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Schizosaccharomyces pombe map3$^+$ Encodes the Putative M-Factor Receptor

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A defect in the *map3* gene of the fission yeast *Schizosaccharomyces pombe* causes h^+ mating-type-specific sterility. This gene was cloned by complementation. Nucleotide sequence analysis showed that it has a coding capacity of 365 amino acids. The deduced *map3* gene product is a putative seven-transmembrane protein and has 20.0% amino acid identity with the a-factor receptor of *Saccharomyces cerevisiae*, encoded by *STE3*. It is also homologous with the *Ustilago maydis* mating pheromone receptors. The *map3* gene is expressed in h^+ cells but not in h^- cells, and the transcripts are induced in response to nitrogen starvation. h^+ cells defective in *map3* do not respond to purified M-factor. When *map3* is expressed ectopically in h^- cells, they apparently acquire the ability to respond to the M-factor produced by themselves. The *gpa1* gene, which encodes the α-subunit of a G-protein presumed to couple with the mating pheromone receptors, is essential for this function of *map3*. These observations strongly suggest that *map3* encodes the M-factor receptor. Furthermore, this study provides strong support for the notion that pheromone signaling is essential for initiation of meiosis in *S. pombe* and that either M-factor signaling or P-factor signaling alone is sufficient.

Considerable progress has been made in the analysis of genes relevant to the mating reaction in fission yeast *Schizosaccharomyces pombe*. Physiological evidence suggested the involvement of diffusible mating pheromones, M-factor secreted by h^- cells and P-factor secreted by h^+ cells, in this reaction (14, 26, 27). The M-factor was purified recently and shown to be structurally similar to *Saccharomyces cerevisiae* **a**-factor (6, 7). It is encoded by two genes named *mfm1* and *mfm2* (7). Similar analyses do not yet exist for the P-factor. The *mam2* gene is likely to encode the P-factor receptor (24); the M-factor receptor gene has not yet been identified. The *gpa1* gene encodes an α-subunit of the G-protein that apparently couples with the mating pheromone receptors (37). Two putative protease genes, *sxa1*, coding for an aspartyl protease, and *sxa2*, coding for a serine carboxypeptidase, have been cloned, and their products are implicated in degradation or processing of the pheromones (22).

Mating-type-specific sterile mutants have been instrumental in elucidation of the mating-pheromone recognition pathway in *S. cerevisiae* (reviewed in reference 29). Mutants defective in STE2 are sterile only if they are **a**, and the gene encodes the α-factor receptor (5, 34), whereas mutants defective in STE3 are sterile only if they are α, and the gene product is the **a**-factor receptor (18, 34). A similar set of mutants demonstrates their usefulness also in *S. pombe*, as shown by identification of the *mam2* gene product as the putative P-factor receptor (24). Egel (11) identified two h^+-specific sterile genes, named *map1* and *map2*, and two h^--specific sterile genes, named *mam1* and *mam2*, in *S. pombe*. A defect in the gene encoding either P-factor or the receptor for M-factor is likely to cause a Map phenotype. The *map1* gene, however, is essential for production of both of them and hence appears to fulfil the requirements for neither a factor nor a receptor gene. This suggests that a saturation mutagenesis for *map* mutants has not been accomplished.

To study the mating pheromone recognition pathway more thoroughly, we set out to expand the range of mating-type-specific sterile mutants of fission yeast. We have identified two new h^+-specific sterile genes, which we have named *map3* and *map4*. We show in this paper that one of them, *map3*, apparently encodes the M-factor receptor. The deduced *map3* gene product is a putative seven-transmembrane protein that has homology with the **a**-factor receptor of *S. cerevisiae*.

MATERIALS AND METHODS

Strains, media, genetic methods, and transformation of *S. pombe*. *S. pombe* strains used in this study are listed in Table 1. Media (9, 17, 30, 45) and general genetic procedures (17, 30) for *S. pombe* have been described previously. Mutagenesis of *S. pombe* cells with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine was carried out essentially as described previously (21). Crosses of mating-deficient strains were performed by protoplast fusion (41). Transformation of *S. pombe* cells was done by an improved lithium method (38).

Isolation of mating-type-specific sterile mutants. Mutagenized cells of homothallic haploid strains were placed on malt extract-agar plates, on which wild-type cells form small colonies and then undergo mating and sporulation because of a limited supply of nitrogen source. Mutants that failed to sporulate were screened by exposure to iodine vapor, which stains spores dark brown (17), and mating-deficient ones were further chosen by microscopic inspection. These mating-deficient mutants were of three kinds: (i) sterile regardless of the mating type, (ii) sterile only in either the h^+ or h^- genetic background, and (iii) deficient in the mating-type gene or in mating-type switching. Mutants in the second group were genetically classified. In addition to *map1* and *map2*, two new genes responsible for h^+-specific sterility were identified and named *map3* and *map4*. Likewise, two

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TABLE 1. *S. pombe* strains used in this study

Strain	Relevant genotype
JY333	h^- *ade6-M216 leu1*
JY334	h^+ *ade6-M216 leu1*
JY362	h^+/h^- *ade6-M210/ade6-M216 leu1/leu1*
JY450	h^{90} *ade6-M216 leu1*
JY505	h^- *ras1::LEU2 ade6-M216 leu1*
JY878	h^{90} *ade6-M216 leu1 ura4-D18*
JZ366	h^{90}/h^{90} *map1/map1 ade6-M210/ade6-M216 leu1/leu1*
JZ396	h^{90} *ste11::ura4*$^+$ *ade6-M216 leu1 ura4-D18*
JZ400	h^- *ste11::ura4*$^+$ *ade6-M216 leu1 ura4-D18*
JZ452	h^- *gpa1::ura4*$^+$ *ade6-M216 leu1 ura4-D18*
JZ453	h^{90} *gpa1::ura4*$^+$ *ade6-M210 leu1 ura4-D18*
JZ728	h^{90} *map3::ura4*$^+$ *ade6-M216 leu1 ura4-D18*
JZ844	h^+ *map3::ura4*$^+$ *ade6-M216 leu1 ura4-D18*
JZ878	h^{90}/h^{90} *map3::ura4*$^+$*/map3::ura4*$^+$ *ade6-M210/ade6-M216 leu1/leu1 ura4-D18/ura4-D18*
ST711	h^{90} *map3-711 ade6-M210 leu1*

novel h^--specific sterility genes were identified and named *mam3* and *mam4*, but their genetic analysis will be described elsewhere.

Cloning of *map3*. *S. pombe* ST711 (h^{90} *map3-711 ade6-M210 leu1*) was transformed with a library constructed with *Sau*3AI partial digests of *S. pombe* genomic DNA in the vector pDB248′ (2). Leu$^+$ transformants were selected and tested for mating and subsequent sporulation by iodine staining. A plasmid recovered from a mating-proficient transformant could retransform ST711 to Map$^+$. This plasmid, pPTS1, carried an *S. pombe* DNA fragment of 7.0 kb, as shown in Fig. 1.

DNA sequence analysis. The DNA sequence of a 3.2-kb *Mlu*I-*Cla*I fragment that carries the *map3* gene was determined by using the dideoxy-chain termination method (40). This DNA fragment was cloned in both orientations into pBluescript KS(+) (Stratagene). Subclones for sequencing were generated by progressive deletion with exonuclease III and S1 nuclease (Takara Shuzo) by the method of Henikoff (19). Single-stranded template DNA was prepared by using the helper M13KO7 bacteriophage. All parts of the sequence shown in Fig. 2 have been determined in both directions at least once. A DNA sequencer (Applied Biosystems 370A) was used for this determination.

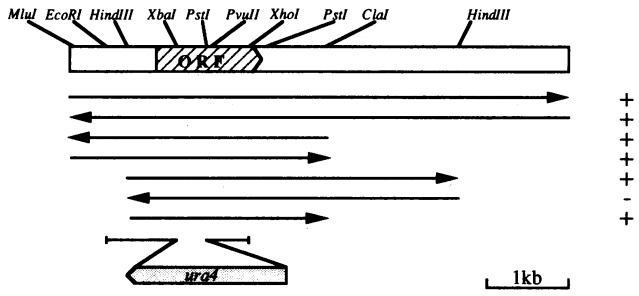

FIG. 1. Restriction map of the *map3* locus and activities of its subclones for complementation. The insert DNA in the original plasmid, pPTS1, is shown as a bar, with the extent and direction of the assigned *map3* ORF indicated by the hatched arrow. Restriction fragments of pPTS1 were subcloned in pDB248′ (2). Each subcloned fragment is represented by an arrow below the bar diagram. The direction of the arrow indicates the fragment orientation with respect to a cryptic promoter on the vector. The ability of each subclone to rescue *map3* deficiency is indicated as + or −. The structure of the linear fragment used to disrupt the *map3* gene in vivo is shown in the lowest line. An *S. pombe ura4*$^+$ cassette (16) was used in this disruption.

Gene disruption. One-step gene disruption (39) of *map3* was carried out. A 0.47-kb *Xba*I-*Pst*I fragment was removed from the open reading frame (ORF), and an *S. pombe ura4*$^+$ cassette (16) was inserted. *S. pombe* JY878 (h^{90} *ade6-M216 leu1 ura4-D18*) was transformed with an *Eco*RI-*Xho*I fragment carrying this disrupted *map3* gene. Most of the Ura$^+$ transformants were deficient in mating. A transformant in which disruption of the *map3* gene could be confirmed by Southern blotting (42) was used for further analysis.

Northern (RNA) blot analysis. *S. pombe* cells were grown to 4 × 10^6/ml in 50 ml of MM+N medium (30) at 30°C. A 30-ml portion of the culture was harvested, and the remainder was collected by centrifugation, resuspended in 30 ml of MM-N medium in which the nitrogen source (NH_4Cl) had been omitted, and cultured for an additional 3.5 h. Total RNA was prepared from these cultures by the procedure described by Elder et al. (13). RNA was denatured with formamide-formaldehyde and was separated by electrophoresis on a 1.1% agarose gel containing 3% formaldehyde. The running buffer contained 20 mM MOPS [3-(*N*-morpholino)propanesulfonic acid], 5 mM sodium acetate, and 1 mM EDTA (pH 7.0). After running, RNA was transferred to a membrane (GeneScreenPlus; DuPont) and hybridized with a probe as described previously (44). A 0.54-kb *Xba*I-*Pvu*II fragment within the *map3* ORF was labeled with [α-^{32}P] dCTP and used as a probe.

Expression analysis by PCR. The cells were grown to exponential phase (5 × 10^6/ml) at 29°C in SSL medium (12) supplemented with adenine and leucine (both at 20 mg/liter). They were resuspended at the same concentration in SSL with adenine and leucine, either with or without a nitrogen source (aspartic acid at 200 mg/liter) and with or without M-factor at 8 U/ml (6). After a further 5 h at 29°C, total RNA was extracted as described previously (30) and subjected to polymerase chain reactions (PCRs) (31) by using a DNA thermal cycler (Perkin-Elmer Cetus) and with materials and conditions recommended by the manufacturer. The products were analyzed by electrophoresis on a 2.6% agarose gel.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence data bases with the accession number D10933.

RESULTS

Characteristics of *map3* mutants. We obtained 60 mating-type-specific sterile isolates from 80,000 mutagenized *S. pombe* cells by the procedure described in Materials and Methods. Of these, 15 were classified as *map1* mutants, 3 as *map2*, 10 as *map3*, and 4 as *map4* by linkage analysis. The rest were *mam* mutants. A homothallic *map3* strain could mate with h^+ but not with h^- tester strains, suggesting that the *map3* function is essential for h^+ cells to mate. Whether this mutant has a defect in production of P-factor or in recognition of M-factor was examined next.

It has been shown that diploid cells of the genotype h^+/h^- *map1/map1* do not sporulate. However, they can be induced to sporulate to some extent if they are exposed to P-factor (11, 24, 27). We therefore mixed an h^{90} *map3* strain with a tester h^{90}/h^{90} *map1/map1* diploid and placed the mixture on sporulation medium. Strains carrying the h^{90} homothallic mating-type allele can assume both h^+ and h^- mating-type

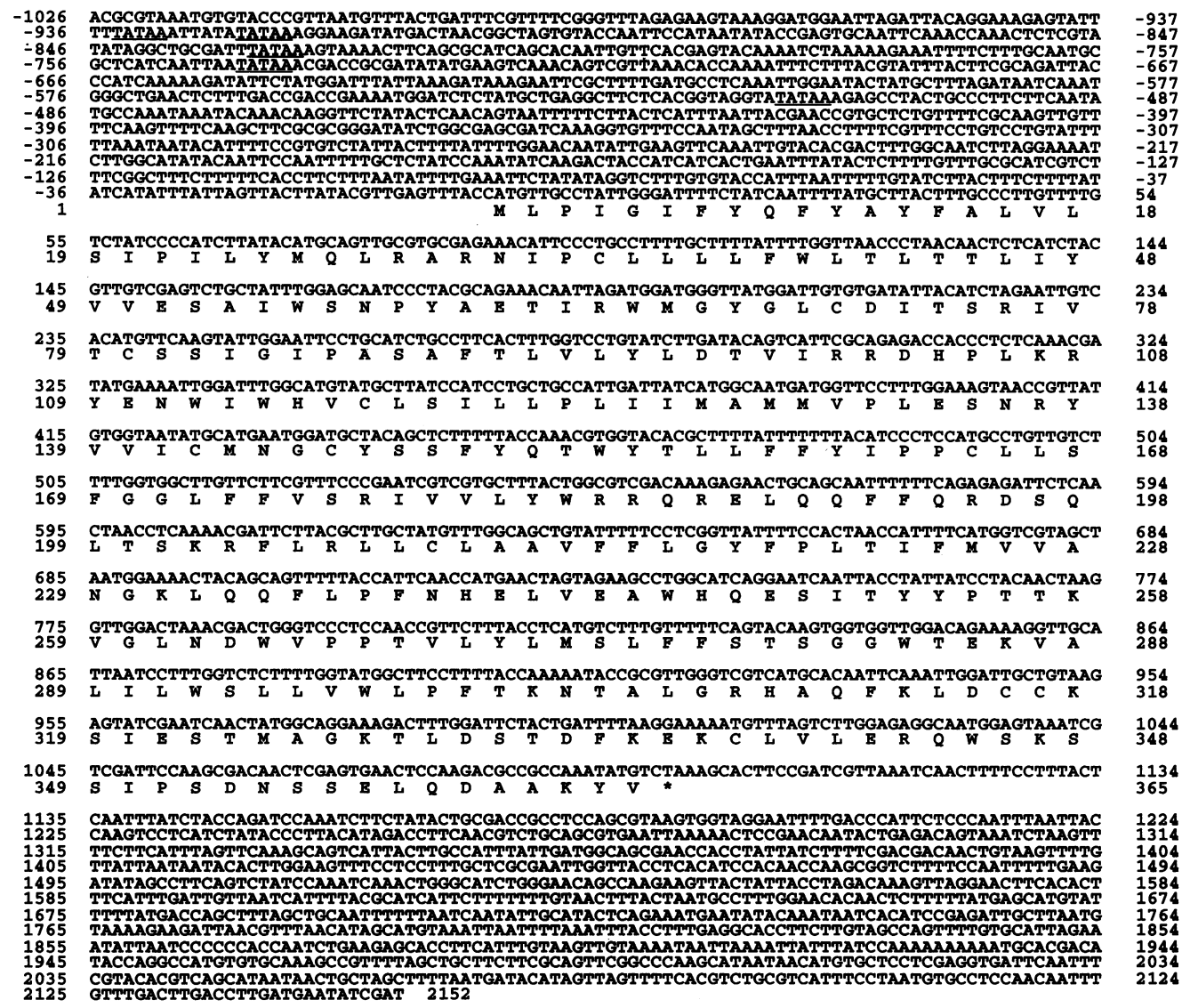

FIG. 2. Nucleotide sequence of the *map3* gene and its deduced amino acid sequence. The sequence of a 3.2-kb *Mlu*I-*Cla*I fragment that carries the entire *map3* gene is shown. Numbering of the nucleotides starts with A in the first methionine codon of the ORF, and that of the amino acid residues starts with this methionine. Possible TATA boxes are underlined.

configurations by frequent rearrangement of the mating-type loci. The *map3* strain induced sporulation of the tester strain at a frequency similar to that observed when an h^+ wild-type strain was mixed with the tester. We thus conclude that the *map3* strain can secrete P-factor.

Cells of an h^{90} *map3* strain placed under nitrogen starvation showed only feeble extension of conjugation tubes, suggesting that they were responding to P-factor but not to M-factor. M-factor has been shown to cause more explicit mating responses than P-factor (14, 26). To confirm this, we mixed the *map3* strain with an h^- *ras1*-disrupted strain, JY505, which secretes M-factor but is itself insensitive to P-factor and does not exhibit any mating response (14, 15, 32). Cells defective in *ras1* have a deformed roundish morphology and are easily distinguishable from *ras1*$^+$ cells (15). Thus, if one observes elongation of conjugation tubes in cells mixed with h^- *ras1* cells, it is an indication that the cells to be tested can respond to M-factor. This was not observed for the *map3* mutant cells (data not shown).

Cloning and sequencing of the *map3* gene. The results described above suggested that *map3* may encode the M-factor receptor. We cloned this gene by complementation, using an *S. pombe* genomic library. The complementing plasmid originally isolated, pPTS1, carried a 7.0-kb *S. pombe* DNA fragment, and subcloning indicated that a 3.2-kb *Mlu*I-*Cla*I fragment covers the entire *map3* gene (Fig. 1).

The nucleotide sequence of the 3.2-kb *Mlu*I-*Cla*I fragment was determined (Fig. 2). A 2.5-kb *Hin*dIII-*Cla*I segment could complement *map3* only when a cryptic promoter from the vector was connected to the *Hin*dIII end. This suggests that sequences necessary for function of the authentic promoter are located within the 0.7-kb *Mlu*I-*Hin*dIII segment. A continuous ORF with a coding capacity of 365 amino acids was found on the *Hin*dIII-*Cla*I segment. The absence of introns in this region was further supported by an analysis of a *map3* cDNA clone isolated by using the PCR techniques (31) (data not shown). A computer search of the NBRF data base revealed that the predicted *map3* gene product has

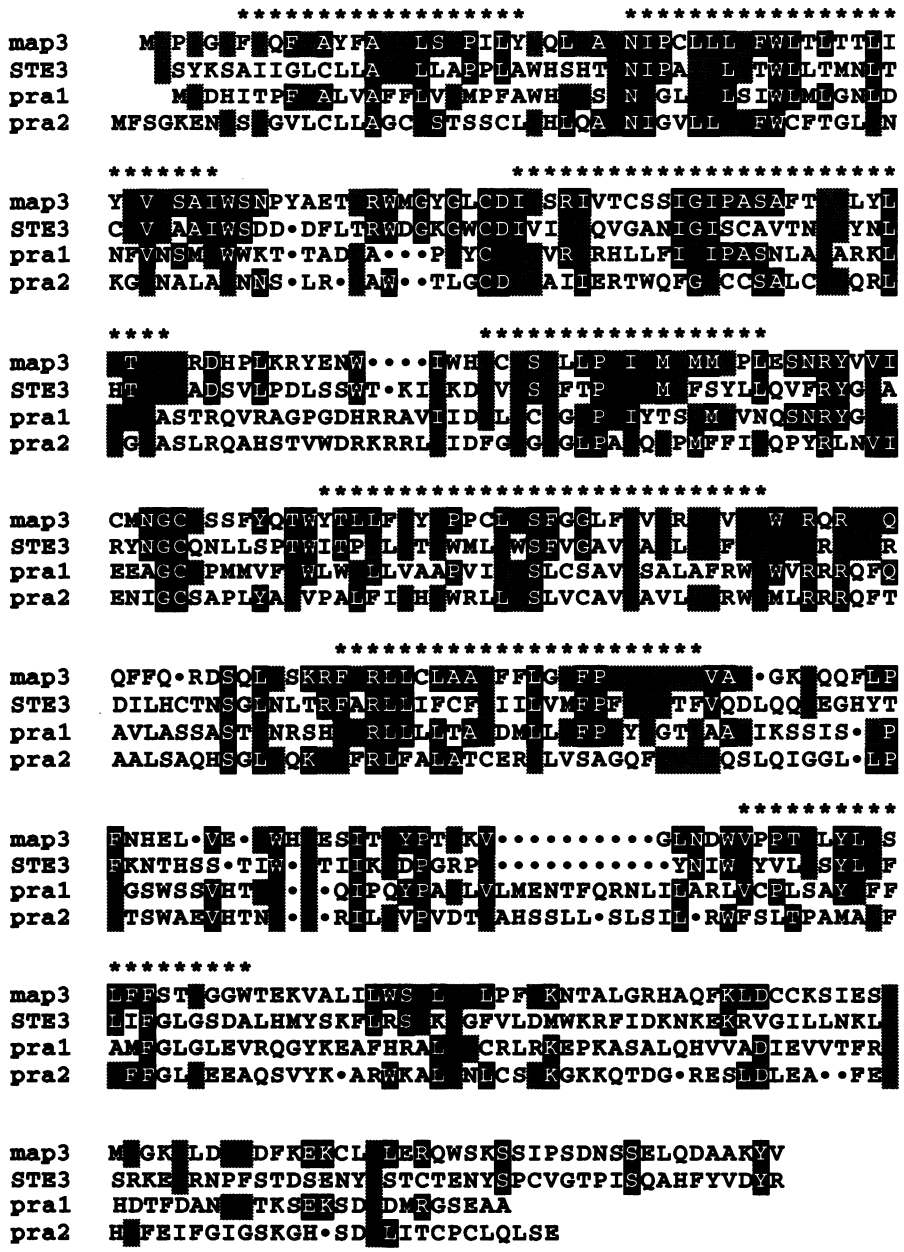

FIG. 3. Comparison of amino acid sequences of the *S. pombe map3* gene product (this study), the *S. cerevisiae STE3* product (18, 34), and the *U. maydis pra1* and *pra2* gene products (4). Amino acids identical between Map3 and at least one of the other three are shown in white against black, and those conserved between them are shaded. Conserved amino acid substitutions are grouped as follows: (V, L, I, M), (F, Y, W), (K, R), (E, D), (Q, N), (S, T), and (A, G). Asterisks indicate possible transmembrane regions.

20.0% amino acid identity with the *S. cerevisiae* a-factor receptor encoded by *STE3* (Fig. 3). It is also homologous with the *Ustilago maydis* mating-pheromone receptors encoded by *pra1* (18.1% amino acid identity) and *pra2* (15.6% identity) (Fig. 3), which have been shown to be homologous to the a-factor receptor (4). Analysis of a hydrophobicity profile of this product indicated that the Map3 protein has seven possible hydrophobic segments that may span a membrane (Fig. 4). Thus, Map3 appears to have characteristics of a receptor protein that couples with a heterotrimeric G-protein (reviewed in reference 9). Like Ste3, Pra1, and Pra2, Map3 has only a small number of N-terminal amino acid residues preceding the hydrophobic domains.

Disruption of *map3*. We disrupted *map3* by inserting the *S. pombe ura4* gene as described in Materials and Methods. The structure of the disrupted allele is illustrated in Fig. 1. The *map3* disruptant showed h^+-specific sterility and behaved like the original *map3* isolates.

To confirm that the disrupted gene is *map3*, we examined allelism between the original *map3* mutation and the null mutation created by the gene disruption. ST711 (h^{90} *map3-711*) was crossed with JZ728 (h^{90} *map3::ura4*$^+$) by protoplast fusion, and sporulation was induced in the resultant diploid cells. No mating-proficient progeny appeared in 13,000 spores tested. This indicates very tight linkage between *map3* and the disrupted gene and strongly suggests that the

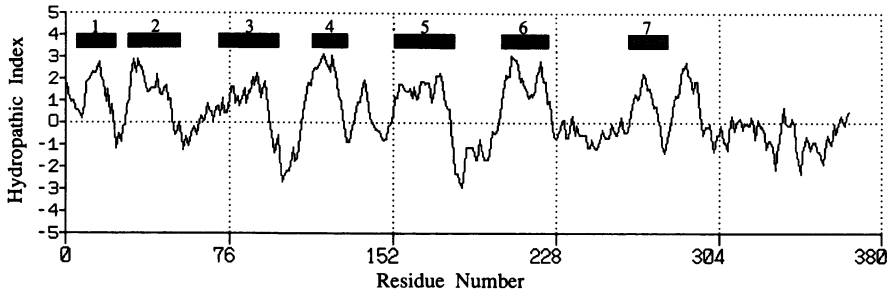

FIG. 4. Hydropathy profile of the deduced Map3 protein. The hydropathic index was calculated by the method of Kyte and Doolittle (25). Seven possible transmembrane domains are indicated.

cloned and disrupted gene is *map3* itself, not a suppressor of it.

An h^{90}/h^{90} *map3*::*ura4*$^+$/*map3*::*ura4*$^+$ diploid strain (JZ878) was constructed by protoplast fusion. This strain was able to sporulate as efficiently as the wild type (data not shown).

***map3* disruptants do not respond to purified M-factor.** Purified M-factor can induce expansion of the cell volume and expression of *mat1-Pm*(*Pi*), one of the two transcription units in the mating-type gene *mat1-P* (23), in h^+ cells under nitrogen-depleted conditions (6, 36). The response of h^+ *map3*-disruptants to pure M-factor was examined according to these two criteria.

Cells defective in *map3* (JZ844) showed no significant increase in cell volume when they were exposed to M-factor at 8 U/ml, whereas *map3*$^+$ cells (JY334) displayed an obvious increase in cell volume under the same conditions (Fig. 5). Induction of *mat1-Pm* by M-factor in the same strains was assayed by PCR analysis as described in Materials and Methods. Expression of *mat1-Pm* could not be detected in JZ844 cells starved for nitrogen in the presence of M-factor, although induction of *mat1-Pm* transcription was obvious in

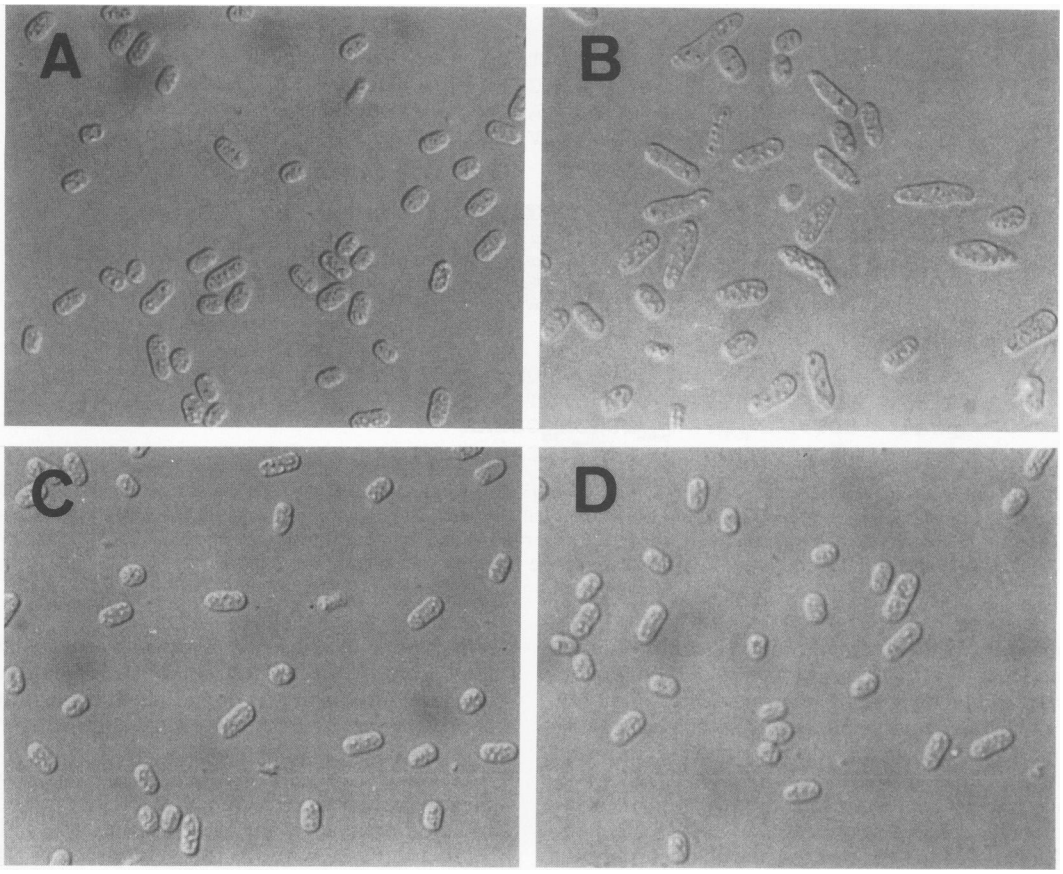

FIG. 5. Analysis of the sensitivity to M-factor by the cell volume assay. Cells of JZ844 (h^+ *map3*::*ura4*$^+$) and JY334 (h^+ *map3*$^+$) were analyzed by using M-factor at 8 U/ml and SSL medium supplemented with adenine and leucine at 20 mg/liter. Details of the assay have been described previously (6). (A) JY334 without M-factor; (B) JY334 with M-factor; (C) JZ844 without M-factor; (D) JZ844 with M-factor. Differential interference micrographs are shown.

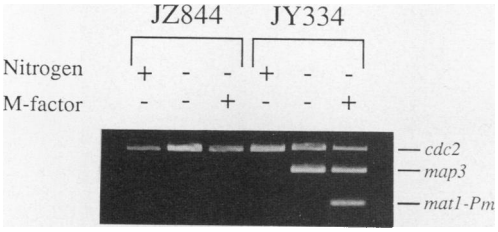

FIG. 6. Analysis of *mat1-Pm* expression by PCR. Total RNA was prepared from either JZ844 or JY334 incubated under the conditions indicated. Three separate reactions were performed on each RNA sample, using oligonucleotide primers specific for either *cdc2*, *map3*, or *mat1-Pm*. The three reaction solutions were then mixed in equal volumes and analyzed by electrophoresis. Primers were selected to generate PCR fragments of 451 bp for *cdc2*, 302 bp for *map3*, and 153 bp for *mat1-Pm*. This assay is not precisely quantitative.

JY334 cells treated similarly (Fig. 6). As controls, expression of *cdc2* in both strains under all the conditions tested and induction of *map3* by nitrogen starvation in JY334 but not JZ844 were confirmed (Fig. 6). Expression of *cdc2* has been shown not to be affected notably by nitrogen starvation (10). Northern blot analysis with a specific probe for *mat1-Pm* also demonstrated that there is no induction of *mat1-Pm* in JZ844 (data not shown).

These results indicate that cells defective in *map3* have no ability to respond to M-factor. This, together with the structural features of the *map3* gene product, strongly suggests that *map3* encodes the receptor for M-factor.

***map3* gene expression: regulation by the mating type, nutritional conditions, and pheromone signaling.** Expression of the *map3* gene was examined by Northern blot analysis. Transcripts of *map3* were judged to be 2.4 kb in length (Fig. 7). We conclude the following from the results summarized in Fig. 7.

Transcripts of *map3* can be seen in h^+, h^{90}, and h^+/h^- cells but not in h^- cells. Nitrogen starvation induces the *map3* transcript, and this induction requires the function of *ste11*, which encodes a transcription factor essential for sexual development (43). The induction is stronger in homothallic (h^{90}) cells than in h^+ cells, given that only about half of the h^{90} cell population is expressing *map3*. Only weak induction of *map3* is seen if the homothallic cells are defective in *gpa1*, which encodes an α-subunit of G-protein that is thought to be coupled with the mating-pheromone receptors (37). Furthermore, the duration of the induction is longer in h^{90} cells than in h^+ cells (Fig. 7B). We therefore conclude that the mating-pheromone signaling is also involved in regulation of *map3* expression.

Artificial expression of *map3* in h^- cells. Plasmid pPTS5 is based on the vector pDB248′ (2) and carries a 2.5-kb *Hin*dIII-*Cla*I fragment that covers the entire *map3* ORF but lacks its promoter. This plasmid can express *map3* from a cryptic promoter carried by the vector, and hence the expression depends on neither the mating type of the cell nor nutritional conditions (data not shown). An h^- strain, JY333, was transformed with pPTS5. This transformant appeared normal when it was growing vegetatively. However, its morphology changed considerably under nitrogen starvation: some cells apparently extended conjugation tubes, and others assumed rather amorphous cell shapes (Fig. 8).

Introduction of a null mutation into either the *ste11* or the

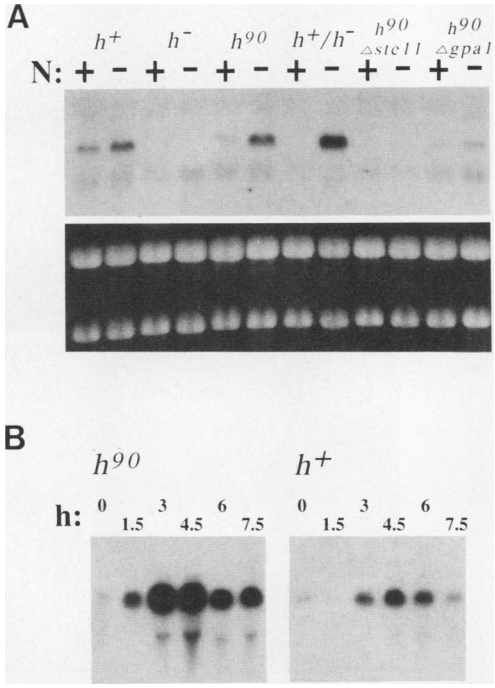

FIG. 7. Expression of the *map3* gene. Transcripts of *map3* were analyzed by Northern blotting. An *Xba*I-*Pvu*II fragment within the *map3* ORF was used as a probe. (A) Total RNA was prepared from heterothallic h^+ haploid cells (JY334), heterothallic h^- haploid cells (JY333), homothallic h^{90} haploid cells (JY450), heterozygous h^+/h^- diploid cells (JY362), and h^{90} cells defective in either *ste11* (JZ396) or *gpa1* (JZ453). The cells were either growing exponentially in the presence of a nitrogen source or starved for nitrogen for 3.5 h. Northern blotting of these RNA preparations (top panel) and ethidium bromide staining that confirms approximately equal loadings of RNA (bottom panel) are shown. The lower faint bands seen in the blotting overlap rRNA and hence appear to be nonspecific. (B) Cells of JY450 (h^{90}) and JY334 (h^+) were transferred to nitrogen-free medium at time zero, and samples were taken at 1.5-h intervals and analyzed by Northern blotting. Ethidium bromide staining confirmed approximately equal loadings of RNA (not shown).

gpa1 gene on the host chromosome blocked this morphological change (Fig. 8). The *ste11* mutation blocks induction of the mating-type genes (43), and the mutant cells do not secrete mating pheromones (28). Thus, the observed morphological change seems to depend on the activity of the mating-pheromone recognition pathway. We conclude that the M-factor receptors produced in a cell carrying pPTS5 can bind M-factor molecules secreted by that cell or by neighboring cells and initiate a mating response. Alternatively, the binding reaction may occur intracellularly. In any case, the observation suggests that an h^- cell is able to respond to M-factor, if the M-factor receptor is expressed ectopically.

Response to either M-factor or P-factor is required for initiation of meiosis. The necessity of pheromone signaling for initiation of meiosis was first suggested by an early observation that *map1/map1* diploid cells are deficient in meiosis but can sporulate if a factor produced by h^+ cells is supplied (11). This notion was subsequently extended by the following observations. (i) The factor is diffusible and may possibly be P-factor itself (27). (ii) Disruption of *mam2*, which encodes the putative P-factor receptor, inhibits sup-

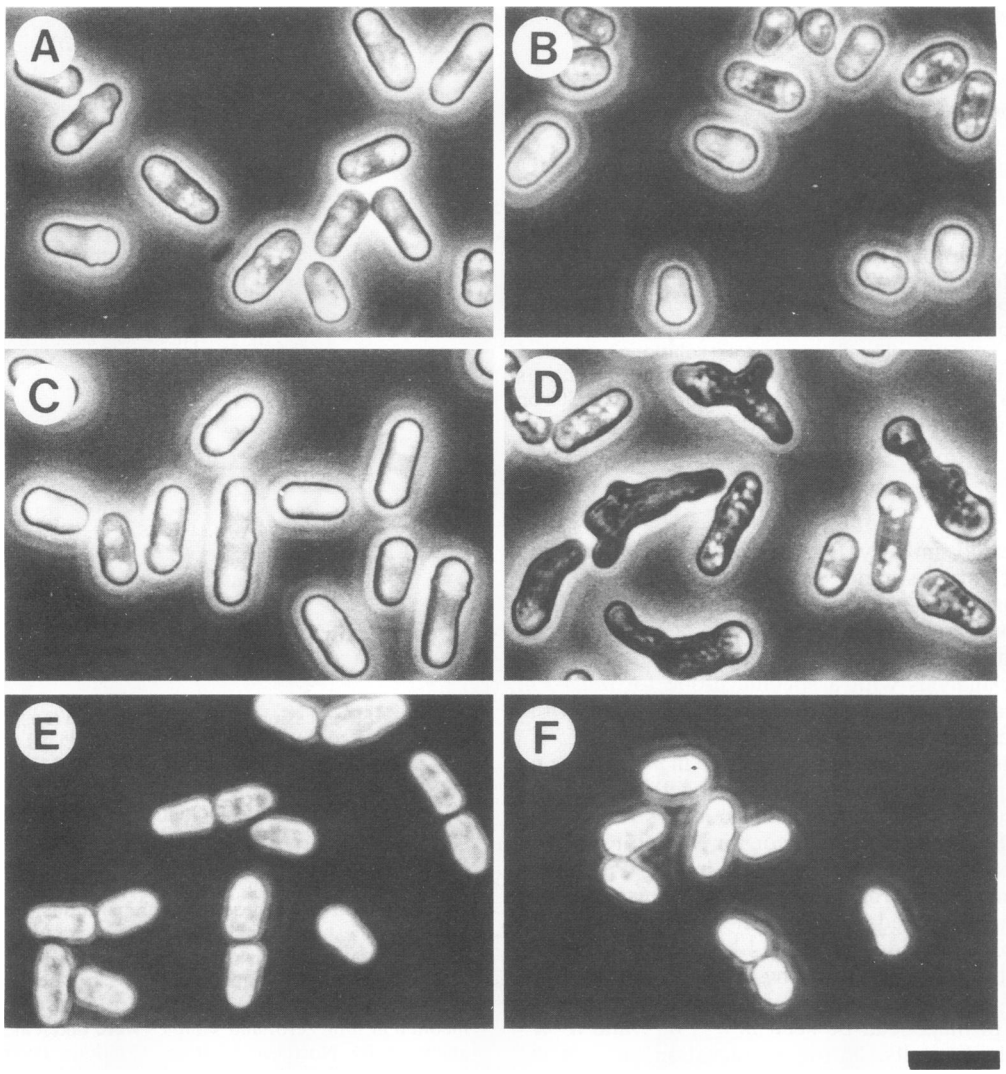

FIG. 8. Phase-contrast micrographs showing the morphology of cells expressing *map3* ectopically. (A) JY333 (h^-) transformed with the vector plasmid, growing in the presence of nitrogen; (B) JY333 transformed with the vector plasmid, starved for nitrogen for 24 h; (C) JY333 transformed with pPTS5 that constitutively expresses *map3*, growing in the presence of nitrogen; (D) JY333 transformed with pPTS5, starved for nitrogen for 24 h; (E) JZ400 (h^- *ste11*) transformed with pPTS5, starved for nitrogen for 24 h; (F) JZ452 (h^- *gpa1*) transformed with pPTS5, starved for nitrogen for 24 h. Bar, 10 μm.

pression of sporulation deficiency by the factor in a *map1*/*map1* diploid (24). (iii) A mutant strain deficient in *gpa1*, which encodes an α-subunit of G-protein apparently coupled with pheromone receptors, is deficient not only in mating but also in meiosis (37).

Once we had identified the gene encoding the putative M-factor receptor, we reexamined the relation between initiation of meiosis and pheromone signaling. The following relevant results were obtained. (i) A *mam2*/*mam2 map3*/*map3* diploid strain was constructed. Although a diploid strain whose genotype is either *mam2*/*mam2* or *map3*/*map3* can sporulate, the double mutant turned out to be unable to undergo meiosis and sporulation (data not shown). (ii) A *map1*/*map1* diploid strain transformed with pPTS5, which expresses *map3* constitutively, is able to sporulate under nitrogen starvation (Fig. 9). From these observations, we can conclude unequivocally that pheromone signaling is essential for initiation of meiosis in *S. pombe* and, further-

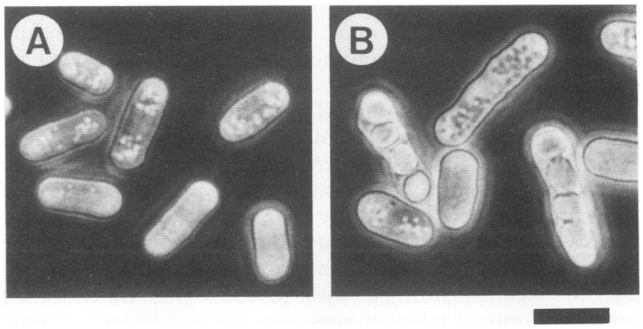

FIG. 9. Suppression of the sporulation deficiency by expression of *map3* in the *map1*/*map1* diploid cells. JZ366 cells (h^{90}/h^{90} *map1*/*map1*) transformed with either the vector (A) or pPTS5 (B) were cultured under nitrogen starvation at 30°C. The phase-contrast micrographs were taken after 24 h incubation. Bar, 10 μm.

more, that either M-factor signaling or P-factor signaling alone is sufficient.

DISCUSSION

This work has shown that the *map3* gene encodes the putative M-factor receptor in *S. pombe*. The deduced *map3* gene product has homology with the **a**-factor receptor of *S. cerevisiae* (18, 34). *S. pombe* M-factor has been shown to be similar to **a**-factor in that it is a small peptide with the C-terminal cysteine residue that is carboxymethylated and *S*-farnesylated (1, 7). Except for the N-terminal tyrosine and the C-terminal cysteine, however, the two peptides show no homology. It has also been shown that the P-factor receptor of *S. pombe* has homology with the α-factor receptor of *S. cerevisiae* (5, 24, 34). Given the distant phylogenetic relationship between the two yeast species (20), it is interesting that they still use similar pheromone receptor systems. *U. maydis* has also been shown to have a similar pheromone-signaling system (4).

The above similarity is puzzling if we consider the difference observed in function of the G-proteins coupled to the mating-pheromone receptors in these two yeasts. In *S. cerevisiae*, the α-subunit of the G-protein encoded by *GPA1* (8, 33) has a regulatory role in induction of mating reactions. It is negatively controlling the function of the βγ complex that transmits the signal downstream (reviewed in reference 29). In *S. pombe*, on the other hand, the α-subunit encoded by *gpa1* is the pheromone signal transmitter and thus functions as a positive factor for mating (37). In addition to this difference in function, *S. pombe* Gpa1 and *S. cerevisiae* Gpa1 are not strikingly homologous in amino acid sequences. We currently have no satisfactory explanation of how these distinct G-proteins have come to couple with similar receptors.

Expression of *map3* was not observed in h^- cells, which appears reasonable in a physiological sense. It was expressed in h^+/h^- diploid cells, and now that pheromone signaling has been shown to be essential for initiation of meiosis, this observation is also comprehensible. Nitrogen starvation induces *map3*, and reinforcement of *map3* induction by pheromone signaling is likely, as mentioned in Results. Thus, the *map3* gene is apparently subject to at least three types of regulation: by the mating type of the cell, by nutritional conditions, and by pheromone signaling. We have shown that induction of *map3* in response to nitrogen starvation requires *ste11*, which encodes a transcription factor for a number of genes involved in sexual development. This induction, however, does not appear to be regulated directly by Ste11, because we find no TR box, which is a binding site of Ste11 (43), in the upstream region of *map3*. It has been shown that *ste11* regulates *mat1-Pc*, which is one of the two transcription units in the *mat1-P* mating-type locus and is essential for conjugation (23). Therefore, although no direct evidence has been obtained yet, *mat1-Pc* is likely to play a key role in the regulation of *map3* transcription.

We previously inferred that the same G-protein, Gpa1, is coupled to both the P-factor receptor and the M-factor receptor (37). In support of this notion, we have observed that h^- cells that ectopically express *map3* are induced to display a mating response. Therefore, the two types of pheromone signaling in *S. pombe* are likely to share a common pathway involving Gpa1. This situation is similar to the one in *S. cerevisiae*, in which exchange of the **a**-factor and α-factor receptors has been more thoroughly studied (3, 35).

Pheromone signaling is essential for the initiation of meiosis in *S. pombe*, in contrast to the situation in *S. cerevisiae*, in which it has no role in meiosis (29). Two *S. pombe* genes referred to in this paper have been shown to be induced intensively in response to pheromone signaling. One is *mat1-Pm*, as described by Nielsen et al. (36), and the other is *sxa2* (22). It is thus likely that a key factor(s) for meiosis is transcriptionally activated by pheromone signaling in *S. pombe*. One obvious possibility is that the factor is *mat1-Pm* itself, because its function has been shown to be essential for meiosis (23). However, this supposition does not appear to be unequivocal, because Nielsen et al. also describe that no *mat1-Pm* induction is seen in *ste6* mutants (36), which are as competent as the wild type in meiosis. More precise quantitative analysis is required to establish the importance of *mat1-Pm* induction in pheromonal regulation of meiosis.

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