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Genetics

Neurospora crassa a mating-type region

(sexual reproduction/vegetative incompatibility/perithecium formation/filamentous fungus)

CHUCK STABEN* AND CHARLES YANOFSKY

Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020

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ABSTRACT **The *a* mating-type region of *Neurospora crassa* controls several major events in both the sexual and asexual phases of the fungal life cycle. This 3235-base-pair DNA segment is not homologous to the comparable genetic region of the *A* mating type. The unique *a* and *A* regions are bordered by nearly identical DNA sequences. The *a* genetic region contains at least two functional segments. One segment encodes a perithecium maturation function that is dependent on the second segment for phenotypic expression. This second *a* segment encodes a spliced mRNA that specifies the mt a-1 polypeptide. This polypeptide appears to be responsible for vegetative incompatibility, mating identity, and perithecium induction. The *a-1* transcript is produced vegetatively and under conditions that induce sexual differentiation. The amino-terminal half of the mt a-1 polypeptide is homologous to the shorter *Schizosaccharomyces pombe* mat-M_c polypeptide. This homology and the properties of *mt a-1* mutants suggest that the a-1 polypeptide segment that is homologous to the mat-M_c polypeptide may be primarily responsible for mating functions, while the distal segment is required for vegetative incompatibility.**

Neurospora crassa is a haploid, heterothallic, filamentous fungus with two stable mating types, designated *a* and *A* (1). The mating-type locus of this ascomycete controls several key events in its life cycle. During vegetative growth *Neurospora* proliferates as a branching mycelium. Under appropriate environmental conditions, strains of either mating type form female sexual structures (protoperithecia and trichogynes) and vegetative spores (conidia). Fusion of the specialized female trichogynes with cells of opposite mating type, generally conidia, initiates sexual reproduction. During the sexual phase, each protoperithecium differentiates into the more complex perithecium, which is composed of maternal and zygotic tissue. The perithecium ultimately harbors many asci, each containing eight haploid ascospores, the products of meiosis. The mating-type locus mediates several processes in addition to mating (2, 3), including secretion of and response to pheromones (4, 5) and vegetative incompatibility (6). Although hyphal filaments fuse freely during vegetative growth, incompatibility loci, including mating type, limit the formation of viable heterokaryons.

Initial molecular characterization of mating-type DNAs from *N. crassa* revealed that the *a* and *A* DNA segments are unrelated (7). These unrelated segments of DNA occupying the same chromosomal location have been named idiomorphs (8). The mating-type genes of *Saccharomyces cerevisiae* (9) and *Schizosaccharomyces pombe* (10) also are nonhomologous. However, the unique *N. crassa* mating-type DNA regions, 3.2 (*a*) and 5.1 (*A*) kilobase pairs (kbp) long are much larger than in *S. cerevisiae* (700 bp) or in *Sc. pombe* (1 kbp). Unlike either yeast, *N. crassa* contains no silent copies of mating-type information, and it does not undergo mating-type switching. There are several self-fertile haploid homothallic species of *Neurospora*. These species either have both *a* and *A* genetic material, or only the *A* sequence (7). Apparently homothallism has been achieved either by inclusion of genetic material from both mating types within the same nucleus, as in yeast, or by activation of sexual reproduction independent of an *a*/*A* interaction.

In this paper, we present the sequence of the *a* genetic region and analyze its functions. The accompanying paper (11) describes a comparable study of the *A* genetic region.

MATERIALS AND METHODS

Strains. *Escherichia coli* strain TG1 was used as the plasmid host. *N. crassa* strains were provided by David D. Perkins (Stanford University). These included *A*, *a*, *fl A*, *fl a*, *tol A*, and strains bearing the a^{m1}, a^{m30}, and a^{m33} mutations (12). (FGSC 2489, 4200, 4317, 4347, 2338, 4564, 6844, and 5382, respectively).

Plasmids and Bacteriophage. Routine subcloning was performed with the Bluescript SK^+ vector from Stratagene (La Jolla, CA). The molecular biology procedures used were standard methods (13). In transformation experiments, we used the hygromycin-resistance marker in pCSN43 (14). Sequencing and transformation were performed with subclones derived from λ phage E9 (7). Single-stranded templates for DNA sequencing were derived from cells bearing phagemids by infection with M13 MK07.

Materials. Restriction endonucleases and DNA modification enzymes were supplied by Bethesda Research Laboratories, New England Biolabs, or United States Biochemical and were used according to the supplier's instructions. Oligonucleotide primers were synthesized on an Applied Biosystems model 380A DNA synthesizer by Mark Rousseau or Lauren Shirvanee (Stanford University). Hygromycin B was from Calbiochem.

Transformation of *N. crassa*. Constructs tested for mating-type activities were introduced into *N. crassa* by cotransformation (15) and selection for hygromycin B resistance (14). The use of transformants in assays of mating-type functions was essentially as described by Glass *et al.* (7).

DNA Sequencing. The *a* DNA segment was divided by subcloning into four primary sequencing targets. Nested deletions of these clones were prepared by the exonuclease III deletion method (16). Single-stranded templates from these clones were sequenced on an Applied Biosystems 370A sequencer according to protocols supplied by the manufacturer. Data were collected by using the UWCG programs (17). Overlapping clones covering both strands in both directions were sequenced.

Polymerase Chain Reaction (PCR) Analysis of Mutant *a* Alleles. Genomic DNAs (18) from *a* mutants were amplified

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Abbreviations: PCR, polymerase chain reaction; ORF, open reading frame.

*Present address: T. H. Morgan School of Biological Sciences, 101 T. H. Morgan Building, University of Kentucky, Lexington, KY 40506-0225.

by the PCR (19) using oligonucleotide primers (bases 2656–2672 and 4629–4610). Amplified fragments were cleaved with *Pst* I and *Eco*RV and inserted into pUC118. Single- or double-stranded templates were sequenced with specific oligonucleotide primers on both strands. These constructs were also used in transformation assays to determine the phenotype corresponding to each cloned segment. Mutations were repaired by replacing a restriction fragment containing a base change with a normal-sequence fragment. The fragment used to repair a^{m1} and a^{m33} was *Nco* I/*Eco*RV (base pairs 4029–4595), and that for a^{m30} was *Bam*HI/*Nru* I (base pairs 3570–3821). Repaired constructs were resequenced to confirm base changes.

Analysis of *a* mRNA. RNA was prepared by a glass-bead disruption/phenol-extraction method (M. S. Sachs and C.Y., unpublished data) from liquid cultures of *N. crassa* grown in Vogel's N or synthetic cross medium (20). Poly(A)$^+$ RNA was prepared by standard methods (13). These RNAs were treated with RQ1 DNase (Promega). One microgram of each RNA was hybridized to oligo(dT) primer and cDNA was synthesized with Moloney murine leukemia virus reverse transcriptase (BRL) as suggested by the supplier. One-tenth of each cDNA was used in PCR amplification reactions with primers (bases 3243–3259) and (bases 4100–4084) corresponding to sequences within the open reading frame segments that span the presumed intron splice junctions. Amplified products were cloned and sequenced by standard methods.

RESULTS

Structure of *a* DNA of *N. crassa*. The complete nucleotide sequence of the unique *a* region and flanking segments was determined.† A region of 3235 bp is unique to *a* strains; in *A* strains, it is replaced by a DNA segment of 5301 bp (Fig. 1) (11). The sequences flanking the unique DNA segments in *a* and *A* strains are virtually identical (see ref. 11).

Two Functional Regions Within Unique *a* DNA. We assessed the vegetative incompatibility, mating identity, and perithecium differentiation functions of segments of *a* DNA by transformation into appropriate strains of *Neurospora*. These assays were based on cotransformation of DNA containing a hygromycin-resistance gene and DNA from different segments of the *a* genetic region (Fig. 1).

When the transformation recipient was a normal *A* strain and the *a* donor DNA was fragment 2 (Fig. 1, nucleotides 2919–4600) or fragment 3 (Fig. 1, nucleotides 1–6582), the frequency of hygromycin-resistant transformants was greatly reduced. In addition, the agar surface of the transformation plates became dark brown, which is indicative of a heterokaryon incompatibility reaction. Consistent with this result, which suggests that transformants of normal *A* strains containing fragment 2 or 3 are nonviable, the hygromycin-resistant colonies that did appear did not contain *a* DNA. If fragment 2 is shortened at either end by 100 bp, the shortened fragment does not cause an incompatibility reaction. Parallel transformation analyses in which the *a* DNA segment used was fragment 1 (Fig. 1, nucleotides 1–2127) gave the same frequency of hygromycin-resistant transformants as the control in which hygromycin-resistant DNA was used alone. In addition, the resulting transformants had no unusual phenotypic characteristics. We conclude that the *a* DNA segment from 2919 to 4600 contains sequences that are necessary for heterokaryon incompatibility.

Fragments of DNA from the *a* idiomorph were successfully cotransformed into a *A* recipient strain that contained the genetic marker *tol*, an unlinked suppressor of mating-type vegetative incompatibility (21). Such transformants were used to assay mating identity and perithecium differentiation functions. These transformants were examined for their ability to mate with *fl A* tester strains. Matings with transformants containing fragment 2 or 3, but not fragment 1, yielded perithecia. Therefore, fragments 2 and 3 bear genetic information that identifies a strain as *a* for mating, as well as expressing vegetative incompatibility. The perithecia produced by mating *tol A*:*a* transformants with *A* strains, and those formed by the transformants themselves on synthetic crossing medium, produce no ascospores. They resemble the barren perithecia typical of matings of strains bearing chromosomal duplications (22). However, *tol A*:*a* transformants containing fragment 3 produced ascospores when crossed to normal *a* strains. This fertility suggests that the short duplicated sequences bordering the unique mating-type region are not the cause of sterility in crosses with *fl A*. Rather, the mating-type DNA segments tested may function properly only when present at their normal chromosomal location. These studies also indicate that *A* is not dominant to *a*, since the *tol A*:*a* transformants initiate mating with both *A* and *a* tester strains.

Homokaryotic *tol A* transformants containing the different *a* DNA segments were transferred to synthetic crossing medium and examined for the differentiation normally associated with mating. Transformants bearing DNA fragment 2 or 3 produced structures resembling perithecia. However, the extent of perithecial development induced by the two fragments was different. Transformants with fragment 2 formed small pseudoperithecia that did not contain ascogenous material, whereas transformants with fragment 3 formed full-sized perithecia that had differentiated beaks and appeared to contain ascogenous tissue. However, these perithecia did not produce spores. Transformants containing fragment 1 had no features that distinguished them from control cultures. However, because this additional DNA segment as part of fragment 3 permitted perithecial maturation to the beak stage, it is likely that some portion of the fragment 1 segment contributes to one or more differentiation functions. In support of this conclusion, deletion of the region from 1615 to 2127 by homologous integration of a disrupted gene fragment prevents ascospore formation when this deletion derivative is crossed by a standard *A* strain.

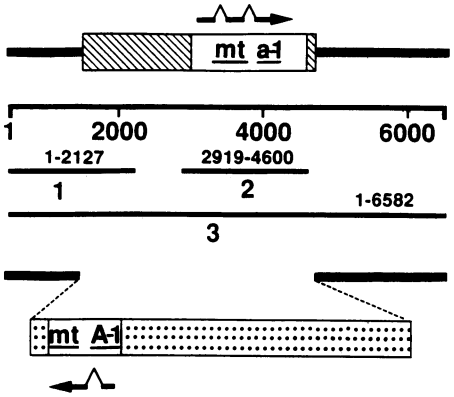

FIG. 1. Comparison of the mating-type regions *a* and *A*. Identical sequences bordering the unique regions (idiomorphs) are indicated by thick lines. The idiomorph sequences are shown in contrasting patterns, with the *mt a-1* and *mt A-1* (11) ORFs indicated. Presumed transcripts are represented by thin lines with arrows. Introns are indicated by indentations in these lines. The nucleotide numbers refer to the *a* idiomorph. Fragments labeled 1, 2, and 3 extend from nucleotides 1–2127, 2919–4600, and 1–6582, respectively (see Fig. 3 for the sequence of fragment 2).

†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M34287).

Structural Features of the *a* Region. The portion of the *a* idiomorph bearing the mating identity and vegetative incompatibility functions contains an open reading frame (ORF) encoding a spliced transcript that could specify a polypeptide of 382 amino acid residues (Fig. 2). We have designated this transcriptional unit *mt a-1*. The *mt a-1* mRNA is produced at very low levels under both nitrogen-rich and nitrogen-limited growth conditions (see below). We have not mapped the initiation or polyadenylylation sites of this mRNA. However, a number of eukaryotic transcription control sequences are upstream of the ORF, including TATAA, ACAAT, and CAAG, as well as a polypyrimidine tract (Fig. 2). Each of these motifs has been found upstream of genes from filamentous fungi (23), although the *mt a-1* upstream segment is atypical in having them all.

We presume that the *a-1* ORF initiates with the methionine codon beginning at nucleotide 3227 and terminates with the TGA codon at nucleotide 4483 (Fig. 1). This open reading frame is interrupted by two introns, 53 and 57 bases long. The borders of the introns and the internal branch sites conform to *Neurospora* consensus sequences (23). Codon usage in this 382-codon ORF is typical of *Neurospora* genes (23). Although there are several ORFs within the first half of the *a* region, none displays *Neurospora* preferred codon usage or is homologous to proteins in the data bases.

A search for known proteins in the Protein Identification Resource, National Biomedical Research Foundation data bank (16), and a search of translation products of DNA data bases (24) identified the *Sc. pombe* mat-M_c polypeptide as the only close homolog of the *a-1* ORF of *Neurospora* (Fig. 3). The homology is within the first half of the mt a-1 polypeptide, although it extends over almost the entire length of the shorter *Sc. pombe* polypeptide. We have not detected homology of the mt a-1 polypeptide to other yeast mating-type polypeptides or to the proposed *N. crassa* mt A-1 polypeptide (11). The predicted mt a-1 polypeptide sequence contains many PEST sequences, which, in other organisms, are associated with protein lability (25). There are 18 prolines in the carboxyl-terminal 100 residues of the predicted mt a-1 polypeptide; similarly there are 23 prolines in the 100-residue transcription activation domain of CTF/NF-1 (26).

***mt a-1* mRNA.** We were unable, using a variety of labeled *mt a-1* DNA probes, to detect *mt a-1* mRNA on Northern blots. To establish that *mt a-1* mRNA was produced from the *mt a-1* region, we prepared cDNAs from poly(A)$^+$ mRNAs and amplified these cDNAs by using the PCR. Four amplified products were present after cDNA synthesis and amplifica-

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2919 CTGCAGGAATCCCCCAAGCCATTCACCGCCCAGTGAATGCTATTCAGGGCCGTGTCAATAGCCATCAATAGACAAAGCGGTTCCACCCAA

3009 AAATCCCGACGTCGTTGCCCCTCCTTATAATCTCCCTCCCTTCCAATTCTTCCTCCTTCCCACTCCTAACATTCCTCCGTCAACATCGCA

3099 ACAATAGTCGACAATCACGACCGATCTTCGCCATCTACTCATTTCTCCTTTCCACAACCAGTCGCCCAAGTCTTTGACAATCTCAAGCAT

3189 CAGCCTTCTTCATCACCAGAGAACGATCAACCGAAACAATGGACGGTAACTCGACACACCCCGCTCCAAACCTCAAGACTACTATGGCTT
                                          MetAspGlyAsnSerThrHisProAlaProAsnLeuLysThrThrMetAla

3279 GGTCGCGCATATCAAACCAACTCGGTCACTGGAATGACCGCAAGGTCATTGCCATTCCTCTGAGCGACTTCCTTAACACCCACCCTGACA
    TrpSerArgIleSerAsnGlnLeuGlyHisTrpAsnAspArgLysValIleAlaIleProLeuSerAspPheLeuAsnThrHisProAsp

3369 TTCAGTCTGGCATCATCGCCGAGTTCAAGTAAGTGTCCTCACCCATTTCTCACCCTACCTTGTACTGACCATTTGCACTAGGAAAGCGAC
    IleGlnSerGlyIleIleAlaGluPheLys                                                    LysAlaThr

3459 TGGCGAAGAGGGCATGTTTGCCCGCGATCCTGAATCATTGGGAATCATGCTTCTTGGTCCCGTCAAGCTGTTCAAGCCCGACAGTGTCGT
     GlyGluGluGlyMetPheAlaArgAspProGluSerLeuGlyIleMetLeuLeuGlyProValLysLeuPheLysProAspSerValVal

3549 CGTCGACGGCAACCTGTTCTGGGATCCCAAGGGCATCCATGCTTCGGCACCCAAGGAGCAGCAGAAGAAGGCCAAGATCCCTCGCCCTCC
     ValAspGlyAsnLeuPheTrpAspProLysGlyIleHisAlaSerAlaProLysGluGlnGlnLysLysAlaLysIleProArgProPro
                          #
3639 CAATGCCTACATCTTGTACCGTAAGGACCATCATCGTGAGATCCGCGAGCAGAATCCCGGACTTCACAATAACGAGATTTGTAAGTTTCT
     AsnAlaTyrIleLeuTyrArgLysAspHisHisArgGluIleArgGluGlnAsnProGlyLeuHisAsnAsnGluIle

3729 TGTCATCATGATCGAAAATCTTTGGCCTTGAGACTAACCTCACTTAGCGGTCATCGTCGGCAACATGTGGCGTGATGAGCAGCCGCACAT
                                          SerValIleValGlyAsnMetTrpArgAspGluGlnProHisIle

3819 TCGCGAGAAATATTTCAACATGTCCAATGAGATCAAGACCAGACTGTTGCTGGAGAATCCCGACTATCGCTACAATCCGCGTCGGTCTCA
      ArgGluLysTyrPheAsnMetSerAsnGluIleLysThrArgLeuLeuLeuGluAsnProAspTyrArgTyrAsnProArgArgSerGln

3909 AGACATTCGCAGGCGCGTCTCGCCGTATCTCAAGATCAAGCTCCTCAACTACGACGTTAATGGCAACCTTCTTTGGGGCACCGTCAACGC
      AspIleArgArgArgValSerProTyrLeuLysIleLysLeuLeuAsnTyrAspValAsnGlyAsnLeuLeuTrpGlyThrValAsnAla
                                                                                (ΔC)
3999 CGAGGATGCTGCGCTGATTCGGACTCACTTCCATGGAGTCGTTCGTGTTGAGGAAATGGATGATGGTTGCAGAATCGTCTGCCGTCCCGT
      GluAspAlaAlaLeuIleArgThrHisPheHisGlyValValArgValGluGluMetAspAspGlyCysArgIleValCysArgProVal
                            A
4089 CGCAGGATCTAGAAAACTTCGCGCCGCCGTTGTCGACACTTGGATGCCTCGCTACACGGTTGACACAACCCCCGTCACCGAGGACGACGA
      AlaGlySerArgLysLeuArgAlaAlaValValAspThrTrpMetProArgTyrThrValAspThrThrProValThrGluAspAspAsp
                         -Ser-
4179 TGCACAGGCTTTCAACTTCAATGATCCCTTGGGCGGTGCTTATTTCCCTTTGAATGAGCACCTCTGGATCACTGTCAACCAAAACCCTCC
      AlaGlnAlaPheAsnPheAsnAspProLeuGlyGlyAlaTyrPheProLeuAsnGluHisLeuTrpIleThrValAsnGlnAsnProPro

4269 CTTCAATGCCCCTCCCCCCAATCCCAACCCACACCTGGATTTCGTTCACCCCGACGGCATGGAGGCAGTTGTTCACAACGTTCAGAACAT
      PheAsnAlaProProProAsnProAsnProHisLeuAspPheValHisProAspGlyMetGluAlaValValHisAsnValGlnAsnMet

4359 GATCGCTCAGGTCCAGGAGGCTAACGAGGCTGCTGCGCTAACGCTACCACCGCCACCACCGCTGCGTCTGCTGTCACTCAGGTTATGGCT
      IleAlaGlnValGlnGluAlaAsnGluAlaAlaAlaLeuThrLeuProProProProProLeuArgLeuLeuSerLeuArgLeuTrpLeu

4449 GATGATACCATTAACCCAGCTCTCATTCCCACTGTGAACACTCATGCATCTGTTCTTCCCTACGTCCATACCATTCCTGACAACGCCACC
      MetIleProLeuThrGlnLeuSerPheProLeuEND

4539 GTTACGCCTTCCGCTACTGGAAACTCGGTTCACGTTGTTACACCCGGTCACCAAGGATATC
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FIG. 2. Sequence of the *mt a-1* functional region. Nucleotides 2919–4600 of the *a* DNA sequence are presented. This is the sequence of fragment 2 in Fig. 1. The presumed amino acid sequence of the encoded mt a-1 polypeptide is shown below the sequence. Gaps in translation correspond to the locations of proposed introns. TATAA, ACAAT, and CAAG sequences are double underlined; the pyrimidine-rich region upstream of the ORF is single underlined. Locations of mutations discussed in the text are indicated above the positions of the changes: #, a^{m30}; ΔC, a^{m1}; and A, a^{m33}. For the last mutation, the nucleotide change and predicted amino acid sequence change are given.

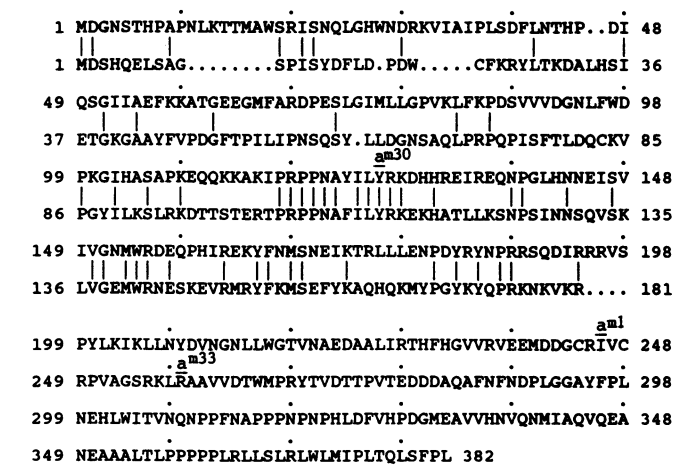

FIG. 3. Homology of the mt a-1 and *Sc. pombe* mat-M_c polypeptides. The *Neurospora* sequence is on the top line. Complete sequences of both polypeptides are shown. The alignment was made with the GAP program (17). Identical residues are denoted by vertical lines. The sequences are 30% identical over the length of the *Sc. pombe* polypeptide, which is shorter (181 residues) than the *Neurospora* polypeptide (382 residues). The positions of the changes in *mt a-1* DNA in mutants a^{m30}, a^{m1}, and a^{m33} are indicated.

tion with primers that flanked the proposed intron locations. The largest product (854 bp) was formed when the mRNA had not been reverse transcribed and when the mRNA was treated with RNase A, suggesting that it was an amplification product of residual DNA in the RNA preparation rather than of unspliced mRNA. The three smaller products were individually cloned and sequenced. The sequence of the 747-bp product corresponded exactly to a mRNA spliced at the predicted splice junctions. This fact reinforces our interpretation of the nature of the transcriptional unit encoding the mt a-1 polypeptide. The 747-bp product was detected in *a* mycelium grown in liquid medium under both high and low nitrogen conditions. The two smaller products contained both specific *a* primer sequences, but the amplified sequences were from other regions of the *Neurospora* genome. We presume that these products were amplification artifacts.

Mutations in the *mt a-1* ORF. We characterized three previously described mating-type mutants (12); each was found to have a sequence change in the *mt a-1* ORF (Figs. 2 and 3). The mutant DNAs were amplified by PCR with primers flanking the *mt a-1* ORF. Amplified products were cloned into pUC118 for sequence analysis and transformation assays. Mutant a^{m1} has a 1-base deletion of C at 4074, causing a frameshift in the *mt a-1* coding region. This change results in loss of mating type and vegetative incompatibility functions. The second mutant, a^{m30}, also lacks both activities. The a^{m30} mutation is an insertion of 212 bp into the *mt a-1* ORF at nucleotide 3653. This insertion introduces an amber codon 10 codons downstream of the insertion, presumably truncating the mt a-1 polypeptide within the sequence most homologous to *Sc. pombe* mat-M_c polypeptide. We have shown that the sequence inserted in a^{m30} is duplicated elsewhere in the genome and is present in the genomes of both *a* and *A*. Initial restriction fragment length polymorphism mapping (27, 28) suggests that the normal location of the insertion sequence is distinct from the mating-type locus. A unique mutant, a^{m33}, is fertile but insensitive to vegetative incompatibility (12). The a^{m33} mutation changes Arg-258 of the mt a-1 polypeptide to Ser. Restoration of the normal *mt a-1* sequence by substitutions of short restriction fragments into the three cloned mutant DNAs yielded DNAs that restored all the activities of wild-type *mt a-1* DNA when examined in transformation assays.

DISCUSSION

DNA hybridization analyses provided the initial evidence demonstrating dissimilarity of the sequences of the *a* and *A* mating-type regions of *N. crassa* (7). The DNA sequence analyses presented in this and the accompanying paper (11) provide a precise description of this dissimilarity. The unlike DNA sequences that occupy the same chromosomal location are referred to as idiomorphs, a term that was introduced to describe this genetic relationship (8). The borders of the idiomorphs and their flanking homologous sequences are sharp, as they are for yeast mating-type cassettes (9, 10). The sequences of the flanking segments are highly conserved (11). The mechanisms responsible for maintaining this conservation are not obvious. The significance of the flanking sequence similarities is discussed in the accompanying paper (11).

The phenotypes of *A* transformants bearing different portions of the *a* idiomorph suggest that there are two functional regions within this genetic segment. One region, *mt a-1*, appears to be responsible for mating identity/perithecium induction/vegetative incompatibility functions, while the second may determine a perithecium differentiation function. The *mt a-1* region contains an ORF that could encode a 382-residue polypeptide. The remaining segment of the *a* idiomorph does not contain any open reading frame that exhibits *Neurospora* codon preferences.

The results of studies with the *N. crassa A* region suggest that the *A* idiomorph also has at least two functional regions (11). Known yeast mating-type DNAs also encode several products. For example, in *Sc. pombe* (10) each mating-type region encodes two polypeptides; two are sufficient for conjugation, but all four are required for meiosis and sporulation. Close linkage of nonhomologous multiple mating-type genes would ensure preservation of heterozygosity for these genes whenever mating was successful.

The similarities of the predicted sequences of the mt a-1 and *Sc. pombe* mat-M_c polypeptides suggest that these polypeptides perform similar functions. However, the yeast protein, which is shorter, lacks the segment of the mt a-1 polypeptide that is probably responsible for vegetative incompatibility. This conclusion is based on the finding that the a^{m33} mutation, which interferes only with vegetative incompatibility, is located beyond the region of homology with the *Sc. pombe* protein. It seems possible, therefore, that the amino-terminal half of the mt a-1 polypeptide is mostly concerned with mating control functions while the vegetative incompatibility function requires the carboxyl-terminal half. The near identity of the PRPPNA(Y/F)ILYRK sequences in the mt a-1 and mat-M_c polypeptides (Fig. 3) suggests that this sequence in these polypeptides is functionally important, presumably for some mating function.

The *mt a-1* ORF is transcribed and spliced under both vegetative and nitrogen-limited conditions. In this respect, its expression is similar to that of its homolog, the *Sc. pombe* *mat-M_c* gene (10).

In addition to the apparent homology of the mt a-1 and *Sc. pombe* mat-M_c polypeptides, mt A-1 appears to be homologous to the MATα1 polypeptide of *S. cerevisiae* (11), and *S. cerevisiae* MATα2 is homologous to *Sc. pombe* Mat-P_i (10). These similarities suggest that these mating-type genes originated in an ancestor of the *Hemiascomycetes* and the *Euascomycetes*. Consistent with this conclusion, homologs of the *Neurospora* mating-type idiomorphs have been detected in related ascomycetes (N. L. Glass, R. L. Metzenberg, and N. Raju, personal communication). It is likely, therefore, that the mating-type genes encode a family of proteins with similar functions, and these functions are conserved within the order. Mating patterns and mechanisms within this order may be explicable as the consequences of

changes in the idiomorphs and changes in the genes and elements with which the mating-type products interact (8).

Note Added in Proof. We have detected sequence similarity of segments of the *N. crassa* mt a-1 and *Sc. pombe* mat-M_c polypeptides to the high mobility group (HMG) proteins and human upstream binding factor (29). These similarities are restricted to the HMG boxes.

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