, which is required for sexual response in Saccharomyces cerevisiae (19, 20), and to the mammalian Cdc42/Rac1-binding kinase, p65PAK (Pak) (21). Our results lead us to propose that signaling pathways mediated by small GTP-binding proteins and protein kinases related to Shk1 are conserved in evolution and participate in regulation of the cytoskeleton and MAPK modules.

MATERIALS AND METHODS

Microbial Manipulation and Analysis. S. pombe strains SP870 (h90 ade6-210 leu1-32 ura4-D18) and SP66 (h90 ade6-210 leu1-32) were provided by D. Beach (Cold Spring Harbor Laboratory). SP870D (h90 ade6-210/ade6-210 leu1-32/leu1-32 ura4-D18/ura4-D18) is a spontaneous diploid derived from SP870 (V. Jung, personal communication). SP206U (h90 ade6-210/ade6-210 leu1-32/leu1-32 ura4-D18/ura4-D18 shk1::ura4/shk1+) was constructed by transformation of SP870D with an Ecl316I-Msc I fragment of shk1::ura4 from plasmid pBSHK1::URA4, SP206UA (h90 ade6-210/ade6-210 leu1-32/leu1-32 ura4-D18/ura4-D18 shk1::ura4::ADE2/shk1+) was constructed by transforming SP206U with a Not I fragment of ura4::ADE2 obtained from pVIN (22). SP42N17 (h90 ade6-210 leu1-32 ura4::adhl-cdc42[T17N]-ADE2) was constructed by transforming the S.

Abbreviations: MAPK, mitogen-activated protein kinase; MAPKK, MAP kinase; MAPKKK, MAPKK kinase; GST, glutathione S-transferase.

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3The sequence has been deposited in the GenBank data base (accession no. L41552.

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pombe strain SP66 with a Not I fragment of ura4-:adh1-cdc42-[T17N]-ADE2 from pVINCECDC42[T17N]. The S. cerevisiae two-hybrid tester strain L40 (MATa ade2 his3 leu2 trp1 lys2::lexA-HIS3 URA3::lexA-lacZ) has been described (23). S. cerevisiae strains AN43-5A (MATa ade1 arg4 leu2-3, 112 trp1 ura3-52 mfa1::FUS1::lacZ his3::FUS1::H153) and AN1016 (MATa leu2-3, 112 ura3-52 trp1 his4 FUS1::lacZ::LEU2) were from A. Neiman and I. Herskowitz (University of California, San Francisco). S. pombe cultures were grown on either rich medium, yeast extract/dextrose/adenine (YEA), or synthetic minimal medium (PM) with appropriate auxotrophic supplements (3). S. cerevisiae cultures were grown on either rich medium, yeast extract/peptone/dextrose, or drop-out medium (DO) with auxotrophic supplements (24). Standard yeast genetic methods were followed (24, 25).

Nucleic Acid Manipulation and Analysis. Plasmids pRD56, pGADGH; pVL11, pLBDCDC42SP, and pALUCDC42 have been described (7, 15, 23, 26). The shkl gene was cloned from a S. pombe genomic bank constructed in plasmid pWH5 (13, 27). pAAUCM contains a ura4-based S. pombe -E. coli shuttle vector used for expressing c-Myc epitope-tagged proteins (28) from the strong S. pombe adh1 promoter (S.M., unpublished work). pARTICM is similar to pAAUCM but contains the S. cerevisiae LEU2 gene as a selectable marker in place of ura4 (15). pSL1508 is a YCP50-based plasmid containing the hyperactive ste11[PS795] mutant gene (from B. Stevenson and G. Sprague, University of Oregon). A BamHI–Kpn I fragment of the STE20 gene was isolated from pSTE20-5 (from E. Leberer, National Research Council, Montreal) and cloned into the corresponding sites of pAAUCM. The resulting plasmid, pAAUCMSTE20, expresses an N-terminal c-Myc epitope-tagged protein lacking the first 69 amino acids of the Ste20 regulatory domain. A Xho I–Spe I fragment of the shkl open reading frame was isolated from pSP206A and cloned into the Xho I–EcoRV sites of pBlueScriptII SK (Stratagene). A Kpn I–Sac I fragment of shkl was then isolated from the resulting plasmid, pBSHSHK1, and cloned into the corresponding sites of pAAUCM, producing pAAUCMSHK1, and into pUC119, to produce pUC119SHK1. A BamHI–EcoRI fragment of shkl was isolated from pUC119SHK1 and cloned into the corresponding sites of pAAUCM to produce pGADSHH1. pGADSTE20 was constructed by cloning a BamHI–Smal I fragment of the STE20 gene into the corresponding sites of pGADGH. pRDSTE20RI, which was used for expression of a Ste20ΔN (residues 496–939) glutathione S-transferase (GST) fusion protein in this study, was provided by A. Neiman and I. Herskowitz. pGSTSHK1AN was constructed by cloning an Xho I–EcoRI fragment of the shkl gene, from pBSHSHK1, into pRDS6, and allows for expression of a Shk1AN (residues 183–540) GST fusion protein in S. cerevisiae. pLBDCDC42N17 was constructed by cloning a BamHI–Xho I PCR-derived fragment of the dominant negative cdc42[T17N] coding sequence into pVL11. A Sal I–Sac I fragment of cdc42[T17N] was isolated from pALUT17N (15) and cloned into the corresponding sites of pVINCE1 (22) to produce pVINCECDC42T17N. PCR was used to generate a BamHI–Sac I deletion fragment of the shkl gene corresponding to the putative Shk1 regulatory domain (amino acid residues 1–262). This fragment was cloned into the corresponding sites of pARTICM to produce the plasmid pARTICMSHK1ΔC. The oligonucleotide primer pair 5′-AAGGATCCGACCTGACGAGATACTCCAGAATTCGCCATAGTGGCAA and 5′-GAGCTCAGATCGAGTAACCTTTCAACACTCTGATT were used to generate a 5′ fragment of the shkl sequence, and the primer pair 5′-ACGGTGACAGTCTAAGTTCAATACTTCTCC and 5′-GGGACGCTAATCTTCAAGTGGCCATAGTGGCAA were used to generate a 3′ fragment of shkl. The 5′ shkl PCR fragment was digested with Sac I and Bgl II, and the 3′ fragment was digested with Bgl II and Kpn I; then the two fragments were ligated together into a Sac I–Kpn I fragment of pBlueScriptII SK, producing pBSHSHK1ΔK'D. A HindIII fragment of the S. pombe ura4 gene was then cloned into the corresponding site of the shkl fragment in pBSSHK1ΔKD. The resulting plasmid, pBSSHK1::URA4, contains a ura4 replacement of the DNA sequence corresponding to amino acid residues 337–391 of the Shk1 kinase (see Fig. 1).

β-Galactosidase Assays. The filter assay for testing two-hybrid interactions was done as described (7). LexA two-hybrid experiments were conducted using LexA DNA-binding domain and Gal4-activating domain pairs of fusion proteins. Liquid β-galactosidase assays were done as described (29).

Mating Assay. Mating assays were done as described (30). Briefly, transformants were grown on PM agar for 6 days to induce sexual activity. Zygoites, asci, and unmated cells within individual clones were quantitated by microscopy.

RESULTS

Cloning and Sequence Analysis of shkl. A fragment of the shkl coding sequence was cloned in a screen for STE20-related genes from fission yeast and rat using the PCR with degenerate oligonucleotide primers based on peptide sequences within the S. cerevisiae Ste20 catalytic domain (MEYM/HRD1KSDN). The PCR-derived fragment was used as a probe to screen a S. pombe genomic library constructed in plasmid pWH5 (27). Six strongly hybridizing transformants were isolated from among ~30,000 transformed bacterial colonies screened. Plasmids were isolated from two of these transformants. pSP206A carried an insert of ~10.5 kb, whereas pSP206B contained an insert ~8 kb in size (data not shown). Dideoxynucleotide chain-termination sequencing indicated that pSP206A and pSP206B inserts each contained identical STE20-related sequences, which we named shkl, for Ste20 homologous kinase. The nucleotide sequence of shkl reveals an intronless open reading frame of 1818 bp that encodes a predicted protein of 540 amino acids. Shkl1 was most closely related structurally to the S. cerevisiae Ste20 (49% identity) (19, 20) and mammalian Pak (41% identity) (21) protein kinases (Fig. 1). These kinases shared significant identity within their catalytic domains (57% identity). In addition, Shkl1 contains, in its putative regulatory domain, a peptide motif similar in structure to the Cdc42/Rac1-binding domain of Pak (Fig. 1). A similar motif is found in the regulatory domain of the S. cerevisiae Ste20 kinase (Fig. 1; ref. 21). Ste20 and Shkl1 share additional regions of limited homology within their regulatory domains not found in Pak.

Phenotype Conferred by Disruption of shkl. A shkl disruption was made by replacing a fragment of shkl, encoding part of the kinase domain, with ura4 (see Materials and Methods). The shkl::ura4 DNA fragment was used to transform the wild-type S. pombe diploid strain SP870D. Diploid transformants carrying a single disrupted copy of shkl1 were identified by Southern blot analysis of genomic DNA digests. Two independent shkl1::ura4/shkl1+ diploids were sporulated, and asci containing four spores were dissected. Spores were incubated on rich medium for 4 days, and the resulting colonies were replica-plated onto selective medium supplemented with or not supplemented with uracil. A representative tetrad analysis is shown in Fig. 2A. Of 46 tetrads analyzed, 40 produced two viable spores, whereas 6 produced only one viable spore. None of the tetrads analyzed produced more than two viable spores, and viable spores were never Ura+. These results demonstrate that the Shkl1 kinase is required for at least one essential cellular function.

We examined further the shkl phenotype by microscopic analysis. Wild-type S. pombe cells are rod-like in morphology (Fig. 2B). shkl spores typically germinated but arrested as spherical cells after one to several rounds of cell division (Fig. 2C). This phenotype is similar to that observed for S. pombe strains carrying cdc42 null mutations (14). Because Shkl1 contains a motif structurally similar to the Cdc42/Rac1-binding domain of the mammalian Pak protein kinase, we
examined whether Shkl and Cdc42 interact physically and functionally.

**Complex Formation Between Shkl and Cdc42.** We used the yeast two-hybrid system to determine whether Shkl and Cdc42 interact physically. Shkl was expressed as a Gal4-activating domain fusion protein, and Cdc42 and Cdc42[T17N] were expressed as fusions to the LexA DNA-binding domain. We detected complex formation between Shkl and wild-type Cdc42 but not between Shkl and the dominant negative Cdc42[T17N] mutant protein. The T17N mutation is analogous to mutations identified at the corresponding positions of yeast and mammalian Ras proteins that result in defective guanine nucleotide exchange (31-33). Our failure to detect an interaction between Shkl and Cdc42[T17N] in the two-hybrid system may indicate that interaction between the two proteins is GTP-dependent and confirms the results of Manser et al. (21), who showed that binding between mammalian Cdc42 and the mammalian Shkl homolog Pak is GTP-dependent. Like Shkl, the structurally similar *S. cerevisiae* Ste20 kinase also interacted with Cdc42 but not with Cdc42[T17N] (data not shown). Neither Shkl nor Ste20 showed detectable interactions with Ras1, Gpa1, Byr2, Sed1, or Sdc2 in the two-hybrid system.

**Functional Interaction Between Shkl, Cdc42, and Ras1 in *S. pombe*.** Genetic experiments were conducted to establish a functional relationship between Shkl and Cdc42. First, we expressed the N-terminal putative regulatory domain of Shkl (ShklΔC) in wild-type *S. pombe* cells. Fig. 2D shows that ShklΔC caused cells to become spherical in morphology. This phenotype was indistinguishable from that resulting from overexpression of the dominant inhibitory Cdc42 mutant protein Cdc42[T17N] (Fig. 2E) and further supports the notion that the N-terminal domain of Shkl is regulatory in function. Additional genetic evidence suggesting a functional interaction between Cdc42 and Shkl came from overexpression of Shkl in *S. pombe* strain overexpressing Cdc42[T17N]. Gain of expressing this dominant inhibitory GTP-binding protein mate at a frequency of about one-fiftieth that of wild-type cells.

Expression of Shkl partially suppressed this mating defect (Table 1).

Cdc42 appears to act downstream of Ras1 in *S. pombe*, and data from two-hybrid experiments suggest that the two proteins are part of a multiprotein complex (15). Unlike Cdc42, Ras1 is not required for cell viability, possibly because Cdc42 has sufficient basal activity to sustain cell viability in the absence of Ras1. Although high-copy expression of Shkl failed to measurably suppress the sporulation or conjugation defects of a *ras1* null mutant, expression of the N-terminal regulatory domain of Shkl markedly attenuated the hypersexual responses (elevated levels of agglutination and projection of conjugation tubes) of a *S. pombe* strain carrying an activated *ras1* [G17V] mutant gene (data not shown). These results are consistent with a role for Shkl in the sexual functions of Ras1. Further evidence for this comes from the functional homology between Shkl and Ste20, as described below.

**Functional Homology Between Shkl and the *S. cerevisiae* Ste20 Protein Kinase.** To examine the functional relatedness of Shkl and Ste20, we constructed the high-copy plasmid pAAUCMSTE20 for expressing Ste20 in *S. pombe*. A *shkl::ADE2/shkl1* diploid strain was transformed with pAAUCMSTE20, and the resulting transformants were subjected to tetrad analysis. Expression of Ste20 restored not only viability to *shkl::ADE2* haploid cells, as determined by the recovery of ADE2+ spores, but also mating and normal morphology (Fig. 2F). These results demonstrate that Shkl and Ste20 are functionally related.

We also examined the function of Shkl in *S. cerevisiae*. In *S. cerevisiae*, the Ste20 kinase is required for signaling from heterotrimeric G protein-coupled peptide mating pheromone receptors to a MAPK module composed of the Ste11 (MAPKK), Ste7 (MAPKK), and Fus3/Kss1 (MAPK) protein kinases (19). Deletion of the Ste20 N-terminal regulatory domain results in a kinase capable of constitutively activating the mating factor response pathway in the absence of mating pheromone (ref. 20; Table 2). Similarly, we found that high-copy expression of a Shkl kinase lacking its N-terminal regulatory domain was capable of inducing the *S. cerevisiae*
Table 1. Overexpression of shkl partially suppresses the mating defect of a S. pombe strain expressing cdc42[T17N]

<table>
<thead>
<tr>
<th>Strain</th>
<th>Overexpressed gene*</th>
<th>Mating, † %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP66</td>
<td>None</td>
<td>53.3 ± 1.9</td>
</tr>
<tr>
<td>SP42N17</td>
<td>cdc42[T17N]</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>shkl</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>cdc42[T17N], cdc42</td>
<td>10.1 ± 0.7</td>
</tr>
</tbody>
</table>

SP66 is a wild-type (cdc42+) S. pombe strain. SP42N17 is isogenic to SP66 but carries an adh1-cdc42[T17N] sequence integrated at the ura4 locus.

*Plasmids used for transformation of SP66 and SP42N17 were pAAUCM (empty vector); PALUCDC42SP, for cdc42 overexpression; and pAAUCMShkl, for shkl overexpression. pAAUCM contains the adh1 promoter sequence, which is also used for overexpression of cdc42[T17N], and its use as a control plasmid provides assurance that the effects observed from shkl overexpression are not the result of promoter competition.

†The quantitative mating assay was done as described (30). Values represent the average of three determinations.

A receptor-coupled G protein and upstream of the pheromone-responsive MAPK module (19). Establishing a similar role for Shkl in S. pombe is complicated by the fact that shkl is an essential gene. However, two results suggest a possible role for Shkl in mating: (i) Shkl partially suppresses the mating defect of a S. pombe strain expressing the dominant inhibitory Cdc42[T17N] mutant protein; and (ii) expression of the Shkl N-terminal putative regulatory domain attenuates the exaggerated sexual responses of a S. pombe strain carrying the activated ras1[G17V] mutant gene.

Although it remains to be demonstrated conclusively, several results lead us to speculate that Shkl may also participate in the Ras1-regulated MAPK module. Shkl is capable of signaling to the pheromone-responsive MAPK module in S. cerevisiae (this report) and is also capable of inducing MAPK activation in cell-free extracts of Xenopus laevis oocytes (S.M. and T.P., unpublished work). Moreover, Ste20, which also signals to the MAPK module in S. cerevisiae, can functionally replace Shkl in S. pombe. Interestingly, recent biochemical studies by Wu and coworkers (31) and by A. Neiman and I. Herskowitz (personal communication) indicate the Byr2 homolog of S. cerevisiae, Ste11, is a Ste20 substrate in vitro.

Fig. 2. Functional characterization of Shkl. (A) shkl is an essential gene. The shkl+/shklD::ura4 diploid strain SP206U was grown on PM medium to induce sporulation. Ascii containing four spores were dissected by micromanipulation, placed on YEA, and incubated for 4 days at 30°C. The resulting colonies were replica-plated onto PM supplemented with (PM + Ura) or without (PM – Ura) uracil. No more than two viable spores were isolated from any four-spore ascus, and no viable spores were ever Ura−. (B) Photomicrograph of the wild-type S. pombe strain SP870. (C) Photomicrograph of shkl− S. pombe cells from culture of sporulated SP206U diploids. (D) Photomicrograph of S. pombe cells transformed with plasmid pART1CMSHK1αC, which overexpresses the Shkl N-terminal regulatory domain. Note similarity to cells in D. (E) Photomicrograph of S. pombe strain SP42N17, which overexpresses the dominant inhibitory cdc42[T17N] gene. (F) Photomicrograph of shkl−/− cells transformed with pAAUCMSTE20, which expresses the S. cerevisiae STE20 gene. shkl−/− STE20* cells are viable, competent for mating, and normal in morphology. (×500.)

DISCUSSION

In S. cerevisiae, the Ste20 protein kinase is required for mating pheromone-induced signal transduction (19, 20). Ste20 appears to function downstream from the mating pheromone

Table 2. S. pombe Shkl kinase activates the S. cerevisiae mating pheromone-responsive MAPK cascade

<table>
<thead>
<tr>
<th>Overexpressed gene</th>
<th>FUS1–lacZ induction,* β-galactosidase units</th>
<th>STE11 †</th>
<th>ste11 †</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4.3 ± 1.4</td>
<td>0.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Ste20ΔN</td>
<td>26.9 ± 3.3</td>
<td>0.8 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>ShklΔN</td>
<td>23.9 ± 6.8</td>
<td>1.0 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Ste11[P279S]</td>
<td>8.8 ± 1.9</td>
<td>14.7 ± 1.1</td>
<td></td>
</tr>
</tbody>
</table>

Ste20ΔN and ShklΔN were expressed as GST fusion proteins from the galactose-inducible GAL1 promoter in plasmids pRDSTE20RI and pRD56SHK1ΔN, respectively. Plasmid pSL1508 was used for expression of the Ste11[P279S] hyperactive mutant protein.

*The congenic STE11 † and ste11 † strains used for this experiment were AN43-5A and AN1016, respectively. Transformants were grown in DO medium lacking uracil (DO-ura) at 30°C to ~5 × 10⁶ cells per ml, then transferred to DO-Ura-glucose containing 2% (wt/vol) galactose, 2% (wt/vol) glycerol, and 1% ethanol, and incubated overnight at 30°C. β-Galactosidase activity was measured as described (29). Units of β-galactosidase were calculated as [1000 × OD420]/[OD600 × time (min) × vol of cells (ml)]. Values represent the averages of at least four determinations.
As we have shown that Shk1 and Cdc42 functionally interact and that Ras1 and Cdc42 functionally interact, we propose that Ras1 has two distinct inputs into the MAPK module: one by direct physical interaction with Byr2 (7, 13) and the second through Shk1, which Ras1 regulates via Cdc42. Our results have led us to propose the model depicted in Fig. 3, in which the Ras1/Cdc42-regulated morphology control pathway in S. pombe also participates in regulation of the Ras1-dependent MAPK cascade, the Byr2 component of which interacts with both Ras1 and Shk1.

As in S. pombe, small GTP-binding proteins have been implicated in morphological regulation in S. cerevisiae. A homolog of Cdc42 is required for normal polarized cell growth in S. cerevisiae, as is a Ras-related GTP-binding protein, Rap1/Bud1 (for review, see ref. 34). Although Ste20 has not as yet been implicated in morphological control in S. cerevisiae, it binds to Cdc42 (this study) and to a mammalian Rho-related GTP-binding protein Rac1 (S.M. and L. Van Aelst, unpublished work) and functionally replaces Shk1 in S. pombe. These results suggest a potential role for Ste20 and/or Ste20-related proteins in the morphological regulation of S. cerevisiae. In this regard, a new gene, Cla4, encoding a protein kinase structurally related to Ste20, has recently been identified in S. cerevisiae. Although deletion of either Cla4 or STE20 alone does not affect cell viability, deletion of both genes is lethal, suggesting that Cla4 and Ste20 perform overlapping, essential cellular functions (35).

Whether Pak or related protein kinases play roles in mammalian cells analogous to the roles of their homologs in yeast—namely, regulation of MAPK modules and cellular morphology—remains to be seen.

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