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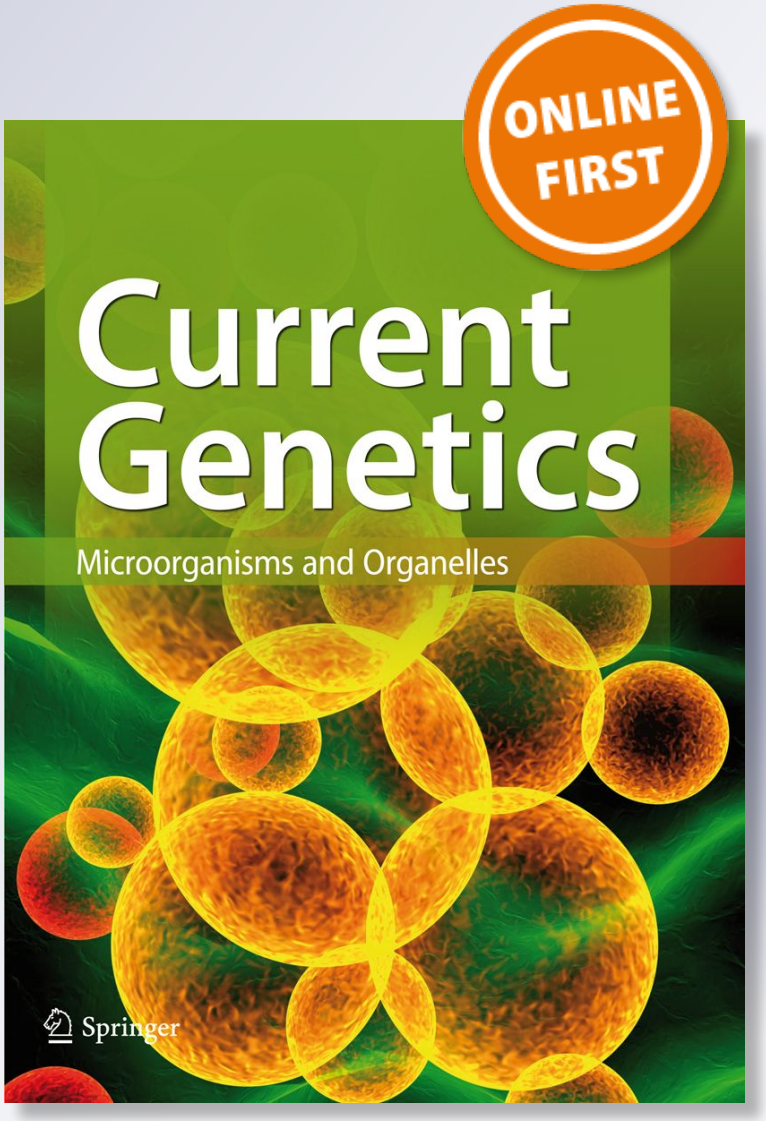
Current Genetics
Microorganisms and Organelles

ISSN 0172-8083

Curr Genet
DOI 10.1007/s00294-019-00968-w



Springer

Current Genetics
https://doi.org/10.1007/s00294-019-00968-w

MINI-REVIEW

The evolution of peptide mating pheromones in fission yeast

Taisuke Seike[1]

Received: 25 March 2019 / Revised: 3 April 2019 / Accepted: 4 April 2019

Abstract
In fungi, sexual reproduction primarily depends on the interaction between peptide pheromones and their receptors. Most ascomycete fungi produce two classes of peptide mating pheromones, a simple peptide and a modified peptide. These peptides are recognized by their corresponding receptors on the surface of cells of the opposite mating type to induce the mating reaction. Pheromone diversification may be associated with reproductive isolation, which restricts gene flow among populations; thus, it remains unclear how pheromones diversify without loss of successful mating. Here, I provide a brief review of recent findings on the 'asymmetric' diversification of peptide pheromones in the fission yeast *Schizosaccharomyces pombe*, and discuss evolution of the mating pheromones in fission yeast.

Keywords Peptide pheromone · Fission yeast · G-protein coupled receptor · Reproductive isolation · Evolution

Introduction

Sexual reproduction in ascomycete fungi commences between cells of two different mating types, with the interaction mediated by pheromones and their corresponding receptors (Kurjan 1993). Most ascomycete fungi produce two classes of peptide mating pheromone: one is a farnesylated peptide pheromone (called 'a-class'), which is hydrophobic; the other is an unmodified peptide pheromone (called 'α-class') (Fraser et al. 2008). The asymmetry in chemical modification of the pheromones is highly conserved across ascomycete fungi (Gonalves-Sá and Murray 2011). Each pheromone plays an important role in species-specific mate recognition, but little is known about the relationship between the diversity and the extent of species-specificity of peptide pheromones among these fungi (Martin et al. 2011).

Recently we have explored the variability in the genes encoding pheromones in 150 wild *Schizosaccharomyces pombe* strains (Seike et al. 2019). This study clearly demonstrated an 'asymmetric' diversification of two pheromones: the amino acid sequences of farnesylated peptide pheromone are completely conserved, whereas those of unmodified pheromone are very diverse among the strains we investigated. Thus, in mate recognition, there is an inherent asymmetry, and the recognition by farnesylated peptide is likely to be more stringent than the recognition by unmodified peptide—perhaps, facilitating more rapid evolution of unmodified pheromone, relative to farnesylated pheromone.

Main text

Mating pathway in *Schizosaccharomyces pombe*

Most ascomycete fungi live in both haploidy and diploidy (Herskowitz 1988; Harari et al. 2018). Usually, the fission yeast *S. pombe*, which is one of the ascomycete fungi, proliferates as haploid cells under vegetative growth. On starvation of nitrogen, two haploid cells of opposite mating types, termed **P**lus (P) and **M**inus (M), mate and form a diploid zygote (Leupold and Hottinguer 1950). P-cells produce P-factor, an α-class unmodified peptide pheromone composed of 23 amino acids, which is recognized by its corresponding G-protein coupled receptor (GPCR) Mam2 on the surface of M-cells (Kitamura and Shimoda 1991; Imai and Yamamoto 1994). By contrast, M-cells produce M-factor, an a-class farnesylated peptide pheromone composed of nine amino acids, which is recognized by its corresponding GPCR Map3 on the surface of P-cells (Davey

Communicated by M. Kupiec.

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Published online: 09 April 2019

1991; Tanaka et al. 1993). The mating reaction of *S. pombe* requires both recognition of P-factor by Mam2 and recognition of M-factor by Map3.

The biosynthetic pathway of the mating pheromones is illustrated in Fig. 1. P-factor is encoded by a single *map2*$^+$ gene and is processed from a precursor polypeptide (Imai and Yamamoto 1994). The precursor contains a signal sequence that targets the polypeptide to the endoplasmic reticulum (ER), two N-linked glycosylation sites, and four coding sequences for the mature pheromone separated by spacer regions (Davey et al. 1994; Imai and Yamamoto 1994). Because the pheromone-coding sequences are slightly different, mature P-factor in the standard laboratory strain is a mixture of three distinct peptides. The biosynthetic pathway of the P-factor precursor is very similar to that of α-factor in the budding yeast *Saccharomyces cerevisiae* (Kurjan and Herskowitz 1982). The N-terminal signal sequence is cleaved by a signal peptidase (probably Sec11). Further processing occurs in the Golgi, where the kexin-related endopeptidase Krp1 cleaves the KKR (Lys-Lys-Arg) motif, and the remaining product is trimmed by a serine carboxypeptidase (probably Kex1) and an aminopeptidase (probably SPAPJ760.01c) to generate mature P-factor (Davey et al. 1994). Lastly, mature P-factor is secreted as a simple peptide by exocytosis (Imai and Yamamoto 1994).

In contrast to P-factor, M-factor is encoded by three redundant *mfm* genes, *mfm1*$^+$, *mfm2*$^+$, and *mfm3*$^+$, and is processed from their respective precursor polypeptides (Davey 1992; Kjaerulff et al. 1994). All three genes generate a precursor containing a single copy of identical M-factor sequence. The structure of the M-factor precursor resembles that of a-factor in *S. cerevisiae* (Michaelis and Barrowman 2012). The precursors synthesized from the three *mfm* genes contain an N-terminal extension and a C-terminal CAAX (Cys, followed by two aliphatic and an arbitrary residue) motif, which serves as a signal for prenylation and carboxyl-methylation (Clarke 1992). These post-translational modifications are carried out by two integral membrane enzymes, Cwp1 and Mam4, which are presumably localized in the ER membrane (Imai et al. 1997; Xue-Franzén et al. 2006). As a result, M-factor peptide has S-farnesylated and carboxyl-methylated modifications, both of which are necessary for its activity and export (Davey 1992; Wang et al. 1994). The precursors undergo several rounds of N-terminal cleavage by as yet unidentified proteolytic enzymes (possibly Irp1) to produce mature M-factor. Lastly, mature M-factor is secreted from the cell by the ATP-binding cassette (ABC) transporter Mam1, but not by the classical secretory pathway (Christensen et al. 1997).

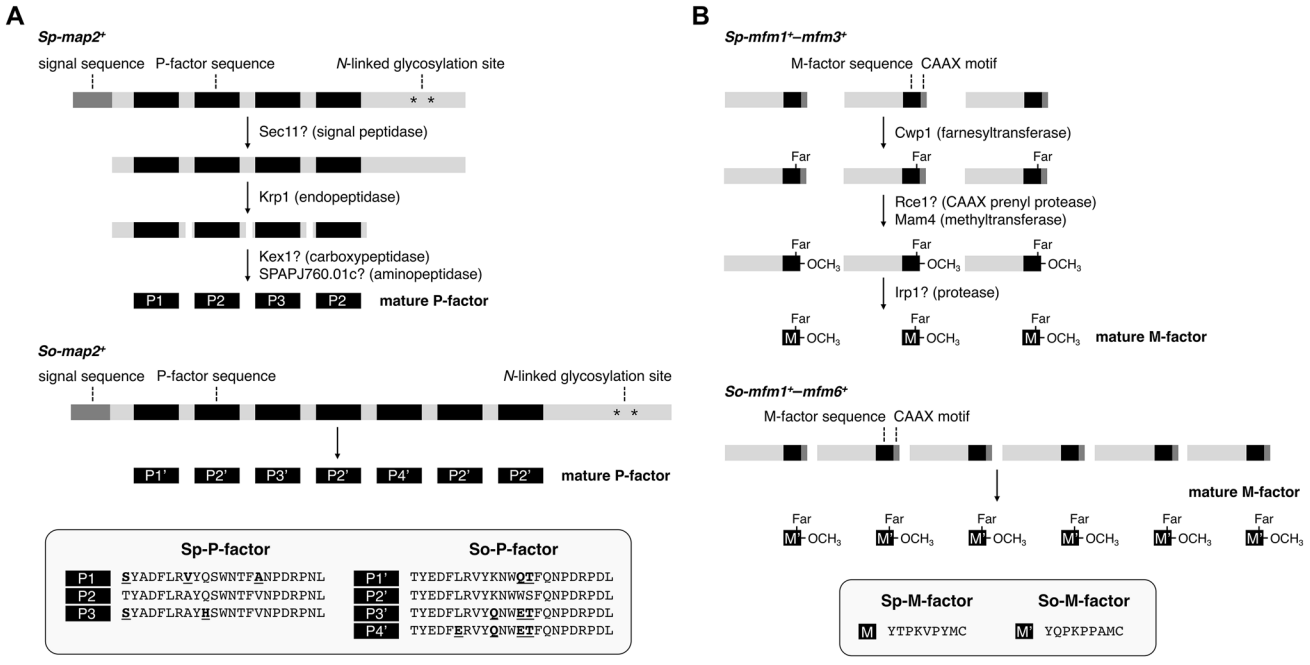

Fig. 1 Biosynthetic pathway and structure of mating pheromones in *S. pombe* and *S. octosporus*. Some of the proteins involved in processing are not well identified in fission yeast. The putative pathway shown here is based largely on that of the budding yeast *S. cerevisiae*. **a** Biosynthetic pathway of P-factor. The signal sequence and P-factor sequences are indicated by gray and black boxes, respectively. The two N-linked glycosylation sites are shown as asterisks. The gene encoding So-P-factor is tentatively termed *So-map2*$^+$. The amino acids that differ within the mature P-factors of each species are underlined in bold (P2: standard sequence). **b** Biosynthetic pathway of M-factor. The CAAX motif and M-factor sequences are indicated by gray and black boxes, respectively. The genes encoding So-M-factor are tentatively termed *So-mfm1*$^+$*–So-mfm6*$^+$. A single mature M-factor is produced by each species. *Far* farnesyl group

Each pheromone is recognized by its corresponding receptor on the surface of cells of the opposite mating type. The P-factor receptor is encoded by the *mam2*$^+$ gene, whereas the M-factor receptor is encoded by the *map3*$^+$ gene (Kitamura and Shimoda 1991; Tanaka et al. 1993). Both Mam2 and Map3 are heterotrimeric GPCRs containing seven transmembrane domains. Binding of the pheromone to its receptor causes dissociation of the trimeric G-protein subunits (Gα, Gβ, and Gγ); activation of the released Gα subunit (Gpa1) then transmits signals through the MAP kinase cascade comprising Byr2 (MAPKKK), Byr1 (MAPKK), and Spk1 (MAPK) (Obara et al. 1991; Barr et al. 1996; Ladds et al. 2005). A consequence of this stimulation is the induction of pheromone-controlled genes (Moretto and van Werven 2017; Pataki et al. 2017). Transmission of the pheromone signal downstream of GPCRs is common to both P- and M-cells (Xue-Franzén et al. 2006). Notably, it is known that there is strong homology between Mam2 and the *S. cerevisiae* α-factor receptor STE2, and between Map3 and the *S. cerevisiae* a-factor receptor STE3 (Burkholder and Hartwell 1985; Nakayama et al. 1985; Hagen et al. 1986).

Diversification of peptide pheromones

In ascomycete fungi, the pheromone–receptor system plays a key role in mating recognition (Gonalves-Sá and Murray 2011; Rogers et al. 2015; Seike et al. 2015). By genetically altering the primary structure of both M-factor and Map3, we previously created a novel fission yeast strain with a pair of opposite sexes that behaved like a new species (i.e., they mated readily with each other, but only very poorly with wild-type strains) (Seike et al. 2012, 2015). In other words, when the novel strain was co-cultured with the wild-type strain in a test tube, the level of inter-species exchange of genetic information was extremely low. This experimental result strongly supported the idea that alterations in a combination of pheromone and receptor might trigger reproductive isolation, which restricts gene flow between individuals of mating partners, by affecting the ability of the cells to recognize each other. If so, however, how can pheromones and receptors diversify while maintaining successful mating in nature?

To find answers to this question, we recently explored the diversity of genes encoding the two pheromones (*map2*, *mfm1*, *mfm2*, and *mfm3*) and their receptors (*mam2* and *map3*) in 150 wild *S. pombe* strains (isolated from more than 22 different countries) with different origins from the standard laboratory strain. The wild strains were obtained from the National BioResource Project (http://yeast.nig.ac.jp/yeast/top.xhtml) and two research groups (Fawcett et al. 2014; Mourier et al. 2015). Extensive survey of the gene sequences clearly showed that there are many nucleotide differences (base substitutions and insertions) among the 150 strains; therefore, the sequences of the pheromones and receptors are relatively diversified. Notably, however, the amino acid sequences of P-factor and Mam2 are very diverse, whereas those of M-factor and Map3 are completely conserved (Seike et al. 2019). We identified six variants of P-factor and five types of Mam2 structure, depending on the region of isolation, but found no differences in the structure of either M-factor or Map3 among the 150 strains that we investigated. Such asymmetric diversification of the two peptide pheromones was also seen in the closely related species *Schizosaccharomyces octosporus* (Seike et al. 2019). Collectively, these observations implied that, in mate recognition, the recognition of Map3 by M-factor may be more important than the recognition of Mam2 by P-factor.

To test this idea, we carried out a series of mating experiments between *S. pombe* and *S. octosporus*. The *S. octosporus* laboratory strain has a single gene encoding P-factor (*So-map2*$^+$), which contains seven tandem coding sequences encoding four slightly different P-factor peptides (Young et al. 2011) (Fig. 1a). On average, 8 of the 23 amino acids of *S. octosporus* P-factor (So-P-factor) differ from the residues of *S. pombe* P-factor (Sp-P-factor). Our experiments showed that (1) the introduction of So-P-factor into an Sp-P-factor-less *S. pombe* strain could partially restore mating ability and vice versa, and (2) all four synthetic So-P-factors had a significant effect on *S. pombe* M-cells (Seike et al. 2019). These experimental data indicate that the specificity of P-factor recognition is more relaxed, allowing cross-reactions to occur even with closely related species. On the other hand, *S. octosporus* has six genes encoding M-factor (*So-mfm1*$^+$–*So-mfm6*$^+$), which contain a single copy of an identical M-factor sequence (Seike et al. 2012). Three of the nine amino acids (Gln2, Pro5, Ala7) of *S. octosporus* M-factor (So-M-factor) differ from those of *S. pombe* M-factor (Sp-M-factor) (Fig. 1b). We found that an *S. pombe* strain producing only So-M-factor instead of Sp-M-factor was completely sterile, and confirmed that synthetic So-M-factor was virtually dysfunctional in *S. pombe* (Seike et al. 2019). In short, the specificity of M-factor recognition is extremely stringent in contrast to that of P-factor. The lack of compatibility of M-factor is likely to be responsible for the reproductive isolation between the two species.

Evolution of peptide pheromones

Our recent finding in *S. pombe*—namely, that recognition is stringent between one pair (M-factor and Map3) but relaxed between the other (P-factor and Mam2)—suggests that the diversity of peptide pheromones has probably resulted from both ‘adaptive’ and ‘non-adaptive’ evolutionary forces. The primary determinant of mating in ascomycete fungi is the specificity of the pheromones and receptors; hence, pheromone diversification must be prevented by strong selective

constraint. Despite these constraints, pheromone sequences show significant diversity (Martin et al. 2011), and only a few pheromone residues are reported to be essential for receptor recognition, while others are less important (Naider and Becker 2004; Seike et al. 2012). In addition, several previous studies have indicated that only one pheromone and receptor pair might be necessary, while the other might eventually be lost (Mayrhofer et al. 2006; Kim et al. 2008; Lee et al. 2008). In periods of preferentially asexual reproduction, the genes for pheromones and their receptors might become obsolete and diverge under relaxed selective constraints (Martin et al. 2011).

At this time, it remains unclear how pheromone diversification is driven in nature, but our recent studies might shed light on the forces that facilitate the evolution of peptide pheromones. I speculate that the more rapid evolution of simple peptide pheromones, relative to their modified peptide counterparts, contributes to reproductive isolation in ascomycete fungi. The gradual process of succession, by which a new version of a pheromone variant replaces the old version, might also facilitate the coevolution of pheromones and receptors, enabling a novel compatible pheromone and receptor pair to spread in a population without loss of successful mating.

Acknowledgements This work was supported by Grant-in-Aid for JSPS Research Fellow (Grant Number JP15J03416) to T.S., Grant-in-Aid for Young Scientists (B) (Grant Number JP17K15181) to T.S., the Sumitomo Foundation (No. 160924) to T.S., and Dr. Yoshifumi Jigami Memorial Fund, The Society of Yeast Scientists to T.S.

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