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MATING TYPE IN FILAMENTOUS FUNGI

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ABSTRACT

Mating type genes regulate sexual compatibility and sexual reproduction in fungi. This review focuses on recent molecular analyses of well-characterized mating systems from representative ascomycete (*Neurospora crassa, Podospora anserina*) and basidiomycete (*Ustilago maydis, Coprinus cinereus, Schizophyllum commune*) fungi. These mating systems include many conserved components, such as gene regulatory polypeptides and pheromone/receptor signal transduction cascades, as well as conserved processes, like self-nonself recognition and controlled nuclear migration. The components' structures and their genetic arrangements in the mating system vary greatly in different fungi. Although similar components and processes are also found in ascomycete yeasts (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*), the filamentous systems exhibit properties not encountered in yeast. Mating type genes act within, and control the development of, spatially differentiated fruiting bodies. The complex mating systems of basidiomycetes, unlike ascomycete systems, involve novel one-to-many specificity in both pheromone-receptor and homeodomain protein interactions.

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INTRODUCTION

Commitment to mating and mate selection are critical decision points in the life cycles of sexually reproducing organisms. The term mating type is used to indicate a genetic regulation of mating specificity and sexual development in many filamentous and nonfilamentous fungi. A phylogenetically broad understanding of mating type is important given the central role of sexual reproduction in fungal life cycles and the critical influence of sex on population genetics and evolution. This review focuses on mating type in filamentous ascomycetes and basidiomycetes. The ascomycetes and basidiomycetes form a monophyletic subgroup within the higher fungi that includes filamentous, nonfilamentous, and dimorphic fungi.

Discussion of fungal mating type evokes thoughts of the ascomycete yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* which serve as invaluable archetypes. The filamentous ascomycete and basidiomycete fungi show a diversity of mating-type systems that has already delivered many surprises, even though it has been only sparsely sampled in phylogenetic terms. Characterization of the determinants of mating type in filamentous fungi has revealed familiar components and regulatory mechanisms initially found in the yeasts. However, these functions are organized in different patterns, almost as if in a "mix and match" design. We highlight similarities and differences by focusing on well-characterized filamentous ascomycetes (*Neurospora crassa*, *Podospora anserina*, *Cochliobolus heterostrophus*), and basidiomycetes (*Ustilago maydis*, *U. hordei*, *Coprinus cinereus*, *Schizophyllum commune*). In representative cases, we describe the mating response, the cellular components

needed for mating, and the structures and functions of the mating-type genes. Space limitations and paucity of molecular information prevent similar consideration of mating in the phyla Chytridomycota and Zygomycota.

Filamentous fungi face developmental challenges and fill ecological niches distinct from those of yeast. The mating systems of these fungi apparently diverged and evolved to meet these needs. For example, unlike unicellular yeasts, filamentous fungi regulate the nuclear composition of their multinucleate mycelium. Filamentous fungi often differentiate multiple distinct vegetative forms, such as asexual spores or specialized infection structures that sense environmental conditions, including the presence of other fungi, to determine whether mating, infection, growth, or some other response would be appropriate. Finally, filamentous fungi, unlike yeast, typically form complex sexual fruiting bodies in which the development of zygotic tissue is carefully coordinated with development of the massive surrounding maternal tissue. In each case, the involvement of the mating-type loci in these pathways must be defined.

Mating type is a genetically determined sexual *compatibility* phenotype. Mating type is defined in terms of heterothallism (self-sterility) in which gamete nuclei *must* come from parents of different mating type. Heterothallic systems range from single-locus, two mating-type scenarios (e.g. *N. crassa*) to multilocus, many-allele combinations (e.g. *S. commune*). In homothallic fungi, each strain is self-fertile, so sexual reproduction can involve genetically identical nuclei and mating type cannot be defined. In contrast, pseudohomothallism refers to systems in which single strains are self-fertile but in which the actual nuclei undergoing fusion are not identical. For example, in *N. tetrasperma* two opposite mating-type nuclei are normally packaged in the same spore, which results in a self-fertile heterokaryon. In another strategy, haploid strains switch mating type, as in *S. cerevisiae*, and descendants of opposite type can then fuse. Finally, many filamentous fungi (imperfect fungi) have no known sexual stage but are closely related to fungi that do have sexual stages and mating-type genes.

MATING IN ASCOMYCETES

All known heterothallic ascomycetes have single-locus, two-allele mating systems. Therefore mating type limits sexual reproduction to crosses between strains of *opposite* specificity. In heterothallic ascomyetes, mating type controls initial fusion of the thalli (plasmogamy) and the subsequent formation of dikaryotic ascogenous hyphae necessary to form zygotes (as in *N. crassa*). In addition, some mating-type genes have associated functions, such as vegetative incompatibility in *N. crassa*, that are not directly related to their sexual

compatibility roles. The complex steps in sexual reproduction for a typical filamentous ascomycete, *N. crassa*, have been reviewed recently (76).

Fundamental Features of Ascomycete Mating Systems

As first characterized in *S. cerevisiae* (10, 43) and *S. pombe* (79), mating relies on a complex signal transduction pathway and on specific gene regulation. Many, but not all, of the signal transduction and regulatory components appear to be widely conserved in fungi (to view, please see the Supplementary Materials Section on our Web site at http://www.AnnualReviews.org for a pathway diagram linked to protein sequences). For example, mating is mediated by hydrophobic peptide pheromones, produced in a mating type–specific manner only by haploid cells. Special processing and export functions may be needed for pheromone secretion. Cells of one mating type respond only to pheromone of the opposite type. The pheromone receptors are transmembrane proteins linked via heterotrimeric G proteins to protein-kinase signal transduction pathways. Interaction between opposite mating types results in a growth response, spatial differentiation, transcription of responsive genes, and close physical interaction, which is mediated by mating type–specific agglutinins. These changes ready the mating partners for cellular fusion (plasmogamy) and subsequent nuclear fusion (karyogamy). In *S. cerevisiae* and *S. pombe*, the mating-type loci encode transcriptional regulatory proteins that specify mating type–specific expression of these functions. The ability of the diploids to differentiate sexual spores results from the novel combination of mating-type regulatory polypeptides formed by fusion of the two haploids. These combinations can repress expression of haploid-specific genes and induce the expression of diploid-specific functions (such as those required for meiosis and ascosporogenesis).

Components of the Mating-Type Recognition Pathways in Filamentous Ascomycetes

There is ample evidence of mating type–specific pheromone production in filamentous ascomycetes. In *N. crassa* males of each mating type produce pheromone to which females only of the opposite mating type respond by orienting the growth of specialized mating hyphae (trichogynes) (13, 14). Mating-type mutants of *N. crassa* do not produce pheromone, suggesting that pheromone production is under mating-type control as in *S. cerevisiae*. *Magnaporthe grisea*, a relative of *Neurospora*, secretes mating type–specific hydrophobic peptides that inhibit formation of the plant infection structure by the opposite mating type. However, the role of the *M. grisea* factors in mating has not yet been established (D Ebbole, personal communication). Probable pheromone biosynthetic genes, which are expressed in a mating type–specific manner, have been identified in *Cryphonectria parasitica* (54), a close relative of *N. crassa*.

Mating type–specific pheromone response pathways and their associated signal transduction cascades are also present in the filamentous ascomycetes. Known fungal pheromone receptors are predicted to have seven transmembrane segments and to interact with a heterotrimeric G protein linked to a protein kinase cascade (10). Pheromone receptors are produced from genes that are transcriptionally regulated by mating-type gene products or, in the basidiomycetes, the receptors are products of the mating-type locus (18, 116). *N. crassa* females respond in a mating type–specific way to pheromones by orienting growth of the trichogyne towards the pheromone source (13, 14). Mating-type mutants of *N. crassa* and dual mating-type strains do not orient growth towards pheromone sources (15). Two Gα-protein encoding genes from *N. crassa* have been characterized: *gna-1* and *gna-2* (109). Disruption of *gna-1* eliminated female fertility and caused other phenotypes (46). Similar G proteins are found in *C. parasitica* (30). *C. parasitica* strains disrupted in *cpg-1* (the *gna-1* homolog) are also female infertile but mate as males; *cpg-2* disruption did not affect fertility. These results suggest that a Gαi protein is a conserved component of the female pheromone response pathway. *M. grisea* has a MAP kinase (*PMK1*) essential for development of infection structures (appressoria) (123). Although this kinase could be a downstream signaling element responsive to the mating type–specific *M. grisea* peptides that inhibit appressoria formation, *PMK1* was not essential for female fertility. This would seem surprising if this kinase were involved in a mating type–specific pheromone response pathway. No analog of the pheromone response factor, such as Ste12p from *S. cerevisiae* or Prf1 from *U. maydis*, has been found in filamentous ascomycetes.

Some mating components found in *S. cerevisiae* have not yet been found in filamentous fungi. For example, export functions analogous to Ste6p, the **a** pheromone transporter, have not been identified, although hydrophobic peptide pheromones probably require a transporter. Mating type–specific proteases to degrade pheromones are found in *S. cerevisiae* (68) and in *S. pombe* (45), but have not been reported from filamentous fungi. *S. cerevisiae* has mating type–specific agglutinins that are necessary for mating in liquid, but not on solid medium (66). Such factors could expedite the adherence of a conidium to the trichogyne in *N. crassa*.

Postfertilization Steps Regulated by Mating Type

At least two postfertilization steps in *N. crassa* depend upon mating type: nuclear transport and dikaryon development. Nuclear transport is a hallmark of post-mating events in the basidiomycetes, but the mechanistic basis for the phenomenon is not known either in the basidiomycetes or in ascomycetes. It seems likely that nuclear transport will depend upon cytoskeletal elements like microtubules or microfilaments and their associated motor proteins; spindle pole

bodies have been implicated in post-meiotic assortment of opposite mating-type nuclei in *P. anserina* (107). A mechanism in which nuclear identity and movement might be related to mating-type polypeptide is suggested by LEF1, an HMG protein similar to MT a-1, which forms a ternary complex with $\beta-$catenin and DNA (12), and enters cell nuclei.

The transport of nuclei into the *N. crassa* ascogonium depends upon mating-type gene function. When heterokaryons formed from *cw* (crosswall-less) strains and the a^{m1} mutant (a frameshift mutation in *mt a-1*) are used as male and female, the ascogonia formed lack crosswalls. This indicates that the a^{m1} mutant nuclei do not genetically contribute the *cw* gene function in the ascogonium, presumably because they cannot be physically delivered to the ascogonium (84).

After the *a* and *A* nuclei arrive in the ascogonium, a series of nuclear divisions generates a syncytial mass of *a* and *A* nuclei. Some mechanism ensures the formation of ascospores only from meioses involving one *a* and one *A* nucleus, because mating type *always* segregates 1:1. This mechanism could be a selective transport of these nuclei into the crozier analogous to hook cell formation in basidiomycetes. Alternatively, nonselective transport could result in abortion of croziers not formed from one *a* and one *A* nucleus. Significant crozier abortion occurs even in normal ascosporogenesis. The phenotypes of mating-type mutants in *N. crassa* (35) and in *Podospora anserina* (127) directly support a role for mating type in postfertilization steps.

Structures and Functions of the mt *Loci from Filamentous Ascomycetes*

The *mt* loci of filamentous fungi contain alternate DNA sequences for which the term idiomorph was coined (73) to denote that they may contain multiple genes and that the genes of alternate mating types bear no obvious allelic relationship to one another. Idiomorphic structure (not allelism) is common to all known fungal mating-type genes, even those from *S. cerevisiae* and *S. pombe*.

The three best characterized mating-type loci from filamentous ascomycetes share similar genes but also exhibit differences. *N. crassa* and *P. anserina mt* loci contain the same basic constellation of genes but *C. heterostrophus* sports a simpler array (76). The *M. grisea* idiomorphs are probably similar to *N. crassa* (53). Gene replacement experiments in *N. crassa* (20), *P. anserina* (83), and *C. heterostrophus* (121) suggest that the idiomorphic regions are necessary and sufficient to specify mating type in each system.

The *N. crassa* mating-type idiomorphs (*mt a* and *mt A*) were the first physically characterized (37). The 5.3-kbp *mt A* idiomorph includes 3 genes (*mt A-1, mt A-2, mt A-3*) (26). The *mt a* idiomorph is 3.2 kbp with one transcription unit (*mt a-1*) (20) (S Chang, CT Badgett & C Staben, unpublished). The

N. crassa idiomorphs are flanked by DNA sequences common to both mating types. Sequence polymorphism outside the idiomorphs is much greater on the centromere proximal than on the centromere distal flank. The 2 kbp of DNA distal to the *mt a* idiomorph is not essential for *a* or *A* mating activities (CT Badgett & C Staben, unpublished). The centromere proximal 1-kbp flanks from different species are from 25% to 90% similar in *a* and *A* (85). In effect, this region is an extension of the idiomorphic DNA that was not appreciated initially. Although this flank of *N. crassa mt a* and *mt A* differ, gene replacement studies demonstrate that the flanking sequence present in *A* does not interfere with *a* mating-type activities (20).

The roles of individual genes within the idiomorphs have been assessed genetically. Mutations within either *mt a-1* (103) or *mt A-1* (34, 90) cause mating defects. A clone bearing only the *mt a-1* gene is sufficient to switch mating type (20), so *mt a-1* must be the only gene essential to mating in the *mt a* idiomorph. An unusual form of directed mutation, RIP (repeat-induced point mutation), that occurs in the *N. crassa* dikaryon during ascosporogenesis (94) can be used as a stage-specific gene disruption technique. The *mt A-1* gene can be disrupted by RIP (35), which suggests that this DNA sequence (and perhaps its product) is not essential beyond the step at which RIP occurs. The *mt a-1* gene has not been inactivated by RIP despite repeated attempts (CT Badgett & C Staben, unpublished). Strains bearing *mt a-1* duplications are always resolved during sexual reproduction (20), which suggests that *mt a-1* has an essential role in the dikaryon. The remainder of the *mt A* idiomorph contains the *mt A-2* and *mt A-3* genes. This region is essential for late steps in ascosporogenesis (35), but it is not clear whether both regions are essential or whether only one of the genes is necessary for ascosporogenesis. The RIP-induced mutant is altered primarily in *mt A-2*. The paucity of mutations in *mt A-3* suggests that *mt A-3*, which should have been altered during RIP, may be essential for activities post-RIP (90).

The *mt* idiomorphs of *P. anserina* are similar to those of *N. crassa*. The *mat+* idiomorph has a single gene (*FPR1*) homologous to *mt a-1*; the *mat−* idiomorph encodes three genes (*FMR1*, *SMR1*, *SMR2*) similar to *mt A-1*, *mt A-2*, and *mt A-3* (24, 25). The roles of the *P. anserina* genes appear to be very similar to those of their *N. crassa* counterparts. Mutation of the mating-type genes in this organism dramatically reduces the number of asci formed in a cross. Progeny from such crosses are uniparental. These observations suggest mating type acts primarily in self-nonself recognition; when this control is absent, nuclei act in a selfish manner. These data also indicate that meiosis and ascosporogenesis do not absolutely depend upon mating-type gene function (127).

The *mt* idiomorphs of *C. heterostrophus* each contain only a single gene. One mating type contains a homolog of *S. cerevisiae* Mat α1p; the second a homolog

of *N. crassa* MT a-1. These genes are necessary and sufficient to confer mating (108). It is not known whether *C. heterostrophus* contains homologs of MT A-2 and MT A-3 outside the *mt* locus. Experiments with *P. anserina* suggest that not all mating-type genes need be present at the *mt* locus, so it would be interesting to know whether *C. heterostrophus* has homologous genes in a different location.

Unusual Properties of the Mating-Type Locus and Ectopic Mating-Type Genes

Physical location in the genome appears to play a special role in the action of the mating-type genes. In many experiments, new mating-type genes are inserted at nonmating-type locations in the genome by transformation. These ectopic mating-type genes are not able, in *N. crassa*, to confer fertility, even though they confer mating properties such as the ability to induce differentiation of large, but barren, perithecia. This limitation has been observed in all combinations of host and transforming DNA: *mt A* or *mt a* transformed into *a*, *A*, a^{m1}, and A^{m64} (wild type and mutant). Heterokaryon experiments with *cw* mutants indicate that nuclei bearing ectopic mating-type genes do not enter the ascogonium (NB Raju & C Staben, unpublished). Ectopic mating-type genes are able to confer fertility in *P. anserina* (83), but a mat^0 deletion strain is more fertile than a wild-type recipient when transformed with a novel mating-type gene. *C. heterostrophus* dual maters are not fertile, but deletion of the resident mating-type idiomorph allows transforming mating-type genes to confer fertility (121). These data suggest an interaction between mating-type genes or a requirement for a specific genetic location that affects steps subsequent to mating. The mechanism of this interaction is not known. Although the phenomenon resembles transvection between alleles during meiosis, as reported for the *N. crassa Asm-1* locus (1), transvection would affect activity at a step much later than that blocked in *N. crassa* ectopic mating-type integrants.

Molecular Mechanisms of Mating-Type Gene Action

The sequences of the genes within the mating-type idiomorphs and initial biochemical characterization of the gene products suggest that the idiomorph products are gene regulatory polypeptides. The genes regulated by mating-type polypeptides and the interactions of the polypeptides with each other or with other factors that confer new properties on mated cells are not yet known.

The *mt a-1* (82) and *mt A-3* (ML Philley & C Staben, unpublished) gene products contain an HMG box domain and bind DNA in vitro. Although the HMG box alone is sufficient for DNA binding in vitro, additional portions of MT a-1 are needed for mating activity in transformation assays. Detailed

analysis of MT a-1 structure and function is incomplete, but either the N-terminal 100 residues or the C-terminal 100 residues can be deleted without destroying mating-type activity. These regions are poorly conserved in related mating-type polypeptides, some of which can confer mating activity when introduced into *N. crassa*. The structures of related sequence-specific HMG box regions (114) can be used to model MT a-1, MT A-3, and their homologs in *P. anserina* and *C. heterostrophus* (11) (to view, please see the Supplementary Materials Section on our Web site at http://www.AnnualReviews.org).

The DNA binding activity of MT a-1 correlates with mating activity in vivo, although which genes are regulated is not known. The DNA sequences bound by MT a-1 center on CAAAG sequences, similar to other HMG box proteins, including hSRY, STE11 from *S. pombe*, and Prf1 from *U. maydis*. HMG polypeptides bind in the minor groove and introduce a bend in the DNA (40). MT a-1 binding sites in the *mt a-1* promoter are not required for a mating-type activity when transformed into *Neurospora* (ML Philley and C Staben, unpublished). Models for development suggest that DNA binding activity or targets must differ in different developmental stages (haploid vs diploid). HMG polypeptides bind calmodulin, which suggests a potential mechanism to regulate MT a-1 activity (41). MT a-1 may also interact with unidentified protein factors. MT A-3 binds to the same DNA fragments bound by MT a-1 in vitro, but it is not known whether MT A-3 or its DNA binding activity are essential to mating in vivo (26).

The MT A-1 polypeptide is similar in sequence to the *S. cerevisiae* α1 polypeptide and is likely to be similar in function. Since *S. cerevisiae* α1p binds DNA in combination with Mcm1p, it seems likely that MT A-1 is also a sequence-specific DNA binding protein that may require a polypeptide cofactor to bind specifically. A homolog of MCM1 from *S. pombe* is also a critical regulator of mating in that fungus, suggesting that the role of an MCM1-like factor in mating may be widely conserved in ascomycetes (124). Mutational analysis of *mt A-1* indicates several distinct functional regions. Residues 1–111 are sufficient for vegetative incompatibility, but residues 1–227 are essential for full mating functions (90). The *P. anserina* FMR1 protein is homologous to MT A-1. Preliminary evidence from yeast two-hybrid experiments indicates that FMR1 contains a transcription activation domain and that SMR2 can interact with FMR1 (127).

The function of the MT A-2 polypeptide is not known. The polypeptide has no obvious relatives in sequence databases other than *Podospora* SMR1. SMR1 and MT A-2 contain acidic regions capable of forming amphipathic helices, which suggested that they could act as transcriptional activator proteins (24). Mutants in *SMR1* yield only uniparental progeny when crossed to wild type, but

biparental progeny can be formed in crosses involving a mutant lacking FPR1. This suggests some physical or genetic interaction that might control nuclear identity in the crozier (127).

In filamentous ascomycetes, two critical pieces of the mating puzzle yet to be identified are the targets of mating-type regulation and an indication of how interactions of the regulatory factors determine cell type in haploids and in the perithecium. Regulatory targets undoubtedly include mating components such as pheromones and pheromone receptors, but they may also include regulators of nuclear transport and fruiting body or zygote development. Some of these targets may include sexual development genes induced during perithecial development (77). Interactions of the regulatory factors may be direct as in the case of *S. cerevisiae* α2-*a1* or may be mediated by other factors.

MATING IN BASIDIOMYCETES

Among fungi, the sexiest must be the basidiomycete species that have evolved-mating systems with two or more specificities at each of two unlinked mating-type loci (55). These extraordinary tetrapolar mating systems engender literally thousands of different mating types within a single species. In addition to tetrapolar mating, heterothallic basidiomycete fungi exhibit two other mating patterns, a bipolar system (two alternate specificities at a single *MAT* locus) that is genetically similar to the mating systems found in ascomycetes and a system with a single *MAT* locus with multiple alternative specificities. In this section, we focus on three tetrapolar species, *U. maydis*, *C. cinereus*, and *S. commune* and one bipolar species, *U. hordei*. After describing the life cycles of these fungi, we consider the role of pheromones in pre- and post-fusion events and the function of homeodomain proteins in multiallelic recognition and subsequent regulation of sexual development.

Cell Type Transitions

The life cycles of the basidiomycete fungi involve cell type transitions between haploid, dikaryotic, and diploid phases. In the smut fungi, haploid budding cells are produced via meiosis during the germination of diploid teliospores. Mating between compatible haploid cells establishes the dikaryon that is obligately dependent on infection of a host plant for growth and teliospore formation. Thus, sexual development and pathogenicity are intricately interconnected phenomena in the smut fungi. Mating is also associated with a dimorphic shift from budding (haploid cells) to filamentous growth (dikaryon) (7, 8).

The mushroom fungi, *C. cinereus* and *S. commune*, grow in two vegetative states: the self-sterile monokaryon (haploid) and the fertile dikaryon. The fertile dikaryon that results from mating between compatible monokaryons has

binucleate cells (one nucleus from each parent) and hook cell connections (also called clamps) at septa. The dikaryon can differentiate fruiting bodies (mushrooms) and karyogamy results in diploid nuclei within basidial cells on the gills of the mushroom. Following meiosis, four basidiospores are formed on each basidium; germination of these spores establishes the vegetative monokaryon. After fusion of monokaryons, the events that establish the dikaryon follow a defined order that is regulated by the genes at the mating-type loci (104). Initially, nuclei are exchanged between mating partners, and these nuclei migrate in a reciprocal fashion through the existing hyphal cells of each monokaryon. The septa dividing cells of the monokaryons break down in advance of the migrating nuclei and the nuclei eventually reach tip cells to establish the dikaryon. Subsequent division of the dikaryotic tip cells occurs by a mechanism that is analogous to crozier formation and that maintains the dikaryotic state. Specifically, the tip cell develops a hook cell and one of the nuclei enters this cell and divides. Division of the remaining nucleus in the tip cell, fusion of the hook cell in a subapical position, and the development of a new septum deposit two different nuclei in the subapical cell.

Mating-Type Loci in Basidiomycetes

U. MAYDIS AND *U. HORDEI* The tetrapolar mating system in *U. maydis* is composed of two loci (*a* and *b*) on separate chromosomes. The two alternate specificities at *a* (*a1* and *a2*) are encoded by idiomorphs of 4.5 kb (*a1*) and 8.0 kb (*a2*) (18, 28). The *a* locus controls the initial fusion of haploid cells and may also contribute to maintenance of the filamentous dikaryon. The *b* locus has 25 naturally occurring specificities and contains two divergently transcribed genes: *bE* (with alleles *bE1-25*) and *bW* (with alleles *bW1-25*). The *bE* and *bW* genes encode homeodomain proteins that regulate the expression of genes required for the filamentous growth and pathogenesis of the dikaryon. Overall, mating partners must have different specificities at *a* and *b* to be compatible and to establish the dikaryotic cell type. In contrast to *U. maydis*, *U. hordei* has a bipolar mating system with two alternate specificities (*MAT-1* and *MAT-2*) at the *MAT* locus. As described in more detail below, the *MAT* locus of *U. hordei* contains subloci related to *a* and *b* in *U. maydis* (4, 5).

C. CINEREUS AND *S. COMMUNE* The tetrapolar mushroom fungi *C. cinereus* and *S. commune* have more complex mating-type loci than the smut fungi. *C. cinereus* and *S. commune* each have two unlinked mating-type factors designated *A* and *B* and each of these factors have subloci designated α and β. In general, the α and β loci are functionally redundant and recombination can occur between the loci (19, 86). The genes at *A* encode homeodomain proteins and the genes at *B* encode pheromones and pheromone receptors. The

fascinating aspect of the *A* and *B* factors is the tremendous number of specificities generated by the subloci. For *S. commune*, the *A* factor has 288 specificities (9 for *A* α, 32 for *A*β) and *B* has 81 versions (9 for *B* α and 9 for *B*β). These combinations can yield over 20,000 mating types (19). For *C. cinereus*, there are an estimated 160 specificities at *A* and 79 at *B* to generate potentially 12,000 different mating types (19). The *A* and *B* mating factors of *C. cinereus* and *S. commune* regulate well-defined events in the establishment of the dikaryon that eventually forms fruiting bodies. The *A*-regulated events include nuclear pairing, hook cell formation, conjugate division of the nuclei in the tip cell, and hook cell septation. The *B* factors regulate nuclear migration and the fusion of the hook cell with the subapical cell.

Pheromone Signaling in Basidiomycetes

Pheromone signaling is important in at least four mating processes in the basidiomycetes: (*a*) cell fusion in the smut fungi; (*b*) establishing and maintaining filamentous growth of the dikaryon in the smut fungi; (*c*) post-fusion nuclear migration after hyphal anastomosis in the mushrooms; and (*d*) fusion of hook cells to maintain the dikaryon in the mushrooms. There may be common underlying mechanisms in these processes because pheromone signaling mediates both cell fusion events (smut fungi) and communication between nuclei and cells in filamentous cell types such as the monokaryon during nuclear migration (mushroom fungi) or a newly established dikaryon (e.g. in *U. maydis*). Pheromone signaling has been reviewed in detail (17, 61, 113).

USTILAGO PHEROMONES CONTROL FUSION AND FILAMENTOUS GROWTH The *a* locus of *U. maydis*, encoding pheromone (*mfa1* and *mfa2*) and pheromone receptor (*pra1* and *pra2*) genes, was cloned by chromosome walking (28) and by a functional assay for dual mating specificity (18). The roles of the *mfa1*, *mfa2*, *pra1*, and *pra2* genes have been established by evaluating the mating behavior of strains carrying insertion mutations in the genes and by transformation of individual genes into strains of opposite mating specificity (18). Insertional inactivation of other genes at the *a2* locus, *rga2* and *lga2*, did not inhibit mating (111). The *a* locus of *U. hordei* was cloned by cross hybridization with the *a* sequences from *U. maydis*. Pheromone and pheromone receptor genes have been identified within these regions (5; J Sherwood, personal communication).

Conjugation tube formation and filamentous growth Mating in the smut fungi begins with the formation of conjugation tubes that grow from the ends of haploid cells in response to pheromone (70, 97, 98). Conjugation tubes eventually fuse at the tips to establish the dikaryon. Pheromone signaling is also important after cell fusion for the maintenance of the filamentous dikaryon.

Diploid strains homozygous for *a* and heterozygous for *b* (e.g. *a2/a2 b1/b2*) display a yeast-like colony morphology, unless mixed with haploid cells that carry an *a* locus of opposite specificity (e.g. *a1*). In this case, a filamentous growth response occurred that was dependent on pheromone stimulation but not cell fusion (18, 101). Pheromone activity was also demonstrated by mixing a haploid strain supplying *a1* pheromone (but defective in the receptor gene *pra1*) with two haploid strains of *a2* specificity (*a2 b1* and *a2 b2*; (101)). A weak filamentous response indicated that pheromone activity alone provoked mating and fusion competence in haploid strains. Pheromones purified from culture medium also triggered conjugation tube formation in haploid strains and induced filamentous growth in diploid strains (101). A role for pheromone signaling after fusion contrasts with the situation in the ascomycete yeasts. The expression of genes required for pheromone production and perception is repressed after fusion in *S. cerevisiae* (10, 61). Clearly, the pheromones of *U. maydis* are involved in two stages of mating, cell fusion and subsequent maintenance of filamentous growth in the dikaryon.

Identification of the pheromone response element Pheromone induces all of the mating-type genes (*mfa1, mfa2, pra1, pra2, lga2, rga2, bE*, and *bW*) 10- to 50-fold (112). The transcripts for the pheromone and receptor genes in a diploid strain (*a1/a2 b1/b2*) are present at levels below those found in uninduced haploid strains. The levels of transcripts for *rga2, bW*, and *bE* were similar to those in a pheromone induced haploid. In contrast, the transcripts for the *lga2* gene were about tenfold higher in the diploid than in pheromone stimulated cells. The results for the *mfa* and *pra* genes indicate that pheromone stimulation is attenuated in strains already heterozygous at *a* and *b*; this regulation may be due to the products of the *b* locus. The increase in *lga2* transcription in the diploid may indicate positive regulation, perhaps by the bE and bW products. The cis-acting element responsible for pheromone stimulation of these genes, the PRE, has the sequence ACAAAGGG. An *mfa1*-GUS reporter gene fusion is pheromone inducible and deletion of the PREs eliminated inducibility. In addition, a synthetic version of the PRE conferred pheromone inducibility on a heat shock promoter from *U. maydis* (112).

Isolation of the pheromone response factor The core sequence of the *U. maydis* PRE (A**CAAAGGG**A) is similar to the consensus sequence recognized by HMG domain polypeptides, including MT a-1 of *N. crassa* (42). Degenerate primers for the DNA binding domain of HMG proteins were used to amplify and isolate a gene called *prf1* that encoded a predicted protein of 840 amino acids (a.a.) with similarity to *ROX1* of *S. cerevisiae*. ROX1 represses anaerobic metabolism genes under aerobic conditions. Transcription of *prf1* was induced

20-fold by pheromone stimulation, and two PRE sequences are found upstream of the gene. Deletion of the *prf1* gene in haploid strains yields mutants that are sterile owing to defects in pheromone production and perception. The *a* mating-type genes are not transcribed at normal basal levels nor induced in *prf1* mutants. It is not clear whether *prf1* is required for basal levels of *b* gene transcription because of the low level of these transcripts in haploid cells. As expected from the induction studies, DNAse I footprint analysis revealed that Prf1 binds to the PRE elements found in both *a1* and *a2* sequences (42).

Elements of the pheromone response pathway In addition to a downstream transcription factor such as Prf1, one expects to find MAP kinases in a pheromone response pathway (61). A homolog of the *STE7* MAPK kinase of *S. cerevisiae* was identified in *U. maydis* (8, 9). This gene, *fuz7*, is required for full levels of filament formation in a mating assay with a wild-type partner. Haploid strains compatible for mating, but each defective for *fuz7*, give a weak mating reaction when mixed, even though it is difficult to detect conjugation tube formation by these strains. Disruption of both copies of the *fuz7* gene in a diploid strain (*a1/a2 b1/b2*) also reduces but does not eliminate filament formation. These results suggest that *fuz7* is partially involved in *a*-dependent events such as conjugation tube formation, and establishment and maintenance of filamentous growth (9). In a search for other components of the pheromone response pathway, Regenfelder et al (87) isolated four genes encoding Gα subunits of heterotrimeric G proteins. Disruption of one of these genes (*gpa3*) caused defects in pheromone response, pheromone induction of *mfa1* and *mfa2* transcription, and mating. Given the possibility that *gpa3* and *fuz7* are both components of a pheromone response pathway in *U. maydis*, it was important to test pheromone induction of *mfa1* and *mfa2* in a *fuz7* mutant. Surprisingly, pheromone response was not affected by the *fuz7* mutation, suggesting that *fuz7* and *gpa3* are not part of the same pheromone response pathway. *Fuz7* may actually be involved in a second phase of signaling that promotes the transition to filamentous growth during mating (87).

Interspecies mating between U. maydis and U. hordei The *a1* mating-type genes encoding pheromone and pheromone receptor from *U. hordei* have been used to manipulate mating between *U. hordei* and *U. maydis*. For example, the *U. hordei a1* genes, when introduced by transformation, will allow *U. maydis* strains to mate with *U. hordei* strains of *MAT-2* (*a2 b2*) but not *MAT-1* (*a1 b1*) specificity. Similarly, transformation of *U. hordei* with the *a1* locus of *U. maydis* allowed transformants to mate with *U. maydis a2*, but not *a1*, strains. These matings result in dikaryons that are not capable of completing the pathogenic phase of the life cycle. These experiments reveal that the *a* locus controls

compatibility between species and that the processes of dikaryon establishment and maintenance are conserved between species (6).

PHEROMONES IN *S. COMMUNE*: NUCLEAR MIGRATION AND HOOK CELL FORMATION Anastomosis of monokaryotic vegetative cells of *S. commune* and *C. cinereus* does not depend on pheromone-based recognition. The *A* and *B* mating factors act to establish and maintain the dikaryon after fusion has occurred. Remarkably, recent work in *S. commune* demonstrated that the *B* factor contains pheromone and pheromone receptor genes, indicating that pheromone signaling controls the *B*-regulated events of reciprocal nuclear migration and hook cell fusion. The breakthrough came from the cloning of the *Bα1* and *Bβ1* loci of *S. commune* by transformation of DNA from a strain of *Bα1β1* specificity into strains of different *B* (*B α2β2*) specificity, and subsequent screening of transformants for activation of the *B*-regulated pathway (99).

B factors encode a pheromone receptor gene and multiple pheromone genes Sequence analysis of the *S. commune Bα1* region revealed a pheromone receptor gene and three pheromone genes: *bar1* (*Bα* receptor 1) and *bap1*, *bap2* and *bap3* (*B α* pheromone) (116). The discovery of these genes at *Bα* led to the following scenario for *B*-regulated events (116). After fusion between monokaryons of different *A* and *B* specificity, secreted pheromones encoded by the fertilizing nucleus act as advance signals to activate receptors encoded by resident nuclei in nearby cells. The pheromone signals prepare the cells for invasion by triggering septal breakdown to allow passage of the fertilizing nuclei. Subsequently, *A* locus regulation occurs and the two nuclei of the mating partners are established in tip cells. These cells are capable of hook cell formation and further *B*-regulated signaling mediates hook cell fusion. This event may be similar to fusion events in other fungi mediated by pheromones and receptors. Pheromone signaling within the dikaryon, e.g. for hook cell fusion, may be similar to the pheromone signaling proposed for the *U. maydis* dikaryon (18).

The *B* factor of *C. cinereus* contains six genes encoding pheromones and three genes encoding pheromone receptors (L Casselton, personal communication).

Multiple specificities and pheromone signaling The *B α* factor of *S. commune* has nine specificities and presumably each encodes a receptor and one or more pheromones. If so, each receptor must distinguish at least eight different nonself pheromones from those encoded by genes of self-specificity. Individual pheromones may also activate more than one receptor because transformation with either the *bap1*(*1*) or *bap1*(*3*) genes from *B α1* activates *B*-regulated events in both *B α2* and *B α3* strains. These findings are striking because in other fungal

mating systems, a receptor is thought to recognize a single pheromone. The *bar1* receptor gene hybridized to DNA from each of the other eight *B* α specificities and one of these hybridizing regions (*B* α*2*) encodes a receptor gene (*bar2*) (116). The *B*β*1* locus, which works independently but redundantly with *B* α, also contains a receptor gene (*bbr1*) and genes for pheromones (*bbp1(1*) and *bbp1*(*2*)) (116). Perhaps, *B* α receptors must not only distinguish among *B* α pheromones but also distinguish self from nonself for *B*β. The prediction is that each specificity has at least one different mating type–specific receptor. There may be analogies in other fungal systems for overlapping specificity in pheromone response. For example, mating among strains of different species in the smut fungi (6) and cross-species receptor recognition in yeast (44) indicates that a receptor in one species may recognize pheromones produced by different species.

A large collection of mutants of *B*-regulated development is available for *S. commune* (for review see 86). Some of the mutations map to the *B* α and *B*β subloci and influence mating specificity; their molecular analysis may clarify pheromone and pheromone receptor gene specificity. Other distinct loci include nine genes that affect nuclear migration, and several others involved in *B*-regulated events; many of these genes are linked to *B*, suggesting a clustering of related functions.

Dikaryon Formation and Homeodomain Proteins

Pheromone signaling is clearly important for establishing the dikaryon in basidiomycetes, but genetic loci encoding homeodomain polypeptides (the *A* loci in the mushroom fungi and the *b* loci in the smut fungi) are also critical determinants of self versus nonself recognition leading to dikaryon establishment and maintenance. Here we focus on two questions. First, how are multiple mating specificities distinguished by protein-protein interactions between homeodomain proteins encoded by different specificities? Second, which genes are controlled by the novel regulatory factor established by dimerization of the homeodomain proteins? The organization of the genes encoding the homeodomain proteins has been reviewed elsewhere (19, 48, 52, 56).

THE bE AND bW PROTEINS OF THE SMUT FUNGI The *b* locus was cloned from *U. maydis* by transformation of DNA from a *b1* strain into a diploid strain homozygous for the *b2* locus (57). Sequence analysis of several alleles and mutational analysis identified the divergently transcribed *bE* and *bW* genes (33, 58, 92). These genes encode proteins with homeodomain motifs (33, 92), which separate variable regions from conserved C-terminal regions (33, 58). The *b* genes from *U. hordei* revealed only two alleles for each of the *bE* and *bW* genes (4, 5). The *b* locus appears to have the same function in both

U. maydis and *U. hordei* and the *b* gene products of *U. hordei* can interact with the *b* products of *U. maydis* to establish a dikaryotic cell type (5, 6). The current view is that the *bE* and *bW* polypeptides encoded by different specificities interact and that two functionally equivalent heterodimeric proteins (e.g. bE1+bW2 and bE2+bW1) regulate dikaryon formation and maintenance.

THE X AND Y PROTEINS OF *S. COMMUNE* The *A* locus of *S. commune* controls formation of the fertile dikaryon by regulating nuclear pairing, hook cell formation, conjugate nuclear division and hook cell septation. The *A α* locus was cloned by chromosome walking (32). The cloned DNA activates *A*-regulated events (e.g. hook cell formation) upon transformation into strains of opposite *A* specificity. Analysis of the *A α1, A α3* and *A α4* specificities revealed genes called *Y* (with alleles *Y1, Y3*, and *Y4*) and *Z* (with alleles *Z3* and *Z4*) that encode homeodomain proteins (although *Z* is missing from the *A α1* locus) (100, 105). A representative *Aβ* sublocus also encodes a predicted polypeptide (gene *AβV6*) with a homeodomain, suggesting that redundant regulatory proteins are encoded at *A α* and *Aβ* (96). Transformation studies indicate that *Y* and *Z* are the only determinants of *A α* mating activity and that *A α* and *Aβ* function independently of each other (88).

THE HD1 AND HD2 PROTEINS OF *C. CINEREUS* The *A* factor in *C. cinereus* also regulates nuclear pairing, clamp cell formation, conjugate nuclear division, and clamp cell septation. The *Aα3* and *Aβ3* subloci of the *A42* mating factor were isolated by chromosome walking (75). The tightly linked *α* and *β* subloci in *C. cinereus* were both isolated in the initial walk. A different factor, *A43*, was isolated by a sib-selection procedure (71). The cloned genes activate A-regulated development (e.g. formation of unfused hook cells) upon transformation into *A6 (α2 β2)* and *A5 (α1 β1)* monokaryons (75). The *A* factors are comprised of two clusters of genes that correspond to the genetically defined *α* and *β* subloci. Extensive analysis of these regions indicates that pairs of genes encoding homeodomain proteins are present within the clusters (59). The archetypical *A* factor would have four gene pairs: *a, b, c*, and *d*, although none of the characterized factors has all eight genes. For example, the *A43* factor has gene pairs *a1-2* and *a2-2* at the *α* complex and gene pair *b1-2* and *b2-2*, along with one member of another pair, *d1-1*, at the *β* complex. Transformation experiments indicate that these genes are capable of triggering *A*-regulated events upon introduction into strains of opposite specificity (60, 110).

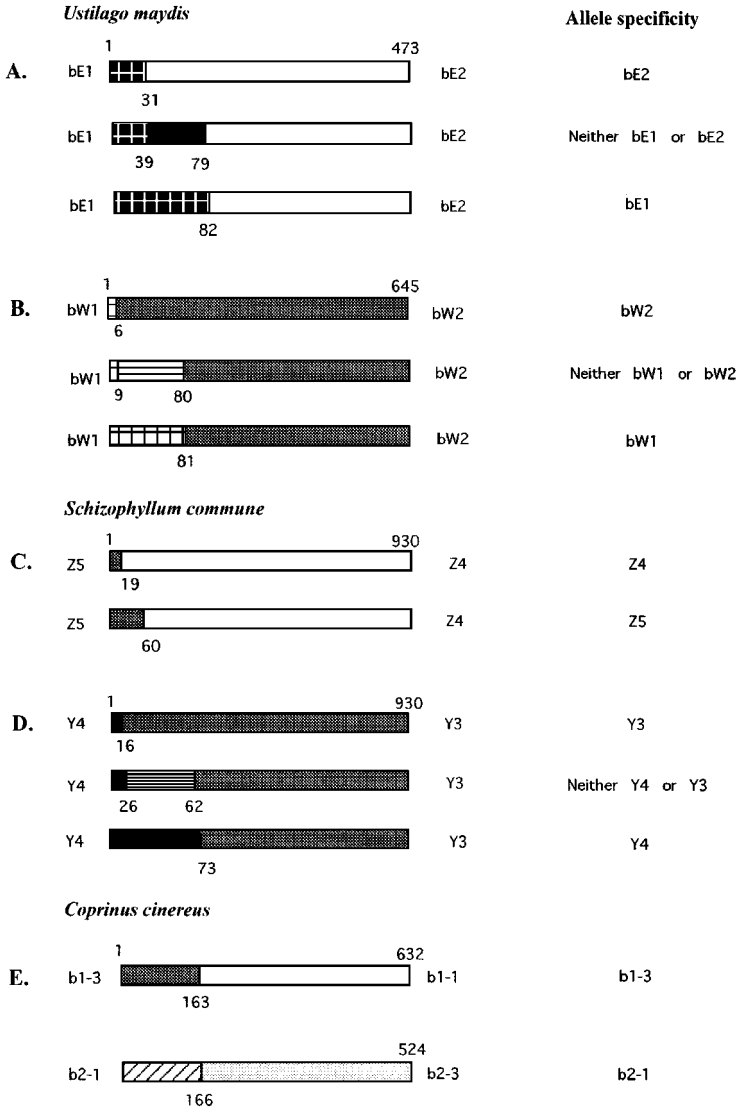
Comparisons of the homeodomain motifs encoded by the genes at *A α* prompted a division of the genes into two classes: HD1 and HD2 (for review see 19). The HD1 class includes the *S. cerevisiae α2* gene, the *S. commune Z* genes, the *U. maydis* and *U. hordei bE* genes, and the *C. cinereus* genes *a-d1*. The HD2

class includes the *S. cerevisiae* **a**1 gene, the *S. commune Y* genes, the *U. maydis* and *U. hordei bW* genes, and the *C. cinereus* genes *a-d2*. Overall, the picture that has emerged is that each pair of HD1 and HD2 proteins, when encoded by factors of different specificity, forms a heterodimer that regulates formation and maintenance of the dikaryon.

DETERMINANTS OF SELF VERSUS NONSELF RECOGNITION The properties of chimeric alleles indicate that the N-terminal portions of the homeodomain mating proteins carry the determinants of specificity (Figure 1) (3, 23, 122, 125). For example, the analysis of 21 chimeric alleles of the *bE1* and *bE2* genes of *U. maydis* identified a region (codons 39 to 79) that was important for specificity (Figure 1*A*) (125). Surprisingly, chimeric alleles with exchange points between codons 39 and 79 had a specificity different from either parental allele. Similar chimeric alleles for *bW1* and *bW2* identified a region between codons 9 and 80. Recombination in this region resulted in chimerics with novel specificity (Figure 1*B*) (A Yee & J Kronstad, unpublished). Mating interactions between strains carrying *bE* and *bW* chimerics revealed that alleles with recombination points in the specificity regions fail to interact with each other. This result suggests that the borders of the specificity regions are important for determining self versus nonself recognition (A Yee & J Kronstad, unpublished).

A series of chimeric genes has also been constructed for the *Z* genes (Figure 1*C*) (122) and the *Y* genes (Figure 1*D*) (126) in *S. commune*. Seven chimeric alleles between *Z5* and *Z4* defined a region important for specificity between codons 19 and 60. Eight chimeric alleles for *Y4* and *Y3* showed that specificity was determined between codons 17 and 72. As with the *bE1/bE2* chimerics (125), *Y4/Y3* chimeric genes with exchange points within the specificity region had specificities different from either parental allele, suggesting that the borders of the specificity region may carry the important determinants of recognition.

Figure 1 Chimeric alleles of the homeodomain mating-type proteins identify N-terminal regions that determine specificity. The positions of the recombination points to generate chimeric alleles of the genes are indicated along with the specificity of the resulting allele. (*A*) Chimerics with recombination events between codons 39 and 79 of the *U. maydis bE1* and *bE2* genes have a specificity that is different from that of either parental allele. (*B*) Chimeric alleles constructed for the *bW1* and *bW2* genes of *U. maydis* define a specificity region between codons 9 and 80. (*C*) For the *Z5* and *Z4* genes of *S. commune*, the first 60 codons are sufficient to confer specificity. (*D*) The *Y4* and *Y3* genes of *S. commune* have a specificity region between codons 26 and 62; recombination events in this region confer a novel specificity. (*E*) Two chimeric alleles have been constructed in *C. cinereus*, one for the HD-1 gene pair *b1-3/b1-1* and one for the HD-2 pair *b2-1/b2-3*. These alleles indicate that specificity is determined in the N-terminal 160 a.a. region for both gene pairs.


Ustilago maydis
Allele specificity
A.
1
473
bE1
bE2
31
bE1
bE2
39
79
bE1
bE2
82
bE2
Neither bE1 or bE2
bE1
B.
1
645
bW1
bW2
6
bW1
bW2
9
80
bW1
bW2
81
bW2
Neither bW1 or bW2
bW1
Schizophyllum commune
C.
1
930
Z5
Z4
19
Z5
Z4
60
Z4
Z5
D.
1
930
Y4
Y3
16
Y4
Y3
26
62
Y4
Y3
73
Y3
Neither Y4 or Y3
Y4
Coprinus cinereus
E.
1
632
b1-3
b1-1
163
b1-3
524
b2-1
b2-3
166
b2-1

In *C. cinereus*, chimeric genes named *b1-3:b1-1* and *b2-1:b2-3* were found to have the specificity of the gene providing the N-terminal sequence (3). For example, the N-terminal 163 a.a. of *b1-3* caused the *b1-3:b1-1* chimeric to behave like *b1-3* and the 166 N-terminal a.a. of *b2-1* conferred *b2-1* specificity on the *b2-1:b2-3* gene (Figure 1*E*).

These analyses of chimeric genes indicate that the N-terminal regions of the homeodomain proteins contain the primary determinants of self versus nonself recognition. In addition, the discovery that some chimerics have specificity different from the parental alleles suggests that new specificities could arise by recombination within the short regions encoding the N-terminal sequences.

DIMERIZATION OF PROTEINS WITH HOMEODOMAIN MOTIFS The specificity of recognition between homeodomain proteins is based on the ability of the proteins encoded by different factors to dimerize via N-terminal regions. Nonself combinations (e.g. bE1 and bW2, Y4 and Z5, or b1-1 and b2-3) are capable of dimerization through N-terminal interactions and self combinations (e.g. bE1 and bW1, Y4 and Z4, or b1-1 and b2-1) are not.

The relationship between specificity and dimerization has been clearly demonstrated for the bE2 and bW2 combination in *U. maydis* (49). The interactions of self and nonself bE and bW polypeptides were determined using the yeast two-hybrid system. The N-terminal variable regions promoted dimerization only in nonself combinations. Mutations in the variable domain of the *bE2* gene were isolated that allowed activation of filamentous growth in the self-combination with *bW2* in *U. maydis*. Most of these mutations resulted in bE2 polypeptides that dimerized with bW2 in the two-hybrid assay, indicating that a change from self to nonself recognition correlates with dimerization. In a reciprocal experiment, mutations in bE2 that allowed dimerization with bW2 in the two-hybrid system were found to activate *b*-regulated development in combination with bW2 in *U. maydis*. An in vitro test for dimerization confirmed that proteins from nonself combinations interact.

The N-terminal regions of the HD1 and HD2 proteins from *C. cinereus* also mediate dimerization. An in vitro GST association assay demonstrated binding of full-length HD1 protein b1-1 to a truncated form of the HD2 protein b2-3, but not to truncated versions of HD1 proteins (b1-1, b1-3, and d1-1) or other HD2 proteins (a2-1, b2-1) (3). Similarly, full-length HD1 protein b1-3 bound truncated HD2 proteins b2-1 and b2-3, but not a2-1 or any of the HD1 proteins. Interaction between b1-3 and b2-3 was unexpected because these proteins are encoded by the same *A* factor (A6). This weak interaction was considered to be nonspecific because no comparable interaction was observed between corresponding incompatible proteins from the *A42* allele (b1-1 and b2-1). Interestingly, the C terminus of b1-3 appeared to be responsible for the binding to b2-3. Experiments with truncated b1-1 and b1-3 proteins demonstrated

that the N-terminal regions were sufficient to mediate mating type–specific dimerization.

The interactions of the Y and Z proteins have also been explored in *S. commune* using the two-hybrid system and in vitro binding assays. Interaction in a two-hybrid assay could be demonstrated for the nonself combination of Y4 and Z5 proteins, but not for Y4 and Z4 proteins encoded by the same *A* factor (69). Truncated versions of Z5 with a N-terminal region as short as 101 a.a. bound to Y4 protein (122). This Z5 sequence includes the specificity region identified by chimeric allele analysis. Similarly, the first 144 a.a. of Y4, which includes the specificity region defined by chimeric alleles, are sufficient to bind to Z3 and Z5, but not Z4 (126). Like bE and bW in *U. maydis*, it appears that the specificity region defined by chimeric allele analysis is important for dimerization.

Recently, the in vitro interactions of full-length, truncated, and internally deleted versions of the Y and Z proteins of *S. commune* have been studied (Y Asada, C Yue, J Wu, G-P Shen, CP Novotny & RC Ulllrich, submitted). Two types of interactions, mating type–dependent and mating type–independent, were found and the protein regions necessary for these interactions were defined. These in vitro interactions raise the possibility that higher order (e.g. tetramers) and self-interactions may occur in vivo between full-length polypeptides. As noted above, binding of *C. cinereus* products from a self-combination (b1-3 and b2-3 from *A*6) was observed, although only one of the interacting proteins (b1-3) was full length (3). The descriptions of specificity and dimerization regions for the homeodomain containing mating-type proteins provide a sketch of the molecular basis of recognition. Polypeptides encoded by nonself combinations of the *bE* and *bW*, *Y* and *Z*, and *HD1* and *HD2* genes interact via an N-terminal region. Self-combinations fail to dimerize via the N-terminal regions and therefore are nonfunctional for subsequent regulation in the dikaryon. Analysis of chimeric alleles indicates that the specificity determinants in the N-terminal sequence may be the borders of specificity regions. Presumably these borders contain key amino acid positions that act as "anti-dimerization" sites to prevent dimerization of self-encoded polypeptides. A complete understanding of the molecular basis of recognition will depend on the characterization of the types of amino acid residues that interact at the dimerization interface(s) in self and nonself combinations and on a three dimensional view of the structures of the N-terminal regions. A detailed discussion of the specificity of recognition has recently appeared (50).

TARGETS OF REGULATION BY HOMEODOMAIN PROTEINS A current challenge is the identification and isolation of genes whose transcription is regulated by the homeodomain mating-type proteins. The approaches to find these genes include isolating mutants defective in mating-type regulated events, screening

for genes differentially expressed during mating, and analyzing the expression of candidate genes at the mating-type loci. In *S. commune*, a number of mutations that interfere with the *A*-regulated pathway (e.g. hook cell formation) have been identified (for review see 86). Similarly, mutations that interfere with mating have been identified in *U. maydis* and *U. hordei* (for reviews see 8, 70), although many of these mutations may interfere with cell fusion and pheromone response.

To specifically target the *b*-regulated pathway in *U. maydis*, pathogenic haploid strains, which bypass the need for cell fusion, have been subjected to mutagenesis (16, 31, 57, 64). Haploids heterozygous at *b* display a weakly filamentous phenotype. Insertion mutations that block filamentous growth may identify targets of *b* gene regulation. A collection of such mutants has been described and the analysis of one insertion mutation led to the isolation of *myp1*, a gene required for full virulence. This gene does not appear to be regulated by the *b* locus (31, 64). Restriction enzyme mediated integration (REMI) has also been used to mutagenize a pathogenic haploid strain of *U. maydis* (16). In this case, a large collection of mutants has been screened for pathogenicity defects and several genes have been characterized. To date, none of these genes has been reported to be regulated by *b*.

Techniques to identify differentially expressed genes have also been applied to find targets of homeodomain mating proteins in *S. commune* and *U. maydis* (52, 117). In the case of *S. commune*, dikaryon-specific transcripts have been identified, but it remains to be seen whether these genes are directly regulated by *A* factor products (93). In *U. maydis*, both differential hybridization and differential display have been used to search for genes specifically expressed in the dikaryon. Although several genes have been cloned and characterized, disruption of these genes does not interfere with *b*-regulated development (52).

Analysis of transcripts for genes at the *a* locus of *U. maydis* revealed a possible influence of the bE/bW heterodimer (112). Transcription of the pheromone-responsive *mfa* and *pra* genes in a haploid strain (*a1 b1*) was much higher than in a diploid strain (*a1/a2 b1/b2*). In contrast, the transcript level for *lga2* was tenfold higher in the diploid. These results suggest that active *b* heterodimer may repress transcription of genes required for fusion and activate the transcription of other genes. The repression of genes for pheromone response by *b* is consistent with earlier observations that heterozygosity at *b* attenuates fusion (64). The possible repression of transcription by the *b* protein heterodimer is reminiscent of the repression by the a1/α2 heterodimer of *S. cerevisiae* (43).

Mating and cAMP

The cAMP pathway plays a role in the switch between budding and filamentous growth in *U. maydis* and *U. hordei* (38; A Lichter & D Mills, submitted). In addition, activation of cAMP-dependent protein kinase (PKA) due to a mutation

in the gene encoding the regulatory subunit (*ubc1*) interferes with mating (38). It is tempting to speculate that recognition of environmental cues via the cAMP pathway acts in concert with mating-type regulation in the formation of the dikaryon. Perhaps phosphorylation by PKA influences the activity of proteins whose expression is regulated by mating-type proteins. Certainly, evidence has been presented for a connection between *A* factor regulation and PKA phosphorylation of a specific protein in *C. cinereus* (106). In addition, cAMP stimulates fruiting body formation in *S. commune* and *C. cinereus* (86). In *U. maydis*, pheromone signaling and environmental signals may be coordinated via common signal transduction components such as the Gα protein encoded by the *gpa3* gene (87). The filamentous cell phenotype of the *gpa3* mutants is certainly similar to the phenotype of *U. maydis* cells defective in adenylyl cyclase or PKA (38; F Dürrenberger, K Wong & J Kronstad, in preparation). Furthermore, a mutation in a gene encoding a Gα subunit results in a filamentous cell phenotype that reverts to budding upon addition of cAMP (A Lichter & D Mills, submitted).

Bipolar Versus Tetrapolar Mating Systems

The characterization of *a* and *b* mating-type sequences at the *MAT* locus of *U. hordei* has led to a molecular explanation for the difference between bipolar and tetrapolar mating systems in the heterobasidiomycetes (4–6). That is, the *a* and *b* sequences are physically and genetically linked in *U. hordei* to establish one giant *MAT* locus; *MAT-1* contains *a1* and *b1* and *MAT-2* contains *a2* and *b2*. Recombination appears to be suppressed in the *MAT* locus because meiotic progeny with *a1 b2* and *a2 b1* combinations have not been found (5), even though recent work suggests that *a* and *b* are separated by 400 to 500 kb (N Lee & J Kronstad, unpublished). The organization of the MAT region is reminiscent of the *MAT* locus in *Chlamydomonas reinhardtii* (27). In this case, a number of genes involved in mating are maintained at *MAT* and recombination is suppressed. A similar clustering of mating-type functions may be present in the *MAT* region of *U. hordei*. Precedent for this idea comes from the work in *S. commune* demonstrating that genes required for dikaryon formation cluster near the *B* factor (86).

MATING-TYPE FUNCTIONS IN NONMATING PROCESSES

It is intriguing that some mating-type loci play roles in processes other than mating. These include vegetative incompatibility, sexual dimorphism, ascospore dimorphism, and virulence. In cases other than vegetative incompatibility, it is not known whether the associated phenomena are encoded by the mating-type genes or by genes tightly linked to them. Association of the mating-type

genes with other functions could have profound effects on the distribution of those characters in the population. This could be particularly true of fungi like *Ustilago*, in which sexual development occurs only in infected plants.

Vegetative incompatibility is a common aspect of fungal mycelial growth that limits the formation of heterokaryons bearing genetically distinct nuclei. In what is essentially the inverse of the heterokaryon compatibility function of mating type in basidiomycetes, the mating-type locus of *N. crassa* has a vegetative incompatibility function that *prevents* the formation of mixed mating-type vegetative heterokaryons. *N. crassa* has at least ten different vegetative incompatibility loci, of which the mating-type locus is but one. The vegetative incompatibility loci other than mating type have no obvious effect on enhancing or restricting sexual compatibility. In fact, the action of the *N. crassa mt* locus as a vegetative incompatibility locus is unusual in ascomycetes and even unusual in the genus *Neurospora*.

When two *N. crassa* strains that differ at mating-type, but not at other vegetative incompatibility loci, fuse under vegetative conditions, the cytoplasm at the fusion site becomes highly vacuolated and the mycelia in this region die. The molecular basis of this reaction is not known. This reaction can be mimicked by the injection of cytoplasm from an *a* strain into an *A* strain (120; J Wilson, personal communication). This suggests that the incompatibility reaction is due to a factor present in *a* cells before they encounter the opposite mating type. The sequence of vegetative incompatibility alleles from *het-c* and *het-e* from *Podospora anserina* (89, 91) and the involvement of adenylate cyclase in incompatibility reactions (67) suggests a role for signal transduction pathways in incompatibility. The *tol* locus is also involved in the mating type associated vegetative incompatibility reaction. Mutation at *tol* (tolerant) allows mixed mating-type heterokaryons to form. *tol* is on chromosome IV, linked closely to *trp-4* (78). Additional *tol* loci may be present, but a recent search for new suppressors yielded only seven new *tol* alleles (115). The vegetative incompatibility activity of MT a-1 (39, 82) and MT A-1 (90) can be separated by mutation from the roles of these polypeptides in fertilization. The MT a-1 vegetative incompatibility function is retained in a mutant that lacks DNA binding activity in vitro and mating activity in vivo, so it is likely that vegetative incompatibility and mating act via biochemically distinct mechanisms (82).

The roles that mating type may play in other processes, such as sexual dimorphism, ascospore dimorphism, and virulence, have been less completely characterized. In *Ascosphaera apis*, limited observations suggest that mating type is associated with male-female dimorphism (102). Such a system would be likely to influence characters like cytoplasmic inheritance as well. Ascospore dimorphism is common in the genus *Sclerotinia* and apparently is related to mating type or to mating-type switching. In *Sclerotinia*, two of the ascospores are typically large and two are small; this seems to correspond to

ascospore mating type (29). Unfortunately, no mating-type genes have been cloned from plectomycetes or discomycetes, although PCR amplification techniques promise facile cloning of mating-type genes (22). Finally, coisogenic strains of *Cryptococcus neoformans* differing at mating type differ greatly in virulence (62). Most strains recovered from patients are the more virulent, α, mating type. The mating-type region of *C. neoformans* is a large idiomorphic region that encodes a pheromone (74). Exposure of a cells to α pheromone induces hyphal growth. In addition, α cells become filamentous and sporulate under certain conditions. These properties may explain the preponderance of α mating type amongst pathogenic infections (119).

MATING-TYPE SWITCHING IN FILAMENTOUS FUNGI

Mating-type switching occurs in several filamentous ascomycetes (*Chromocrea spinulosa, Sclerotinia sclerotiorum, Glomerella cingulata*) (80) and in the basidiomycete *Agrocybe aegerita* (63). In *G. cingulata*, switching appears to be unidirectional and dependent upon a gene distinct from mating type that may be functionally similar to the *HO* gene of *S. cerevisiae* (21, 118). Switching in these systems has not been characterized at the molecular level.

QUESTIONS CONCERNING MATING TYPE AND EVOLUTION

A discussion of mating type evokes many evolutionary and population genetics questions in addition to questions of mating mechanisms. For example, how did mating systems arise and how have they come to exhibit their current diversity? What adaptive advantages do the diverse systems offer and do mating systems relate to speciation? Finally, a longstanding problem has been the relationship of mating type to asexuality in fungi.

Origin and Diversity of Mating-Type Regulatory Systems

All mating systems include related, but not identical, sets of gene-regulatory polypeptides and signal transduction cascades. The "set" of regulators encoded by the loci is not constant (76). For example, *C. heterostrophus* has only the MT a-1 and MT A-1 homologs; there is no evidence for MT A-2. *S. cerevisiae* and *S. pombe* have even more divergent sets of mating-type genes. Even within closely related fungi, mating-type polypeptide sequences have diverged rapidly. For example, one finds only 20% similarity between FPR1 and MT a-1. In contrast, glyceraldehyde-3-phosphate dehydrogenase genes from *P. anserina* and *N. crassa* are 80% similar and many homologous proteins are over 50% similar. This suggests that there are different evolutionary constraints acting on the mating-type genes than upon housekeeping genes. Surprisingly, these

diverse polypeptides show broad cross-species activity. The mating-type genes of *P. anserina, N. crassa*, and *C. heterostrophus* can function in the opposite species (2), although this cross-species activity does not appear to extend to *S. pombe* (ML Philley & C Staben, unpublished).

The origins of different mating systems and the selective pressures involved in their evolution may be best understood by studying closely related species with different mating systems. One example is the genus *Neurospora*, which has heterothallic, pseudohomothallic, and homothallic species. Individual mating-type genes from *Neurospora* are similar to one another (37) and in the cases tested, they function normally when introduced into *N. crassa* by introgression (47, 72). Pseudohomothallic *N. tetrasperma* apparently arose within a group of heterothallic ancestral *Neurospora* (85). Because the mating-type locus of at least one ancestral species, *N. crassa*, has a vegetative incompatibility function, one would expect that the heterokaryotic *N. tetrasperma* would either lose this mating-type function or become unable to induce incompatibility in response to *a/A* interaction. In fact, the *tol* locus of *N. tetrasperma* does not prevent mixed mating-type heterokaryons from forming when introgressed into *N. crassa* (47).

The homothallic Neurospora differ at mating type; all known species have a sequence that hybridizes at high stringency to *mt A*, but only *N. terricola* has an obvious *mt* a homolog. The roles of mating-type genes present in homothallic Neurospora species are not clear. These mating-type genes could be irrelevant remnants inherited from heterothallic ancestors, or they could regulate homothallic sexual development. The *mt A-1* mating-type gene from the homothallic species *N. africana* is similar in sequence to that of *N. crassa*, and it can function with *mt A-1* specificity when transformed into *N. crassa* (36). Many different arrangements of mating-type genes could result in homothallism, including linkage of two mating-type genes or even fusion of the coding regions. The nonheterothallic species of Neurospora (particularly the homothallic species) tend to occur at the extremes of Neurospora's ecological range, so the variant mating systems are presumed to be adaptations that ensure sexual reproduction and ascospore production when encounters between unlike genotypes are rare. Characterization of the mating-type loci and evolutionary genetics of homothallic Neurospora and related *Sordariaciae* should shed light on the origins of variant mating systems within this group and on evolutionary mechanisms that may operate in other fungi.

Role of the mt Loci in Variant Life Cycles, Speciation, and Asexuality

The roles of the mating-type loci in variant life cycles and their relationship to speciation events have been explored, but much remains to be learned in these areas. Interspecific crosses between species like *N. crassa* and *N. sitophila*

suggest that there are only a few chromosomal segments likely to be involved in the species barriers (81). Clearly, in Neurospora, the mating-type genes do not act as primary species barriers. Nevertheless, it is possible that mating type controls genes, such as pheromone genes, that are involved in species barriers. Curiously, many cross-species matings result in fertilization but subsequent formation of barren perithecia. One would expect, given the enormous investment in the differentiation of macroscopic females, that formation of barren perithecia would be the most expensive sexual mistake that a filamentous fungus could make. Therefore, it seems surprising that barriers to cross-species fertilization do not arise coincidentally with barriers to zygote formation and differentiation.

Asexuality in fungi is interesting from an applied perspective because many plant pathogenic fungi are asexual, which prevents genetic analysis. Loss of a mating-type gene is one mechanism that could render a fungus asexual, but this does not appear to be the case for some imperfect ascomycetes. *Bipolaris sacchari*, an asexual relative of *C. heterostrophus*, contains a functional mating-type gene, and its asexuality is not remedied by transformation with mating-type genes (95). It seems likely that most asexual fungi are clonal derivatives that diverged from sexually reproducing populations and that loss of sexuality may occur in many different ways. Loss of female fertility is quite widespread among plant pathogens, and may be the most frequent first step taken toward asexuality (65).

CONCLUSIONS AND FUTURE PROSPECTS

The mating pathways in many fungi have now been sketched onto frameworks similar to those first delineated in *S. cerevisiae* and *S. pombe*. Although a partial cast of characters, and some aspects of their roles, can be discerned, many challenges remain. It is frustrating that we know least about aspects of mating-type action in filamentous fungi that are very different from simple yeast systems. Unlike yeast, which are limited in spatial differentiation, mating type in filamentous fungi acts in very specialized cells: the crozier, the hook cell, and fruiting bodies. Unlike ascomycetes in which recognition is one-to-one, basidiomycetes have evolved novel one-to-many specificity in pheromone signaling and in homeodomain protein interaction; unraveling this specificity poses an enormous challenge. Future work must flesh out details of expected pathways, dissect cell type determination, and sexual structure formation at the molecular level, and relate these findings to broad evolutionary questions.

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Literature Cited

1. Aramayo R, Metzenberg RL. 1996. Meiotic transvection in fungi. *Cell* 86:103–13
2. Arnaise S, Zickler D, Glass NL. 1993. Heterologous expression of mating-type genes in filamentous fungi. *Proc. Natl. Acad. Sci. USA* 90:6616–20
3. Asante-Owusu RN, Banham AH, Bohnert HU, Mellor EJ, Casselton LA. 1996. Heterodimerization between two classes of homeodomain proteins in the mushroom *Coprinus cinereus* brings together potential DNA-binding and activation domains. *Gene* 172:25–31
4. Bakkeren G, Kronstad JW. 1993. Conservation of the *b* mating-type gene complex among bipolar and tetrapolar smut fungi. *Plant Cell* 5:123–36
5. Bakkeren G, Kronstad JW. 1994. Linkage of mating-type loci distinguishes bipolar from tetrapolar mating in basidiomycetous smut fungi. *Proc. Natl. Acad. Sci. USA* 91:7085–89
6. Bakkeren G, Kronstad JW. 1996. The pheromone cell signalling components of the *Ustilago a* mating type loci determine intercompatibility between species. *Genetics* 143:1601–13
7. Banuett F. 1992. *Ustilago maydis*, the delightful blight. *Trends Genet.* 8:174–80
8. Banuett F. 1995. Genetics of *Ustilago maydis*, a fungal pathogen that induces tumors in maize. *Annu. Rev. Genet.* 29:179–208
9. Banuett F, Herskowitz I. 1994. Identification of fuz7, a *Ustilago maydis* MEK/MAPKK homolog required for a-locus-dependent and -independent steps in the fungal life cycle. *Genes Dev.* 8:1367–78
10. Bardwell L, Cook JG, Inouye CJ, Thorner J. 1994. Signal propagation and regulation in the mating pheromone response pathway of the yeast *Saccharomyces cerevisiae*. *Dev. Biol.* 166:363–79
11. Baxevanis AD, Bryant SH, Landsman D. 1995. Homology model building of the HMG-1 box structural domain. *Nucleic Acids Res.* 23:1019–29
12. Behrens J, von Kries JP, Kuhl M, Bruhn L, Wedlich D, et al. 1996. Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature* 382:638–42
13. Bistis GN. 1981. Chemotropic interactions between trichogynes and conidia of opposite mating-type in *Neurospora crassa*. *Mycologia* 73:959–75
14. Bistis GN. 1983. Evidence for diffusible mating-type specific trichogyne attractants in *Neurospora crassa*. *Exp. Mycol.* 7:292–95
15. Bistis GN. 1996. Trichogynes and fertilization in uni- and bimating type colonies of *Neurospora tetrasperma*. *Fungal Genet. Biol.* 20:93–98
16. Bölker M, Bohnert HU, Braun KH, Gorl J, Kahmann R. 1995. Tagging pathogenicity genes in *Ustilago maydis* by restriction enzyme-mediated integration (REMI). *Mol. Gen. Genet.* 248:547–52
17. Bölker M, Kahmann R. 1993. Sexual pheromones and mating responses in fungi. *Plant Cell* 5:1461–69
18. Bölker M, Urban M, Kahmann R. 1992. The *a* mating type locus of *U. maydis* specifies cell signaling components. *Cell* 68:441–50
19. Casselton L, Kües U. 1994. Mating-type genes in homobasidiomycetes. In *The Mycota*, ed. JW Wessels, F Meinhardt, 1:307–21. Berlin: Springer-Verlag
20. Chang S, Staben C. 1994. Directed replacement of *mt A* by *mt a-1* effects a mating type switch in *Neurospora crassa*. *Genetics* 138:75–81
21. Chilton SJP, Wheeler HE. 1949. Genetics of Glomerella. VII. Mutation and segregation in plus cultures. *Am. J. Bot.* 36:717–21
22. Christiansen SK, Yoder OC, Turgeon BG. 1997. Efficient cloning of ascomycete mating type genes by PCR amplification of the conserved MAT HMG box. *Fungal Genet. Biol.* 21:118–30
23. Dahl M, Bölker M, Gillissen B, Swauwecker F, Schroeer B, Kahmann R. 1991. The *b* locus of *Ustilago maydis:* molecular analysis of allele specificity. In *Advances in Moleular Genetics of*

Plant-Microbe Interactions, ed. HH Verma, 1:264–71. Dordrecht: Kluwer
24. Debuchy R, Arnaise S, Lecellier G. 1993. The *mat-* allele of *Podospora anserina* contains three regulatory genes required for the development of fertilized female organs. *Mol. Gen. Genet.* 241:667–73
25. Debuchy R, Coppin E. 1992. The mating types of *Podospora anserina:* functional analysis and sequence of the fertilization domains. *Mol. Gen.Genet.* 233:113–21
26. Ferreira AV, Saupe S, Glass NL. 1996. Transcriptional analysis of the *mt A* idiomorph of *Neurospora crassa* identifies two genes in addition to *mt A-1*. *Mol. Gen. Genet.* 250:767–74
27. Ferris PJ, Goodenough UW. 1994. The mating-type locus of *Chlamydomonas reinhardtii* contains highly rearranged DNA sequences. *Cell* 76:1135–45
28. Froeliger E, Leong SA. 1991. The *a* mating-type alleles of *Ustilago maydis* are idiomorphs. *Gene* 100:113–22
29. Fujii H, Uhm JY. 1988. *Sclerotinia trifoliorum* cause of rots of *Trifolium* spp. *Adv. Plant Pathol.* 6:233–47
30. Gao S, Nuss DL. 1996. Distinct roles for two G protein alpha subunits in fungal virulence, morphology, and reproduction revealed by targeted gene disruption. *Proc. Natl. Acad. Sci. USA* 93:14122–27
31. Giasson L, Kronstad JW. 1995. Mutations in the *myp1* gene of *Ustilago maydis* attenuate mycelial growth and virulence. *Genetics* 141:491–501
32. Giasson L, Specht CA, Milgrim C, Novotny CP, Ullrich RC. 1989. Cloning and comparison of A alpha mating-type alleles of the basidiomycete *Schizophyllum commune*. *Mol. Gen. Genet.* 218:72–77
33. Gillissen B, Bergemann J, Sandmann C, Schroeer B, Bölker M, Kahmann R. 1992. A two-component regulatory system for self/nonself recognition in *Ustilago maydis*. *Cell* 68:647–57
34. Glass NL, Grotelueschen J, Metzenberg RL. 1990. *Neurospora crassa A* mating-type region. *Proc. Natl. Acad. Sci. USA* 87:4912–16
35. Glass NL, Lee L. 1992. Isolation of *Neurospora crassa A* mating type mutants by repeat induced point (RIP) mutation. *Genetics* 132:125–33
36. Glass NL, Smith ML. 1994. Structure and function of a mating-type gene from the homothallic species *Neurospora africana*. *Mol. Gen. Genet.* 244:401–9
37. Glass NL, Vollmer SJ, Staben C, Metzenberg RL, Yanofsky C. 1988. DNAs of the two mating-type alleles of *Neurospora crassa* are highly dissimilar. *Science* 241:570–73
38. Gold S, Duncan G, Barrett K, Kronstad J. 1994. cAMP regulates morphogenesis in the fungal pathogen *Ustilago maydis*. *Genes Dev.* 8:2805–16
39. Griffiths AJF, DeLange AM. 1978. Mutations of the a mating-type gene in *Neurospora crassa*. *Genetics* 88:239–54
40. Grosschedl R, Giese K, Pagel J. 1994. HMG domain proteins: architectural elements in the assembly of nucleoprotein structures. *Trends Genet.* 10:94–100
41. Harley VR, Lovell-Badge R, Goodfellow PN, Hextall PJ. 1996. The HMG box of SRY is a calmodulin binding domain. *FEBS Lett.* 391:24–28
42. Hartmann HA, Kahmann R, Bölker M. 1996. The pheromone response factor coordinates filamentous growth and pathogenicity in *Ustilago maydis*. *EMBO J.* 15:1632–41
43. Herskowitz I, Rine J, Strathern J. 1992. Mating-type determination and mating-type interconversion in *Saccharomyces cerevisiae*. In *The Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression*, ed. EW Jones, JR Pringel, JR Broach, 2:583–656. Plainview, NY: Cold Spring Harbor Lab. Press
44. Hisatomi T, Yanagishima N, Sakurai A, Kobayashi H. 1988. Interspecific actions of alpha mating pheromones on the a mating-type cells of three Saccharomyces yeasts. *Curr. Genet.* 13:25–27
45. Imai Y, Yamamoto M. 1992. *Schizosaccharomyces pombe* sxa1+ and sxa2+ encode putative proteases involved in the mating response. *Mol. Cell Biol.* 12:1827–34
46. Ivey FD, Hodge PN, Turner GE, Borkovich KA. 1996. The Gαi homologue *gna-1* controls multiple differentiation pathways in *Neurospora crassa*. *Mol. Cell Biol.* 7:1283–97
47. Jacobson DJ. 1992. Control of mating type heterokaryon incompatibility by the *tol* gene in *Neurospora crassa* and *Neurospora tetrasperma*. *Genome* 35:347–53
48. Kämper J, Bölker M, Kahmann R. 1995. Mating-type genes in the heterobasidiomycetes. In *The Mycota*, ed. MJ Wessels, 1:323–32. Berlin: Springer-Verlag
49. Kämper J, Reichmann M, Romeis T, Bölker M, Kahmann R. 1995. Multiallelic recognition: nonself-dependent dimerization of the bE and bW homeodomain proteins in *Ustilago maydis*. *Cell* 81:73–83
50. Kahmann R, Bölker M. 1996. Self/nonself recognition in fungi: old mysteries

and simple solutions. *Cell* 85:145–48
51. Kahmann R, Romeis T, Bölker M, Kämper J. 1995. Control of mating and development in *Ustilago maydis*. *Curr. Opin. Genet. Dev.* 5:559–64
52. Kahmann R, Romeis T, Hartmann HA, Bohnert HU, Bölker M, Kämper J. 1996. Control of mating, filamentous growth, and pathogenicity in *Ustilago maydis*. In *Biology of Plant-Microbe Interactions*, ed. G Stacey, B Mullin, PM Gresshoff, pp. 239–44. St. Paul, MN: Int. Soc. Plant-Microbe Interact.
53. Kang S, Chumley FG, Valent B. 1994. Isolation of the mating-type genes of the phytopathogenic fungus *Magnaporthe grisea* using genomic subtraction. *Genetics* 138:289–96
54. Kazmiercazk P, Pfeiffer P, Zhang L, van Alfen NK. 1996. Transcriptional repression of specific host genes by the mycovirus *Cryphonectria* hypovirus 1. *J. Virol.* 70:1137–42
55. Koltin Y, Stamberg J, Lemke PA. 1972. Genetic structure and evolution of the incompatibility factors in higher fungi. *Bacteriol. Rev.* 36:156–71
56. Kronstad J. 1996. Pathogenesis and sexual development of the smut fungi. In *Plant-Microbe Interactions*, ed. KG Stacey, NT Keen, 1:141–86. New York: Chapman & Hall
57. Kronstad JW, Leong SA. 1989. Isolation of two alleles of the *b* locus of *Ustilago maydis*. *Proc. Natl. Acad. Sci. USA* 86:978–82
58. Kronstad JW, Leong SA. 1990. The b mating-type locus of *Ustilago maydis* contains variable and constant regions. *Genes Dev.* 4:1384–95
59. Kües U, Casselton LA. 1993. The origin of multiple mating types in mushrooms. *J. Cell Sci.* 104:227–30
60. Kües U, Richardson WVJ, Tymon AM, Mutasa ES, Gottgens B, et al. 1992. The combination of dissimilar alleles of the Aα and Aβ gene complexes, whose proteins contain homeodomain motifs, determines sexual development in the mushroom *Coprinus cinereus*. *Genes Dev.* 6:568–77
61. Kurjan J. 1993. The pheromone response pathway in *Saccharomyces cerevisiae*. *Annu. Rev. Genet.* 27:147–79
62. Kwon-Chung KJ, Edman JC, Wickes BL. 1992. Genetic association of mating types and virulence in *Cryptococcus neoformans*. *Infect. Immun.* 60:602–5
63. Labarere J, Noel T. 1992. Mating type switching in the tetrapolar basidiomycete *Agrocybe aegerita*. *Genetics* 131:307–19
64. Laity C, Giasson L, Campbell R, Kronstad J. 1995. Heterozygosity at the *b* mating-type locus attenuates fusion in *Ustilago maydis*. *Curr. Genet.* 27:451–59
65. Leslie JF, Klein KK. 1996. Female fertility and mating type effects on effective population size and evolution in filamentous fungi. *Genetics* 144:557–67
66. Lipke PN, Kurjan J. 1992. Sexual agglutination in budding yeasts: structure, function, and regulation of adhesion glycoproteins. *Microbiol. Rev.* 56:180–94
67. Loubradou G, Begueret J, Turcq B. 1996. An additional copy of the adenylate cyclase-encoding gene relieves developmental defects produced by a mutation in a vegetative incompatibility-controlling gene in *Podospora anserina*. *Gene* 170:119–23
68. MacKay VL, Welch SK, Insley MY, Manney TR, Holly J, et al. 1988. The *Saccharomyces cerevisiae BAR1* gene encodes an exported protein with homology to pepsin. *Proc. Natl. Acad. Sci. USA* 85:55–59
69. Magae Y, Novotny C, Ullrich R. 1995. Interaction of the A alpha Y and Z mating-type homeodomain proteins of *Schizophyllum commune* detected by the two-hybrid system. *Biochem. Biophys. Res. Commun.* 211:1071–76
70. Martinez-Espinoza AD, Gerhardt SA, Sherwood JE. 1993. Morphological and mutational analysis of mating in *Ustilago hordei*. *Exp. Mycol.* 17:200–14
71. May G, LeChevanton L, Pukkila PJ. 1991. Molecular analysis of the *Coprinus cinereus* mating type *A* factor demonstrates an unexpectedly complex structure. *Genetics* 128:529–38
72. Metzenberg RL, Ahlgren SK. 1973. Behaviour of *Neurospora tetrasperma* mating type genes introgressed into *N. crassa*. *Can. J. Gen. Cytol.* 15:571–76
73. Metzenberg RL, Glass NL. 1990. Mating type and mating strategies in *Neurospora*. *BioEssays* 12:53–59
74. Moore TD, Edman JC. 1993. The α-mating type locus of *Cryptococcus neoformans* contains a peptide pheromone gene. *Mol. Cell Biol.* 13:1962–70
75. Mutasa E, Tymon AM, Gottgens B, Mellon FM, Little PFR, Casselton LA. 1990. Molecular organization of an A mating type factor of the basidiomycete fungus *Coprinus cinereus*. *Curr. Genet.* 18:223–29
76. Nelson MA. 1996. Mating systems in ascomycetes: a romp in the sac. *Trends Genet.* 12:69–74

77. Nelson MA, Metzenberg RL. 1992. Sexual development genes of *Neurospora crassa. Genetics* 132:149–62
78. Newmeyer D. 1970. A suppressor of the heterokaryon-incompatibility associated with mating type in *Neurospora crassa. Can. J. Gen. Cytol.* 12:914–26
79. Nielsen O, Davey J. 1995. Pheromone communication in the fission yeast *Schizosaccharomyces pombe. Semin. Cell Biol.* 6:95–104
80. Perkins DD. 1987. Mating-type switching in filamentous ascomycetes. *Genetics* 115:215–16
81. Perkins DD, Bojko M. 1992. The basis of decreased recombination in certain outcrosses of *Neurospora crassa. Genome* 35:503–9
82. Philley ML, Staben C. 1994. Functional analyses of the *Neurospora crassa* MT a-1 mating type polypeptide. *Genetics* 137:715–22
83. Picard M, Debuchy R, Coppin E. 1991. Cloning the mating types of the heterothallic fungus *Podospora anserina:* developmental features of haploid transformants carrying both mating types. *Genetics* 128:539–47
84. Raju NB. 1992. Genetic control of the sexual cycle in *Neurospora. Mycol. Res.* 96:241–62
85. Randall TA, Metzenberg RL. 1995. Species-specific and mating type-specific DNA regions adjacent to mating type idiomorphs in the genus *Neurospora. Genetics* 141:119–36
86. Raper C. 1983. Controls for development and differentiation of the dikaryon in basidiomycetes. In *Secondary Metabolism and Differentiation in Fungi,* ed. JWAC Bennet, pp. 195–238. New York: Dekker
87. Regenfelder E, Spellig T, Hartmann A, Lauenstein S, Bölker M, Kahmann R. 1997. G proteins in *Ustilago maydis:* transmission of multiple signals? *EMBO J.* 16:1934–42
88. Robertson C, Bartholomew KA, Novotny CP, Ullrich RC. 1996. Deletion of the *Schizophyllum commune Aα* locus: the roles of the A*α* Y and Z mating-type genes. *Genetics* 144:1437–44
89. Saupe S, Descamps C, Turcq B, Begueret J. 1994. Inactivation of the *Podospora anserina* vegetative incompatibility locus *het-c,* whose product resembles a glycolipid transfer protein, drastically impairs ascospore production. *Proc. Natl. Acad. Sci. USA* 91:5927–31
90. Saupe S, Stenberg L, Shiu KT, Griffiths AJF, Glass NL. 1996. The molecular nature of mutations in the *mt A-1* gene of the *Neurospora crassa A* idiomorph and their relation to mating-type function. *Mol. Gen. Genet.* 250:115–22
91. Saupe S, Turcq B, Begueret J. 1995. A gene responsible for vegetative incompatibility in the fungus *Podospora anserina* encodes a protein with a GTP-binding motif and G*β* homologous domain. *Gene* 162:135–39
92. Schulz B, Banuett F, Dahl M, Schlesinger R, Schafer W, et al. 1990. The *b* alleles of *Ustilago maydis,* whose combinations program pathogenic development, code for polypeptides containing a homeodomain-related motif. *Cell* 60:295–306
93. Schuren F, Wessels JGH. 1990. Two genes specifically expressed in fruiting dikaryons of *Schizophyllum commune:* homologies with a gene not regulated by mating-type genes. *Gene* 90:199–205
94. Selker EU, Cambareri EB, Jensen BC, Haack KR. 1987. Rearrangement of duplicated DNA in specialized cells of *Neurospora. Cell* 51:741–52
95. Sharon A, Yamaguchi K, Christiansen S, Horwitz BA, Yoder OC, Turgeon BG. 1996. An asexual fungus has the potential for sexual development. *Mol. Gen.Genet.* 251:60–68
96. Shen G-P, Park D-C, Ullrich RC, Novotny CP. 1996. Characterization of a *Schizophyllum* gene with *Aα6* mating-type activity. *Curr. Genet.* 29:136–42
97. Snetselaar K. 1993. Microscopic observation of *Ustilago maydis* mating interactions. *Exp. Mycol.* 17:345–55
98. Snetselaar K, Mims CW. 1992. Sporidial fusion and infection of maize seedlings by the smut fungus *Ustilago maydis. Mycologia* 84:193–203
99. Specht CA. 1995. Isolation of the B alpha and B beta mating-type loci of *Schizophyllum commune. Curr. Genet.* 28:374–79
100. Specht CA, Stankis MM, Giasson L, Novotny CP, Ullrich RC. 1992. Functional analysis of the homeodomain-related proteins of the *Aα* locus of *Schizophyllum commune. Proc. Natl. Acad. Sci. USA* 89:7174–78
101. Spellig T, Bölker M, Lottspeich F, Frank RW, Kahmann R. 1994. Pheromones trigger filamentous growth in *Ustilago maydis. EMBO J.* 13:1620–27
102. Spiltoir CF. 1955. Life cycle of *Ascosphaera apis* (*Pericystis apis*). *Am. J. Bot.* 42:501–8
103. Staben C, Yanofsky. 1990. *Neurospora crassa a* mating type region. *Proc. Natl. Acad. Sci. USA* 87:4917–21

104. Stankis MM, Specht CA, Giasson L. 1990. Sexual incompatibility in *Schizophyllum commune:* from classical genetics to a molecular view. *Semin. Dev. Biol.* 1:195–206
105. Stankis MM, Specht CA, Yang H, Giasson L, Ullrich R, Novotny CP. 1992. The *Aα* mating locus of *Schizophyllum commune* encodes two dissimilar multiallelic proteins. *Proc. Natl. Acad. Sci. USA* 89:7169–73
106. Swamy S, Uno I, Ishikawa T. 1985. Regulation of cyclic AMP-dependent phosphorylation of cellular proteins by the incompatibility factors of *Coprinus cinereus. J. Gen. Appl. Microbiol.* 31:339–46
107. Thompson-Coffe C, Zickler D. 1994. How the cytoskeleton recognizes and sorts nuclei of opposite mating type during the sexual cycle in filamentous ascomycetes. *Dev. Biol.* 165:257–71
108. Turgeon BG, Bohlmann H, Ciuffetti LM, Christiansen SK, Yang G, et al. 1993. Cloning and analysis of the mating type genes from *Cochliobolus heterostrophus. Mol. Gen. Genet.* 238:270–84
109. Turner GE, Borkovich KA. 1993. Identification of a G protein *α* subunit from *Neurospora crassa* that is a member of the Gi family. *J. Biol. Chem.* 268:14805–11
110. Tymon AM, Khes U, Richardson WVJ, Casselton LA. 1992. A fungal mating type protein that regulates sexual and asexual development contains a POU-related domain. *EMBO J.* 11:1805–13
111. Urban M, Kahmann R, Bölker M. 1996. The biallelic *a* mating type locus of *Ustilago maydis:* remnants of an additional pheromone gene indicate evolution from a multiallelic ancestor. *Mol. Gen. Genet.* 250:414–20
112. Urban M, Kahmann R, Bölker M. 1996. Identification of the pheromone response element in *Ustilago maydis. Mol. Gen. Genet.* 251:31–37
113. Vaillancourt L, Raper CA. 1996. Pheromones and pheromone receptors as mating-type determinants in basidiomycetes. In *Genetic Engineering, Principles and Methods*, ed. JK Setlow, 18:219–47. New York: Plenum
114. van Houte LP, Chuprina VP, van der Wetering M, Boelens R, et al. 1995. Solution structure of the sequence-specific HMG box of the lymphocyte transcriptional activator Sox-4. *J. Biol. Chem.* 270:30516–24
115. Vellani TS, Griffiths AJF, Glass NL. 1993. New mutations that suppress mating-type vegetative incompatibility in *Neurospora crassa. Genome* 37:249–55
116. Wendland J, Vaillancourt LJ, Hegner J, Lengeler KB, Laddison KJ, et al. 1995. The mating type locus B*α*1 of *Schizophyllum commune* contains a pheromone receptor gene and putative pheromone genes. *EMBO J.* 14:5271–78
117. Wessels JG. 1987. Mating-type genes and the control of expression of fruiting genes in basidiomycetes. *Antonie Van Leeuwenhoek* 53:307–16
118. Wheeler HE. 1950. Genetics of Glomerella. VIII. A genetic basis for the occurence of minus mutants. *Am. J. Bot.* 37:304–12
119. Wickes BL, Mayorga ME, Edman U, Edman JC. 1996. Dimorphism and haploid fruiting in *Cryptococcus neoformans:* association with *α*-mating type. *Proc. Natl. Acad. Sci. USA* 93:7327–31
120. Wilson JF, Garnjobst L, Tatuem EL. 1961. Heterokaryon incompatibility in *Neurospora crassa*-microinjection studies. *Am. J. Bot.* 48:299–305
121. Wirsel S, Turgeon BG, Yoder OC. 1996. Deletion of the *Cochliobolus heterostrophus* mating-type (*MAT*) locus promotes the function of *MAT* transgenes. *Curr. Genet.* 29:241–49
122. Wu J, Ullrich RC, Novotny CP. 1996. Regions in the Z5 mating gene of *Schizophyllum commune* involved in Y-Z binding and recognition. *Mol. Gen. Genet.* 252:739–45
123. Xu J-R, Hamer JE. 1996. MAP kinase and cAMP signaling regulate infection structure formation and pathogenic growth in the rice blast fungus *Magnaporthe grisea. Genes Dev.* 10:2696–706
124. Yabana M, Yamamoto M. 1996. *Schizosaccharomyces pombe map1+* encodes a MADS-box-family protein required for cell-type-specific gene expression. *Mol. Cell Biol.* 16:3420–28
125. Yee AR, Kronstad JW. 1993. Construction of chimeric alleles with altered specificity at the *b* incompatibility locus of *Ustilago maydis. Proc. Natl. Acad. Sci. USA* 90:664–68
126. Yue C, Osier M, Novotny CP, Ullrich RC. 1997. The specificity determinant of the Y mating-type proteins of *Schizophyllum commune* is also essential for Y-Z protein binding. *Genetics* 145:253–60
127. Zickler D, Arnaise S, Coppin E, Debuchy R, Picard M. 1995. Altered mating-type identity in the fungus *Podospora anserina* leads to selfish nuclei, uniparental progeny, and haploid meiosis. *Genetics* 140:493–503