Cloning the Mating Types of the Heterothallic Fungus
_Podospora anserina_: Developmental Features of Haploid Transformants Carrying Both Mating Types

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ABSTRACT

DNAs that encode the mating-type functions (mat+ and mat−) of the filamentous fungus _Podospora anserina_ were cloned with the use of the mating-type A probe from _Neurospora crassa_. Cloning the full mat information was ascertained through gene replacement experiments. Molecular and functional analyses of haploid transformants carrying both mating types lead to several striking conclusions. Mat+ mat− strains are dual mated. However, the resident mat information is dominant to the mat− information added by transformation with respect to fruiting body development and ascus production. Moreover, when dual mating mat+ mat− strains are crossed to mat+ or mat− testers, there is strong selection, after fertilization, that leads to the loss from the mat+ mat− nucleus of the mat− information that matches that of the tester. Finally, the mat locus contains at least two domains, one sufficient for fertilization, the other necessary for sporulation.

Several laboratories began studying the mating-type loci of filamentous fungi at the molecular level by cloning the mat genes from ascomycetes such as _N. crassa_ (Vollmer and Yanofsky 1986; Glass et al. 1988; Metzenberg and Glass 1990; Staben and Yanofsky 1990; Glass, Grotelueschen and Metzenberg 1990) and from basidiomycetes, e.g., _Schizophyllum commune_ (Giaisson et al. 1989), _U. maydis_ (Kronstad and Leong 1989; Schulz et al. 1990) and _Coprinus cinereus_ (Mutasa et al. 1990). The mechanism by which mating-type genes control sexual reproduction has been intensively studied in the yeast _Saccharomyces cerevisiae_ (reviewed by Herskowitz 1989).

Here we describe the cloning of the mat+ and mat− loci of the filamentous ascomycete _Podospora anserina_. We cloned the _Podospora_ mat− DNA by using the _Neurospora_ mating-type A DNA as a probe. Transformation experiments yielded haploid strains carrying both mat+ and mat− information. Molecular and physiological analyses of these unnatural strains give new insights into the genetic control of heterothallism in filamentous fungi. The data are discussed with respect to the general model for the actions of the _S. cerevisiae_ MAT locus and to the biology of filamentous fungi.

MATERIALS AND METHODS

Fungal strains and transformations: Genetics and biological properties of _P. anserina_ were first described by Rizet and Engelmann (1949) and have been reviewed by Esser (1974). The ascii contain four spores, each formed around two non-sister nuclei after a post-meiotic mitosis. A
few asci contain five spores among which two are smaller and develop around one nucleus. These uninucleate spores give rise to homocaryotic mycelia, which have been used for molecular and genetic analysis. When it was necessary, whole asci were investigated in order to know more precisely what happened during (or before) meiosis. The mating type (mat) is controlled by two haplotypes mat+ and mat− that display a 98% second division segregation so that the five-spore ascus generally contain a mat+ and a mat− small spore. The 193 mutation, unlinked to mat, which prevents spore and mycelium pigmentation, exhibits a high first division segregation (Picard 1971).

The recipient strains for transformation experiments carried either the leu-1 mutation or the ure3-6 mutation (Razanamparany and Bégueuet 1986). The first one was used for transformations with cosmids from the mat− library: in this case, the pHSU8 vector (Debuchi et al. 1988) carries the opal suppressor tRNA su8-1 (Debuchi and Brygo 1985) which suppresses the leu-1 mutation. The second one was used for transformations with cosmids from the mat+ library: in that case, the cosmid vector (pHC79-ura5) carries the ura5+ gene (Bégueuet et al. 1984). The (ura+) transformants were crossed to ura5-6 tester strains while the (leu+) transformants were crossed to tester strains carrying the 193 mutation, which allows one to analyze easily the inheritance of the sub-1 selective marker. Furthermore, the transforming sub-1 marker suppresses the spore color mutation 193 in such a way that the three kinds of genotypes are easily scored: 193 sub+ (white spores), 193 sub-1 (green spores) and 193* (black spores).

Transformation experiments were performed as described (Brygo and Debuchi 1985) with the exception that the spheroplasts were heat shocked (5 min at 48°) before adding the DNA (Berges and Barreau 1989).

**Bacterial strains**: Cloning and plasmid preparations were done with both *Escherichia coli* BJ 5183 (Hanahan 1983) or HB 101 (Boyer and Roulland-Dussoux 1969).

**Plasmids and vectors**: The plasmid pMTAG-2 contains a 1.2-kb EcoRI-BclI fragment from the A mating-type specific region of *N. crassa* (Glass et al. 1988). The plasmid pCSN4 has a 1.9-kb BamHI-EcoRV fragment from the α mating-type-specific region of *N. crassa* (Glass et al. 1988). The pHSU8 vector (Debuchi et al. 1988) contains the sub-1 gene that suppresses both the leu-1 and the 193 spore color UGA mutations. The pHC79-ura5 vector (Turcq, Denayrolles and Bégueuet 1990) contains the ura5+ gene.

**Genomic libraries**: The *P. anserina* mat− lambda library (Debuchi et al. 1988) and the *P. anserina* mat+ cosmid library (Picard et al. 1987) were described previously. The *P. anserina* mat+ cosmid library was constructed by Turcq, Denayrolles and Bégueuet (1990).

**Genomic DNA preparation and analysis**: Genomic DNA was prepared from lyophilized mycelium according to a miniprep method (Coppin-Raynal, Picard and Arnaise 1989). Genomic DNA was treated overnight with restriction enzymes. DNA fragments were separated on 0.5% agarose gels and transferred to Hybond Nylon membrane (Amersham Corporation) by the Southern method.

**Hybridization procedures**: Hybridizations with *N. crassa* A and a probes were performed at low stringency. The 1.2-kb EcoRI-BamHI fragment from pMTAG-2 (A idiomorph) and the 1.9-kb EcoRV-EcoRV fragment from pCSN4 (α idiomorph) were gel purified, isolated by freeze squeeze and randomly labeled (Feinberg and Vogelstein 1983, 1984); activities were 2-10^6 cpm/μg and 10^6 cpm/μg, respectively. Nylon filters were prehybridized overnight at 37° in 45% formamide, 5 × SSC, 0.1% bovine serum albumin, 0.1% Ficoll 40, 0.1% polyvinylpyrrolidone 350, 20 μg/ml salmon sperm. The probe was added and filters were incubated for 48 hr, then washed in 2 × SSC containing 0.1% SDS for 2 hr at 37°. High stringency hybridizations performed for screening the cosmid libraries and for analyzing genomic DNAs from the transformants were as described by Maitis, Fritsu and Sambrook (1982) except that BLOTTO (Johnson et al. 1984) was used instead of Denhardt's solution.

**RESULTS**

Cloning the mating-type specific regions: Genomic DNA from mat+ and mat− strains was probed at low stringency with the A and α mating type specific regions of *N. crassa*. The A specific probe hybridizes to a mat− specific sequence on a 18-kb EcoRI fragment (Figure 1). In the case of the α specific probe, short exposure of the blot revealed a 13-kb EcoRI fragment present in both mat+ and mat− strains. Overexposure of the blot revealed very faint additional fragments that made a reliable search for a mating type-specific fragment impossible (Figure 1).

The mat− specific sequence was detected in a bacteriophage library of a mat− strain probed at low stringency with the A-specific DNA. The insert of the corresponding phage (λ9-1, Figure 2) was used as a probe to screen a mat− cosmid library and allowed the isolation of four cosmids: N8, N9, N10 and N11. The deduced physical map of the genome in this region and of the cosmids is shown in Figure 2A. The 18-kb EcoRI fragment present in cosmids N9, N10 and N11 hybridized with the A-specific probe (data not shown), confirming its identity with the genomic DNA fragment that hybridized with the same probe (Figure 1). Hybridization at high stringency of the 18-kb EcoRI fragment with the total DNA of a mat− strain indicates that no similar sequence is present on
a different sized EcoRI fragment (data not shown).

Hybridization of the 18-kb EcoRI DNA fragment from the mat- strain with the DNA from a mat+ strain reveals a 22-kb EcoRI fragment (data not shown). Therefore, besides its mat- specific region, the 18-kb fragment has some sequences present in both mat+ and mat- genome. We supposed that these common sequences were linked to the mat-specific region and could be used to identify cloned DNA from the mat locus of a mat- strain. Cosmid U1 of a mat- library hybridizes to the 18-kb EcoRI probe. This cosmid contains an EcoRI fragment of 22 kb (Figure 2B). Total DNA of mat+ strains does not contain any other fragments that hybridize with the 22-kb EcoRI fragment of U1 (data not shown).

**Mat- fertilization functions are encoded in the 18-kb EcoRI fragment:** Cosmids N8, N9, N10 and N11 (see Figure 2) transform the leu1-1 mat+ strain to leucine prototrophy with similar efficiency. Ten transformants bearing each cosmid were tested for fertilization and ascospore production (Table 1). All N8 primary transformants mated only as mat+: they did not cross with the mat+ tester strain. However, most N9 (6/10), N10 (9/10) and N11 (8/10) transformants induce formation of perithecia in both mat+ and mat- tester strains (Table 1). These data confirm that the DNA from a Podospora mat- strain isolated with the Neurospora A probe confer the ability to mate with a mat+ strain on a mat+ recipient. The gene(s) necessary for fertilization is (are) totally included in the 18-kb EcoRI fragment; it is the only fragment common to N9, N10 and N11. Transformation with the 18-kb fragment subcloned in pHSU8 confirms that this fragment contains mat- DNA; 54 of 84 transformants tested mate with mat+ and mat- testers.

Transformants bearing cosmids N9, N10 or N11 that display a mat+ mat- phenotype were expected to contain a tandem duplication of mat DNA, one copy mat+ and one mat-. Their EcoRI restricted genomic DNA was fractionated by agarose gel electrophoresis and the gel blots were hybridized with radiolabelled N9, N10 or N11 cosmids. The EcoRI restriction maps of the mat+ and mat- idiomorphs and adjacent regions differ as shown in Figure 2. The mat+ information is in a 22-kb fragment, which is replaced by two fragments of 4.3 and 18 kb in mat- DNA. This polymorphism is seen in blots of DNA from the mat+ and mat- untransformed strains in Figure 3. The Southern analysis of N9B2 and N11B1 transformants is shown in Figure 3, lanes A1 and Ba. The two
transformants contain the 22-kb fragment specific to the resident \textit{mat+} DNA and the 4.3- and 18-kb fragments specific to the transforming \textit{mat-} DNA as well as the 7.8-kb EcoRI fragment corresponding to integrated cosmid vector. The vector fragment was close to (N11) or superimposed (N9) on a genomic fragment (see the map in Figure 2). The presence of the vector was checked by using the labeled vector without insert as a probe. Besides the polymorphic mating-type bands and the vector band, the pattern of the transformants corresponded to the pattern of the untransformed strain. No additional fragment was found, indicating that cosmid DNA had integrated by homologous recombination at the resident locus. Subsequent genetic analysis of the transformants confirmed that the transforming DNA had integrated at \textit{mat}. The integration event gave rise to a tandem duplication, one copy containing the \textit{mat-} information, the other containing the \textit{mat+} information. All the transformants that were submitted to Southern analysis (four N9, two N10 and five N11 transformants) bore tandem duplications. Furthermore, molecular analysis of one transformant that exhibited the \textit{mat+} phenotype alone (as the recipient strain) indicated that only the \textit{mat+} 22-kb fragment was present; the 4.3- and 18-kb fragments were not detected. This suggests that a part of the cosmid sufficient to complement the \textit{leu1-1} mutation had integrated, but without the \textit{mat-} information.

**Dual \textit{mat+}, \textit{mat-}* information can be inherited through a cross to a \textit{mat-} strain:** Primary transformants contain both transformed and untransformed nuclei. In order to obtain homocaryotic transformed strains, it is necessary to go through sexual reproduction (in Podospora, uninucleate microconidia are unable to germinate and act only as male gametes in the fertilization process). Most primary transformants that display the \textit{mat+ mat-}* phenotype\(^1\) are fully fertile when crossed to a \textit{mat-} strain (see Table 1). The transforming marker (\textit{su8-1}) suppresses the spore color mutation 193, so crosses were performed with a 193 \textit{mat-} (white spores) strain. Ten to 30 uninucleate spores displaying the 193 \textit{su8-1} (green spores) phenotype were isolated from each cross. Analysis showed that, in all cases, cosmids integrated at (or very near) the \textit{mat+} locus. In fact, most spores carried the selective (\textit{su8-1}) marker and displayed the dual \textit{mat+ mat-}* phenotype.

Southern blots were performed on DNA from progeny exhibiting this \textit{mat+ su mat-}* phenotype. In the case of N9 transformants, the restriction pattern did not differ from that of the primary transformants shown in Figure 3, lanes 1 and 2 for N9B2. Although they remain dual maters, progeny of the two N11 transformants that have been analyzed lost the 4.3-kb EcoRI fragment (data not shown). Therefore, the \textit{mat+} and \textit{mat-}* information, artificially associated in the same nucleus, can be inherited through a cross to a \textit{mat-} strain and, as expected from transformation with N10, the 4.3-kb EcoRI fragment is not essential for the dual mating phenotype.

The \textit{mat-}* information can be lost through a cross to a \textit{mat-} strain: Although \textit{mat-}* can be inherited through a cross, extensive analysis of offspring from \textit{mat+ su8-1 mat-}* \(\times\) \textit{mat-} crosses (described in the previous paragraph), revealed that some spores lost the \textit{mat-} phenotype. These strains are in no way different from a \textit{mat+} reference strain. These \textit{mat-} strains belong to two classes: those which retained and those which lost the \textit{su8-1} marker. The second class is probably due to recombination/excision of tandemly duplicated sequences that occurs mostly before premeiotic replication in Podospora (COPI-N-RAYNAL, PICARD and ARNAISE 1989). Molecular analysis of the first class of strains, which no longer expressed the \textit{mat-} information although still containing the transformation marker, showed that they retained the vector part of the cosmid (data not shown). The mechanisms giving rise to these progeny are under study and will be discussed elsewhere. The results of \textit{mat+ mat-}* \(\times\) \textit{mat-} crosses are summarized in Figure 5 (left part, progeny class 1 to 3).

The 4.3-kb EcoRI fragment contains information necessary for peritheicum development and ascus production: As noted above (see also Table 1), most transformants obtained with N9, N10 and N11 cosmids mate with \textit{mat+} strains as well as with \textit{mat-} strains, in the sense that fertilization occurred. Furthermore, all transformants produced many asci when crossed to a \textit{mat-} strain. The transformants could be divided into two classes on the basis of their behavior when they were crossed to a \textit{mat+} strain. None of the nine N10 transformants gave rise to progeny in this kind of cross; in contrast, the N9 (five among six) and N11 (six among eight) transformants gave a few fertile perithecia when crossed to \textit{mat+} strains (see Table 1). Fertile perithecia represented less than 1% of those formed but the fertile perithecia contained a normal number of asci with viable spores. We do not know why so few perithecia were fertile when the (N9 and N11) \textit{mat+ mat-}* transformants were crossed to a \textit{mat+} strain while all perithecia were fertile when the same transformants were crossed to a \textit{mat-} strain. However, low fertility was inherited by the \textit{mat+ mat-}* progeny in the N9 secondary (purified) transformants. Although secondary N11 transformants that lost the 4.3-kb EcoRI fragment and retained the 18-kb EcoRI fragment were still able to fertilize a \textit{mat+} strain, they no longer generated fertile perithecia. These obser-

\(^1\) The star indicates that \textit{mat} DNA was added by transformation. For instance, \textit{mat+ mat-}* indicates that a recipient \textit{mat+} was transformed with a cosmid carrying the \textit{mat-} information.
vations, along with the fact that N10 transformants never gave rise to fertile perithecia in a cross to mat+ strains, suggest that the 4.3-kb EcoRI fragment contains information necessary for ascus production. In fact, the only difference between N9 and N10 cosmids is that the first contains and the second lacks this fragment (see Figure 2).

The mat+* information can replace the mat+ information through a cross to a mat+ strain: Progeny from the few fertile perithecia of mat+ mat- mat- mat- crosses were analyzed genetically. All three N9 and two of four N11 transformants gave rise to a minority of spores that still expressed the selective (su8-I) marker. However, most asci had lost this marker. Genetic analysis showed that these spores no longer expressed the mat+ information but, instead, behaved as mat- strains. They mated perfectly with mat+ strains (as female and as male), and gave rise to fertile perithecia whose progeny were identical to those from classical mat+ mat- crosses. The results of mat+ mat- mat- mat- crosses are summarized in Figure 5 (right part).

Several mat- progeny from the cross of a mat+ su8-1 mat- mat- transformant to a mat+ strain were submitted to molecular analysis (Figure 3, lanes 3 and b). The loss of the 22-kb mat+ and 7.8-kb vector DNA fragments indicated that the resident mat+ information was excised together with the vector DNA containing the su8-I selective marker. The 7.8-kb fragment in lane 3 corresponded only to genomic DNA since it did not hybridize to a vector probe. An event during the cross led to the replacement of the mat+ resident information by the mat- transforming sequence. We confirmed that the mat- sequence had integrated in the mat+ chromosomal context by using a restriction fragment length polymorphism that distinguishes DNA from mat+ and mat- origins (COPPIN-RAYNAL, PICARD and ARNAISE 1989). DNA probes from that region reveal a 2.2-kb EcoRV fragment in mat+ DNA and a 2.4-kb fragment in mat- DNA (Figure 4). Only the 2.2-kb mat+ specific band was detected in N9B2 mat+ mat- mat- and in its mat- progeny (Figure 4). The same results were obtained with N11B1. This control eliminates the possibility that the mat- spores are contaminants.

Therefore, through this two-step process (transformation followed by recombination/excision) it is possible to obtain a mat- strain from a mat+ strain. This observation proves that cosmids N9 and N11 contain all the specific sequences necessary to confer the mat- identity. These sequences lie in the 4.3- and 18-kb EcoRI fragments which are the only ones shared by these two cosmids.

The mat+ su8-1 mat-* strains are not true homothallic strains: Homocaryotic strains from uninucleate spores that carry information for both mating types look like self-fertile (homothallic) strains with respect to the fertilization process. In fact, at the end of growth, the plates are covered with fertilized perithecia. This self-mating makes it very difficult to use these strains as female partners in crosses. Apparently, self-fertilization is so efficient that very few female organs remain available to male gametes of another strain. Therefore, at the level of fertilization, these mat+ mat- strains, like heterocaryotic strains where the mat+ and mat- information are expressed in two nuclei, are self-maters. However, only the heterocaryotic strains give progeny; the unnatural homocaryotic mat+ mat- transformants are self-sterile. Further studies, especially cytological data, will clarify this block in the development of fertilized perithecia.

A converse story for mat+: The U1 cosmid isolated from the mat+ library was used to transform a recipient mat- strain. In this case, the selective marker carried by the cosmid was the Podospora ura5- gene (BEGUERET et al. 1984) and the recipient was ura5-.

Among 28 (ura5+) transformants, 11 exhibited only primary and purified transformant; lane 3, N9B2 mat+ mat-* progeny. The polymorphic 2.2- and 2.4-kb fragments are indicated.

Several mat- progeny from the cross of a mat+ su8-1 mat- mat- transformant to a mat+ strain were submitted to molecular analysis (Figure 3, lanes 3 and b). The loss of the 22-kb mat+ and 7.8-kb vector DNA fragments indicated that the resident mat+ information was excised together with the vector DNA containing the su8-I selective marker. The 7.8-kb fragment in lane 3 corresponded only to genomic DNA since it did not hybridize to a vector probe. An event during the cross led to the replacement of the mat+ resident information by the mat- transforming sequence. We confirmed that the mat- sequence had integrated in the mat+ chromosomal context by using a restriction fragment length polymorphism that distinguishes DNA from mat+ and mat- origins (COPPIN-RAYNAL, PICARD and ARNAISE 1989). DNA probes from that region reveal a 2.2-kb EcoRV fragment in mat+ DNA and a 2.4-kb fragment in mat- DNA (Figure 4). Only the 2.2-kb mat+ specific band was detected in N9B2 mat+ mat- mat- and in its mat- progeny (Figure 4). The same results were obtained with N11B1. This control eliminates the possibility that the mat- spores are contaminants.

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homologous and ectopic transformants. In the latter case, as the ectopic \textit{mat\textsuperscript{+}} segregated independently of the \textit{mat} locus, spores containing two copies of \textit{mat}\textsuperscript{+} information (\textit{mat\textsuperscript{+}, mat\textsuperscript{+*}}) were recovered. They did not display any phenotype different from standard \textit{mat\textsuperscript{+}} strains. As for the homologous transformants, recombination/excision events occurred during crosses giving rise to \textit{mat\textsuperscript{−}} spores (when they were crossed to a \textit{mat\textsuperscript{+}} strain) and to \textit{mat\textsuperscript{+}} (when they were crossed to a \textit{mat\textsuperscript{−}} strain). The \textit{mat\textsuperscript{−}} spores correspond to a replacement of the resident \textit{mat\textsuperscript{−}} information by the \textit{mat\textsuperscript{+}} information borne by the cosmid.

The data obtained with \textit{mat\textsuperscript{+}} are very similar to those described for \textit{mat\textsuperscript{−}}. Although DNA from \textit{mat\textsuperscript{+*}} \textit{mat\textsuperscript{−}} transformants has not been analyzed, the \textit{mat\textsuperscript{+}} data can be summarized in the following five points. First, the ectopic expression of \textit{mat\textsuperscript{+}} and the gene replacement experiments indicate that the U1 cosmid contains all information necessary for \textit{mat}\textsuperscript{+} activities. Second, two copies of \textit{mat\textsuperscript{+}} DNA do not disturb the life cycle of the fungus. Third, the \textit{mat\textsuperscript{+*} mat\textsuperscript{−}} strains are dual maters. Fourth, ascus production needs more information than fertilization does as evidenced by one of the ectopic transformants. Fifth, with respect to sporulation (perithecium development and ascus production) there is a better expression of the resident \textit{mat\textsuperscript{−}} information than fertilization does as evidenced by the \textit{mat\textsuperscript{+}} information borne by the cosmid.

The data obtained with \textit{mat\textsuperscript{+}} are very similar to those described for \textit{mat\textsuperscript{−}}. Although DNA from \textit{mat\textsuperscript{+*}} \textit{mat\textsuperscript{−}} transformants has not been analyzed, the \textit{mat\textsuperscript{+}} data can be summarized in the following five points. First, the ectopic expression of \textit{mat\textsuperscript{+}} and the gene replacement experiments indicate that the U1 cosmid contains all information necessary for \textit{mat}\textsuperscript{+} activities. Second, two copies of \textit{mat\textsuperscript{+}} DNA do not disturb the life cycle of the fungus. Third, the \textit{mat\textsuperscript{+*} mat\textsuperscript{−}} strains are dual maters. Fourth, ascus production needs more information than fertilization does as evidenced by one of the ectopic transformants. Fifth, with respect to sporulation (perithecium development and ascus production) there is a better expression of the resident DNA than of the transforming DNA.

There is only one difference in our results with \textit{mat\textsuperscript{+}} and \textit{mat\textsuperscript{−}} transformants. While we did not obtain progeny from the self-fertilized peritheca in the case of \textit{mat\textsuperscript{+} mat\textsuperscript{−}} strains, one of the U1 homologous transformants was partly self-fertile. The spores from this selfing \textit{mat\textsuperscript{+} mat\textsuperscript{−}} strain kept the same \textit{mat\textsuperscript{+*} mat\textsuperscript{−}} phenotype as the parental strain, which closely resembles homothallism. Further experiments are needed to understand why \textit{mat\textsuperscript{+*} mat\textsuperscript{−}} and \textit{mat\textsuperscript{+}} \textit{mat\textsuperscript{−}} strains do not behave in the same way with regard to selfing.

DISCUSSION

The \textit{A} specific probe from \textit{N. crassa} permitted the cloning of \textit{P. anserina} DNA that encodes \textit{mat\textsuperscript{−}} functions. The cloned fragment contains all sequences necessary to \textit{mat\textsuperscript{−}} activities (mating, perithecium development and ascus production) as evidenced by \textit{mat\textsuperscript{+}} to \textit{mat\textsuperscript{−}} substitution experiments.

DNA encoding the \textit{Podospora} \textit{mat\textsuperscript{+}} determinants was identified in a genomic library using a \textit{mat\textsuperscript{−}} probe containing DNA sequences common to \textit{mat\textsuperscript{+}} and \textit{mat\textsuperscript{−}} genomes. \textit{Mat\textsuperscript{−}} to \textit{mat\textsuperscript{+}} replacement experiments showed that the cloned sequences contain all information required for \textit{mat\textsuperscript{+}} activities.

Probing total DNA from \textit{mat\textsuperscript{+}} and \textit{mat\textsuperscript{−}} strains with DNA fragments encoding either \textit{mat\textsuperscript{+}} or \textit{mat\textsuperscript{−}} activities revealed that these sequences are present only as a single copy in each haploid genome. Therefore, unlike yeasts (\textit{NASMYTH and TATCHELL}, 1980; \textit{STRATHERN et al.}, 1980; \textit{BEACH}, 1983), \textit{Podospora} contains no silent mating information. \textit{Podospora} shares this feature with the four characterized heterothallic filamentous fungi: the ascomycete \textit{N. crassa} (\textit{GLASS et al.}, 1988) and the basidiomycetes \textit{S. commune} (\textit{GIASSON et al.}, 1989), \textit{U. maydis} (\textit{KRONSTAD and LEONG}, 1989; \textit{SCHULZ et al.}, 1990) and \textit{C. cinereus} (\textit{MUTANA et al.}, 1990).

The \textit{A} specific probe of \textit{Neurospora} hybridizes to a 13-kb \textit{EcoRI} fragment present in both \textit{mat\textsuperscript{+}} and \textit{mat\textsuperscript{−}} \textit{Podospora} genomic DNAs. The \textit{EcoRI} restriction map of 84-kb flanking the \textit{mat\textsuperscript{+}/mat\textsuperscript{−}} region does not reveal any 13-kb fragment (Figure 2). Therefore, \textit{Podospora} contains a sequence, related to the \textit{Neurospora} \textit{a} idiomorph, which is not \textit{mat} specific. This \textit{a} related sequence may correspond to one of the numerous incompatibility genes identified in \textit{Podospora} (\textit{RIZET and ESSEY}, 1953; \textit{BERNET}, 1967). In fact, the \textit{mt} \textit{a}-1 polypeptide is not only responsible for mating functions but also for vegetative incompatibility. These two activities might be controlled by two different domains of the polypeptide (\textit{STABEN and YANOFSKY}, 1990). Furthermore, the lack of \textit{mat\textsuperscript{+}/a} hybridization is well explained by preliminary sequence comparisons (R. \textit{DEBUCHY} and E. \textit{COPPIN}, unpublished data). Although weak homology was found at the DNA level, extensive similarity was detected at the level of the translation products. This indicates that \textit{mat\textsuperscript{+}} may be the functional counterpart of \textit{mt} \textit{a} although DNA sequences have strongly diverged.

The transformation experiments described in this paper give new insights into the genetic control of mating types in heterothallic ascomycetes. The data can be discussed according to four ideas.

\textit{Mat\textsuperscript{+}} \textit{mat\textsuperscript{−}} \textit{strains are dual maters:} \textit{Neurospora} and \textit{Podospora} mating differ strikingly from mating in two other heterothallic organisms where information for both mating types in the same nucleus is documented. In \textit{S. cerevisiae}, the presence of active \textit{MATa} and \textit{MATa} information in the same haploid or diploid nucleus leads to sterility (the cells are unable to mate) (\textit{HABER and GEORGE}, 1979; \textit{KLAR, VOGEL} and MacLeod 1979). However, in the alga \textit{C. reinhardtii}, diploids heterozygous at the mating-type locus (\textit{mt\textsuperscript{+}/mt\textsuperscript{−}}) behave as \textit{mt\textsuperscript{−}} cells (\textit{EBERSOLD}, 1967). The sterile phenotype of yeasts heterozygous at \textit{MAT} is well understood and explained through the regulatory activities of the \textit{MATa}2 and \textit{MATa}1 genes (see \textit{HESKOWITZ}, 1989, for a review). In \textit{Chlamydomonas}, it is not known why \textit{mt} \textsuperscript{−} is dominant to \textit{mt}\textsuperscript{+}. In \textit{Neurospora}, transformants that contain both \textit{A} and \textit{a} mating-type sequences mate as both \textit{A} and \textit{a}, although exhibiting a growth inhibited phenotype unless vegetative incompatibility associated with mating-type is suppressed (\textit{GLASS et al.}, 1988; \textit{STABEN} and \textit{YANOFSKY}, 1988; \textit{STRATHERN et al.}, 1980; \textit{BEACH}, 1983).

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1990). In Podospora, the mat+ mat− strains grow as well as reference strains and mate as both mat+ and mat−.

It is now clear that the mat loci of filamentous fungi contain regulatory genes (Glass, Grotelueschen and Metzenberg 1990; Staben and Yanofsky 1990; R. Deubcy and E. Coppin, unpublished data). However these regulatory genes might not act in the same way as in yeasts. In fact, filamentous fungi have other means at their disposal in order to regulate matings since they develop sexual organs whatever the mating type.

Ascus production is dependent upon information beyond that necessary for fertilization: Transformation of a mat+ recipient with the three cosmids N9, N10 and N11 from a mat− library generates two kinds of transformants: those able to mate as mat− and to give fertile perithecia when crossed to mat+ and those able to mate as mat− but unable to give progeny when crossed to mat+ (Table 1). Comparison of the cosmid maps (Figure 2) leads to the conclusion that complete information for fertilization lies in the 18-kb EcoRI fragment while the 4.3-kb EcoRI fragment contains information required for sporulation, i.e. all the processes happening between fertilization and ascus production. Here again, the traditional nomenclature that represents mating-types as alleles is inadequate. The data presented in this paper indicate the existence of several (at least two) closely linked sexual-related functions in the mating-type locus region. This situation is like that in S. cerevisiae (Strathern, Hicks and Herskowitz 1981), Schizosaccharomyces pombe (Kelly et al. 1988), Chlamydomonas (Galloway and Goodenough 1985) and Neurospora (Staben and Yanofsky 1990; Glass, Grotelueschen and Metzenberg 1990). It is also tempting to speculate that bipolar fungi (whose mating types are specified by one locus) have information at one locus that tetrapolar basidiomyces (whose mating-type functions are encoded by two complex loci) have in two loci.

Resident information for ascus production is dominant to information added by transformation: The ten transformants (four N9 and six N11, Table 1) that mate as both mat+ and mat−, give only a few fertile perithecia when crossed to mat+ but all the perithecia are fertile when the cross is performed to a mat− strain. Conversely, the resident mat− information is expressed better than mat+ when mat− recipients are transformed with mat+. Neurospora transformants behave in the same way, at least when A is the resident information and a the additional information (Staben and Yanofsky 1990).

Therefore, the mating-type information required for ascus production does not follow the rule of the apparent coexpression observed for the fertilization information. Further genetic and molecular data are needed to understand this situation. The infertility of self-mating Podospora and Neurospora transformants also distinguishes these ascomycetes from the basidiomycete U. maydis, where heterozygosity at the b locus in a haploid nucleus allows the fungus to complete the sexual stage of its life cycle (Kronstad and Leong 1989).

Perithecial development, a glow in the black box? Figure 5 summarizes the different kinds of progeny observed when a mat+ mat− strain is crossed either to a mat+ or a mat− tester. The striking fact is that there is a strong selection which goes in the opposite directions for the two crosses. When the cross is performed with a mat− strain, progeny containing the transformant chromosome always express full mat+ function. The mat− information is either lost or kept in its original state (good mater, poor ascus production). When the cross is performed with a mat+ strain, the resident mat+ information of the mat+ mat− transformant is either lost or transmitted with a new status (good mater, poor ascus producer). Therefore, the tandemly duplicated DNA formed by homologous recombination of the cosmid in the mat locus is the target of different kinds of events that change the structure and (or) the expression of the dual information. We presume that within the developing peritheium, those nuclei in which the DNA rearrangements preserve the information necessary for fertility with the tester partner are the ones that undergo caryogamy, meiosis and ascus development.

After more than 40 years and despite increasingly precise cytological analysis, the developing peritheium remains a black box (Zickler 1973; Simonet and Zickler 1978). After fertilization, the male and the female nuclei divide, giving rise to a heterocaryotic syncytium inside the fruiting body. Then dicar-
yotic cells emerge from the syncytium. These diploids divide in such a way as to preserve the dicaryotic structure except in the apex where caryogamy occurs. Each diploid cell that will undergo meiosis and sporulation processes to give rise to an ascus contains mat+ and mat− information. However it is not known whether the mat+ and mat− nuclei recognize each other at the time of dicaryon formation or at the time of caryogamy. Furthermore, nobody knows how this recognition is achieved. Cytological analysis of the sterile perithecia induced in mat+ mat− × mat+ mat− crosses or after self-fertilization of the mat+ mat− strains may enlighten us with respect to this old problem.

Structural and functional analyses of the mat+ and mat− information required for mating and perithecial development should help determine whether the mat genes of filamentous fungi are structural or regulatory genes (or both). Comparison of the Podospora mating-type genes to those of other fungi should help explain how these genes and the control of sexual reproduction evolved among ascomycetes and basidiomycetes.

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