

Mutations in Mating-Type Genes of the Heterothallic Fungus *Podospora anserina* Lead to Self-Fertility

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ABSTRACT

The heterothallic fungus *Podospora anserina* has two mating-type alleles termed *mat+* and *mat-*. The *mat+* sequence contains one gene, *FPR1*, while *mat-* contains three genes: *FMRI*, *SMR1*, and *SMR2*. *FPR1* and *FMRI* are required for fertilization, which is followed by mitotic divisions of the two parental nuclei inside the female organ. This leads to the formation of plurinucleate cells containing a mixture of parental *mat+* and *mat-* nuclei. Further development requires a recognition between *mat+* and *mat-* nuclei before migration of the *mat+/mat-* pairs into specialized hyphae in which karyogamy, meiosis, and ascospore formation take place. *FPR1*, *FMRI*, and *SMR2* control this internuclear recognition step. Initial development of the dikaryotic stage is supposed to require *SMR1*; disruption of *SMR1* results in barren perithecia. In a systematic search for suppressors restoring fertility, we isolated 15 suppressors—all of them mutations in the mating-type genes. These *fmr1*, *smr2*, and *fpr1* mutants, as well as the strains disrupted for *FMRI*, *SMR2*, and *FPR1*, are weakly self-fertile. They are able to act as the male partner on a strain of the same mating type and give a mixture of biparental and uniparental progeny when crossed with a wild-type strain of opposite mating type. These observations lead us to propose that *SMR2*, *FMRI*, and *FPR1* act as activators and repressors of fertilization and internuclear recognition functions.

THE mating-type alleles, which were defined as controlling fertilization, appear to control some additional events after fertilization in the fungus *Podospora anserina* (ZICKLER *et al.* 1995). In heterothallic filamentous ascomycetes such as *P. anserina*, in which each nucleus contains a single copy of one of two mutually exclusive mating-type loci, sexual reproduction begins with recognition between a female organ (the ascogonium) and a male cell (the microconidium) of opposite mating type. This recognition event leads to fertilization, during which the male nucleus is imported into the ascogonium. However, karyogamy between nuclei of opposite mating type does not take place immediately. Instead, the male nucleus undergoes several mitotic divisions resulting in the formation, inside the female organ, of plurinucleate cells containing both male and female nuclei. Therefore, nuclei of male and female origin (of opposite mating type) must recognize each other before they are isolated within the cell that gives rise to the ascus. This internuclear recognition, called IR (DEBUCHY 1999), is thus also associated with a transition from a syncytial stage to a cellular stage, which requires that the two nuclei of opposite mating types migrate from the syncytial cell into a specialized hypha (the ascogenous hypha) where they divide mitotically, maintaining a strict ratio of 1:1 of each parental nucleus.

Eventually pairs of nuclei fuse and meiosis ensues immediately, resulting in the expected Mendelian ratio of each mating-type allele in the progeny.

The mating-type locus consists of two exclusive alleles, *mat+* and *mat-*. The *mat+* sequence contains a sole gene, *FPR1*, and the *mat-* sequence contains three genes: *FMRI*, *SMR1*, and *SMR2* (DEBUCHY and COPPIN 1992; DEBUCHY *et al.* 1993). These genes, except *SMR1*, encode regulatory proteins related to two well-known transcription factor families, the HMG family and the proteins related to MAT α 1 of *Saccharomyces cerevisiae*. *SMR1* does not display any functionally characterized motif; however, it contains a highly conserved region also present in *matA-2* of *Neurospora crassa* (FERREIRA *et al.* 1996) and *SmatA-2* of *Sordaria macrospora* (PÖGGELER *et al.* 1997). This region has been proposed to define a new family of transcription factors (DEBUCHY *et al.* 1993).

Mutations in the C terminus of *FMRI* or *SMR2* lead to the formation of uninucleate ascogenous hyphae and progeny in which the *mat+* parent is absent. This phenotype has been interpreted as resulting from an altered property of mutant *mat-* nuclei, which become able to proceed alone through the developmental steps that are possible only for two compatible nuclei in the wild-type strains. It was suggested that wild-type *FMRI* and *SMR2* ensure that *mat-* nuclei express a property required for their recognition by *mat+* nuclei (ZICKLER *et al.* 1995; ARNAISE *et al.* 1997). This property has been termed nuclear identity. However, the nature of the nuclear identity alteration observed in *fmr1* or *smr2* mu-

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tants, as well as the reasons for uninucleate ascogenous hypha formation, remain unclear. Similarly, *fpr1* mutants that have conserved the fertilization domain lead to selfish *mat+* mutant nuclei equivalent to the selfish *mat-* mutant nuclei (ZICKLER *et al.* 1995). Disruption of *SMR1* does not prevent the mutant strain from crossing with a *mat+* wild-type strain, but perithecia are barren and no dikaryotic specialized hyphae are formed. It has been proposed that *SMR1* is required for the recovery from a developmental arrest resulting from IR (COPPIN and DEBUCHY 2000).

We have undertaken a systematic search for suppressor genes allowing the formation of progeny in a cross between a disrupted *SMR1* strain and a *mat+* strain. Such mutations should permit the by-pass of the requirement for *SMR1*. Surprisingly, the 15 mutants obtained mapped in *FMRI*, *SMR2*, or *FPR1*. All mutants show developmental phenotypes similar to those previously described for the *mat* mutants, but they are self-fertile, a feature that proved to have been overlooked in former *mat* mutants. This suggests that the wild-type *mat-* genes *FMRI* and *SMR2* are required for the repression of *mat+* functions in addition to being necessary for the expression of *mat-* functions, whereas *FPR1* has converse effects on *mat+* and *mat-* functions. On the basis of these dual functions, we propose a model for the selfish behavior of *mat* mutant nuclei.

MATERIALS AND METHODS

***P. anserina* culture and genetic analysis:** The characteristics of *P. anserina* were first described by RIZET and ENGELMANN (1949) and then reviewed by ESSER (1974). Most asci contain four binucleate ascospores. A few asci contain five ascospores: three binucleate and two smaller uninucleate ascospores that produce homokaryons. Each binucleate ascospore is formed around two nonsister nuclei after a postmeiotic mitosis. Binucleate ascospores are generally self-fertile, because the two alleles that control mating types display a 98% segregation at the second division of meiosis. The *136* mutation, which prevents full ascospore and mycelium pigmentation, exhibits a first division segregation; 98% of the asci contain two green and two black ascospores (MARCOU and PICARD 1967). The uniparental progeny are composed of asci and of scattered ascospores containing markers from one parent only. For instance, in a cross between a *mat-* mutant strain and a *mat+* *136* strain, uniparental *mat-* asci contain four black ascospores while biparental asci contain two black and two green ascospores. Uniparental scattered ascospores, which can be binucleate or uninucleate, are black. These scattered ascospores belong to asci that contain at least one viable ascospore while the others have undergone abortive development, most often because they contain aneuploid nuclei (ZICKLER *et al.* 1995). When the frequency of asci containing four black ascospores was <20% of the total progeny, a systematic analysis of the segregation of the *mat* marker was performed for 20–30 asci to determine if the asci containing four black ascospores were due to segregation at the second division of the *136* marker (which is recessive) or to uniparental progeny.

Crosses were performed by spermatization (spraying of microconidia onto strains of opposite mating type). When crosses were performed to separate male and female functions, the

strains to be used as male or female partner were grown separately on petri dishes containing minimal medium, and the female partner was incubated at 27° in the light to allow formation of the female organs. On the sixth day of culture, microconidia of the strain to be used as the male partner were recovered with 2 ml of sterile water and spread on mycelia of the female partner strain.

Nomenclature: To simplify the nomenclature, the disrupted genes *FMRI::ura5*, *SMR1::ura5*, and *SMR2::ura5* were named *fmr1-r*, *smr1-r*, and *smr2-r*, respectively. The *FPR1::ura5* disrupted gene (see below) was named *fpr1-r* for the same reason. All the new mutants obtained in *FMRI*, *SMR1*, *SMR2*, and *FPR1* are lowercase. The wild-type ectopic copy of a gene in a strain is noted in uppercase.

***mat* mutant strains previously obtained:** Gene disruptions were introduced at the *mat-* idiomorph by homologous recombination and the resulting strains called *smr1-r*, *smr2-r*, and *fmr1-r* (ARNAISE *et al.* 1997). The *fpr1-1* mutant strain was obtained by transformation of the Δmat^{SK} strain (see below) with a 1727-bp *NcoI-NcoI* fragment containing a truncated *FPR1* gene coding for a polypeptide lacking the 121 C-terminal amino acids. This *fpr1-1* mutant gene was integrated at an ectopic site (ZICKLER *et al.* 1995). In the *fpr1-1* and *fmr1-r* mutants, the N-terminal part of the polypeptide necessary for fertilization was unchanged. The *mat+* *SMR1* *SMR2* was obtained by transforming a *mat+* strain with a plasmid containing both *SMR1* and *SMR2* sequences and the *ble* gene conferring resistance to phleomycin (ARNAISE *et al.* 1997).

Disruption of the *FPR1* gene: *FPR1* was disrupted by insertion of the *ura5* gene from *P. anserina* (TURCQ and BÉGUERET 1987) downstream of the region necessary for fertilization. The plasmid puraN14EP with the disrupted *FPR1* was used to transform a *mat+* *ura5-6* strain. Previous analyses of the *fpr1-1* ectopic mutant showed that sporulation efficiency was affected when the mutant was crossed with a *mat-* strain (ZICKLER *et al.* 1995). Therefore, the sporulation phenotypes of 390 transformants were tested in crosses with a *mat-* strain. Primary [*ura*⁺] transformants were crossed with a *mat-* *ura5-6* strain. A *ura5-6* × *ura5-6* cross leads to barren perithecia, while a *ura*⁺ × *ura5-6* cross is fully fertile. Consequently, progeny can result only from the transformed nuclei. Ten transformants exhibited a decrease in sporulation efficiency. Since primary transformants contain both transformed and untransformed nuclei, they were purified by crossing with a tester strain of opposite mating type and by selecting homokaryotic transformed strains in the progeny. Crosses of these purified transformants with a *mat-* *ura5-6* strain showed that the [*ura*⁺] phenotype segregated with the *mat+* locus. Four of them were submitted to Southern blot analysis. Three displayed rearrangement in the *FPR1* region and one was shown to have the expected disruption of the *FPR1* gene. This latter transformant was named *fpr1-r*.

Construction of the *mat+*/*smr1-r* strain: The *mat+*/*smr1-r* strain is a heterokaryotic strain issued from binucleate ascospores and therefore contains a mixture of *mat+* and *smr1-r* nuclei. It was obtained from a cross between a *mat+* *SMR1* *SMR2* strain and a *smr1-r* strain. *SMR1* in the *mat+* nucleus can complement the *smr1-r* disruption present in the partner and allows the production of progeny (ARNAISE *et al.* 1997). *mat+*/*smr1-r* strains were issued from binucleate ascospores that did not display resistance to phleomycin and thus did not carry ectopic *SMR1* and *SMR2* genes. The genotype of these binucleate ascospores was confirmed by crossing them with the *mat-* and the *mat+* tester strains.

Selection of *smr1-r* revertants: The heterokaryotic strain *mat+*/*smr1-r* differentiates perithecia that do not sporulate. To obtain sporulation-competent revertants, the *mat+*/*smr1-r* strain was inoculated on 3-cm cellophane discs plated on solid

medium (M2) and grown for 2 days at 27°. The thalli were treated by ultraviolet irradiation at 100, 150, 200, and 300 J/m² (240, 120, 240, and 120 thalli, respectively). Eighty untreated thalli were reserved as a control for spontaneous reversion. The discs were then transferred onto new M2 dishes and put back in the light at 27°. After about 1 week, perithecia were formed and a systematic search for appearance of ascospores or asci was undertaken. Fifteen independent sporulation sectors were obtained. For each of them, all the scattered ascospores or the asci were picked up. A, B, and C strains correspond to different progeny from different *mat+* *SMR1 SMR2* × *smr1-r* crosses. Revertant strains were termed *smr1-r su* or *mat+ su*, according to the nucleus that contains the suppressor.

Construction of the strains used for complementation of suppressors: To obtain the *mat+* *SMR1* strain, transformants resistant to hygromycin were recovered upon transformation of the wild-type *mat+* strain with a pCBSMR1 plasmid containing *SMR1* (see construction below). To determine whether the hygromycin-resistant transformants carried a functional *SMR1*, they were crossed with a *smr1-r* strain. Sterility of this strain is complemented by a functional *SMR1* either in the *mat-* or in the *mat+* partner.

The *mat+* *SMR2* strain was obtained by transforming a *mat+* strain with the plasmid pCBSMR2, containing *SMR2* (COPPIN and DEBUCHY 2000).

The *mat+* *FMRI* strain was selected as a hygromycin-resistant transformant upon transformation of the *mat+* strain with the pCBFMR1 plasmid that contains *FMRI* (see construction below). Introduction of a *FMRI* transgene into a *mat+* strain induces self-fertilization. The postfertilization function was examined by introgressing the ectopic *FMRI* gene in a *fmr1-r* strain.

The *mat-* *FPRI* strain was obtained by crossing a Δmat^{SK} *RIR5* strain, in which the *RIR5* fragment carries *FPRI*, with a *mat-* strain. The Δmat^{SK} *RIR5* itself was obtained through the following steps: the Δmat^{SK} strain is a strain deleted from a large part of the *mat+* specific sequences (COPPIN *et al.* 1993). A *leu1-1* Δmat^{SK} strain was transformed with the pHMTTP plasmid containing the whole *mat-* information and the *su8-1* tRNA gene that encodes a *leu1-1* suppressor (DEBUCHY and COPPIN 1992). The *leu1+* *mat-* resulting strain was then cotransformed with the KSR1R5 plasmid (containing the entire *FPRI* gene) and the pUT703 plasmid containing the *ble* gene that confers phleomycin resistance. This transformant was crossed with a *mat-* strain and a Δmat^{SK} *RIR5* was obtained among their progeny.

Construction of the strains used for selfing test: The $\Delta pah1$ mutation is a deletion in the homeobox gene *pah1* (ARNAISE *et al.* 2001). The nature of the *IncA* mutation is unknown but this mutation confers a female sterile and a "super male" phenotype (MARCOU *et al.* 1993 and reference therein). The $\Delta pah1$ *smr1-r fmr1* ^{$\Delta 95-107$} *SMR1 SMR2* strain was obtained by a cross between *smr1-r fmr1* ^{$\Delta 95-107$} *FMRI* and *mat+* $\Delta pah1$ *SMR1 SMR2*. The *SMR1 SMR2* sequence, integrated at an ectopic position (see above), and the *pah1* gene segregated independently of the *mat* locus. The *smr1-r fmr1* ^{$\Delta 95-107$} *FMRI* strain was obtained by transforming the *smr1-r fmr1* ^{$\Delta 95-107$} (R10) strain with the pCBFMR1 plasmid (which contains *FMRI*). The *IncA* Δmat and the $\Delta pah1$ Δmat strains were obtained by a cross between a Δmat^{SK} *RIR5* (see above) and either the *IncA* or the $\Delta pah1$ strain of opposite mating type.

Counting of ascospores: The entire progeny issued from a cross on a petri dish was recovered on its lid. For an ascospore density <50,000 per lid, the ascospores present on a quarter of the lid surface were counted and this number was multiplied by four to obtain the total progeny of the cross. For lids containing >100,000 ascospores, the progeny were estimated by visual comparison with two reference lids chosen for their

difference in ascospore density: the ascospores present on an eighth of the lid surface were counted and this number was multiplied by eight to obtain the total progeny present on each lid.

Counting of microconidia and perithecia: The relevant strains were grown on petri dishes containing minimal synthetic medium (M2) and incubated at 27° in the dark. The microconidia were recovered after 6 days of culture by washing the surface of the mycelia with 2 ml of sterile water. This allowed us to recover 1 ml of microconidia suspension, which was counted under the microscope in a hemacytometer chamber. To test their fertilization ability, 1 ml of microconidia suspension (after dilutions) was spread on wild-type mycelia used as female partners, which were previously grown on M2 medium at 27° during 6 days in the light to allow formation of female organs. Perithecia were counted 5 days after fertilization.

Light microscopy preparations: These were performed as previously described by ZICKLER *et al.* (1995).

Bacterial strains, plasmids, and plasmid constructions: Cloning and plasmid preparations were done in either *Escherichia coli* HB101 (BOYER and ROULLAND-DUSSOIX 1969) or DH5 α (HANAHAN 1983).

pCBSMR1 is based on pCB1004 (CARROLL *et al.* 1994) and contains *SMR1* on a 2.1-kb *AsaI-EcoRI* fragment derived from pULP68 (COPPIN and DEBUCHY 2000). pCBSMR2 is based on pCB1004 and contains *SMR2* on a 2.5-kb *EcoRI-PstI* fragment derived from pULP (COPPIN and DEBUCHY 2000). pCBFMR1 is based on pCB1004 and contains *FMRI* on a 2.3-kb *EcoRI-XbaI* fragment derived from pULP (DEBUCHY *et al.* 1993).

Plasmid puraN14EP contains the *FPRI* gene with a disruption downstream of the region encoding the HMG domain. It is based on pucEP, which contains the 20-kb *EcoRI-PstI* fragment encompassing the *mat+* idiomorph (DEBUCHY and COPPIN 1992). Plasmid pucEP has been partially digested with *NcoI*, molecules with one cut have been isolated on an agarose gel, treated with the Klenow enzyme, and ligated with a 1.6-kb blunt-end fragment containing the *ura5* gene. This blunt-end fragment was obtained by S1 digestion of the 1.6-kb *EcoRI* fragment of pPAura5-1 (TURCQ and BÉGUERET 1987). Restriction digests of recombinant plasmids allow us to identify insertion of the *ura5* gene in the *NcoI* site of pucEP corresponding to residue 282 of *FPRI*.

The plasmid KSR1R5 is a Bluescript derivative carrying the 4.1-kb *EcoRI-EcoRV* fragment of the *mat+* locus containing the whole *FPRI* gene (DEBUCHY and COPPIN 1992).

DNA procedures and sequencing: Genomic DNA was prepared using the rapid petri dish-grown mycelia method (LECELLIER and SILAR 1994). The *SMR2* gene from the 11 revertants from class 2 was amplified using polymerase chain reaction (PCR) with the pair of primers, 278028 (5'-GATAT TATTCTGCCACTCCC-3') and 1884 (5'-CTGAACCAACGTC TGGTGC-3'). The *FPRI* gene from the three revertants of class 3 was amplified by PCR with the pair of primers, E1 (5'-TCAATCTCAGCATCCGAGAC-3') and F13 (5'-GCCGAA GTGATCAGAATTGA-3'). The *FMRI* gene from the unique revertant of class 1 was amplified by PCR with the pair of primers, 765 (5'-GTTTGCCTTCATTTCATCCC-3') and 2526 (5'-GACCTCCCGCCCTCGGTCGG-3'). The amplification products were then sequenced using the ABI PRISM Ready Reaction DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, CA), with an automatic sequencing machine (373 A DNA sequencer; Applied Biosystems).

RESULTS

Revertants of the *smr1-r* developmental arrest belong to three different classes of mating-type phenotypes:

smr1-r corresponds to a disruption of the coding sequence of the *SMR1* gene leading to barren perithecia in a *smr1-r* and *mat+* strain cross. The heterokaryotic strain *smr1-r/mat+* was used to select revertants able to sporulate, as described in MATERIALS AND METHODS, and 15 sporulating sectors were obtained after mutagenesis. For each sector, all the ascospores that had germinated (1–27) were crossed by *mat-* and a *mat+* tester strains. This allowed us to group the revertants into three classes according to their mating-type phenotype. In the first class, all ascospores of a given sector were unable to mate with strains of either mating type (1 revertant: R10). In the second class all ascospores were *mat-* (11 revertants: R1–R7 and R11–R14) and in the third class all ascospores were *mat+* (3 revertants: R8, R9, and R15). Each sporulating sector was considered to be the progeny of one mutation event and only one ascospore of each sporulating sector was analyzed further.

The suppressor of the first class resulted from a mutation in the *FMRI* gene: To characterize the molecular event leading to the inability to mate with the *mat-* or *mat+* tester strains, the structure of the mating-type locus of the R10 strain was determined by PCR analysis. The two pairs of primers specific for the *FPR1* gene gave no amplification product, but an amplification product smaller than expected was obtained with the two pairs of primers specific for the *FMRI* gene. Sequencing of this amplification product revealed a deletion of 31 bp, 282 bp downstream of the ATG in the *FMRI* gene. This mutation corresponded to an in-frame deletion of 13 amino acids (95–107) just after the $\alpha 1$ domain (Figure 1); it was named *fmr1* Δ^{95-107} and its corresponding strain, *smr1-r fmr1* Δ^{95-107} . A wild-type copy of the *FMRI* gene, introduced by transformation, can complement the fertilization defect of the *smr1-r fmr1* Δ^{95-107} strain. This confirms that the mutant phenotype of the R10 strain was caused by the mutation in the *FMRI* gene and indicates that the *fmr1* Δ^{95-107} mutation is recessive.

The suppressors of the second class resulted from mutations in the *SMR2* gene: The revertants with the *mat-* mating type were crossed with the 136 *mat+* tester strain. The 136 mutation is a spore color marker allowing easy detection of uniparental progeny (see MATERIALS AND METHODS). The sporulation efficiency was decreased compared to a wild-type cross and the progeny was exclusively uniparental *mat-*. To ascertain that the reversion events were not a loss of the insertion of the *ura5* gene that disrupts the *SMR1* gene, PCR experiments were performed with two primers in the *SMR1* gene flanking the *ura5* gene. In all the revertants we amplified a fragment of the expected size for an intact insertion of the *ura5* gene. Thus, all the revertants had the *smr1-r* mutation and an extragenic suppressor mutation (*su*) in their *mat-* nucleus. *SMR1* can fulfill its function whatever its location, in either a *mat-* or a *mat+* nucleus (ARNAISE *et al.* 1997). To determine the phenotype of the suppressors, we complemented the

SMR1 disruption by crossing each revertant (*smr1-r su*) by a *mat+* 136 strain with an ectopic *SMR1* gene. All the suppressors led to *mat-* uniparental and biparental progeny (Table 1). For the suppressors R2, R11, and R13 the biparental progeny are quantitatively comparable to those of a wild-type cross. However, these suppressors also gave 0.3–6% uniparental progeny, a feature never observed in a wild-type cross (10^6 asci were analyzed; S. ARNAISE, unpublished data). For the other suppressors the progeny were decreased (one-tenth of the wild-type progeny) and the percentage of uniparental progeny was 23–99%. To localize the suppressors, the biparental progeny of the *smr1-r su* \times *mat+* *SMR1* cross were further analyzed. Depending on the revertant, 8–86 *mat-* ascospores, which had not received the wild-type *SMR1* gene from the *mat+* *SMR1* parent, were crossed with a *mat+* tester strain. These crosses produced only uniparental *mat-* progeny, as observed in the initial test cross of the revertant. Thus, for all the revertants, the suppressor phenotype cosegregated with *mat-*.

Since these suppressors displayed phenotypes similar to those of *smr2* or *fmr1* mutants, and were close to the *mat-* locus, we then tested if they could be alleles of *SMR2* or *FMRI*. We therefore constructed *smr1-r su SMR2* and *smr1-r su FMRI* strains, introducing by crosses a *SMR2* or a *FMRI* ectopic gene in each revertant strain. Because *smr1-r su* strains give only uniparental progeny in crosses with a *mat+* strain, *smr1-r* must first be complemented by *SMR1* to allow segregation of *FMRI* or *SMR2* from the *mat+* strain with the suppressor of the revertant strains. *smr1-r su SMR1* strains were obtained from a cross between *smr1-r su* and *mat+* *SMR1*. *SMR2* or *FMRI* transgenes were then associated with the mutant *mat-* idiomorph by crossing the *smr1-r su SMR1* strains with a *mat+* *SMR2* or a *mat+* *FMRI* strain.

All the *smr1-r su SMR2* strains displayed the phenotype of a *smr1-r* strain (sterile), while the *smr1-r su FMRI* strains displayed the phenotype of a *smr1-r su* strain (uniparental progeny). Thus, in all the revertants the wild-type *SMR2* gene complemented the suppressor, in contrast to *FMRI*. Sequencing of the *SMR2* gene in each revertant revealed either missense (R1, R2, R3, R3, R4, R5, R6, R11, R13, and R14) or nonsense (R12) mutations in *SMR2* (Table 1 and Figure 1), except for the R7 mutant, which resulted from a mutation in the first base of the second intron (*smr2*³⁹⁸). The R3 and R6, R4 and R14, and R5 and R11 carry the same mutations. These mutations could not preexist in the strain before mutagenesis because, for each pair of revertants, the mutagenized strain was different (Table 1). Among the eight different *SMR2* mutations five were localized in the HMG domain of the protein and two upstream of this domain. Each revertant was further named using the number of the mutated residue (for example, R1 was named *smr1-r smr2*^{126T}).

The suppressors of the third class resulted from mutations in the *FPR1* gene: Since the revertants of the third

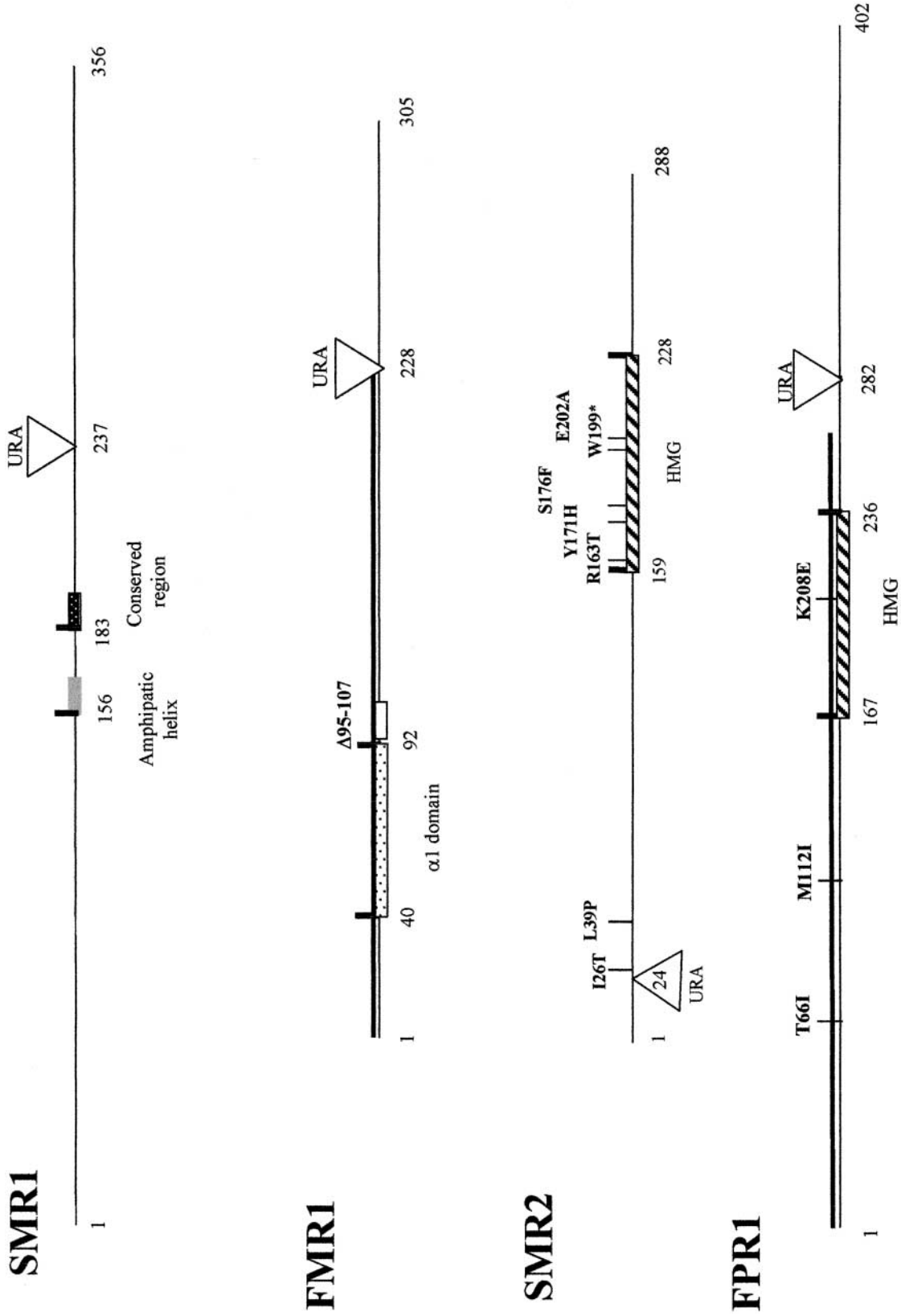


FIGURE 1.—SMR1, FMR1, SMR2, and FPR1 protein structures and positions of mutations for each revertant. Triangle, position of the *ura5* gene insertion. Shaded box, amphipathic helix; black dotted box, conserved region between SMR1 and SMATA-1 of *S. macrospora* and MATA-1 of *N. crassa*; diagonally hatched box, HMG boxes; dotted box, $\alpha 1$ motif; thin vertical bars, position of the mutated residues in the revertants; thick vertical bars, amino acid numbers; open box, deletion *fmr1* ^{$\Delta 95-107$} in the R10 revertant; asterisk (*), stop; thick horizontal bars, N-terminal portions of FPR1 and FMR1 required for fertilization. Amino acids are numbered from the putative ATG.

TABLE 1
Suppressors of the *smr1-r* mutation resulting from mutations in *SMR2*

Revertant	Strain used for mutagenesis	Progeny of R × <i>mat+</i> <i>SMR1</i>		Mutation at the nucleotide level ^b	Mutation at the amino acid level ^c
		Total ascospores ^a	% uniparental		
R1	A	28,000	41	T → C bp 77	I → T residue 26
R3	A	19,600	83	T → C bp 116	L → P residue 39
R6	B	23,300	76	T → C bp 116	L → P residue 39
R7	B	13,400	77	G → A bp 398	1st base of 2 d intron
R2	A	240,000	0.6	C → T bp 601	R → T residue 163 HMG
R4	B	8,808	23	T → C bp 625	Y → H residue 171 HMG
R14	C	34,200	47	T → C bp 625	Y → H residue 171 HMG
R13	A	240,000	0.3	C → T bp 641	S → F residue 176 HMG
R12	A	21,800	99	G → A bp 764	Stop in HMG residue 199
R5	B	18,600	77	G → A bp 771	E → A residue 202 HMG
R11	A	120,000	6	G → A bp 771	E → A residue 202 HMG
Wild type		240,000	0		

^a The progeny correspond to the entire progeny of one cross performed by spermatization as described in MATERIALS AND METHODS.

^b Numeration starts at the A of the translation initiation codon.

^c Numeration starts at the first methionine.

class (R8, R9, and R15) were *mat+*, they were crossed with a *smr1-r* strain to determine if they contained a suppressor mutation. These crosses gave an exclusively uniparental *mat+* progeny, confirming the presence of a suppressor mutation in the *mat+* nucleus. All these suppressors led to both *mat+* uniparental progeny and biparental progeny when crossed to a *mat-* 136 strain. Except for R8, the uniparental progeny represented <1% of the total progeny (Table 2). To localize the suppressor mutations each *mat+* *su* revertant was crossed with a *smr1-r SMR1* strain and we analyzed 15 *mat-* ascospores that had not received the wild-type *SMR1* gene from the *smr1-r SMR1* parent. In all cases, all *mat-* progeny displayed the *smr1-r* phenotype (sterile) upon a cross with a *mat+* tester strain. Thus the suppressor mutations are genetically linked to the *mat+* locus. To determine if they were alleles of the *FPR1* gene, we

made the *mat+* *su FPR1* strains by crossing the *mat+* *su* strains with the *mat-* *FPR1* strain. The *mat+* *su FPR1* strains were unable to produce progeny when crossed with a *smr1-r* strain. Thus, for the three revertants, a wild-type *FPR1* gene complemented the suppressors. Sequencing of the *FPR1* gene in each revertant revealed missense mutations in *FPR1*, one in the HMG domain of the protein and two upstream of this domain (Table 2 and Figure 1). The three mutations are thus localized in the N-terminal portion, previously described as necessary for fertilization. However, the fertilization event did not seem affected in the three mutants as shown by their male fertility test (data not shown).

The *fmr1*, *smr2*, and *fpr1* mutant strains are self-fertile:

All *fmr1*, *smr2*, and *fpr1* mutant strains obtained from the search for *smr1-r* revertants are self-fertile (Table 3). This self-fertility was detected primarily when the

TABLE 2
***fpr1* mutant strains**

Strain	Strain used for mutagenesis	Progeny of R × <i>mat-</i>		Mutation at the nucleotide level ^a	Mutation at the amino acid level ^a
		Total ascospores ^a	% uniparental		
R15	C	120,000	0.5	C → T 195	T → I residue 66
R8	A	11,300	26	G → A 334	M → I residue 112
R9	A	120,000	0.4	A → G 708	K → E residue 208 HMG
<i>fpr1-r</i>		120,000	0.4	Insertion of the <i>URA</i> gene at 792	
Wild type		120,000	0		

The number of ascospores in the wild-type cross is half of the number of ascospores in the wild-type cross in Table 1. This is explained by variation from one experiment to another. All crosses of each table were done under the same conditions.

^a See Table 1.

TABLE 3
Self-fertility of the *fmr1*, *smr2*, *fpr1*, and *smr1* mutant strains

Strain	Perithecia ^a	Ascospores ^a	Strain	Perithecia ^b	Ascospores ^b
<i>fmr1-r</i>	36	10	<i>smr1-r fmr1Δ⁹⁵⁻¹⁰⁷</i>	1	0
<i>fmr1-r Δpah1</i>	580	100	<i>smr1-r fmr1Δ⁹⁵⁻¹⁰⁷ Δpah1</i>	6	15
<i>fpr1-r</i>	38	0	<i>smr1-r fmr1Δ⁹⁵⁻¹⁰⁷ Δpah1 SMR1^d</i>	0	
<i>fpr1^{M112I}</i>	88	50	<i>smr1-r</i>	0	
<i>fpr1^{K208E}</i>	120	>100	<i>smr1-r Δpah1</i>	0	
<i>fpr1^{T66I}</i>	124	>100	Δ mat	4	0
<i>fpr1^{T66I} Δpah1</i>	524	>100	Δ mat Δ pah1	0	
<i>smr2-r</i>	14	7	Wild-type <i>mat+</i>	14	0
<i>smr2-r Δpah1</i>	50	50	<i>Δpah1</i>	0	
<i>smr1-r smr2^{L39P} c</i>	15	24			

^a No. of perithecia or ascospores per petri dish.

^b No. of perithecia or ascospores observed from 13 petri dishes.

^c All other *smr2* mutant strains, which have been obtained as suppressors of *smr1-r*, have similar phenotypes.

^d The strain carries also a second copy of the *SMR2* gene linked to the *SMR1* ectopic gene.

mating-type mutation was associated with the Δ *pah1* mutation for the *fpr1^{T66I}* strains (R15). The Δ *pah1* mutation is a loss of function of the homeobox gene *pah1*, which is a repressor of microconidiogenesis (ARNAISE *et al.* 2001). The Δ *pah1* mutant strain produces 40 times more microconidia than a wild-type strain, suggesting that the self-fertility is increased when the number of microconidia is enhanced. This prompted us to reexamine the previously described *fmr1-r* and *smr2-r* phenotypes (ARNAISE *et al.* 1997) and to test the *fpr1-r* disruption. In fact, these mutants are also self-fertile. Their selfing is weak, as only 1–300 perithecia are formed per petri dish, compared to the 5000 perithecia seen in a wild-type cross made in the same condition. The number of ascospores produced is also very low; a maximum of 100 ascospores are produced, while a wild-type cross produces at least 100,000 ascospores per petri dish. In the case of *smr1-r fmr1 Δ ⁹⁵⁻¹⁰⁷* (R10), self-fertility can be detected only when the mating-type mutation is associated with the Δ *pah1* mutation. In fact, self-fertility is enhanced when the mating-type mutation is associated with the Δ *pah1* mutation in *fpr1^{T66I}* (R15), *fmr1-r*, and *smr2-r*. All ascospores produced by selfing have the phenotype of the strain from which they issued, indicating that no cross contamination has occurred. The germination frequency of these ascospores is low (35–85%) as compared to the near 100% of the wild-type ascospores. The *fmr1 Δ ⁹⁵⁻¹⁰⁷* or *smr2* mutations are associated with the *smr1-r* mutation; therefore, we cannot separate them by crossing. To differentiate the phenotype of the revertants from the phenotype of the suppressors, we introduced a wild-type copy of the *SMR1* gene in the revertants by crossing. No differences in the self-fertility phenotype were observed between the revertant strains (*smr1-r smr2* or *smr1-r fmr1 Δ ⁹⁵⁻¹⁰⁷*) and the strains complemented for *SMR1* (suppressor alone strains; data not shown).

All the *fmr1*, *smr2*, and *fpr1* mutant strains can act as male partners on a strain of the same mating type: We have tested whether the self-fertile mutant strains can fertilize a wild-type strain of the same mating type. The results of these crosses are shown in Tables 4 and 5. No fertilization can be detected when the single mating-type mutants are used. When the mating-type mutations are associated either with the Δ *pah1* mutation or with the *IncA* mutation, fertilization events can be detected. The *IncA* mutant produces 1000 times more microconidia than wild type. The role of the *IncA* gene is unknown. It is likely that, in both *IncA* and Δ *pah1 mat* mutant strains, the increase in the number of fertilization events is a consequence of the increase of the number of micro-

TABLE 4
fmr1 and *smr2* mutant strains crossed with a *mat*– wild-type strain

Male partner	Perithecia	Female partner <i>mat</i> –	
		Ascospores observed	Ascospores analyzed
<i>fmr1-r</i>	3 ^a	0 ^a	
<i>fmr1-r Δpah1</i>	50 ^a	14 ^a	5
<i>fmr1-r IncA</i>	1809 ^a	104 ^a	15
<i>smr2-r</i>	0 ^a		
<i>smr2-r Δpah1</i>	3 ^a	0 ^a	
<i>smr2-r IncA</i>	216 ^a	10 ^a	9
<i>mat</i> – Δ <i>pah1</i>	0 ^a		
<i>mat</i> – <i>IncA</i>	1 ^b	0 ^b	

^a No. of perithecia or ascospores from one cross performed by spermatization as described in MATERIALS AND METHODS.

^b This number has been obtained from three crosses performed by spermatization as described in MATERIALS AND METHODS.

TABLE 5

fpr1 mutant strains crossed with a *mat+* wild-type strain

Male partner	Female partner <i>mat+</i>	
	Perithecia ^a	Ascospores ^a
<i>fpr1</i> ^{T66l}	10	0
<i>fpr1</i> ^{T66l} Δ <i>pah1</i>	50	0
<i>fpr1</i> ^{T66l} <i>IncA</i>	600	0
<i>mat+</i> <i>IncA</i>	0	0

^a See Table 4.

conidia. This hypothesis is supported by the correlation between the number of microconidia that are produced by Δ *pah1* and *IncA* mutant strains and the increase observed in the number of perithecia resulting from the association of either mutation with mating-type mutations. This assumption was verified by diluting microconidia of a Δ *pah1 fmr1-r* strain before crossing with a *mat-* strain. Fertility decreases with the number of microconidia (data not shown). In all the crosses of *smr2* or *fmr1* with *mat-* or *fpr1* with *mat+*, all the progeny have the phenotype of the mating-type mutant parent. This indicates that the mating-type mutant nucleus is able to proceed through the developmental steps leading to ascospore formation.

A wild-type copy of the *SMR2* gene, introduced by crossing in the *smr2-r* mutant, complemented the two phenotypes: selfing and crossing with a strain of the same mating type. This *smr2-r SMR2* strain is incapable of selfing or of crossing with a *mat-* strain (data not shown).

Self-fertile perithecia contain mainly uninucleated croziers and exhibit haploid meioses: Although rare, the perithecia formed by selfing of *smr2*, *fmr1*, and *fpr1* are similar to those of wild type in morphology, but they remain mainly barren. Wild-type perithecia develop after fertilization of a female reproductive organ by a male nucleus carrying a nucleus whose mating type differs from that of the female. The female organ is plurinucleate and both male and female nuclei undergo several mitotic divisions in a common cytoplasm before a pair of nuclei of opposite mating type are isolated in a crozier cell. Thus, these nuclei must recognize their partners among many others to form a correct pair. Partitioning of nuclei in the crozier is followed by synchronous mitosis and two daughter nuclei of opposite mating type are isolated in the upper cell of the crozier by formation of two septa (Figure 2a; small arrow). These nuclei will fuse as the cell begins polarized growth to become an ascus (Figure 2a; long arrow). Wild-type fruiting bodies thus contain 100–200 asci within which meiosis and sporulation occur. When almost all asci from a perithecium contain four ascospores, there are almost no remaining croziers.

smr2, *fmr1*, and *fpr1* mutant fruiting bodies contain

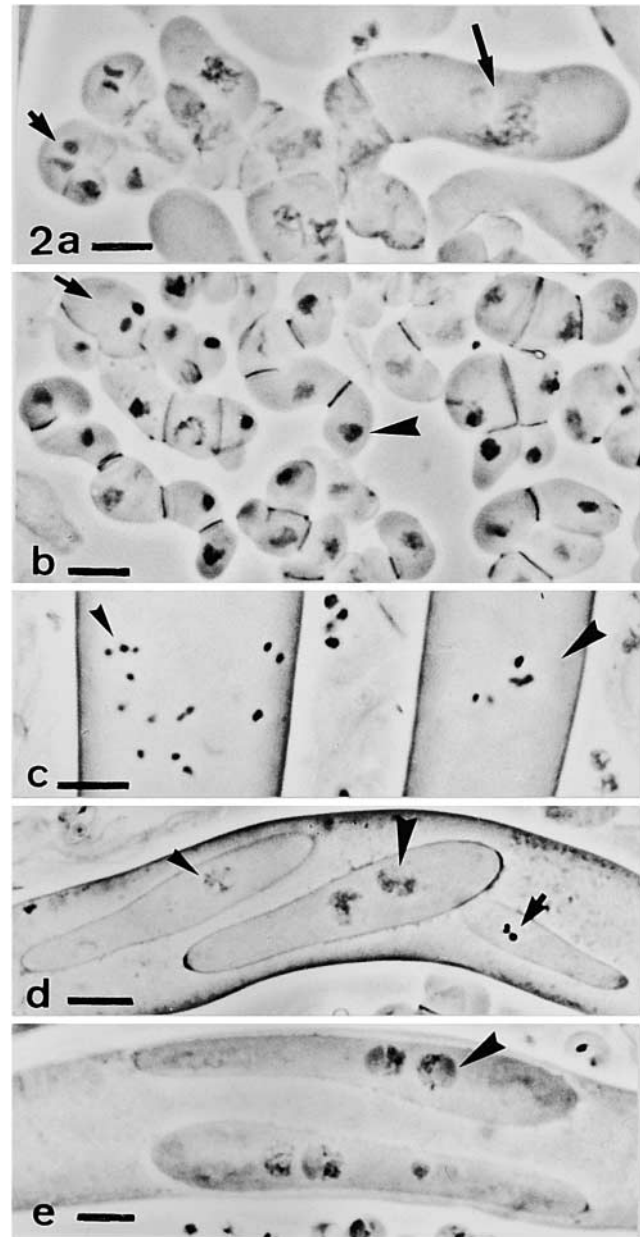


FIGURE 2.—Perithecium development in wild type and mutants. (a) Wild-type young perithecium with four-nucleated croziers (small arrow) and asci in prophase I (long arrow). (b) Δ *pah1 fpr1*^{T66l} croziers. All are uninucleate (arrowhead) except one, which shows four nuclei (arrow). (c) Two asci of *fpr1*^{T66l} (R15). The ascus on the right shows a metaphase I with 7 chromosomes (large arrowhead) and the ascus on the left shows metaphase II nuclei with 14 chromosomes in total (small arrowhead). (d) *fpr1*^{T66l} ascus with four ascospores. The middle ascospore contains two normal nuclei (large arrowhead). Nuclei from the two other ascospores are abnormal (smaller, arrowhead and arrow) due to aneuploidy. (e) Δ *pah1 fpr1*^{T66l} ascus with two ascospores each containing two nuclei (arrowhead points to the nucleolus). Bar, 5 μ m.

either no or only a few asci (1–50) and their development is abnormal in several respects.

1. Perithecia are always smaller than wild-type peri-

- thecia and a quarter of them contain only sterile paraphyses.
- Another quarter, moreover, contain a few (2–30) uninucleate hook-shaped cells that are probably croziers, since some of their upper cells evolve into asci.
 - All other perithecia contain hundreds of “croziers,” which almost never evolve into asci; instead, their nuclei divide and after septation their cells form long rows of more or less elongated cells that completely fill the perithecia. Moreover, in contrast to wild-type croziers (Figure 2a), almost all mutant croziers are uninucleate (Figure 2b). Half of those perithecia never differentiate asci.
 - When the upper cell of a uninucleate crozier forms an ascus, its nucleus enters meiosis. In those haploid meioses, the seven chromosomes are randomly distributed to the anaphase I poles, resulting in aberrant numbers of chromosomes in the four nuclei issued from the second division (Figure 2c). As some asci nonetheless proceed through postmeiotic mitosis (14 chromosomes are seen in such metaphases instead of the 4×7 expected in normal meiosis) and sporulation, the vast majority of ascospores are abnormal (Figure 2d). However, some asci contain four uninucleate or two binucleate (Figure 2e) wild-type-shaped ascospores that might result from early centromere cleavage at metaphase I giving rise to four nuclei with seven chromosomes.

A very small amount (<0.1%) of diploid croziers are also observed, either because septation does not occur after mitosis in a uninucleate crozier or because two nuclei are isolated within a crozier (see arrow in Figure 2b). These meioses are normal and give rise to four binucleate ascospores. Occasionally, the entire ascus yields a single giant spore.

Those three categories of perithecia are seen in all mutants. The number of perithecia and especially the number of large perithecia increase in the double mutants *smr2* Δ *pah1*, *fmr1* Δ *pah1*, and *fpr1* Δ *pah1*. However, their development is similar to what is observed with the single mutants. The phenotype of perithecia in crosses of *fmr1-r* Δ *pah1* or *fmr1-r* *IncA* with the wild-type *mat*[–] strain are similar to those of the self-fertile perithecia.

Phenotypes of strains deleted from a part of or from the entire fertilization domain: The *smr1-r fmr1* ^{Δ 95-107} mutant strain (R10) carries a deletion of the *FMR1* gene in the domain necessary for fertilization while the Δ *mat* strain is deleted for the entire *mat*⁺ idiomorph (COPPIN *et al.* 1993). These two strains display a similar phenotype. They are unable to cross either with a *mat*[–] or a *mat*⁺ tester strain. Nevertheless, in a Δ *pah1* or in a *IncA* context, where the number of microconidia is enhanced, these two strains are able to act as the male partners for *mat*⁺ and *mat*[–] strains. The progeny are uniparental *mat* mutants, which indicates that only the *mat* mutant

nucleus is able to enter the ascogenous hyphae and participate in ascospore formation (Table 6).

DISCUSSION

FPR1, FMR1, and SMR2 are required for the repression and activation of functions involved in fertilization and internuclear recognition: To date, neither wild-type strains of *P. anserina* nor any *mat* mutant has been found to be capable of fertilizing a strain of the same mating type. In contrast, *fpr1* mutants described in this study are capable of selfing as well as of fertilizing a tester strain of the same mating type. Our interpretation is that these *fpr1* mutant strains express the *mat*[–] genes required for fertilization. This implies that the wild-type FPR1 acts as a direct or indirect negative regulator of *mat*[–] fertilization genes (Figure 3). An equivalent phenotype has been observed with *mat*[–] strains with mutations in *FMR1* and *SMR2*. The self-fertile phenotype of *fmr1* and *smr2* mutant strains is hardly detectable in a wild-type context but it becomes obvious when the microconidium number is increased, as, for instance, in Δ *pah1* strains (ARNAISE *et al.* 2001). This feature has delayed the detection of self-fertility in the initial examination of the *fmr1-r* and *smr2-r* mutants (ARNAISE *et al.* 1997) because the Δ *pah1* strain was not yet available. The self-fertile phenotype of *fmr1* and *smr2* mutant strains suggests that they have lost the ability to repress the *mat*⁺ fertilization genes and that the wild-type *FMR1* and *SMR2* are direct or indirect negative regulators of the *mat*⁺ genes required for fertilization (Figure 3). It is possible that the repressor activity of FMR1 and SMR2 on *mat*⁺ functions results from the formation of a FMR1/SMR2 heterodimer, as suggested by the interaction found between FMR1 and SMR2 employing the yeast two-hybrid system (E. COPPIN and R. DEBUCHY, unpublished results). In contrast to *FMR1*, *SMR2* is not required for the expression of *mat*[–] mating type (DEBUCHY *et al.* 1993), but *SMR2* appears to be a gene involved in fertilization in the sense that it is required during fertilization in *mat*[–] strains to avoid self-fertilization (Figure 3). *SMR2* was not found to be expressed in the mycelium (COPPIN and DEBUCHY 2000), which suggests that this gene should be specifically expressed in *mat*[–] protoperithecia and microconidia, in addition to fruiting bodies where its transcription has been demonstrated (COPPIN and DEBUCHY 2000).

In selfing perithecia of all *fpr1*, *fmr1*, and *smr2* mutants, as well as in crosses between these mutants and a strain of the same mating type, fertilization is followed by the development of uninucleate croziers. Haploid meioses occur occasionally in these ascogenous hyphae and can lead to the formation of progeny consisting exclusively of the *mat* mutant nucleus. It is noticeable that the wild-type nucleus is never found in such progeny. Self-fertilization of *fpr1*, *fmr1*, or *smr2* mutants suggests a straightforward explanation for this phenotype.

TABLE 6
fmr1^{Δ95-107} and Δ*mat* crossed by wild type

Male partner	Female partner					
	<i>mat</i> ⁻			<i>mat</i> ⁺		
	Perithecia ^a	Ascospores observed ^a	Ascospores analyzed ^a	Perithecia ^a	Ascospores observed ^a	Ascospores analyzed ^a
<i>smr1-r fmr1</i> ^{Δ95-107}	5	0		9	0	
<i>smr1-r fmr1</i> ^{Δ95-107} Δ <i>pah1</i>	152	3	2	247	14	8
Δ <i>mat</i>	7	0		13	0	
Δ <i>mat</i> Δ <i>pah1</i>	500	4	1	400	59	23
Δ <i>mat IncA</i> ^b	62 ± 19	0		750 ± 164	50	13
<i>mat</i> ⁺	5,000	240,000		0		

^a See Table 4.

^b The numbers are the mean values of six independent experiments.

We propose that the mutant nucleus expresses both nuclear identities, leading to self-recognition. Self-recognition would be sufficient to promote the developmental stages normally followed by two compatible nuclei. As for fertilization, wild-type *FPR1* probably acts as a repressor of the *mat*⁻ IR functions, as well as being an activator of *mat*⁺ IR functions (Figure 3). Conversely, *FMR1* and *SMR2* are probably repressors of the *mat*⁺ IR functions and activators of the *mat*⁻ IR functions (Figure 3). Most of the *fpr1*, *fmr1*, and *smr2* mutant genes remain competent to activate their specific IR functions but all of them have lost their repressor activity. Nuclear self-recognition may also explain the uniparental progeny that are produced in crosses of *fpr1* and *smr2* mutant strains to *mat*⁻ and *mat*⁺ wild-type strains, respectively. This phenotype was reported initially by ZICKLER *et al.* (1995) for *fmr1-1*, *smr2-1*, and *fpr1-1* strains in crosses

to wild-type strains, but the authors did not propose any model for the formation of the uniparental progeny. We now can propose that the expression of the opposite nuclear identity in the *mat* mutant nucleus could trigger self-recognition, although the mutant nucleus remains competent for pairing with a compatible nucleus. If the mutant nucleus self-recognizes, it migrates alone into the ascogenous hyphae, ignoring its wild-type partner and eventually producing uniparental progeny. If the mutant nucleus pairs with a compatible nucleus, the pair follows the wild-type developmental pathway and yields biparental progeny. According to this model, the ratio of uniparental progeny mirrors the competition between self-recognition and pairing with a compatible nucleus. A high level of uniparental progeny could be attributed to a complete loss of repression of the alternative IR functions, resulting mostly in mutant nucleus

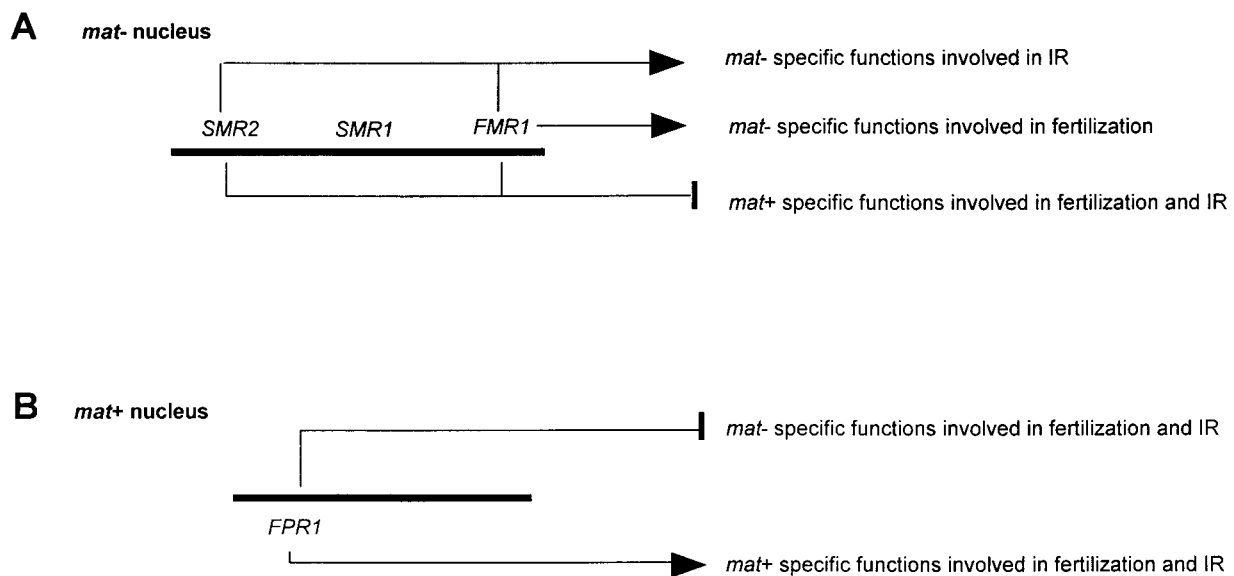


FIGURE 3.—Control of fertilization and IR in *P. anserina*. (A) In a *mat*⁻ nucleus where only *mat*⁻ specific functions are expressed. (B) In a *mat*⁺ nucleus where only *mat*⁺ specific functions are expressed.

self-recognition (*e.g.*, the R12 mutant, Table 1). A low level of uniparental progeny could indicate that the repression of the alternative IR functions is mildly affected, which would favor pairing with a compatible nucleus instead of self-recognition (*e.g.*, the R13 mutant, Table 1).

The hypothesis of the repression of alternative fertilization and IR functions by *mat* genes is supported by the phenotype of the Δmat and *fmr1* ^{$\Delta 95-107$} strains. The Δmat strain carries a complete deletion of the *mat+* information (COPPIN *et al.* 1993). The *fmr1* ^{$\Delta 95-107$} mutant has a deletion within the domain that was previously characterized as necessary for the control of fertilization (DEBUCHY and COPPIN 1992). Both strains are nevertheless able to fertilize a *mat+* or *mat-* wild-type strain, but the number of perithecia is 500–1000 times lower than the number of perithecia obtained in a wild-type cross, and these crosses yield no progeny (Table 6). The number of perithecia is increased and some ascospores are obtained when the number of microconidia is increased (in a $\Delta pah1$ or in an *IncA* context, Table 6). These observations indicate that the fertilization and IR *mat+* and *mat-* target genes are expressed at a low level in a nonrepressed, nonactivated context. As self-fertility is not observed in wild-type strains, the *mat+* and *mat-* functions should be utterly shut off in *mat-* and *mat+* strains, respectively.

The type of *smr1-r* suppressor mutations and their implications in regard to fertilization and IR: Mutageneses were performed during the vegetative state and expression of the *smr-1* suppressors was necessary during the sexual cycle. This strategy requires that the suppressor mutations allow fertilization to start the sexual cycle. The excess of mutations in *SMR2* is in agreement with the observation that this gene is not necessary for the expression of *mat-* fertilization functions. As expected, none of the three mutations in *FPR1* alters fertilization. In contrast to the *smr2* and *fpr1* mutations, *fmr1* ^{$\Delta 95-107$} has lost wild-type expression of the fertilization function and is supposed to have retained only the nonrepressed, nonactivated basal expression of these functions. Our experiments suggest that this basal expression is not sufficient to promote the sexual cycle unless *fmr1* ^{$\Delta 95-107$} is associated with a mutation increasing the number of microconidia (Table 6). The most likely explanation for the presence of *fmr1* ^{$\Delta 95-107$} among the suppressors is that it is a spontaneous mutation that occurred after fertilization instead of during mutagenesis.

How can nuclei expressing both nuclear identities bypass *smr1-r* developmental arrest? It has been proposed that IR is followed by a developmental arrest (COPPIN and DEBUCHY 2000). Although this developmental arrest is not visible in a wild-type cross, it has been demonstrated by a lethal phenotype in ascospores that express the three IR genes. *SMR1* allows these ascospores to recover from the lethal phenotype (COPPIN and DEBUCHY 2000). The developmental arrest is re-

vealed in a *smr1-r* \times *mat+* cross by a block in the development of the perithecia (ARNAISE *et al.* 1997). According to this model, self-recognition of *fpr1*, *fmr1*, or *smr2* mutant nuclei should result in a developmental arrest, while these nuclei can bypass the need for *SMR1* and proceed through the developmental steps leading to a progeny. The reason for this by-pass is unknown. However, *fmr1*, *smr2*, or *fpr1* mutants never give any biparental progeny in a cross in a *smr1-r* context, although these mutants are able to give at least some biparental asci in a cross in which a functional *SMR1* is present in either parent. This result confirms that *SMR1* is required for the recovery from the developmental arrest following IR between two compatible nuclei and it suggests that self-recognition might generate a mild form of developmental arrest that can be overcome without *SMR1*.

Comparisons with other fungi: Little is known about the functions of mating-type genes in other filamentous ascomycetes. *N. crassa* has the same mating-type structure as *P. anserina*, but mutation analyses suggest that *matA-2* (similar to *SMR1*) and *matA-3* (similar to *SMR2*) have different functions from their counterparts in *P. anserina* (FERREIRA *et al.* 1998). In contrast, the expression of *matA-1* and *mata-1* in *P. anserina*, as well as the expression of *FMR1* or *FPR1* in *N. crassa*, suggest that fertilization is controlled in the same way in both fungi (ARNAISE *et al.* 1993). However, no self-mating or uniparental progeny have ever been reported in *N. crassa* as a result of mutations in *mata-1* or *matA-1* genes. It is possible that the basal expression of the target genes involved in fertilization and IR is so low that no repressor activity is required to obtain a complete extinction of the *A* and *a* target genes in *a* and *A* strains, respectively. The yeast *S. cerevisiae* offers a well-documented example that seems more related to *P. anserina*. The **a** mating functions are constitutive. In α cells, the **a**-specific genes are repressed by *MAT- α 2* while the α -specific genes are induced by the *MAT- α 1* gene product. This combination of repression and activation of target genes involved in mating is reminiscent of the events that occur during fertilization in the *mat-* strain of *P. anserina* (see Figure 3A), and the similarity of *FMR1* with *MAT- α 1* (DEBUCHY and COPPIN 1992) makes the similarity of the two systems even more striking. However, *SMR2* and *MAT- α 2* belong to different regulatory protein families; *SMR2* is a HMG protein (DEBUCHY *et al.* 1993) and *MAT- α 2* contains a homeodomain (SHEPHERD *et al.* 1984). Nevertheless, it is not known if *SMR2* is a direct or an indirect repressor of *mat+* fertilization functions and we cannot exclude that it activates the expression of a repressor similar to *MAT- α 2*. The *mat+* mating system shows no similarity with the **a** mating system of yeast. In contrast to the **a** mating system, *mat+* fertilization functions are not constitutively expressed, since their expression requires the presence of *FPR1* (DEBUCHY and COPPIN 1992). However, the *mat+* fertilization system operates in a similar way to the yeast α mating system, except

that *FPR1* alone is sufficient to control the activation and repression of *mat+* and *mat-* fertilization functions, respectively. We cannot exclude that, as for *SMR2*, the repressor effect of *FPR1* is mediated by the activation of a repressor gene.

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