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Mapping of the *Cryptococcus neoformans MAT*α Locus: Presence of Mating Type-Specific Mitogen-Activated Protein Kinase Cascade Homologs

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In this study we investigated the relationship between the *MAT*α locus of *Cryptococcus neoformans* and several *MAT*α-specific mitogen-activated protein (MAP) kinase signal transduction cascade genes, including *STE12*α, *STE11*α, and *STE20*α. To resolve the location of the genes, we screened a cosmid library of the *MAT*α strain B-4500 (JEC21), which was chosen for the *C. neoformans* genome project. We isolated several overlapping cosmids spanning a region of about 71 kb covering the entire *MAT*α locus. It was found that *STE12*α, *STE11*α, and *STE20*α are imbedded within the locus rather than closely linked to the locus. Furthermore, three copies of *MF*α, the mating type α-pheromone gene, a *MAT*α-specific myosin gene, and a pheromone receptor (*CPR*α) were identified within the locus. We created a physical map, based on the restriction enzyme *Bam*HI, and identified both borders of the *MAT*α locus. The *MAT*α locus of *C. neoformans* is approximately 50 kb in size and is one of the largest mating type loci reported among fungi with a one-locus, two-allele mating system.

Cryptococcus neoformans is the etiologic agent of cryptococcosis, which is one of the most serious fungal diseases encountered worldwide. Although *C. neoformans* primarily affects patients with impaired immune systems, people with no known underlying immunodeficiencies are also affected (9).

C. neoformans is a bipolar heterothallic fungus in which completion of the meiotic cycle is dependent upon interaction between cells of *MAT*α and *MAT***a** types. Although segregation patterns of the meiotic products yield *MAT*α and *MAT***a** progeny in the expected 1:1 ratio for a bipolar heterothallic fungus (12), *MAT*α strains are found far more frequently than *MAT***a** strains in clinical as well as environmental isolates among serotype D strains (10). Among serotype A strains, *MAT*α has thus far been the only mating type to be found regardless of isolate source (13). To investigate the relationship between mating type and virulence, Kwon-Chung et al. (11) constructed a pair of congenic *MAT*α (B-4500) and *MAT***a** (B-4476) strains of serotype D for genetic analysis. In a mouse systemic infection model, both the parental strain and F_1 progeny of *MAT*α type were found to be significantly more virulent than the *MAT***a** parental strain and its F_1 progeny (11). Therefore, molecular and pathobiological studies of *C. neoformans* serotype D isolates have since been carried out mostly with *MAT*α strains (3, 20).

In 1993, Moore and Edman (16) identified the *MAT*α locus by employing a difference cloning method using the congenic strains B-4500 (JEC21, *MAT*α) and B-4476 (JEC20, *MAT***a**). The *MAT*α locus was marked by the presence of the *MF*α gene, which encodes a pheromone precursor. Analysis of *MAT*α-specific phage and cosmid clones led them to conclude that the *MAT*α locus was 35 to 45 kb in size. Subsequently, Wickes et al. discovered haploid fruiting to be a *MAT*α-specific phenomenon in *C. neoformans* (26). Molecular analysis of haploid fruiting in *MAT*α strains resulted in the cloning of the *STE12*α gene, a homolog of the *Saccharomyces cerevisiae* transcriptional activator *STE12*, and the *STE11*α gene (26), a homolog of the *S. cerevisiae STE11* (MEKK) gene (19, 22). Recently, Wang and Heitman isolated *STE20*α (accession no. AF162330), a homolog of *S. cerevisiae STE20*, from H99, a serotype A *MAT*α strain of *C. neoformans*. Although these genes were reported to be specific for *MAT*α strains, their genomic locations have not been clearly determined. Additionally, *STE12*α was not found in the *MAT*α-specific region (26) previously reported by Moore and Edman (16), nor was a *MAT*α-specific receptor of the *MAT***a** pheromone found, a gene which should be mating type specific.

The *STE12*α gene recently has been deleted from both serotype D and serotype A strains, and the gene was found to be essential for haploid fruiting but not for mating (4, 28). Furthermore, *STE12*α was reported to regulate several virulence associated genes in serotype D *C. neoformans* (4). Mating type-specific mitogen-activated protein (MAP) kinase genes of the signal transduction cascade have not been reported in any other fungi. Since the importance of these genes in the pathobiology of *C. neoformans* is becoming increasingly evident (4; D. L. Clarke, U. Edman, G. L. Woodlee, C. M. McClelland, T. S. Seymour, J. C. Edman, and B. L. Wickes, unpublished data), we have attempted to determine their genomic locations.

In this paper, we present a physical map of the B-4500 *MAT*α locus which was obtained by analysis of overlapping subclones isolated from a cosmid library of B-4500. The *MAT*α locus, which spans 50 kb, contained several mating type α-specific homologs of the pheromone response MAP kinase signal transduction cascade genes, multiple copies of *MF*α, and one copy of the pheromone receptor gene, *CPR*α. Unexpectedly, a myosin gene and a homolog of *S. cerevisiae* translation initiation factor, *PRT1*, specific to the *MAT*α strain, were also discovered within the locus.

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TABLE 1. *E. coli* plasmids and cosmids used in this study

Plasmid or cosmid	Construction	Source or reference
pNH7	Rescued plasmid with 8.5-kb insert, containing *STE12*α	26
pC12-1.3	1.3-kb *Bam*HI fragment in pBluescript KS(−)	This work
pC12-6.5	6.5-kb *Bam*HI fragment in pBluescript KS(−)	This work
pC12-17	12-kb *Bam*HI fragment in pBC KS$^-$	This work
pC12-4.5	4.5-kb *Bam*HI fragment in pBC KS$^-$	This work
pSCC93-3.8	3.8-kb *Bam*HI fragment in pBluescript KS(−)	This work
pC12-13	13-kb *Bam*HI fragment in pBC KS$^-$	This work
pSCC93-20	21-kb *Bam*HI fragment in pBC KS$^-$	This work

MATERIALS AND METHODS

C. neoformans* strains, *Escherichia coli* plasmids and cosmids, and growth conditions.** The *C. neoformans* strains B-4500 (JEC21, *MAT*α) and B-4476 (JEC20, *MATa**) are isogenic strains (11). The strains were maintained on YEPD agar medium (1% yeast extract, 2% peptone, 2% dextrose, 1.5% agar) and grown on MIN medium (0.67% yeast nitrogen base without amino acids, 2% glucose) before DNA was extracted. *E. coli* plasmids and cosmids used in this study are listed in Table 1.

Molecular techniques. Standard methods described by Sambrook et al. (21) were used for transformation of *E. coli* and DNA analysis. Genomic DNA was extracted from *C. neoformans* essentially as described by Pitkin et al. (18). All Southern blots (except the myosin blot) were hybridized and washed under stringent conditions at 65°C. The myosin blot was hybridized under less stringent conditions at 45°C. For subcloning of DNA fragments >10 kb from the isolated cosmids (containing an ampicillin resistance cassette), 5 μg of cosmid DNA was digested to completion, ethanol precipitated, washed, and resuspended in 20 μl of H_2O. The DNA fragments were cloned into a linearized and dephosphorylated plasmid (pBC KS$^-$) containing a cassette for chloramphenicol resistance (Stratagene, La Jolla, Calif.). This cloning strategy eliminated clones derived from religation of the cosmid backbone, since the cosmid selectable marker is ampicillin and recircularized vectors will not grow on chloramphenicol. Positive clones were identified by using colony hybridization and an appropriate DNA sequence as a probe. For sequencing, 0.5 to 1.0 μg of DNA was used with the DNA Sequencing Kit ABI PRISM and an ABI377 DNA sequencer from Perkin-Elmer (PE Biosystems, Warrington, England).

CHEF analysis. Contour-clamped homogeneous electric field (CHEF) blots were prepared as previously described (27) and analyzed by Southern hybridization using probes generated from various regions of the *MAT* locus. The probes of *MAT*α and *MAT***a** have previously been described (5). The probes FUR and MK were generated by PCR, using primers derived from partial sequence analysis of regions III and VI, respectively (see Fig. 4). The oligonucleotide pairs 5′-AATGGGGGAAAACGCACGAG-3′ with 5′-CTTCTAAGGACTTGCGG TTCTCAACTC-3′ and 5′-CCTGCACGGAAAATATCCAC-3′ with 5′-GCAA GATATATGGACCCCTG-3′ were used to isolate FUR and MK, respectively.

RESULTS

Creation of a physical map and localization of *STE12*α within the *MAT*α locus. Previous studies suggested that the location of *STE12*α and *STE11*α was closely linked to the *MAT*α locus (26). Subsequently, *STE20*α, an α-specific homolog of *S. cerevisiae STE20*, was cloned by Wang and Heitman (accession no. AF162330) although its location in relation to the *MAT*α locus has not been reported. To establish the physical relationship between these MAP kinase signal transduction cascade genes and the *MAT*α locus in *C. neoformans*, the sequence downstream of *STE12*α was first used as a probe (Fig. 1, probe I) to screen a cosmid library of B-4500. Several overlapping cosmids were isolated. A physical map of these cosmids was created by using the restriction enzyme *Bam*HI (Fig. 1). Southern blot analysis using a PCR-derived probe of *MFα1*, the original marker for the *MAT*α locus described by Moore and Edman (16), detected a 13-kb *Bam*HI band in several of our cosmids (data not shown). These data indicated that *STE12*α is physically linked to the *MFα1* gene, although the exact position of *MFα1* could not be determined at this stage. To investigate the neighboring sequence of *STE12*α, we subcloned and sequenced the 5.5-kb *Bam*HI fragment (Fig. 1) which contained the downstream sequence of the *STE12*α gene. We identified a 3.4-kb sequence about 1 kb downstream of *STE12*α with high similarity to the gene encoding a mitochondrial RNA polymerase of *S. cerevisiae* (*RPO41*, accession no. M17539) (7). To determine whether this sequence was shared in both mating types, *Bam*HI-digested genomic DNAs from B-4500 and B-4476 were each hybridized with a probe from this gene (Fig. 1, probe II). The hybridization pattern revealed a single band with different sizes in each mating type (see Fig. 4J). Another probe derived from a 3-kb *Bam*HI fragment, which was located next to the 5.5-kb fragment outside the conserved *RPO41* gene (Fig. 1, probe III), also showed a signal in both mating types (data not shown). When several different regions upstream of *STE12*α (left side of *STE12*α in Fig. 1) were used as probes, they all showed an α-specific pattern (see below). It was therefore clear that not only was *STE12*α physically associated with the *MFα1* gene but it was actually located at one end of the *MAT*α locus, close to the boundary.

After the identification of one end of the *MAT*α locus, a physical map of the entire region was created in order to determine the location of other α-specific genes within the locus. Using the 3.8-kb *Bam*HI fragment located at the left side of the map (Fig. 1) as a hybridization probe to genomic B-4500 and B-4476 DNA, we realized that this probe hybridized to a 4.5-kb fragment present only in the α-mating type (data not shown). These data suggested that our initial cosmids did not

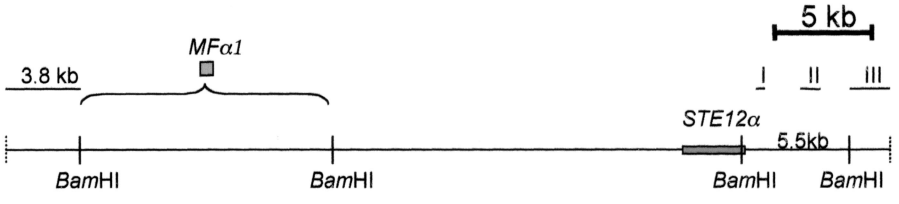

FIG. 1. Physical map of the overlapping cosmids digested by *Bam*HI. Cosmids were isolated by using probe I to screen a cosmid library of B-4500 (JEC21). *STE12*α is located on the right side of the restriction map, and *MFα1* is localized on the 13-kb *Bam*HI fragment at the left side. The exact position of *MFα1* could not be determined at this stage. Shaded bars are locations of genes. I, II, and III represent the regions used as probes, hybridizing to genomic DNA of both mating types.

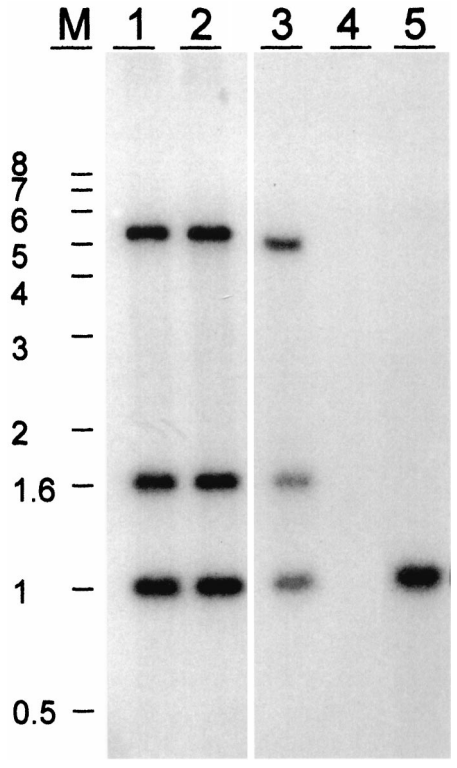

FIG. 2. Southern blot analysis of *MF*α genes. Cosmid DNA and genomic DNA of B-4500 and B-4476 digested with *Hae*II were hybridized with the *MFα1* probe. Lanes 1 and 2 show three *MF*α bands detected in the DNA of the cosmids C12 and C18. The same banding pattern was observed with genomic DNA of B-4500 (lane 3), whereas no signal was obtained with the genomic DNA of B-4476 (lane 4). A single band of 1 kb was detected with DNA of cosmid C5-3 (lane 5).

contain the sequence present in the other end of the *MAT*α locus. Another indication for the lack of the entire *MAT*α locus in our first cosmids was that the banding patterns of *Hae*II-digested cosmid DNA and B-4500 genomic DNA hybridized with *MFα1* were not identical. While three distinct bands of 1, 1.6, and 5 kb were present in the genomic DNA of B-4500, only a single band of 1 kb was detected in the cosmid DNA (Fig. 2). To isolate overlapping cosmids which covered the entire *MAT*α locus, we performed a second screening of the cosmid library by using the 3.8-kb *Bam*HI probe. Several cosmids extending to the left side of our previous map were identified. Southern blot analysis identified two new cosmids having the same hybridization pattern as B-4500 genomic DNA when the *MFα1* coding region was used as a probe (Fig. 2, lanes 1 and 2). A new map containing all the overlapping cosmids is depicted in Fig. 4. With the information on one border of the *MAT*α locus in hand, an attempt was made to identify the opposite border. We generated several probes using DNA fragments starting from the left side of the new map and hybridized them against genomic DNA of B-4500 and B-4476 digested with *Bam*HI (see Fig. 4). Fragments I, II, and III hybridized to both mating types, whereas fragment IV hybridized only to the DNA of the α-mating type. When several other different regions from the new extended map (right side from fragment IV) were used as probes, they all showed a *MAT*α-specific pattern (see below). These data suggested that we identified the other boundary of the *MAT*α locus. Confirmation of the mating type junction location was obtained by hybridization to CHEF blots of *C. neoformans* chromosomal DNA isolated from the two strains B-4476 and B-4500. Each pheromone probe, *MFα1* and *MFa* (5), hybridized exclusively to its corresponding 2.5-Mb *MAT* chromosome (Fig. 3). Probes adjacent to the locus junction hybridized to the *MAT* chromosomes from both mating types. These data, in conjunction with the cosmid hybridizations, demonstrate that the α-specific probes as well as the common **a**/α junction probes reside on the *MAT* locus-containing chromosome in both mating types.

The physical maps of all isolated cosmids covered about 71 kb of the B-4500 genome. To rule out the presence of artifacts which could have been generated during the cloning process, Southern blot analysis containing all the *Bam*HI-digested overlapping cosmids and B-4500 genomic DNA was carried out. DNA fragments representing each *Bam*HI fragment of the cosmid map were used as probes in these analyses. In each case, excluding the 1.3-kb fragment on the left side, we obtained a matching signal with the predicted size in both genomic and cosmid DNA (data not shown). Using the 1.3-kb fragment from the left border (Fig. 4, probe I), we detected a 1.6-kb band in the genomic DNA. These data indicated that the genomic sequence was truncated around the 1.3-kb region in the cosmid clone. Each *Bam*HI fragment of the overlapping cosmids was subcloned, and both ends were sequenced. PCR primers designed adjacent to each *Bam*HI site were used to amplify DNA across each *Bam*HI site using cosmid and genomic DNA as template. Matching PCR fragments were obtained in all cases (data not shown). The arrangement of the *Bam*HI fragments in the cosmids, therefore, is free from any artifact and reflects the actual position in the genome.

Localization of other mating type α strain-specific genes in the *MAT*α locus. After isolation of overlapping cosmids spanning the entire *MAT*α locus, further analysis of the locus was made in relation to other α-specific genes. We characterized regions near all the *Bam*HI sites in the map. Southern blottings were used to demonstrate the mating type specificity of each characterized region (Fig. 4). The positions of already known mating type-specific genes inside the locus were determined, and new genes were discovered. Sequence data suggested the existence of a sequence encoding a putative GTP-binding protein (accession no. AC019018) between the 1.3- and 6.5-kb *Bam*HI fragments (Fig. 4A) located in the genomes of B-4500 and B-4476. From the *Hae*II-digested genomic DNA of B-4500 (Fig. 2), we predicted that there were three copies of the *MF*α gene in the *MAT*α locus. By analyzing our cosmid clones, we located all three copies of the *MF*α genes. The previously reported *MF*α gene (16) was located on the 13-kb *Bam*HI fragment (Fig. 4D) and was renamed *MFα1* (accession no. S56460). The other two *MF*α genes were adjacent to each other and were located in the 17-kb *Bam*HI fragment near the left boundary of the *MAT*α locus (Fig. 4B). We designated these two genes *MFα2/3*. A putative eukaryotic translation initiation factor (accession no. J02674), a homolog of *S. cerevisiae PRT1*, was located between the 4.5- and 13-kb fragments (Fig. 4C). The *STE11*α gene (accession no. AF294841) was found (Fig. 4E) in the 13-kb fragment where *MFα1* is located (Fig. 4D). The genes on the 21-kb *Bam*HI fragment that have been thus far identified include a *MAT*α-specific myosin (accession no. AF267642) gene (Fig. 4F) and *STE20*α (accession no. AF162330) (Fig. 4G). In the case of myosin, an additional band was detected in both *MAT*α and *MAT***a** strains, under low stringency and washing conditions (Fig. 4F). We also identified the *CPR*α gene (accession no. AF259519), a homolog of the *Coprinus cinereus* pheromone receptor (Fig. 4H) located about 1 kb upstream of *STE12*α (accession no. AF012924) (Fig. 4I). The 5.5-kb fragment beyond the right border of the *MAT*α locus contained an RNA polymerase gene

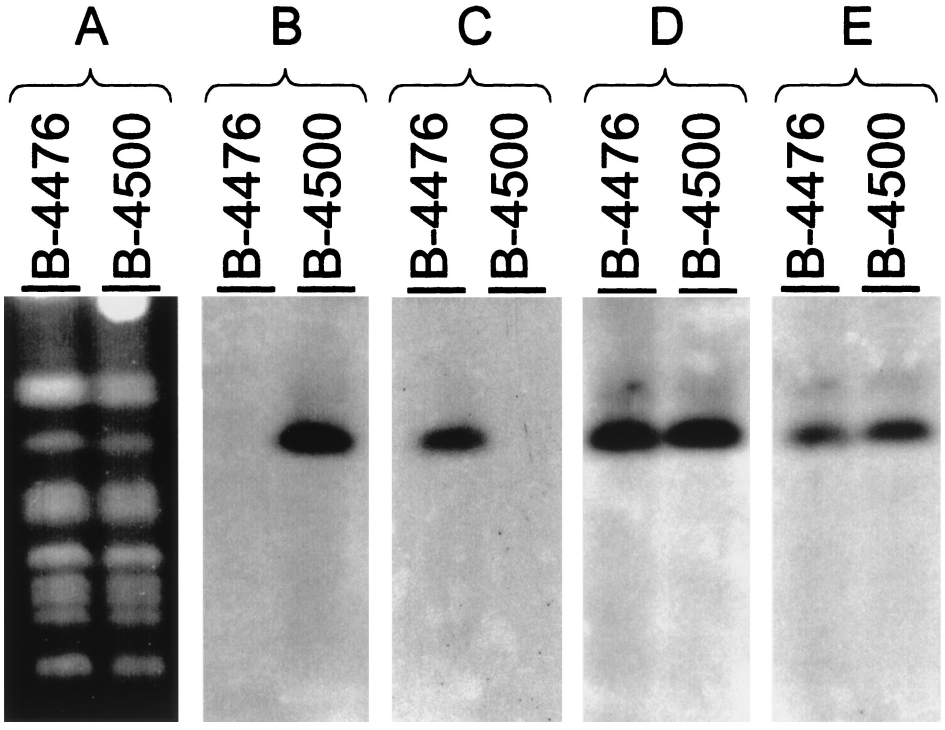

FIG. 3. Southern hybridization with various probes to a CHEF panel of strains B-4476 and B-4500. Shown are the karyotype (A), hybridizations with the *MAT*α probe (B) and the *MAT***a** probe (C) recently described by Chaturvedi et al. (5), and hybridizations with FUR (D) and MK (E) (see Materials and Methods).

(accession no. AF295125) (Fig. 4J). Therefore, the entire *MAT*α locus of *C. neoformans* var. *neoformans* appears to span about 50 kb on chromosome 3 (27).

DISCUSSION

It is known for *S. cerevisiae* that *STE20*, *STE11*, and *STE12*, components of the MAP kinase pathway that signal the mating pheromone response, are also involved in filamentous morphogenesis in diploid as well as haploid cells (reviewed in reference 15). These genes are required for mating since mutations in these genes cause sterility. Unlike those in *C. neoformans*, however, the components of the *S. cerevisiae* MAP kinase pathway which respond to pheromone have no association with mating type. The link between mating type, virulence, and haploid (monokaryotic) fruiting in *C. neoformans* is unique to this species. It is even more unusual to find that some genes of the MAP kinase pathway are mating type specific. Recent studies have shown that *STE12*α (4) and *STE11*α (D. L. Clarke, U. Edman, G. L. Woodlee, C. M. McClelland, T. S. Seymour, J. C. Edman, and B. L. Wickes, unpublished data) play important roles in virulence in the mouse model. In order to clarify the physical relationship between the α-specific genes and the *MAT*α locus, we cloned the entire *MAT*α locus and constructed its physical map.

A previous report by Moore and Edman (16) indicated that the entire *MAT*α locus may span between 35 and 70 kb and that one *MF*α gene (*MF*α*1*) was located within the locus. The present study revealed that the locus is approximately 50 kb in size. We detected 5 to 7 kb of flanking sequence at both ends of the locus which is shared between strains of *MAT*α and *MAT***a**, reflecting the boundary of the *MAT* locus. The *MAT*α locus harbored two additional *MF*α genes, *MF*α*2* and *MF*α*3*, and a putative α-pheromone receptor, *CPR*α. The presence of the pheromone genes and pheromone receptor(s) in the mating type locus has been frequently recognized in several heterothallic fungi, such as *C. cinereus*, *Schizophyllum commune*, and *Ustilago maydis* (2, 17, 25).

The three homologs of the *S. cerevisiae* pheromone response MAP kinase signal transduction cascade genes, *STE11*α, *STE12*α, and *STE20*α, were found to be within a 24-kb region near *MF*α*1*. Between *STE20*α and *STE11*α, a *MAT*α-specific sequence homologous to myosin was identified. The existence of MAP kinase signal transduction cascade genes, a mating type-specific gene of a molecular motor, and a translation initiation factor in a *MAT* locus have never been reported for any other fungi. These MAP kinase signal transduction cascade genes may play important roles in pathogenicity in addition to the mating of the fungus. In fact, a recent study of the role of *STE12*α suggested that this gene regulates virulence-associated genes, such as the *CAP* genes and *CNLAC1* gene in serotype D strains of *C. neoformans* (4). What sets *STE12*α apart from *STE12* of *S. cerevisiae* is that *STE12*α is not essential for mating but essential for haploid fruiting (4). Such a phenomenon was not expected since *STE12*α is part of the *MAT*α locus. While *STE12*α is not essential for mating, *ste12*α mutants are reduced in mating efficacy by approximately 100-fold (4).

Characterization of the remaining MAP kinase signal transduction genes within the *MAT*α locus should clarify whether *STE12*α functions in the same pathway as the other two kinases. Sequence analysis of the pheromone receptor which was found adjacent to *STE12*α showed a high degree of homology to seven previously characterized transmembrane pheromone receptors from other fungi (e.g., *C. cinereus*, *U. maydis*, *S. commune*) (2, 17, 25). The role of *CPR*α in mating as well as in cryptococcal pathogenicity is presently being characterized in our laboratory.

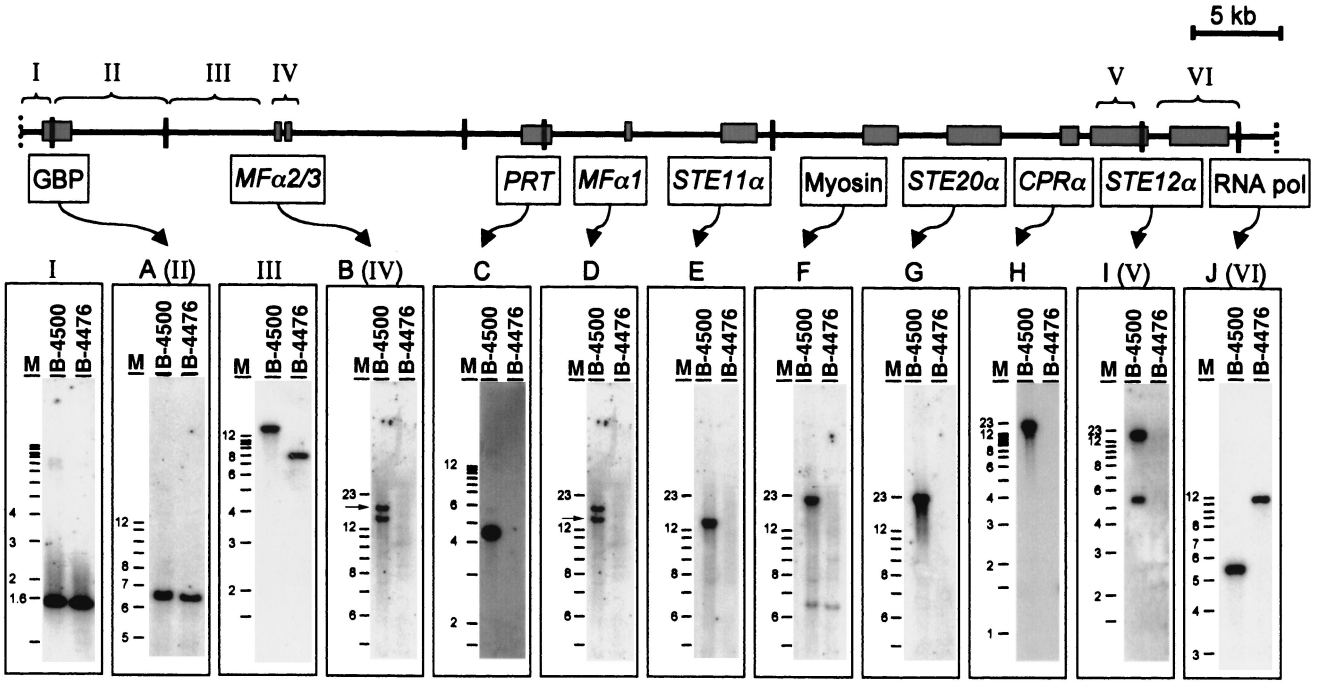

FIG. 4. Overview of the *MAT*α locus as well as identification and localization of the genes within the locus. Overlapping cosmids covering the whole mating type locus were isolated and analyzed. Using Southern blot techniques and several probes, the mating type-specific (between IV and V) and shared (I to III, VI) sequences were identified. The brackets mark the regions of the probes, indicated by roman letters. Specific probes were used to hybridize with *Bam*HI-digested genomic DNA of B-4500 (*MAT*α) and B-4476 (*MAT***a**) to confirm their mating type specificity. Probes A (GTP binding protein) and J (RNA polymerase) are located outside the mating type locus which hybridized with the DNA of both mating types. (B) *MF*α*2/3* are located on the 17-kb *Bam*HI fragment, followed by a mating type-specific translation initiation factor, a homolog of the *S. cerevisiae PRT1* (C). Previously described *MF*α*1* (D) is located on the same 13-kb *Bam*HI fragment as *STE11*α (E). The largest *Bam*HI fragment is ~21 kb in size and contains a myosin gene (F), *STE20*α (G), the pheromone receptor *CPR*α (H), and *STE12*α (I).

Discovery of *MAT*α-specific myosin in the *MAT* locus is unprecedented and unexpected. Since myosin is an important protein for maintenance of morphological structure, this gene may be responsible for the ability to form hyphae during mating or during haploid fruiting, which is specific to mating type α strains. An additional smaller band was detected in both mating types with the myosin probe, perhaps due to cross-hybridization, since low-stringency conditions were used. These bands may suggest the presence of an additional non-mating type-specific myosin gene. Functional analysis of the mating type-specific myosin gene should reveal its role in morphogenesis.

The cloning of the complete *MAT*α locus not only will allow us to analyze the function of remaining genes located within the locus, such as the translation initiation factor, but also will allow us to identify their counterparts in the *MAT***a** locus. A comparison of the genomic arrangement between the two loci may lead to a further understanding of the mating system and its role in pathobiology of *C. neoformans*. Our recent characterization of the **a** mating type-specific pheromone receptor gene, *CPRa* (M. Karos, Y. C. Chang, and K. J. Kwon-Chung, Abstr. 100th Gen. Meet. Am. Soc. Microbiol., abstr. F76, 2000), and *STE12a* (Y. C. Chang and K. J. Kwon-Chung, Abstr. 99th Gen. Meet. Am. Soc. Microbiol., abstr. F63, 1999) revealed that the relative arrangement of these two genes in the *MAT***a** locus is different from that of the *MAT*α homologs (data not shown). It is known from the genomic arrangement of mating loci in other organisms that rearrangement of homologous genes in opposite mating types serves as a preventive measure for recombination or gene conversion, thus protecting the genetic organization of the locus (6). Another important feature of mating type loci is to make sure that the locus is inherited as a single intact unit in order to maintain heterothallism. In fact, conservation of mating type genes within and between species has been well documented for other heterothallic fungi, such as *Pyrenopeziza brassicae* and *Tapesia yallundae*, which are plant pathogenic discomycetes (23).

It is possible that an essential gene within the locus may ensure the stable inheritance of the locus. The *PRT1* homolog, present in the *C. neoformans MAT*α locus, may serve this function since this gene is essential in *S. cerevisiae* (8). If this assumption is correct, there must be a gene in the *MAT***a** locus which has the same function as the *PRT1* gene. Analysis of the *MAT***a** locus will clarify this question.

The *MAT*α locus in *C. neoformans* is one of the largest *MAT* loci among fungi with a one-locus, two-allele heterothallism. It is known that the *MAT* locus of *Ustilago hordei*, a bipolar fungus, is even larger, spanning a region of about 500 kb (14). Though bipolar in phenotype, the *MAT* locus of *U. hordei*, however, is composed of two distinct but closely linked loci, **a** and **b**. The **a** locus encodes mating-type specific pheromones (*Uhmfa*) as well as the pheromone receptors (*Uhpra*). The **b** locus is multiallelic and contains two divergently transcribed genes, **b***E* (*b*East) and **b***W* (*b*West). Furthermore, the **b** locus governs pathogenicity and completion of the life cycle. The **a** and **b** loci are separated by a spacer region in which recombination is probably suppressed, and thus, the bipolarity of the species is maintained (14). The *MAT* loci in *U. maydis* or *S. commune* are much more complex. *U. maydis* has, in contrast, a tetrapolar mating system because the **a** and **b** loci are on separate chromosomes and therefore segregate independently during meiosis (1), whereas *S. commune* has, in addition to its

tetrapolar mating system, a multiallelic *B*α locus (24). Therefore, the *MAT*α locus in *C. neoformans* is not only different from MAT loci in these fungi in its organization but also is one of the largest known mating type loci. The presence of numerous mating type-specific genes in the *MAT* locus distinguishes *C. neoformans* from all other heterothallic fungi thus far investigated.

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