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Mating Types and Sexual Development in Filamentous Ascomycetes

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INTRODUCTION

Almost a century ago, the discovery of heterothallism in fungi brought to light the existence of what was later termed mating types. In 1904, Blakeslee (13) demonstrated that in some members of the Mucorinae (zygomycetes), fertile pairings were only possible between two different single-spore strains. The cultures could be divided into two sexually compatible groups, indistinguishable morphologically, defining two signs, + and −. Following this observation, heterothallism was discovered in other fungi, in particular in filamentous ascomycetes (32), and the determinants of mating type were subjected to genetic analysis. It was first demonstrated in *Ascobolus magnificus* and *Ascobolus carbonarius* (28) and then in several *Neurospora* species (89) that mating type corresponded to two allelic forms of a single locus and, accordingly, that the heterothallism was bipolar (105). The initial confusion between sign and sex (male and female) was alleviated when it was shown that heterothallism was independent of sexual differentiation. In fact, strains of each mating type produced receptor and donor elements, that is to say female organs and fertilizing elements, but the two strains contributing a compatible pair of organs were always of opposite mating types (51). A compatible interaction initiated the development of fruiting bodies that produced ascospores. The point in the multistep sexual process at which the mating type alleles function was determined in the following decades. As early as 1952, Zickler (111) reported that in *Bombardia lunata*, donor cells produced a substance that was able to specifically attract the receptor cells of opposite mating type. This observation was the first indirect evidence that the mating types control a hormonal mechanism mediating the fusion of sexually compatible cells. Additional physiological experiments carried out with *Ascobolus stercorarius* (12) suggested that the mating types might have another function at a subsequent stage of the sexual process, during the development of the fruiting bodies. This dual role of the mating types, suspected very early, has been confirmed by molecular approaches. Molecular analyses of mating types in filamentous ascomycetes began only in 1988 with the cloning of *A* and *a* mating types from *Neurospora crassa* (46), several years after the complete DNA sequence of the **a** and α mating types of *Saccharomyces cerevisiae* had been determined (4). Meanwhile, the mating systems of *S. cerevisiae* and *Schizosaccharomyces pombe* had been extensively analyzed and regulatory models had already been proposed (reviewed in references 34, 54, and 77). In the two yeasts, each of the mating-type loci encodes transcriptional factors controlling the expression of cell-type-specific genes. In particular, they mediate conjugation by controlling the production of mating pheromones and their receptors. They also control entry into meiosis. In spite of these basic similarities, the number and nature of the transcription factors and the wiring of the regulatory circuits vary strikingly between the two yeast mating systems. The discovery of new regulatory pathways was expected with the extension of the analysis to the mating systems of the filamentous ascomycetes, where the sexual process is more elaborate than that of the single-cell yeasts. In these multicellular organisms, sexual reproduction requires differentiation of reproductive structures and a complex developmental process.

The purpose of this review is to summarize what we know about the mating types and their role in the sexual development of the filamentous ascomycetes. A considerable amount of data about numerous species has accumulated during half a century of genetic and physiological investigation. The advent of molecular biology resulted in an explanation, in molecular terms, of what was often already known in physiological terms and has taken the analysis one step further. We refer readers to previous reviews devoted to the subject for an overview of the advances in our knowledge in the last 6 years (42, 44, 69, 71). In the few mating systems which have been subjected to molecular analysis to date, there are common features but also variations in the number of genes involved, their nature, and their functional role. Bipolar heterothallism is only one of the reproductive strategies found in filamentous ascomycetes. Many species are homothallic: homokaryotic haploid strains are self-fertile and complete the sexual cycle without seeking a mate. In the two yeasts which are basically heterothallic, a homothallic breeding system is achieved through a mating-type switching. Information for both mating types is present in silent loci, and a transposition event moves one of the silent copies into the active mating-type locus, allowing its expression. Molecular data have indicated that the switching model of yeast is inappropriate to explain homothallism in filamentous ascomycetes. Studies to determine how sexual reproduction is controlled in species with no genetically definable mating types are under way. In contrast to the mating types of the well-studied filamentous ascomycetes, which are extremely stable, unstable mating types have been found in several species. Future investigations of the mating systems in some of these species will probably reveal a new organization of mating-type information. Finally, many species related to the filamentous ascomycetes reproduce only vegetatively and appear to have abandoned sexual reproduction. It is of great interest to determine the cause of this failure to mate in asexual species. These are the principal points that will be developed in the present review. First, we will outline the sexual cycle of filamentous ascomycetes and underline its specificities. The review will then follow in chronological order with a presentation of the state of knowledge before and after the advent of the molecular approach. We will attempt to point out some of the exciting prospects that this still recent field of investigation holds for future studies. In conclusion, parallels will be presented with the mating systems of yeasts and basidiomycetes to emphasize the diversity of the responses used to solve the same fascinating biological problem: how to distinguish self from nonself and yield sexual progeny.

SEXUAL CYCLE OF FILAMENTOUS ASCOMYCETES

Only the principal events of the sexual cycle will be described. We will stress the characteristics that distinguish the sexual cycle of filamentous ascomycetes from that of yeasts and basidiomycetes. We refer readers who wish to learn more about the cytology of sexual development to the reviews by Raju (83, 85). During their vegetative phase, the filamentous

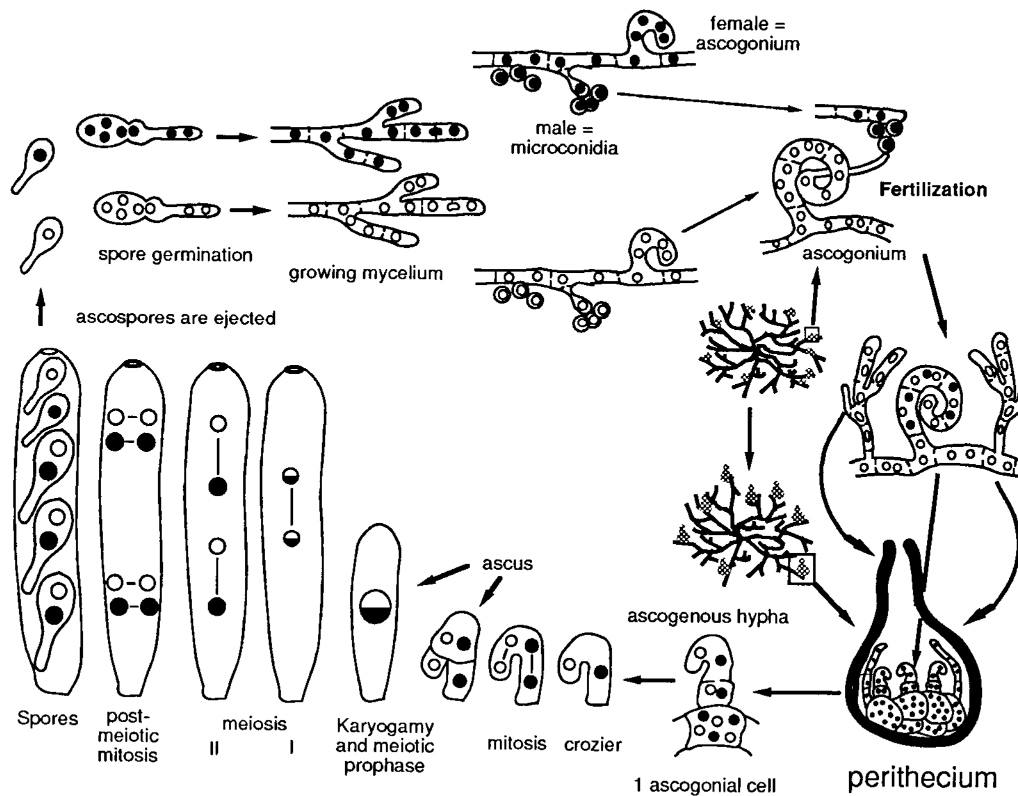

FIG. 1. Life cycle of *P. anserina*.

ascomycetes form a network of partially septate hyphae containing haploid nuclei. The life cycle of *Podospora anserina* is presented as an example in Fig. 1. The sexual cycle begins with the differentiation of female reproductive structures, the ascogonia, under appropriate environmental conditions, including light and starvation for specific nutrients. Ascogonia arise as lateral coiled hyphae with, in many species, an apical receptive hyphal element, the trichogyne. Ascogonia can be naked or quickly enclosed by nearby hyphae, forming a protoperithecium. In heterothallic species, the trichogyne fuses with a male element of the opposite mating type. The donor cells may be microconidia, macroconidia, or hyphae. Fertilization consists of entry of the fertilizing nucleus into the primary ascogonium cell. This fertilization event initiates the development of the fruiting body in which asci are formed through a complex developmental process. Traditional classification of the filamentous ascomycetes based on fruiting-body structure has been largely supported by the molecular phylogenetic studies, except for the loculoascomycetes (96). Depending on their morphology, fruiting bodies are called perithecia (pyrenomycetes), apothecia (discomycetes), cleistothecia (plectomycetes), or pseudothecia (loculoascomycetes). What happens in the fruiting bodies, whatever their morphology, is very similar in all the filamentous ascomycetes.

After fertilization, the male and female nuclei do not fuse. Karyogamy is postponed. Each nucleus proliferates, populating the growing fruiting bodies with numerous multinucleate cells. Then pairs of parental nuclei of opposite mating type migrate into specialized cells, the ascogenous hyphae, which give rise to croziers. In the crozier, the two parental nuclei undergo a coordinate mitosis which yields, after septum formation, uninucleate basal and lateral cells and a binucleate cell which can differentiate into an ascus mother cell, in which karyogamy takes place. The binucleate cell is the only diploid cell in the entire life cycle. The diploid nucleus proceeds directly into meiosis followed by one (or several) postmeiotic mitoses depending on the species and finally by the formation of ascospores. The basal and lateral cell can fuse and divide again, leading to a new crozier. Several asci are thus produced from each initial dikaryotic cell.

Some well-studied species like *P. anserina*, *Neurospora tetrasperma*, and *Gelasinospora tetrasperma* are pseudohomothallic. Their asci contain four spores in which two nuclei of opposite mating types are regularly enclosed. As a consequence, cultures from single ascospores are self-fertile heterokaryons. Nevertheless, in contrast to the true homothallic species, each haploid nucleus contains one of the two alternative mating types. A comparative cytological study of the diverse programs found in four pseudohomothallic species has been done recently (84) and shows that pseudohomothallic and heterothallic species differ in the program of ascus development. It is noteworthy that in pseudohomothallic species, homokaryotic cultures of each mating type can be easily obtained. They are issued from the low percentage of five-spored asci in which two small uninucleate ascospores of each mating type are substituted for one large binucleate ascospore (Fig. 1).

The life cycle of filamentous ascomycetes is geared to produce hundreds of asci from a single fertilization event. Simultaneously, it raises a crucial biological problem: the haploid male and female nuclei of different mating types must “find each other” within a common cytoplasm before differentiation of ascogenous hyphae occurs. One of the main contributions of the molecular analysis of the mating types has been the demonstration of their involvement in recognition between the

nuclei at this step of sexual development. Surprisingly, the cytological picture for crozier and ascus initiation is identical in homothallic species, although the female and male nuclei do not display genetically different mating types. One of the exciting prospects of the studies presently under way in homothallic species is to understand how the pairing of the two parental nuclei is achieved.

ROLES OF THE MATING TYPES: WHAT WAS KNOWN BEFORE THE MOLECULAR APPROACH?

Control of a Hormonal Mechanism

The main role of the mating types, common to all studied ascomycetes, is to control the recognition mechanism leading to fertilization. The first evidence of the existence of a pheromone receptor mechanism came from direct examination of fertilization. Zickler (111) observed that the growing trichogyne of *B. lunata* changed its direction in the vicinity of a spermatium and grew toward this fertilizing element. Filtrates of spermatial suspensions had the same attracting effect only if they were from the opposite mating type. Similar observations were carried out by Bistis, first in *A. stercorarius* (8) and then in *N. crassa* (10). In *A. stercorarius*, the trichogyne was seen to grow toward the oidium and to change its direction when the oidium was relocated. The fertilizing element thus produced a substance which controlled both the growth and direction of growth of the trichogyne. In *N. crassa*, the behavior of individual protoperithecia was observed after adding drops of a macro- or microconidial suspension from a donor of the opposite mating type to an adjacent agar block. Within 2.5 h, the trichogyne began to grow toward the conidia and fusion took place in approximately an additional 2 h. When both macro- and microconidia competed, no preference was observed in the orientation of the trichogyne. The substance secreted by the fertilizing elements was trapped in blocks of activated charcoal placed onto a donor mycelial culture (11). The blocks were then placed on test mycelia that were examined under a microscope. The trichogynes responded by growing to and around the blocks only in inter-mating-type combinations. This result demonstrated the presence of a pheromone-like substance that was able to attract the trichogynes. The observation that the *a* mating-type mutants isolated by Griffiths and DeLange (49) failed to display interactions with *A* mating-type testers (10) was the first evidence that the attraction is controlled by the mating types in *N. crassa*.

Chemical factors other than sex pheromones have also been shown to function in the sexual cycle of some filamentous ascomycetes. They promote morphogenetic changes associated with sexual reproduction. A morphogen was identified in ethanol extracts of sexually fertile paired cultures of the pathogen *Pyrenopeziza brassicae*. The extracts were found to trigger the development of sterile apothecia and to simultaneously inhibit asexual sporulation (57). When added to mated cultures, they induced an increase in the number of mature apothecia produced and in their speed of maturation (90). The biologically active sexual factor present in the extracts is a mixture of lipid compounds. Its effect is not specific, since it can influence the sexual development of other fungi, both ascomycetes and basidiomycetes (91). Attempts to characterize chemical factors capable of triggering the development of perithecia without mating in *Nectria haematococca* have failed. Nevertheless, lipid extracts from 10-day-mated cultures, as well as linoleic acid and other related unsaturated fatty acids, were found to enhance the production of fertile perithecia in crosses (30). Differentiation of barren fruiting bodies in homokaryotic cultures of filamentous ascomycetes was observed not only in the presence of morphogens but also in mutants. One such mutant, called *sfp1-1*, was described in *Magnaporthe grisea* (97), and two mutants, *vacua* (35) and *modE* (29), were described in *P. anserina*. The *modE* mutation, selected as a functional suppressor of vegetative incompatibility, was found to alter a gene encoding a heat shock protein (66a). In the presence of some mutations or by adding morphogens, it is thus possible to uncouple mating and fruiting, which thus appear to be two independent processes. Nevertheless, under natural conditions when the sexual process is initiated by fertilization, development of the female organs and differentiation of the inner fertile tissues are concomitant. It can be assumed that synthesis of a morphogen that stimulates fruiting, which is inoperative or repressed during vegetative phase, may be induced after mating has occurred. A lipid factor stimulating fruiting was also characterized in the homothallic fungus *Aspergillus nidulans*. It acts as a precocious sexual inducer and is therefore called *psi* (15). In a culture initiated from asexual spores, mycelial growth occurs first, asexual spores differentiate next, and fruiting bodies appear afterward. When the *psi* factor is added to the culture medium, fruiting bodies appear prematurely and asexual sporulation is inhibited.

In *N. crassa* a morphogen (probably peptidic) competent to induce the formation of perithecia in single-mating-type cultures was also identified in extracts from mated cultures (102). Interestingly, in spite of the absence of fertilization, a few asci which carried genetic markers from the parent were produced. This unexplained observation is now gaining renewed significance, with the functional model recently proposed for the mating-type genes of *P. anserina* (see below).

Control of Sexual Differentiation and Sexual Development

Fertilization requires the fusion of a male element that can donate a nucleus with a cell of the female organ which can accept this nucleus (for example, the trichogenous extension of an ascogonium). In species like *P. anserina* or *N. crassa*, the mating-type alleles are not the factors directly responsible for the morphogenesis of sexual structures, at least until fertilization. The *N. crassa* mating-type mutants isolated still produce viable conidia and ascogonia with trichogynes (10). The ability to differentiate sexual structures in the absence of mating type was confirmed later in *P. anserina* by deleting the mating-type sequences (22). In contrast, in other species, such as *Ascobolus* species, no ascogonia differentiate until competent vegetative cells of the opposite mating type are allowed to interact. Bistis (8) observed the sequence of developmental stages of an ascogonium in *A. stercorarius* after transferring an oidium or a hyphal fragment (male element) to the vicinity of a mycelium of the opposite mating type. The male element undergoes a physiological change termed sexual activation (9). This activation is completed 2 to 4 h after the first contact with a mycelium of the opposite mating type. Subsequently, some of the mycelial hyphae are induced to differentiate ascogonia. Illegitimate fusion between trichogynes and oidia of like mating types was performed (12). An oidium controlling the growth of a trichogyne of the opposite mating type was replaced by an oidium of the same mating type as the female organ. This oidium had been previously sexually activated in a compatible confrontation. Illegitimate fusion occurred in both $a \times a$ and $A \times A$ confrontations. This resulted in the formation of fruiting bodies which began to enlarge but did not produce asci. Cytological examinations indicated that "their development apparently ceases sometime during the development of the ascogenous hyphae." Therefore, as early as 1963, Bistis and

Raper concluded that "some conditions. . .had prevented the fruiting bodies from developing to maturity" and thus gave the first experimental evidence of involvement of the mating types after initiation of mating (12).

Vegetative Functions Linked to Mating Types

Incompatibility. It has long been known that the mating-type locus of *N. crassa* is bifunctional: it controls not only mating (the $A \times a$ cross is fertile) but also vegetative incompatibility ($A + a$ heterokaryons are inhibited in growth). The two phenotypes have never been separated by genetic recombination (74) but have been separated by mutations. First, the mutation *tolerant* or *tol* was found to suppress heterokaryon incompatibility of the mating-type alleles without affecting their ability to cross (73). Second, by using the incompatibility function, mutations affecting either the *a* or the *A* mating-type locus were selected (48, 49). The incompatibility and fertility functions were inactivated simultaneously by the mutation event(s) in all but one of the mutants, a^{m33}. This mutant lost its incompatibility function but retained the ability to produce fertile perithecia in crosses. In fact, the question of whether fertility and incompatibility are determined by two very closely linked genes or a single gene with two functions remained unanswered until the advent of molecular analysis.

Cellular death. A historic observation that the *P. anserina mat+* wild-type strain exhibited a longer life span than the *mat−* strain suggested that the mating types might be involved in senescence (86). It was recently found that the increased life span of *mat+* strains was independent of the mating-type information, since an increased life span was maintained when the *mat+* information was deleted (22). A gene closely linked to the mating-type locus is thus responsible for the longevity phenotype. Another phenomenon of cellular death, premature death syndrome, was also assumed to be under mating-type control (7). This syndrome was specifically observed when the *AS1-4* mutation, which affects a gene encoding a cytosolic ribosomal protein (27), was associated with the *mat−* haplotype. A widespread search for recombination events permitted the dissociation of the gene responsible for premature death, named *rmp*, from the *mat−* mating type (21).

Others. A difference in ascospore size in two organisms, *Sclerotinia trifolorium* (100) and *Chromocrea spinulosa* (68), was found to be associated with a specific mating behavior. Genetic analysis demonstrated that the same genetic locus was responsible for both spore size dimorphism and mating type. Whether there is one single gene with two functions or two linked genes at this locus has not been determined. The genetic instability of this locus, its most interesting property, is discussed in the next section.

Unconventional Mating Types

In the well-studied heterothallic filamentous ascomycetes like *N. crassa*, no novel mating type has ever been found in nature or has arisen in the laboratory: heterothallism is thus considered to be strictly biallelic (74). A recent investigation of the mating system of *Glomerella cingulata* (the sexual form of *Colletotrichum gloeosporioides*) has revealed an exception to this rule. Crosses were carried out between five heterothallic isolates in all possible combinations. Mating tests performed on single ascospore progeny from each successful cross have indicated that mating is controlled by a locus with multiple alleles (19a). To date, this situation is unique in filamentous ascomycetes.

The mating types are also extremely stable. An interconversion between one mating type and its alternative allele has never been reported in any of the best-analyzed species. Unidirectional reversal of mating type was nevertheless observed in several species which are basically heterothallic: *Chromocrea spinulosa* (68) (Pyrenomycetes), *Sclerotinia trifolorium* (100, 101) (Discomycetes), and *Botrytinia fuckeliana*, the sexual form of *Botrytis cinerea* (36) (Discomycetes). Perkins has already pointed out the original feature of these unstable mating types (79). As an illustration, we will present the experimental data that gave evidence for the mating-type change in two organisms.

Sclerotinia trifolorium. As reported above, in *S. trifolorium*, as in *C. spinulosa*, ascospore size dimorphism is associated with the mating type (100). Ordered tetrad analysis demonstrated a 4:4 segregation of large and small ascospores. Strains derived from small spores are self-sterile, whereas strains derived from large spores are self-fertile. Asci issued from self fertilization of large spores again contain four small spores (self-sterile) and four large spores (self-fertile). When mycelia obtained from the small spores are spermatized with microconidia of the large-spore strains, fertile apothecia are produced with the same 4 large:4 small type of asci. However, when small-spore strains are spermatized between themselves, no apothecia are recovered. The segregation of one pair of alleles responsible for both mating type and spore size, designated *L*/*S*, may account for these data (101). Thus, *S. trifolorium* can be considered a bipolar heterothallic fungus. The self-fertility of the strains issued from *L* spores may be due to the occurrence of the *S* mating type in the *L* cultures: there is a change from *L* to *S*. The stability of the *S* allele excludes the possibility of the reciprocal change from *S* to *L*. The change from the *L* to the *S* mating type might occur randomly. Then some sterile *L* mycelium would be expected, either because the mating-type change has occurred in early mycelium development, resulting in the whole mycelium of *S* mating type, or because no change has yet occurred. An interesting possibility is that the change from the *L* to the *S* mating type takes place specifically in the apothecia, ensuring that mycelium from *L* ascospores will be always self-fertile.

Botrytinia fuckeliana. The instability of the mating type has recently been more clearly demonstrated in *B. fuckeliana*, which is responsible for the "grey mould" disease. Mating experiments performed previously with field and monoascospore isolates classified the strains into two compatibility groups (36). Consequently, *B. fuckeliana* appears to be a bipolar heterothallic fungus, and the two mating-type alleles have been named *MAT1-1* and *MAT1-2*. Accordingly, a Mendelian segregation of *MAT1-1*:*MAT1-2* was observed in random ascospore progeny. Nevertheless, strains sexually compatible with both the *MAT1-1* and *MAT1-2* testers, i.e., homothallic and self-fertile, were also recovered and were designated *MAT1-1/2*. This phenotype was not due to inclusion of two nuclei of opposite mating types within an ascospore. Analysis of ordered eight-ascospore tetrads confirmed the normal 4 *MAT1-1*:4 *MAT1-2* segregation in most asci examined and the appearance of the homothallic *MAT1-1/2* phenotype in about 6% of the asci (37). This phenotype always replaced the *MAT1-2* allele. It might be due to the occurrence of the *MAT1-1* mating type in the ascospore which should carry the *MAT1-2* allele. The change is thus apparently unidirectional.

Comments. As already discussed by Perkins, the number of eight-spored species with unstable mating types may have been underestimated (79). The presence of self-fertile cultures derived from single ascospores may be interpreted as true homothallism if tetrad analysis has not been performed. The occurrence of [minus] variants in [plus] single-ascospore cultures of *Glomerella* (isolated from *Ipomoea* [19]) has subse-

quently been explained by a reversal of mating type (79). However, an alternative interpretation can be proposed. Although it was demonstrated that the [minus] and [plus] phenotypes were determined by a pair of alleles called *B*/*b*, these probably do not correspond to different mating types. The *Glomerella* isolate studied was homothallic, as suggested by the ability of the single-ascospore cultures to produce fertile perithecia. The [plus] and [minus] phenotypes were defined on the basis of macroscopic mating characteristics: [plus] cultures produced fertile perithecia in large glomerate masses, whereas [minus] cultures produced scattered, poorly fertile perithecia. Accordingly, the *B*/*b* pair of alleles may not determine a mating specificity but a more general sexual character, "the arrangement of perithecia" (18). The gene concerned may be involved in the initiation and/or the development of perithecia. Nevertheless, the constant occurrence of [minus] variants in the ascospore progenies of some [plus] cultures remains a particularly interesting problem related to sexual reproduction. The genotypic instability was shown to depend on a "mutator gene" segregating as a Mendelian character in its active and inactive forms (104). Molecular investigation would help to determine the nature of this mutator and to elucidate the mechanism of the plus-to-minus change. Molecular analysis also appears necessary to understand the unstable mating systems. They are fundamentally different from the classical heterothallic and homothallic systems and might correspond to a very particular organization of the mating-type genes in these species. Mating-type instability in filamentous ascomycetes is reminiscent of the mating-type switching described in yeast (54). Similarly, it might involve the transposition of information from a silent cassette to an active site. However, the fact that switching is unidirectional and irreversible suggests that only one mating type would be present as a silent copy: the *S* mating type in *S. sclerotinium* and the *MAT1-1* mating type in *B. fuckeliana.*

MOLECULAR APPROACH

The Mating-Type Loci of Filamentous Ascomycetes Consist of Unrelated and Unique Sequences

The dissimilarity of the two mating-type alleles was the first observation identified by their molecular analysis. The *MAT***a** and *MAT*α loci of *Saccharomyces cerevisiae* were found to contain a unique sequence of several hundreds of nucleotide pairs (4). In *Schizosaccharomyces pombe*, the extent of dissimilarity is more than 1 kb (61). It is even more pronounced in filamentous ascomycetes and can be up to several kilobases. Four different strategies were used to isolate the mating-type loci from four different filamentous ascomycetes.

N. crassa. The mating types were first cloned in *N. crassa* (46). The *A* locus was present on a cosmid found to complement the thermosensitive *un-3* mutation, a locus closely linked genetically to the mating-type locus. The presence of a functional *A* allele on this cosmid was assessed by its ability to confer *A* mating behavior by DNA-mediated transformation of an a^{m} sterile mutant. The sequence with *A* specificity was unique to *A* cells. It was flanked by sequences common to *a* and *A* strains, which were used to isolate the homologous region from a genomic library of an *a* strain. Subsequent DNA sequencing determined more precisely the amount of heterology: there is 5.1 kb of *A*-specific sequence (40) and 3.2 kb of *a*-specific sequence (94).

P. anserina. Since *P. anserina* and *N. crassa* belong to the same family, the Sordariaceae, their mating types were expected to display some homology. In fact, probing of the genomic DNA of *P. anserina* with the *A* sequence of *N. crassa* at low stringency gave a signal specific for the *mat*− strain. By using heterologous hybridization, we isolated three overlapping cosmids which were found to confer both dual-mating and self-mating phenotypes when introduced into a *mat*+ recipient. The homologous mat^{+} locus was identified in a *mat*+ cosmid library (82). The amount of dissimilarity between *mat*+ and *mat*− regions can only be estimated, since the *mat*+ locus has not been totally sequenced: *mat*+ corresponds to 3.8 ± 0.2 kb, and *mat*− corresponds to 4.7 ± 0.2 kb (25).

C. heterostrophus. The mating-type locus of *Cochliobolus heterostrophus* was cloned by functional complementation (99). The selection was based on the assumption, subsequently confirmed, that introduction of the *MAT-1* information into a *MAT-2* recipient would lead to self-mating. Of the 250 tested transformants obtained upon transformation with a cosmid library, 1 displayed this phenotype. In *C. heterostrophus*, *MAT-1* and *MAT-2* contain 1,297 and 1,171 bp of dissimilar DNA, respectively.

M. grisea. Since nonhomology was observed between the opposite mating types of ascomycetes, a genomic substraction method was used to isolate the mating-type genes of *Magnaporthe grisea* (60). DNA segments specific to the *MAT1-1* locus were cloned. A subfragment of one clone that hybridized specifically to DNA from all *MAT1-1* strains allowed the isolation of overlapping cosmid clones. One cosmid introduced into a *MAT1-2* recipient yielded transformants able to self-mate. Subsequent cloning of the cosmid carrying *MAT1-2* and cross-hybridization experiments indicated that *MAT1-1* and *MAT1-2* are approximately 2.5 and 3.5 kb, respectively.

Comments. The lack of sequence similarity between the two alternate mating types is a common property in the four filamentous ascomycetes studied so far. The term "idiomorph" was proposed to denote sequences unrelated in structure although present at the same homologous locus (69). The two mating types can still be referred to as alleles in their initial definition as characters mutually exclusive in meiosis. The more general term "haplotype" can also be used.

The lack of additional copies of mating-type sequences outside the mating-type locus is a characteristic that distinguishes these filamentous ascomycetes from the yeasts. Each haploid strain contains unique sequences specific to either mating type. The absence of a silent copy prevents a mechanism of mating-type switching and explains the great stability of the mating types. However, the unstable mating types described above may exhibit a different physical organization.

The Mating-Type Loci of *P. anserina*, *N. crassa*, and *C. heterostrophus* Contain One or Several Genes with Multiple Functions

In 1978, in their paper describing mutations of the *a* mating type (49), Griffiths and DeLange posed a crucial question: "Although successful mating requires heterozygosity for the mating types alleles, the extent of their involvement in the mating process and subsequent events is not known. Do these genes simply generate a signal for the initiation of mating or is there continuing involvement at later stages of mating and even meiosis?" As mentioned in a previous section, the involvement of mating type after fertilization was already suspected by Bistis and Raper in 1963 (12). A definite answer to this question was provided by the functional analysis of the cloned mating-type genes. The organization of the mating-type loci of *N. crassa*, *P. anserina*, and *C. heterostrophus* is presented in Fig. 2.

P. anserina. The two genes responsible for mating specificity, *FPR1* and *FMR1* (fertilization plus and minus regulators) in *P.*

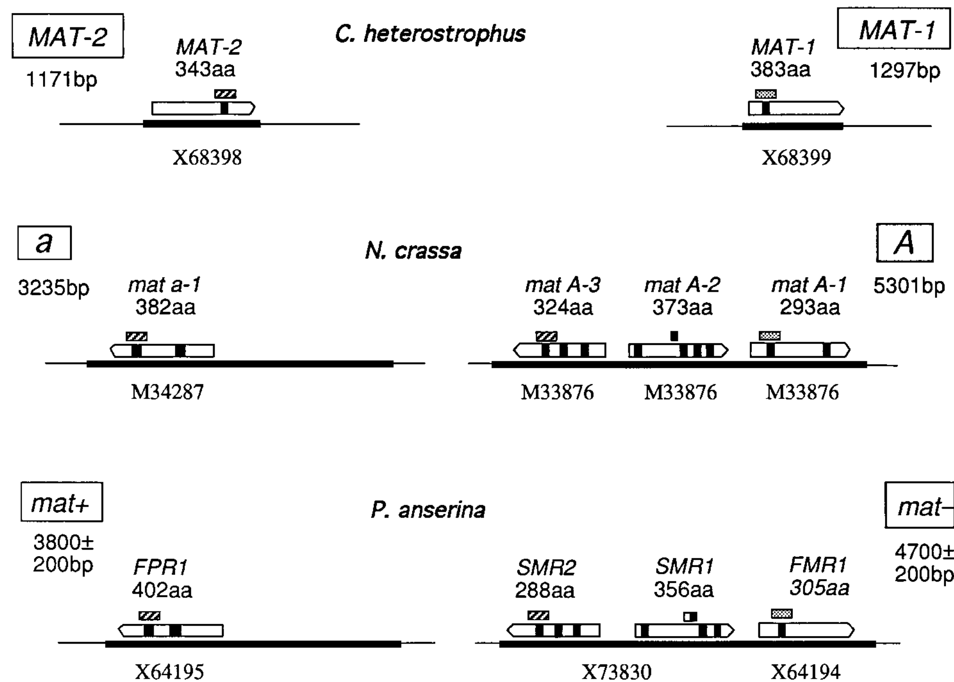

FIG. 2. Comparison of the mating-type loci in *C. heterostrophus* (99), *N. crassa* (38, 40, 94), and *P. anserina* (24, 25). The sizes (in base pairs) correspond to the unique sequences (idiomorphs) indicated by thick lines. The bordering identical sequences are indicated by thin lines. Arrows interrupted by solid boxes represent the coding sequences of the identified genes and the introns, respectively. The database numbers are indicated under each gene. The approximate position of the sequence encoding the putative DNA binding motifs is indicated by a box above each gene. This sequence is generally interrupted by an intron. Symbols: hatched boxes, HMG proteins; stippled boxes, protein with a α1 domain; solid boxes, 19-aa peptide conserved in SMR1 and Mat A-2; open box, putative amphipathic helix found in SMR1. Introns and their spliced junctions predicted on the basis of sequence data were in most cases confirmed by cDNA sequencing and/or transcript analyses. In *C. heterostrophus*, the *MAT-2* gene contains a unique intron (64a) instead of two, as initially suggested (99). Similarly, the *FMR1* gene of *P. anserina* contains only one intron (24) and not two (25). The first intron of FPR1 contains 52 nucleotides (21a) instead of 163 (25).

anserina were identified first (25). Then a combination of molecular and genetic data allowed the characterization of the other genes present in the remaining region and required after fertilization. The construction of mutants with the mating-type locus deleted (Δ*mat*) by gene replacement facilitated functional analyses (22). In this manner, the mating function of a transforming DNA could be determined without interference with the resident mating type. The functions of mating types carrying different mutations in the putative open reading frames (ORFs) were tested, and two additional genes, *SMR1* and *SMR2* (Fig. 2), were thus identified unambiguously in the *mat*− idiomorph (24). While *FMR1* is sufficient to induce fertilization, both *SMR1* and *SMR2*, together with *FMR1*, are necessary to promote the development of perithecia to maturity. In contrast, *FMR1* is the only gene present in the *mat*+ idiomorph: a fragment encompassing *FPR1* but lacking the 1.5-kb adjacent idiomorphic sequences restored the fertility of the Δ*mat* strain. Such an analysis was feasible in *P. anserina* because the mating-type genes are fully functional at ectopic positions (22).

N. crassa. In *N. crassa*, *mat a-1* and *mat A-1* are the two genes responsible for *a* and *A* mating specificity, respectively (40, 94). In contrast to *P. anserina*, ectopic mating types are only partially active in *N. crassa*. Mating-type information, introduced by transformation, retains its complete activity only when it replaces the information present at the resident locus (46). Therefore, gene replacement of the *A* idiomorph by cloned *a* DNA was necessary to demonstrate that the *mat a-1* gene is the sole determinant of the *a* mating type (16). The *a* idiomorph of *N. crassa* contains 1.5 kb of apparently nonessential DNA, as does the *mat*+ idiomorph of *P. anserina*. Identification of other genes present in the *A* idiomorph was difficult because of the inability to complement RIP mutations that confer sterility and are localized outside *mat A-1*, with transforming DNA integrated ectopically (41). Those genes were finally characterized at the transcriptional level. Two cDNAs with opposite orientation derived from the *mat A-1* adjacent region were amplified by reverse transcription-PCR, and the two corresponding transcripts were found by Northern blot analysis (38). The two genes were called *mat A-2* and *mat A-3* (Fig. 2).

C. heterostrophus. The *mat* idiomorphs of *C. heterostrophus* are smaller than those of *N. crassa* and *P. anserina* (1.2 to 1.3 kb) and contain a single gene (Fig. 2). Each of these genes, *MAT-1* and *MAT-2*, confers mating specificity. Their probable role after fertilization was suggested by an indirect argument. The introduction of a mating-type transgene into a strain of alternate mating type yields poorly fertile transformants: when function of the transgenic mating type is required (in a cross or a self-mating), the number of pseudothecia is normal but the number of ascospores produced is small (99). "Inactivation" of the transgenic *mat* information by the resident mating type will be discussed below. Whatever its explanation, the phenotype of the dual maters indicates that the mating-type function is also necessary after mating for completion of the sexual cycle and production of an abundant progeny.

The *mat* Genes May Encode Transcriptional Factors

Computer sequence analysis of the putative proteins encoded by the *mat* genes has revealed conserved regions that correspond, or may correspond, to DNA binding motifs. The MAT proteins are conserved in *N. crassa* and *P. anserina*,

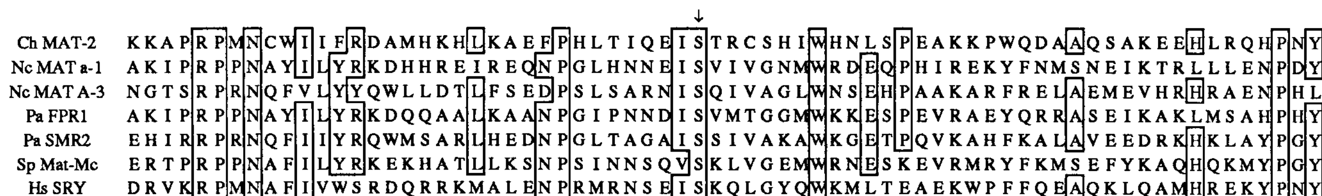

FIG. 3. Comparison of the HMG DNA binding domains of the proteins involved in sexual reproduction. The amino acid sequence alignments are as follows: *C. heterostrophus*, MAT2 (99); *N. crassa*, MAT a-1 (94) and MAT A-3 (38); *P. anserina*, FPR1 (25) and SMR2 (24); *S. pombe*, Mat1-Mc (61); *Homo sapiens*, SRY (93). The arrow indicates conserved position of introns in the HMG motif of the three filamentous ascomycetes. Identical residues are boxed when present in at least five proteins.

which both belong to the family Sordariaceae (pyrenomycetes), but their similarities to proteins of *C. heterostrophus* (loculoascomycetes) are restricted to the DNA binding motifs. The MAT proteins can be divided into three families according to their common motifs (Fig. 2).

HMG domain proteins. A high-mobility-group (HMG) domain (reviewed in reference 50) was found in five MAT proteins (Fig. 3). Strikingly, in all of these, it is interrupted by an intron at precisely the same amino acid position. The HMG domain was initially identified in abundant nonhistone components of chromatin, such as HMG1, in specific regulators of transcription, and more recently in the protein encoded by *SRY*, the gene responsible for male sex determination in mammals (93). Two other HMG domain proteins involved in the mating process of a fungus are known: the proteins encoded by *matMc* (61) and *ste11* (95) in fission yeast. HMG domains are authentic DNA binding domains which can fold independently of the rest of the polypeptide. Although specific DNA target sequences have been determined in some cases, sequence specificity is limited and HMG boxes tend to recognize altered DNA structures. Their binding induces DNA bending (reviewed in reference 64). In *N. crassa*, DNA binding was assayed for the MAT a-1 protein purified after expression in *E. coli* (81). The MAT a-1 protein was able to retard the migration of DNA fragments from both *a* and *A* idiomorphs and particularly from the region 5′ to its own coding sequence. The core of the specific target sequence was 5′-CTTTG-3′. Assays performed with in-frame deletions within the *mat a-1* gene showed that the HMG domain was necessary and sufficient for binding to DNA.

All five MAT proteins with HMG domains also have an acidic proline-rich C-terminal tail similar to regions found in transcriptional transactivator proteins (52). The terminal acidic region of FPR1 was nevertheless dispensable for *mat+* mating ability, since a transgenic Δ*mat* strain containing the truncated *FPR1* gene devoid of that region could still fertilize a *mat−* tester (110). However, a reduction of the number of progeny was obtained, which suggests a possible role of the C-terminal region in postfertilization functions. In contrast, in *N. crassa*, deletion of the last 188 amino acids (aa) of the MAT a-1 polypeptide completely abolishes the mating function (81). The a^{m1} mutation that creates a frameshift at codon 246 has a similar effect (94). The more drastic effect observed for the *N. crassa* MAT a-1 protein may be due to the larger size of the deleted or altered region (188 and 136 aa compared to 120 aa for FPR1).

α1 domain proteins. A subregion of the FMR1 (*P. anserina*), MAT A-1 (*N. crassa*), and MAT-1 (*C. heterostrophus*) polypeptides displays similarity to the protein encoded by the *MATα1* gene in *S. cerevisiae* and has been called the α1 domain (Fig. 4). The MATα1 protein is a transcription activator that cooperates with the MCM1 protein to recognize the promoter of several α-specific genes (47). A short region within the α1 domain of MATα1 has been proposed to be involved in the interaction with MCM1 in *S. cerevisiae* (108). The *S. pombe* Mat-Pc protein has also been reported to contain this short region (Fig. 4), which could be involved in interaction with Map1, a protein related to MCM1 (76). The conservation of the α1 domain in some MAT proteins of filamentous ascomycetes suggests that these may also act as transcriptional activators and may cooperate with another transcriptional factor related to MCM1, which remains to be identified.

The C-terminal part of MAT A-1, FMR1, and MAT-1 is relatively acidic. In *P. anserina*, an FMR1 polypeptide, with the carboxy-terminal 112 aa removed, retains *mat* fertilization function but loses its postfertilization function (25). This acidic C-terminal region appears to be dispensable for mating activity but necessary for full completion of the sexual cycle and production of abundant progeny. Interestingly, the acidic region has been shown to participate in the interaction with the SMR2 protein, as revealed in the yeast two-hybrid system (25a). In *N. crassa*, the region encompassing the first 85 aa is sufficient to induce a very low fertilization ability: the A^{m99} mutant forms very few perithecia when used as a female parent in a cross but is completely sterile when used as a male parent (87). The entire polypeptide is necessary for male mating activity and for optimal activity as a female parent.

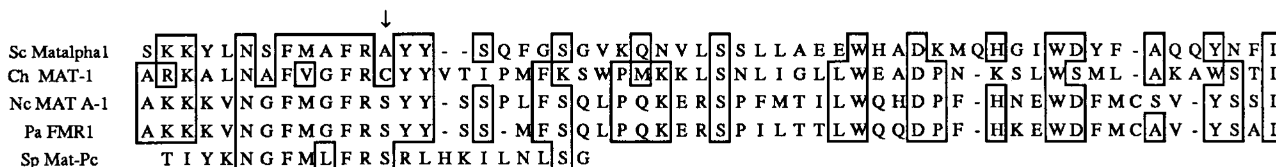

FIG. 4. Comparison of the α1 domain of fungal MAT proteins. The amino acid sequence alignments are as follows: *S. cerevisiae*, Matα1 (4); *C. heterostrophus*, MAT-1 (99); *N. crassa*, MAT A-1 (43); *P. anserina*, FMR1 (25); *S. pombe*, Mat1-Pc (the region proposed to interact with Map1) (76). The arrow indicates conserved position of introns in the α1 domain of the three filamentous ascomycetes. Identical residues are boxed when present in at least three proteins. Gaps (−) were introduced when required for optimal alignments.

Nc MAT A-2	N W H V D H T L H P L R R V P G T P W H K F F G N L E V
Pa SMR1	Y Y H G E K L S H P L R Q L P G N P W H K F F G N F P E

FIG. 5. Highly conserved motif found in MAT A-2 (*N. crassa*) (38) and SMR1 (*P. anserina*) (24). Identical amino acids are boxed.

Proteins with a hypothetical new DNA binding motif. The *N. crassa* MAT A-2 and *P. anserina* SMR1 proteins contain a sequence, with 14 identical and 5 similar amino acids, that has no significant similarity to other characterized DNA binding domains (Fig. 5). The hypothesis that this could correspond to a new DNA binding domain requires molecular confirmation. In SMR1, immediately adjacent to the common region, there is a sequence of 20 residues that could potentially form an acidic amphipathic α-helix. This region corresponds to a gap in the alignment with MAT A-2.

Heterologous expression. Heterologous transformation assays demonstrated that the *mat* genes involved in fertilization and sharing a motif are functional homologs. *FPR1* and *mat a-1*, as well as *FMR1* and *mat A-1*, are interchangeable between *P. anserina* and *N. crassa* for fertilization (3). However, in *P. anserina*, in which ectopic *mat* information is active, only immature perithecia were recovered. Similarly, the *MAT-1* and *MAT-2* genes of *C. heterostrophus* retain their fertilization function in *P. anserina* (1a).

Comments. The overall analysis suggests that the mating-type genes in *N. crassa*, *P. anserina*, and *C. heterostrophus* encode transcriptional regulators, although DNA binding has been demonstrated for only one protein, MAT a-1. Since it is established that the mating types regulate a pheromone response, the *mat* genes controlling mating ability, *mat A-1/FMR1/MAT-1* and *mat a-1/FPR1/MAT-2*, are assumed to induce expression of the genes encoding the sexual pheromones and their receptors. The involvement of the mating types in postfertilization functions indicates that the *mat* genes may control other regulatory networks activated during development of the fruiting bodies. The pheromone/receptor signal may also still be required after fertilization. In *C. heterostrophus*, two genes, one for each mating type, can control the whole sexual cycle, whereas in *N. crassa* and *P. anserina*, two additional genes are necessary. We will see below how this apparent discrepancy can be explained.

The *mat a-1* and *mat A-1* Genes Control Both Mating and Incompatibility Functions in *N. crassa*

Cloning of the mating types provided an answer to an old question: are there two genes, one for incompatibility and one for mating, or a single gene for both functions? The hypothesis that there was a single gene was confirmed by showing that three *a* mutants, previously selected for loss of incompatibility, have a sequence change in the *mat a-1* ORF (94). Similarly, all the A^m mutations conferring vegetative compatibility and sterility were localized within the *mat A-1* ORF (40). This result demonstrated that the *mat a-1* and *mat A-1* gene products were bifunctional.

Functional dissection of the MAT a-1 polypeptide (382 aa) has permitted Philley and Staben (81) to characterize short regions required either for incompatibility or for mating. The HMG domain necessary for mating was shown to be dispensable for vegetative incompatibility. In contrast, a deletion of a short domain (positions 216 to 220) in the C-terminal acidic region abolished incompatibility without affecting mating. The region required for vegetative incompatibility is likely to extend further, because a missense mutation, a^{m33}, creating an arginine-to-serine change at position 258, was demonstrated to be responsible for loss of incompatibility function with maintenance of sexual function (94). It has been proposed that MAT a-1 controls mating via the DNA binding activity of its HMG domain while its C-terminal part controls vegetative incompatibility via an interaction with another protein (81).

Functional dissection of the MAT A-1 polypeptide (293 aa) has not resulted in a clear dissection of two separate domains for incompatibility and for mating. The sterile mutants of *mat A-1*, selected for loss of vegetative incompatibility, correspond to small deletions or insertions creating frameshift mutations upstream of position 200 in the coding sequence (40). This indicates that the C-terminal part of the polypeptide is required for both mating and incompatibility functions. Interestingly, when both *mat A-1* and *mat a-1* are expressed in the same nucleus (in transformants), incompatibility is more efficiently induced than when they are expressed in separate nuclei (in heterokaryons). This result was shown by the finding that a plasmid carrying the A^{m54} allele, deficient in heterokaryon incompatibility, was nevertheless able to trigger vegetative incompatibility when introduced into an *a* recipient (87). Transformation of an *a* recipient with a truncated *mat A-1* gene that encodes only the first 111 aa can also elicit the incompatibility reaction, thus defining this region as the minimal functional region.

In Addition to Their Role in Cell-Cell Recognition at Fertilization, the Mating-Type Genes Control Recognition between Nuclei

Apart from the genes necessary for fertilization in *N. crassa* and *P. anserina* (*mat a-1/FPR1* and *mat A-1/FMR1*), two additional genes are required for subsequent development of the fertilized perithecia (*mat A-2/SMR1* and *mat A-3/SMR2*). The postfertilization step under mating-type control was determined in *P. anserina* by studying the effect of mutations in each of the four *mat* genes. A mutant *mat*+ strain was obtained upon transformation of a strain with the mating type deleted with a *FPR1* gene mutagenized in vitro and expressed at an ectopic site (110). Mutations were introduced at the *mat*− resident locus by gene replacement (2). In the *FPR1* and *FMR1* mutants, the N-terminal part of the polypeptide necessary for fertilization was unchanged. The sexual behavior of each *mat* mutant was examined in crosses with a tester of the opposite mating type. Mutations in *SMR1* were shown to cause sterility, whereas mutations in the two other *mat*− genes, *FMR1* and *SMR2*, and in the single *mat*+ gene, *FPR1*, led to a reduction in the number of progeny (1% or less of a wild-type progeny). The wild-type *mat* allele was absent in most of the recovered asci, most of which contained only the mutant *mat* allele. They thus corresponded to uniparental progeny. Normally, the croziers contain one *mat*+ and one *mat*− nucleus of each parent, as indicated by the *mat*+/*mat*− segregation in asci (Fig. 1). The recovery of abnormal progeny was correlated with aberrant cytological features: perithecia were filled with hundreds of croziers containing a single nucleus, which occasionally were able to undergo a haploid meiosis. The study indicated overall that the rare progeny arose from homokaryotic ascogenous hyphae with one or a pair of *mat* mutant nuclei. It has been concluded that a wild-type nucleus recognizes its opposite-mating-type partner inside the multinucleate cell before the pair migrates into the ascogenous hypha. *FMR1*, *SMR2*, and *FPR1* were assumed to control the recognition between nuclei of opposite mating types. However, several lines of evidence suggest that *SMR1* is not involved in this process. It could be necessary for the development of biparental ascogenous hy-

phae (2, 110). It has been proposed that *FMR1* and *SMR2* control the expression of proteins specific to *mat*− nuclei while *FPR1* governs the expression of *mat*+ nucleus-specific proteins. These proteins are supposed to mediate the recognition between *mat*+ and *mat*− nuclei via interactions with the cytoskeleton (2, 110). It is assumed that the loss of these proteins in nuclei with mutations in the mating-type genes leads to the loss of cytoskeletal attachment and thus to random migration of these nuclei inside the multinucleate cell. Some of these nuclei may enter the ascogenous hyphae, which then start their development, resulting in uniparental progeny with the mutant mating-type allele. If the *mat* genes involved in nuclear recognition are required for a proper biparental cellular state, they are not required for the subsequent developmental program since the ascogenous hyphae are able to undergo meiosis whatever their nuclear content: two nuclei of opposite mating type, two identical nuclei, and even only one nucleus (110).

The model of nuclear recognition implies that *FMR1*, *SMR2*, and *FPR1* are expressed in a nucleus-restricted way. This hypothesis was tested through internuclear complementation tests (2). In these experiments, the *mat*− mutants have been crossed with a *mat*+ strain carrying the wild-type *mat*− genes and complementation of the defective *mat*− nuclei by their *mat*+ partners with a *mat* gene has been assessed by the restoration of a normal biparental progeny. The failure of internuclear complementation for *SMR2* indicated that its expression is nucleus limited, suggesting that it may indeed control the expression of target genes specific to *mat* nuclei. The interaction found between FMR1 and SMR2 in the yeast two-hybrid system and the similarity of the *FMR1* and *SMR2* mutant phenotypes led the authors to propose that *FMR1* is also involved in nuclear recognition. Although *FMR1* gave a partial internuclear complementation, this has been attributed to an artifact induced by the interaction of its product with SMR2. *FMR1* and *SMR2* were termed nuclear identity genes on the basis of their function in the nuclear recognition process (2). Preliminary data indicate that *FPR1* could be also considered a nuclear identity gene which would be the counterpart of *FMR1* and *SMR2* for the *mat*+ nucleus (1a). The internuclear complementation test showed that the expression of *SMR1* was not nucleus limited, indicating that *SMR1* is not a bona fide *mat* gene, since it can fulfill its function whatever its location, in either a *mat*− or a *mat*+ nucleus, and even when present in both nuclei.

The data obtained in *P. anserina* provides a simple explanation for the minimal structure of the *MAT-1* locus in *C. heterostrophus*. The *MAT-1* gene product alone may fulfill the function controlled by the *FMR1*/*SMR2* heterodimer and the gene equivalent to *SMR1* may be localized outside the *mat* locus.

In *N. crassa*, RIP mutations within the resident region encompassing both *mat A-2* and *mat A-3* strongly affect fertility but the few asci that are produced correspond to conventional biparental progeny (41). Consequently, the functional model proposed in *P. anserina* does not seem to be relevant to *N. crassa*. However, recovery of uniparental progeny was already reported in *N. crassa*, after artificial induction of the sexual cycle in a single mating-type culture (102). The inducer was a sex factor present in the mycelial extracts from mated cultures. This factor may induce steps of the sexual development downstream of fertilization and upstream of formation of the crozier. The association of two identical nuclei in the ascogenous hyphae is not completely prohibited but is infrequent, as indicated by the small number of asci that were produced.

Target Genes

The molecular data suggest that the mating-type genes of filamentous ascomycetes encode factors which may control the transcription of target genes acting upstream of a regulatory cascade. The physiological data show that the *mat* genes, which control mating, must induce the expression of genes encoding the pheromones and their receptors. These target genes have not yet been characterized in species with molecularly well-studied mating systems. However, in the heterothallic fungus *Cryphonectria parasitica*, the causal agent of chestnut blight, the putative structural genes for the pheromone precursors specific for each mating type have been isolated. This experiment was done as part of a more general study carried out to understand the mechanism by which a mycovirus perturbs gene expression in *C. parasitica*, and so induces hypovirulence of this pathogen. The viral infection causes a transcriptional down regulation of fungal host genes. Among those genes, *vir2* was found to be necessary for sexual sporulation (109). The genomic DNA of the transcribed *vir2* and adjacent regions contained four small in-frame ORFs. The first encodes a 23-aa polypeptide with a C-terminal CAAX motif, a prenylation signal common to all known fungal sex pheromones. An identical ORF was identified in a gene related to *vir2* called *vir1*. The *vir1* and *vir2* mRNAs are expressed in a mating-type-specific manner since they were detected only in *Mat-2* strains. They are thus assumed to act as mating factors. Their counterpart in the *Mat-1* strains was recently cloned (108a). The gene contains a long ORF that encodes a putative polypeptide displaying structural similarities to the precursor of α-factor of *S. cerevisiae* and of the *p*-pheromone of *S. pombe*. It is present as a unique copy and is specifically expressed in *Mat-1* strains. However, like its counterparts, it is present in the genome of both *Mat-1* and *Mat-2* strains, as expected for genes under control of the mating types. A null mutation of the *vir2* gene in *C. parasitica* is still capable of inducing the formation of perithecia (109). We can postulate that the second gene, *vir1*, may induce the expression of the pheromone response. More surprising is the finding that the perithecia are barren. The fact that the absence of a pheromone alters the sexual development suggests that some steps after fertilization may be dependent on the pheromone response.

What Happens in a Heterothallic Filamentous Fungus when Both Mating-Type Alleles Are Associated in the Same Haploid Nucleus?

The artificial association of both mating types in the same nucleus was first carried out in *N. crassa*, before the cloning of *A* and *a* mating types. This *A*/*a* strain resulted from a duplication of the mating-type chromosome region produced by an insertional translocation (78). The duplication caused a drastic inhibition of growth due to vegetative heterokaryon incompatibility and the formation of barren perithecia in crosses with a standard tester. Incompatibility was probably not responsible for the lack of ascospore production, since this defect was not suppressed by the *tol* incompatibility suppressor. Similarly, the transgenic *A*/*a* strains, obtained more recently through transformation, produce nonfertile perithecia (40, 94). In *N. crassa*, a strain carrying both mating types is thus able to self-mate (it is a dual mater) but unable to produce progeny. The sterility can be explained by the poor expression of the *mat* information localized at ectopic positions, as discussed in the previous paragraphs. Nevertheless, such an explanation seems inappropriate for the duplicated *A*/*a* strain resulting from a translocation that concerns a large chromosomal region surrounding the *mat* locus. Moreover, in *P. anserina* (82) and *C. heterostrophus* (99),

the transgenic dual maters are similarly poorly fertile, although in those species, the mating-type genes are fully active outside their resident locus (22, 107). As in *N. crassa*, self-mating results in barren fruiting bodies which can sometimes produce a very few ascospores. Thus, in the three fungi, the resident mating-type genes interfere with expression of the transgene. In *P. anserina*, different mating-type associations were assayed by constructing a set of transformants carrying both mating types at ectopic positions, with the help of the Δ*mat* mutant. Expression of each mating type was tested separately in crosses with a tester of the complementary mating type (1b). Those experiments showed that there is also an interference between transgenic mating types. What was observed was a competition between the two mating types: the one that is better expressed (the "dominant") gave fertile perithecia, whereas its partner mostly gave immature perithecia and few perithecia producing few ascospores. "Inactivation" is reversible since the "inactivated" mating type recovered its full expression when separated from its competitor in the Δ*mat* context. A scale of efficiency can be established, with the resident mating type being in general the most efficient. Transgenic *mat*+ and *mat*− informations may display comparable efficiency when associated and may both give moderate progeny in crosses with partners of opposite mating types. The progeny is considered moderate if compared with the optimal progeny obtained when each transgenic mating type is placed in a Δ*mat* background. Efficiencies are additive: when two transgenic *mat*+ informations (localized at different positions) displaying the same efficiency as a transgenic *mat*− information are combined, the resulting strain is a more efficient *mat*+ partner in cross (21a). The *mat*+ mating type has become "dominant" over its *mat*− partner. The analysis can be interpreted overall within the framework of the nuclear identity concept. The association of both mating types within the same nucleus may cause an identity conflict and so impair the formation of the dikaryotic ascogenous hyphae leading to asci. Two observations are in agreement with this interpretation. First, uninucleate croziers were observed cytologically in the perithecia resulting from self-fertilization of a dual mater (109a). Second, in crosses involving the "inactivated" mating type of several dual maters, uniparental progeny were recovered at low frequency (21a).

Homothallic and Asexual Fungi Carry Functional Mating-Type Information

The availability of cloned mating-type genes has made it possible to answer the main question concerning the differences between heterothallic species and homothallic and asexual species: the absence of genetically defined mating types in homothallic species and the absence of sexual reproduction in asexual species are not due to the lack of mating-type sequences. In all the species in which they have been sought, sequences similar to mating types have been identified. They were functional each time they were tested in transformation assays.

Asexual fungi. *Bipolaris sacchari*, the only asexual fungus tested for the presence of a mating-type sequence, has a homolog of the *MAT-2* gene of the related species *C. heterostrophus* (88). The coding sequences of both genes as well as their 5′ and 3′ noncoding sequences are 97 to 98% identical. Introduction of the *MAT-2*-like gene of *B. sacchari* into a *C. heterostrophus MAT-1* strain induces self-mating, while introduction of *MAT-1* and *MAT-2* genes of *C. heterostrophus* into *B. sacchari* does not induce sexual reproduction. Thus, the lack of mating in *B. sacchari* is not due to the lack of mating-type genes but is probably due to a defect in at least one of their target genes. Interestingly, some *B. sacchari* transformants carrying multiple copies of *C. heterostrophus MAT-1* or *MAT-2* can form barren pseudothecia in a cross with a *C. heterostrophus* partner. This observation, still unexplained, raises the exciting prospect of genetic manipulation in fungi naturally devoid of sexuality.

Homothallic fungi. Genomic DNAs of numerous homothallic species have been hybridized with diverse *mat* genes from their heterothallic relatives. The most extensive analysis has been performed in the genus *Neurospora* and in related genera. Two situations can be distinguished (Table 1). In four of the five tested homothallic *Neurospora* species, only hybridization to the *A*-specific mating-type probe of *N. crassa* was detected (46). In contrast, *N. terricola* and the other members of the family Sordariaceae (*Gelasinospora*, *Sordaria*, and *Anixiella*) contain sequences similar to both the *A* and *a* mating-type sequences present as a single copy (20, 43). The conservation of the *A* and *a* mating-type loci was studied in more detail in several species by using a series of probes spanning the entire 5,301-bp *A* and 3,235-bp *a* idiomorphs and their flanking sequences. *N. africana* (45), *Anixiella sublineata* (6), and six *Gelasinospora* species (6) were found to contain sequences that are similar in composition and organization to the *A* idiomorph of *N. crassa*. In contrast, in all the species that hybridized with *a*-specific probes, the *a* idiomorph lacks about 700 bp belonging to the region with no defined function. In contrast, the region encompassing the *mat a-1* gene is present. *N. terricola*, the only *Neurospora* species that contains sequences similar to both *A* and *a*, has a shorter *a* idiomorph, as do *Anixiella* and *Gelasinospora* species, and also a shorter *A* idiomorph; the border region corresponding to the *mat A-3* gene is missing (6).

These mating-type sequences not only are remnants of an ancient heterothallic state but probably also play a role in sexual development. The *N. africana* homolog of the *N. crassa mat A-1* gene was cloned and sequenced (45). The two genes are highly conserved (91% identity at the DNA level), and the *N. africana* gene confers both mating specificity and vegetative incompatibility in *N. crassa*. A cosmid containing the putative mating types of *Sordaria macrospora* was isolated by probing a cosmid genomic library with *A* and *a* sequences of *N. crassa*. When introduced into a *mat*+ or a *mat*− strain of *P. anserina*, the cosmid induced the formation of barren perithecia. The observation of self-fertilization, whatever the recipient, indicates the presence of both *FPR1* and *FMR1* functional homologs for fertilization at the *S. macrospora* mating-type locus (82a).

Initiation of the sexual cycle is the step that mainly distinguishes heterothallic and homothallic species. Heterothallic species require a partner for mating, whereas homothallic species are able to self-mate. Apart from this difference, the process of sexual development is identical in both types (83). Homothallic as well as heterothallic filamentous ascomycetes require dikaryotic ascogenous hyphae. However, in heterothallic species, the two nuclei have opposite mating types and there is a recognition mechanism operating before the migration of the nuclear pairs into the ascogenous hyphae. Does a similar mechanism also operate in homothallic species? In these species, all nuclei contain the same genetic information. If they contain a functional set of mating-type genes with their relevant target genes, as in heterothallic species, a dual nuclear identity should be expressed in each nucleus. The phenotype of dual maters constructed through transformation in *C. heterostrophus* and *P. anserina* (see the previous section) indicates that the expression of opposite mating types in the same nucleus is not compatible with a wild-type progress of the sexual cycle. Therefore, a mechanism allowing alternate expression of

TABLE 1. Hybridization of genomic DNA from heterothallic and homothallic members of the Sordariaceae to *A* and *a* mating-type probes of *N. crassa*

Species	Reference(s)	Mating system[a]	Hybridization		Structural information
			a probe (*mat a-1*)	*A* probe (*mat A-1*)	
Neurospora	43, 46				
N. discreta		Het	+ or −[b]	− or +	
N. sitophila		Het	+ or −	− or +	
N. intermedia		Het	+ or −	− or +	
N. africana		Hom	−	+	*mat A-1*-like gene (45)
N. dodgei		Hom	−	+	
N. galapagosensis		Hom	−	+	
N. lineolata		Hom	−	+	
Five new isolates		Hom	−	+	
N. terricola		Hom	+	+	No *mat A-3*-like sequence (6)
Gelasinospora	43				
G. calospora		Hom	+	+	*mat A-1-*, *mat A-2-*, *mat A-3*-like sequence; Δ700 bp in *mat a* (6)
G. reticulospora		Hom	+	+	*mat A-1-*, *mat A-2-*, *mat A-3*-like sequence; Δ700 bp in *mat a* (6)
Strain S23		Hom	+	+	*mat A-1-*, *mat A-2-*, *mat A-3*-like sequence; Δ700 bp in *mat a* (6)
32 new isolates		Hom	+	+	
Three new isolates		Het	+ or −	− or +	
Sordaria	43				
S. fimicola		Hom	+	+	
S. macrospora		Hom	+	+	
S. brevicolis	20	Hom	+	+	
Anixiella sublineata	43	Hom	+	+	

[a] Het, heterothallic; Hom, homothallic.
[b] Each haploid strain contains sequences hybridizing with either *A* or *a* but never with both.

either mating type should exist in homothallic filamentous ascomycetes. Consequently, although individual nuclei contain both mating-type informations, they could be functionally heterothallic as already proposed (43). The switching mechanism of yeast is excluded since the homothallic filamentous ascomycetes so far analyzed carry a single copy of each mating type, which precludes the existence of additional unexpressed cassettes. Switching the expression of mating types could occur through transcriptional regulation or through specific rearrangements at the DNA level. For instance in *Salmonella*, alternate expression of the two flagellin genes operates through inversion of a DNA segment including a promoter (92). This explanation does not seem appropriate for homothallic *Neurospora* species lacking *mat a-1*-related sequences (Table 1), although it cannot be excluded that these species contain a second mating type that has sufficiently diverged from *a* to escape detection by hybridization. As an alternative to the nuclear recognition hypothesis, it can be postulated that nuclei pair randomly. However, genetic analysis of homothallic species like *S. macrospora* does not support this hypothesis. If there was a random coupling of nuclei, outcrossing with a tester carrying a genetic marker should give three types of asci within a single perithecium: the biparental asci exhibiting a 4:4 segregation of the genetic marker and both uniparental types of asci. This situation is never observed in *S. macrospora* (56). Nuclei that pair in ascogenous hyphae seem to recognize each other as being different, whatever the mechanism of this recognition—because they express different mating types, because they are from a female or a male origin, and/or because they are differentially imprinted as suggested by Perkins (78a).

With the advancement of molecular characterization, we hope that at least some questions about homothallism will be soon answered. Recent data already suggest that homothallism could in fact correspond to multiple strategies. Preliminary hybridization experiments carried out with the homothallic species *Podospora setosa* indicate an organization of the mating-type information more complex than in *S. macrospora*. As reported above, in *S. macrospora* the mating-type genes are localized at the same genetic locus. In *P. setosa*, apart from a complex locus carrying genes related to *FPR1*, *FMR1*, *SMR1*, and *SMR2*, three additional unlinked copies hybridizing with *FPR1* were identified (23a).

From Heterothallism to Homothallism and the Origin of Mating Types

Filamentous ascomycetes have adopted different reproductive strategies: heterothallic, homothallic, and, less frequently, pseudohomothallic species can be found within the same genus. This diversity raises the problem of the evolutionary relation among the different mating systems: is homothallism ancestral to heterothallism or vice versa? Several lines of evidence suggest that evolution from heterothallism to homothallism is the most likely scenario.

The mating types of *N. crassa* and *P. anserina*, which have a very similar structural organization (Fig. 2), may have diverged from a common ancestral structure. Consequently, they might have evolved from a heterothallic ancestor. Homothallism would have appeared afterward, and thus independently, in both genera. A model based on population genetics established to explain the evolution of mating systems in the family Sordariaceae is also in agreement with this scenario (70). According to this model, the conditions required for homothallism to invade a heterothallic population are more easily achieved than the reverse situation. The repeated occurrence of homothallism within numerous genera and the predominance of homothallism in filamentous ascomycetes suggest that

this mating system has a great selective advantage (80). In homothallism, sexuality is maintained but heterokaryosis is most often lost. The sexual cycle is achieved without seeking a mate and provides long-lived ascospores, which can counterbalance the absence of vegetative spores (conidia), which are frequently observed in homothallic species. The ability to self-mate may be an important adaptation feature, as emphasized by the fact that self-mating can be achieved by an alternative mechanism, pseudohomothallism.

In heterothallic species, the allelic mating-type sequences have been termed idiomorphs based on their apparent unrelated structure and uncommon descent (69). The presence of dissimilar sequences at the same locus can occur by various chromosomal events from a structure where these two sequences are adjacent on the chromosome. Such an ancestor would be a homothallic species. However, this model is in conflict with population genetics models which suggest that evolution from homothallism to heterothallism is improbable (70). Therefore, another explanation has to be found for the origin of the mating-type sequences and heterothallism. We speculate that heterothallism has evolved from an asexual species to which it brought the advantage of the sexual cycle and that the DNA sequence of mating types arose from a common sequence by mutation. A prediction of this hypothesis is that the mating types may have conserved a trace of this common sequence. *mat A-3* and *mat a-1* in *N. crassa* (or *FPR1* and *SMR2* in *P. anserina*) display this common sequence trace: both encode HMG proteins and have the same orientation and position in the idiomorphs. The two additional genes present in the *N. crassa A* idiomorph (or *mat* in *P. anserina*) may have been recruited by one of the diverging sequences. However, the large idiomorphic noncoding sequence present in *mat a* and *mat+* idiomorphs suggests a more adequate hypothesis: the ancestors of *mat A-2/SMR1* and *mat A-1/FMR1* were present in the initial locus; they have acquired their present functions in one of the mating-type alleles, while mutations have generated the large noncoding sequence in the other allele. The comparison of *MAT-1* and *MAT-2* sequences of *C. heterostrophus* reveals a large number of identical nucleotides, which supports the hypothesis of a common origin (99). A similar search for DNA identities between the large noncoding sequence of *a/mat+* and the opposite mating type may help to assess the validity of this hypothesis.

FUNGI HAVE ADOPTED MULTIPLE MATING STRATEGIES

The molecular investigation of mating systems, initiated in the two yeasts *S. cerevisiae* and *S. pombe*, is now well advanced in several filamentous ascomycetes, a heterobasidiomycete, *Ustilago maydis*, and two homobasidiomycetes, *Coprinus cinereus* and *Schizophyllum commune* (reviewed in reference 62). Heterothallism is bipolar and is generally controlled by a biallelic locus in ascomycetes, whereas it is often tetrapolar and controlled by two structurally complex loci which are multiallelic and can contain subloci in basidiomycetes. Consequently, thousands of mating types can be found in single species of basidiomycetes, whereas there are only two alternative mating types in ascomycetes. In all fungi, the mating types control both the recognition between two compatible partners and the subsequent process giving rise to a sexual progeny. Beyond this unifying scheme, there are fundamental differences in the way in which mating-type functions are achieved. These differences, discussed below, are outlined in Fig. 6. In this oversimplified presentation, only the mating-type genes will be considered. We will not mention the numerous other regulatory proteins encoded outside the mating-type loci which act in combination with the MAT proteins, well characterized in the two yeasts but still unknown in the filamentous ascomycetes.

Control of Mating

In ascomycetes and heterobasidiomycetes, the choice of the mating partner is mediated by a pheromone signal. When haploid cells (or organs) come into contact, they detect each other's presence in response to the mating pheromone produced by cells of the unlike mating type. This double pheromone-receptor interaction initiates a signal transduction pathway that changes the pattern of gene expression. The pheromone response pathway in *S. cerevisiae* (55, 77) and *S. pombe* (75) is well described. The first discrepancy between the different mating systems concerns the way in which the differential production of a pheromone-receptor couple is achieved. In yeasts, the regulatory proteins encoded by the mating-type genes are responsible for the specific production of a pheromone and the alternate receptor in each mating partner (Fig. 6A and B). In contrast, in the heterobasidiomycete *U. maydis*, a pheromone-receptor pair is directly encoded by each alternative allele of the *a* mating-type locus (Fig. 6C) (14). The situation is similar in the homobasidiomycete *Schizophyllum commune*, in which the *B* locus encodes multiple pheromone-receptor sets (103). It is noteworthy that mycelia in homobasidiomycetes can fuse, whatever their mating types: the recognition of self versus nonself is shifted from a cellular to a nuclear level (see below). A second discrepancy between the different mating systems concerns the mode of action of the MAT regulatory proteins. In *S. cerevisiae*, no *mat*-encoded gene is required to confer *a* specificity (54). In fact, the **a**-specific genes, among them the **a**-pheromone and the cell surface receptor that recognizes the α-factor, are expressed constitutively (Fig. 6A). Their expression is nevertheless restricted to the **a** cells because in α cells, the **a** specific genes are repressed by the α2 repressor while the α-specific genes are induced by the α1 activator. Consequently, cells lacking mating-type functions exhibit an **a** mating specificity. In contrast, in *S. pombe* (Fig. 6B), the proteins encoded by *Mat1-Mc* in *M* cells and *Mat1-Pc* in *P* cells positively regulate the expression of the specific target genes allowing mating (reference 76 and references therein). When the mating-type locus is deleted, the components of the pheromone signal are no longer induced and the strain is sterile (61). The situation may be similar in filamentous ascomycetes, as suggested by the sterility of the *mat* deletion mutants (22, 107). Moreover, the same kind of MAT regulatory proteins, i.e., an HMG protein and a protein with similarities to MATα1, are required for mating in filamentous ascomycetes and in *S. pombe* (compared Fig. 6 with Fig. 2).

While it is known that pheromones in yeasts and basidiomycetes are short lipopeptides or peptides, the chemical structure of the pheromones involved in fertilization in filamentous ascomycetes remains to be determined. Nevertheless, experiments performed by Bistis, as reported above, indicate that diffusible pheromones do exist in *N. crassa* and *A. stercorarius* (8, 10). Moreover, a gene for a peptide pheromone has been identified in *Cryphonectria parasitica*, although its function in fertilization has not yet been established (108a).

It is not known whether the expression of the receptor and pheromone genes is controlled in filamentous fungi as it is in yeasts. In yeasts, there is a mutual stimulation of the two mating partners since each expresses both receptor and pheromone. Filamentous ascomycetes are anisogamous, and one of the mating partners acts as a male while the other acts as a

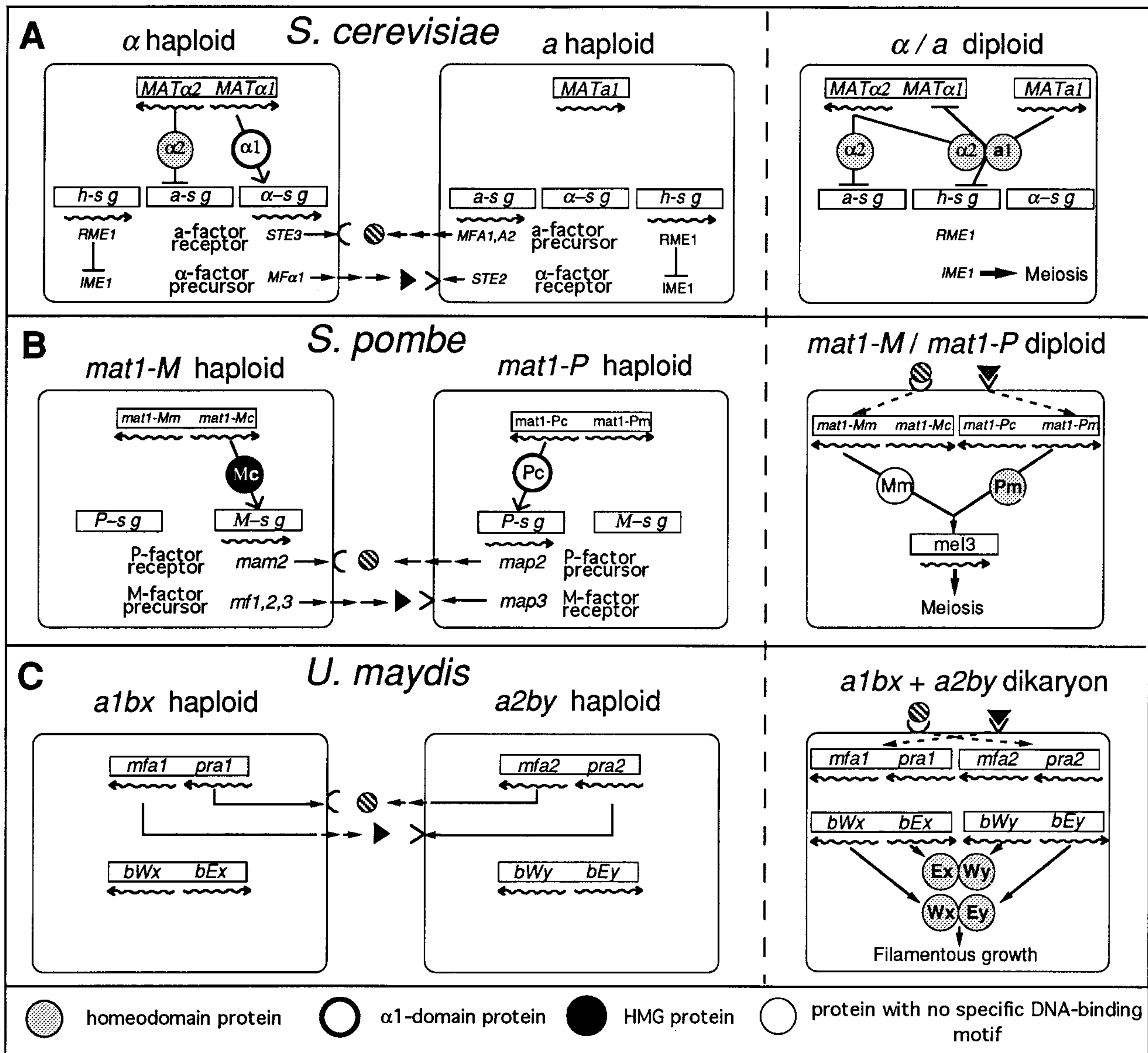

FIG. 6. Model for mating-type functions in *S. cerevisiae* (A), *S. pombe* (B), and *U. maydis* (C). The wavy lines indicate gene expression. Lines with terminal bars indicate inhibition of gene expression, and arrows indicate activation of gene expression. Dotted arrows indicate indirect activation of gene expression via a pheromone signal. Abbreviations: *a-sg*, *α-sg*, and *h-sg*, genes specifically expressed in **a**, α, and haploid cells in *S. cerevisiae*, respectively; *M-sg* and *P-sg*, genes specifically expressed in *M* and *P* cells in *S. pombe*, respectively. The circles represent the encoded proteins.

female. Investigations of filamentous fungi conducted by Bistis indicate that the female organs should express the receptor gene to sense the pheromone expressed by the fertilizing element (8, 10). These experiments do not indicate if the female organs express the pheromone gene and if the fertilizing element has the sensing capacity of the female organ. We speculate that this scenario is probably not the case, at least in homothallic and pseudohomothallic species. In these fungi, concomitant expression of receptor and pheromone genes in the same cell would result in the two reciprocal pheromone-receptor interactions and in the improper activation of the pheromone response pathway. Moreover, female organs of homothallic species are expected to sense the pheromone emitted by the fertilizing element without interference by their own pheromone production. It is therefore likely that the expression of pheromone and receptor genes is under differential control in the various tissues of filamentous ascomycetes. Pheromones (either the pheromone used for fertilization or another one) may also play a role after mating, as proposed by Egel (33). This hypothesis is supported by the finding that deletion of a pheromone-encoding gene in *C. parasitica* prevents the development of perithecia (109). These observations indicate that the expression of the pheromone and receptor genes in filamentous ascomycetes may require a much more complex control than observed in yeasts.

Control Subsequent to Mating

a1-α2 yeast model. After mating, the two haploid nuclei of opposite mating types joined within a single cell have different behaviors depending on the organism. In yeasts, they fuse immediately to form a diploid cell. In basidiomycetes, the

dikaryotic state is maintained in vegetative hyphae. Nuclear fusion occurs only in specific tissues of the fruiting bodies which differentiate in the dikaryotic mycelium. The new pathway required for maintaining the dikaryotic state in basidiomycetes and the diploidy in *S. cerevisiae* is achieved by the association of two dissimilar homeodomain proteins, each encoded by one alternative mating-type allele. The MATα2-MAT**a**1 pair of *S. cerevisiae* is the archetypal active heterodimer. It indirectly activates meiosis by repressing the haploid-specific genes (Fig. 6A). In *U. maydis*, two homeodomain monomers, bE and bW, are encoded by each *b* allele. These proteins can only interact when they arise from different *b* alleles. Two active heterodimers are thus produced for each compatible combination (Fig. 6C). Since 18 different *b* alleles have been identified so far, 300 active heterodimers can be formed, although a single one is sufficient to trigger sexual development (reviewed in references 58 and 59). The functional redundancy is still higher in homobasidiomycetes (63). The *A* mating-type locus of *Coprinus cinereus* and *Schizophyllum commune* encode several pairs of dissimilar homeodomain proteins (designated HD1 and HD2 in *C. cinereus*). Moreover, in both organisms, the *A* locus is multiallelic: in *S. commune* more than 100 different mating types may exist in nature. It is not known whether the heterodimers formed in basidiomycetes act as repressors, as do the MATα2-MAT**a**1 heterodimers of *S. cerevisiae*. In *S. pombe*, although direct interaction between the proteins encoded by the *mat1-Pm* and *mat1-Mm* genes (Fig. 6B) has not so far been reported, expression of both genes is required to activate meiosis (106).

Interestingly, the genes necessary for mating in *S. pombe* (*mat1-Mc* and *mat1-Pc*) and in *U. maydis* (the *a* genes) are also required after cell fusion for entry into meiosis and filamentous growth, respectively. This result was explained by the fact that in both organisms the transcription of the genes necessary after mating is pheromone dependent (53, 106).

The biological situation is very different in heterothallic filamentous ascomycetes. After fertilization, the male and female nuclei divide inside the female organ, leading to multinucleate cells containing a mixture of nuclei of opposite mating types that pair and finally fuse in ascogenous hyphae. How two nuclei of opposite mating types can be sorted from a mixture of nuclei is the crucial question to answer. This step is essential to the efficiency and accuracy of sexual reproduction. It could be assumed that each mating type encodes half of a heteromeric transcription factor similar to **a**1/α2 which would control the expression of genes required for the development of the ascogenous hyphae. Although this model may explain why dicaryotic ascogenous hyphae contain exclusively nuclei of opposite mating types, several lines of evidence argue against the **a**1/α2 model in filamentous ascomycetes. In *P. anserina*, the strongest evidence is the failure to detect any interaction between the proteins encoded by opposite-mating-type genes in the yeast two-hybrid system while interaction between the two *mat*− transcription factors, FMR1 and SMR2, and dimerization of the *mat*+ transcription factor FPR1 were successfully detected (25a).

Nuclear recognition model. Experiments performed with *P. anserina* have led to another model, in which the mating-type proteins control the recognition between nuclei of opposite mating types inside multinucleate cells instead of the development of the ascogenous hyphae (110). The challenge in this model is to understand the molecular basis for the internuclear recognition. The recognition between *mat*+ and *mat*− nuclei might be mediated by the cytoskeleton. It has been proposed that two nuclei of opposite mating types are physically linked, possibly by astral microtubule bridges as observed in later steps of the sexual cycle (98). The linked nuclei would then migrate inside the multinucleate cell toward emerging ascogenous hyphae. The ascogenous hyphae would develop, whatever nuclei they contained. Wild-type ascogenous hyphae contain two nuclei of opposite mating types, but in that case an additional mating-type gene, *SMR1*, would be required for their development. SMR1 would be necessary for the dissociation of the cytoskeleton bridge linking the two nuclei. The ascogenous hyphae could develop even if they contained one nucleus or two identical nuclei (110). Nuclei with a mating-type mutation may lose their link to the cytoskeleton and could enter randomly into the ascogenous hyphae. As expected, these uniparental ascogenous hyphae do not require *SMR1* for their development.

As an initial step in the understanding of the role of the cytoskeleton in sexual development, immunofluorescence microscopy has been used to characterize the cytoskeleton during the sexual cycle of several filamentous ascomycetes. In particular, comparative analyses of croziers were performed by using antibodies specific for the spindle pole bodies (SPB) in *N. crassa*, *N. tetrasperma*, and *P. anserina*, which have bipolar mating systems, and in a homothallic species with no genetically defined mating type, *Sordaria macrospora* (98). In these fungi, the SPB localized on the outer face of the nuclear membrane is the microtubule organizing center. The SPB of the three heterothallic species are reactive with 4′,6-diamidino-2-phenylindole (DAPI), a stain specific for chromatin, while those of *S. macrospora* are not. This result suggests the presence of DNA close to the nuclear envelope in contact with the SPB in the heterothallic species. This putative DNA-SPB association might be involved in the recognition between nuclei of opposite mating types.

Nuclear recognition in homobasidiomycetes? Fusion of two hyphae which have different alleles at the *B* locus results in the bilateral migration of nuclei from one mate to the other, to form a specialized hypha containing one nucleus from each mate. These events require a mechanism for discriminating between the invading nucleus and the resident nuclei, as well as septal modifications to permit passage of the invading nucleus. The molecular analysis of different alleles of the *B* locus of *S. commune* has shown that they contain different pheromone and receptor genes (103). It has been proposed that interaction of a pheromone with a receptor of a compatible strain would produce an advance signal which would induce the dissolution of the septum and allow nucleus migration from cell to cell. However, the mechanism for nuclear discrimination is yet unexplained. This mechanism could be similar to the nuclear recognition observed in the filamentous ascomycetes. It has been demonstrated that a strain transformed with a pheromone gene from a compatible strain becomes capable of donating migrating nuclei to a partner in test matings (103). This result has led Kothe to propose that the induction of the pheromone response pathway prepares the cytoskeleton machinery to accept the migrating nucleus (62). Another intriguing possibility would be that the pheromone molecules are used as nuclear identity markers for discrimination between resident and migrating nuclei.

PROSPECTS

Cloning of New Mating-Type Genes

Mating types of heterothallic ascomycetes have been cloned through diverse strategies: complementation of a mating-type-linked gene (46), heterologous hybridization (82), transformation of a strain of one mating type by a library of the opposite

mating-type strain and screening for self-fertilizing transformants (99), and genomic subtraction (60). Cloned mating types were found to be functionally conserved in different filamentous ascomycetes (3, 107), and heterologous expression should also be considered in a cloning project. More recently, a fast procedure has been developed for cloning *mat* genes in a broad spectrum of species. The sequence conservation of the HMG boxes within the MAT proteins has been used to design primers for PCR amplification (1). Positive results were obtained with DNA from numerous sexual and asexual species of loculoascomycetes and from pyrenomycetes, in which the mating-type genes had not been previously molecularly characterized. Subsequent genetic analyses have confirmed that the HMG-specific products represent authentic mating-type genes. In *Tapesia yallundae*, a heterothallic discomycete (31), a similar approach has been successfully used. The identified PCR product may correspond to the HMG fragment of a mating-type gene since it gives a hybridization signal on DNA from one mating-type isolate and no signal from DNA of an isolate of the opposite mating type (29a). The variety of strategies that have been successful for cloning mating types suggest that the mating types of any fungal species should be within the range of investigators.

Downstream of the Mating-Type Genes

Nucleus-specific labelling. New tools have been developed in cellular biology which should open the way for new observations in ascomycete development. In particular, the advent of green highly fluorescent protein (23) should allow testing of the *P. anserina* model for the mating-type gene function. The fusion of the green highly fluorescent protein gene with one of the mating-type genes, whose expression is restricted to its own nucleus, should result in specific nuclear labelling at the stage of nuclear recognition.

Toward identification of mating-type target genes. It is now established that in several heterothallic and homothallic filamentous ascomycetes, the genes present at the mating-type locus encode transcription factors which are required for fertilization and further steps of the development of the fruiting body.

The first identification of genes regulated by the products of the mating types has been done in *N. crassa* (72). Subtractive hybridization was used to isolate genes that are transcribed preferentially during the formation of the protoperithecia. Many such sexual development (*sdv*) genes were found to respond to null mutations in *mat A-1* by forming either a lower or a higher level of transcripts. However, it is not known whether this control is direct or mediated through other effectors. The role of most *sdv* genes is unknown, except for the *sdv-10* gene (renamed *asd-1*), which is required for the formation of the external tissues of the fruiting body and for the development of the asci. Additional target genes could be characterized by the subtractive hybridization strategy by using wild-type perithecia and perithecia from crosses involving *mat* mutants.

In *P. anserina*, the characterization of the *mat* target genes has been attempted genetically. An initial method was based on the observation that mutations in mating-type genes controlling internuclear recognition led to uniparental asci. Accordingly, mutations in the target genes of the mating-type proteins should also result in uniparental asci. Therefore, a search for uniparental asci in the progeny of a wild-type cross could be used to isolate mutations in a mating-type target gene. Although a few uniparental asci (easily detected by using ascospore color markers) were obtained from a wild-type cross after UV irradiation, no mutation was identified. Genetic analysis of these asci suggested that they result from wild-type nuclei which have escaped the control of the mating-type genes (1a). A uniparental ascus has been found by chance in *N. crassa*, but, as in *P. anserina*, all ascospores from this ascus contained wild-type nuclei (5). These results indicate that the natural occurrence of uniparental progeny, although rare, is much more common than is mutation in a putative target gene. The second method is based on the selection of suppressors of mutations in mating-type genes. This is the usual means of identifying genes encoding proteins that can restore or bypass the function impaired in the original mutant. The suppressor mutations could affect the target genes of the relevant mating-type gene. Suppressors of the *SMR1* mutation which blocks the development of the fruiting body have been obtained in *P. anserina* and are being analyzed (1a). Suppressors of the mating-type RIP mutant of *N. crassa* (41) would be also good candidates to find mating-type target genes in this organism.

A selection system which allows the identification of heterologous DNA binding sites has been developed in *S. cerevisiae* (65). This method takes advantage of the fact that the transcriptional activation domain of GAL4 fused to a specific DNA binding domain can activate the expression only of a reporter gene that contains the cognate DNA binding site in its promoter. By fusing a MAT protein containing a putative DNA binding domain to GAL4, cloning of promoter regions of the relevant target genes should be feasible. A similar rationale has been successfully used to clone target genes of the *Drosophila* Ultrabithorax homeoproteins (67) to identify *Aspergillus brlA* response elements (17). This observation suggests that the improved method proposed by Liu et al. (65) is a valuable tool for cloning unknown target genes of a transcription factor.

Beyond the Mating Types

In the filamentous ascomycetes, the two parental reproductive nuclei have a very particular fate: they divide, pair, and divide again before fusing (Fig. 1). Beyond its apparent specificity, the sexual development of these organisms raises fundamental biological problems with the opportunity of a thorough investigation at both the genetic and molecular levels. Study of the mating types, in particular in *P. anserina*, has brought to light the problem of internuclear recognition. Simultaneously, it has raised another problem: how is the development of the ascogenous hyphae triggered? The signal depends neither on the mating-type genes nor on the recognition between two nuclei (see above). The checkpoint which couples gene expression to the morphogenesis of ascogenous hyphae may be the entry of nuclei, of whatever type, into the ascogenous hyphae. Such a mechanism supposes that the activation of certain developmental genes could depend on the attainment of certain landmark events in morphogenesis. This phenomenon has already been described in prokaryotes (for a review, see reference 66) and proposed in the development of the conidiophore in *Aspergillus nidulans* (39). The development of ascogenous hyphae may be another example of this control.

Control of the recognition between different nuclei in a syncytium is of considerable interest both in terms of its mechanism and with respect to the evolutionary history of the eukaryotes. As emphasized by Denis and Mignot (26), the syncytial state may have been an intermediary form in the evolution toward organized multicellular eukaryotes. The recognition between different haploid nuclei within a syncytium may also be considered the first step of the sexual cycle at a time when fertilization and its corollary cell-to-cell communication had not yet arisen.

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REFERENCES

1. **Arie, T., S. K. Christiansen, O. C. Yoder, and B. G. Turgeon.** 1997. Efficient cloning of ascomycete mating-type genes by PCR amplification of the conserved *MAT* HMG box. Fungal Genet. Biol. **21:**118–130.
1a. **Arnaise, S.** Unpublished data.
1b. **Arnaise, S., and E. Coppin.** Unpublished data.
2. **Arnaise, S., R. Debuchy, and M. Picard.** What is a *bone fide* mating-type gene? Internuclear complementation of *mat* mutants in *Podospora anserina*. Mol. Gen. Genet., in press.
3. **Arnaise, S., D. Zickler, and N. L. Glass.** 1993. Heterologous expression of mating-type genes in filamentous fungi. Proc. Natl. Acad. Sci. USA **90:**6616–6620.
4. **Astell, C. R., L. Ahlstrom-Jonasson, M. Smith, K. Tatchell, K. A. Nasmyth, and B. D. Hall.** 1981. The sequence of the DNAs coding for the mating-type loci of *Saccharomyces cerevisiae*. Cell **27:**15–23.
5. **Barry, E.** 1996. Uniparental progeny in *Neurospora crassa*. Fungal Genet. Newsl. **43:**14.
6. **Beatty, N. P., M. L. Smith, and N. L. Glass.** 1994. Molecular characterization of mating-type loci in selected homothallic species of *Neurospora*, *Gelasinospora* and *Anixiella*. Mycol. Res. **98:**1309–1316.
7. **Belcour, L., O. Begel, and M. Picard.** 1991. A site-specific deletion in mitochondrial DNA in *Podospora anserina* is under the control of nuclear genes. Proc. Natl. Acad. Sci. USA **88:**3579–3583.
8. **Bistis, G. N.** 1956. Sexuality in *Ascobolus stercorarius*. I. Morphology of the ascogonium; plasmogamy; evidence for a sexual hormonal mechanism. Am. J. Bot. **6:**389–394.
9. **Bistis, G. N.** 1957. Sexuality in *Ascobolus stercorarius*. II. Preliminary experiments on various aspects of the sexual process. Am. J. Bot. **44:**436–443.
10. **Bistis, G. N.** 1981. Chemotropic interactions between trichogynes and conidia of opposite mating type in *Neurospora crassa*. Mycologia **73:**959–975.
11. **Bistis, G. N.** 1983. Evidence for diffusible, mating-type-specific trichogyne attractants in *Neurospora crassa*. Exp. Mycol. **7:**292–295.
12. **Bistis, G. N., and J. R. Raper.** 1963. Heterothallism and sexuality in *Ascobolus stercorarius*. Am. J. Bot. **50:**880–891.
13. **Blakeslee, A. F.** 1904. Sexual reproduction in the Mucorineae. Proc. Am. Acad. Arts Sci. **40:**205–319.
14. **Bölker, M., M. Urban, and R. Kahman.** 1992. The *a* mating type locus of *U. maydis* specifies cell signaling components. Cell **68:**441–450.
15. **Champe, S. P., P. Rao, and A. Chang.** 1987. An endogenous inducer of sexual development in *Aspergillus nidulans*. J. Gen. Microbiol. **133:**1383–1387.
16. **Chang, S., and C. Staben.** 1994. Directed replacement of *mtA* by *mta-1* effects a mating-type switch in *Neurospora crassa*. Genetics **138:**75–81.
17. **Chang, Y. C., and W. E. Timberlake.** 1992. Identification of *Aspergillus brlA* response elements (BREs) by genetic selection in yeast. Genetics **133:**29–38.
18. **Chilton, S. J. P., and H. E. Wheeler.** 1948. Genetics of *Glomerella*. VI. Linkage. Am. J. Bot. **36:**270–273.
19. **Chilton, S. J. P., and H. E. Wheeler.** 1949. Genetics of *Glomerella*. VII. Mutation and segregation in plus cultures. Am. J. Bot. **36:**717–721.
19a. **Cisar, C. R., and D. O. TeBeest.** Personal communication.
20. **Cisar, C. R., D. O. TeBeest, and F. W. Spiegel.** 1994. Sequence similarity of mating-type idiomorphs: a method which detects similarity among the Sordariaceae fails to detect similar sequences in other filamentous ascomycetes. Mycologia **86:**540–546.
21. **Contamine, V., G. Lecellier, L. Belcour, and M. Picard.** 1996. Premature death in *Podospora anserina*: sporadic accumulation of the deleted mitochondrial genome, translational parameters and innocuity of the mating types. Genetics **144:**541–555.
21a. **Coppin, E.** Unpublished data.
22. **Coppin, E., S. Arnaise, V. Contamine, and M. Picard.** 1993. Deletion of the mating-type sequences in *Podospora anserina* abolishes mating without affecting vegetative functions and sexual differentiation. Mol. Gen. Genet. **241:**409–414.
23. **Cormack, B. P., R. H. Valdivia, and S. Falkow.** 1996. FACS-optimized mutants of the green fluorescent protein (GFP). Gene **173:**33–38.
23a. **Debuchy, R.** Unpublished data.
24. **Debuchy, R., S. Arnaise, and G. Lecellier.** 1993. The *mat*− allele of *Podospora anserina* contains three regulatory genes required for the development of fertilized female organs. Mol. Gen. Genet. **241:**667–673.
25. **Debuchy, R., and E. Coppin.** 1992. The mating types of *Podospora anserina*: functional analysis and sequence of the fertilization domains. Mol. Gen. Genet. **233:**113–121.
25a. **Debuchy, R., and E. Coppin.** Unpublished data.
26. **Denis, H., and J.-P. Mignot.** 1994. L'origine des métazoaires. Med. Sci. **5:**551–563.
27. **Dequard-Chablat, M., and C. H. Sellem.** 1994. The S12 ribosomal protein of *Podospora anserina* belongs to the S19 bacterial family and controls the mitochondrial genome integrity through cytoplasmic translation. J. Biol. Chem. **269:**14951–14956.
28. **Dodge, B. O.** 1920. The life history of *Ascobolus magnificus*. Mycologia **12:**115–134.
29. **Durrens, P.** 1983. *Podospora* mutant defective in glucose-dependent growth control. J. Bacteriol. **154:**702–707.
29a. **Dyer, P.** Personal communication.
30. **Dyer, P., D. S. Ingram, and K. Johnstone.** 1993. Evidence for the involvement of linoleic acid and other endogenous lipid factors in perithecial development of *Nectria haematococca* mating population VI. Mycol. Res. **97:**485–496.
31. **Dyer, P. S., P. Nicholson, H. N. Rezanoor, J. A. Lucas, and J. F. Pederby.** 1993. Two-allele heterothallism in *Tapesia yallundae* the teleomorph of the cereal eyespot pathogen *Pseudocercosporella herpotrichoides*. Physiol. Mol. Plant Pathol. **43:**403–414.
32. **Edgerton, C. W.** 1914. Plus and minus strain in an ascomycete. Science **35:**151.
33. **Egel, R.** 1992. Sexual identity and smut. Nature **357:**23–24.
34. **Egel, R., O. Nielsen, and D. Weilguny.** 1990. Sexual differentiation in fission yeast. Trends Genet. **6:**369–373.
35. **Esser, K., and D. Graw.** 1980. Homokaryotic fruiting in the bipolar-incompatible ascomycete *Podospora anserina*. Mycologia **72:**534–541.
36. **Faretra, F., E. Antonacci, and S. Pollastro.** 1988. Sexual behaviour and mating system of *Botrytinia fuckeliana*, teleomorph of *Botrytis cinerea*. J. Gen. Microbiol. **134:**2543–2550.
37. **Faretra, F., and S. Pollastro.** 1996. Genetic studies of the phytopathogenic fungus *Botrytinia fuckeliana* (*Botrytis cinerea*) by analysis of ordered tetrads. Mycol. Res. **100:**620–624.
38. **Ferreira, A. V. B., S. Saupe, and N. L. Glass.** 1996. Transcriptional analysis of the *mt A* idiomorph of *Neurospora crassa* identifies two genes in addition to *mt A-1*. Mol. Gen. Genet. **250:**767–774.
39. **Fischer, R., and W. E. Timberlake.** 1995. *Aspergillus nidulans apsA* (anucleate primary sterigmata) encodes a coiled-coil protein required for nuclear positioning and completion of asexual development. J. Cell Biol. **128:**485–498.
40. **Glass, N. L., J. Grotelueschen, and R. L. Metzenberg.** 1990. *Neurospora crassa A* mating-type region. Proc. Natl. Acad. Sci. USA **87:**4912–4916.
41. **Glass, N. L., and L. Lee.** 1992. Isolation of *Neurospora crassa A* mating-type mutants by repeat induced point (RIP) mutation. Genetics **132:**125–133.
42. **Glass, N. L., and I. A. J. Lorimer.** 1991. Ascomycetes mating types, p. 193–216. *In* J. W. Bennett and L. L. Lasure (ed.), More gene manipulations in fungi. Academic Press, Inc., New York, N.Y.
43. **Glass, N. L., R. Metzenberg, and N. B. Raju.** 1990. Homothallic Sordariaceae from nature: the absence of strains containing only the *a* mating type sequence. Exp. Mycol. **14:**274–289.
44. **Glass, N. L., and M. A. Nelson.** 1994. Mating-types in ascomycetes, p. 295–306. *In* J. D. H. Wessels and F. Meinhardt (ed.), The mycota. I. Growth, differentiation and sexuality. Springer-Verlag KG, Berlin, Germany.
45. **Glass, N. L., and M. L. Smith.** 1994. Structure and function of a mating-type gene from the homothallic species *Neurospora africana*. Mol. Gen. Genet. **244:**401–409.
46. **Glass, N. L., S. J. Vollmer, C. Staben, J. Grotelueschen, R. L. Metzenberg, and C. Yanofsky.** 1988. DNAs of the two mating-type alleles of *Neurospora crassa* are highly dissimilar. Science **241:**570–573.
47. **Grayhack, E. J.** 1992. The yeast α1 and MCM1 proteins bind a single strand of their duplex DNA recognition site. Mol. Cell. Biol. **12:**3573–3582.
48. **Griffiths, A. J. F.** 1982. Null mutants of the *A* and *a* mating type alleles of *Neurospora crassa*. Can. J. Genet. Cytol. **24:**167–176.
49. **Griffiths, A. J. F., and A. M. DeLange.** 1978. Mutations of *a* the mating-type gene in *Neurospora crassa*. Genetics **88:**239–254.
50. **Grosschedl, R., K. Giese, and J. Pagel.** 1994. HMG domain proteins: architectural elements in the assembly of nucleoprotein structures. Trends Genet. **10:**94–100.
51. **Gwynne-Vaughan, H. C. I., and H. S. Williamson.** 1932. The cytology and development of *Ascobolus magnificus*. Ann. Bot. **46:**653–670.
52. **Hahn, S.** 1993. Structure and function of acidic transcription activators. Cell **72:**481–483.
53. **Hartmann, H. A., R. Kahmann, and M. Bolker.** 1996. The pheromone response factor coordinates filamentous growth and pathogenicity in *Ustilago maydis*. EMBO J. **15:**1632–1641.
54. **Herskowitz, I.** 1989. A regulatory hierarchy for cell specialization in yeast. Nature **342:**749–757.
55. **Herskowitz, I.** 1995. MAP kinase pathways in yeast: for mating and more. Cell **80:**187–197.

56. **Heslot, H.** 1958. Contribution à l'étude cytogénétique et génétique des Sordariacées. Rev. Cytol. Biol. Veg. **19:**180.
57. **Ilott, T. W., D. S. Ingram, and C. J. Rawlinson.** 1986. Evidence of a chemical factor involved in the control of sexual development in the light leaf spot fungus, *Pyrenopeziza brassicae* (ascomycotina). Trans. Br. Mycol. Soc. **87:**303–308.
58. **Kahmann, R., and M. Bölker.** 1996. Self/nonself recognition in fungi: old mysteries and simple solutions. Cell **85:**145–148.
59. **Kahmann, R., T. Romeis, M. Bölker, and J. Kämper.** 1995. Control of mating and development in *Ustilago maydis*. Curr. Opin. Genet. Dev. **5:** 559–564.
60. **Kang, S. C., F. G. Chumley, and B. Valent.** 1994. Isolation of the mating-type genes of the phytopathogenic fungus *Magnaporthe grisea* using genomic subtraction. Genetics **138:**289–296.
61. **Kelly, M., J. Burke, M. Smith, A. Klar, and D. Beach.** 1988. Four mating-type genes control sexual differentiation in the fission yeast. EMBO J. **7:**1537–1547.
62. **Kothe, E.** 1996. Tetrapolar fungal mating types: sexes by the thousands. FEMS Microbiol. Rev. **18:**65–87.
63. **Kuës, U., and L. A. Casselton.** 1992. The origin of multiple mating types in mushrooms. J. Cell Sci. **104:**227–230.
64. **Landsman, D., and M. Bustin.** 1993. A signature for the HMG-1 box DNA-binding proteins. Bioessays **15:**539–546.
64a. **Leubner-Metzger, G., B. A. Horwitz, O. C. Yoder, and B. G. Turgeon.** Transcripts at the mating type locus of *Cochliobolus heterostrophus*. Mol. Gen. Genet., in press.
65. **Liu, J., T. E. Wilson, J. Milbrandt, and M. Johnston.** 1993. Identifying DNA-binding sites and analyzing DNA-binding domains using a yeast selection system. Methods **5:**125–137.
66. **Losick, R., and L. Shapiro.** 1993. Checkpoints that couple gene expression to morphogenesis. Science **262:**1227–1228.
66a. **Loubradou, G., J. Béqueret, and B. Turcq.** A mutation in a HSP90 gene affects the sexual cycle and suppresses vegetative incompatibility in the fungus *Podospora anserina*. Genetics, in press.
67. **Mastick, G. S., R. McKay, T. Oligino, K. Donovan, and A. J. Lopez.** 1995. Identification of target genes regulated by homeotic proteins in *Drosophila melanogaster* through genetic selection of ultrabithorax protein-binding sites in yeast. Genetics **139:**349–363.
68. **Mathieson, M. J.** 1952. Ascospore dimorphism and mating type in *Chromocrea spinulosa* (Fuckel) Petch n. comb. Ann. Bot. **16:**449–466.
69. **Metzenberg, R. L., and N. L. Glass.** 1990. Mating-type and mating strategies in *Neurospora*. Bioessays **12:**53–59.
70. **Nauta, M. J., and R. F. Hoekstra.** 1992. Evolution of reproductive systems in filamentous ascomycetes. I. Evolution of mating types. Heredity **68:**405–410.
71. **Nelson, M. A.** 1996. Mating systems in ascomycetes: a romp in the sac. Trends Genet. **12:**69–74.
72. **Nelson, M. A., and R. L. Metzenberg.** 1992. Sexual development genes of *Neurospora crassa*. Genetics **132:**149–162.
73. **Newmeyer, D.** 1970. A suppressor of the heterokaryon-incompatibility associated with mating type in *Neurospora crassa*. Can. J. Genet. Cytol. **12:** 914–926.
74. **Newmeyer, D., H. B. Howe, and D. R. Galeazzi.** 1973. A search for complexity at the mating-type locus of *Neurospora crassa*. Can. J. Genet. Cytol. **15:**577–585.
75. **Nielsen, O., and J. Davey.** 1995. Pheromone communication in the fission yeast *Schizosaccharomyces pombe*. Semin. Cell Biol. **6:**95–104.
76. **Nielsen, O., T. Friis, and S. Kjærulff.** 1996. The *Schizosaccharomyces pombe map1* gene encodes an SRF/MCM1-related protein required for *P*-cell specific gene expression. Mol. Gen. Genet. **253:**253–387.
77. **Oehlen, B., and F. R. Cross.** 1994. Signal transduction in the budding yeast *Saccharomyces cerevisiae*. Curr. Opin. Cell Biol. **6:**836–841.
78. **Perkins, D.** 1972. An insertional translocation in *Neurospora* that generates duplication heterozygous for mating type. Genetics **71:**25–51.
78a. **Perkins, D.** Personal communication.
79. **Perkins, D. D.** 1987. Mating-type switching in filamentous Ascomycetes. Genetics **115:**215–216.
80. **Perkins, D. D.** 1991. In praise of diversity, p. 3–26. *In* J. W. Bennett and L. L. Lasure (ed.), More gene manipulations in fungi. Academic Press, Inc., New York, N.Y.
81. **Philley, M. L., and C. Staben.** 1994. Functional analyses of *Neurospora crassa* MT a-1 mating-type polypeptide. Genetics **137:**715–722.
82. **Picard, M., R. Debuchy, and E. Coppin.** 1991. Cloning the mating types of the heterothallic fungus *Podospora anserina*: developmental features of haploid transformants carrying both mating types. Genetics **128:**539–547.
82a. **Pöggeler, S., S. Rish, U. Kück, and H. D. Osiewacz.** 1997. Mating-type genes from the homothallic fungus *Sordaria macrospora* are functionally expressed in a heterothallic ascomycete. Genetics **147:**567–580.
83. **Raju, N. B.** 1992. Genetic control of the sexual cycle in *Neurospora*. Mycol. Res. **96:**241–262.
84. **Raju, N. B., and D. D. Perkins.** 1994. Diverse programs of ascus development in pseudohomothallic species of *Neurospora*, *Gelasinospora*, and *Podospora*. Dev. Genet. **15:**104–118.
85. **Raju, N. B.** 1980. Meiosis and ascospore genesis in *Neurospora*. Eur. J. Cell Biol. **23:**208–223.
86. **Rizet, G.** 1953. Sur la longévité des souches de *Podospora anserina*. C. R. Acad. Sci. **237:**1106–1109.
87. **Saupe, S., L. Stenberg, K. T. Shiu, A. J. F. Griffiths, and N. L. Glass.** 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:**115–122.
88. **Sharon, A., K. Yamaguchi, S. Christiansen, B. A. Horwitz, O. C. Yoder, and B. G. Turgeon.** 1996. An asexual fungus has the potential for sexual development. Mol. Gen. Genet. **251:**60–68.
89. **Shear, C. L., and B. O. Dodge.** 1927. Life histories and heterothallism of the red bread mould fungi of the *Monilia sitipholia* group. J. Agric. Res. **34:** 1019–1042.
90. **Siddiq, A., K. Johnstone, and D. S. Ingram.** 1990. Evidence for the production during mating of factors involved in suppression of asexual sporulation and the induction of ascocarp formation in *Pyrenopeziza brassicae*. Mycol. Res. **96:**757–765.
91. **Siddiq, A. A., D. S. Ingram, K. Johnstone, J. Friend, and A. M. Ashby.** 1989. The control of asexual and sexual development by morphogens in fungal pathogens. Aspects Appl. Biol. **23:**417–426.
92. **Simon, M., J. Zieg, M. Silverman, G. Mandel, and R. Doolittle.** 1980. Phase variation: evolution of a controlling element. Science **209:**1370–1374.
93. **Sinclair, A. H., P. Berta, M. S. Palmer, J. R. Hawkins, B. L. Griffiths, J. S. Matthijs, J. W. Foster, A.-M. Frischauf, R. Lovell-Badge, and P. N. Goodfellow.** 1990. A gene from he human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature **34:**240–244.
94. **Staben, C., and C. Yanofsky.** 1990. *Neurospora crassa mt a* mating-type region. Proc. Natl. Acad. Sci. USA **87:**4917–4921.
95. **Sugimoto, A., Y. Iino, T. Maeda, Y. Watanabe, and M. Yamamoto.** 1991. *Schizosaccharomyces pombe ste11+* encodes a transcription factor with an HMG motif that is a critical regulator of sexual development. Genes Dev. **5:**1990–1999.
96. **Taylor, J. W.** 1995. Molecular phylogenetic classification of fungi. Arch. Med. Res. **26:**307–314.
97. **Tharreau, D., J.-L. Notteghem, and M.-H. Lebrun.** 1997. Mutations affecting perithecium development and sporulation in *Magnaporthe grisea*. Fungal Genet. Biol. **21:**206–213.
98. **Thompson-Coffe, C., and D. Zickler.** 1994. How the cytoskeleton recognizes and sorts nuclei of opposite mating type during the sexual cycle in filamentous ascomycetes. Dev. Biol. **165:**257–271.
99. **Turgeon, B. G., H. Bohlmann, L. M. Ciuffetti, S. K. Christiansen, G. Yang, W. Schäfer, and O. C. Yoder.** 1993. Cloning and analysis of the mating-type genes from *Cochliobolus heterostrophus*. Mol. Gen. Genet. **238:**270–284.
100. **Uhm, J. Y., and H. Fujii.** 1983. Ascospore dimorphism in *Sclerotinia trifolorium* and cultural characters of strains from different-sized spores. Phytopathology **73:**565–569.
101. **Uhm, J. Y., and H. Fujii.** 1983. Heterothallism and mating-type mutation in *Sclerotinia trifolorium*. Phytopathology **73:**569–572.
102. **Vigfusson, N. V., and R. J. Cano.** 1974. Artificial induction of the sexual cycle of *Neurospora crassa*. Nature **249:**383–385.
103. **Wendland, J., L. J. Vaillancourt, J. Hegner, K. B. Lengeler, K. J. Laddison, C. A. Specht, C. A. Raper, and E. Kothe.** 1995. The mating-type locus *B* α *1* of *Schizophyllum commune* contains a pheromone receptor gene and putative pheromone genes. EMBO J. **14:**5271–5278.
104. **Wheeler, H. E.** 1950. Genetics of *Glomerella*. VIII. A genetic basis for the occurrence of minus mutants. Am. J. Bot. **37:**304–312.
105. **Whitehouse, H. L. K.** 1949. Heterothallism and sex in the fungi. Biol. Rev. **24:**411–447.
106. **Willer, M., L. Hoffmann, U. Styrkarsdottir, R. Egel, J. Davey, and O. Nielsen.** 1995. Two-step activation of meiosis by the *mat1* locus in *Schizosaccharomyces pombe*. Mol. Cell. Biol. **15:**4964–4970.
107. **Wirsel, S., B. G. Turgeon, and O. C. Yoder.** 1996. Deletion of the *Cochliobolus heterostrophus* mating-type (*MAT*) locus promotes the function of *MAT* transgenes. Curr. Genet. **29:**241–249.
108. **Yuan, Y.-I. O., I. L. Stroke, and S. Fields.** 1993. Coupling of cell identity to signal response in yeast: interaction between the α1 and STE12 proteins. Genes Dev. **7:**1584–1597.
108a. **Zhang, L., A. Baasiri, and N. K. Van Alfen.** Personal communication.
109. **Zhang, L., A. C. L. Churchill, P. Kazmierczak, D.-H. Kim, and N. K. Van Alfen.** 1993. Hypovirulence-associated traits induced by a mycovirus of *Cryphonectria parasitica* are mimicked by targeted inactivation of a host gene. Mol. Cell. Biol. **13:**7782–7792.
109a. **Zickler, D.** Personal communication.
110. **Zickler, D., S. Arnaise, E. Coppin, R. Debuchy, and M. Picard.** 1995. Altered mating-type identity in the fungus *Podospora anserina* leads to selfish nuclei, uniparental progeny, and haploid meiosis. Genetics **140:**493–503.
111. **Zickler, H.** 1952. Zur entwicklungsgeschichte des askomyzeten *Bombardia lunata*. Arch. Protistenkd. **98:**1–70.