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Sexual Pheromones and Mating Responses in Fungi

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INTRODUCTION

Sexual development in fungi is initiated by the fusion of haploid cells that are morphologically indistinguishable. However, they can fuse only if they are of different mating types. In general, the term "mating type" defines all specific activities of a cell that are necessary to go from the haploid to the diploid stage and to continue on to meiosis. The mating type is determined by the genetic information present at the mating-type (*mat*) locus. Diverse systems have evolved to ensure the maintenance of different sexes of either two or multiple mating types. In many fungal species, specific cell recognition and fusion are mediated by diffusible peptide mating factors. These secreted peptides have been termed pheromones by analogy with the secreted chemical substances of insects and mammals, which can elicit specific responses in very low doses. In this review, we will focus on some of these fungal peptide pheromones and their functions within the sexual cycle. The study of these pheromones has allowed insights into fields as diverse as protein modification and trafficking, receptor–ligand interactions, signal transduction, and cell cycle regulation.

By far the best-understood system with respect to the genetic and molecular mechanisms of pheromone production and pheromone response is that of the yeast *Saccharomyces cerevisiae*. The first hint of the existence of peptide pheromones in this system came from the observation that a diffusible substance can act at a distance and that this effect is cell type specific (Levi, 1956). The mating reaction of two haploid cells involves a complicated set of reactions to ensure the coordinated expression of a number of genes that are necessary to mediate the controlled fusion of cells and the subsequent karyogamy of nuclei. Being in the same phase of the cell cycle is a prerequisite for nuclear fusion; therefore, cell cycle arrest in G1 is one of the primary responses to pheromones in yeast. This response provides the opportunity to investigate how a signal elicited by extracellular pheromones is passed into the nucleus, where it affects the cell cycle.

The pheromone receptors belong to the large family of G-protein–coupled seven-transmembrane receptors, and the yeast system has proved to be a useful model system for studying these receptors and their signal transduction chain. The enzymes involved in modification and translocation of the prenylated **a**-factor of yeast have attracted interest because prenylation of eukaryotic proteins such as RAS oncoprotein has been implicated in tumorigenesis of many types of cancer. Agents that interfere with the activity of prenyltransferase may have therapeutic value, and the mating system of yeast can be used for the screening of potential tumor-suppressing substances.

In recent years, evidence has emerged that peptide pheromones are ubiquitous in the fungal kingdom. They have been found in fungal species in which there had been no hint of their existence from physiological studies. There are also indications that pheromones not only are involved in cell–cell recognition but also are necessary for postfusion events, such as induction of meiosis in *Schizosaccharomyces pombe* and maintenance of the filamentous state in *Ustilago maydis*. Because morphogenetic transitions are sometimes associated with pathogenesis, it would be interesting to determine whether pheromones are involved, either directly or indirectly, in such transitions. In medically important fungi, the receptors that sense the pheromones could serve as potential targets for chemotherapy.

In many filamentous fungi, however, cell fusion can occur without the need for specific mating factors. In these organisms, which often have multiple mating types, the decision to undergo sexual development is initiated after cell fusion by combinatorial interactions of regulatory proteins. The molecular analysis of such mating-type loci revealed that they encode polypeptides that are related to the homeodomain proteins of higher eukaryotes. These proteins are thought to act as transcriptional regulators in specific pairwise combinations. The analysis of such multiallelic mating-type genes is likely to reveal the molecular mechanisms of self/nonself discrimination. These systems have been reviewed recently (Kües and Casselton, 1992). Interestingly, in some species, such as *U. maydis*, mixed types of mating-type determination exist, in which pheromone-mediated cell–cell recognition is coupled with a multiallelic incompatibility system based on the action of homeodomain proteins.

SACCHAROMYCES CEREVISIAE

Mechanisms of pheromone production and response have, as mentioned above, been best studied in yeast, whose mating

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system has been the topic of a number of recent reviews (Cross et al., 1988; Fields, 1990; Marsh et al., 1991; Kurjan, 1992). Because it is the signal transduction chain involved in pheromone response that has been most extensively reviewed, we will concentrate on those aspects of yeast mating that can either serve as a paradigm for the understanding of other fungal mating systems or differ specifically from those found in other systems.

In *S. cerevisiae*, two different mating types, **a** and α, exist. The mating type is determined by a single genetic locus, *MAT*, which codes for regulatory proteins that control the expression of a number of haploid- and **a** or α mating type–specific genes. Among the genes that are differentially expressed in **a** and α cells are those that are essential for specific cell–cell recognition and fusion, i.e., the pheromone and receptor genes. Because the loss of these gene products causes a mating defect, many of the genes involved in conjugation were identified by complementing sterile mutants. After an **a** and an α cell fuse, expression of all haploid-specific genes and of **a** or α mating type–specific genes is shut off, and the diploid-specific genes that are necessary for meiosis and sporulation are expressed. This change in gene expression is achieved by combinatorial interactions of the *MAT* gene products and proceeds in a hierarchical fashion (Herskowitz, 1989).

Cell culture supernatants from both α and **a** cells can specifically induce cell cycle arrest and morphological changes in cells of opposite mating types. The purification of the secreted activities allowed the identification and characterization of both mating factors. α-factor is a 13–amino acid peptide (Stötzler et al., 1976) that is secreted by the classic secretory pathway. Two genes, *MFα1* and *MFα2*, code for precursors of 165 and 120 amino acids that contain tandem copies of the α-factor sequence (Kurjan and Herskowitz, 1982; Singh et al., 1983). The precursors are processed in a series of events that give rise to the mature α-factor, whose sequence is shown in Table 1.

Table 1. Structure of Mating Pheromones

Species	Pheromone	Structure[a]
S. cerevisiae	α-factor	Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr-OH
S. cerevisiae	**a**-factor[b,c]	Tyr-Ile-Ile-Lys-Gly-Val/Leu-Phe-Trp-Asp-Pro-Ala-Cys-OCH_3
S. kluyveri	α^{sk2} pheromone	Trp-His-Trp-Leu-Ser-Phe-Ser-Lys-Gly-Glu-Pro-Met-Tyr-OH
S. pombe	M-factor[c]	Tyr-Thr-Pro-Lys-Val-Pro-Tyr-Met-Cys-OCH_3
R. toruloides	Rhodotorucine *A*[c]	Tyr-Pro-Glu-Ile-Ser-Trp-Thr-Arg-Asn-Gly-Cys-OH
T. mesenterica	Tremerogen *A*-10[c]	Glu-His-Asp-Pro-Ser-Ala-Pro-Gly-Asn-Gly-Tyr-Cys-OCH_3 (CH_2OH)
T. mesenterica	Tremerogen *a*-13[c]	Glu-Gly-Gly-Gly-Asn-Arg-Gly-Asp-Pro-Ser-Gly-Val-Cys-OH
T. brasiliensis	Tremerogen A-9291-I[d]	Asp-Ser-Gly-Ser/Gly-Ser-Arg-Asp-Pro-Gly-Ala-Ser-Ser-Gly-Gly-Cys-OCH_3 (CH_2OH)

[a] The references for the structures are as follows: α-factor, Stötzler et al. (1976); **a**-factor, Anderegg et al. (1988); α^{sk2} pheromone, Sakurai et al. (1984); M-factor, Davey (1992); Rhodotorucine *A*, Kamiya et al. (1978); tremerogen *A*-10, Sakagami et al. (1979); tremerogen *a*-13, Sakagami et al. (1981); tremerogen A-9291-I, Ishibashi et al. (1984).

[b] Ambiguity in the amino acid sequence of the *S. cerevisiae* **a**-factor, as indicated by a slash, results from sequence differences at this position between the two pheromone genes.

[c] The farnesyl group of **a**-factor and the other farnesylated pheromones are linked by a thioether to the C-terminal cysteine.

[d] Microheterogeneity in the amino acid sequence of *T. brasiliensis* tremerogen A-9291-I, as shown by a slash, may indicate the existence of more than one gene for this factor.

Activity of the secreted mating factor can be scored by spotting purified or synthetic peptide on a lawn of **a** cells. Activity manifests itself as a cell-free zone ("halo") around the spot of α-factor (Sprague, 1991). This effect is due to specific growth inhibition, with cells arresting in the G1 phase of the cell cycle. Cells responding to the mating factor also show a morphological change, forming a projection and appearing pear shaped ("shmoo") (Cross et al., 1988). After a while, arrested cells can recover from growth inhibition by desensitization of the signal transduction pathway and by producing an α-factor–degrading activity, the *BAR1* gene product (MacKay et al., 1988). Several modified α-factor peptides carrying different deletions were synthesized and tested for biological activity. Peptides truncated at the N terminus were inactive and acted as antagonists, suggesting that binding determinants reside in the C terminus. Peptides truncated at the C terminus were also inactive; surprisingly, they acted as synergists when applied together with wild-type peptide. This finding may indicate that residues near the N terminus are involved in signal transduction, but a convincing molecular explanation for the observed synergism does not yet exist (Eriotou-Bargiota et al., 1992).

Because of its hydrophobicity, **a**-factor was much more recalcitrant to purification than α-factor. The hydrophobic nature of **a**-factor was found to be the result of a post-translational modification. The C-terminal cysteine of the 12–amino acid peptide carries a farnesyl group (Anderegg et al., 1988). This type of peptide modification was first identified in the mating factor of the basidiomycetous yeast *Rhodosporidium toruloides* (see below) and consists of a C_{15} polyprenyl moiety (see Table 1). The **a**-factor is encoded by two genes, *MFa1* and *MFa2*, which code for 34– and 36–amino acid precursors (Brake et al., 1985; Michaelis and Herskowitz, 1988). The sequences of the mature **a**-factor pheromones specified by *MFa1* and *MFa2* differ at one position (Table 1). The **a**-factor precursors contain the specific C-terminal signal sequence for prenylation, the CAAX motif (where C stands for cysteine, A for an aliphatic amino acid, and X for any amino acid). This sequence specifies prenylation and carboxymethylation of the cysteine and proteolytic cleavage of the last three amino acids (reviewed in Clarke, 1992; Schafer and Rine, 1992).

The yeast system has also been useful to determine the order of these reactions and to identify the enzymes that are involved. Two genes, *RAM1* and *RAM2*, encode polypeptide components of the farnesyltransferase that prenylates both **a**-factor and RAS protein (Powers et al., 1986; He et al., 1991). Carboxymethylation of the C-terminal cysteine is catalyzed by the *STE14* gene product (Hrycyna et al., 1991). The maturation of **a**-factor occurs in a defined order: farnesylation of the cysteine is prerequisite for the second step, the C-terminal proteolytic cleavage of the last three amino acids. The last step is the carboxymethylation of the cysteine. Recent reviews cover progress in the field of protein prenylation (Glomset et al., 1990; Clarke, 1992; Schafer and Rine, 1992).

The biological significance of the C-terminal methylester and the farnesyl group of **a**-factor has been tested with synthetic peptides in which these groups have been replaced by other substituents (Marcus et al., 1991). Removal of the farnesyl or methyl group resulted in significant reduction but not complete loss of activity. The farnesyl group could be replaced by other hydrophobic side chains, resulting in pheromones that were equally active or even more active than wild-type **a**-factor (Marcus et al., 1991). The secretion of the farnesylated **a**-factor is independent of the classic secretory pathway. Its translocation across the membrane is mediated by the yeast *STE6* gene product, which shows homology to eukaryotic membrane glycoproteins conferring multiple drug resistance (Kuchler et al., 1989). A *ste6* yeast mutant can be functionally complemented by the mouse wild-type *mdr3* gene, which encodes a P-glycoprotein functioning as an ATP-driven drug efflux pump (Raymond et al., 1992). This indicates that the **a**-factor transporter belongs to a family of ATP-dependent transport proteins.

Recognition of pheromones occurs through binding to specific receptors. The pheromone receptors of yeast have been cloned and sequenced. Their characterization revealed that both, although unrelated in primary sequence, belong to the large family of receptors with seven hydrophobic, potential membrane-spanning domains (Burkholder and Hartwell, 1985; Nakayama et al., 1985; Hagen et al., 1986). Rhodopsin and odorant and adrenergic receptors are other members of this family. These receptors are known to be coupled to heterotrimeric GTP binding proteins (G-proteins). Binding of the specific ligand to the receptor induces the α subunit of the G-protein to exchange the bound GDP for GTP. This leads to dissociation of the trimeric protein into the GTP-bound α subunit, which in most cases is the active component, and the dimer of β and γ subunits. In yeast, both pheromone receptors activate a common response pathway and are coupled to the same G-protein (Bender and Sprague, 1986; Nakayama et al., 1987).

In contrast to many other systems, the active component of the activated G-protein in *S. cerevisiae* has been demonstrated to be the dimer of β and γ subunits (Leberer et al., 1992a). This was the first demonstration that the βγ dimer can play an active role in signal transduction, a phenomenon that has long been discussed for other G-proteins and for which there is now evidence in mammalian systems as well (Federman et al., 1992). Recent results indicate that the primary candidate for direct activation by the βγ dimer of the G-protein might be the protein kinase STE20 (Leberer et al., 1992b).

The subsequent steps of the signal transduction cascade eliciting the pheromone response involve a set of different protein kinases and a transcription factor. These elements have been reviewed extensively elsewhere (Marsh et al., 1991; Sprague, 1991; Kurjan, 1992). The primary response to pheromone is cell cycle arrest in the G1 phase, the second response is the change in morphology (projection or shmoo formation), and the third is the induction of a number of genes that are involved in the fusion process. Among these genes are the pheromone genes themselves: the *STE6* gene, which is involved in transport of the **a**-factor; the pheromone receptor genes; and genes encoding **a**- and α-specific agglutinins,

which are not essential for fusion but which help the cells to coagulate (Lipke and Kurjan, 1992). The fusion process itself requires a highly organized temporal and spatial expression of genes leading to pronounced changes in the organization of cytoskeletal elements in preparation for the fusion event. The pheromone receptors and the **a**-factor transporter accumulate in the tip of the projection that is directed toward the mating partner (Kuchler et al., 1993). In addition to the poor solubility of **a**-factor, this is likely to produce a steep gradient of pheromone concentration, with the highest amount defining the fusion site. This might allow cells to direct their fusion sites and to fuse preferentially with cells that secrete the highest amounts of pheromone, a process referred to as courtship partner discrimination (Jackson and Hartwell, 1990). This effect could also explain the failure of added purified pheromone to restore the mating defect of **a**-factor mutant strains (Michaelis and Herskowitz, 1988).

In yeast, fusion of haploid cells is followed immediately by nuclear fusion. Diploid yeast cells are able to continue vegetative growth by budding. They undergo meiosis only if they are starved for nitrogen. In the diploid stage, most of the genes involved in pheromone production and response, such as the pheromone and receptor genes, are shut off (Herskowitz, 1989). Therefore, diploid **a**/α cells do not secrete mating factor or respond to mating factor. Thus, the diffusible mating factors of *S. cerevisiae* appear to function only in the process of cell–cell recognition during the fusion of haploid cells.

SCHIZOSACCHAROMYCES POMBE

Cells of the fission yeast *S. pombe* exist in one of two mating types, plus (P) or minus (M). Mating between cells of opposite types occur only under conditions of nitrogen starvation and is mediated by pheromones that induce conjugation tube formation and cell fusion. In contrast to *S. cerevisiae,* the resulting diploid cell undergoes meiosis and sporulation immediately. Mating-type identity and sexual differentiation are determined by four genes that are located at the active mating-type locus, *mat1* (Kelly et al., 1988). Each mating-type allele contains a pair of genes, which are termed *mat1-Mc* and *mat1-Mm* at the minus locus and *mat1-Pc* and *mat1-Pm* at the plus locus. The *mat1-Mc* and *mat1-Pc* genes are necessary for conjugation, whereas the *mat1-Mm* and *mat1-Pm* genes (alternatively termed *mat1-Mi* and *mat1-Pi*) are essential for meiosis. The mat1-Mc and mat1-Pm proteins display significant similarity to DNA binding proteins, which suggests that they act as transcriptional regulators. The molecular function of the other two gene products is not yet known. All four genes are needed for sexual differentiation. Sporulation and meiosis are achieved by a complicated network involving negative feedback loops acting on the protein kinase pat1, which is a general inhibitor of sexual functions (reviewed in Egel et al., 1990; Hayles and Nurse, 1992).

The pheromone secreted by M-cells has been purified and sequenced. The M-factor is a farnesylated and carboxymethylated nonapeptide that is processed from 42- and 44-amino acid precursors encoded by two genes, *mfm1* and *mfm2* (Davey, 1992). Production of the pheromone is induced by nitrogen starvation, and the P-cell response to purified M-factor is seen only under low nitrogen conditions. The P-factor has not yet been purified.

Genes encoding the pheromone receptors have been identifed for both mating types by isolating mating type–specific sterile mutants. The M-factor receptor, which is expressed only in P-cells, is similar to both the yeast STE3 **a**-factor receptor and the pheromone receptors of *U. maydis*, *pra1* and *pra2* (Tanaka et al., 1993). These receptors have in common that they all recognize farnesylated mating factors, and it will be interesting to determine whether a domain exists within these related receptors that is responsible for recognition of the farnesyl moiety. The farnesyl group is proposed to direct membrane targeting and interaction with other membrane-localized proteins. The exact function of the farnesyl moiety, however, is still unknown, and different models for direct or indirect interaction of proteins or receptors with the prenyl group have been discussed (Schafer and Rine, 1992). The receptor specific for the P-factor resembles the α-factor receptor of yeast (Kitamura and Shimoda, 1991), which might suggest that the as yet uncharacterized P-factor might be an unmodified peptide similar to the yeast α-factor.

In contrast to yeast, in which mating factors function only to achieve cell fusion, in *S. pombe* the pheromones serve an additional function. Continued activation of the pheromone response pathway is necessary to proceed through meiosis (Leupold et al., 1989).

NEUROSPORA CRASSA

In the filamentous ascomycete *Neurospora crassa*, two different mating types, termed *A* and *a*, exist. The mating-type locus confers sexual identity as well as heterokaryon incompatibility. The mating-type genes, therefore, fulfill contradictory functions: if mycelial cells of opposite mating types fuse, the resulting dikaryon is nonviable due to heterokaryon incompatibility. However, when the fungus enters the sexual cycle, specialized organs are formed that produce trichogynes (the female organ) and microconidia (the male cells). This specialization is affected by environmental conditions, such as medium composition and temperature. Specific recognition between microconidia and trichogynes of opposite mating types is mediated by pheromones (Bistis, 1983). The dikaryon produced by this sexual mating event is viable because the heterokaryon incompatibility associated with mating type is specifically suppressed by the action of a tolerance (*tol*) gene in these specialized cells (Newmeyer, 1970). The heterokaryon differentiates to form ascogenous hyphae. In these structures,

nuclei divide in synchrony, and, at some point, nuclear fusion takes place. This is followed by meiosis and formation of ascospores (reviewed in Metzenberg and Glass, 1990).

Both mating-type alleles, *A* and *a*, have been cloned, and their molecular organization reveals that they are defined by large regions of nonhomologous DNA. The term idiomorph has been introduced to denote these dissimilar regions, which occupy the same locus on their chromosome but are not related by sequence (Glass et al., 1988). In each mating-type allele, a single gene has been identified, termed *mt A-1* and *mt a-1*, which confers sexual identity and heterokaryon incompatibility (Glass et al., 1990; Staben and Yanofsky, 1990). The amino acid sequence of the *mt A-1* gene product shows some homology to the MATα1 protein of *S. cerevisiae*, and the *mt a-1* gene product is similar to the *S. pombe mat1-Mc* gene product. It is, therefore, likely that the genes encode regulatory proteins. The mating type–specific expression of pheromones involved in mating is thought to be controlled by the regulatory genes *mt a-1* and *mt A-1* (Glass et al., 1990). Interestingly, the putative pheromone gene present in the *A* mating type is physically linked to the *A* mating type locus and may be part of the locus; no evidence is available on the nature or linkage of an analogous locus in the *a* mating type (R.J. Metzenberg, personal communication).

RHODOSPORIDIUM TORULOIDES

In the basidiomycetous yeast *Rhodosporidium toruloides*, the mating reaction between compatible *A* and *a* cells is initiated by the formation of long conjugation tubes that are directed toward the mating partner (Abe et al., 1975). The mating interaction appears to be asymmetric and sequential: first, *A* cells induce conjugation tubes in *a* cells, then the growing tip of the *a* cells induces formation of conjugation tubes in *A* cells, and eventually, cells fuse at the tips of conjugation tubes (Abe et al., 1975). A diffusible mating hormone, termed Rhodotorucine *A*, was purified from the *R. toruloides* strain *A* (Kamiya et al., 1978). The purified pheromone arrests *a* cells in G1 and induces conjugation tubes in *a* cells. Analysis of the mating factor revealed that it is a short lipopeptide. The SH group of the C-terminal cysteine of the undecapeptide was shown to be covalently linked to a farnesyl group through a thioether bond (Kamiya et al., 1978; Table 1). This was the first demonstration of this type of protein modification, which has since been found in so many other fungal mating factors as well as in other eukaryotic proteins. The exact function of the farnesyl group remains unclear. In Rhodotorucine *A*, the carboxyl group of the terminal cysteine is unmodified. This is in contrast to other pheromones, in which this group is often methylated.

Genetic analysis has revealed that three genes encode Rhodotorucine *A*. They all code for tandem copies with either three (*RHA3*), four (*RHA1*), or five (*RHA2*) repeats of the undecapeptide sequence (Akada et al., 1989a, 1989b). The genes are present only in *A* cells, indicating that they are part of the mating-type locus, which has not yet been defined physically. Processing seems to occur at a lysine residue that is located between the repeats (Akada et al., 1989b). The Rhodotorucine *A* precursor is the first example of a peptide in which internal CAAX boxes can serve as substrates for prenylation (Schafer and Rine, 1992). It is not clear, however, whether farnesylation occurs before the tandem copies are cleaved.

CRYPTOCOCCUS NEOFORMANS

The opportunistic fungal pathogen *Cryptococcus neoformans* is a basidiomycetous yeast that causes a serious meningitis in patients who are immune deficient. Formation of the dikaryon is accompanied by a morphogenic transition from yeastlike to hyphal growth. Interestingly, strains of the mating type MATα are more virulent than those that are MAT**a** (Kwon-Chung et al., 1992). The MATα locus has been isolated by difference cloning; molecular analysis revealed the presence of a very large (35 to 40 kb) region of DNA that is present only in MATα strains (Moore and Edman, 1993). Within this region, a gene was identified that induces hyphal growth when transformed into MAT**a** strains but not MATα strains. The small size of this gene and the presence of a CAAX motif at its C terminus, as shown in Figure 1, indicate that it is likely to encode a pheromone precursor (Moore and Edman, 1993). It is not yet clear how the pheromone precursor is processed, and it

Species	Gene	Structure
C. neoformans	MFα	MDAFTAIFTTFFSAATSSSEAPRNQEAHPGGMTL**CVIA**
U. maydis	mfa1	MLSIFAQTTQTSASEPQQSPTAPQGRDNGSPIGYSS**CVVA**
U. maydis	mfa2	MLSIFETVAAAAPVTVAETQQASNNENRGQPGYY**CLIA**

Figure 1. Amino Acid Sequences of Pheromone Precursors.

Sequences are described by Moore and Edman (1993) and by Bölker et al. (1992). Bold letters indicate the CAAX motif. The structures of the mature pheromones are not known.

remains to be seen whether the pheromone itself affects virulence.

TREMELLA MESENTERICA AND *TREMELLA BRASILIENSIS*

In the basidiomycete *Tremella mesenterica*, a jelly fungus, diffusible hormones, called tremerogens, induce conjugation tube formation (Bandoni, 1965). *T. mesenterica a* cells produce the tremerogen *a*-13, and *A* cells produce the tremerogen *A*-10 mating factor. The genes coding for the precursors of these pheromones have not yet been identified. Both pheromones are farnesylated, but they show some differences in their modifications: Tremerogen *a*-13 is a farnesylated 13–amino acid lipopeptide whose cysteine is not carboxymethylated (Sakagami et al., 1981; Table 1), whereas the decapeptide tremerogen *A*-10 contains a farnesylated and carboxymethylated cysteine and the farnesyl group contains an alcohol group (Sakagami, 1979; Table 1).

Synthetic analogs of this pheromone have been synthesized and tested for their biological activity (Fujino et al., 1980). Removal of the carboxymethyl group was shown to destroy biological activity. Substitution of the carboxymethyl group with an amido group restores function. The farnesyl moiety could be replaced by other highly lipophilic side chains without affecting activity. Surprisingly, farnesyl analogs consisting of four to five prenyl groups were substantially more active than tremerogen *A*-10, whose normal farnesyl group contains three prenyl groups. In contrast to yeast **a**-factor, secretion of the tremerogen *A*-10 seems to occur by the classic secretory pathway because it can be inhibited by monensin, an inhibitor of Golgi transport (Miyakawa et al., 1985).

In *T. brasiliensis*, the tremerogen A-9291-I has been identified as a 15–amino acid peptide whose C-terminal cysteine is carboxymethylated and carries an oxidized farnesyl group (Ishibashi et al., 1984; Table 1). During purification, a second activity was detected with much less specific activity. Further analysis revealed this activity to correspond to a noncarboxymethylated species of the pheromone. This indicates that the carboxymethyl group is important for full activity. It has been speculated that the inactive mating factor could result from a specific enzymatic activity that renders the pheromone inactive by demethylating it (Ishibashi et al., 1984).

USTILAGO MAYDIS

The Basidiomycete fungus *U. maydis* has a mating system that is controlled by two unlinked genetic loci, *a* and *b*. The *a* locus, which exists in two alleles (*a1* and *a2*), controls fusion of haploid cells. The multiallelic *b* locus encodes two regulatory proteins that govern sexual development. Together with *a*, the *b* locus controls the transition from yeastlike growth of haploid cells to hyphal growth of the dikaryon as well as maintenance of the dikaryon (for review, see Banuett, 1992). The *a* locus is thus necessary both for the cell fusion event and for filament maintenance (Banuett and Herskowitz, 1989).

The cloning and the molecular analysis of the *a* mating-type locus revealed a novel strategy of mating-type determination. The *a1* and *a2* alleles are characterized by large regions of sequence dissimilarity (4.5 and 8 kb, respectively), very much like the *a* and *A* alleles of *N. crassa* (Froeliger and Leong, 1991; Bölker et al., 1992). Within these dissimilar sequences, two genes have been identified that are crucial for mating-type determination. A short gene was found in each allele, with coding capacities of 40 amino acids in *a1* (*mfa1*) and 38 amino acids in *a2* (*mfa2*). These genes were implicated to encode pheromone precursors because of their size and because a CAAX box was found at the C terminus of both hypothetical proteins, suggesting that the peptides are subject to post-translational prenylation (Figure 1; Bölker et al., 1992). The amino acid sequences of the other genes at the *a* locus, *pra1* and *pra2*, were found to be similar to that of the yeast *STE3* gene, the **a**-factor receptor, suggesting that *pra1* and *pra2* encode specific pheromone receptors.

It was possible to test these assertions by making use of the unique mating behavior of *U. maydis*: diploid cells that carry both the *a1* and the *a2* allele but are homozygous at *b* can readily fuse with haploid cells carrying a second *b* allele (Holliday, 1961). Haploid strains carrying a mutant pheromone gene are sterile when crossed with compatible haploid strains; their mating defect can be alleviated, however, if they are crossed to diploid strains heterozygous for *a*. This indicates that the product of the *mfa* gene is secreted and can be supplied by the diploid mating partner. Strains mutant for the pheromone receptor gene are also sterile; however, this phenotype cannot be complemented by strains heterozygous for *a* (Bölker et al., 1992).

This is the first example of a mating-type locus containing the structural genes for components that are involved directly in cell–cell communication. This finding was particularly surprising because in *U. maydis*, the existence of diffusible mating factors had not been inferred from observation. The presence of these genes has prompted the search for a direct demonstration of pheromone activity. A biological test was devised that is based on the observation that cocultivation of a receptor mutant that is unable to mate, but that can still produce *a1*-specific pheromone, with a diploid *a2/a2 b1/b2* strain induces a transition from yeastlike to filamentous growth. This change in morphology is thought to occur only if both the pheromone response pathway and the regulatory cascade controlled by the *b* locus are activated. A partially purified activity secreted by *a1* cells was also able to induce filament formation in this diploid strain (T. Spellig, M. Bölker, and R. Kahmann, unpublished results). The same fraction, however, did not affect the morphology of an *a1* strain heterozygous for *b*, which illustrates that the observed effect is pheromone specific. Because activation of the pheromone receptor is prerequisite for filamentous growth, in the experiments mentioned above, the naturally

occurring filamentous dikaryon is also likely to depend on the continuous activation of the pheromone response pathway.

The pheromones of *U. maydis* can, therefore, be viewed as autoinducers triggering an autocrine response. Consistent with this function of the *a* locus after mating is the finding that expression of the pheromone genes is not repressed after fusion has occurred (M. Bölker, unpublished results). The situation is more complex, however, because the filamentous dikaryon must be heterozygous at *a* and *b* for its stable maintenance and further development. The multiallelic *b* locus codes for two homeodomain-related proteins, termed *bE* and *bW*, which are presumed to control sexual development by certain combinatorial interactions and to act as transcription factors (Kronstad and Leong, 1990; Schulz et al., 1990; Gillissen et al., 1992). The pathways triggered in response to the pheromone and the transcriptional regulation by the homeodomain proteins must at some point converge to elicit the morphogenic transition. It will be interesting to discover how this is achieved in molecular terms.

USTILAGO HORDEI

The phytopathogenic fungus *U. hordei* is closely related to *U. maydis* but has a mating-type system with only two mating types, *MAT-1* and *MAT-2* (formerly designated *A* and *a*). Although there is some evidence for the existence of diffusible mating factors, the cloning of mating-type alleles revealed the presence of "*b*"-like homeodomain proteins. These proteins are similar to those found in *U. maydis* and can functionally replace the *U. maydis* proteins (Bakkeren and Kronstad, 1993). The most likely scenario for the mating behavior of *U. hordei* is that the genes that trigger dikaryon-specific gene expression are genetically linked to the genes coding for cell signaling components.

PERSPECTIVE

Pheromones occur mostly in fungi in which single cells have to fuse: in yeasts, which exist always as single cells; in basidiomycetous fungi, where the transition between a haploid unicellular phase and the filamentous dikaryotic phase is associated with mating; and in ascomycetes, during the fusion of specialized organs. The fusion of mycelial growing cells is often promiscuous, as in basidiomycetes, and in these systems, the particular combinations of transcription factors combined in a single cell may be decisive for further development. In ascomycetes, fusion of mycelial cells often results in cell death due to the action of heterokaryon incompatibility genes. It is not clear whether diffusible substances and/or specific receptors play an additional role in these systems.

The specific pheromones and their receptors are essential for cell–cell recognition and for the control of the complex fusion reaction. Many fungal pheromones are highly hydrophobic due to modification of the C-terminal cysteine by a farnesyl group. Only the α-factor of the yeast *S. cerevisiae* and the α pheromone of the closely related yeast *S. kluyveri* (Sakurai et al., 1984) have been found to be unmodified peptide mating factors. It is not clear why yeast produces these two different types of pheromone. Yeast can grow in quite different habitats—in liquid culture or as colonies on the surface of fruits, leaves, or solid media—and the different chemical properties of the mating factors might be well adapted to these varying conditions. The problem of the low solubility of farnesylated mating factors in aqueous solution might be alleviated if their diffusion were to occur in lipid films on hydrophobic surfaces. This interesting possibility awaits investigation.

It is also not clear why pheromone precursor genes tend to exist in several copies. Higher copy numbers could simply guarantee high expression levels of pheromone, which would be advantageous during partner selection. To have several copies would also allow the production of pheromones that differ in sequence, as has been observed in some cases. It is conceivable that these alterations affect pheromone stability or play a role in yet unknown processes of recognition.

The growing number of fungal mating-type loci that have been analyzed makes it possible to compare different systems of mating-type determination. Two fundamentally different strategies appear to have been adopted to specify cell type. In *S. cerevisiae* and *S. pombe*, the expression of specific pheromones is under transcriptional control because copies of both cell type–specific pheromone genes are present in the same cell. In all other species in which pheromone precursor genes have been identified, they are located in nonhomologous regions or are closely linked to these regions. In *U. maydis*, the genes coding for the receptors have also been found in these regions of dissimilarity. It is not clear if these different mating-type systems are all derived from a common ancestor and, if so, how the archetype of mating-type determination was organized. It is obvious, however, that the pheromonal regulation of mating has adopted different functions in the context of sexual differentiation and development and is not only used to sense the presence of the other mating partner. The potential involvement of pheromones in virulence of both plant and human pathogens might provide new opportunities to study these processes at the molecular level. Because certain development events are initiated only after mating, pheromone receptors are becoming potential new targets for pharmaceutical drugs that could be used in the control of fungal infection.

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