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Functional Homology of Protein Kinases Required for Sexual Differentiation in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* Suggests a Conserved Signal Transduction Module in Eukaryotic Organisms

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We present genetic evidence that three presumptive protein kinases of *Schizosaccharomyces pombe*, byr2, byr1, and spk1 that are structurally related to protein kinases of *Saccharomyces cerevisiae*, STE11, STE7, and FUS3, respectively, are also functionally related. In some cases, introduction of the heterologous protein kinase into a mutant was sufficient for complementation. In other cases (as in a *ste11*$^-$ mutant of *S. cerevisiae*), expression of two *S. pombe* protein kinases (*byr2* and *byr1*) was required to observe complementation, suggesting that byr2 and byr1 act cooperatively. Complementation in *S. pombe* mutants is observed as restoration of sporulation and conjugation and in *S. cerevisiae* as restoration of conjugation, pheromone-induced cell cycle arrest, and pheromone-induced transcription of the *FUS1* gene. We also show that the *S. pombe* kinases bear a similar relationship to the mating pheromone receptor apparatus as do their *S. cerevisiae* counterparts. Our results indicate that pheromone-induced signal transduction employs a conserved set of kinases in these two evolutionarily distant yeasts despite an apparently significant difference in function of the heterotrimeric G proteins. We suggest that the STE11/byr2, STE7/byr1, and FUS3/spk1 kinases comprise a signal transduction module that may be conserved in higher eukaryotes. Consistent with this hypothesis, we show that a mammalian mitogen-activated protein (MAP) kinase, ERK2, can partially replace spk1 function in *S. pombe*.

INTRODUCTION

Signal transduction, the process whereby changes in the extracellular milieu induce programmed cellular responses, is fundamental to the interaction of all living things with their environment. Due to the relative ease with which yeast can be analyzed by genetic methods, the sexual differentiation pathways induced by mating pheromones have been favored for the study of signal transduction. A convergence of information about the sexual differentiation pathways of two highly diverged yeasts, the fission yeast, *Schizosaccharomyces pombe*, and the budding yeast, *Saccharomyces cerevisiae*, has led to the experiments described in this report.

Haploid cells of the yeast *S. cerevisiae* conjugate in response to the **a** and α mating pheromones secreted by *MAT***a** and *MAT*α cells, respectively (for reviews see Cross *et al.*, 1988; Marsh *et al.*, 1991). These pheromones bind to membrane receptors that have seven transmembrane spanning regions (Burkholder and Hartwell, 1985; Nakayama *et al.*, 1985; Hagen *et al.*, 1986). The binding of pheromones to their respective receptors is thought to trigger the dissociation of a heterotrimeric G protein that mediates the sexual response. Disruption

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Table 1. Percent identity between protein kinases

	STE7	byr1	STE11	byr2	cdc2	FUS3	KSS1	spk1	ERK1	ERK2
STE7	100									
byr1	**43.7**	100								
STE11	26.4	25.1	100							
byr2	28.1	30.9	**42.3**	100						
cdc2	25.8	25.7	28.5	29.2	100					
FUS3	31.9	29.0	27.5	27.1	40.6	100				
KSS1	29.2	25.8	26.3	25.5	40.8	**56.9**	100			
spk1	24.0	23.1	26.7	31.0	39.9	**55.2**	**60.5**	100		
ERK1	25.0	23.1	24.3	26.1	36.0	**51.2**	**50.7**	**51.1**	100	
ERK2	26.5	25.0	23.2	25.4	36.0	**52.0**	**53.2**	**54.0**	**84.9**	100

Data were derived with the GAP program (Needleman and Wunsch, 1970) of the GCG package (Devereux *et al.*, 1984). The same gap weight and gap length weight (default values for the program) were used for each comparison.

of *GPA1*, encoding the G_α subunit, leads to a constitutive activation of the mating pathway and a terminal state of sexual differentiation (Dietzel and Kurjan, 1987a; Miyajima *et al.*, 1987; Jahng *et al.*, 1988). Disruption of either the *STE4* or *STE18* genes, encoding the G_β or G_γ subunits, respectively, results in a block in the response pathway and hence sterility (Whiteway *et al.*, 1989). The effector target of the heterotrimeric G protein is unknown, but several genes, including *STE5*, *STE7*, *STE11*, *FUS3*, *KSS1*, and *STE12*, encode products that likely function downstream of the G protein (for review see Marsh *et al.*, 1991). *STE7*, *STE11*, *FUS3*, and *KSS1* are each predicted to encode protein kinases (Teague *et al.*, 1986; Courchesne *et al.*, 1989; Elion *et al.*, 1990; Rhodes *et al.*, 1990). FUS3 and KSS1 have redundant function (Elion *et al.*, 1991) and are members of the same branch of the protein kinase family (see DISCUSSION).

In *S. pombe*, as in *S. cerevisiae*, cells of opposite mating type conjugate in response to mating factors (Friedmann and Egel, 1978; Fukui *et al.*, 1986a; Leupold, 1987; Leupold *et al.*, 1989; Davey, 1991, 1992). Cells of the "+" mating type produce P factor and cells of the "−" mating type produce M factor. *mam2*, the gene encoding a receptor for P factor, has been cloned and found to encode a protein with seven transmembrane spanning domains homologous to the STE2 mating pheromone receptor of *S. cerevisiae* (Kitamura and Shimoda, 1991). As discussed below, pheromone response in *S. pombe* also involves a G protein.

There are several major differences between the two yeasts. Unlike *S. cerevisiae*, starvation is a prerequisite for mating in *S. pombe*. Upon starvation, synthesis of several components of the mating pathway are induced (Shimoda *et al.*, 1987; Kelly *et al.*, 1988; Kitamura and Shimoda, 1991; Sugimoto *et al.*, 1991). In contrast to *S. cerevisiae*, *S. pombe* cells typically undergo immediate meiosis and sporulation after conjugation. This process requires many of the same signal transduction components as does the mating response (for review see Egel *et al.*, 1990). The G proteins of the two yeasts offer a striking functional contrast. As noted above, the G_α subunit of *S. cerevisiae* inhibits sexual differentiation, and the $G_{\beta\gamma}$ subunits mediate the response. In *S. pombe*, it is the G_α subunit, encoded by *gpa1*, that appears to mediate response and thereby induce sexual differentiation (Obara *et al.*, 1991). Finally, in *S. pombe* the *ras1* gene, a homolog of the mammalian and *S. cerevisiae* *RAS* genes, is required for both conjugation and sporulation (Fukui *et al.*, 1986b; Nadin-Davis *et al.*, 1986). In contrast, the *RAS* genes of *S. cerevisiae* do not participate in conjugation.

Two *S. pombe* genes have been identified that, when present in cells in multiple copies, can partially restore the sexual defects of $ras1^-$ cells. Both *byr1* and *byr2* (for bypass of *ras1*) can restore sporulation competence to $ras1^-/ras1^-$ diploid cells (Nadin-Davis and Nasim, 1988, 1990; Wang *et al.*, 1991), and both can very weakly suppress the conjugal defects of $ras1^-$ haploid cells (Xu *et al.*, 1992). Disruption of either *byr* gene leads to an apparently absolute defect in sporulation and conjugation that is not restored by overexpression of wild-type *ras1* or the activated $ras1^{val17}$. Overexpression of *byr1* restores sporulation to $byr2^-/byr2^-$ diploid cells, but overexpression of *byr2* does not restore sporulation to $byr1^-/byr1^-$ diploid cells (Wang *et al.*, 1991). These epistasis relationships place byr1 and byr2 downstream of ras1, and byr1 downstream of byr2. *ras1*, *byr1*, and *byr2* each correspond to previously discovered *S. pombe* sterile (*ste*) loci: *ras1* to *ste5*, *byr1* to *ste1*, and *byr2* to *ste8* (Lund *et al.*, 1987; Nadin-Davis and Nasim, 1990; Wang *et al.*, 1991).

The *byr1* and *byr2* genes are each predicted to encode protein kinases (Nadin-Davis and Nasim, 1988; Wang *et al.*, 1991). byr1 is most closely related to the *S. cerevisiae* STE7 kinase, whereas byr2 is most closely related

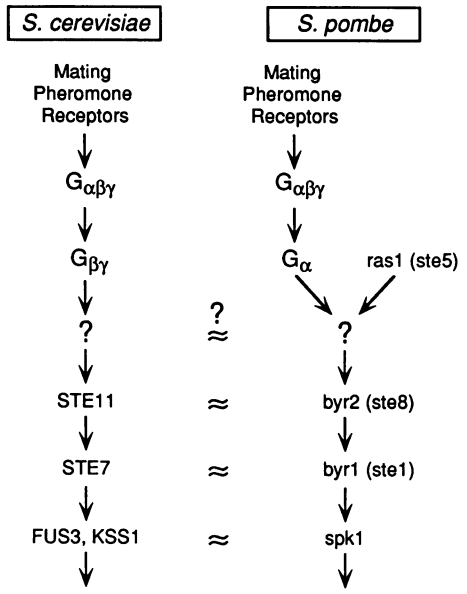

Figure 1. Proposed interactions of signal transducers in the *S. cerevisiae* and *S. pombe* pheromone response pathways. The interactions depicted are the minimal relationships suggested by genetic and biochemical data. More complex interactions are possible. Interactions that are not well established are denoted by question marks (?), as are hypothetical homologies between signaling molecules. The nature of the relationship between ras1 and the pheromone receptor apparatus in *S. pombe* is, at present, unclear.

to STE11 (see Table 1; Neiman and Stevenson, unpublished observations). An additional gene, *spk1*, has recently been discovered in *S. pombe* that also encodes a protein kinase required for conjugation (Toda *et al.*, 1991). The spk1 protein is most closely related to the *S. cerevisiae* KSS1 and FUS3 kinases, and to the family of ERK/MAP kinases of higher eukaryotes (Toda *et al.*, 1991; see also Table 1). The ERK/MAP kinases are activated by extracellular growth and differentiation factors (Ray and Sturgill, 1987, 1988; Hoshi *et al.*, 1988; Boulton *et al.*, 1990a,b; Boulton *et al.*, 1991). This activation has recently been shown to be RAS dependent (Thomas, 1992; Thomas *et al.*, 1992; Wood *et al.*, 1992).

These relationships among the protein kinases of budding and fission yeast have led us to the hypothesis depicted in Figure 1, that a conserved set of protein kinases act in concerted fashion in *S. pombe* and *S. cerevisiae*. In this report we have tested various aspects of this model. We show that byr2 shares conserved function with STE11, as does byr1 with STE7, and spk1 with FUS3 and KSS1. Moreover, elements of the regulation of the kinases have been conserved in the two yeasts. Finally, we provide evidence that the *S. pombe* kinases function downstream of the G protein as do their *S. cerevisiae* counterparts. Similarities between these pathways and the signalling pathways of higher eukaryotes suggest a protein kinase module has been conserved in eukaryotic cells. Consistent with this hypothesis, we demonstrate a partial conservation of function between spk1 and a mammalian mitogen-activated protein (MAP) kinase, ERK2.

MATERIALS AND METHODS

Microbial Manipulation and Analysis

S. cerevisiae strains are listed in Table 2. Standard media and genetic methods were used (Mortimer and Hawthorne, 1969; Hicks and Herskowitz, 1976; Rose *et al.*, 1990) unless otherwise noted. AN1012 and AN1016 were constructed by transformation of strains IH1770 and IH1768, respectively. The *FUS1::lacZ* gene is on an integrating plasmid, carrying a *LEU2* marker, which was integrated into the chromosome at *FUS1*. AN1015 was constructed by one step gene replacement of *FUS3* (Rothstein, 1983) in strain AN37-4C-S. AN42-2A was constructed by one step gene replacement of *FUS3* and *KSS1* in congenic *MATa* and *MATα* strains (IH1783 and IH1784); these two strains were mated to create AN42, and AN42-2A was obtained as segregant after sporulation and dissection of AN42.

S. pombe strains used in this study are listed in Table 3. Strains were grown on either rich medium, YEA, or synthetic minimal medium, PM, with appropriate auxotrophic supplements (Nadin-Davis *et al.*, 1986). Cells were transformed by the lithium acetate procedure (Ito *et al.*, 1983) and transformants selected by growth on PM agar plates. PM lacking NH_4Cl and containing only 1% glucose (PM-N) was used for induction of mating and sporulation.

Nucleic Acid Manipulation and Analysis

Plasmid pSTE7 is pJD7 (a gift of Stan Fields) and carries the chromosomal *STE7* gene in a YEp24 vector. pSTE11 is pSTE11.1 (Chaleff

Table 2. *S. cerevisiae* strains used in this study

Strain	Genotype	Source
AN1012	*MATa his4-519 ura3-52 leu2 trp1 ste7Δ2 FUS1::lacZ (LEU2)*	This study
IH1770	*MATa his4-519 ura3-52 leu2 trp1 ste7Δ2*	Laboratory collection
IH1768	*MATa his4-519 ura3-52 leu2 trp1 ste11Δ1*	Laboratory collection
AN40-5A	*MATa his4-519 ura3-52 leu2 trp1 ste7Δ2 ste11Δ1*	This study
AN42-2A	*MATa his4-519 ura3-52 leu2 trp1 fus3::URA3 kss1::URA3*	This study
AN37-4C-S	*MATa ura3-52 leu2 trp1 ade2 met1 HMLa HMRa bar1-1*	Laboratory collection
AN1015	*MATa ura3-52 leu2 trp1 ade2 met1 HMLa HMRa bar1-1 fus3::URA3*	This study
AN1016	*MATa his4-519 ura3-52 leu2 trp1 ste11Δ1 FUS1::lacZ (LEU2)*	This study
5815-7-1	*MATα ura3 his3 hop1-1*	N. Hollingsworth

Table 3. *S. pombe* strains used in this study

Strain	Relevant genotype	Source
SP258	*h⁺ ade6-216*	D. Beach
SP870	*h⁹⁰ ade6-210 leu1-32 ura4-D18*	D. Beach
SPBU	*h⁹⁰ ade6-210 leu1-32 ura4-D18 byr1::ura4*	Wang *et al.*, 1991
SPGL	*h⁹⁰ ade6-210 leu1-32 ura4-D18 gpa1::LEU2*	This study
SPGLD	*h⁹⁰ ade6-210 leu1-32 ura4-D18 gpa1::LEU2/ h⁹⁰ ade6-210 leu1-32 ura4-D18 gpa1::LEU2*	This study
SPKU	*h⁹⁰ leu1 ura4 spk1::ura4*	T. Toda
SPKUD	*h⁹⁰ leu1 ura4 spk1::ura4/h⁹⁰ leu1 ura4 spk1::ura4*	This study
SPRUD	*h⁹⁰ ade6-210 leu1-32 ura4-D18 ras1::ura4/ h⁹⁰ ade6-210 leu1-32 ura4-D18 ras1::ura4*	Wang *et al.*, 1991
SPSU	*h⁹⁰ ade6-210 leu1-32 ura4-D18 byr2::ura4*	Wang *et al.*, 1991

and Tatchell, 1985), which carries the chromosomal *STE11* locus in YEp13. pFUS3-2μ-TRP was made by cloning a 3.7 kb *Bam*HI-*Sal* I fragment of pYEE81 (Elion *et al.*, 1990) carrying *FUS3* into the polylinker of pRS424 (a gift of J. Li). pbyr1 was made by cloning a 2.0 kb *Sal* I-*Kpn* I fragment carrying the *byr1* gene from pART3-BYR—the *byr1* gene cloned into pART1 (McLeod *et al.*, 1987) (a gift of S. Nadin-Davis)—into pART2-2μ to create pART2-2μ-byr1. Plasmid pART2-2μ was made by replacing the 1.2 kb *Eco*RI fragment of pART2 (McLeod *et al.*, 1987), containing the *S. pombe* ARS and stabilizer regions, with a 2.2 kb *Eco*RI fragment of YEp24 containing the 2 μ origin. This expression vector has *URA3* as a selectable marker and carries the *S. pombe adh* promoter. pSPK1 is pADH-spk1 (Toda *et al.*, 1991), which carries the *spk1* gene under the control of the *S. pombe adh* promoter. pbyr2 was made by cloning a 2.0 kb *Sal* I-*Sac* I fragment of the *byr2* open reading frame, which was isolated from pAIS1 (Wang *et al.*, 1991), into the corresponding sites of pAD5, a plasmid derived from pAD4Δ (Ballester *et al.*, 1989). pAD5 contains the *LEU2* gene, a fragment of the 2μ sequence, the ampicillin resistance gene, and a fragment containing the *S. cerevisiae ADH1* promoter. Immediately downstream of the *ADH1* promoter is a sequence encoding the Lerner epitope tag (Field *et al.*, 1988). The *byr2* fragment was cloned in frame with the Lerner epitope encoding sequence, producing the plasmid pbyr2.

A fragment of the *S. pombe gpa1* gene (Obara *et al.*, 1991) was cloned from yeast genomic DNA by use of the polymerase chain reaction (PCR) with the oligonucleotide primers 5′-ATTCGGACTGTCGACATTTGCCAGGT and 5′-AGTTACACGGAGCTCCGAAGAAGCTA. The 2.3 kb *gpa1* fragment was digested with *Sal* I and *Sac* I and then ligated into the corresponding sites of pUC119, producing the plasmid pUC119GPA1. pUC119GPA1 was digested with *Xho* I and *Spe* I to remove a 1.0-kb fragment containing most of the *gpa1* open reading frame. This fragment was replaced by a 2.0 kb *Sal* I-*Xba* I fragment of the *S. cerevisiae LEU2* gene, which was isolated from the plasmid pJJ250 (Jones and Prakash, 1990), producing the plasmid pUC119GPA1::LEU2.

The *STE7* open reading frame was cloned from *S. cerevisiae* genomic DNA by PCR with the oligonucleotide primers 5′-GTATATTGGCTGCAGGTCATGTTTCAACGA and 5′-TAACTAATGGGATCCCATGCATTCAATGG. The 1.6 kb *STE7* fragment was digested with *Pst* I and *Bam*HI, then cloned into the corresponding sites of the *S. pombe-E. coli* shuttle vector pART1 (McCleod *et al.*, 1987), producing the plasmid pART1STE7. In this plasmid, *STE7* is expressed from the *S. pombe adh* promoter.

pST23, a *S. pombe* expression vector containing a genomic clone of the *spk1* gene, was the kind gift of Takashi Toda (Toda *et al.*, 1991). pART1ERK2 was constructed by cloning a *Pst* I-*Eco*RV fragment of ERK2, isolated from pSKERK2 (a gift from George Yancopoulis), into the *Pst* I-*Sma* I sites of pART1.

For construction of pAAAUSTE11, the *Acc*I site 5′ of the STE11 open reading frame, contained in pRS316STE11, was converted to a *Sal* I site by oligonucleotide mediated site directed mutagenesis. A 2.3-kb fragment containing the *STE11* open reading frame was isolated by digestion with *Sal* I and *Bam*HI, then subcloned into the vector pAAUN (Xu *et al.*, 1990), resulting in the plasmid pADHSTE11. The *Sph* I-*Bam*HI *adh-STE11* fragment was isolated from this plasmid and used to replace the *Sal* I-*Bam*HI fragment of JC99 from pALU99 (Eric Chang, unpublished results), producing the plasmid pALUSTE11. A 2.2 kb *S. cerevisiae ADE2* gene fragment, isolated from pASZ11 (Stotz and Linder, 1990) was then cloned into the *Bam*HI site of pALUSTE11, resulting in the plasmid pAAAUSTE11. The *ADE2* gene complements the *ade6⁻* mutation of *S. pombe* (Marcus, unpublished results). pAAAUSTE11 can thus be used to transform *ade6⁻* and *ura4⁻ S. pombe* strains.

The plasmids pALR, pART1BYR1, and pAIS1 have been described previously (Wang *et al.*, 1991). The plasmid pAUCMBYR1, which expresses an *N*-terminal c-myc epitope (Evan *et al.*, 1985) tagged byr1 protein, was made by replacing the *Sph* I-*Sac* I *adh-byr2* fragment of pAIS1 with an *adh-byr1* fragment isolated from pART1CMBYR1, a derivative of pART1BYR1. The vector pAAAUBYR2 was made by cloning the 2.2 kb *ADE2* fragment into the *Sac* I site of pAIS1. Like pAAAUSTE11, this plasmid can be used to transform both *ade6⁻* and *ura4⁻ S. pombe* strains.

For construction of pAIS1^{Ser209}, the proline at position 209 of *byr2* was converted to a serine by use of PCR according to the method described by Ho *et al.* (1989). Two PCR reactions were done initially. The first reaction used the primers 5′-TTATACGTTGTCGACCCACTTTCCTG (primer A) and 5′-TCGCTAGG**A**-**GA**TCGCTGGTA (primer B1). The second reaction used the primers 5′-TACCAGCGA**TCT**CCTAGCGA (primer B2) and 5′-CGAATTCGAGCTCGCTTATG (primer C). In both reactions, the plasmid pAIS1 was used as template DNA. The partially overlapping *byr2* fragments generated from the two initial PCR reactions were gel purified, then used as templates in the final PCR reaction, which used only primers A and C. The resulting *byr2* fragment was gel purified, digested with *Sal* I and *Sac* I, and used to replace the wild-type *byr2* fragment of pAIS1, producing plasmid pAIS1^{Ser209}. The Pro209 → Ser mutation was confirmed by dideoxy nucleotide sequencing (Sanger *et al.*, 1977).

Construction of S. pombe gpa1 and spk1 Null Mutants

The plasmid pUC119GPA1::LEU2 was digested with *Sal* I and *Sac* I to release a 2.9 kb *gpa1::LEU2* fragment, which was used to transform the *S. pombe* strain SP870. Sterile transformants were identified by

iodine vapor staining and the *gpa1::LEU2* disruption confirmed by PCR. The resulting strain was named SPGL. The isogenic homozygous *gpa1⁻/gpa1⁻* diploid strain, SPGLD, was isolated by use of the lithium acetate transformation process, followed by selection of diploids on YEA plates containing phloxin B.

The *spk1⁻h⁹⁰* strain, SPKU, was a gift from Takashi Toda. The isogenic *spk1⁻/spk1⁻* diploid strain, SPKUD, was isolated by use of the lithium acetate transformation process, followed by selection of diploids on YEA plates containing phloxin B.

Quantitative S. cerevisiae Mating Assays

Quantitative matings were performed essentially as described previously (Neiman *et al.*, 1990). The strain to be tested was grown in synthetic media with an equal number of cells of the mating tester strain 5815-7-1 and then filtered onto a 0.45 μm nitrocellulose filter (Whatman, Clifton, NJ). The filter was incubated on a yeast extract, peptone, dextrose (YEPD) plate at 30°C for 4 h. Cells were resuspended in 5 ml of SD by 30 s vigorous vortexing followed by sonication for 5 s. Serial dilutions were plated on YEPD plates for total colony forming units and on SD-His or SD minimal plates to select for diploids, depending on the mating tester used. Mating frequency was calculated as diploids/total colonies. Under these conditions, wild-type cells mated with an efficiency of ~20%.

Quantitative S. pombe Sporulation and Mating Assays

Sporulation and conjugation of *S. pombe* strains was measured by modification of the liquid assays described by Obara *et al.* (1991). Cultures were grown in PM medium to a density of 1×10^6 to 8×10^6 cells/ml. Cells were then pelleted by centrifugation, washed once with PM-N, and resuspended in PM-N at 5×10^6 cells/ml for sporulation assays and 2×10^7 cells/ml for conjugation assays. The frequency of sporulation was determined by microscopic quantitation of asci and spores after a 36- to 48-h incubation in PM-N, while the frequency of conjugation, as determined by quantitation of zygotes and asci, was measured after a 24-h incubation. Under these assay conditions, wild-type cells sporulate with an efficiency of 80–90% and conjugate with an efficiency of ~15%–20%.

The quantitative auxotrophic mating assay was performed essentially as previously described (Xu *et al.*, 1992). Briefly, SPGL transformants were mated with SP258, which is an h⁻ strain. Strains were cultured separately for 2 d in PMA medium, then 5×10^7 cells of each mating type were mixed and cultured for another 4 h in the same medium. The mixture was spread onto PMA plates for 24 h and replicaplated onto PM plates for 5 d to select for growth of diploid cells by adenine prototrophy. Diploid colonies were verified by a second round of replicaplating onto PM plates, followed by microscopic examination.

β-Galactosidase Assays

Fresh overnight cultures of strains to be tested were diluted 1:20 in selective medium and grown to optical density $(OD)_{600}$ of 0.3. Aliquots were removed at this point and assayed for β-galactosidase activity. Synthetic α-factor (Sigma, St. Louis, MO) was added to a final concentration of 25 μg/ml, and the cultures were grown for 5 h and assayed for β-galactosidase activity. Assays were performed as described by Stern *et al.* (1984). Units of β-galactosidase activity were calculated as $[1000 \times OD_{420}]/[OD_{600} \times \text{time (min)} \times \text{vol cells (ml)}]$.

Halo Assays

Fresh overnight cultures grown in media selective for the plasmid (if any) were diluted 10 000-fold and 0.1 ml were spread as a lawn on a YEPD plate. A filter disk (Schleicher and Schuell, Keene, NH) containing 5 μg of synthetic α-factor (Sigma) was then placed in the middle of the plate and the plates were incubated for 2 d at 30°C.

Nomenclature

Based on previously established nomenclature, *S. cerevisiae* wild-type genes are designated by italicized uppercase letters, whereas mutant genes are designated by lowercase italicized letters. *S. pombe* genes, both wild-type and mutant, are designated by lowercase italicized letters. For clarity, we have used the minus (−) superscript to further denote both *S. cerevisiae* and *S. pombe* mutant genes. *S. cerevisiae* proteins are designated by uppercase roman letters, whereas *S. pombe* proteins are designated by lowercase roman letters.

RESULTS

Cross Complementation of STE11 and byr2 Genes

To test the functional significance of the structural similarities between the different kinases, we tested the ability of the *S. pombe* and *S. cerevisiae* genes to function like their structural homologs when expressed in the heterologous host. Disruption of the *byr2* locus leaves *S. pombe* cells defective in conjugation and sporulation (Wang *et al.*, 1991). Transformation of the *byr2* null haploid strain with a multicopy plasmid expressing the *S. cerevisiae STE11* gene restored conjugation to near wild-type levels (Table 4). Plasmids expressing *S. pombe byr1* or *S. cerevisiae STE7* could not suppress the conjugation defect of *byr2⁻* cells.

Reciprocal experiments were performed in *S. cerevisiae*. A *ste11⁻* strain of *S. cerevisiae* (IH1768) was transformed with various plasmids and quantitative mating assays were performed on transformants (Table 5). We could not detect suppression of the *ste11⁻* conjugal defect when the *S. pombe byr2* gene was expressed alone, nor did expression of *byr1* alone suffice. However, coexpression of *byr2* with *byr1* allowed significant levels of conjugation, almost 10^4-fold above either gene alone.

Table 4. Expression of *STE11* can complement the mating defect of a *S. pombe byr2⁻* strain

S. pombe genotype[a]	Expressed gene[b]	Mating frequency[c]	n
byr2⁻	*byr2*	1.0	3
	STE11	0.33	4
	STE7	$<10^{-5}$	2
	byr1	$<10^{-5}$	2
	None	$<10^{-5}$	2

[a] The *byr2⁻* strain was SPSU.

[b] Plasmids used for transformation of SPSU were pAAAUBYR2, for *byr2* expression; pAAAUSTE11, for *STE11* expression; pART1STE7, for *STE7* expression; and pART1BYR1, for *byr1* expression.

[c] The quantitative *S. pombe* mating assay was based on microscopy as described in MATERIALS AND METHODS. All values are expressed as mating frequency relative to SPSU transformed with pAAAUBYR2, which was normalized to a frequency of 1.0. The frequencies reported are the average over n experiments.

Table 5. Coexpression of *byr2 and byr1* can complement the mating defect of a *S. cerevisiae ste11*$^-$ strain

S. cerevisiae genotype[a]	Expressed gene(s)[b]	Mating frequency[c]	n
ste11$^-$	*STE11*	1.0	4
	byr2	$<10^{-6}$	3
	byr2, byr1	9.0×10^{-3}	5
	byr1	$<10^{-6}$	2
	byr2, STE7	3.0×10^{-4}	3
	STE7	$<10^{-6}$	1
	byr2, FUS3	$<10^{-6}$	1
	byr2, spk1	$<10^{-6}$	1
	None	$<10^{-6}$	2

[a] The *ste11*$^-$ strain was IH1768.

[b] Plasmids used for transformation of IH1768 were pSTE11, for *STE11* expression; pbyr2, for *byr2* expression; pSTE7, for *STE7* expression; pFUS3-2μ-TRP, for *FUS3* expression; and pspk1, for *spk1* expression.

[c] The quantitative *S. cerevisiae* mating assay is described in MATERIALS AND METHODS. The mating tester used was 5815-7-1. All values are expressed as mating frequency relative to IH1768 transformed with pSTE11, which was normalized to a frequency of 1.0. The frequencies reported are the average over n experiments.

The observation of significant suppression of the conjugation defect only upon coexpression suggests a cooperative interaction between byr2 and byr1, a relationship that we will encounter again and discuss below. Overexpression of *STE7* with *byr2* also restored some mating competence, suggesting that these two gene products can also interact, though perhaps not as strongly as byr2 and byr1. The levels of mating observed are still very much lower than wild-type levels. Note that coexpression of *S. pombe spk1* or *S. cerevisiae FUS3* with *byr2* had no effect.

Cross Complementation of STE7 and byr1 Genes

To test the functional relatedness of byr1 and STE7, we transformed a *ste7*$^-$ *S. cerevisiae* strain with various plasmids and measured the ability of the transformants to mate. Expression of *byr1* alone was sufficient to restore mating at least 6×10^3-fold over background levels, although mating was still at least a 100-fold lower than observed in the same strain carrying a plasmid borne *STE7* gene (see Table 6). Expression of *STE11, byr2, spk1,* or *FUS3* alone was without effect. Coexpression of *byr1* and *byr2* or *byr1* and *STE11* did not have any clear additional effect on mating activity, nor did coexpression of *byr1* with either *spk1* or *FUS3*. Also included in Table 6 are results which show that the *S. pombe* byr1, byr2 pair of kinases can restore mating ability to the *S. cerevisiae ste7*$^-$ *ste11*$^-$ double mutant. This experiment demonstrates that the function of the *S. pombe* kinases does not depend on the presence of either of the STE kinases in *S. cerevisiae* and suggests cooperative interaction between byr1 and byr2.

We next examined the ability of plasmids to suppress conjugal defects in *byr1*$^-$ *S. pombe* cells. No suppression was observed consequent to expression of *STE7, STE11* or *byr2* alone (Table 7). However, a dramatic improvement in conjugation, close to wild-type levels was seen when *STE7* was coexpressed with either *STE11* or *byr2*. These results support the notion that *STE7* and *byr1* share activities. The data again indicate a cooperative interaction between STE7 and either byr2 or STE11.

Evidence for Conserved Regulation of the byr and STE Kinases

The above studies do not address whether the *S. pombe* byr kinases are properly regulated when expressed in *S. cerevisiae*. This question can be partly answered by using a reporter system for the activity of the pheromone dependent pathway. Expression of the *FUS1* gene is induced in wild-type *S. cerevisiae* cells upon pheromone stimulation (McCaffrey *et al.*, 1987; Trueheart *et al.*, 1987). By creating a fusion between the promoter of *FUS1* and the β-galactosidase coding sequences, one can

Table 6. Expression of *byr1* can complement the mating defect of a *S. cerevisiae ste7*$^-$ strain

S. cerevisiae genotype[a]	Expressed gene(s)[b]	Mating frequency[c]	n
ste7$^-$	*STE7*	1.0	6
	byr1	6.4×10^{-3}	5
	byr1, byr2	1.8×10^{-2}	4
	byr1, STE11	6.3×10^{-3}	3
	byr1, spk1	1.8×10^{-2}	3
	byr1, FUS3	2.0×10^{-3}	3
	byr2	$<10^{-6}$	2
	STE11	$<10^{-6}$	2
	spk1	$<10^{-6}$	1
	FUS3	$<10^{-6}$	2
	None	$<10^{-6}$	4
ste7$^-$ *ste11*$^-$	*STE7, STE11*	1.0	3
	STE7, byr2	2.7×10^{-3}	3
	byr1, STE11	2.6×10^{-3}	3
	byr1, byr2	2.7×10^{-2}	3
	STE7	$<10^{-6}$	1
	STE11	$<10^{-6}$	1
	None	$<10^{-6}$	3

[a] The *ste7*$^-$ strain was IH1770. The *ste7*$^-$ *ste11*$^-$ strain was AN40-5A.

[b] Plasmids used for transformation were pSTE11, for *STE11* expression; pbyr2, for *byr2* expression; pSTE7, for *STE7* expression; pFUS3-2μ-TRP, for *FUS3* expression; and pspk1, for *spk1* expression.

[c] The quantitative *S. cerevisiae* mating assay is described in MATERIALS AND METHODS. The mating tester used was 5815-7-1. All values are expressed as mating frequency relative to IH1770 transformed with pSTE7 or AN40-5A transformed with pSTE7 and pSTE11, which were normalized to a frequency of 1.0. The frequencies reported are the average over n experiments.

Table 7. Coexpression of *STE7 and STE11* can complement the mating defect of a *S. pombe byr1*$^-$ strain

S. pombe genotype[a]	Expressed gene(s)[b]	Mating frequency[c]	n
byr1$^-$	*byr1*	1.0	2
	STE7	$<10^{-5}$	2
	STE7, STE11	0.19	4
	STE7, byr2	0.14	4
	STE11	$<10^{-5}$	2
	byr2	$<10^{-5}$	2
	None	$<10^{-5}$	2

[a] The *byr1*$^-$ strain was SPBU.

[b] Plasmids used for transformation of SPBU were pART1BYR1, for byr1 expression; pART1STE7, for *STE7* expression; pAAAUSTE11 for *STE11* expression; and pAAAUBYR2, for *byr2* expression.

[c] The quantitative *S. pombe* mating assay was based on microscopy as described in MATERIALS AND METHODS. All values are expressed as mating frequency relative to SPBU transformed with pART1BYR1, which was normalized to a frequency of 1.0. The frequencies are reported as averages over n experiments.

readily assay the responsiveness of the cell exposed to pheromone (McCaffrey *et al.*, 1987; Trueheart *et al.*, 1987). We therefore constructed *S. cerevisiae* strains with the *FUS1-lacZ* gene that carried disruptions of either *STE7* or *STE11*. We transformed these strains with either a control plasmid or with plasmids expressing either *byr1*, *byr2*, or *STE7* and then exposed these cells to pheromone (see Table 8). In an effectively wild-type background, basal levels of β-galactosidase were low but measurable. Upon stimulation, expression increased >200-fold. *ste7*$^-$ or *ste11*$^-$ mutants had unmeasurable basal levels and were not inducible. Introduction of *byr1* into a *ste7*$^-$ background did not measurably increase basal levels but rendered the *FUS1-lacZ* transcription unit responsive to pheromone. Expression of *S. pombe byr2* or *byr1* alone in a *S. cerevisiae ste11*$^-$ strain did not restore inducibility. Expression of *byr2* and *byr1* together restored both basal levels and inducibility in the *ste11*$^-$ strain. Although β-galactosidase levels do not reach the wild-type induced levels, these results indicate that some regulation of this pathway is maintained when the *S. pombe* genes substitute for their *S. cerevisiae* homologs. These results are also consistent with our previous observations on the cooperativity between byr1 and byr2.

As a further test of the conservation of regulation, we introduced a mutation in *byr2* resulting in the substitution of proline for serine at position 209 of the encoded protein. This mutation lies in a region of homology between STE11 and byr2 that is outside the kinase catalytic domain. The analogous mutation in STE11 (proline to serine at position 279) renders this kinase constitutively activated (Stevenson *et al.*, 1992). As shown in Table 10, expression of the mutant *byr2* resulted in somewhat better suppression of the sporulation defects of a *gpa1*$^-$ *S. pombe* strain than did expression of the wild-type *byr2* gene. We also found that *S. pombe* cells expressing *byr2*^{Ser209} agglutinated more readily than cells expressing wild-type *byr2*, and unlike overexpression of the wild-type gene, overexpression of the mutant *byr2* could even induce low-frequency haploid sporulation.

Cross Complementation of spk1 and FUS3

To test the ability of the *S. pombe spk1* and *S. cerevisiae FUS3* kinases to share function, we constructed a *S. cerevisiae* strain lacking both *FUS3* and *KSS1* (AN42-2A). This strain was transformed with various plasmids and the mating activity of the transformants observed. Expression of the *S. pombe* gene *spk1* was nearly as effective as expression of *FUS3* in restoring full conjugal activity (Table 9). Control plasmids, or plasmids expressing *byr1*, *byr2*, *STE7*, or *STE11*, were ineffective. We conclude that spk1 shares function with KSS1 and FUS3 that is unique to this set of kinases.

The *S. cerevisiae FUS3* and *KSS1* genes have overlapping but not completely redundant functions (Elion *et al.*, 1991). Strains which are mutant in *FUS3* but wild-type for *KSS1* are still competent for the transcriptional response to mating pheromone, as measured by *FUS1* induction. *fus3*$^-$ mutants, however, fail to arrest their growth in the presence of mating pheromone. This observation suggests that FUS3 has at least one substrate required for cell-cycle arrest that is not shared with

Table 8. Expression of *byr1* or *byr2* can restore pheromone responsiveness to the *FUS1* promoter

Strain[a]	Expressed genes[b]	Units β-galactosidase[c]	
		Basal	Induced
ste7$^-$	*STE7*	0.2	42
	byr1	<0.05	2.5
	None	<0.05	<0.05
ste11$^-$	*byr2*	<0.05	<0.05
	byr2, byr1	0.5	4
	byr2, STE7	0.1	0.3
	byr1	<0.05	<0.05
	None	<0.05	<0.05

[a] The *ste7*$^-$ strain was AN1012 and the *ste11*$^-$ strain was AN1016.

[b] Plasmids used for transformation of AN1012 were pSTE7 for *STE7* expression, and pbyr1 for *byr1* expression. Plasmids used for transformation of AN1016 were pbyr1, for *byr1* expression; pbyr2, for *byr2* expression and pSTE7, for *STE7* expression.

[c] The β-galactosidase assay is described in MATERIALS AND METHODS. Induced level is the activity 3 h after addition of α-factor for AN1012, and 1.5 h after addition for AN1016. Values reported are averages of three experiments.

Table 9. Expression of *spk1* can complement the mating defect of a *S. cerevisiae fus3⁻ kss1⁻* strain

S. cerevisiae genotype[a]	Expressed genes[b]	Mating frequency[c]	n
fus3⁻ kss1⁻	*FUS3*	1.0	3
	spk1	0.4	3
	byr2	$<10^{-6}$	1
	STE11	$<10^{-6}$	1
	STE7	$<10^{-6}$	1
	None	$<10^{-5}$	3

[a] The *fus3⁻ kss1⁻* strain was AN42-2A.

[b] Plasmids used for transformation of AN42-2A were pSTE11, for *STE11* expression; pbyr2, for *byr2* expression; pSTE7, for *STE7* expression; pFUS3-2μ-TRP, for *FUS3* expression; and pspk1, for *spk1* expression.

[c] The quantitative *S. cerevisiae* mating assay is described in MATERIALS AND METHODS. The mating tester used was 5815-7-1. All values are expressed as mating frequency relative to AN42-2A transformed with pFUS3-2μ-TRP, which was normalized to a frequency of 1.0. The frequencies reported are the average over n experiments.

KSS1. We have tested whether spk1 is more homologous to FUS3 or KSS1 in this regard. A strain lacking *FUS3* was transformed with a plasmid expressing *spk1*, and its ability to arrest growth in response to mating pheromone was monitored by a halo assay (Figure 2). We found that *spk1* is able to restore growth arrest to *fus3⁻* mutants nearly as well as wild-type *FUS3*. These results indicate that spk1 shares a function with FUS3 that it does not share with KSS1 and suggest the possibility that the specific substrate of FUS3 that is involved in cell-cycle arrest might be conserved in *S. pombe*.

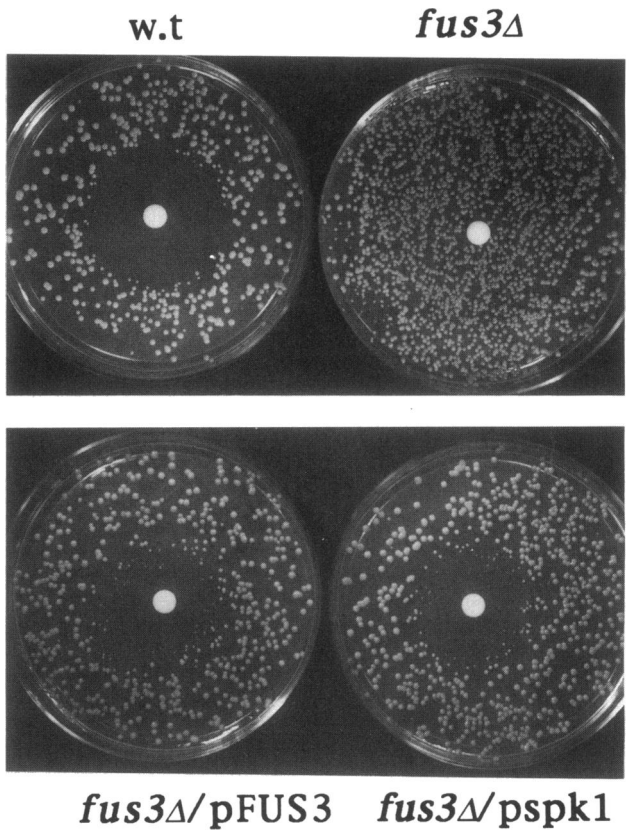

Figure 2. *spk1* complements the *fus3⁻* cell-cycle arrest defect. *fus3⁻* mutants do not arrest in response to α-factor. Expression of either *FUS3* or *spk1* restores arrest to a *fus3⁻* strain, as evidenced by the restoration of a halo around an α-factor treated disk. Strains used are AN37-4C-S (w.t.); AN1015 (*fus3*Δ); AN1015/pFUS3; and AN1015/pspk1.

A Mammalian MAP Kinase, ERK2, Partially Replaces spk1 Function in S. pombe

It was previously reported that *S. pombe* cells disrupted for *spk1* are defective in conjugation (Toda *et al.*, 1991). We have confirmed this and have determined further that *spk1⁻* mutants are also defective in sporulation (Table 10). To test the functional relatedness of a yeast MAP kinase and a structurally related mammalian homolog, we expressed the rat p42^{mapk} encoding gene, *ERK2*, in *S. pombe spk1⁻* haploid and diploid strains. Although *ERK2* did not complement the conjugation defect of the *spk1⁻* haploid strain, it restored sporulation to a substantial degree when expressed in the *spk1⁻* diploid strain (Table 10). We conclude that spk1 and ERK2 are partially conserved in function.

Epistasis Relationships Among the byr1, byr2, gpa1, ras1, and spk1 Genes

To establish the relationships between the byr kinases and the mating pheromone receptor apparatus of *S. pombe*, we tested the ability of high level expression of either *byr* gene to bypass defects in *gpa1⁻* strains. The *gpa1* gene, which is required for conjugation and sporulation, encodes the G_α subunit of a putative hetero-

Table 10. Expression of mammalian *ERK2* can complement the sporulation defect of a *S. pombe spk1⁻* strain

Diploid strain[a]	Expressed gene[b]	Percent sporulation[c] Expt. 1	Expt. 2	Expt. 3
spk1⁻	None	<0.01	<0.01	<0.01
	spk1	81	87	86
	ERK2	8.7	8.2	8.9

[a] The *spk1⁻/spk1⁻* diploid strain used in this experiment was SPKUD.

[b] Plasmids used for transformation of SPKUD were pST23, for *spk1* expression, and pART1ERK2, for *ERK2* expression.

[c] The sporulation assay is described in MATERIALS AND METHODS.

trimeric G protein thought to mediate the signal that pheromone is bound to its receptor (Obara *et al.*, 1991). In *S. pombe*, the pathway leading to meiosis and sporulation is dependent upon pheromone mediated signaling (Leupold *et al.*, 1989; Kitamura and Shimoda, 1991; Obara *et al.*, 1991). The requirement for the integrity of this pathway is less stringent for sporulation than for conjugation. Therefore, we first sought to establish epistasis relationships between *gpa1* and the *byr* genes by monitoring sporulation. We disrupted the *gpa1* gene in the strain SP870 with the *LEU2* marker to yield strain SPGL. SP870 is an h^{90} (homothallic) strain that readily undergoes mating type switching and hence is capable of self-mating and subsequent sporulation. In contrast, SPGL is sterile, due to the loss of *gpa1*. The *gpa1*$^-$/*gpa1*$^-$ diploid strain SPGLD, which is unable to sporulate, was isolated from a culture of SPGL as described in MATERIALS AND METHODS. We transformed SPGLD with plasmids that allowed for overexpression of either *byr1* or *byr2* and monitored sporulation by microscopy. Quantitative results from microscopic examination are shown in Table 11. For comparison, quantitative suppression of a *ras1*$^-$ strain is also shown in Table 11. The sporulation defect of the *gpa1*$^-$ strain is more severe than for the *ras1*$^-$ strain. Both *byr* plasmids, as well as a plasmid expressing the *S. cerevisiae STE11* gene, readily restored sporulation upon transformation into the *gpa1*$^-$ and *ras1*$^-$ strains. We conclude that the *byr1* and *byr2* genes encode products that act downstream from both *ras1*, as previously shown (Nadin-Davis and Nasim, 1988; Wang *et al.*, 1991) and from the putative heterotrimeric G protein that mediates mating pheromone signal transduction in *S. pombe*.

We have previously observed weak suppression of the conjugation defect of a *ras1*$^-$ strain by overexpression of *byr1*, and to a lesser degree, by *byr2* (Xu *et al.*, 1992). Overexpression of *byr1* similarly provided weak suppression of the conjugation defect of the *gpa1*$^-$ strain, just above our limit of detection, thus confirming that byr1 functions downstream of gpa1. Suppression of the conjugation defect of the *gpa1*$^-$ mutant by overexpression of *byr2* was not detectable in our assay.

As already noted, *spk1*$^-$ strains are incapable of either conjugation or sporulation. Overexpression of the *ras1*, *ras1*^{val17}, *gpa1*, *byr2*, *byr2*^{Ser209}, or *byr1* genes did not result in suppression of these phenotypes. This result is consistent with a model in which spk1 acts downstream of ras1, gpa1, and the byr kinases. Expression of *spk1* on a multicopy plasmid was not able to suppress the conjugation and sporulation defects of *byr1*$^-$, *byr2*$^-$, *gpa1*$^-$, or *ras1*$^-$ strains. This result is consistent with the above model if either spk1 is tightly regulated or elements upstream of spk1 have functions that are essential in conjugation and sporulation that are not shared by spk1.

Table 11. Partial suppression of *S. pombe ras1*$^-$ and *gpa1*$^-$ sporulation defects by *S. pombe byr* genes and *S. cerevisiae STE11* gene

Diploid strain[a]	Expressed gene[b]	Sporulation frequency[c]
WT	None	1.0
ras1$^-$	None	3.6×10^{-2}
	byr1	0.38
	byr2	0.37
	STE11	0.67
gpa1$^-$	None	$<3.0 \times 10^{-5}$
	byr1	4.8×10^{-2}
	byr2	4.6×10^{-2}
	byr2^{Ser209}	0.10
	STE11	0.11

[a] The *ras1*$^-$/*ras1*$^-$ diploid strain used in this experiment was SPRUD; the *gpa1*$^-$/*gpa1*$^-$ diploid strain was SPGLD. Wild-type sporulation was determined by transforming SPRUD with the plasmid pALR, which contains a single copy of the wild-type *S. pombe* ras1 gene.

[b] Plasmids used for transformation of SPRUD were pART1BYR1, for byr1 expression; pAAAUBYR2, for *byr2* expression; and pAAAUSTE11, for *STE11* expression. Plasmids used for transformation of SPGLD were pAUCMBYR1, for *byr1* expression; pAIS1, for *byr2* expression; pAIS1^{ser209}, for *byr2*^{Ser209} expression; and pAAAUSTE11, for *STE11* expression.

[c] The sporulation assay is described in MATERIALS AND METHODS. Under these assay conditions, 80–90% of wild-type cells sporulate. All values are expressed as sporulation frequency relative to wild type, which was normalized to a frequency of 1.0, and represent the average of at least two determinations.

DISCUSSION

Conservation of the Kinases

The byr2, byr1, and spk1 kinases of *S. pombe* are structurally related to the STE11, STE7, and FUS3 (and KSS1) kinases of *S. cerevisiae*, respectively (see Table 1). We therefore tested the conservation of their function by cross complementation. Expression of the appropriate *S. cerevisiae* kinase in *S. pombe* mutants restores sporulation and conjugation, and expression of the appropriate *S. pombe* kinases in mutants of *S. cerevisiae* restores conjugation, pheromone-induced growth arrest, and pheromone induction of *FUS1* expression. These studies suggest that homologous pairs of kinases have conserved substrate recognition and perhaps elements of regulation.

It is striking that, unassisted, expression of the *S. pombe byr2* gene failed to complement the *ste11*$^-$ *S. cerevisiae* strain, and that, unassisted, expression of the *S. cerevisiae STE7* gene failed to complement the *byr1*$^-$ *S. pombe* strain. In the former case, multicopy plasmids expressing *byr2* and either *byr1* or *STE7* were sufficient to complement the *ste11*$^-$ mutation; in the latter, multicopy plasmids expressing *STE7* and either *STE11* or *byr2* were sufficient to complement the *byr1*$^-$ *S. pombe* strain. Our interpretation of these results is that the in-

teraction of the byr2/STE11 kinases with the byr1/STE7 kinases is cooperative but that the coupling between byr2 and STE7 may be particularly weak. These relationships can be readily understood if the byr2/STE11 proteins physically interact with the byr1/STE7 proteins. In preliminary and unpublished studies, Printen and Sprague (personal communication) have found that STE7 and STE11 appear to form a complex with each other when tested in a genetic assay for physical association (Fields and Song, 1989).

Data on *FUS1* induction support the idea that some degree of proper regulation of the *S. pombe* byr kinases by the pheromone response pathway occurs in *S. cerevisiae*. Whereas expression of *byr1* in a *S. cerevisiae ste7*$^-$ strain did not detectably increase the basal level of expression from the *FUS1* promoter, a substantial degree of induction occurred in response to mating pheromone. Coexpression of *byr2* and *byr1* in the *S. cerevisiae ste11*$^-$ mutant resulted in similar effects, relatively low levels of *FUS1* basal expression, and a marked induction in response to mating pheromone. These results suggest that the activities of the byr kinases are regulated by upstream elements in the pheromone response pathway of *S. cerevisiae*.

The degree of cross complementation observed between the *S. pombe* byr and *S. cerevisiae* STE kinases was sometimes rather weak, particularly considering that we expressed the heterologous kinases from multicopy plasmids, often from strong promoters. Since we have not quantitated levels of expression of the various kinases in their hosts, and we cannot yet assay their biochemical interactions, the possibility exists that this imperfect complementation is due to poor expression. Alternatively, there may not have been complete conservation of function. This hypothetically diverged function may be in regulation, substrate recognition, or both.

Order of Function of the Kinases

Studies of genetic epistasis relationships in *S. pombe* have suggested that byr1 acts downstream of byr2 and that byr2 acts downstream of ras1 (Wang *et al.*, 1991). In this report we have demonstrated a similar relationship between the byr kinases and gpa1, the G_α homolog required for mating pheromone-induced responses in *S. pombe*. Overexpression of the *byr* genes overcomes the sporulation defects of both *ras1*$^-$ and *gpa1*$^-$ cells. We have placed spk1 downstream of the byr kinases. Strains lacking *spk1*, like strains lacking the *byr* genes, are defective in both conjugation (Toda *et al.*, 1991) and sporulation (this report). Overexpression of *ras1*^{val17}, *byr2*, or *byr1* cannot suppress these defects. From this data, we have tentatively placed spk1 acting downstream of gpa1, ras1, byr2, and byr1. This speculation is strengthened by comparison with *S. cerevisiae* but is by no means proven.

Recent studies have provided evidence for ordering the relationships between the *S. cerevisiae* STE11, STE7, and FUS3/KSS1 protein kinases. Hyperactive alleles of *STE11* have been identified that partially suppress the sterility resulting from deletion of the *STE4* gene (Stevenson *et al.*, 1992), which encodes the G_β subunit of the heterotrimeric G protein (Whiteway *et al.*, 1989). Both STE7 and FUS3 are phosphorylated in response to mating pheromone, and the activated STE11 is capable of inducing phosphorylation of both the STE7 and FUS3 protein kinases in the absence of mating pheromone (Gartner *et al.*, 1992; Stevenson *et al.*, 1992). Phosphorylation of STE7 is dependent on both STE11 and FUS3/KSS1, whereas phosphorylation of FUS3 is dependent on both the STE11 and STE7 kinases (Gartner *et al.*, 1992; Stevenson *et al.*, 1992). Phosphorylation of STE7 was induced by the hyperactive alleles of *STE11* even in the *ste4* deletion mutant (Stevenson *et al.*, 1992). These results suggest a possible order of interaction of these protein kinases: STE11 upon STE7 and FUS3 (or KSS1), and more complex interactions between these kinases are suggested.

The dependence of FUS3/KSS1 phosphorylation on STE7 and the reciprocal dependence of STE7 phosphorylation on FUS3/KSS1 has led to the proposal that these two *S. cerevisiae* kinases are interdependent for activation (Gartner *et al.*, 1992). A similar relationship between the *S. pombe* byr1 and spk1 kinases could account for some of our difficulties in establishing clear epistasis relationships with spk1. In any event, we note that the linear interactions between the kinases depicted in Figure 1 are only the minimal relationships needed to satisfy the genetic data.

Role of ras1 in S. pombe Sexual Differentiation

One of the most striking differences between the sexual differentiation pathways of *S. pombe* and *S. cerevisiae* concerns the role of RAS. The products encoded by the *S. cerevisiae RAS* genes are not required for conjugation in that yeast. On the other hand, *ras1* is required for sexual differentiation in *S. pombe*. It has recently been shown that the *ras1* gene product is required for pheromone-induced transcription of the *S. pombe mat1-Pm* gene, suggesting that ras1 may be involved in transduction of the pheromone response signal (Nielsen *et al.*, 1992). As already noted, both ras1 (Nadin-Davis and Nasim, 1988, 1990; Wang *et al.*, 1991) and gpa1 (this study) function upstream of the byr kinases in *S. pombe*. We have further determined that hypersexual phenotypes resulting from overexpression of the activated *ras1*^{val17} allele in *S. pombe* are blocked by deletion of the *gpa1* gene (Marcus, unpublished observations), suggesting that gpa1 functions either downstream of, or at the same level as, ras1 in the *S. pombe* pheromone response pathway. Additional results (unpublished

data) support this conclusion. The precise relationship between ras1 and gpa1 remains to be determined, but the data presently available suggest that ras1 acts at or near the level of the mating pheromone receptor apparatus.

Speculations on the Generality of our Findings

Given the divergence between *S. pombe* and *S. cerevisiae* in the pheromone-induced signaling pathways at the level of the heterotrimeric G proteins and RAS, it is surprising to find these pathways converge once more at the level of the protein kinases. Our results raise the question of whether STE11, STE7, and FUS3-related kinases act generally as a conserved functional module. It is evident both from the study of the sexual differentiation pathways and the RAS dependent pathways in both organisms that signal transduction pathways do not remain intact throughout evolution. Nevertheless, it is also apparent that certain groupings of signal transduction components do remain conserved. One conserved module comprises RAS and its regulators, the proteins homologous to the *S. cerevisiae* CDC25 protein and the GAP-like proteins. Another comprises the receptors of the serpentine class and the heterotrimeric G proteins they regulate. We propose that the triumvirate of kinases, represented in *S. pombe* by byr2, byr1, and spk1, and in *S. cerevisiae* by STE11, STE7, and FUS3 or KSS1, represent another conserved functional module.

The structural homology of the spk1, FUS3, and KSS1 kinases to the mammalian MAP/ERK kinases has already been noted (Boulton *et al.*, 1990b, 1991; Toda *et al.*, 1991). In this report, we have shown that spk1 and a mammalian MAP kinase, ERK2, are also functionally related. *ERK2* was capable of suppressing only the sporulation defect, but not the conjugation defect, of *S. pombe spk1*$^-$ mutants. Thus the ability of ERK2 to interact with the *S. pombe* sexual response pathway has only been weakly conserved. Suppression of sporulation defects in *S. pombe* appears to be substantially easier than suppression of defects in conjugation (Xu *et al.*, 1992). Suppression of sporulation is, therefore, a highly useful assay for the functional analysis of weakly conserved homologs of *S. pombe* signal transduction factors.

We have recently identified genes encoding structural homologs of byr1/STE7 in higher eukaryotes (Neiman, unpublished observations). Furthermore, other investigators have recently determined, by use of partial protein sequencing analysis, that purified activators of MAP kinase from mouse (Crews and Erikson, 1992) and *Xenopus* (Kosako *et al.*, 1992) are structurally related to byr1 and STE7. If our proposal is correct that the three yeast kinases indeed represent a conserved grouping in eukaryotic signal transduction, then we shall find a structural homolog of STE11/byr2 in vertebrates as the third part of a kinase module that includes the ERK/MAP kinases. Indeed, a protein kinase structurally homologous to STE11/byr2 has been isolated from tobacco (Machida, personal communication), which demonstrates that this kinase family is not limited to yeasts.

Parallels between the RAS dependent signaling pathways of metazoans and *S. pombe* can perhaps be taken one step further (Figure 3). The RAS dependence of the tyrosine kinase mediated pathways in metazoans is now reasonably established (Smith *et al.*, 1986; Deshpande and Kung, 1987; Korn *et al.*, 1987; Feig and Cooper, 1988; Rubin, 1991; Sternberg and Horvitz, 1991). A common mechanism for RAS action in metazoans can thus be anticipated. Investigators have shown that MAP/ERK kinases are activated by factors acting through receptor tyrosine kinases (Ray and Sturgill, 1987, 1988; Hoshi *et al.*, 1988; Boulton *et al.*, 1990a,b, 1991), and this activation is RAS dependent (Thomas, 1992; Thomas *et al.*, 1992; Wood *et al.*, 1992). In *S. pombe*, spk1, the kinase closely related to the MAP/ERK kinases, is likely to be involved in a RAS dependent signal cascade. As discussed above, the mating pheromone receptor apparatus and ras1 potentially operate at or near the same point in the sexual differentiation pathway. All these observations could be unified by the hypothesis that the mating pheromone pathway in *S. pombe* and the tyrosine kinase mediated pathways in metazoans have at least one common biochemical ef-

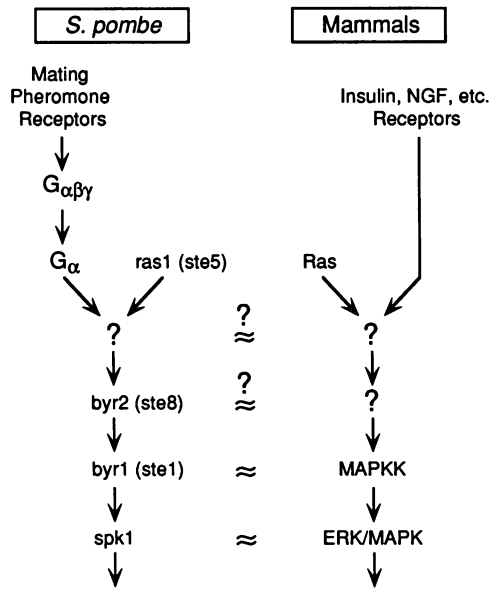

Figure 3. Proposed interactions of signal transducers in a hypothetical conserved eukaryotic signal transduction pathway. Interactions that are not established are denoted by question marks (?), as are hypothetical homologies between signaling molecules. The nature of the relationship between ras1 and the pheromone receptor apparatus in *S. pombe* and between Ras and tyrosine kinase receptors in mammals is, at present, unclear.

fector that is dependent upon RAS and that activates the kinase cascade.

Note added in proof. In an independent study, Styrkársdóttir *et al.* (Mol. Gen. Genet., 1992, *235*, 122–130), have shown that the *S. cerevisiae STE11* gene suppresses the *S. pombe byr2*$^-$ mutation. In addition, Crews *et al.* (Science, 1992, *258*, 478–480) have recently cloned a murine MAPK/ERK activator encoding cDNA and found it to encode a predicted protein kinase with substantial homology to the *S. pombe* byr1 kinase.

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