Directed Replacement of *mt A* by *mt a-1* Effects a Mating Type Switch in *Neurospora crassa*

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ABSTRACT

To test the functions of *a* mating type genes, we developed an efficient strategy to select transformants of *Neurospora crassa* in which resident *A* mating type DNA was replaced by cloned DNA from the *mt a* idiomorph. Cloned *a* idiomorphic DNA could specify all functions, including fertility, of *a* mating type, but only when it replaced *A* DNA at the mating type locus. Only the *mt a-1* region of the *a* idiomorph was necessary in order to specify *a* mating type. Gene replacement events involved the homologous sequences flanking the unique mating type idiomorphic DNA, resulting in apparently isogenic *a* and *A* strains. These isogenic strains were fertile when crossed with one another, indicating that no determinants outside the transforming DNA are necessary for fertility as *a* and that no host sequences of *A* strains interfere with fertility as *a*. One *a* replacement strain bore a duplication of the transforming *mt a-1* and *hph* DNA. The duplication strain had unexpected properties. Although mating type segregated 1:1 in crosses of this strain to *A*, the duplicated regions were efficiently altered during the sexual process to generate a single copy in the progeny. No progeny were recovered that had undergone RIP (repeat induced point mutation) sufficient to inactivate the *mt a-1* gene. We infer that the *mt a-1* gene is necessary and sufficient to specify *a* mating type identity in all vegetative and sexual activities. *Mt a-1* may also play an essential role in ascosporeogenesis after fertilization.

NEUROSPORA *crassa* is a haploid, heterothallic filamentous fungus. A single mating type locus specifies mating type. The two alternate mating types, *a* and *A*, specify cell type during both vegetative and sexual development. During vegetative growth, mating type acts as a vegetative incompatibility locus, which prevents the formation of viable *a/A* heterokaryons. Mating type has many roles in sexual development. All vegetative forms (mycelia, macroconidia and microconidia) can act as males, which presumably secrete mating type-specific pheromones (BISTIS 1981, 1983). These pheromones attract specialized hyphae of differentiated females of the opposite mating type, resulting in fertilization. Ensuing fruiting body development and ascosporeogenesis are very complex (RAJU 1980, 1992). However, it is clear that both the *a* (RAJU 1992) and *A* (GLASS and LEE 1992) idiomorphs have post-fertilization activities.

The single mating type locus appears to be the sole genetic determinant of mating type. DNA from the mating type locus of each of the two mating types has been cloned and characterized (GLASS *et al.* 1988, 1990; STABEN and YANOFSKY 1990). Each mating type locus contains a unique DNA segment called an idiomorph. Transformation assays established that the cloned idiomorphic DNAs encoded both the vegetative incompatibility and mating type activities. In a typical assay, a mutant that lacks all mating activity is transformed with cloned idiomorph DNA. Such transformants regain both vegetative incompatibility and the ability to mate (defined as production of perithecia). In the case of *a*

mating type, both properties depend upon a short segment (bases 2923–4596, unique to *a* strains) from within the *mt a* idiomorph DNA that contains the *mt a-1* gene. The *mt a-1* gene encodes the MT *a-1* polypeptide, which has a DNA-binding activity correlated with mating function (PHILLEY and STABEN 1994). Transformation assays did not establish any function associated with idiomorphic *a* DNA other than that of the *mt a-1* region.

Although ectopic transforming *a* or *A* idiomorphic DNA can confer the ability to mate upon a recipient, transformants do not produce ascospores as the ectopically integrated, transforming mating type. Transformants of strains tolerant to mating type vegetative incompatibility (*a^{m23};tol,a;tol,A*) do produce ascospores as the original mating type, indicating that such transformants are not simply sterile. The lack of fertility conferred by mating type transformation in *N. crassa* contrasts with fertility conferred by ectopic mating type genes in both *Podospora anserina* (PICARD *et al.* 1991) and *Cochliobolus heterostrophus* (TURGEON *et al.* 1993). Although ectopic mating type genes in these other fungi do not confer as great a degree of fertility as does the intact, resident mating type idiomorph, the cloned idiomorphic DNA segments clearly contain information necessary to specify *all* functions of mating type in the respective fungi.

The inability to demonstrate fertility due to transformation with cloned DNA from the *N. crassa* mating type loci suggested the possibility that the cloned DNAs did not include all determinants necessary to specify mating type. Therefore, we developed a method to replace the

resident mating type DNA with transforming DNA. We determined that the cloned *a* idiomorph segment specified all functions essential for *a* mating type identity. We extended our replacement analyses to determine whether regions of the *a* idiomorph, outside *mt a-1*, were required for fertility or whether *mt a-1* was the sole determinant of *a* mating type.

MATERIALS AND METHODS

Strains and media: Media for *N. crassa* and manipulations followed standard procedures (DAVIS and DESERRES 1970). Hygromycin B solution was from Calbiochem.

The cloned *mt a* DNA in pCSN27 derived from the benomyl-resistant strain, FGSC#3460. The genotypes of other strains, listed by FGSC stock number (genotypes in parentheses) were: 1946 (*tol a*), 2338 (*tol A*), 2489 (*A*), 3831 (*ff-1 A*), 4200 (*a*), 4317 (*fl A*), 4347 (*fl a*), 4570 (*A^{m44} un-3 ad-3A nic-2 cyh-1*), 5382 (*a^{m33}*) and 6844 (*a^{m30} ad-3B cyh-1*). Strains were obtained from DAVID PERKINS or from the Fungal Genetic Stock Center.

Plasmid DNA constructs: The two replacement constructs, pCSN61 and pCSK25, were derived from pCSN27 (STABEN and YANOFSKY 1990). Plasmid pCSN61 was constructed by digesting pCSN27 with *EcoRI* and *SacII*, creating blunt ends with the Klenow fragment of DNA polymerase I, and inserting the blunt-ended *Sall-MluI* fragment bearing the *hph* gene of pCSN43 (STABEN *et al.*, 1988). Plasmid pCSK25 was constructed by digestion of pCSN27 with *PstI*, blunting the ends, digestion with *SphI*, and insertion of the *EcoRV-SphI* fragment of pCSN44 bearing the *hph* gene of pCSN44. Therefore, pCSK25 contains the normal centromere-distal flank of *mt a*, up to and including the *SphI* site at position 1409 of the normal sequence. Genomic DNA of *A* strains does not appear to contain an *SphI* site in a similar position. DNA sequences of these constructs and base numbers referenced in the text can be related to the DNA sequence of the *mt a* idiomorph and its flanks deposited in GenBank (M54787). Standard techniques for DNA manipulations were used throughout (SAMBROOK *et al.* 1989).

Neurospora transformation and selection: We used a standard protocol for transformation of *N. crassa* (VOLLMER and YANOFSKY 1986), modified for selection of hygromycin-resistant cells (STABEN *et al.* 1988). In general, 500- μ l aliquots of transformation reactions, expected to yield 1000 individual transformants, were plated on selective media. Each of the replacement strains was derived from a separate transformation and is expected to be an independent event. Transformants were transferred to hygromycin medium in small slant tubes and tested for mating type. Those that mated as *a* were subcultured and crossed to *A* tester strains.

Mating type assays: Mating type was usually assayed by transferring a suspension of conidia to *fl, A* or *fl, a* tester lawns grown on synthetic cross medium. Strains of opposite mating type formed perithecia within 7 days at room temperature (22°). These perithecia ejected spores, which were collected and analyzed. To determine whether isogenic strains could mate, similar assays were done with the *A* parent and the replacement strains. To be certain that the replacement strains could act as females, plates of the replacement strains were fertilized with *ff-1* (female sterile), *A* conidia in separate assays.

Southern mapping: DNA was isolated (LEE *et al.* 1988), restriction endonuclease digested, and Southern blotted (SAMBROOK *et al.* 1989). Probes were prepared by random primer labeling of specific restriction fragments isolated from low melting point agarose gels.

RESULTS

Replacement of the *A* idiomorph by cloned *mt a* DNA:

We developed a method to select replacements of resident *A* idiomorphic DNA by cloned *a* DNA. This strategy relied upon counter-selection of ectopic integrants due to the vegetative incompatibility reaction that results when transformants that retained *mt A-1* at the mating type locus integrated *mt a-1* at an ectopic location. The *mt a* DNA could be integrated at the mating type locus by either a homologous double crossover or a gene conversion event. Gene conversion seems unlikely to occur over such an extensive region of non-homology. The consequences of a double crossover integration at mating type and nonhomologous integration at an ectopic site are compared in Figure 1A. The constructs that we used for replacement are diagrammed in Figure 1B.

Transformation experiments indicated that the vegetative incompatibility selection was an efficient means of identifying gene replacements at mating type. For example, transformation of spheroplasts from a *a* strain with 1 μ g of a hygromycin resistance vector, pCSN43 (STABEN *et al.* 1988), yielded approximately 1000 drug resistant transformants. Transformation of the *a* strain with pCSN61 or pCSK25 in which the same *hph* gene neighbored the *mt a-1* gene also yielded 1000 transformants/ μ g DNA. Although transformation of spheroplasts made from a *A* strain with pCSN43 yielded 1000 transformants/ μ g, transformation with pCSN61 or pCSK25 yielded 1–10 viable transformant/ μ g (Figure 2). The *A*:pCSN61 and *A*:pCSK25 transformation plates also became discolored, which is typical of *N. crassa* cultures undergoing a vegetative incompatibility reaction. Most viable transformants had not switched to *a* mating type (Table 1). We presume that in these transformants the *mt a-1* gene on the plasmid had been interrupted or inactivated during integration.

Genetic characterization of the gene replacement strains: Four viable transformants (CSN61-2, CSN61-10, CSN61-20 and CSK25-18) appeared to be replacements because they mated only as *a* (Table 1). The *mt a* idiomorphs introduced by replacement with pCSN61 and pCSK25 all functioned genetically as if they had integrated at the mating type locus. Replacement strains generated fertile perithecia when crossed to *fl, A* tester strains. Replacement strains also produced ascospores when crossed with their (presumed) perfectly isogenic parent *A* strain. The replacement strains were fertile as either male or female. Female fertility of the replacement strains was confirmed by determining that they were fertile even in crosses with a *A* female sterile mutant strain (*A, ff-1*). Perithecia formed efficiently and were morphologically normal.

Ascospores from crosses with the *fl, A* tester or with the isogenic *A* parent showed 50:50 segregation of *A* and *a* mating type. All of the *a* ascospores yielded hygromycin resistant mycelia; none of the *A* ascospores produced

FIGURE 1.—(A) Gene replacement and selection strategy. During the course of gene replacement, the transforming *mt a-1-hph* DNA replaces the resident *A* mating type DNA at the mating type locus. This results in a viable transformant in which mating type has been switched. The *mt a-1* DNA is tightly linked to *hph* DNA. The genome of the transformant is derived largely from the original *A* host. The DNA immediately flanking mating type may be derived either from the *A* host or the cloned *a* flanking DNA, depending upon the site at which replacement initiates. Ectopic integration leads to an inviable, dual mating type, transformant. (B) Restriction Maps of pCSN61, pCSK25 replacement constructs. The map of pCSN27 shows the locations of restriction sites used in the construction of pCSN61 and pCSK25 as well as sites common to all three plasmids (*SacI*, *SphI* and *HindIII*). Both pCSN61 and pCSK25 contain additional *SacI*, *EcoRI* and *PstI* sites within the *hph* DNA segment that were omitted for simplicity. These maps are oriented such that base 1 of the *mt a* DNA sequence from GenBank is at the left. The *mt* locus is on the right arm of chromosome; these maps are reversed such that the left of the map is actually centromere-distal on these maps and on those in Figure 4.

hygromycin-resistant mycelia (Table 2). These properties are expected of strains in which the resident *A* idiomorph were replaced by the cloned *a* idiomorph DNA.

Molecular characterization of *a* mating type replacement strains: The physical maps of the mating type locus DNA of the *a* replacement strains were consistent with replacement of the resident *A* idiomorph by the transforming *mt a-hph* pCSN61 or pCSK25 constructs. Each *a* replacement strain bore only *a* idiomorphic DNA, no *mt A* DNA. We were unable to detect any *mt A* DNA by polymerase chain reaction with *mt A-1*-specific primers that detected 1% contamination of genomic DNA from a *a* strain with genomic DNA from

FIGURE 2.—Typical gene replacement transformations. Wild-type recipients of both mating types have been transformed with the DNA constructs indicated. Transformants were selected on hygromycin medium as described in materials and methods.

TABLE 1

Frequencies of transformation with various plasmids in *a* and *A* strains

Transforming DNA	Recipient	
	<i>a</i>	<i>A</i>
pCSN43	1000/μg	1000/μg
pCSN61	1000/μg	30/3 μg
		3 <i>a</i>
		30 <i>A</i>
pCSK25	1000/μg	25/5 μg
		1 <i>a</i>
		24 <i>A</i>

TABLE 2

Segregation properties of replaced *a* mating type idiomorph

Strain	<i>a</i> ,hyg ^R	<i>a</i> ,hyg ^S	<i>A</i> ,hyg ^R	<i>A</i> ,hyg ^S
CSN61-2	50	0	0	57
CSN61-10	37	0	0	29
CSN61-20	9	0	0	10
CSK25-18	29	0	0	27

a *A* strain in control experiments. Each *a* replacement strain had also integrated the *hph* gene (Figure 3). We have generated maps of the mating type loci of the replacements (Figure 4) consistent with restriction and hybridization data (Figure 5 contains representative data).

The restriction map of isolate CSN61-2 differed from the other CSN61 isolates. The restriction pattern of this isolate (Figures 3, 5 and 6) suggested that it bears a direct duplication of a large portion of the transforming *mt a* DNA. Some of its properties differ from the other strains and they are discussed separately below.

Mapping data cannot determine whether the replace-

FIGURE 3.—Physical map of *a* replacement strain mating type DNA. Genomic DNAs from *A*, *a*, CSN61-2, CSN61-10, CSN61-20 and CSK25-18 were digested with *EcoRV* and *SphI*, electrophoresed, blotted, and probed as described. From left to right, panels were probed with: with the *mt A-1* region of pMTAG-2, the *mt a-1* region of pCSN4, the *hph* region of pCSN43.

ment events occurred through homologous recombination of the flanking sequences (double crossover) or by gene conversion. However, restriction analyses (Figures 4 and 5) indicated that replacement events preserved the normal structure of the DNA bordering the idiomorph. The DNA within 1 kbp flanking the mating type idiomorph on the centromere distal side was derived from the *A* host. This conclusion is based on the presence in the replacement strains of an *EcoRV* restriction site found in the *A* host DNA, but not in the cloned or genomic DNA from *a* strains (Figures 4 and 5, left). Because of the limited sequence polymorphism in the centromere proximal border (approximately 1%, data not shown), we were not able to determine the origin of the DNA on the centromere-proximal flank of the idiomorph.

The *mt a-1* gene is sufficient to specify *a* mating type:

The gene replacement experiments indicated that the cloned *a* idiomorph was sufficient to confer all *a* mating type properties. The *a* replacement strains were vegetatively incompatible with *A* strains and fertile as either *a* males or as *a* females (data not shown). The replacement strains also produced *a* pheromone and responded to *A* pheromone (G. BISTIS, personal communication). We also concluded that bases 1665–2127 (*SacII-EcoRI*) of the *a* idiomorph, which had been replaced by the *hph* gene in the construction of pCSN61, were not essential for mating and fertility as *a*. We extended this conclusion by constructing pCSK25, which contained only 1600 bases of the *a* idiomorph. The fully fertile and functional pCSK25 replacement strain defines functionally sufficient *a* mating type idiomorph as the *mt a-1* region (bases 2923–4596). However, the ability of pCSK25 to confer fertility as the *a* mating type depended upon integration at the mating type locus. These plasmids can be integrated ectopically into the genomes of hosts that do not undergo the mating type vegetative incompatibility response. However, neither

pCSK25 nor pCSN61 conferred fertility as a *a* strain when inserted ectopically by transformation into *a*^{m30}, *tol*, *A*, or *A*^{m44} recipients (not shown).

Properties of the CSN61-2 *a* duplication strain:

Strain CSN61-2 appeared to bear a duplication of a major portion of the transforming DNA generated during the replacement event (Figures 3, 5 and 6). This strain proved stable to repeated conidial passage with no observed change in the physical map of its mating type DNA or its hygromycin resistance properties.

The duplication strain had unexpected properties in sexual crosses. First, we observed that CSN61-2 generated an unusually high frequency of small, apparently aborted, perithecia, which contained no ascospores. However, perithecia that did mature produced normal ascospores. We determined the mating type and hygromycin resistance of 107 progeny from this cross. Mating type and hygromycin resistance segregated 1:1 among the random spores. No progeny had a null or mutant mating type, nor were any *a* progeny hyg^s. However, the *a* progeny that we examined by restriction enzyme mapping had undergone rearrangements in the mating type DNA (Figure 6). The parent CSN61-2 DNA contained a 4.0-kbp *EcoRI-EcoRV* fragment that its *a* progeny did not contain. From hybridizations with other probes and with other restriction endonuclease digests (not shown), we concluded that CSN61-2 contained a direct duplication of the *mt a-1* and *hph* sequences and that the 4.0-kbp DNA fragment in *EcoRI-EcoRV* DNA fragment represented the core of this duplication (Figure 4). Neither duplicated *a* DNA nor duplicated *hph* DNA was transmitted to *a* progeny of CSN61-2. The restriction maps of the DNA of these *a* progeny were consistent with the resolution of the apparent duplication by recombination such that a single copy of the *mt a-1* and *hph* DNA remained at the mating type locus (Figure 6).

DISCUSSION

Vegetative incompatibility selection: Selection against vegetative incompatibility was a very effective means of selecting for gene replacement at the mating type locus. We recovered fifty-four viable transformants from a pool that would have contained 8000 transformants in the absence of the vegetative incompatibility selection. Four of these transformants were mating type replacements. This suggests that the frequency of homologous integration at mating type was 1 in 2000, much lower than that at the *am* locus (approximately 1/3) (FREDERICK *et al.* 1989; FREDERICK and KINSEY 1990). However, in the mating type replacement events, unlike those at *am*, recombination or conversion occurred on a substrate bearing large nonhomologous regions in each DNA segment. In addition, in comparison to replacements at *am*, the homologous targeting sequences that we used were shorter, and include regions of slight sequence variation in *a* and *A* strains (1–5%, estimated).

FIGURE 4.—Inferred restriction maps of *a*, *A* and replacement strains. In these maps, the idiormorphic regions are represented by the boxes and flanking DNA by the solid lines. The left of this diagram corresponds to the centromere-distal side of the locus. The *mt a* idiormorphs are aligned by their common centromere-distal borders, with the exception of the duplication strain, CSN61-2. Solid arrowhead indicates an *EcoRV* site, gray arrowhead an *EcoRI* site, open arrowheads an *SphI* site. Note that the DNA flanking *mt a* lacks an *EcoRV* site found in the flank of *mt A*, which is shown in parenthesis in the map of wild-type *mt a*. Probes A, B and C were used in Figures 5 and 6.

FIGURE 5.—Physical mapping of *a* replacement strain DNA. Genomic DNA was digested, electrophoresed and probed as described in MATERIALS AND METHODS and Figure 3. DNA was digested with either *EcoRV* (lanes 1–5) or both *EcoRI* and *EcoRV* (lanes 6–10). Probes are center (centromere distal) and left (centromere proximal), indicated as probes B and C, respectively, in Figure 4 and the *hph* gene from pCSN43. Additional digests and hybridizations (not shown) were consistent with the physical maps (Figure 4).

Alternatively, there may be a selection against certain types of replacements at the mating type locus that lowers the observed frequency.

We anticipate that any construct that encodes *a* mating type vegetative incompatibility and contains DNA flanking the mating type idiormorph can be integrated at the mating type locus of *A* strains and selected by our

FIGURE 6.—Analysis of CSN61-2 and its meiotic progeny. Indicated genomic DNAs were digested with both *EcoRI* and *EcoRV* and probed with the entire 6.5-kbp *a* idiormorph and its centromere proximal and distal flanks contained in the transforming DNA constructs (probe A, Figure 4). Lane 1 contained DNA from CSN61-2, while lanes 2–6 contained DNA from random meiotic progeny of *a* mating type, all of which were hygromycin-resistant. Note that the digestion pattern of the DNA from the meiotic progeny is indistinguishable from that of CSN61-10 or CSN61-20 (Figure 5). DNA size markers are indicated at right.

method. A similar stratagem could be useful in mapping the functional regions within the *A* idiomorph by replacement and selection in *a* hosts. Functional mapping of *A* determinants of ascosporeogenesis has been hindered by inability to complement mutations in these functions with ectopically integrated transforming DNA (GLASS and LEE 1992).

Characteristics of replaced mating type genes: Replacement of resident *A* mating type DNA by cloned *a* DNA completely switched the mating type of the recipients. This result indicated that the cloned *a* idiomorph DNA was sufficient to specify mating type when present at the mating type locus. Fertility of the replacement strains contrasts with the infertility of strains bearing mating type DNA integrated ectopically (GLASS *et al.* 1990; STABEN and YANOFSKY 1990).

One can construct many explanations for the difference in fertility between the replacement strains and those bearing ectopic mating type genes. The difference between ectopic and resident mating type genes suggests that *cis*-acting sequences control mating type expression at the mating type locus. Because ectopic mating type genes can control the induction of perithecia, we suspect that the essential difference between ectopic and resident genes is relevant to a post-fertilization activity. Because the ectopic mating type genes do not function in any cell background (*a* mutants, *A* mutants, or *tolerant* mutants of either mating type), we conclude that interference of the resident mating type gene products with the ectopic gene function is not likely. The *a*-specific idiomorph flanks have been substituted by similar sequences from the *A* background in the fertile *a* replacement strains. This suggests that no mating-type specific, flanking elements are required for *a* fertility. Initial deletion analyses (T. C. BADGETT, S. CHANG and C. STABEN, unpublished) suggest that none of the centromere-proximal sequences in pCSK25 or pCSN61 are essential for fertility. Therefore, we favor the hypothesis that residence at the mating type locus confers a special property upon genes that is not found at other locations.

We suggest that ectopic mating type genes may not be properly regulated to fill post-fertilization roles. The mating type locus may confer properties that allow mating type genes to be expressed at times, such as during chromatin condensation in meiosis, when expression from ectopic sites might generally be repressed. In *P. anserina*, mating type DNA transformed into ectopic sites of a strain with a normal, but opposite, resident mating type locus confers fertility. However, these dual mating type strains are much less fertile (as the ectopic mating type) than wild type strains. One result in *P. anserina* that apparently contradicts the expression hypothesis above is that ectopic mating type genes confer normal fertility when inserted into a strain that lacks all mating type DNA sequences (COPPIN *et al.* 1993). In-

terpretation of infertility associated with ectopic mating type genes will rely on generation of a *N. crassa* strain lacking any mating type idiomorphic DNA and characterization of mating type gene function during ascosporeogenesis.

Nonessential idiomorphic DNA sequences: All *a* idiomorphic DNA sequences between residues 1409 and 2923 can be replaced by the *hph* gene from pCSN43 without eliminating fertility. This region contains several open reading frames over 100 amino acids in length, although none show convincing similarities to known or potential polypeptides in databases (ALTSCHUL *et al.* 1990). We have not detected mRNA from this region under any culture condition (S. CHANG and C. STABEN, unpublished). This region of the *a* idiomorph either encodes gene products that are not essential for mating and not expressed under the conditions we have assayed, or it may not encode any gene products. This nonessential fragment may evolve rapidly in the genus *Neurospora* and its relatives, resulting in great sequence divergence in this region.

Nonessential DNA is also found in the mating type idiomorphs of other fungi. The *P. anserina* *mat+* idiomorph is similar in size to the *N. crassa* *a* idiomorph, and the region outside *FPR1* (the *mt a-1* homolog), as in *N. crassa*, is also not essential for fertility (DEBUCHY and COPPIN 1992). The *C. heterostrophus* idiomorph contains only a 1.2-kbp *MAT1-2* region (TURGEON *et al.* 1993) homologous to *mt a-1*. The *Saccharomyces cerevisiae* *MATa* allele (or idiomorph) also contains a nonessential DNA region, *mata2*. This gene encodes a polypeptide, but has no known function (HERSKOWITZ 1989). The presence of nonessential DNA at fungal mating type loci is intriguing from both a functional and an evolutionary perspective.

Potential roles of DNA flanking the mating type idiomorph: Many loci that control mating in fungi are complex and contain multiple genes. Although additional determinants of mating type could flank the known idiomorphs, they must not be essential for fertility as *a*. If these determinants were essential, the replacement strains would not be fertile. The DNA in these strains other than the *a* idiomorph and its immediate flanks were derived from the *A* parent. Also, any determinants of mating or additional idiomorphic regions in the genome of *A* strains that remained in the replacement strain did not interfere with *a* function. Isogenic strains that differ *only* at mating type were fertile when crossed.

Properties of the *a* duplication strain: The strain that bore a duplication of the *mt a-1* and *hph* sequences generated both abortive and fertile perithecia. The duplicated DNA segment was resolved into a single copy at high efficiency during sexual crosses. Direct duplications of this sort are known to recombine premeiotically at high efficiency (SELKER *et al.* 1987). Duplications are

also subject to the RIP process, a process that mutates both copies of a duplication after fertilization, but before diploid formation) (SELKER *et al.* 1987; CAMBARERI *et al.* 1989). Either recombination or RIP can alter the same duplex substrate. Southern analysis of five *a* progeny showed that, in each case, the duplication was resolved to a single copy (Figure 6). We did not find any ascospores that had a null mating type. We have not assayed for sequence changes due to RIP in the remaining *mt a* segments. However, we infer from our inability to recover null mating type mutants from these crosses that mutation of *mt a* by RIP may prevent formation of viable ascospore progeny, consistent with the presumed post-fertilization roles of *a*. Inactivation by RIP could also account for the high frequency of small, apparently aborted, perithecia formed in crosses with the duplication parent.

Functions of the *a* idiomorph: The *mt a-1* region specified all known functions of the *a* mating type: vegetative incompatibility, pheromone production, pheromone response, induction of perithecia, and production of ascospores. The functions of *mt a-1* during ascosporeogenesis can be inferred from genetic data. First, mutant *a^{m1}* nuclei do not enter ascogenous hyphae (RAJU 1992). (The *a^{m1}* mutant is a null *mt a-1* mutant (STABEN and YANOFSKY 1990).) Second, dikaryotic (*a/A*) ascogenous hyphae develop from the fertilized ascogonium. The fertilized ascogonium is syncytial and contains many *a* and *A* nuclei, so some mating type-dependent mechanism must ensure that only dikaryons formed from *a* and *A* nuclei enter meiosis and give rise to ascospores.

The product of the *mt a-1* gene appears to be a multifunctional DNA-binding polypeptide, MT a-1 (PHILLEY and STABEN 1994). This putative DNA-binding polypeptide has the potential to control many target genes essential for the complex developmental pathways regulated by *a* mating type. All *a* mating type functions must be encoded by the *mt a-1* region (bases 2923–4596) and must be a function of MT a-1 activity. Deletions beyond base 2923 lack *mt a-1* activity in transformation assays, and would presumably lack activity in replacement strains. (Such replacements cannot be selected by using our strategy.) Because the mating type genes play so many different roles in orchestrating the morphologically complex sexual development in *N. crassa*, we anticipate that the activities of MT a-1 are modified during development to assume changing regulatory roles. However, it is clear that MT a-1 is the central molecule in determination of *a* properties.

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