Fungal Genetics and Biology **27,** 146–152 (1999)
Article ID fgbi.1999.1129, available online at http://www.idealibrary.com on IDEAL®

REVIEW

Mating Types and Pheromone Recognition in the Homobasidiomycete *Schizophyllum commune*

Erika Kothe[1]

Microbial Phytopathology, Friedrich-Schiller-University, Beutenbergstr. 11, 07745 Jena, Germany

Accepted for publication March 19, 1999

This review is dedicated to Jos Wessels on occasion of his retirement.

Kothe, E. 1999. Mating types and pheromone recognition in the homobasidiomycete *Schizophyllum commune. Fungal Genetics and Biology* 27, 146–152. In the homobasidiomycete *Schizophyllum commune* the mating type genes of the *B* locus encode pheromones and pheromone receptors in multiple allelic specificities. Interaction of non-self pheromones and receptors leads to induction of *B*-regulated development easily scored in *S. commune* by the "flat" phenotype which lacks aerial mycelium formation and shows aberrant hyphal morphology. In contrast, self pheromones are not recognized and *B*-regulated development is not induced. Natural and mutant alleles of receptors have been analyzed for their specificity in transformation assays, and parts of the receptor involved in ligand discrimination can be described. The biological role of pheromone response in *S. commune* is assumed to be connected to nuclear migration based on the observation that wild-type cells with a receptor gene of different specificity lead to cells capable of nuclear uptake. Other possible roles for pheromone function are discussed.

Index Descriptors: mating type genes; basidiomycetes; fungal pheromones; ligand recognition; natural and mutant alleles.

Pheromones are defined as small, diffusible substances that are exchanged between different individuals of one species (for reviews including basidiomycete pheromones see Bölker and Kahmann, 1993; Vaillancourt and Raper, 1996). Pheromones have been identified in different fungal taxa including ascomycetes like yeast and the heterobasidiomycetes *Rhodosporidium toruloides* and *Tremella* (Ishibashi *et al.,* 1984; Kamiya *et al.,* 1978; Kurjan, 1993; Sakagami *et al.,* 1981) and were found to be encoded by mating type genes in *Ustilago maydis* (Bölker *et al.,* 1992) where fusion of the mating partners and filamentous growth are dependent on pheromone exchange (Banuett and Herskowitz, 1989; Spellig *et al.,* 1994).

In homobasidiomycetes, pheromones were not expected since plasmogamy occurs between all mating types. The four different reactions observed in matings between monosporous isolates of tetrapolar homobasidiomycetes such as *Schizophyllum commune* rather led to the conclusion that interactions of intracellular components are the basis of recognition (Kühn and Parag, 1972). However, recently genes coding for pheromones and their receptors have been identified in the *B* mating type loci of *S. commune* and *Coprinus cinereus* (O'Shea *et al.,* 1998; Vaillancourt *et al.,* 1997; Wendland *et al.,* 1995). Thus, pheromones are major components in mating type determination in homobasidiomycetes (see also Casselton, 1997; Kronstad and Staben, 1997).

I. FUNGAL PHEROMONE SYSTEMS

In the yeast *Saccharomyces cerevisiae,* one mating type secretes a peptide pheromone that is recognized by the other and induces G1 arrest (Kurjan, 1993). When the two

[1] To whom correspondence should be addressed. Fax: +49(3641)657633. E-mail: ekothe@pmail.hki-jena.de.

1087-1845/99 $30.00

cells fuse they are both in G1 phase and can start the new cell division cycle synchronously. One mating type expresses a receptor, Ste2, that recognizes a peptide pheromone of the other mating type, and the other mating type receptor, Ste3, is activated by a farnesylated and carboxymethylated lipopeptide (Kurjan, 1993). The receptor belongs to a class of receptors with seven transmembrane domains which is known to be present in all kingdoms of life; a few examples are the phytochrome system in plants, bacteriorhodopsin in prokaryotes, rhodopsin in animals, and the mammalian odorant, adrenergic, or bradykinin receptors.

In basidiomycetes, the two allelic pheromone mating type loci of *U. maydis, a1* and *a2,* have been analyzed in detail (Bölker *et al.,* 1992; Urban *et al.,* 1996a). Each locus contains one receptor (named, respectively, *pra1* and *pra2*) and one active pheromone gene (*mfa1* and *mfa2,* respectively). The pheromone genes code for precursors 40 amino acids long for *mfa1* and 38 amino acids long for *mfa2* pheromones, respectively. These precursors are truncated, carboxymethylated, and farnesylated to form the 13 and 9 amino acid active ligands (Spellig *et al.,* 1994). This situation is similar to the a-factor pheromone of *Saccharomyces cerevisiae* which is also farnesylated. The farnesylation signal consensus sequence C-a-a-X (for cysteine, two aliphatic amino acids, and a terminal amino acid) is found at the C-terminus of the peptide sequence immediately preceding the stop codon. At the cysteine residue the peptide chain is truncated and the cysteine is modified by carboxymethylation and addition of the farnesyl tail (Clarke, 1992).

In yeast, the G-protein-linked receptors transmit the pheromone signal via a MAP kinase cascade, including *STE7, STE11,* and *STE20* gene products culminating in the activation of a specific transcription factor, Ste12 (Kurjan *et al.,* 1993). A similar system has been described for the fission yeast *Schizosaccharomyces pombe* (Aono *et al.,* 1994) and the heterobasidiomycete *U. maydis,* where several G-proteins have been analyzed (Regenfelder *et al.,* 1997). One, Gpa3, seems to participate in signal transduction following pheromone stimulation (see also Bölker, 1998). In this heterobasidiomycete, one gene that possibly plays a role in signal transduction, *fuz7,* that was thought to be a functional homologue to the yeast *STE7* gene. Most important, a transcription factor able to activate target genes has been identified. This pheromone response factor, Prf, binds to pheromone response elements, *PRE,* that are present in the promoter regions of target genes including the pheromones (Hartmann *et al.,* 1996; Urban *et al.,* 1996b).

II. ISOLATION AND FUNCTION OF *S. COMMUNE* MATING TYPE GENES

In tetrapolar basidiomycetes four different reactions can be distinguished upon mating, e.g., in the homobasidiomycete *S. commune* (Fig. 1). The term tetrapolar describes this phenomenon which was attributed to two separate mating type factors, called *A* and *B* in homobasidiomycetes (Kniep, 1920, 1928). The four different reactions include (1) compatible matings between two isolates with different *A* and *B* mating type genes, which is necessary for fruitbody formation and spore production; (2) incompatible matings between isolates with identical A and B mating type genes, resulting in growth indistinguishable from the monokaryotic strains; and (3,4) two semicompatible mating reactions where the mating type genes of the confronted mycelia differ either in *A* alone (also called a common-*B* reaction) or in *B* alone (also known as common-*A* reaction). Upon plasmogamy, which is observed regardless of mating type, nuclear migration is induced to transport the mate's nuclei throughout the entire mycelium as a prerequisite to nuclear pairing. In a compatible mating the nuclei pair and perform conjugate divisions forming clamps which ensure the proper distribution of both genetically distinct nuclei in the daughter cells. Karyogamy takes place within the fruitbody hymenium

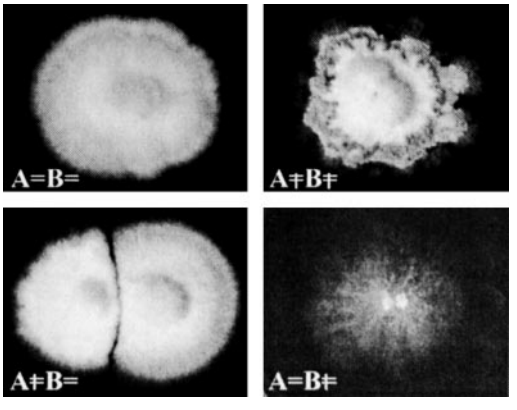

FIG. 1. Tetrapolar mating interactions of *Schizophyllum commune.* Monosporous isolates representing the offspring of one fruitbody were mated. Identical *A* and *B* factors lead to growth indistinguishable from monokaryon (*A*= *B*=), different *A* as well as *B* factors result in fertile dikaryotic mycelium (*A*≠ *B*≠), whereas semicompatible crosses with different *A* loci can lead to the formation of a barrage reaction seen in the zone separating the two monokaryotic strains (*A*≠ *B*=). In semicompatible crosses with different *B* factors, *S. commune* shows a distinct "flat" phenotype with lack of aerial mycelium (A= *B*≠).

immediately before meiosis. If only the *A* factors are different, part of this developmental pathway is switched on. Nuclear pairing and clamp cell formation can be seen only at the mating zone between the two isolates, but clamp cell fusion and nuclear migration are missing. The latter event apparently is under control of the *B* factor as seen in matings of strains different only in *B*, not in *A* (Raper, 1983, 1966).

The first mating type genes in basidiomycetes were identified in *S. commune* (Giasson *et al.*, 1989). In this fungus, both *A* and *B* factors are found to be composed of two separate genetic loci, *Aα* and *Aβ*, and *Bα* and *Bβ* for *A* and *B*, respectively. For all four loci multiple alleles exist in nature which makes this mating type system the most extensive known so far. Nine *Aα* allelic specificities are found and *Aβ* is estimated to have 32 specificities based on recovery frequencies of different alleles from nature. In both *Bα* and *Bβ* nine allelic forms are found (Koltin *et al.*, 1972; Raper *et al.*, 1958). In combination, this leads to 23,328 different possible mating types for *S. commune.* It is thought that this large number stimulates outbreeding and thus has evolved to promote heterozygosity in the natural population (Raper, 1966).

Sequence analysis of the *Aα* locus has identified two divergently transcribed genes termed *y* and *z* (Specht *et al.*, 1992; Stankis *et al.*, 1992). Heterodimers formed between the gene products Y and Z are active only when the two subunits are from different allelic specificities (Magae *et al.*, 1995). The heterodimer is a homeodomain-containing transcription factor (Luo *et al.*, 1994) able to induce expression of downstream target genes. In the *Aβ* locus gene products were found homologous to Y and Z, e.g., the *Aβ6* gene product V is similar to Y (Shen *et al.*, 1996). The same homeodomain-containing transcription factor gene arrangement is seen in the single *b* mating type locus of the heterobasidiomycete *Ustilago maydis* (Gillisen *et al.*, 1992; Schulz *et al.*, 1990). In this tetrapolar fungus, the multiallelic *b* locus with the genes b_E and b_W (for east and west) is homologous to *A* of *S. commune.* The third basidiomycete that has been investigated in detail is the homobasidiomycetous mushroom *C. cinereus* (Casselton and Kües, 1994). Here, three to four closely linked units of divergently transcribed homeodomain transcription factor genes are found in the *A* locus (Casselton, 1998; Kües and Casselton, 1993; Kües *et al.*, 1992; Tymon *et al.*, 1992).

The second mating type locus in all three model basidiomycetes has been found to contain genes coding for pheromones and their receptors. For the heterobasidiomycete *U. maydis* this was not unexpected since this organism can grow in the yeast form and directed growth of mating types toward each other is seen prior to plasmogamy (Snetselaar *et al.*, 1996).

In *S. commune* the *B* mating type loci *Bα* and *Bβ* have also been isolated (Specht, 1996), yielding the *Bα1* and *Bβ1* mating type genes which were sequenced (Wendland *et al.*, 1995; Vaillancourt *et al.*, 1997). This revealed four genes present in both the *Bα1* and the *Bβ1* mating type loci, one coding for a receptor and three others encoding putative pheromones (Fig. 2). The pheromone receptor genes were identified by sequence similarity with other pheromone receptor genes, in particular the *STE3* receptor gene in *S. cerevisiae* and *pra1* and *pra2* receptor genes of *U. maydis.* The putative pheromone genes were functionally characterized by induction of *B*-specific development in a recipient strain upon transformation.

III. RECOGNITION OF MULTIPLE PHEROMONES

The pheromone genes were characterized by open reading frames that encoded putative pheromone precursors which all ended with a sequence that is reminiscent of the farnesylation signal C-a-a-X of yeast, containing a cysteine 4 amino acids from the stop codon and, in most cases, the cysteine followed by two aliphatic amino acids. In yeast, the terminal amino acid of the C-a-a-X box specifies the isoprenoid ligand that is attached to the sulfur alcohol of the cysteine; i.e., serine, alanine, glutamine, or

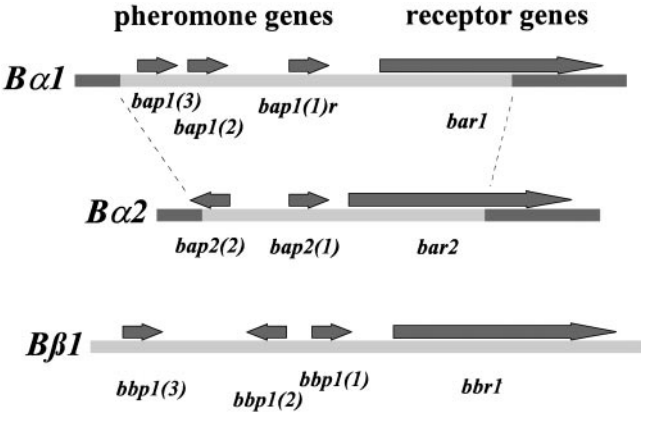

FIG. 2. Genomic *Bα* and *Bβ* loci of *S. commune.* Comparison of sequences of the allelic *Bα1* and *Bα2* loci has revealed homologous flanks (darker shade) bordering the heterologous mating type loci.

methionine as terminal amino acids specify a farnesyl ligand, while leucine as the terminal amino acid would specify the addition of a geranylgeranyl component (Caldwell *et al.*, 1995; Clarke, 1992; Omer and Gibbs, 1994). In *S. commune* eight pheromone genes have been identified so far located in the *Bα1, Bα2,* and *Bβ1* mating type loci (Fig. 3).

Since in nature nine *Bα* and nine *Bβ* alleles are found, the activation pattern for each gene was tested. The receptor gene *bar1* for the *Bα1* locus activated *B*-regulated development in all eight specificities differing from self. The same is true for *bbr1,* the receptor gene in the *Bβ1* locus. This shows that the receptors are able to recognize pheromone(s) in each recipient strain. The pheromone genes showed a distinct activation pattern in that every gene was able to induce *B*-regulated development in at least one recipient. In every recipient strain one receptor gene is found which implies that a single pheromone is able to activate more than one receptor and that one receptor is able to distinguish between multiple pheromones—no activation is seen with self pheromones, while some of the non-self pheromones induce pheromone response via the same single receptor (Wendland *et al.,* 1995).

By comparing *S. commune* and *C. cinereus* pheromone sequences, a putative amino-terminal processing site was postulated to consist of a conserved amino acid sequence element of two charged residues: (E, D, N, or Q) and (R or H) (O'Shea *et al.,* 1998; Casselton and Olesnicky, 1998). Analysis of the eight *S. commune* pheromones shows two such sites for Bap1(2), which is highlighted by spacing in Fig. 3. If the second processing site is used, a mature pheromone of 5 amino acids results, which might explain the capacity of this specific pheromone to induce pheromone response only in one other mating type.

For one gene, *bap1(1)* of the *Bα1* mating type locus, expression analyses have shown an orientation different from that previously assumed (Raudaskoski *et al.,* 1998). The gene that has been identified in the expression analysis is termed *bap1(1)r* for distinction. The gene product ends in the motif -CXXX as a signal for isoprenylation. In most other *S. commune* pheromones, -CVVA was found at the terminus, which specifies farnesylation in yeast. Three other C-termini were found, for *bap1(2)* -CVRG, for *bap2(1)* -CDKA, and for *bap2(2)* -CDYQ (Hegner *et al.,* 1999; Wendland *et al.,* 1995). In the mushroom *C. cinereus* three arrays of one pheromone receptor and two pheromone genes are arranged head to tail in the *B* locus (Casselton, 1998; O'Shea *et al.,* 1998).

IV. NATURAL AND MUTANT ALLELES

In addition to the *Bα1* locus, three more loci have been analyzed so far. In an allelic *Bα1* strain that was isolated from a different source, the respective *bar1* gene was sequenced. No amino acid differences were observed in the part of the receptor that is thought to include the pheromone binding sites on the extracellular face or transmembrane domains. Differences in the long intracellular C-terminus, however, allowed the determination of the point at which the *B* locus ends. The locus is defined by a sequence that must be different between different mating types and similar between identical mating types to suppress recombination between different alleles, thus avoiding the loss of former identity in a new composition of pheromones with a non-self receptor. Thus, the sequence different between the two mates is not included in the mating type locus but represents one flank of the *Bα* locus (Wendland and Kothe, 1996).

```
Bap1(1)r                                          MLANVAPPAQLNGALPRNG.EREGGSDCTAWCVVA*
Bap1(2)                              MRSRASAEGIAVLGLRRRGESPVCRRRNVVVCFWG.DRSCV.EREG.CVRG*
Bap1(3)                              MDDFAEFFPTLVLDEPEVARRPARDAEVLAILADA.ERPGGSNCTAWCVVA*
Bap2(1)                                                        MDVTGH.......CDKA*
Bap2(2)                                                  MANRTPACGK.QRFGPGHVQDNAAICDYQ*
Bbp1(1)  MDAFTAMFPELFPIEEGLEDALVGSDTSAASASATHTSPASTDTF DDADILAILADA.EHWR.GGNTTAHGWCVVA*
Bbp1(2)           MDAFTDFSILADGLASLGDESSHTILAEFSPSILDGPFVADSAPLTEAPC.NHDQIADY...GSYCVVA*
Bbp1(3)    MASSVLARPGPSTVLPAMTRPPPPMAHRAAATPSFARSAQPQT DDA VLALLANA.EHTEAGEETTARGWCVVA*
```

FIG. 3. Amino acid sequences of pheromones encoded in the *Bα1* (Wendland *et al.,* 1995), *Bα2* (Siebert-Bartholmei and Kothe, unpublished; Genebank Accession No. AF 102785) and *Bβ1* (Vaillancourt *et al.,* 1997) loci of *S. commune.* N-terminal putative processing sites are highlighted by spacing, putative farnesylation motifs including 4 amino acid and a stop codon (*) are underlined.

Very interesting results came from the investigation of mutants that had been introduced in the *B* genes in screens for constant activation of *B*-regulated development and their reversion (Raper and Raper, 1973). It has been postulated that the original self-recognizing mutant was obtained by altering one of the pheromones such that it now activates the "self" receptor. Such an alteration was verified by sequence analysis (Fowler *et al.*, 1998). Interestingly, all possible variations for reversion have been found in the secondary mutants that have lost the self-activating phenotype. Deletion of the mutated pheromone and the receptor, deletion of the whole locus, and alteration of the receptor were all found in sequence analyses of the different classes of secondary mutants obtained previously (Fowler *et al.*, 1998). The deletion of the entire locus will prove helpful in future research as it makes investigation of the properties of a single gene possible without the background of natural alleles and genes found in a wild-type recipient strain.

V. PHEROMONE RESPONSE IN HOMOBASIDIOMYCETES

The identification of mating pheromones in both *S. commune* and *C. cinereus* prompted the question: what is the function of these pheromones in homobasidiomycetes? Attraction of mates has not been reported, although some evidence for directed growth might be found at the microscopical level (Raudaskoski, 1998). However, another possible function is seen with respect to the events induced by *B*. These include especially the induction and continuation of nuclear migration throughout the mate's mycelium. A role for a diffusible pheromone might be to induce the cells of the mate for the production of nuclear transport machinery as was postulated earlier for the role of pheromones in homobasidiomycetes (Kothe, 1996; Wendland *et al.*, 1995). This role of pheromones is now well established as evidenced by the different behavior of transformants containing either additional receptor or additional pheromone genes. Pheromone transformants are able to induce *B*-regulated development in a mate which is attributed to the diffusible nature of a secreted pheromone. The receptor, however, will stay in the membrane of the transformed cell and no activation of the mate is seen. This different behavior can be scored as occurrence of the "flat" phenotype in the transformant only. Another possibility to look for different behavior of pheromone vs. receptor transformants is scoring unilateral nuclear migration in crosses in which the mate has different A and the same B as the transformation recipient. In such a cross between recipient and tester strain formation of the barrage reaction is expected (common-*B* reaction). If a receptor transformant was crossed to the tester strain, nuclear migration is induced only in the transformant, which now is able to take up the tester's nuclei and then is able to form clamp connections in dikaryotic hyphae. The tester strain is not able to take up nuclei and therefore will not be able to develop dikaryotic hyphae and clamp connections. The transformant thus can be scored as unilateral nuclear acceptor. If a pheromone transformant and the tester strain are opposed, the diffusible pheromone induces nuclear uptake in the tester strain and therefore a bidirectional, symmetric nuclear migration with subsequent dikaryon and clamp formation is seen. From this observation the nature of mutations can be predicted, which was confirmed in molecular analyses (Fowler *et al.*, 1998).

Another function that has been attributed to the *B* genes is clamp cell fusion. Here, directional growth is seen, which leads to bending of the clamp cell and ultimately to the close contact of clamp cell and penultimate cell, which is necessary for clamp cell fusion. This could elegantly be explained by directional growth in the clamp cells of dikaryons toward a source of pheromone. It would not explain, however, the similar structure of unfused pseudoclamps in forced matings, in which the mates differ only in their *A* factors or in strains transformed with *A* genes. In addition, not all basidiomycetes need clamp connections to maintain dikaryosis (Salo *et al.*, 1989).

Another phenomenon that is still not understood is nuclear identity. In the dikaryon the two different nuclei of both parent strains are paired. To ensure pairing of the two different nuclei, the two nuclei must be recognized, even after they were located in a common cellular compartment. Thus, the identity of each nucleus must be maintained even after a long passage in a common, dikaryotic mycelium. Since the only genetic loci that need to be different in a mating are the mating type loci, this information should be expected to reside within the mating type loci themselves. The *A* loci seem more likely to permit such a function because the *A* loci are thought to control nuclear pairing, but possibly both mating type loci are involved in this process.

A very interesting assumption is that the effect of pheromones on receptors is linked to the nuclear distance in dikaryons. It has been shown that in dikaryotic aerial hyphae the two nuclei are further apart than in submerged hyphae which correlates with a "monokaryon-like" gene

expression pattern in aerial hyphae of dikaryons. A model has been proposed that involves the interaction of pheromones and receptors in such a process (Schuurs *et al.*, 1998). It is expected that the receptors and pheromones are translated in the vicinity of the nucleus in which they are encoded. Then, the position of insertion of the receptor in the membrane marks the position of this specific nucleus which could be linked to this membrane patch by cytoskeleton structures. Receptor activation is then transformed into positioning of the nucleus in the vicinity of the induced receptors, which permits a force to be generated that moves the two nuclei together. In aerial mycelium, where diffusion of pheromones is inhibited by the aerial environment, this force would be weakened and thus separation of the nuclei is seen. This scenario is very interesting and should be tested in detail. It may open a new route to understanding the specific properties of dikaryons specific to fungi and at the same time allow insight into molecular imprinting mechanisms that have been postulated but are not understood in a wide spectrum of taxa.

A screen for genes differentially regulated by the *B* genes may answer the question for a role of pheromones in *Schizophyllum* development. One gene specifically downregulated by *B* has been identified by differential RNA display (Lengeler and Kothe, 1999). This gene, *brt1,* codes for a putative translational initiation inhibitor, which might indicate that this transcriptionally regulated gene is involved in triggering translational control which in eukaryotes is a main control mechanism of development.

ACKNOWLEDGMENT

The author thanks K. K. Klein for critical reading of the manuscript.

REFERENCES

Aono, T., Yanai, H., Miki, F., Davej, J., and Shimoda, C. 1994. Mating pheromone-induced expression of the *mat1-Pm* gene of *Schizosaccharomyces pombe:* Identification of signaling components and characterization of upstream controlling elements. *Yeast* **10:** 757–770.

Banuett, F., and Herskowitz, I. 1989. Different *a* alleles of *Ustilago maydis* are necessary for maintenance of filamentous growth but not for meiosis. *Proc. Natl. Acad. Sci. USA* **86:** 5878–5882.

Bölker, M. 1998. Sex and crime: Heterotrimeric G proteins in fungal mating and pathogenesis. *Fungal Genet. Biol.* **25:** 143–156.

Bölker, M., and Kahmann, R. 1993. Sexual pheromones and mating responses in fungi. *Plant Cell* **5:** 1461–1469.

Bölker, M., Urban, M., and Kahmann, R. 1992. The *a* mating type locus of *U. maydis* specifies cell signaling components. *Cell* **68:** 441–450.

Caldwell, G. A., Naider, F., and Becker, J. M. 1995. Fungal lipopeptide mating pheromones: A model system for the study of protein prenylation. *Microbiol. Rev.* **59:** 406–422.

Casselton, L. A. 1997. Molecular recognition in fungal mating. *Endeavour* **21:** 159–163.

Casselton, L. A. 1998. The mating type genes of the homobasidiomycete fungi. In *Proceedings of the Fourth Conference on the Genetics and Cellular Biology of Basidiomycetes* (L. J. L. D. van Griensven and J. Visser, Eds.), pp. 20–26. Mushroom Experimental Station, Horst, The Netherlands.

Casselton, L. A., and Kües, U. 1994. Mating-type genes in homobasidiomycetes. In *The mycota, Vol. 1, Growth, Differentiation and Development* (J. G. H. Wessels and F. Meinhardt, Eds.), pp 307–321. Springer Verlag, Heidelberg.

Casselton, L. A., and Olesnicky, N. S. 1998. Molecular genetics of mating recognition in basidiomycete fungi. *Microbiol. Mol. Biol. Rev.* **62:** 55–70.

Clarke, S. 1992. Protein isoprenylation and methylation at carboxyl-terminal cysteine residues. *Annu. Rev. Biochem.* **61:** 355–386.

Fowler, T. J., Mitton, M. F., and Raper, C. A. 1998. Gene mutations affecting specificity of pheromone/receptor mating interactions in *Schizophyllum commune.* In *Proceedings of the Fourth Conference on the Genetics and Cellular Biology of Basidiomycetes.* (L. J. L. D. van Griensven and J. Visser, Eds.), pp. 130–134. Mushroom Experimental Station, Horst, The Netherlands.

Giasson, L., Specht, C. A., Milgrim, C., Novotny, C. P., and Ullrich, R. C. 1989. Cloning and comparison of *Aα* mating-type alleles of the basidiomycete *Schizophyllum commune. Mol. Gen. Genet.* **218:** 72–77.

Gillissen, B., Bergemann, J., Sandmann, C., Schroeer, B., Bölker, M., and Kahmann, R. 1992. A two-component regulatory system for self/nonself recognition in *Ustilago maydis. Cell* **68:** 647–657.

Hartmann, H. A., Kahmann, R., and Bölker, M. 1996. The pheromone response factor coordinates filamentous growth and pathogenic development in *Ustilago maydis. EMBO J.* **15:** 1632–1641.

Hegner, J., Siebert-Bartholmei, C., and Kothe, E. 1999. Ligand recognition in multi-allelic pheromone receptors from the basidiomycete *Schizophyllum commune* studied in yeast. *Fungal Genet. Biol.*, in press.

Ishibashi, Y., Sakagami, Y., Isogai, A., and Suzuki, A. 1984. Structure of tremerogens A-9291-I and A-9291-VIII: Peptide sex hormones of *Tremella brasiliensis. Biochemistry* **23:** 1399–1404.

Kamiya, Y., Sakurai, A., Tamura, S., Takahashi, N., Abe, K., Tsuchiya, E., and Fukui, S. 1978. Structure of rhodotorucine A, a novel lipopeptide, inducing mating tube formation in *Rhodosporidium toruloides. Biochem. Biophys. Res. Commun.* **83:** 1077–1083.

Kniep, H. 1920. Über morphologische und physiologische Geschlechtsdifferenzierung (Untersuchungen an Basidiomyzeten). *Verhandlungen der Physikalisch-Medizinischen Gesellschaft in Würzburg* **46:** 1–18.

Kniep, H. 1928. *Die Sexualität der niederen Pflanzen.* Gustav Fischer Verlag, Jena.

Koltin, Y., Stamberg, J., and Lemke, P. A. 1972. Genetic structure and evolution of the incompatibility factors in higher fungi. *Bacteriol. Rev.* **36:** 156–171.

Kothe, E. 1996. Tetrapolar mating types: sexes by the thousands. *FEMS Microbiol. Rev.* **18:** 65–87.

Kronstad, J. W., and Staben, C. 1997. Mating type in filamentous fungi. *Annu. Rev. Genet.* **31:** 245–276.

Kües, U., and Casselton, L. A. 1993. The origin of multiple mating-types in mushrooms. *J. Cell Sci.* **104:** 227–230.

Kües, U., Richardson, W. V. J., Tymon, A. M., Mutasa, E. S., Göttgens, B., Gaubatz, S., Gregoriades, A., and Casselton, L. A. 1992. The combination of dissimilar alleles of the *Aα* and *Aβ* complexes, whose proteins contain homeo domain motifs, determines sexual development in the mushroom *Coprinus cinereus. Genes Dev.* **6:** 568–577.

Kühn, J., and Parag, Y. 1972. Protein-subunit aggregation model for self-incompatibility in higher fungi. *J. Theor. Biol.* **35:** 77–91.

Kurjan, J. 1993. The pheromone response pathway in *Saccharomyces cerevisiae. Annu. Rev. Genet.* **27:** 147–179.

Lengeler, K. B., and Kothe, E. 1999. Identification and characterization of *brt1*, a gene down-regulated by mating factor *B* in *Schizophyllum commune. Curr. Genet.*, in press.

Luo, Y., Ullrich, R. C., and Novotny, C. P. 1994. Only one of the paired *Schizophyllum commune Aα* mating-type, putative homeobox genes encodes a homeodomain essential for *Aα*-regulated development. *Mol. Gen. Genet.* **244:** 318–324.

Magae, Y., Novotny, C. P., and Ullrich, R. C. 1995. Interaction of the *Aα* Y and Z mating type homeodomain proteins of *Schizophyllum commune* detected by the two-hybrid system. *Biochem. Biophys. Res. Commun.* **211:** 1071–1076.

Omer, C. A., and Gibbs, J. B. 1994. Protein prenylation in eukaryotic microorganisms: Genetics, biology and biochemistry. *Mol. Microbiol.* **11:** 219–225.

O'Shea, S. F., Chaure, P. T., Halsall, J. R., Olesnicky, N. S., Leibbrandt, A., Connerton, I. F., and Casselton, L. A. 1998. A large pheromone and receptor gene complex determines multiple *B* mating type specificities in *Coprinus cinereus. Genetics* **148:** 1081–1090.

Raper, C. A. 1983. Controls for development and differentiation of the dikaryon in basidiomycetes. In *Secondary Metabolism and Differentiation in Fungi* (J. Bennett and A. Ciegler, Eds.), pp. 195–238. Dekker, New York.

Raper, C. A., and Raper, J. R. 1973. Mutational analysis of a regulatory gene for morphogenesis in *Schizophyllum. Proc. Natl. Acad. Sci. USA* **70:** 1427–1431.

Raper, J. R. 1966. *Genetics of sexuality in higher fungi.* Ronald Press, New York.

Raper, J. R., Krongelb, G. S., and Baxter, M. G. 1958b. The number and distribution of incompatibility factors in *Schizophyllum commune. Am. Nat.* **92:** 221–232.

Raudaskoski, M. 1998. The relationship between *B*-mating-type genes and nuclear migration in *Schizophyllum commune. Fungal Genet. Biol.* **24:** 207–227.

Raudaskoski, M., Färdig, M., and Uuskallio, M. 1998. The structure of pheromone and receptor gene transcripts in *Bα1* and *Bβ1* mating-type loci of *Schizophyllum commune.* In *Proceedings of the Fourth Conference on the Genetics and Cellular Biology of Basidiomycetes* (L. J. L. D. van Griensven and J. Visser, Eds.), pp. 119–124. Mushroom Experimental Station, Horst, The Netherlands.

Regenfelder, E., Spellig, T., Hartmann, A., Lauenstein, S., Bölker, M., and Kahmann, R. 1997. G proteins in *Ustilago maydis:* Transmission of multiple signals? *EMBO J.* **16:** 1934–1942.

Sakagami, Y., Yoshida, M., Isogai, A., and Suzuki, A. 1981. Peptidyl sex hormones inducing conjugation tube formation in compatible mating type cells of *Tremella mesenterica. Science* **212:** 1525–1527.

Salo, V., Niini, S. S., Virtanen, I., and Raudaskoski, M. 1989. Comparative immuno-cytochemistry of the cytoskeleton in filamentous fungi with dikaryotic und multinucleate hyphae. *J. Cell Sci.* **94:** 11–24.

Schulz, B., Banuett, F., Dahl, M., Schlesinger, R., Schäfer, W., Martin, T., Herskowitz, I., and Kahmann, R. 1990. The *b* alleles of *U. maydis*, whose combinations program pathogenic development, code for polypeptides containing a homeodomain-related motif. *Cell* **60:** 295–306.

Schuurs, T. A., Dalstra, H. J. P., Scheer, J. M. J., and Wessels, J. G. H. 1998. Positioning of nuclei in the secondary mycelium of *Schizophyllum commune* in relation to differential gene expression. *Fungal Genet. Biol.* **23:** 150–161.

Shen, G.-P., Park, D.-C., Ullrich, R. C., and Novotny, C. P. 1996. Cloning and characterization of a *Schizophyllum* gene with *Aβ6* mating-type activity. *Curr. Genet.* **29:** 136–142.

Snetselaar, K. M., Bölker, M., and Kahmann, R. 1996. *Ustilago maydis* mating hyphae orient their growth toward pheromone sources. *Fungal Genet. Biol.* **20:** 299–312.

Specht, C. A. 1996. Isolation of the *Bα* and *Bβ* mating type loci of *Schizophyllum commune. Curr. Genet.* **28:** 374–379.

Specht, C. A., Stankis, M. M., Giasson, L., Novotny, C. P., and Ullrich, R. C. 1992. Functional analysis of the homeodomain-related proteins of the *Aα* locus of *Schizophyllum commune. Proc. Natl. Acad. Sci. USA* **89:** 7174–7178.

Spellig, T., Bölker, M., Lottspeich, F., Frank, R. W., and Kahmann, R. 1994. Pheromones trigger filamentous growth in *Ustilago maydis. EMBO J.* **13:** 1620–1627.

Stankis, M. M., Specht, C. A., Yang, H., Giasson, L., Ullrich, R. C., and Novotny, C. P. 1992. The *Aα* mating locus of *Schizophyllum commune* encodes two dissimilar multiallelic homeodomain proteins. *Proc. Natl. Acad. Sci. USA* **89:** 7169–7173.

Tymon, A. M., Kües, U., Richardson, W. V. J., and Casselton, L. A. 1992. A fungal mating type protein that regulates sexual and asexual development contains a POU-related domain. *EMBO J.* **11:** 1805–1813.

Urban, M., Kahmann, R., and Bölker, M. 1996a. The biallelic *a* mating type locus of *Ustilago maydis:* Remnants of an additional pheromone gene indicate evolution from a multiallelic ancestor. *Mol. Gen. Genet.* **250:** 414–420.

Urban, M., Kahmann, R., and Bölker, M. 1996b. Identification of the pheromone responsive element in *Ustilago maydis. Mol. Gen. Genet.* **251:** 31–37.

Vaillancourt, L. J., and Raper, C. A. 1996. Pheromones and pheromone receptors as mating-type determinants in basidiomycetes. In *Genetic Engineering* (J. K. Setlow, Ed.), Vol. 18, pp. 219–247. Plenum Press, New York.

Vaillancourt, L. J., Raudaskoski, M., Specht, C. A., and Raper, C. A. 1997. Multiple genes encoding pheromones and a pheromone receptors define the *Bβ1* mating-type specificity in *Schizophyllum commune. Genetics* **146:** 541–551.

Wendland, J., and Kothe, E. 1996. Allelic divergence at the *Bα1* pheromone receptor genes of *Schizophyllum commune. FEMS Microbiol. Lett.* **145:** 451–455.

Wendland, J., Vaillancourt, L. J., Hegner, J., Lengeler, K. B., Laddison, K. J., Specht, C. A., Raper, C. A., and Kothe, E. 1995. The mating type locus *Bα1* of *Schizophyllum commune* contains a pheromone receptor and putative pheromone genes. *EMBO J.* **14:** 5172–5178.