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

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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.

**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 α 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 α 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. Upon mating, the resulting diploid zygote normally undergoes meiosis and sporulation immediately. 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 α 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. 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
programme is started when nitrogen starvation causes a reduction in the concentration of cAMP. 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. On the other hand, in cells in which the cAMP level is elevated the sexual differentiation process is repressed. 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. The ste11 gene encodes a transcription factor that may mediate transcription in response to reduced cAMP.

Another key component controlling the processes of mating and meiosis is the pat1 (or ran1) gene, which encodes a 52 kDa protein kinase. 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 and if pat1 function is completely lost, the cells attempt to undergo meiosis directly from the haploid stage. Sexual differentiation of wild-type cells may be caused by relief of the repression exerted by the pat1 protein kinase. 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, 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.

### 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. 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 locus.
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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.

The mat1-Pc gene together with an unlinked gene, map1, controls the mating activities of P cells18,20,21. 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-factor20 (Fig. 2).

The mat1 locus also controls entry into meiosis5. 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 meiosis52. Curiously, transcription of mat1-Pm, and hence entry into meiosis, requires a pheromone signal53. 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 gene18. 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-factor21. 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. Wilier 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 fusion3 appears to be regulated by pheromones (D. Weilguny and O. Nielsen, unpublished). Expression of the ssa2 gene (see below) may also depend on a pheromone signal24. Presumably, a general transcription factor is responsible for induction of pheromone-controlled genes exists in S. pombe (corresponding to the STE12 gene product in budding yeast25). Since an ste11-binding site is found upstream of the mat1-Pm gene14, 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 pat15 alleles are inactivated18.

Pheromones and receptors

P and M cells do not produce similar amounts of pheromone: M-factor is much more readily detectable than P-factor4. 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 unaffected26. 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-factor29. 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 nanopetide in which the C-terminal cysteine residue is carboxy-methylated and S-farnesylated27. These modifications are also found in the a-factor of S. cerevisiae28. Two structural genes for M-factor, mfm1 and mfm2, have been isolated27. 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-
The precursors encoded by M-factor and S. pombe M-factor and S. cerevisiae α-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 receptor29 (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 gene30,31. 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 α- 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 conclusion29. M strains carrying a deleted mam2 gene are unable to respond to P-fac- tor, 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 charac- terized, is likely to encode the M-factor receptor32. This protein also has seven potential transmem- brane domains and other similarities to the budding yeast α-factor receptor (the STE3 gene product31). 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 internal- ization 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-factor24, respectively, suggesting that mech- anisms 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 Gα, Gβ and Gγ subunits. Activation of the receptor by ligand binding leads to replacement of GDP with GTP on the Gα subunit and dissociation of Gβ and Gγ subunits. In most mammalian signal transduction systems, the free Gα 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 Gβγ subunit13,33. This has been inferred from the behaviour of yeast cells carrying mutations in the genes for the various subunits: whereas cells without the Gβγ subunit are un- responsive to pheromone, cells lacking the Gα subunit are permanently activated even in the absence of pheromone. The same heterotrimeric G protein is coupled to the receptors for both α-factor and α-factor.

In S. pombe, only the Gα subunit of the G protein coupled to the mating factor receptors has been identified34. It is homologous to rat G11α (37% identity) and to S. cerevisiae Gα (34% identity). This fission yeast Gα 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 Gα subunit in S. pombe, as in mammalian cells. The behaviour of strains carrying the gpa1-1244 mutation supports the idea that pheromone-receptor interaction causes replacement of GTP with GDP on the Gα subunit34. By analogy with other G proteins, this allele is presumed to encode a Gα protein that is stuck in the activated, GTP-

![FIGURE 2](image-url)

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.
that are generally believed to act as molecular transduction.

because they cannot respond morphologically 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. However, this response still requires the presence of pheromone, suggesting that activation of ras1 does not substitute for the pheromone signal. Therefore, unlike \(G_a\), 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 ras\(^{val7}\) 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 ras2 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 mitotic block caused by mutations in ste8 can be bypassed by artificial expression of the mat1-Pm gene44. 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. The fact that byr1 mutants do not form conjugation tubes when exposed to pheromone26 is consistent with the involvement of byr1 in the fission yeast response. Overexpression of byr1 can overcome the mitotic defect of ste8 mutant strains, whereas overexpression of ste8 does not rescue meiosis in byr1 diplo. 15, 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 mitotic defect of ras1 strains, suggesting that these kinases function downstream of ras1 in the pathway. Finally, the spkl gene encodes a KSS1/FUS3 homologue47. Cells in which this spkl gene is disrupted are sterile. However, it is not clear where in the pathway spkl functions relative to ste8 and byr1.

Recent findings indicate that the protein kinase cascade involved in the fission yeast response in yeast mutants 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 proliferation18. Interestingly, these proteins are very homologous to the budding yeast FUS3/KSS1 kinases and the fission yeast spkl kinase. Furthermore, an activator of mammalian MAP kinase is a protein kinase that is similar to spkl (Ref. 49). Hence, this array of protein kinases in signal transduction may be widely conserved among eukaryotes.

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