Signal transduction during mating and meiosis in S. pombe

Nielsen, Susanne Odette Olschansky; Nielsen, Olaf

Published in:
Trends in Cell Biology

Publication date:
1993

Document Version
Early version, also known as pre-print

Citation for published version (APA):
Nielsen, O., & Nielsen, O. (1993). Signal transduction during mating and meiosis in S. pombe. Trends in Cell Biology, 3(2), 60-5.

Signal transduction during mating and meiosis in *S. pombe*

Olaf Nielsen

When starved, the fission yeast Schizosaccharomyces pombe responds by producing mating factors or pheromones that signal to cells of the opposite sex to initiate mating. Like its distant relative Saccharomyces cerevisiae, cells of the two mating types of S. pombe each produce a distinct pheromone that binds to receptors on the opposite cell type to induce the morphological changes required for mating. While the pathways are basically very similar in the two yeasts, pheromone signalling in S. pombe differs in several important ways from that of the more familiar budding yeast. In this article, Olaf Nielsen describes the pheromones and their effects in S. pombe, and compares the signalling pathways of the two yeasts.

Cells communicate with each other through a variety of signalling molecules such as growth factors, hormones and neurotransmitters. The process by which a cell recognizes a particular signalling molecule and responds to it is referred to as signal transduction. In recent years, the two yeast species *S. cerevisiae* and *S. pombe* have proven very useful for unravelling signal transduction processes. These species are unicellular eukaryotes that have evolved a means of sexual differentiation during which two haploid cells, one of each mating type, fuse to produce a diploid cell. Before fusing, the cells communicate through small diffusible peptides called pheromones. The pheromones enable the yeast cell to locate a partner of the opposite sex and initiate a chain of events that ultimately leads to mating. Identification of the components participating in this pathway has been facilitated by the use of genetic analysis to obtain recessive mutants defective in various steps of the response. In the budding yeast *S. cerevisiae*, this signal transduction system has been studied intensively for a number of years and a general picture of the pathway is emerging (see Ref. 1 for a review). In the remotely related fission yeast *S. pombe*, the components of the pheromone communication system are now also being characterized and, although the pathway resembles that of *S. cerevisiae* in many respects (see Table 1), certain features are more similar to signal transduction processes found in higher eukaryotes.

Olaf Nielsen is at the Institute of Genetics, University of Copenhagen, Øster Farimagsgade 2A, DK-1353 Copenhagen K, Denmark.

Haploid versus diploid strategy

The pheromone-response system of *S. pombe* is best explained in the context of the *S. cerevisiae* system. First, however, one has to appreciate the different life cycles of the two yeasts (Fig. 1). *S. cerevisiae* is essentially a diploid organism: newly germinated haploid spores respond immediately to a potential mating partner to form a diploid, and since cells of both **a** and α mating type germinate from the same ascus, mating usually takes place during one of the first mitotic cell cycles. This means that all components of the pheromone communication system in **a** and α cells are expressed during vegetative haploid growth. Once a zygote has been formed, the cell shuts off the communication system. This diploid cell type is the major vegetative form in budding yeast. When a diploid cell is deprived of nutrients it undergoes meiosis and sporulation, thereby completing the sexual differentiation pathway. Therefore, pheromone communication and meiosis occur at distinct stages in the life cycle of a budding yeast.

By contrast, *S. pombe* is essentially a haploid organism (Fig. 1). In this yeast, the mating activities are latent in the germinating spores and their progeny cells. Only at the end of the vegetative phase when the nutrients are limiting do the haploid cells – of mating type *P* (for plus) and *M* (for minus) – begin to communicate by pheromones and subsequently to mate[2–4]. Upon mating, the resulting diploid zygote normally undergoes meiosis and sporulation immediately[5,6]. Therefore, the diploid stage is very transient, and *S. pombe* has not evolved a specific mechanism to turn off the signalling system in diploids. In the laboratory it is possible to create mitotically dividing diploid strains, and they still express the pheromone communication system upon nutritional depletion.

The fact that the pheromone communication system of *S. pombe* is induced only upon starvation restricts the types of experiments that can be conducted in fission yeast compared with budding yeast. For example, if α-factor is added to vegetatively growing haploid **a** cells of *S. cerevisiae*, they will arrest in the G1 phase of the cell cycle in a state competent to mate. This is not possible with *S. pombe*. To make the cells responsive to pheromone, one has to starve them of a nitrogen source and this in itself causes G1 arrest[7]. Furthermore, the identification of components of the signalling system (e.g. purification of the pheromones) is complicated by the fact that they are expressed only in starved, nondividing cells.

Control of sexual differentiation

How does *S. pombe* recognize unfavourable nutritional conditions and respond to them by initiating mating and subsequent meiosis? This sensing process is not particularly well understood. It has been suggested that the sexual differentiation

TABLE 1 – *S. POMBE* GENES INVOLVED IN PHEROMONE SIGNAL TRANSDUCTION AND THEIR *S. CEREVISIAE* HOMOLOGUES

S. pombe			*S. cerevisiae*		
Gene	**Protein function**	**Refs**	**Gene**	**Protein function**	**Refs**
byr1 (=ste1)	Protein kinase	46,50	*STE7*	Protein kinase	41,42
gap1 (=sar1)	ras1 downregulator	39,40	*IRA1, IRA2*	–[a]	
gpa1	G_α subunit	34	*GPA1 (=SCG1)*	G_α subunit	33,51
mam2	*P*-factor receptor	29	*STE2*	α-Factor receptor	30,31
map1	*P* mating-type control	21	–		
map3	*M*-factor receptor	32	*STE3*	a-Factor receptor	31
mat1-Mc	*M* mating-type control	20	–[b]		
mat1-Mm	Control of meiosis	20	–[b]		
mat1-Pc	*P* mating-type control	20	–[b]		
mat1-Pm	Control of meiosis	20	–[b]		
mei3	Inducer of meiosis	22	–		
mfm1	*M*-factor	27	*MFA1*	a-Factor	28
mfm2	*M*-factor	27	*MFA2*	a-Factor	28
pat1 (=ran1)	Kinase, repressor of pathway	15	–		
ral2	ras1 activator	37	–		
ras1 (=ste5)	GTPase	35	*RAS1, RAS2*	–[a]	
spk1	MAP kinase	47	*FUS3/KSS1*	MAP kinase	43
ste8 (=byr2)	Protein kinase	44,45	*STE11*	Protein kinase	41,42
ste6	ras1 activator	38	*CDC25*	–[a]	
ste11	Transcription factor	14	–		
sxa1	*M*-factor protease	24	*BAR1*	α-Factor protease	
sxa2	*P*-factor protease	24	*PRC1*	Carboxypeptidase Y	

[a]The RAS pathway of *S. cerevisiae* is not directly involved in pheromone signal transduction.
[b]The *MAT* genes of *S. cerevisiae* are not homologous to the *S. pombe mat1* genes.

programme is started when nitrogen starvation causes a reduction in the concentration of cAMP[8]. Observations of mutants with altered cAMP metabolism lend support to this notion. Strains that have a lowered level of cAMP are generally derepressed for mating and meiosis[9–11]. On the other hand, in cells in which the cAMP level is elevated the sexual differentiation process is repressed[11,12]. This repression by cAMP is apparently executed at the level of gene expression. Many of the genes required during the sexual differentiation process are transcriptionally induced by nitrogen starvation and this induction can be prevented by the addition of cAMP[8,13,14]. The *ste11* gene encodes a transcription factor that may mediate transcription in response to reduced cAMP[14].

Another key component controlling the processes of mating and meiosis is the *pat1* (or *ran1*) gene, which encodes a 52 kDa protein kinase[15]. Loss of *pat1* function, for example by inactivation of a temperature-sensitive allele, leads to unscheduled sexual differentiation. When the pat1 kinase is partially inactivated, mating begins in rich media[13,16], and if pat1 function is completely lost, the cells attempt to undergo meiosis directly from the haploid stage[16,17]. Sexual differentiation of wild-type cells may be caused by relief of the repression exerted by the pat1 protein kinase[18]. The relationship between pat1-mediated and cAMP-mediated repression of the sexual pathway is unclear. However, hyperactivation of cAMP-dependent protein kinase can compensate for lack of pat1 kinase[12,13], suggesting that the two enzymes share key substrates whose phosphorylation prevents sexual differentiation. Consistent with this idea is the observation that *pat1* inactivation apparently causes induction of those genes in the sexual pathway that can be repressed by cAMP[18].

The mating-type locus

Cells of *P* and *M* mating type differ from each other only in their mating potential, i.e. the two cell types secrete and respond to different pheromones (Fig. 2). The pheromone secreted by *P* cells is called *P*-factor and that produced by *M* cells is called *M*-factor[4,19]. When a cell is exposed to pheromone from the opposite sex it responds by extending a conjugation tube towards the pheromone source. If this elongation leads to cell–cell contact, the cells fuse to form a zygote.

The mating type of a particular cell is determined by the allele it carries at its mating-type

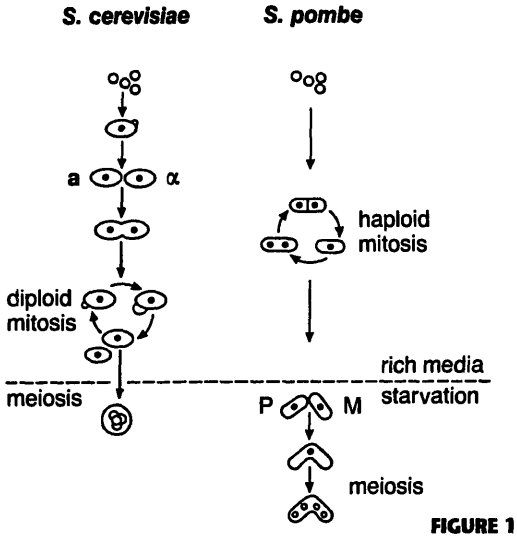

FIGURE 1

Comparison of the life cycles of *S. cerevisiae* and *S. pombe*. In *S. cerevisiae*, mating between a and α cells is induced immediately after spore germination. The resulting diploid cells grow mitotically until they are starved, when they undergo meiosis and produce an ascus containing four spores. In *S. pombe*, the haploid cells grow mitotically as long as nutrients are sufficient. *P* and *M* cells are induced to mate only when starved. The resulting diploid zygote normally enters meiosis and sporulation immediately.

locus, *mat1*. A cell that carries the *mat1-P* allele displays the *P* mating type, whereas a cell with the *mat1-M* allele has the *M* mating type[20]. Each allele of *mat1* contains two genes: *mat1-Pc* and *mat1-Pm* in *P* cells, and *mat1-Mc* and *mat1-Mm* in *M* cells.

The *mat1-Pc* gene together with an unlinked gene, *map1*, controls the mating activities of *P* cells[18,20,21]. Cells carrying mutations in either of these genes are unable to produce the *P*-factor pheromone and cannot respond to *M*-factor. Similarly, the *mat1-Mc* gene controls the ability of *M* cells to produce *M*-factor and respond to *P*-factor[20] (Fig. 2).

The *mat1* locus also controls entry into meiosis[5]. A diploid cell can enter meiosis only if it carries both *mat1-P* and *mat1-M*, which is the case in the newly formed zygote. This is because expression of the *mat1-Pm* and *mat1-Mm* genes in the diploid is required for transcription of *mei3*, a gene whose expression triggers meiosis[22]. Curiously, transcription of *mat1-Pm*, and hence entry into meiosis, requires a pheromone signal[23]. It is not obvious why this cell–cell communication system is also used to control the intracellular decision to enter meiosis. It may provide a means of ensuring that *mat1-Pm* is transcribed only in the presence of potential mating partners. Furthermore, since the *mat1-Pm* gene product is already made in prezygotic *P* cells under the influence of *M*-factor, the zygotes are primed to synthesize the mei3 protein immediately and become committed to meiosis.

Cells defective in the pheromone communication system cannot enter meiosis because they do not express the *mat1-Pm* gene[18]. In diploid *mat1-Pc*$^-$ and *map1*$^-$ mutants, the cells cannot send the *P*-factor signal and do not receive the *M*-factor signal (see Fig. 2). However, the cells can still synthesize *M*-factor and respond to *P*-factor. Hence, a pheromone signal can be generated if the cells are exposed to *P*-factor, and indeed these mutants undergo meiosis when in the vicinity of cells producing *P*-factor[21]. On the other hand, in diploid *mat1-Mc*$^-$ mutant cells, the ability to send the *M*-factor signal and to receive the *P*-factor signal is affected (Fig. 2). These cells can still recognize *M*-factor, and meiosis can be restored if *M*-factor is provided externally (M. Willer and O. Nielsen, unpublished). Hence, *P*-factor and *M*-factor appear to trigger the same intracellular signal in *S. pombe*.

In addition to the *mat1-Pm* gene needed for entry into meiosis, other genes required for mating are expected to be pheromone controlled. Indeed, the *fus1* gene required for cell–cell fusion[3] appears to be regulated by pheromones (D. Weilguny and O. Nielsen, unpublished). Expression of the *sxa2* gene (see below) may also depend on a pheromone signal[24]. Presumably, a general transcription factor that is responsible for induction of pheromone-controlled genes exists in *S. pombe* (corresponding to the *STE12* gene product in budding yeast[25]). Since an ste11-binding site is found upstream of the *mat1-Pm* gene[14], the ste11 transcription factor is probably involved in activation of pheromone-dependent transcription. In addition, the pat1 protein kinase apparently represses this class of genes. This follows from the observation that *mat1-Pm* transcription becomes independent of a pheromone signal when *pat1*^{ts} alleles are inactivated[18].

Pheromones and receptors

P and *M* cells do not produce similar amounts of pheromone: *M*-factor is much more readily detectable than *P*-factor[4]. Indeed, *P*-factor has not yet been purified, and its existence is inferred only from the fact that, from a distance, *P* cells can induce morphological changes in *M* cells and meiosis in diploid *mat1-Pc*$^-$ or *map1*$^-$ mutants (see above). In general, *M* appears to be the most spontaneously expressed mating type in *S. pombe*. A number of mutations in the *ste* genes have been described that abolish sexual differentiation. Most of these mutants no longer produce *P*-factor, whereas *M*-factor production is unaffected[26]. Since these *ste*$^-$ mutants are also unable to receive a pheromone signal, it has been suggested that *P* cells require an *M*-factor pheromone signal in order to produce *P*-factor[26]. In other words, *M* cells initiate cell–cell courtship. Although appealing, it has not been possible to substantiate this idea experimentally.

M-factor is a nanopeptide in which the C-terminal cysteine residue is carboxy-methylated and S-farnesylated[27]. These modifications are also found in the **a**-factor of *S. cerevisiae*[28]. Two structural genes for *M*-factor, *mfm1* and *mfm2*, have been isolated[27]. They are 71% identical in their coding region and have an intron in the same position. These genes are expressed in cells of *M* mating type only under conditions of nutritional starvation. Both encode precursor proteins, with N-terminal extensions of 30 and 32 residues, re-

spectively. The cleavage site between these extensions and the mature *M*-factor (-Lys-Asn/Tyr-) is also similar to that found in **a**-factor precursor. The precursors encoded by *mfm1* and *mfm2* both have C-terminal extensions that contain consensus sequences for farnesylation on cysteine. Although there is little sequence homology between *S. pombe* *M*-factor and *S. cerevisiae* **a**-factor, the overall similarity of their precursors and of their modifications indicates that the enzymes responsible for their processing and secretion might be conserved between the two yeasts.

The *mam2* gene encodes the *P*-factor receptor[29] (Fig. 3). The product of this gene is a hydrophobic protein of 348 amino acids that is homologous to the α-factor receptor encoded by the *S. cerevisiae* *STE2* gene[30,31]. The sequence of the mam2 protein contains seven possible membrane-spanning domains as well as a hydrophilic C-terminal region. These features are characteristic of receptors of the rhodopsin family, to which the budding yeast **a**- and α-factor receptors also belong. The *mam2* gene is expressed only in *M* cells, suggesting that it is positively regulated by the *mat1-Mc* gene product. Since purified *P*-factor is not available, it has not been possible to demonstrate directly that the *mam2* gene product actually binds pheromone. However, the behaviour of *mam2*$^-$ mutants strongly supports this conclusion[29]. *M* strains carrying a deleted *mam2* gene are unable to respond to *P*-factor, and therefore they are sterile. Furthermore, the *P*-factor-induced meiosis in diploid cells that lack *mat1-Pc* or *map1* function (see above) also depends on the *mam2* product. In cells of the *P* mating type, loss of mam2 function has no effect.

The *map3* gene, which has recently been characterized, is likely to encode the *M*-factor receptor[32]. This protein also has seven potential transmembrane domains and other similarities to the budding yeast **a**-factor receptor (the *STE3* gene product[31]). Furthermore, *map3* is expressed only in *P* cells, and disruption of the gene renders the cells unresponsive to *M*-factor. A specific mechanism for attenuation of the pheromone response by internalization of ligand-bound receptors has not been reported. However, mutations in at least two genes, *sxa1* and *sxa2*, cause supersensitivity to *M*-factor and *P*-factor[24], respectively, suggesting that mechanisms of desensitization exist. Both genes encode putative proteases.

Receptors of the rhodopsin family are known to interact with heterotrimeric G proteins, which are composed of G_α, G_β and G_γ subunits. Activation of the receptor by ligand binding leads to replacement of GDP with GTP on the G_α subunit and dissociation of G_α and $G_{\beta\gamma}$ subunits. In most mammalian signal transduction systems, the free G_α subunit is then responsible for activating the downstream signalling pathway. In *S. cerevisiae*, on the other hand, the pheromone signalling is mediated by the free $G_{\beta\gamma}$ subunit[1,33]. This has been inferred from the behaviour of yeast cells carrying mutations in the genes for the various subunits: whereas cells without the $G_{\beta\gamma}$ subunit are unresponsive to pheromone, cells lacking the G_α subunit are permanently activated even in the absence of pheromone. The same heterotrimeric G protein is coupled to the receptors for both **a**-factor and α-factor.

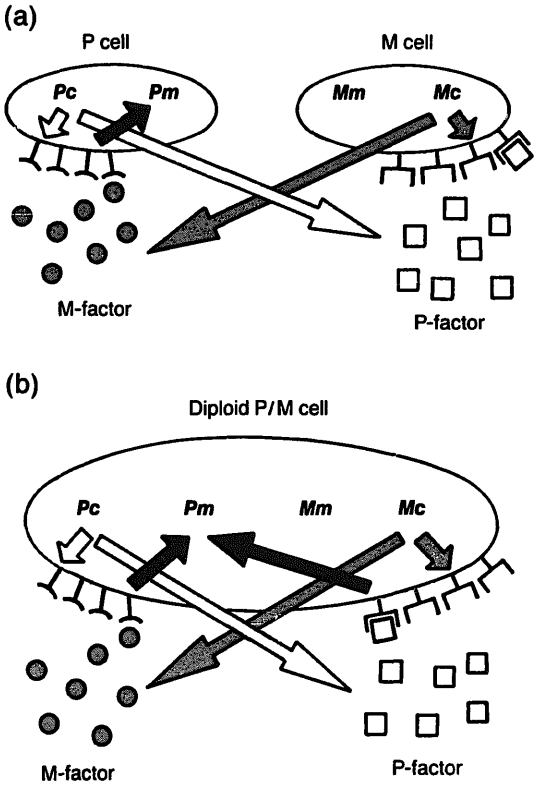

FIGURE 2

Pheromone communication in haploid and diploid cells of *S. pombe*. (a) In *P* cells the *mat1-Pc* gene (together with *map1*) controls production of the *P*-factor pheromone and the synthesis of the *M*-factor receptor. In *M* cells the *mat1-Mc* gene similarly controls production of *M*-factor and the synthesis of *P*-factor receptor. (b) In diploid *P/M* cells the pheromone communication system is still active in both directions. This enables the cell to generate an autonomous pheromone signal, which allows expression of the *mat1-Pm* gene. Expression of *mat1-Pm* together with *mat1-Mm*, which does not require a pheromone signal (M. Willer and O. Nielsen, unpublished), induces expression of *mei3*, which encodes a meiotic activator.

In *S. pombe*, only the G_α subunit of the G protein coupled to the mating factor receptors has been identified[34]. It is homologous to rat $G_{i1\alpha}$ (37% identity) and to *S. cerevisiae* G_α (34% identity). This fission yeast G_α subunit is encoded by the *gpa1* gene. Disruption of *gpa1* confers sterility on both *P* and *M* cells due to their inability to respond to pheromones. Thus, the signal is transduced through the G_α subunit in *S. pombe*, as in mammalian cells. The behaviour of strains carrying the *gpa1*-L244 mutation supports the idea that pheromone–receptor interaction causes replacement of GDP with GTP on the G_α subunit[34]. By analogy with other G proteins, this allele is presumed to encode a G_α protein that is stuck in the activated, GTP-

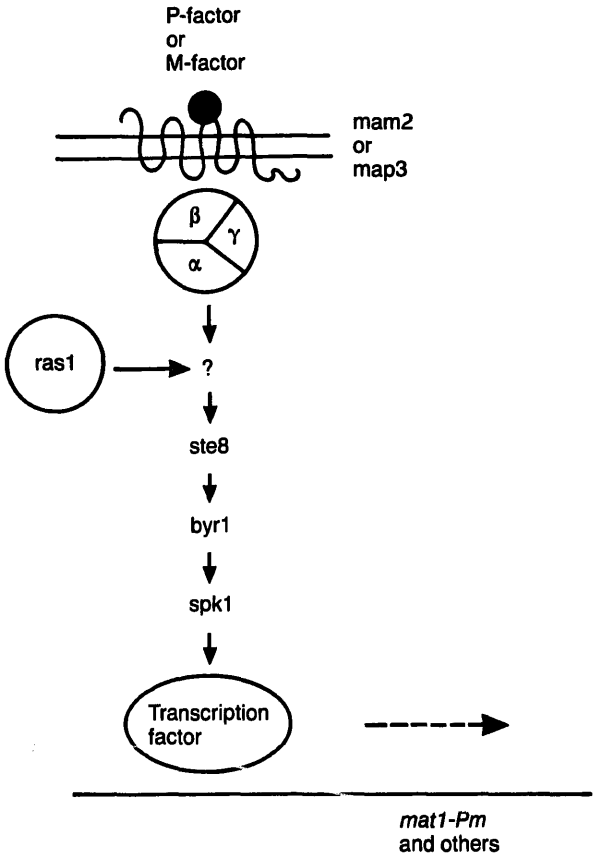

FIGURE 3

The signal transduction pathway in *S. pombe*. The receptors for *P*-factor and *M*-factor are encoded by the *mam2* and *map3* genes, respectively. Both receptors are thought to interact with the same heterotrimeric G protein, of which only the G_α subunit has been described. When the receptor is occupied, the free G_α subunit activates an intracellular effector, the identity of which is currently unknown. Three protein kinases, encoded by the genes *ste8*, *byr1* and *spk1*, function downstream. This pathway ultimately activates a transcription factor that recognizes pheromone-controlled genes such as *mat1-Pm*. The signal transduction system functions only if the ras1 protein has been activated.

bound configuration, unable to form a complex with $G_{\beta\gamma}$. When starved of nitrogen, *gpa1*-L244 mutant cells of both mating types behave as if they are constantly receiving a pheromone signal.

ras1 function

In fission yeast, a single homologue of mammalian *ras* oncogenes, *ras1*, has been identified. Strains with mutated *ras1* are unable to mate[35,36] because they cannot respond morphologically to pheromone[19]. Furthermore, strains of *P* mating type that carry a mutation in *ras1* are unable to induce transcription of the *mat1-Pm* gene in response to *M*-factor[23]. These observations suggest that ras1 is required for pheromone signal transduction.

Members of the Ras protein family are GTPases that are generally believed to act as molecular switches in many signal transduction pathways. According to the model, Ras is inactive in the GDP-bound form, whereas the GTP-bound configuration is active. An incoming signal activates the Ras protein by promoting substitution of GTP for GDP. The GTP-bound form transmits the signal to an effector molecule and then hydrolyses the GTP to return to the basal GDP-bound state. *S. pombe* strains harbouring an activated *ras1*^{val17} allele that is unable to hydrolyse GTP are hypersensitive to mating pheromones and form exaggerated conjugation tubes[35,36]. Also, these cells display a much stronger induction of the *mat1-Pm* gene than normal[23]. However, this response still requires the presence of pheromone, suggesting that activation of ras1 does not substitute for the pheromone signal. Therefore, unlike G_α, the ras1 protein is not a direct conveyor of the pheromone signal (Fig. 3).

How then does the ras1 protein contribute to the signal transduction process? It has been proposed that ras1 activation plays a role in establishing a cellular state competent to respond to pheromone, by monitoring nitrogen limitation[37]. However, the *ras1*^{val17} mutation does not permit pheromone-induced *mat1-Pm* transcription independently of nitrogen starvation[23], indicating that ras1 activation is not the only effect of nitrogen starvation.

The *ste6* gene encodes a homologue of ras activators that catalyse the GDP–GTP exchange[38]. Genetic evidence suggests that the *ral2* gene may code for an additional activator of ras1 (Ref. 37). The product of the *gap1* gene[39] (also referred to as *sar1* (Ref. 40), on the other hand, downregulates ras1 activity by catalysing hydrolysis of ras1-bound GTP, and *gap1*$^-$ mutants, too, are hypersensitive to pheromone. To define the role of ras1 activation in signal transduction, the relative contributions of these various modulators of ras1 activity clearly have to be determined.

Downstream components: a cascade of kinases

What is the target of the activated receptor? As in *S. cerevisiae*, nothing is known about the immediate downstream target of the G protein in *S. pombe*. It is not even clear whether this reaction involves direct protein–protein interactions or the synthesis of a second messenger.

In budding yeast, three consecutive protein kinase activities are involved in the intracellular transmission of the pheromone signal. The first two kinases are encoded by the *STE11* and *STE7* genes, respectively[41,42]. The third kinase function is mediated by the products of the *FUS3* and *KSS1* genes, which are functionally redundant[43].

In *S. pombe*, homologues of all three kinases have been identified (Fig. 3), and *ste8* (the *STE11* homologue) has been demonstrated to be required for signal transduction[44]. Cells carrying mutations in *ste8* cannot induce transcription of the *mat1-Pm* gene in response to pheromone. The budding yeast *STE11* gene can complement mutations in the *ste8* gene of *S. pombe*, demonstrating that this function has been conserved in evolution. The *ste8* gene belongs to the class of genes that, when mutated,

cause defects in both mating and meiosis. The meiotic block caused by mutations in *ste8* can be bypassed by artificial expression of the *mat1-Pm* gene[44]. This observation demonstrates that *ste8*$^-$ mutants are defective in meiosis because they cannot induce *mat1-Pm* in response to pheromone.

The fission yeast *byr1* gene is the homologue of *STE7* in *S. cerevisiae*[1]. The fact that *byr1*$^-$ mutants do not form conjugation tubes when exposed to pheromone[26] is consistent with the involvement of byr1 in the pheromone response. Overexpression of *byr1* can overcome the meiotic defect of *ste8*$^-$ mutant strains, whereas overexpression of *ste8* does not rescue meiosis in *byr1*$^-$ diploids[45], indicating that ste8 functions upstream of byr1 like the situation with their *S. cerevisiae* counterparts. Overexpression of *ste8* (Ref. 45) and of *byr1* (Ref. 46) can suppress the meiotic defect of *ras1*$^-$ strains, suggesting that these kinases function downstream of ras1 in the pathway. Finally, the *spk1* gene encodes a *KSS1/FUS3* homologue[47]. Cells in which the *spk1* gene is disrupted are sterile. However, it is not clear where in the pathway spk1 functions relative to ste8 and byr1.

Recent findings indicate that the protein kinase cascade involved in the pheromone response in yeasts may be closely related to signal transduction pathways in higher eukaryotes. The MAP kinases, a family of highly conserved protein kinases are involved in growth-factor-stimulated cell proliferation[48]. Interestingly, these proteins are very homologous to the budding yeast FUS3/KSS1 kinases and the fission yeast spk1 kinase. Furthermore, an activator of mammalian MAP kinase is a protein kinase that is similar to *S. pombe* byr1 (Ref. 49). Hence, this array of protein kinases in signal transduction may be widely conserved among eukaryotes.

References

1 MARSH, L., NEIMAN, A. M. and HERSKOWITZ, I. (1991) *Annu. Rev. Cell Biol.* 7, 699–728
2 EGEL, R., WEILGUNY, D. and NIELSEN, O. (1990) *Trends Genet.* 6, 369–373
3 BRESCH, C., MÜLLER, G. and EGEL, R. (1968) *Mol. Gen. Genet.* 102, 301–306
4 LEUPOLD, U. (1987) *Curr. Genet.* 12, 543–545
5 EGEL, R. (1973) *Mol. Gen. Genet.* 121, 277–284
6 McLEOD, M. (1989) *BioEssays* 11, 9–14
7 NURSE, P. and BISSETT, Y. (1981) *Nature* 292, 558–560
8 WATANABE, Y., IINO, Y., FURUHATA, K., SHIMODA, C. and YAMAMOTO, M. (1988) *EMBO J.* 7, 761–767
9 MAEDA, T., MOCHIZUKI, N. and YAMAMOTO, M. (1990) *Proc. Natl Acad. Sci. USA* 87, 7814–7818
10 KAWAMUKAI, M., FERGUSON, K., WIGLER, M. and YOUNG, D. (1991) *Cell Regul.* 2, 155–164
11 MOCHIZUKI, N. and YAMAMOTO, M. (1992) *Mol. Gen. Genet.* 233, 17–24
12 DeVOTI, J., SEYDOUX, G., BEACH, D. and McLEOD, M. (1991) *EMBO J.* 10, 3759–3768
13 BEACH, D., RODGERS, L. and GOULD, J. (1985) *Curr. Genet.* 10, 297–311
14 SUGIMOTO, A., IINO, Y., MAEDA, T., WATANABE, Y. and YAMAMOTO, M. (1991) *Genes Dev.* 5, 1990–1999
15 McLEOD, M. and BEACH, D. (1988) *Nature* 332, 509–514
16 NURSE, P. (1985) *Mol. Gen. Genet.* 198, 497–502
17 IINO, Y. and YAMAMOTO, M. (1985) *Mol. Gen. Genet.* 198, 416–421
18 NIELSEN, O. and EGEL, R. (1990) *EMBO J.* 9, 1401–1406
19 FUKUI, Y., KAZIRO, Y. and YAMAMOTO, M. (1986) *EMBO J.* 5, 1991–1993
20 KELLY, M., BURKE, J., SMITH, M., KLAR, A. and BEACH, D. (1988) *EMBO J.* 7, 1537–1547
21 LEUPOLD, U., NIELSEN, O. and EGEL, R. (1989) *Curr. Genet.* 15, 403–405
22 McLEOD, M., STEIN, M. and BEACH, D. (1987) *EMBO J.* 6, 729–736
23 NIELSEN, O., DAVEY, J. and EGEL, R. (1992) *EMBO J.* 11, 1391–1395
24 IMAI, Y. and YAMAMOTO, M. (1992) *Mol. Cell. Biol.* 12, 1827–1834
25 DOLAN, J. W. and FIELDS, S. (1990) *Genes Dev.* 4, 492–502
26 LEUPOLD, U., SIPICZKI, M. and EGEL, R. (1991) *Curr. Genet.* 20, 79–85
27 DAVEY, J. (1992) *EMBO J.* 11, 951–960
28 MICHAELIS, S. and HERSKOWITZ, I. (1988) *Mol. Cell. Biol.* 8, 1309–1328
29 KITAMURA, K. and SHIMODA, C. (1991) *EMBO J.* 10, 3743–3751
30 BURKHOLDER, A. C. and HARTWELL, L. C. (1985) *Nucleic Acids Res.* 13, 8463–8475
31 NAKAYAMA, N., MIYAJIMA, A. and ARAI, K. (1985) *EMBO J.* 4, 2643–2648
32 TANAKA, K., DAVEY, J., IMAI, Y. and YAMAMOTO, M. *Mol. Cell. Biol.* (in press)
33 MIYAJIMA, I. *et al.* (1987) *Cell* 50, 1011–1019
34 OBARA, T., NAKAFUKU, M., YAMAMOTO, M. and KAZIRO, Y. (1991) *Proc. Natl Acad. Sci. USA* 88, 5877–5881
35 FUKUI, Y., KOZASA, T., KAZIRO, Y., TAKEDA, T. and YAMAMOTO, M. (1986) *Cell* 44, 329–336
36 NADIN-DAVIS, S. A., NASIM, A. and BEACH, D. (1986) *EMBO J.* 5, 2963–2971
37 FUKUI, Y., MIYAKE, S., SATOH, M. and YAMAMOTO, M. (1989) *Mol. Cell. Biol.* 9, 5617–5622
38 HUGHES, D. A., FUKUI, Y. and YAMAMOTO, M. (1990) *Nature* 344, 355–357
39 IMAI, Y., MIYAKE, S., HUGHES, D. A. and YAMAMOTO, M. (1991) *Mol. Cell. Biol.* 11, 3088–3094
40 WANG, Y., BOGUSKI, M., RIGGS, M., RODGERS, L. and WIGLER, M. (1991) *Cell Regul.* 2, 453–465
41 STEVENSON, B. J., RHODES, N., ERREDE, B. and SPRAGUE, G. F., JR (1992) *Genes Dev.* 6, 1293–1304
42 CARINS, B. R., RAMER, S. W. and KORNBERG, R. D. (1992) *Genes Dev.* 6, 1305–1318
43 GARTNER, A., NASMYTH, K. and AMMERER, G. (1992) *Genes Dev.* 6, 1280–1292
44 STYRKARSDOTTIR, U., EGEL, R. and NIELSEN, O. (1992) *Mol. Gen. Genet.* 235, 122–130
45 WANG, Y., XU, H-P., RIGGS, M., RODGERS, L. and WIGLER, M. (1991) *Mol. Cell. Biol.* 11, 3554–3563
46 NADIN-DAVIS, S. A. and NASIM, A. (1988) *EMBO J.* 7, 985–993
47 TODA, T., SHIMANUKI, M. and YANAGIDA, M. (1991) *Genes Dev.* 5, 60–73
48 PELECH, S. L. and SANGHERA, J. S. (1992) *Science* 257, 1355–1356
49 CREWS, C. M., ALESSANDRINI, A. and ERIKSON, R. L. (1992) *Science* 258, 478–480
50 NADIN-DAVIS, S. A. and NASIM, A. (1990) *Mol. Cell. Biol.* 10, 549–560
51 DIETZEL, C. and KURJAN, J. (1987) *Cell* 50, 1001–1010

Acknowledgements

I thank present and former members of the fission yeast group at the Institute of Genetics in Copenhagen for stimulating discussions. I am grateful to Richard Egel for comments on the manuscript and to Martin Willer for preparing the figures. Work in our laboratory is supported by the Danish Center for Microbiology.