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Two-Step Activation of Meiosis by the *mat1* Locus in *Schizosaccharomyces pombe*

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The *mat1* locus is a key regulator of both conjugation and meiosis in the fission yeast *Schizosaccharomyces pombe*. Two alternative DNA segments of this locus, *mat1-P* and *mat1-M*, specify the haploid cell types (*Plus* and *Minus*). Each segment includes two genes: *mat1-P* includes *mat1-Pc* and *mat1-Pm*, while *mat1-M* includes *mat1-Mc* and *mat1-Mm*. The *mat1-Pc* and *mat1-Mc* genes are responsible for establishing the pheromone communication system that mediates conjugation between *P* and *M* cells, while all four *mat1* genes are required for meiosis in diploid *P*/*M* cells. Our understanding of the initiation of meiosis is based largely on indirect observations, and a more precise investigation of these events was required to define the interaction between the *mat1* genes. Here we resolve this issue using synthetic pheromones and *P*/*M* strains with mutations in either *mat1-Pc* or *mat1-Mc*. Our results suggest a model in which the *mat1* locus plays two roles in controlling meiosis. In the first instance, the *mat1-Pc* and *mat1-Mc* functions are required to produce the mating pheromones and receptors that allow the generation of a pheromone signal. This signal is required to induce the expression of *mat1-Pm* and *mat1-Mm*. This appears to be the major pheromone-dependent step in controlling meiosis since ectopic expression of these genes allows meiosis in the absence of *mat1-Pc* and *mat1-Mc*. The *mat1-Pm* and *mat1-Mm* products complete the initiation of meiosis by activating transcription of the *mei3* gene.

The fission yeast *Schizosaccharomyces pombe* is capable of undergoing sexual differentiation in response to nutritional starvation. In this process, the haploid cells first become diploid by mating and subsequently the diploid cells enter meiosis and sporulation (2, 3, 5, 23). Haploid cells of *S. pombe* exist in one of two mating types, either *Plus* (*P*) or *Minus* (*M*). The mating type of a particular cell is determined by which of two alternative DNA segments is carried at the *mat1* locus (1). The *mat1-P* segment contains the *mat1-Pc* gene that specifies the *P* mating type, while the *mat1-Mc* gene of the *mat1-M* segment specifies the *M* mating type (17). These genes mediate conjugation by controlling the production of mating pheromones and their receptors. *P* cells produce P-factor, an unmodified peptide of 23 amino acids (16), and respond to M-factor by expressing Map3, the M-factor receptor (44). *M* cells express the P-factor receptor (Mam2) (18) and release M-factor, a nanopeptide in which the C-terminal cysteine is carboxy methylated and farnesylated (4). The receptors are seven-span membrane proteins that couple to a heterotrimeric G protein, and pheromone stimulation leads to activation of a signalling pathway that is homologous to the mitogen-activated protein kinase pathway in mammalian cells (35, 36).

The *mat1* locus also controls entry into meiosis. This process is restricted to diploid cells carrying both *mat1-P* and *mat1-M* (*P*/*M* cells). Unlike conjugation, however, which requires only *mat1-Pc* and *mat1-Mc*, meiosis also requires expression of the *mat1-Pm* and *mat1-Mm* genes (sometimes referred to as *mat1-Pi* and *mat1-Mi*) (6, 10, 17, 31). All four gene products are present in a diploid *P*/*M* cell following starvation, and this allows expression of the *mei3* gene, which encodes a direct inducer of meiosis (30). In contrast, *mat1-Pm* and *mat1-Mm* are not expressed in diploid *P*/*M* strains during vegetative growth (17).

Several lines of indirect evidence suggest that entry into meiosis—like conjugation—requires a pheromone signal in *S. pombe*. Mutation of the pheromone response pathway, for example, prevents meiosis in *P*/*M* cells (12, 26, 32, 39), and the meiotic defect of *mat1-Pc*$^-$ and *mat1-Mc*$^-$ mutants is also consistent with this idea. Diploid strains with these mutations express only one of the two mating activities, and so, while a normal *P*/*M* strain produces both pheromones and both receptors, these mutants produce only one pheromone and only one, the incompatible, receptor. Mutants lacking *mat1-Pc* produce M-factor and the P-factor receptor, while disruption of *mat1-Mc* produces a strain that expresses the M-factor receptor but releases P-factor. Strains lacking *map1* have a phenotype similar to that of the *mat1-Pc* mutants (7).

The meiotic defect of the various mating type-specific mutants can be overcome by treatments that are expected to restore a pheromone signal. The *P*-specific mutants, for example, are rescued by exposure to a diffusible substance released by wild-type *P* cells (7, 25, 31). This substance is probably P-factor, since it requires P-factor receptor for activity (18). These mutants are also rescued by constitutive expression of the M-factor receptor, presumably via stimulation by the endogenously produced M-factor (44).

The simplest explanation for these observations is that entry into meiosis requires pheromone signalling and that signalling by either M-factor or P-factor is sufficient for this initiation. Unfortunately, the indirect nature of the current evidence does not preclude other explanations and a more direct demonstration of the link between signalling and meiosis is required. Furthermore, it is necessary to define what role(s) pheromone signalling plays in controlling entry into meiosis. At least one of the meiotic genes, *mat1-Pm*, is known to be pheromone de-

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TABLE 1. *S. pombe* strains used

Strain	Genotype	Source or reference
EG282	h^{90}	23
EG397	h^{90} *pat1-114*	38
EG410	h^{90} *mat1-Pc* JM161	38[a]
EG422	*mat1-M Δmat2,3::LEU2 pat1-114*	This study
EG432	*mat1-P Δmat2,3::LEU2 leu1 ura4-D18*	37
EG443-2n	h^{90} *mat1-Pm B102 pat1-114* (diploid)	This study[b]
EG444-2n	h^{90} *mat1-Mm* B406 *pat1-114* (diploid)	This study[c]
EG530	*mat1-M Δmat2,3::LEU2 ade6-M26 ura4-D18*	This study
EG571	*mat1-M int-H1::ura4*$^+$ *Δmat2,3::LEU2 leu1-32 ura4-D18 ade6-M26*	This study
EG572	*mat1-M Δmat2,3::LEU2 ura4-D18*	This study
EG575	*mat1-Mc*^{opal7} *int-H1::ura4*$^+$ *Δmat2,3::LEU2 leu1-32 ura4-D18 ade6-M26*	This study
EG586	*mat1-M Δmat2,3::LEU2 pat1-114 ade6 ura4-D18*	This study
EG587	*mat1-M Δmat2,3::LEU2 pat1-114 ura4-D18*	This study
EG590	*mat1-Mc*^{opal7} *int-H1::ura4*$^+$ *Δmat2,3::LEU2 pat1-114 ura4-D18 ade6*	This study
EG591	*mat1-Pc*^{opal5} *Δmat2,3::LEU2 pat1-114 ura4-D18*	This study
EG665	EG590 × EG591	This study
EG670	EG432 × EG575	This study
EG699	*mat1-M Δmat2,3::LEU2 sxa2*	19
EG767-2n	*mat1-Pc JM161 Δmat2,3::leu2*$^-$ *leu1 ura4-D18* (diploid)	This study[a,d]
EG811	*mat1-M Δmat2,3::LEU2 sxa2 ura4-D18*	This study
EG812	*mat1-M int-H1::ura4*$^+$ *sxa2 ade6*	This study
EG817	*mat1-Pc*^{opal5} *Δmat2,3::LEU2 ura4-D18*	This study
EG818	*mat1-Pc*^{opal5} *Δmat2,3::LEU2 sxa2 ura4-D18*	This study
EG819	*EG817 × EG571*	This study
EG820	*EG818 × EG812*	This study

[a] The JM161 mutant is described in reference 31.
[b] The B102 mutant is described in reference 6.
[c] The B406 mutant is described in reference 10.
[d] The *Δmat2,3::leu2*$^-$ mutant is described in reference 20. All other mutants are referred to in the text.

pendent (37), but the comparable study with *mat1-Mm* has not been performed, and the pheromone dependence of other meiotic events has not been determined.

We have now resolved these issues using synthetic pheromones and mitotically stable *P/M* strains that carry mutations in either *mat1-Pc* or *mat1-Mc*. We show directly that meiosis is pheromone dependent, as the addition of synthetic P-factor induces meiosis in the *mat1-Pc*$^-$ strain while M-factor induces meiosis in the *mat1-Mc*$^-$ strain. We also demonstrate that expression of the *mat1-Mm* gene, like that of *mat1-Pm* (37), is pheromone dependent. Furthermore, the induction of these two genes appears to be the major pheromone-dependent step in control of meiosis, since the simultaneous ectopic expression of *mat1-Pm* and *mat1-Mm* allows cells to undergo meiosis independently of *mat1-Pc* and *mat1-Mc*. Our results confirm a model in which the *mat1* locus plays two roles in controlling entry into meiosis. In the first instance, the *mat1-Pc* and *mat1-Mc* functions control the production of mating pheromones and receptors and mediate the generation of a pheromone signal. This signal induces the expression of *mat1-Pm* and *mat1-Mm*, and these complete the initiation of meiosis by activating transcription of the *mei3* gene, which activates meiosis.

MATERIALS AND METHODS

Genetic procedures and culture media. The *S. pombe* strains used in this study are listed in Table 1. Standard *S. pombe* procedures were done according to those in reference 33. Protoplast fusion was essentially as described in reference 34. For physiological experiments defined minimal medium (PM) or minimal medium lacking a nitrogen source (PM−N) was used (2). Synthetic pheromones were prepared as described elsewhere (16, 45). Standard DNA manipulations were done according to the instructions in reference 41. PCR amplification was performed as described previously (22).

Construction of stable diploid strains. The two diploid *P/M mat1-Pc*$^-$ strains were constructed basically in the same way, the only difference being that one strain is *sxa2*$^+$/*sxa2*$^+$ (EG819) while the other is *sxa2*$^-$/*sxa2*$^-$ (EG820). Both strains were obtained by protoplast fusion of a *mat1-M* strain and a *mat1-Pc*^{opal5} strain, selecting for uracil-adenine prototrophs (EG819, EG817 × EG571; EG820, EG818 × EG812). The *M* strains (EG571 and EG812) contain the *ura4*$^+$ gene inserted into a *Bgl*II linker in the H1 box of *mat1*, which abolishes generation of the double-strand break that initiates mating-type switching (43). The *mat1-Pc*^{opal5} strains (EG817 and EG818) were made by transforming the *mat1-M* strains EG572 and EG811, respectively, with the *mat2-Pc*^{opal5} replicating donor plasmid previously described (17). After switching of the *mat1-Pc*^{opal5} cassette into *mat1* on the chromosome, the cells were grown under nonselective conditions in order to allow plasmid loss.

The diploid *P/M mat1-Mc*$^-$ strain EG670 was made by protoplast fusion of strains EG575 and EG432, selecting for uracil-adenine prototrophs. EG575 was obtained by gene replacement in EG530 with a 10-kb *Xho*I-*Cla*I fragment containing a *mat1-M* cassette with an opal stop codon (codon 7) in the *mat1-Mc* gene (17) and the 1.8-kb *ura4*$^+$ fragment inserted in the H1 box (see above).

EG665 was made by protoplast fusion of strains EG590 and EG591, selecting for uracil-adenine prototrophs. EG590 was constructed basically the same way as EG575 (see above) by replacing the *mat1-M* locus of EG586 with the *Xho*I-*Cla*I *mat1-Mc*^{opal7} *int-H1::ura4*$^+$ fragment. EG591 was constructed in the same way as EG817 and EG818 were. The *mat1-M* locus of EG587 was switched to *mat1-Pc*^{opal5} by a replicating donor plasmid which was subsequently lost.

Induction of meiosis. In pheromone-dependent meiosis experiments, cells were grown in PM to a density of 5×10^6 cells per ml and then transferred to PM−N and either P-factor (EG820) or M-factor (EG670) was added at 300 U/ml (see references 4 and 16 for unit definition). The cells were incubated overnight at 30°C and photographed through Nomarski optics. In *pat1-114* induction experiments, cells were grown in PM to 5×10^6 cells per ml, shifted to 30°C, and incubated overnight. Ectopic expression of *mat1-Pm* and *mat1-Mm* from the thiamine-repressible *nmt1* promoter in EG767-2n cells was induced in the following way. Cells were grown in PM plus 2 μM thiamine to a density of 5×10^6 cells per ml, washed, and resuspended in PM−N. The culture was divided in two, and thiamine was added to one half. After incubation overnight the cells were inspected in the microscope. Plasmid pmat1-Mm was obtained by inserting the *mat1-Mm* gene as a 0.25-kb *Nde*I fragment (from pMiadh [17]) after the *nmt1* promoter of the *LEU2* vector pREP1 (27). Plasmid pmat1-Pm, which contains the *mat1-Pm* open reading frame controlled by the *nmt1* promoter of the *ura4* vector pREP2 (28), has been described previously (42).

Northern (RNA) analysis. In general cells were grown in PM at 30°C to a density of 5×10^6 cell per ml, harvested, and resuspended in PM or PM−N at the same density. When indicated, synthetic pheromone was added at 300 U/ml. After 5 h of incubation at 30°C, total RNA was isolated as described elsewhere

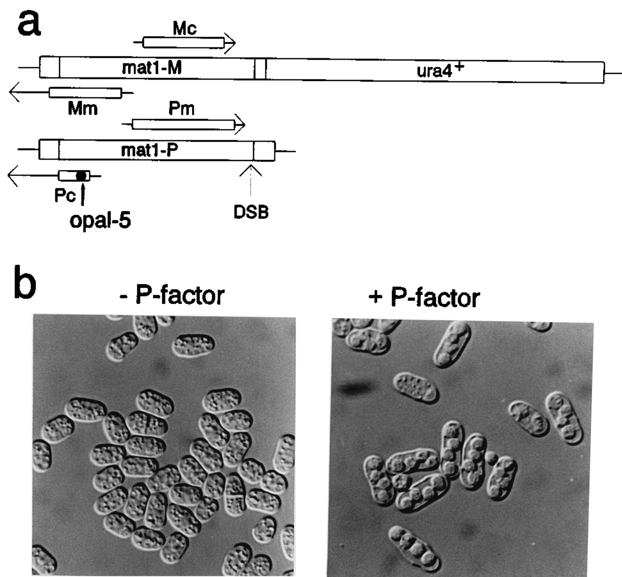

FIG. 1. P-factor-induced meiosis and sporulation in diploid *P/M mat1-Pc*$^-$ cells. (a) *mat1* configuration of the diploid *P/M mat1-Pc*$^-$ strains EG819 (*sxa2*$^+$/*sxa2*$^+$) and EG820 (*sxa2*$^-$/*sxa2*$^-$). The four *mat1* genes *mat1-Mc* (Mc), *mat1-Mm* (Mm), *mat1-Pc* (Pc), and *mat1-Pm* (Pm) are indicated. The *mat1-Pc* gene contains a nonsense mutation at the fifth codon (opal-5). Mating-type switching is prevented by insertion of the *ura4* gene near the site of the DNA double-strand break (DSB) at *mat1-M*. (b) A culture of EG820 was starved of nitrogen and divided into halves, and synthetic P-factor was added to one of these. Only the culture to which P-factor had been added entered meiosis and sporulation.

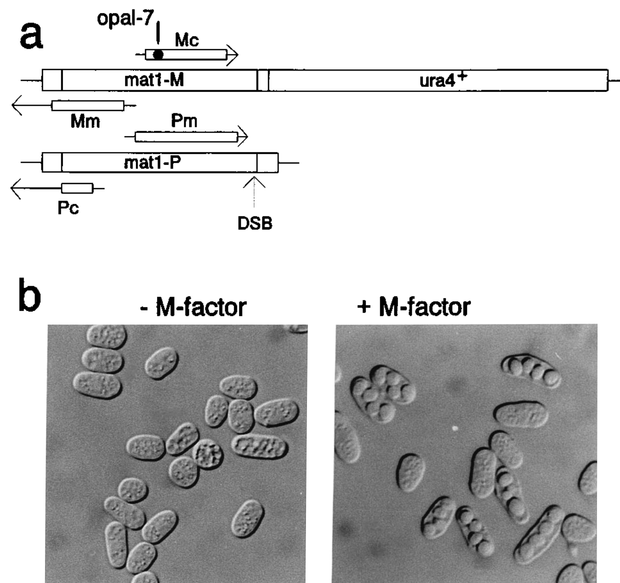

FIG. 2. M-factor-induced meiosis and sporulation in diploid *P/M mat1-Mc*$^-$ cells. (a) *mat1* configuration of the diploid *P/M mat1-Mc*$^-$ strain EG670. Gene symbols are as in Fig. 1. The *mat1-Mc* gene contains a nonsense mutation at the seventh codon (opal-7). (b) A culture of EG670 was starved of nitrogen and divided into halves, and synthetic M-factor was added to one of these. Only the culture to which M-factor had been added entered meiosis and sporulation.

(38). In the case of *pat1-114* strains, the cells were grown at 23°C to the same density, half of the culture was transferred to 30°C, and RNA was isolated after 5 h. In experiments where transcription of *mat1-Pm* and *mat1-Mm* was induced from the *nmt1* promoter (see above), the RNA was isolated after 16 h of incubation in thiamine-free medium. Ten micrograms of total RNA was run in each lane on 1.5% formaldehyde gels, blotted onto Hybond-N membranes (Amersham), and hybridized to RNA probes as described previously (38).

Single-stranded ^{32}P-labelled RNA probes were transcribed from pGEM plasmids (Promega) according to the manufacturer's instructions. The probes specific for *mat1-Pm* and *cdc2* were described previously (38). The *mat1-Mm*-specific probe was transcribed from a 0.5-kb *Eco*RI-*Nsi*I fragment from *mat-M* (17) cloned into pGEM4. This probe extends into the H2 homology box and therefore also recognizes the *mat1-Pc* transcript (see Fig. 1a and 3b). The *mei3* probe was transcribed from a 326-bp *mei3* fragment inserted in pGEM4 between *Bam*HI and *Sph*I. This fragment was cloned by PCR using the primers 5′-GCGCGGATCCAGTACTAACACTAAC and 5′-GCCGCATGCTTAGCGAGAGGTGTTG (30) (*Bam*HI and *Sph*I sites are underlined).

RESULTS

Entry into meiosis requires a pheromone signal. The genes *mat1-Pc* and *map1* control *P* cell mating activities, and genetic analysis has shown that these functions are also required in diploid *P/M* cell lines for entry into meiosis. The meiotic defect of mutants in these genes can be rescued by a diffusible extracellular substance produced by wild-type *P* cells (7, 25, 31). These and other observations discussed above suggest that this *map1* substance is the P-factor mating pheromone. Since the structure of P-factor was recently determined (16), we wanted to investigate whether synthetic P-factor could induce meiosis in *P/M mat1-Pc*$^-$ cells.

We constructed a stable diploid *P/M* strain with a nonsense mutation in the fifth codon of the *mat1-Pc* gene (EG819) (Fig. 1a). Diploid cells of *S. pombe* still undergo efficient mating-type switching on both homologs (8); a culture is therefore a mixture of *P/P*, *M/M*, and *P/M* cells, and only the last class can be induced to enter meiosis. In order to overcome this problem, we deleted the *mat2-P* and *mat3-M* donor cassettes in both homologs, thus preventing mating-type switching (21). However, even in the absence of the silent donor loci, the *mat1* locus of diploids tends to become homozygous by *mat1-mat1 trans* switching events (data not shown). We therefore inserted the *ura4* gene in the H1 homology box at *mat1* in one homolog, thereby eliminating formation of double-strand break (43) and reducing gene conversion significantly (9). As expected, this strain (EG819) (Table 1) had a stable *P/M mat1-Pc*$^-$ phenotype and was meiosis deficient. Furthermore, meiosis could be restored if the cells were mixed with wild-type *P* cells (data not shown).

Upon pheromone stimulation, *S. pombe M* cells secrete a very potent protease that degrades P-factor (15), and synthetic P-factor has a lasting physiological effect only on *M* cells defective in the *sxa2* gene encoding this protease (16). Our stable diploid *P/M mat1-Pc*$^-$ strain EG819 was also expected to produce the Sxa2 protease, and indeed we observed a very limited induction of sporulation after adding synthetic P-factor to the cells (data not shown). We therefore constructed a derivative of the *P/M mat1-Pc*$^-$ strain homozygous for the *sxa2* mutation (EG820) (Table 1). In this strain meiosis and sporulation could be efficiently induced by the addition of synthetic P-factor (Fig. 1b). We conclude that the *map1* substance capable of inducing meiosis in diploid *mat1-Pc*$^-$ cells is identical to the P-factor pheromone.

Wild-type diploid *P/M* cells can presumably generate this pheromone signal themselves before proceeding into meiosis. Furthermore, diploid *P/M mat1-Mc*$^-$ cells are expected to be defective in meiosis because they produce neither M-factor nor receptor for P-factor and hence cannot generate a pheromone signal. Therefore, meiosis is expected to be restored if M-factor is added. In order to test this hypothesis, we constructed a stable diploid *P/M* strain with a nonsense mutation in the seventh codon of the *mat1-Mc* gene (EG670) (Fig. 2a). As expected, this strain was meiosis deficient, but if M-factor was added, the cells proceeded efficiently into meiosis and sporu-

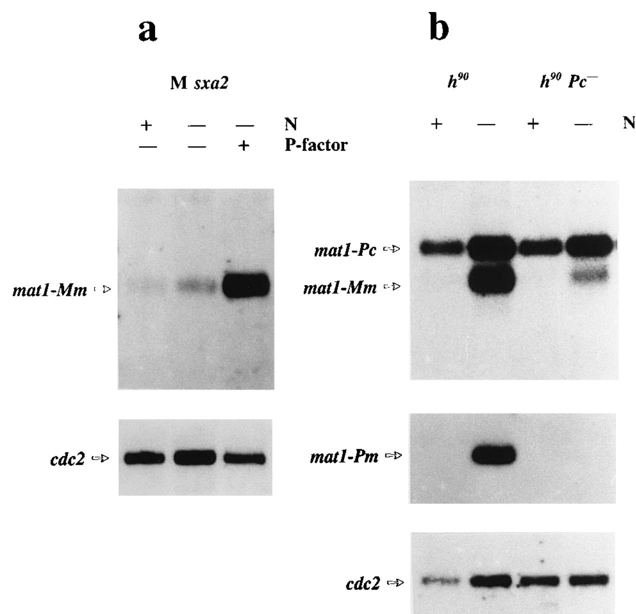

FIG. 3. Pheromone induction of the *mat1-Mm* gene. (a) The *M sxa2* strain EG699 was grown under three different culture conditions: nitrogen source present, nitrogen starvation, and nitrogen starvation in the presence of P-factor. The ability to transcribe the *mat1-Mm* gene was monitored by Northern analysis (upper panel). Expression is stimulated by starvation and strongly induced if pheromone is also added. The same blot was hybridized to a *cdc2*-specific probe in order to demonstrate that approximately equal amounts of RNA were loaded (lower panel). (b) Comparison of an h^{90} wild-type strain (EG282) and an h^{90} *mat1-Pc*$^-$ mutant strain (EG410) defective in pheromone communication. In the *mat1-Pc*$^-$ strain the *M* cells transcribe *mat1-Mm* at a lower level (top panel), and the *P* cells do not transcribe *mat1-Pm* (middle panel). The membrane was also hybridized to the *cdc2* control probe (bottom panel). The *mat1-Mm* probe also recognizes the *mat1-Pc* transcript.

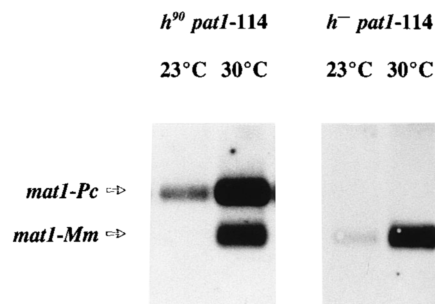

FIG. 4. Induction of the *mat1-Mm* gene by temperature shift in *pat1-114* mutants. The h^{90} *pat1-114* strain EG397 and the h^- *pat1-114* strain EG422 were grown at 23°C or temperature shifted to 30°C for 5 h, and the ability to transcribe *mat1-Mm* was examined. The probe also recognizes the *pat1*-controlled *mat1-Pc* transcript present in the h^{90} strain.

lation (Fig. 2b). These results demonstrate that a pheromone signal is required for entry into meiosis and, furthermore, that the signal can be generated by either P-factor or M-factor.

The *mat1-Mm* gene is pheromone controlled. The previous observations suggest that at least one function required for entry into meiosis depends on a pheromone signal. A candidate for such a function is expression of the *mat1-Pm* gene in the *mat1-P* locus. This gene was previously shown to be required for entry into meiosis (6, 17), and *P* cells need to be stimulated with M-factor in order to transcribe it (37). The corresponding gene from the *mat1-M* locus, *mat1-Mm*, is also required for entry into meiosis (10, 17), but whether it is pheromone controlled as well has not been investigated. We therefore tested this, and indeed we found that the addition of P-factor to *M sxa2* cells strongly induced the level of the *mat1-Mm* transcript (Fig. 3a). However, as opposed to the *mat1-Pm* gene (37) (see also Fig. 3b), *mat1-Mm* is induced to an intermediate level already when the cells are starved of nitrogen (Fig. 3a, middle lane). The contribution from a pheromone signal to *mat1-Mm* expression is not confined to *sxa2*$^-$ cells. In a homothallic h^{90} *mat1-Pc*$^-$ strain, the *M* cells clearly express *mat1-Mm* at a lower level than in a corresponding *mat1-Pc*$^+$ strain (Fig. 3b). Since the *mat1-Pc* gene has no function in *M* cells (17), this lower expression is most likely a consequence of the failure of the *P* cells to produce P-factor (25). Therefore, both *mat1-Pm* and *mat1-Mm* represent pheromone-controlled functions needed for entry into meiosis.

Expression of *mat1-Pm* and *mat1-Mm* is sufficient for transcription of *mei3*. As both *mat1-Pm* and *mat1-Mm* are required for entry into meiosis, the transcription of these genes could explain the requirement of a pheromone signal for entry into meiosis. To investigate this possibility further, we sought to express *mat1-Pm* and *mat1-Mm* independently of the pheromone signal.

In order to do so, we utilized the temperature-sensitive *pat1-114* mutation (14). This mutant can be manipulated to derepress different steps of the differentiation program by incubation at different temperatures (2). At 30°C transcription of the *mat1-Pm* gene becomes independent of both nitrogen starvation and a pheromone signal (38). We have found that this is true for the *mat1-Mm* gene as well (Fig. 4). When shifted to 30°C, an h^- *pat1-114* strain expresses this gene at a high level even in the absence of P-factor. We therefore constructed a stable diploid *P/M mat1-Pc*$^-$ *mat1-Mc*$^-$ strain homozygous for the *pat1-114* mutation (EG665) (Fig. 5a). When shifted to 30°C, this strain induced expression of *mat1-Pm* and *mat1-Mm* as expected, and more than 90% of the cells entered meiosis (data not shown).

As the only function of the *mat1* locus during the initiation of meiosis is activation of the *mei3* gene, a direct inducer of meiosis (30), we used expression of *mei3* as a monitor for entry into meiosis. We found that transcription of *mei3* was induced in EG665 when shifted to 30°C (Fig. 5b), confirming that meiosis has become independent of *mat1-Pc* and *mat1-Mc*. However, both *mat1-Pm* and *mat1-Mm* were required for this induction, since *pat1-114* strains defective in either *mat1-Pm* or *mat1-Mm* failed to express *mei3* (Fig. 5b) and did not undergo meiosis at all when shifted to 30°C. This observation also shows that *mei3* expression is not directly controlled by *pat1.*

The previous experiment demonstrated that *pat1*-driven expression of *mat1-Pm* and *mat1-Mm* allowed transcription of *mei3* in the complete absence of the pheromone communication system controlled by *mat1-Pc* and *mat1-Mc*. However, the possibility that additional *pat1*-controlled genes are required for activation of *mei3* exists. In order to test this possibility, we proceeded to express *mat1-Pm* and *mat1-Mm* from the inducible *nmt1* promoter in a diploid *pat1*$^+$ strain devoid of *mat1-Pc* and *mat1-Mc* (EG767-2n) (Table 1). This also caused induction of the *mei3* gene (Fig. 6a) and allowed entry into meiosis (Fig. 6b), although only 15% of the cells sporulated in this case. In *S. pombe* most plasmids are highly unstable (see reference 40 for a discussion), and the nonsporulating cells presumably had lost one of the plasmids. In conclusion, *mat1* activation of meiosis is mediated through expression of *mat1-Pm* and *mat1-Mm.*

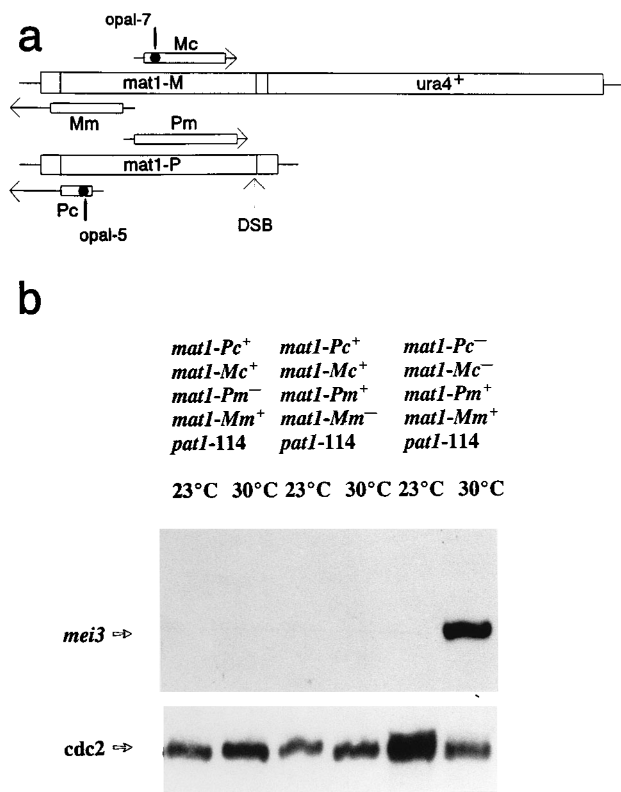

FIG. 5. Expression of *mat1-Pm* and *mat1-Mm* causes induction of *mei3*. (a) *mat1* configuration of the diploid *P/M mat1-Pc*$^-$/*mat1-Mc*$^-$ *pat1-114/pat1-114* strain EG665. Gene symbols are as in Fig. 1. The *mat1-Pc* gene contains a nonsense mutation at the fifth codon (opal-5), whereas the *mat1-Mc* gene contains a nonsense mutation at the seventh codon (opal-7). (b) EG665 cells were grown at 23°C or temperature shifted to 30°C for 5 h, and the ability to transcribe *mei3* was examined (rightmost pair of lanes). A strong induction is observed when the cells are shifted to 30°C. Cells defective in *mat1-Pm* (EG443-2n; leftmost pair of lanes) or *mat1-Mm* (EG444-2n; middle pair of lanes) fail to induce *mei3* at 30°C. The membrane was also hybridized to the *cdc2* probe (lower panel).

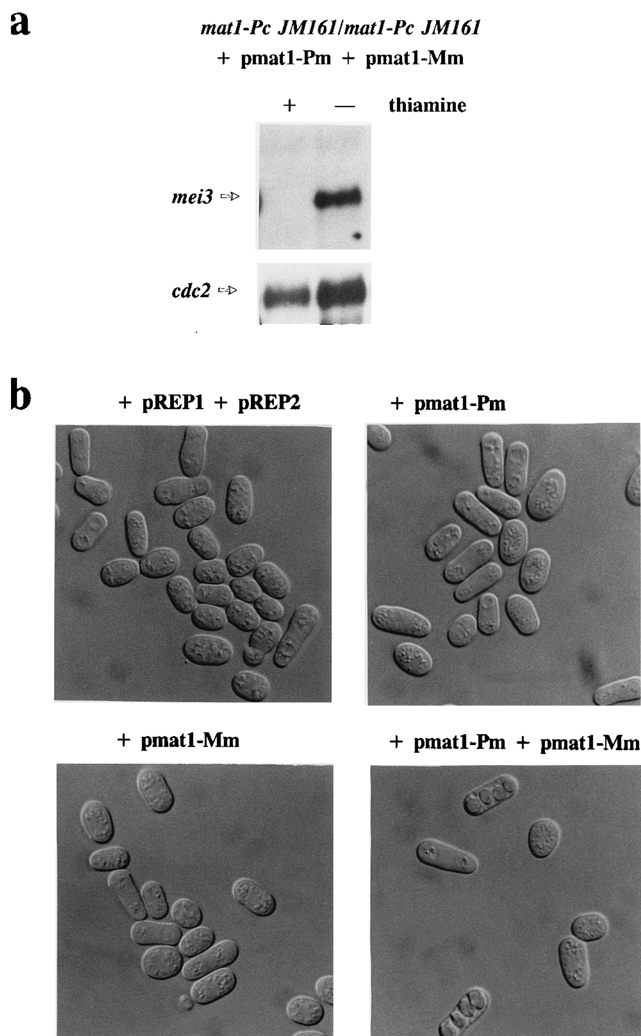

FIG. 6. Activation of meiosis by ectopic expression of *mat1-Pm* and *mat1-Mm*. (a) The diploid *mat1-Pc*$^-$ strain EG767-2n was transformed with plasmids pmat1-Pm and pmat1-Mm, and the cells were starved in the presence (+) or absence (−) of thiamine. Thiamine depletion causes induction of *mat1-Pm* and *mat1-Mm*, which in turn activates *mei3* transcription (upper panel). The membrane was also hybridized to the *cdc2* probe (lower panel). (b) EG767-2n cells transformed with both pmat1-Pm and pmat1-Mm sporulated when starved in the absence of thiamine, whereas cells containing only pmat1-Pm or pmat1-Mm or the vectors pREP1 and pREP2 failed to do so.

DISCUSSION

S. pombe cells ensure that entry into meiosis occurs from the diploid state by monitoring heterozygosity at the mating-type locus. A haploid cell manipulated to contain both the *mat1-P* and the *mat1-M* loci will thus attempt to undergo meiosis and sporulation when starved (1). Previous genetic analysis has shown that all four products encoded by *mat1-P* and *mat1-M* are required for meiosis (17). We have now more closely defined the roles of these genes and show that the products control meiosis in two steps (Fig. 7). First, the *mat1-Pc* and *mat1-Mc* genes establish a pheromone communication system that allows a pheromone signal to be generated. This signal activates transcription of *mat1-Pm* and *mat1-Mm*, leading to induction of *mei3* and entry into meiosis.

The pheromone communication system was presumably developed to allow haploid cells to identify a mating partner, and its requirement for entry into meiosis is probably best understood in the context of the haploid life strategy of the fission yeast. The diploid state in *S. pombe* is essentially confined to the zygote, which normally proceeds directly into meiosis. Since both *mat1-Pm* (38) and *mat1-Mm* (Fig. 3) are pheromone induced, these genes are already expressed in the two prezygotic mating partners (Fig. 7a). Consequently, when the cells fuse, *mat1-Pm* and *mat1-Mm* come together in the zygote and cause activation of *mei3* immediately. Pheromone control of *mat1-Pm* and *mat1-Mm* therefore ensures that their products are made only in the presence of a potential mating partner and that the resulting zygote is committed to meiosis. As a result, only a very small fraction of the newly formed zygotes can resume mitosis as diploids if nutritional starvation is relieved. If the coupling of conjugation and meiosis is lost, by mutation of *mat1-Pm*, for example, then a 100-fold greater fraction of the zygotes can return to diploid mitosis (6).

Wild-type diploid cell lines generally sporulate easily (24), and this form is probably not important in nature. Unlike budding yeast (13), diploid *S. pombe* cells do not possess specific mechanisms that prevent mating-type switching (8) or pheromone communication (this study). A diploid cell with a *P/M* configuration at *mat1* will enter azygotic meiosis when starved, and in order to activate *mei3*, it needs to transcribe *mat1-Pm* and *mat1-Mm*. This requires that the cell generate a

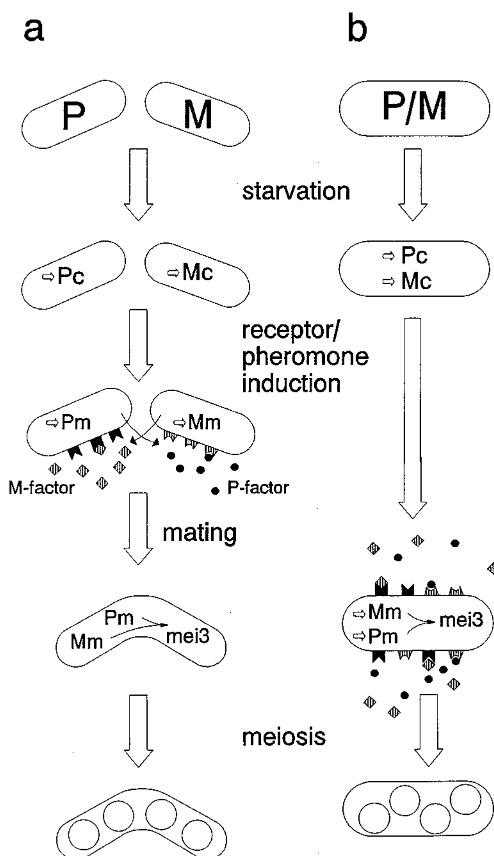

FIG. 7. Model for two-step activation of meiosis in *S. pombe*. (a) In haploid wild-type cells starvation will induce the *mat1-Pc* gene (Pc) in *P* cells and the *mat1-Mc* gene (Mc) in *M* cells. This will activate the pheromone communication system. In response to a pheromone signal, the *mat1-Pm* gene (Pm) will be transcribed in *P* cells and the *mat1-Mm* gene (Mm) will be transcribed in *M* cells. Upon successful conjugation these two gene products come together in the zygote, and this activates the *mei3* gene, which triggers meiosis and sporulation resulting in a zygotic ascus. (b) In a vegetative diploid *P/M* cell starvation will cause induction of both *mat1-Pc* and *mat1-Mc*, and the cell will generate a pheromone signal itself and activate *mat1-Pm* and *mat1-Mm*. This induces *mei3* and activates meiosis, generating an azygotic ascus.

pheromone signal itself (Fig. 7b). Mutant strains defective in *mat1-Pc* or *mat1-Mc* are unable to generate this signal, and hence meiosis becomes dependent on externally added P-factor (Fig. 1b) or M-factor (Fig. 2b). This is the first direct demonstration that meiosis can be activated by pheromones, and it provides a framework for understanding why mutants in the signal transduction pathway cannot undergo meiosis. The stable diploid strains that sporulate when exposed to P- or M-factor represent useful tools in *S. pombe* pheromone research, and they have recently been utilized in the development of a halo assay for pheromone production (11).

The direct activator of meiosis is the *mei3* gene product (30). Forced expression of *mei3* in vegetatively growing cells causes meiosis presumably by completely inhibiting the *pat1*-encoded protein kinase. Purified Mei3 specifically abolishes kinase activity of purified Pat1 in vitro (29). Our results show that expression of *mat1-Pm* and *mat1-Mm* is sufficient to activate *mei3* (Fig. 5b and 6a). Therefore, the only meiotic function of *mat1-Pc* and *mat1-Mc* is to mediate expression of *mat1-Pm* and *mat1-Mm*, and furthermore, this appears to be the only step for which a pheromone signal is required during entry into meiosis. The *JM56* allele of the *mat1-Pc* gene was reported to have a leaky phenotype preventing conjugation but still allowing meiosis (17, 31). This could be explained if the *JM56* mutant reduced *P*-specific pheromone activities and if conjugation were more sensitive to this than meiosis.

We found that nitrogen starvation is still required in order to drive *mei3* transcription by ectopic expression of *mat1-Pm* and *mat1-Mm* (Fig. 6). Nitrogen starvation presumably stabilizes the *mat1-Pm* and *mat1-Mm* transcripts, since these are much less abundant in this strain when nitrogen is present (data not shown). Hence, increased stability of specific RNAs during sexual differentiation may be an important consequence of nitrogen depletion.

The Pat1 protein kinase appears to be a master regulator of sexual differentiation in *S. pombe*. Experiments with the temperature-sensitive *pat1-114* allele suggest that Pat1 represses the differentiation process at at least three different levels. When *pat1-114* is partially inactivated, mating is allowed in rich medium (14). Thus, Pat1 has a function in nutritional sensing. Upon further *pat1-114* inactivation, pheromone-controlled genes like *mat1-Pm* and *mat1-Mm* are activated (Fig. 4) (37, 38). Finally, when the Pat1 activity is completely lost, the cells undergo meiosis. In wild-type cells this is accomplished by the Mei3 protein (29, 30).

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