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Published in:
Trends in Cell Biology

Publication date:
1993

Document version
Early version, also known as pre-print

Citation for published version (APA):
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.

**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 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 mate2-4. Upon mating, the resulting diploid zygote normally undergoes meiosis and sporulation immediately5,6. 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 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 arrest7. 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein function</th>
<th>Refs</th>
<th>Gene</th>
<th>Protein function</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>byr1 (=steD)</td>
<td>Protein kinase</td>
<td>46,50</td>
<td>STE7</td>
<td>Protein kinase</td>
<td>41,42</td>
</tr>
<tr>
<td>gap1 (=sar1)</td>
<td>ras1 downregulator</td>
<td>39,40</td>
<td>IRA1, IRA2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>gap1</td>
<td>Gα subunit</td>
<td>34</td>
<td>GPA1 (=SCG1)</td>
<td>Gα subunit</td>
<td>33,51</td>
</tr>
<tr>
<td>mam2</td>
<td>P-factor receptor</td>
<td>29</td>
<td>STE2</td>
<td>α-factor receptor</td>
<td>30,31</td>
</tr>
<tr>
<td>map1</td>
<td>P mating-type control</td>
<td>21</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>map3</td>
<td>M-factor receptor</td>
<td>32</td>
<td>STE3</td>
<td>a-Factor receptor</td>
<td>31</td>
</tr>
<tr>
<td>mat1-Mc</td>
<td>M mating-type control</td>
<td>20</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>mat1-Mm</td>
<td>Control of meiosis</td>
<td>20</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>mat1-Pc</td>
<td>P mating-type control</td>
<td>20</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>mat1-Pm</td>
<td>Control of meiosis</td>
<td>20</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>met3</td>
<td>Inducer of meiosis</td>
<td>22</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>mm1</td>
<td>M-factor</td>
<td>27</td>
<td>MFA1</td>
<td>a-Factor</td>
<td>28</td>
</tr>
<tr>
<td>mm2</td>
<td>M-factor</td>
<td>27</td>
<td>MFA2</td>
<td>a-Factor</td>
<td>28</td>
</tr>
<tr>
<td>pat1 (=ran1)</td>
<td>Kinase, repressor of pathway</td>
<td>15</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>rap2</td>
<td>ras1 activator</td>
<td>3/7</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ras1 (=ste5)</td>
<td>GTPase</td>
<td>35</td>
<td>RAS1, RAS2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>sph1</td>
<td>MAP kinase</td>
<td>47</td>
<td>FUS3/KSS1</td>
<td>MAP kinase</td>
<td>43</td>
</tr>
<tr>
<td>ste8 (=byr2)</td>
<td>Protein kinase</td>
<td>44,45</td>
<td>STE11</td>
<td>Protein kinase</td>
<td>41,42</td>
</tr>
<tr>
<td>ste6</td>
<td>ras1 activator</td>
<td>38</td>
<td>CDC25</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ste11</td>
<td>Transcription factor</td>
<td>14</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>sxα1</td>
<td>M-factor protease</td>
<td>24</td>
<td>BAR1</td>
<td>α-Factor protease</td>
<td>–</td>
</tr>
<tr>
<td>sxα2</td>
<td>P-factor protease</td>
<td>24</td>
<td>PRC1</td>
<td>Carboxypeptidase Y</td>
<td>–</td>
</tr>
</tbody>
</table>

*The RAS pathway of S. cerevisiae is not directly involved in pheromone signal transduction.
*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. 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.
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. 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. 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 (Fig. 2).

The mat1 locus also controls entry into meiosis. 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. Curiously, transcription of mat1-Pm, and hence entry into meiosis, requires a pheromone signal. It is not obvious why this cell-to-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. 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. 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. These cells can still recognize M-factor, and meiosis is 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 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. 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 yeast). Since an ste11-binding site is found upstream of the mat1-Pm gene, 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 alleles are inactivated.

Pheromones and receptors

P and M cells do not produce similar amounts of pheromone: M-factor is much more readily detectable than P-factor. 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. 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. 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. Two structural genes for M-factor, mfn1 and mfn2, have been isolated. They are 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-

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 spor germination. The resulting diploid cells grow mitotically until they are starved, when they undergo meiosis and produce an asc 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 precursors encoded by M-factor and between the two yeasts. There is little sequence homology between sequences for farnesylation on cysteine. Although have C-terminal extensions that contain consensus similarities 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 (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. 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 conclusion. 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. This protein also has seven potential transmembrane domains and other similarities to the budding yeast α-factor receptor (the STE3 gene product). 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, 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 Ga, Gβ, and Gγ subunits. Activation of the receptor by ligand binding leads to replacement of GDP with GTP on the Ga subunit and dissociation of Ga and Gβγ subunits. In most mammalian signal transduction systems, the free Ga 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 subunit. 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 unresponsive to pheromone, cells lacking the Ga 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 identified. It is homologous to rat Ga1 (37% identity) and to S. cerevisiae Gaα (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-I244 mutation supports the idea that pheromone-receptor interaction causes replacement of GDP with GTP on the Gα subunit. By analogy with other G proteins, this allele is presumed to encode a Gα protein that is stuck in the activated, GTP-
bound configuration, unable to form a complex with \( G_\alpha \). 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\(^{39} \). 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 \( ras1^{ma17} \) 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 \( G_\alpha \), 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\(^{23} \). However, the \( ras1^{ma17} \) 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 \( gap1^{11} \) 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. 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 pheromone is consistent with the involvement of byr1 in the pheromone response. Overexpression of byr1 can overcome the meiotic defects of ste8 mutant strains, whereas overexpression of ste8 does not rescue meiosis in byr1 diploids, 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 defects of ras1 strains, suggesting that these kinases function downstream of ras1 in the pathway. Furthermore, the spkl gene encodes a KSS1/FUS3 homologue. Cells in which the 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 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. Interestingly, these proteins are very homologous to the budding yeast FUS3/KSS1 kinases involved in growth-factor-stimulated cell proliferation. The MAP kinases, a family of highly conserved protein kinases are involved in signal transduction pathways in higher eukaryotes. The MAP kinases, a family of highly conserved protein kinases are involved in signal transduction pathways in higher eukaryotes.

References


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.