The fission yeast mating pheromone P-factor: its molecular structure, gene structure, and ability to induce gene expression and G₁ arrest in the mating partner

Yoshiyuki Imai^{1,2} and Masayuki Yamamoto¹⁻³

¹Division of Cell Proliferation, National Institute for Basic Biology, Okazaki, Aichi 444, Japan; ²Department of Biophysics and Biochemistry, School of Science, University of Tokyo, Hongo, Tokyo 113, Japan

Schizosaccharomyces pombe h^+ cells secrete a diffusible mating pheromone called P-factor. Here we show that the *map2* gene, a defect of which confers h^+ -specific sterility, encodes the precursor of P-factor. We purified P-factor from cells overexpressing *map2* and determined its amino acid sequence. P-factor is a peptide of 23 residues, with the sequence Thr-Tyr-Ala-Asp-Phe-Leu-Arg-Ala-Tyr-Gln-Ser-Trp-Asn-Thr-Phe-Val-Asn-Pro-Asp-Arg-Pro-Asn-Leu. A synthetic peptide of this sequence gave the same specific activity and chromatographic profile as the purified P-factor, suggesting that P-factor is unmodified. h^- cells starved for nutrition showed a morphological response to P-factor. Transcription of the *sxa2* gene, which encodes a protease thought to degrade P-factor, was activated in these cells. The *cyr1* null mutant, which lacks adenylyl cyclase and has little intracellular cAMP, was susceptible to P-factor even in the presence of nutrients. Combination of the *cyr1* and *sxa2* mutations enhanced this susceptibility. P-factor induced not only responses toward mating but also arrest of the cell cycle at the G₁ phase in h^- *cyr1 sxa2* cells. This proves that the *S. pombe* mating pheromone has the ability to arrest cell cycle progression, which has previously been obscured by the usual requirement for mating of nutritional starvation and subsequent growth arrest.

[Key Words: *Schizosaccharomyces pombe*; mating type-specific sterility; peptide sequence; synthetic P-factor; gene induction; cell cycle arrest]

Received September 21, 1993; accepted in revised form December 16, 1993.

Yeasts use diffusible pheromones in cell-cell communication prior to mating. Mating pheromones known to date are short polypeptides, some of which are modified (for review, see Cross et al. 1988). The fission yeast *Schizosaccharomyces pombe* has two mating types, called h^+ and h^- , and cells of the opposite mating types mate and form diploid zygotes when they are starved for nutrition. Physiological evidence has suggested that both h^+ and h^- cells secrete pheromones, named P-factor and M-factor, respectively (Fukui et al. 1986a; Leupold 1987). M-factor has been purified and shown to be a nonapeptide with the carboxy-terminal cysteine residue both S-farnesylated and carboxy-methylated (Davey 1991, 1992). Two genes coding for M-factor, named *mfm1* and *mfm2*, have been identified (Davey 1992). The molecular nature of P-factor, however, has not yet been clarified. An interesting question concerning the fission yeast pheromones is whether they have a cytostatic ac-

tivity. Although a- and α -factors of *Saccharomyces cerevisiae* clearly arrest cells of the opposite mating type at the G₁ phase (Buckling-Throm et al. 1973; for review, see Cross et al. 1988; Marsh et al. 1991), the above question has been unsolved because *S. pombe* cells communicate by mating pheromones only in the absence of nutrition, which automatically leads them to G₁ arrest.

Mating type-specific sterility genes are likely to encode peptides or proteins involved specifically in cell-cell interaction during mating. Four h^+ -specific sterility genes, *map1* through *map4*, and four h^- -specific sterility genes, *mam1* through *mam4*, have been identified in *S. pombe* (Egel 1973; Tanaka et al. 1993; Y. Imai and M. Yamamoto, unpubl.), and some of them have been characterized. The *map3* and *mam2* genes encode the pheromone receptors, *map3* for M-factor and *mam2* for P-factor (Kitamura and Shimoda 1991; Tanaka et al. 1993). The *map4* and *mam3* genes encode putative agglutinins (Y. Imai and M. Yamamoto, unpubl.). In addition to the above mating type-specific genes, which show relatively clear phenotypes, two genes are known to cause leaky

³Corresponding author.

mating type-specific sterility when disrupted. These genes, named *sxa1* and *sxa2*, encode putative proteases that possibly degrade M-factor and P-factor, respectively (Imai and Yamamoto 1992). Their defects make the cells hypersensitive to the pheromone and block them from proper mating.

From physiological analysis, we suspected that the *map2* gene, which is required for production of P-factor, may encode P-factor itself. DNA sequence analysis of *map2* supported this notion. We then purified P-factor from a *map2*-overexpressing strain. Determination of its amino acid sequence unequivocally confirmed that *map2* is the structural gene for P-factor. Synthesized P-factor showed the same biological activities as the purified one. In addition to presenting these results, we will demonstrate here that P-factor has a potential to arrest the cell cycle of *h⁻* cells at the G₁ phase. Some aspects of gene regulation by P-factor signaling will also be discussed.

Results

Cloning and disruption of the *map2* gene

Mutations in the *map2* gene confer *h⁺*-specific sterility (Egel 1973). Analysis of the *map2* mutants by the same bioassay as we employed previously (Tanaka et al. 1993) indicated that they are able to respond to M-factor but do not produce a detectable amount of P-factor (data not

shown). We thus suspected the possibility that *map2* encodes P-factor and set out to clone this gene. As described in the Materials and methods, we obtained five clones with overlapping inserts that could complement a *map2* mutation. One of these clones, pST621-5, was studied further. pST621-5 contained a 5.4-kb insert, a restriction map of which is shown in Figure 1. A 1.7-kb *SpeI*-*Scal* fragment was sufficient for complementation of *map2* and an intronless 603-bp open reading frame (ORF) was found on it (Fig. 1). One step disruption of the ORF on the chromosome was carried out in a homothallic haploid strain, as detailed in the Materials and methods and Figure 1. Successful disruption was confirmed by Southern hybridization analysis (data not shown). The disruptant (JZ800) showed essentially the same phenotypes as the original *map2* isolates. Tight linkage was observed between *map2* and the disrupted gene in genetic crosses (data not shown), indicating that the cloned gene is indeed *map2*.

The map2 gene product is a protein with a putative signal sequence and repeats of 34 amino acid residues

The predicted *map2* gene product (Map2) had 201 amino acid residues, and its amino terminus was hydrophobic, suggesting that this region might serve as a signal sequence (Fig. 1). Map2 carried four tandem copies of 34 amino acid residues, each of which terminated with trib-

Figure 1. A restriction map, gene disruption, and the nucleotide sequence of *map2*. (a) A restriction map of the insertion in pST621-5, which can complement the *map2* mutation, is shown. An arrow indicates the direction and extent of the *map2* ORF. The ability of subclones derived from pST621-5 to complement *map2* is shown as (+) or (-) on the right. The structure of a linear DNA fragment used for disruption of the *map2* gene is depicted at the bottom. Restriction sites are abbreviated as follows: (E) *EcoRI*; (EV) *EcoRV*; (Nd) *NdeI*; (P) *PstI*; (Sc) *Scal*; (Sp) *SpeI*. (b) The nucleotide sequence of the *map2* gene and the deduced amino acid sequence of its product. The sequence of a 1.7-kb *SpeI*-*Scal* fragment carrying the *map2* ORF is shown. Numbering starts at the first methionine codon of the ORF. Putative TATA boxes are underlined. Possible N-linked glycosylation sites are doubly underlined. Sequences that are considered to become mature P-factor are boxed and possible cleavage signals, Lys and Lys-Lys-Arg, are shown in white against black. The *NdeI* and *EcoRI* sites that are the termini of the DNA fragment deleted for gene disruption are marked with dots. The accession number of this nucleotide sequence in the GSDB, DDBJ, EMBL, and NCBI data bases is D26072.

asic residues Lys-Lys-Arg. Two possible *N*-linked glycosylation sites were seen near the carboxyl terminus of Map2. Glycosylation has been shown to be important for the transport of the α -factor precursor through the secretory pathway in *S. cerevisiae* (Julius et al. 1984a; Caplan et al. 1991). Thus, all of these features are consistent with the suggestion that Map2 is a P-factor precursor. The features of Map2 are schematically shown in Figure 2, together with those of the *S. cerevisiae* α -factor precursor for comparison. A cluster of acidic amino acids was seen at position 161–172 of Map2, although its meaning is unclear.

Expression of the *map2* gene

Expression of *map2* was examined in various strains. The *map2* gene was transcribed into a 1.2-kb transcript. Transcription of *map2* was induced by nitrogen starvation in haploid heterothallic h^+ cells, haploid homothallic h^{90} cells and diploid h^+/h^- cells, but was not seen in haploid heterothallic h^- cells (Fig. 3). Transcriptional activation of *map2* was much stronger in h^{90} cells than in h^+ cells, suggesting that *map2* expression is enhanced by pheromone signaling.

Ectopic expression of *map2* makes h^- cells secrete P-factor

Expression of *map2* was not detectable in h^- cells. We examined phenotypes of an h^- strain that ectopically expressed *map2*. To make the results clearer, two types of *ras1* mutants were used here. JZ318 (h^- *ras1*^{Val-17}) was used as a responder to P-factor because *ras1*^{Val-17} mutants are hypersensitive to the mating pheromones (Fukui et al. 1986b; Nadin-Davis et al. 1986). JZ838 (h^- *ras1*⁻), which is insensitive to the pheromone and has a round cell shape easily distinguishable from the wild type, was used as the host strain in which *map2* was ectopically expressed. If JZ318 cells were mixed with JZ838 cells transformed with the vector plasmid, they showed no response. However, if JZ318 cells were mixed with JZ838 cells transformed with pADMP2, which constitutively expressed *map2* (see Materials and methods), they extended conjugation tubes (Fig. 4). This observation suggests again that *map2* is the structural gene for P-factor, and furthermore, that processing and secretion of P-factor can be properly performed even in h^- cells.

Figure 2. Comparison of the structural features of the *map2* gene product (a) and the *S. cerevisiae* Mfa1 gene product, one of the α -factor precursors (b). Gray boxes represent putative signal peptides, black boxes represent basic residues that may serve as cleavage signals, and hatched boxes represent sequences that correspond to mature pheromones. Possible *N*-linked glycosylation sites are shown by arrowheads.

Figure 3. Dependence of *map2* gene expression on the mating type, nitrogen starvation, and pheromone signaling. JY1 (h^-) (lanes 1,2), JY2 (h^+) (lanes 3,4), JY3 (h^{90}) (lanes 5,6), and JY919 (h^+/h^-) (lanes 7,8) were cultured in PM medium. When they were grown to log phase ($4\text{--}6 \times 10^6$ cells/ml), half of the culture was harvested (lanes 1,3,5,7). The remainder was transferred to PM-N medium, which lacked the nitrogen source, and incubated for 4 hr more before the cells were collected (lanes 2,4,6,8). Total RNA was prepared from these samples. Ten micrograms of each RNA preparation was separated in formaldehyde-agarose gel electrophoresis, blotted onto a membrane, and analyzed with a hybridization probe carrying the *map2* ORF. rRNA stained with ethidium bromide (bottom panel) indicates that approximately the same amount of RNA was loaded in each lane.

The *sxa2* mutant is the most sensitive to P-factor among *S. pombe* strains currently available

The response of h^- cells to P-factor is much weaker than that of h^+ cells to M-factor (Fukui et al. 1986a), and the use of h^- wild-type cells as the responder to detect P-factor was not very appropriate. To improve the assay procedure for P-factor, we compared three mutants that apparently were hypersensitive to the pheromone, namely *ras1*^{Val-17} (Fukui et al. 1986b; Nadin-Davis et al. 1986), *gap1* (Imai et al. 1991) and *sxa2* (Imai and Yamamoto 1992) mutants. Among them, the *sxa2* mutant extended conjugation tubes in the presence of the smallest amount of P-factor (Fig. 5). By use of this mutant, a semi-quantitative assay of P-factor was possible, as described in the Materials and methods. One unit of P-factor was defined as the minimal amount that induces detectable elongation of conjugation tubes in h^- *sxa2* cells in a 1-ml culture. The wild-type cells slightly increased their cell volume but did not elongate obvious conjugation

Figure 4. Ecotopic expression of the *map2* gene induces P-factor production in h^- cells. JZ838 (h^- *ras1*) was transformed with either pADMP2 (A), which overexpresses *map2*, or a vector pART1 (B), and was mixed with JZ318 (h^- *ras1*^{Val-17}) hypersensitive to P-factor. After 24 hr of incubation on SPA plates, photographs were taken under the phase contrast microscope. JZ838 appears as cells with roundish morphology in both panels A and B. JZ318 obviously responds to the pheromone and elongates conjugation tubes in panel A. Bar, 10 μ m.

tubes even when they were exposed to 100 U/ml of P-factor (data not shown).

Purification of P-factor and determination of its structure

Overexpression of *map2* in h^+ cells increased the activity of secreted P-factor (data not shown). We therefore used an h^+ strain overexpressing *map2* as the source for purification of P-factor. The supernatant of a late-log culture of JZ836 transformed with pADMP2 was filtered and processed as described in the Materials and methods (Fig. 6). About 200 μ g of purified P-factor, with a specific activity of about 6 U/ μ g, was obtained from 1-liter culture. Amino acid sequencing of the P-factor preparation was done by automated Edman degradation and the peptide sequence shown in Figure 6d was deduced (data not shown). The deduced sequence of 23 amino acids matches the second and fourth repeats of the predicted *map2* gene product (Fig. 1). A comparison of the mature P-factor and Map2 suggests that basic residues, namely Lys and Lys-Lys-Arg, are the likely cleavage signals of the P-factor precursor (Figs. 1,2).

A synthetic peptide exhibits the P-factor activity

A peptide that has the same sequence as the purified P-factor was synthesized by use of an automated synthesizer. The synthesized P-factor showed an activity to induce elongation of conjugation tubes in JZ606 (*sxa2*), and its specific activity was about 6 U/ μ g of peptide (data not shown), a value similar to that of the purified P-factor. The retention time of the synthesized peptide in the reverse phase column chromatography was indistinguishable from that of the purified P-factor, and the mixture of

the two migrated in a single peak (Fig. 6e–g). These results strongly suggested that they are identical and that natural P-factor is unmodified. We therefore used synthetic P-factor in the subsequent experiments.

Transcriptional activation of the *sxa2* gene by P-factor

M-factor induces transcription of the mating-type gene *mat1-Pi* in h^+ cells under nitrogen starvation (Nielsen et al. 1992). This has been the only clear example of gene expression regulated by pheromone signaling in *S. pombe*. We previously pointed out the possibility that the *sxa2* gene is inducible in h^- cells by P-factor (Imai and Yamamoto 1992). To examine this possibility, we added synthetic P-factor to h^- cells starved for nitrogen. The following results were obtained. Transcription of the *sxa2* gene was strongly induced in 2 hr and was down-regulated in 4 hr (Fig. 7a). If the amount of P-factor applied was increased, *sxa2* was induced more strongly (Fig. 7b). The transcriptional activation of *sxa2* by P-factor required the function of *ras1* and *gpa1* (Fig. 7b), both of which have been shown to be essential for the mating response (Fukui et al. 1986b; Nadin-Davis et al. 1986; Obara et al. 1991).

Nutritional starvation was required for the induction of *sxa2* (Imai and Yamamoto 1992). When we added P-factor to h^- cells growing exponentially in rich medium, we saw no induction of *sxa2* until very weak induction started at 6 hr, which was due to gradual starvation (Fig. 7c). However, if P-factor was added to the *cyr1* mutant (JZ300) growing exponentially, the *sxa2* gene was strongly induced in 1 hr and down-regulated in 4 hr. The *cyr1* gene encodes adenyl cyclase (Yamawaki-Kataoka et al. 1989; Young et al. 1989). Mutants defective in *cyr1* have no detectable cAMP (Maeda et al.

Figure 5. Morphological changes of h^- *sxa2* cells induced by the addition of P-factor. JZ606 (h^- *sxa2*) was cultured in YPD medium overnight and shifted to MEL medium, as described in Materials and methods. After 6 hr of incubation, 3 units of purified P-factor was added to 1 ml of the culture and incubation was continued for 24 hr more. Photographs were taken under the phase contrast microscope. In panel A, JZ606 elongates conjugation tubes in response to P-factor. (B) Control experiment in which P-factor was omitted. Bar, 10 μ m.

Figure 6. Purification of P-factor. (a) A typical pattern obtained in Amberlite XAD-2 chromatography. JZ836 transformed with pADMP2 was cultured in SD medium to late log phase and the medium was processed as described in the Materials and methods. Materials adsorbed to XAD-2 were eluted by a stepwise gradient of methanol and monitored by OD₂₈₀. Fractions carrying the P-factor activity are indicated by a gray box. (b) A typical pattern obtained in gel filtration with Sephadex LH-20. The fractions obtained in the previous step were pooled, concentrated, and subjected to gel filtration as in the Materials and methods. Active fractions are indicated by a gray box. (c) The pattern obtained in reverse-phase column chromatography using PepRPC HR10/10, which gave the P-factor preparation subjected to amino acid analysis. The fractions obtained in the previous step were pooled, concentrated, and loaded on a column of PepRPC HR10/10, as detailed in the Materials and methods. The active peak is indicated by a gray box. (d) The amino acid sequence of the purified P-factor, determined by automated Edman degradation. (e–g) Demonstration of the identical retention time of the purified and synthesized P-factor in reverse-phase column chromatography. A column of PepRPC HR5/5 (Pharmacia) was run under the same conditions as described for PepRPC HR10/10, except that the flow rate was 0.7 ml/min. Elution patterns of 10 µg of the purified P-factor (e), 10 µg of the synthesized P-factor (f), and their mixture (g) are shown. We deliberately display here the results obtained with a purified P-factor preparation that still contains some impurities, because the peak of the impurities apparently serves as a nice internal marker. The same kind of experiments have been repeated using a purer preparation of P-factor.

1990; Kawamukai et al. 1991) and genes required for sexual development are ectopically derepressed in them (Sugimoto et al. 1991). Our observation suggests that the P-factor signaling requires the function of genes that is repressed via the cAMP pathway when the nutritional conditions are favorable for vegetative growth.

P-factor has a potential to induce growth arrest at the G₁ phase in the recipient cell

M-factor has been shown to induce no obvious growth arrest in vegetatively growing *h⁺* cells (Davey 1991). Similarly, addition of P-factor to growing *h⁻* wild-type cells caused no growth delay (Fig. 8a). This is not surprising, because pheromone signaling calls for nutritional starvation as a prerequisite. To circumvent this problem, we tested mutant strains as the acceptor of P-factor. Addition of P-factor to either *cyr1⁻* or *sxa2⁻* vegetative cells caused no obvious growth delay (Fig. 8b,c). However, the *cyr1 sxa2* double mutant (JZ916) arrested growth in response to P-factor, doubling the cell number once after the addition (Fig. 8d). This arrest was

transient, and the cells began to repropagate several hours after the arrest (Fig. 8d). The growth recovery occurred in the medium that still contained a sufficient amount of P-factor to cause the cell-cycle arrest in fresh cells (data not shown, see Discussion). The pheromone-treated cells saturated at a lower cell density than the control that received no P-factor. Different concentrations of P-factor (0.2, 2, 20 µg/ml) gave essentially the same results (Fig. 8d).

Cell morphology of JZ916 suffering the growth arrest by P-factor was examined (Fig. 9). Cell elongation and an increase in the cell volume were observed 3 hr after the addition of P-factor. After 9 hr, almost all cells elongated conjugation tubes and the cell volume reached its maximum. After this point the cells started to divide again and gradually resumed the normal morphology. The saturated culture was a mixture of normally shaped cells and elongated cells.

The DNA content of the arrested JZ916 cells was monitored by flow cytometry (Fig. 10). JZ916 cells growing exponentially showed G₂-equivalent DNA content (2C), as has been documented with vegetative *S. pombe* cells

Figure 7. Transcriptional activation of the *sxa2* gene by P-factor. (a) JY333 (*h⁻* wild type) grown to 4×10^6 cells/ml in PM medium was shifted to nitrogen-free PM-N medium, and synthetic P-factor was added to a final concentration of 2 μ g (12 U)/ml. Cells were harvested at intervals and RNA was prepared from each aliquot. Expression of *sxa2* was examined by Northern blotting. (b) JY333, JZ838 (*ras1*), and JZ452 (*gpa1*) were subjected to nitrogen starvation as above and treated with various concentrations of P-factor. After 2 hr of incubation, cells were harvested and expression of *sxa2* was examined. (c) JY333 and JZ300 (*cyr1*) were grown to log phase $2\text{--}3 \times 10^6$ cells/ml in medium with ample nitrogen (SD), and P-factor was added to a final concentration of 2 μ g/ml. Cells were harvested at intervals and *sxa2* expression was examined. In each lane throughout the panels, 10 μ g of RNA denatured with formamide was loaded. rRNA stained with ethidium bromide, shown under each lane, proves loading of approximately the same amount of RNA in each lane.

(Bostock 1970). Cells carrying G_1 -equivalent DNA content (1C) began to accumulate 1 hr after the addition of P-factor. After 4–6 hr, most cells were with 1C. After 9 hr, 2C cells emerged again, and cell division restarted. The saturated culture was a mixture of cells with 1C and 2C (Fig. 10a). If no P-factor was added, cells with 1C did not appear during the incubation, and the saturated culture contained only 2C cells under the current experimental conditions (Fig. 10b).

Discussion

The pair of mating pheromones and the pair of mating

pheromone receptors in the fission yeast have now been identified. A comparison of them with their counterparts in the budding yeast *S. cerevisiae*, which is only distantly related to *S. pombe*, reveals high similarity between the two systems. As we and others discussed previously, the P-factor receptor (Mam2) (Kitamura and Shimoda 1991) has homology with the α -factor receptor (STE2) (Burkholder and Hartwell 1985; Nakayama et al. 1985), and the M-factor receptor (Map3) (Tanaka et al. 1993) has homology with the a-factor receptor (STE3) (Nakayama et al. 1985; Hagen et al. 1986). M-factor (Davey 1992) and a-factor (Anderegg et al. 1988) share common structural features, although their peptide se-

Figure 8. Effects of P-factor on cell growth of various strains. JY333 (wild type) (a), JZ300 (*cyr1*) (b), JY606 (*sxa2*) (c), and JZ916 (*cyr1 sxa2*) (d) were grown in SD medium at 30°C. When the cell concentration reached $3\text{--}4 \times 10^6$ cells/ml, each culture was divided into portions, and they received various concentrations of P-factor. Cultivation was continued, and the cell growth was chased by counting the cell number under the microscope. Concentration of P-factor added: (●) 0 μ g/ml, (△) 0.2 μ g/ml, (□) 2 μ g/ml, and (○) 20 μ g/ml. JZ300 and JZ916 grew more slowly than the other two strains because of their defect in *cyr1* (Maeda et al. 1990; Kawamukai et al. 1991).

Figure 9. Cell morphology of *cyr1 sxa2* cells exposed to P-factor. JZ916 treated with (a) or without (b) 2 µg/ml P-factor was chronologically analyzed. The samples correspond to open squares (a) and closed circles (b) in Fig. 8d. Photographs taken under the Nomarski differential interference microscope are shown. Bar, 20 µm.

quences differ considerably. They both have a cysteine residue at the carboxyl terminus, which is S-farnesylated and carboxy-methylated. Their precursors terminate with the CAAX motif and have no signal sequence.

Similarity between *S. pombe* P-factor and *S. cerevisiae* α -factor (Stotzler et al. 1976; Kurjan and Herskowitz 1982; Singh et al. 1983) became evident in this study. They both are produced from a large precursor that car-

ries a signal sequence, basic amino acid residues for cleavage signals, possible N-linked glycosylation sites and repeats of the mature pheromone sequence. Although P-factor and α -factor have quite different amino acid sequences, they are both simple peptides.

Well-characterized yeast mating pheromones, including *S. cerevisiae* α -factor and a-factor and *S. pombe* M-factor, are known to be encoded by more than one gene. However, P-factor is likely to be encoded only by *map2*, because disruption of the *map2* gene confers complete sterility and Southern hybridization analysis suggests no homolog of *map2* on the *S. pombe* genome (data not shown). Thus, *map2* appears to be an exceptional pheromone gene that is not redundant and can be identified as a sterility gene.

The predicted *map2* gene product has four repetitive units. The second and fourth units can generate the P-factor sequence identified by the analysis of the purified preparation. The first and third units encode peptides slightly different from it. Although potentially three species of P-factor can be produced from Map2, one of them, which we purified, apparently predominates the others in quantity. The fate of the other two species is unclear. It is possible that the minor species are less active or less stable and have eluded our detection. More precise biochemical analysis is required to reach a firm conclusion.

h⁻ cells in which *map2* was ectopically expressed secreted P-factor, suggesting that enzymes required for the processing of the P-factor precursor exist both in *h⁺* and *h⁻* cells. The processing of the P-factor precursor may be analogous to the case of *S. cerevisiae* α -factor. At least three enzymes are involved in processing of α -factor precursors: KEX2, an endopeptidase that cleaves the carboxy-terminal side of Lys-Arg (Julius et al. 1984b); KEX1, a carboxypeptidase that removes basic residues from the

Figure 10. Flow cytometric analysis of *cyr1 sxa2* cells exposed to P-factor. Samples taken from the same series of JZ916 cultures as analyzed in Figs. 8d and 9 were fixed with 70% ethanol, stained with propidium iodide, and subjected to flow cytometry. (a) JZ916 grown in the presence of 2 µg/ml P-factor, and (b) with no P-factor. The main peaks at 0 hr (0h) in panels (a) and (b) correspond to 2C (G_2 -equivalent) DNA content, whereas the main peak at 6 hr (6h) in a corresponds to 1C (G_1 -equivalent) DNA content. Note that the peaks tend to drift toward the right as the cell volume gets larger, even if the DNA content is constant.

carboxyl terminus [Dmochowska et al. 1987]; and STE13, a dipeptidyl aminopeptidase that removes dipeptides (X-Ala) from the amino terminus [Julius et al. 1983]. Whether *S. pombe* has counterparts of these processing enzymes remains to be elucidated.

The *sxa2* gene encodes a putative serine carboxypeptidase that is likely to be secreted [Imai and Yamamoto 1992]. We have suggested that the *sxa2* gene product may be involved in degradation of P-factor, because a defect in *sxa2* confers hypersensitivity to P-factor. Here we demonstrated that the *sxa2* mutant is sensitive to P-factor at least 100 times more than the wild type and that expression of *sxa2* depends on the acceptance of P-factor. The ability of Sxa2 to inactivate P-factor seems very strong. Once *sxa2* is induced and Sxa2 is secreted in response to P-factor, this protease will extensively degrade P-factor and protect the recipient cells from excessive response. In RNA blot analysis, we found that induction of *sxa2* was transient, probably because generated Sxa2 protease rapidly degraded P-factor, and the signaling was extinguished. However, because adaptation occurs even in the *sxa2* mutant cells, degradation of P-factor cannot be the only mechanism to downregulate pheromone signaling in *S. pombe* (see below). Sxa2 protease resembles *S. cerevisiae* BAR1 [MacKay et al. 1988], a protease that is thought to degrade α -factor, in many aspects, but curiously enough Sxa2 is a serine carboxypeptidase, whereas BAR1 is an aspartyl protease.

In *S. pombe*, the mating response occurs only under starvation for nutrients. Many genes essential for the mating response are transcriptionally activated by nitrogen starvation. In other words, they are expressed only in cells no longer growing. Thus, it is impossible to examine by physiological means whether the mating pheromones can block the cell cycle in *S. pombe*. However, by the use of synthetic P-factor and two types of mutations, namely *cyr1*, which allows ectopic expression of the genes required for mating, and *sxa2*, which reduces down-regulation of the signaling, we have demonstrated that P-factor has a potential to arrest the cell cycle of h^- cells at the G_1 phase. Thus, the pheromones of *S. pombe*, at least P-factor, appear to share the basic characteristics with the *S. cerevisiae* pheromones, which arrest the cell cycle of the partner at Start, a point in late G_1 prior to the initiation of DNA synthesis [Buckling-Throm et al. 1973]. In *S. cerevisiae*, the pheromone signaling apparently prohibits G_1 progression by preventing the function of G_1 cyclins (see March et al. 1991). It is very intriguing to see whether *S. pombe* employs a similar mechanism.

The two pheromone receptors encoded by *mam2* and *map3* both have potential seven transmembrane domains [Kitamura and Shimoda 1991; Tanaka et al. 1993], and are thought to be coupled with a single G-protein, the α subunit of which is encoded by *gpa1* [Obara et al. 1991]. Seven transmembrane receptors coupled with G-proteins generally are subjected to a regulation called adaptation, which diminishes the signaling after a certain duration even if the ligand continuously stays on its receptor. This phenomenon appears to be controlled by

various factors, which include phosphorylation of receptors and G-proteins [for review, see Dohlman et al. 1991; Marsh et al. 1991]. We observed that the *cyr1 sxa2* mutant was arrested at the G_1 phase in response to P-factor but the arrest was transient. This cannot be explained by the degradation of P-factor, because the strain lacked Sxa2 protease and the growth recovery indeed occurred in the presence of P-factor. Thus *S. pombe* is quite likely to have an adaptation mechanism similar to other G-protein systems.

Our observations will give the following view. In wild-type *S. pombe* cells growing in rich medium, the level of intracellular cAMP is high and many proteins necessary for pheromone signaling are not made. Only under nutritional starvation and a concomitant decrease in the level of intracellular cAMP do the proteins become available and the signaling pathway operational. P-factor is now able to induce various changes in h^- cells, including alteration of the cell morphology toward mating, arrest of the cell cycle at the G_1 phase and transcriptional activation of the *sxa2* gene to down-regulate the signaling. The enforcement of G_1 arrest to nutritionally starved cells by the mating pheromone probably is meaningful to assure efficient mating, because *S. pombe* cells can enter the resting state from both G_1 and G_2 phases, depending on the nutritional conditions [Bostock 1970].

Materials and methods

Yeast strains, genetic procedure, and media

S. pombe strains used in this study are listed in Table 1. General genetic procedures for *S. pombe* have been described [Gutz et al. 1974]. Sterile mutants were crossed by protoplast fusion [Sipiczki and Ferenczy 1977]. A lithium method was used for transformation of *S. pombe* [Okazaki et al. 1990]. A qualitative assay of the mating efficiency was done by staining colonies with iodine vapor, which stains spores dark brown. Yeast media YPD and SD [Sherman et al. 1986], SSA [Egel and Egel-Mitani 1974], SPA and MEL [Gutz et al. 1974], and PM and its nitrogen-free version PM-N [Beach et al. 1985; Watanabe et al. 1988] were used. SD used here contained only 1% glucose.

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

Strain	Genotype
JY1	h^- wild type
JY2	h^+ wild type
JY3	h^{90} wild type
JY333	$h^- ade6-M216 leu1$
JY878	$h^{90} ade6-M216 leu1 ura4-D18$
JY919	$h^+ / h^- ade6-M210/ade6-M216$
JZ300	$h^- cyr1::ura4^+ ade6-M216 leu1 ura4-D18$
JZ318	$h^- ras1^{Val-17} << LEU2 ade6-M216 leu1$
JZ361	$h^+ mat2,3::LEU2 ras1^{Val-17} << LEU2 ade6-M216 leu1 his2$
JZ452	$h^- gpa1::ura4^+ ade6-M216 leu1 ura4-D18$
JZ606	$h^- sxa2::ura4^+ ade6-M216 leu1 ura4-D18$
JZ731	$h^{90} map2-621 ade6-M216 leu1$
JZ800	$h^{90} map2::ura4^+ ade6-M216 leu1 ura4-D18$
JZ836	$h^+ ras1::ura4^+ ade6-M216 leu1 ura4-D18$
JZ838	$h^- ras1::ura4^+ ade6-M216 leu1 ura4-D18$
JZ916	$h^- cyr1::ura4^+ sxa2::ura4^+ ade6-M216 leu1 ura4-D18$

Cloning and sequencing of the map2 gene

Essentially the same protocol was followed as we employed when we cloned *map3* (Tanaka et al. 1993). JZ731 (*h⁹⁰ map2-621 ade6-M210 leu1*) was transformed with an *S. pombe* genomic library made of *Sau3AI* partial digests of the chromosome and the vector pDB248' (Beach et al. 1982). Colonies of the transformants on synthetic sporulation agar (SSA) plates were stained with iodine vapor to screen for mating and sporulation positives. Plasmids were recovered from positive colonies into *E. coli*. We thus obtained five candidate plasmids with overlapping inserts, which were subsequently proven to carry the *map2* gene. A 1.7-kb *SpeI*–*SalI* fragment carrying *map2* was cloned into pUC119 and unidirectional deletions of the insert DNA were generated using Exonuclease III and S1 nuclease (Takara Shuzo) (Henikoff 1984). Single-stranded DNA was prepared from the deletion series by infection of a helper bacteriophage M13KO7. The nucleotide sequence of *map2* was determined by the dideoxy chain termination method (Sanger et al. 1977) with the Sequenase kit (U.S. Biochemical Corp.) and [α -³²P]dCTP. All the sequence shown in Figure 1 was determined in both orientations.

Disruption of the map2 gene

A 0.45-kb *NdeI*–*EcoRI* fragment was eliminated from the cloned *map2* ORF and the *S. pombe ura4⁺* cassette (Grimm et al. 1988) was inserted. An *EcoRV*–*NdeI* fragment carrying the disrupted *map2* ORF (Fig. 1) was used to transform a homothallic haploid strain JY878. Most of the stable *Ura⁺* transformants were sterile and the proper replacement of the wild-type allele with the disrupted *map2* construct was confirmed by Southern blot analysis (Southern 1979) of their genomic DNA.

Preparation and Northern blot analysis of RNA from S. pombe

S. pombe cells ($1\text{--}5 \times 10^8$) were collected by centrifugation and broken by vortexing vigorously with glass beads in a 0.2 M Tris-HCl (pH 7.5) containing 0.5 M NaCl, 0.01 M EDTA, and 1% SDS. After extraction with phenol–chloroform (1:1), RNA was recovered by ethanol precipitation. Ten micrograms of each RNA preparation was denatured with formamide, separated by formaldehyde gel electrophoresis (Sambrook et al. 1989) and blotted to a membrane (Hybond-N, Amersham). Probe DNA containing either the *map2* or the *sxa2* sequence was labeled with [α -³²P]dCTP by random priming.

Assay of P-factor

JZ606 (*h⁻ sxa2*) cells, which are hypersensitive to P-factor (Imai and Yamamoto 1992; this study), were grown overnight in YPD medium. They were collected by centrifugation, resuspended in malt-extraction liquid (MEL) medium at a concentration of $2\text{--}4 \times 10^6$ cells/ml, and incubated for 6 hr. A small volume of P-factor suspension in methanol ($<10 \mu\text{l}$) was added to each 1-ml aliquot of the JZ606 culture. After incubation for 24 hr, cell morphology was inspected microscopically. The P-factor activity was detectable by elongation of conjugation tubes in JZ606. Every incubation was done at 30°C with aeration. A semi-quantitative estimation of the P-factor concentration was done as follows. Serial twofold dilutions of a P-factor sample were prepared, and the lowest concentration that can cause a visible response in JZ606 cells was defined as 1 U/ml. One unit turned out to be roughly 170 ng for both purified and synthesized P-factor.

Construction of a plasmid, pADMP2, that overexpresses map2

The expression vector pART1 (McLeod et al. 1987) is based on pUC118 and carries the *S. pombe adh* promoter, *ars1*, and the *S. cerevisiae LEU2* gene, which can complement *S. pombe leu1*. A *map2*-overexpressing plasmid pADMP2 was constructed by insertion of a 0.8-kb *DraI* fragment that covers the *map2* ORF into the *SmaI* site of pART1. The *map2* gene on pADMP2 was constitutively expressed from the *adh* promoter.

Purification of P-factor

The polystyrene resin Amerlite XAD-2 (Organo) was washed with excess water, packed into a column, and washed with 6 column volumes each of methanol and water at a flow rate of 1–2 column volumes/hr. Washed resin was pooled in water and sterilized by autoclaving. JZ836 (*h⁺ ras1*) transformed with pADMP2 was grown to late log-phase ($2\text{--}4 \times 10^7$ cells/ml) in SD medium. Empirically, *ras1* strains produced more mating pheromones than the wild type. The supernatant of the culture was filtrated through a 0.2- μm pore-sized filter (Nalgene). After filtrating, 0.1 volume of the resin was added to the filtrate, and the mix was incubated for 20–24 hr at 30°C with gentle shaking. Then the resin was washed several times with water and packed into a column. The column was washed with 40% methanol, and the P-factor fraction was eluted with 80% methanol (3 column volumes each, with a flow rate of 1–2 volumes/hr). The eluted material was dried in a rotatory evaporator and dissolved in a small volume of methanol. Insoluble materials were removed by centrifugation. The sample was loaded on a column (1.1 $\times$ 90 cm) of Sephadex LH-20 (Pharmacia) in methanol and was eluted at a flow rate of 6 ml/hr. Each fraction (2.2 ml) was dried, dissolved in a small volume of methanol, and assayed for the P-factor activity. Active fractions were pooled, dried, and dissolved in a small volume of 25% (vol/vol) acetonitrile, 0.1% (vol/vol) trifluoroacetic acid (TFA). Insoluble materials were removed and the sample was loaded on a reverse phase FPLC column (1.0 $\times$ 10 cm) PepRPC HR 10/10 (Pharmacia). The column was washed with 25% acetonitrile, 0.1% TFA for 15 min, and developed with a linear gradient from 25% acetonitrile, 0.1% TFA to 32.2% acetonitrile, 0.1% TFA for 30 min with a flow rate of 2.8 ml/min. Each fraction was dried, dissolved in a small volume of methanol, and assayed for the P-factor activity. The purified P-factor could be stably stored either in methanol at -20°C or dried at 4°C .

Peptide sequence analysis

Automated Edman degradation analysis of the FPLC-purified P-factor (1 nmole) was carried out by use of a gas-phase sequenator Model 130A (Applied Biosystems).

Synthetic peptide

Synthetic P-factor was prepared by a solid phase method using an automated synthesizer Model 430A-03 (Applied Biosystems). The peptide was kept in methanol at a concentration of 10 mg/ml.

Flow cytometric analysis

Cells ($2\text{--}3 \times 10^6$) were collected by centrifugation, washed with water, and resuspended in 0.3 ml water. Then 0.7 ml of ethanol was added, and the cells were left overnight at -20°C . The fixed cells were collected, washed with 0.5 ml of 0.2 M Tris-HCl, 0.05

M EDTA (pH 6.8), and resuspended in 0.5 ml of the same solution. The cells were sonicated and RNase A was added to the sample to the final concentration of 0.2 mg/ml. After incubation at 37°C for 4 hr, the cells were collected and resuspended at the same concentration in 0.2 M Tris-HCl, 0.05 M EDTA (pH 6.8) containing 2 µg/ml propidium iodide. After incubating for at least 8 hr at 4°C, the cells were analyzed with a flow cytometer Model EPICS-C (Coulter).

Acknowledgments

We thank Dr. C. Shimoda for an *S. pombe* strain, Ms. Y. Sakurai for assistance in peptide sequence analysis, Mr. T. Usui and Dr. T. Beppu for aid in flow cytometry, and Dr. J. Davey for helpful comments. This work was supported by grants-in-aid from the Ministry of Education, Science, and Culture of Japan. P-factor was kindly synthesized by Drs. M. Tomita and E. Oda, Faculty of Pharmaceutical Sciences, Showa University, under the support of a grant for Cancer Research from the same Ministry.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Anderegg, R.J., R. Betz, S.A. Carr, J.W. Crabb, and W. Duntze. 1988. Structure of *Saccharomyces cerevisiae* mating hormone α -factor. *J. Biol. Chem.* **263**: 18236–18240.
- Beach, D., M. Piper, and P. Nurse. 1982. Construction of a *Schizosaccharomyces pombe* gene bank in a yeast bacterial shuttle vector and its use to isolate genes by complementation. *Mol. Gen. Genet.* **187**: 326–329.
- Beach, D., L. Rodgers, and J. Gould. 1985. *RAN1*⁺ controls the transition from mitotic division to meiosis in fission yeast. *Curr. Genet.* **10**: 297–311.
- Bostock, C.J. 1970. DNA synthesis in the fission yeast *Schizosaccharomyces pombe*. *Exp. Cell. Res.* **60**: 16–26.
- Bucking-Throm, E., W. Duntze, L.H. Hartwell, and T.R. Manney. 1973. Reversible arrest of haploid yeast cells at the initiation of DNA synthesis by a diffusible sex factor. *Exp. Cell. Res.* **76**: 99–110.
- Burkholder, A.C. and L.H. Hartwell. 1985. The yeast α -factor receptor: Structural properties deduced from the sequence of the *STE2* gene. *Nucleic Acids Res.* **13**: 8463–8475.
- Caplan, S., R. Green, J. Rocco, and J. Kurhan. 1991. Glycosylation and structure of the yeast *MF α 1* α -factor precursor is important for efficient transport through secretory pathway. *J. Bacteriol.* **173**: 627–635.
- Cross, F., L.H. Hartwell, C. Jackson, and J.B. Konopka. 1988. Conjugation in *Saccharomyces cerevisiae*. *Annu. Rev. Cell Biol.* **4**: 430–457.
- Davey, J. 1991. Isolation and quantitation of M-factor, a diffusible mating factor from the fission yeast *Schizosaccharomyces pombe*. *Yeast* **7**: 357–366.
- . 1992. Mating pheromones of the fission yeast *Schizosaccharomyces pombe*: Purification and structural characterization of M-factor and isolation and analysis of two genes encoding the pheromone. *EMBO J.* **11**: 951–960.
- Dmochowska, A., D. Dignard, D. Henning, D.Y. Thomas, and H. Bussey. 1987. Yeast *KEX1* gene encodes a putative protease with a carboxypeptidase B-like function involved in killer toxin and α -factor precursor processing. *Cell* **50**: 573–584.
- Dohlman, H.G., J. Thorner, M.G. Caron, and R.J. Lefkowitz. 1991. Model systems for the study of seven-transmembrane segment receptors. *Annu. Rev. Biochem.* **60**: 653–688.
- Egel, R. 1973. Genes involved in mating type expression of fission yeast. *Mol. Gen. Genet.* **122**: 339–343.
- Egel, R. and M. Egel-Mitani. 1974. Premeiotic DNA synthesis in fission yeast. *Exp. Cell Res.* **88**: 127–134.
- Fukui, Y., Y. Kaziro, and M. Yamamoto. 1986a. Mating pheromone-like diffusible factor released by *Schizosaccharomyces pombe*. *EMBO J.* **5**: 1991–1993.
- Fukui, Y., T. Kozasa, Y. Kaziro, T. Takeda, and M. Yamamoto. 1986b. Role of a *ras* homolog in the life cycle of *Schizosaccharomyces pombe*. *Cell* **44**: 329–336.
- Grimm, C., J. Kohli, J. Murray, and K. Maundrell. 1988. Genetic engineering of *Schizosaccharomyces pombe*: A system for gene disruption and replacement using the *ura4* gene as a selectable marker. *Mol. Gen. Genet.* **215**: 81–86.
- Gutz, H., H. Heslot, U. Leupold, and N. Loprieno. 1974. *Schizosaccharomyces pombe*. In *Handbook of genetics* (ed. R.D. King), vol. 1, pp. 395–446. Plenum Publishing Co., New York.
- Hagen, D.C., G. McCaffrey, and G.F. Sprague, Jr. 1986. Evidence the yeast *STE3* gene encodes a receptor for the peptide pheromone α factor: Gene sequence and implications for the structure of the presumed receptor. *Proc. Natl. Acad. Sci.* **83**: 1418–1422.
- Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene* **28**: 351–359.
- Imai, Y. and M. Yamamoto. 1992. *Schizosaccharomyces pombe* *sxa1*⁺ and *sxa2*⁺ encode putative proteases involved in the mating response. *Mol. Cell. Biol.* **12**: 1827–1834.
- Imai, Y., S. Miyake, D.A. Hughes, and M. Yamamoto. 1991. Identification of a GTPase-activating protein homolog in *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* **11**: 3088–3094.
- Julius, D., L. Blair, A. Brake, G. Sprague, and J. Thorner. 1983. Yeast α -factor is processed from a larger precursor polypeptide: The essential role of a membrane-bound dipeptidyl aminopeptidase. *Cell* **32**: 839–852.
- Julius, D., R. Scheckman, and J. Thorner. 1984a. Glycosylation and processing of prepro- α -factor through the yeast secretory pathway. *Cell* **36**: 309–318.
- Julius, D., A. Brake, L. Blair, R. Kunisawa, and J. Thorner. 1984b. Isolation of the putative structural gene for the lysine-arginine cleaving endopeptidase required for processing of yeast prepro- α -factor. *Cell* **37**: 1075–1089.
- Kawamukai, M., K. Ferguson, M. Wigler, and D. Young. 1991. Genetic and biochemical analysis of the adenylyl cyclase of *Schizosaccharomyces pombe*. *Cell Reg.* **2**: 155–164.
- Kitamura, K. and C. Shimoda. 1991. The *Schizosaccharomyces pombe* *mam2* gene encodes a putative pheromone receptor which has a significant homology with the *Saccharomyces cerevisiae* Ste2 protein. *EMBO J.* **10**: 3743–3751.
- Kurjan, J. and I. Herskowitz. 1982. Structure of a yeast pheromone gene (*MF α*): A putative α -factor precursor contains four tandem copies of mature α -factor. *Cell* **39**: 933–943.
- Leupold, U. 1987. Sex appeal in fission yeast. *Curr. Genet.* **12**: 543–545.
- MacKay, V.L., S.K. Welch, M.Y. Insley, T.R. Manney, J. Holly, G.C. Saari, and M.L. Parker. 1988. The *Saccharomyces cerevisiae* *BARI* gene encodes an exported protein with homology to pepsin. *Proc. Natl. Acad. Sci.* **85**: 55–59.
- Maeda, T., N. Mochizuki, and M. Yamamoto. 1990. Adenylyl cyclase is dispensable for vegetative cell growth in the fission yeast *Schizosaccharomyces pombe*. *Proc. Natl. Acad. Sci.* **87**: 7814–7818.
- Marsh, L., A. Neiman, and I. Herskowitz. 1991. Signal trans-

- duction during pheromone response in yeast. *Annu. Rev. Cell Biol.* **7**: 699–728.
- McLeod, M., M. Stein, and D. Beach. 1987. The product of the *mei3⁺* gene, expressed under control of the mating-type locus, induces meiosis and sporulation in fission yeast. *EMBO J.* **6**: 729–736.
- Nadin-Davis, S.A., A. Nasim, and D. Beach. 1986. Involvement of *ras* in sexual differentiation but not in growth control in fission yeast. *EMBO J.* **5**: 2963–2971.
- Nakayama, N., A. Miyajima, and K. Arai. 1985. Nucleotide sequences of *STE2* and *STE3*, cell type-specific sterile genes from *Saccharomyces cerevisiae*. *EMBO J.* **4**: 2643–2648.
- Nielsen, O., J. Davey, and R. Egel. 1992. The *ras1* function of *Schizosaccharomyces pombe* mediates pheromone-induced transcription. *EMBO J.* **11**: 1391–1395.
- Obara, T., M. Nakafuku, M. Yamamoto, and Y. Kaziro. 1991. Isolation and characterization of a gene encoding a G-protein α subunit from *Schizosaccharomyces pombe*: Involvement in mating and sporulation pathways. *Proc. Natl. Acad. Sci.* **88**: 5877–5881.
- Okazaki, K., N. Okazaki, K. Kume, S. Jinno, K. Tanaka, and H. Okayama. 1990. High-frequency transformation method and library transducing vectors for cloning mammalian cDNAs by *trans*-complementation of *Schizosaccharomyces pombe*. *Nucleic Acids Res.* **18**: 6485–6489.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.* **74**: 5463–5467.
- Sherman, F., G. Fink, and J. Hicks. 1986. *Methods in yeast genetics: Laboratory course manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Singh, A., E.Y. Chen, J.M. Lugovoy, C.N. Chang, R.A. Hitzman, and P.H. Seeburg. 1983. *Saccharomyces cerevisiae* contains two discrete genes coding for the α -factor pheromone. *Nucleic Acids Res.* **11**: 4049–4063.
- Sipiczki, M. and L. Ferenczy. 1977. Protoplast fusion of *Schizosaccharomyces pombe* auxotrophic mutants of identical mating-type. *Mol. Gen. Genet.* **151**: 77–81.
- Southern, E. 1979. Gel electrophoresis of restriction fragments. *Methods Enzymol.* **68**: 152–176.
- Stotzler, D., J. Kiltz, and W. Duntze. 1976. Primary structure of α -factor peptide from *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **69**: 397–400.
- Sugimoto, A., Y. Iino, T. Maeda, Y. Watanabe, and M. Yamamoto. 1991. *Schizosaccharomyces pombe ste11⁺* encodes a transcription factor with an HMG motif that is a critical regulator of sexual development. *Genes & Dev.* **5**: 1990–1999.
- Tanaka, K., J. Davey, Y. Imai, and M. Yamamoto. 1993. *Schizosaccharomyces pombe map3⁺* encodes the putative M-factor receptor. *Mol. Cell. Biol.* **13**: 80–88.
- Watanabe, Y., Y. Iino, K. Furukata, C. Shimoda, and M. Yamamoto. 1988. The *S. pombe mei2* gene encoding a crucial molecule for commitment to meiosis is under the regulation of cAMP. *EMBO J.* **7**: 761–767.
- Yamawaki-Kataoka, Y., T. Tamaoki, H.-R. Choe, H. Tanaka, and T. Kataoka. 1989. Adenylate cyclase in yeast: A comparison of the genes from *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **86**: 5693–5697.
- Young, D., M. Riggs, J. Field, A. Vojtek, D. Broek, and M. Wigler. 1989. The adenylyl cyclase gene from *Schizosaccharomyces pombe*. *Proc. Natl. Acad. Sci.* **86**: 7989–7993.

The fission yeast mating pheromone P-factor: its molecular structure, gene structure, and ability to induce gene expression and G1 arrest in the mating partner.

Y Imai and M Yamamoto

Genes Dev. 1994, **8**:

Access the most recent version at doi:[10.1101/gad.8.3.328](https://doi.org/10.1101/gad.8.3.328)

References

This article cites 47 articles, 13 of which can be accessed free at:
<http://genesdev.cshlp.org/content/8/3/328.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

Focused on your science.

USC
SCIENTIFIC
SIDE BY SCIENCE