Three Mating Type-Like Loci in Candida glabrata
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Candida glabrata, the second most prevalent Candida species colonizing humans, possesses three mating type-like (MTL) loci (MTL1, MTL2, and MTL3). These loci contain pairs of MTL genes with their respective coding regions on complementary Crick and Watson DNA strands. Each pair of genes is separated by a shared intergenic promoter region, the same configuration found at the mating type loci of Saccharomyces cerevisiae. Two of the MTL loci, MTL1 and MTL2, contain either the MTLa1/MTLa2 configuration or the MTLa1/MTLo2 configuration in different strains. All but one of the 38 tested C. glabrata strains were either aααα or aααα. One test strain was αααα. Based on the mating type genotype, the MTL genes at the MTL1 or MTL2 loci, and the size of the XbaI fragment harboring MTL1 or MTL2, four classes of C. glabrata strains (I, II, III, and IV) were distinguished. Northern analysis revealed that strains were either a-expressors or α-expressors and that expression always reflected the genotype of either the MTL1 or MTL2 locus, depending on the class. The expression pattern in each class, therefore, is similar to that observed in S. cerevisiae, which harbors two silent cassette loci, HMR and HML, and the expression locus MAT. High-frequency phenotypic switching between core phenotypes in an α-expressing, but not in an a-expressing, strain modulated the level of MTL expression, suggesting a possible relationship between core phenotypic switching and mating.

Candida glabrata is the second most prevalent Candida species in humans (15, 17, 32, 38, 41). Although it is genetically far more related to Saccharomyces cerevisiae than to Candida albicans (3, 42, 48), it mimics in many respects the pathogenic capabilities of C. albicans, the most prevalent Candida species, residing as a commensal in healthy individuals and causing vaginitis (15, 43, 44) and bloodstream infections (40). Recently, it was demonstrated that in the elderly, C. glabrata has emerged as the major commensal (32). The prominence of C. glabrata as a pathogen is of particular clinical concern because it is naturally resistant to azole drug therapy (4, 16, 20, 36).

Until recently, it was generally assumed that C. glabrata did not undergo the bud-hypha transition, and no reports had been published on phenotypic switching, two developmental programs that contribute to the pathogenic success of C. albicans. However, recent studies have demonstrated that C. glabrata forms pseudohyphae (12, 26, 27, 39), forms noncompartmentalized tubes distinct from true hyphae (27), and undergoes high-frequency phenotypic switching (26, 27). C. glabrata, therefore, possesses developmental programs at least as complex as those of C. albicans. Recently, it was demonstrated that C. albicans, which is diploid, possesses the mating type-like (MTL) loci MTLα and MTLα (21) and that homozygous MTLα and homozgoyous MTLα strains will mate both in vivo (22) and in vitro (35). It was subsequently demonstrated that while most strains of C. albicans are heterozygous for mating type at the MTL locus and do not undergo white-opaque switching, homozygous strains do undergo the transition (33, 37), and the opaque phenotype dramatically facilitates mating (34, 37). Given the pathogenic similarities of C. albicans and C. glabrata and the developmental correlates between the two species, we have searched for and identified the mating type genes of C. glabrata. We demonstrate that C. glabrata harbors three loci containing mating type-like genes, that two loci, MTL1 and MTL2, can contain either an MTLα1-MTLα2 or an MTLα1-MTLα2 configuration in different strains, and that based on expression patterns, C. glabrata strains can be distinguished as either a mating type or a mating type. Our results further indicate that in any one class, only one of the three MTL loci serves as the mating type expression locus.

MATERIALS AND METHODS

Maintenance and growth of strains. Thirty-eight different clinical isolates of C. glabrata previously genetically fingerprinted with the C. glabrata DNA fingerprinting probe CgI2 (30) are described in Table 1. All strains were maintained on YPD agar slants (1.5% [weight/vol] agar, 2% [weight/vol] Bacto Peptone, 2% [weight/vol] glucose, 1% [weight/vol] yeast extract). For experimental purposes, cells were streaked on fresh YPD agar plates and incubated for 3 to 4 days at 25°C prior to use. For monitoring phenotypic switching, cells were plated on YPD agar containing 1 mM CuSO4 (26).

Isolation and sequence analysis of MTL loci. Based on homology comparisons with the MATα2p or MTLα2p from the species S. cerevisiae, Klyveromyces lactu, and C. albicans by using the multiple-alignement editor of Clustal W software developed by Michele Clamp (www.cmbi.kur.nl/bioinf/tools/clustalw.shtml), two highly conserved amino acid sequences containing homeobox do-...
Isolation of a conserved domain of C. glabrata MAT2p.

Based on homology comparisons between MATa2p and MATa2p of S. cerevisiae, C. albicans, and K. lactis, two highly conserved regions were identified. Assuming that these sequences would also be conserved in C. glabrata based on the high level of genetic relatedness between it and S. cerevisiae (3, 42), degenerate primers that encompassed approximately 125 bp of the MATa2 ORF of S. cerevisiae were designed. Using these primers and C. glabrata DNA, three distinct PCR products of 450, 240, and 125 bp were identified. Sequence analysis of the recombinant plasmids containing the PCR products revealed that the 125- and 450-bp inserts contained uninterrupted ORFs flanked by highly conserved sequences. A BLAST-based sequence similarity search revealed that the 450-bp ORF did not encode a MATa2p but rather encoded a protein homologous to a JUN activation domain binding protein or morphogenetic factor in humans and plants, respectively (6, 8). However, the BLAST search of the 125-bp PCR product revealed that it was derived from a locus homologous to MATa2. The deduced amino acid sequence of the 125-bp PCR product suggested that the ORF encoded 41 amino acids with identities of 69, 57, and 50% and similarities of 82, 77, and 65% to the MATa2p or MATa2p of S. cerevisiae, K. lactis, and C. albicans, respectively.

Isolation and characterization of the MTLa1 and MTLa2 loci.

The 125-bp fragment was used in turn as a probe to screen a C. glabrata EMBL3a λ library constructed from genomic DNA of strain 7549 (30) for the gene locus. Fifty-five putative clones were identified and tested for the presence of a 125-bp MATa2-like insert by PCR. One of these clones, λMP1.1, was used to determine the sequence of a 2,587-bp insert by custom primer walking in both directions (Fig. 1A and 2A). A BLAST-type search of the sequence identified a 312-bp S. cerevisiae MATa1p-like protein-coding sequence and a 510-bp S. cerevisiae MATa2p/MATa2p-like protein-coding sequence separated by a 180-bp putative promoter region. The locus containing these two coding sequences and intergenic sequence will be referred to as mating type-like locus 1 (MTL1). The S. cerevisiae MATa1-like coding sequence in MTL1, which we will refer to as MTLa1, was 126 bp shorter than the S. cerevisiae MATa1 coding region. The deduced 104-amino-acid sequence had 28% overall similarity with S. cerevisiae MATa1p (Fig. 3A). The carboxy-terminal 57 amino acids showed a high level of similarity with the carboxy-terminal regions of C. albicans MATa1p (21), K. lactis MATa1p (1), and S. cerevisiae MATa1p (2) (Fig. 3A). The S. cerevisiae MATa2/MATa2-like coding sequence in MTL1 was 150 bp longer than the S. cerevisiae MATa2 coding region. The deduced 170-amino-acid sequence had an overall identity of 26% with both S. cerevisiae MATa2p and S. cerevisiae MATa2p. The carboxy-terminal 123 amino acids exhibited 31% identity with the carboxy-terminal 119 amino acids of both S. cerevisiae MATa2p and S. cerevisiae MATa2p (Fig. 3B). The carboxy-terminal two-thirds of MATa2p is identical to the full-length MATa2p in S. cerevisiae (2). Align-
ment of MTL1a2p with S. cerevisiae MATa2p and MATα2p revealed a region of highest similarity in the carboxy-terminal end, spanning 60 amino acids (Fig. 3B). This region includes a homeodomain signature sequence (WVXNNRRR) (Fig. 3B) that is a near match with that of C. glabrata and S. cerevisiae. This region has been implicated in ternary complex formation with DNA and MATα1p in S. cerevisiae (29). The two protein-coding regions in MTL1 of C. glabrata strain 7549 will be referred to as MTL1a1 and MTL1a2, respectively (Fig. 1A).

No potential methionine initiation amino acid could be identified in frame in either MTL1a1p or MTL1a2p. Sequencing of three independent 5’ rapid amplification of cDNA ends (5’-RACE)-derived PCR products of MTL1a1 mRNA revealed an additional 21-bp sequence upstream of the first isoleucine codon (Fig. 2A). 5’-RACE-derived PCR products of MTL1a2 mRNA revealed an additional 15-bp sequence upstream of the first glutamine codon (Fig. 2B). 5’-RACE analysis, therefore, did not identify any typical AUG-type initiation codons or distinguish whether MTL1a1 or MTL1a2 transcripts are transcriptionally functional. The MTL1a1 and MTL1a2 ORFs were positioned on complementary Crick and Watson DNA strands, suggesting divergent transcription from the intervening promoter region, a configuration similar to that in S. cerevisiae (Fig. 1A) (2, 18).

The 3’ end of MTL1a1 was flanked by the gene MRA1 (Fig. 1A), which encodes a multicopy suppressor of RAS1 in S. cerevisiae (http://genome-www.stanford.edu/saccharomyces/). The 3’ end of MTL1a2 was flanked by the undefined sequence FLR (Fig. 1A). In S. cerevisiae, the functional MATα locus is flanked by BUD5 at the 3’ end of MATα1 and by TAF2 at the 3’ end of MATα2 (Fig. 1A), while the silent HMα and HML loci are flanked by YCRWDDt012/YCR097W-a and YCL065C/HCL065W, respectively (http://genome-www.stanford.edu/saccharomyces/). The intergenic promoter region between MTL1a1 and MTL1a2 was similar in size to that for MATα in S. cerevisiae (Fig. 1), but the sequence of the MTL1a1-MTL1a2 intergenic region was dissimilar to that of S. cerevisiae MATα1-MATα2, except for the presence of two putative binding sites for TBPI, one at the 3’ end of each of the presumed overlapping promoters for MTL1a1 and MTL1a2 (Fig. 1A).

To identify a DNA fragment containing a second MTLα locus in strain 7549, a negative PCR selection strategy, involving two primer pairs for the MTL1a flanking regions, was used to screen 55 primary lambda clones. The two primer pairs used, MLFLF3-MLFLR3 and FLR2-MLFLR2, represented the 3’ ends of MTL1a1 and MTL1a2, respectively (Table 2). Lambda clone λ1.1 was selected for sequencing, and a total of 1,960 nucleotides were determined by primer walking. Analysis of the nucleotide sequence revealed that the DNA fragment harbored an MTL1a ORF and an MTL1a2 ORF on Crick and Watson strands and that they were flanked by sequences distinct from those flanking MTL1. We therefore designated this DNA fragment MTL2.

The full-length MTL1a2 ORF, which included 472 nucleotides flanking the 3’ end, was identical to MTL1a1. A stretch of 347 nucleotides at the 3’ end of MTL1a2 represented a unique flanking sequence of MTL1a. The full-length ORFs of both MTL1a2 and MTL1a2 were identical except for the last 11 codons of the former and 20 codons of the latter, which are unique prior to the same TGA stop codon. Thus, MTL1a2p is 9 amino acids longer than MTL1a2p. The MTL1a2a flanking region, which spans the 3’ end of MTL1a2, contained a short sequence homologous to S. cerevisiae and C. albicans Nep1p.

### Table 2. Primers used in this study

| Primer       | Sequence                                                                 |
|--------------|--------------------------------------------------------------------------|
| MP2F2        | 5’-TGGTTGCAAAGAAYANNAGAAAYCCNTA-3’                                       |
| MP2R1        | 5’-TTTTAGGTCTCTCATTGATGCACCA-3’                                         |
| MFLR1        | 5’-GCACTAAGGGTAGTCTGAATTA-3’                                            |
| MFLF3        | 5’-TTTTTCTTTTCTTCTTATTGACCCAAGAAGTGGTGAGA-3’                            |
| A1RACE1      | 5’-AACTACACACACATTGCACCGCCTG-3’                                         |
| CGPF1        | 5’-ATGGGACATCGCTGAGGCCAGA-3’                                            |
| PIRACE1      | 5’-GGGAAGTTGAGTTGGTGGTGAATTA-3’                                         |
| MP2R1        | 5’-CTGAGAGAATGACGGAGAGTGTA-3’                                           |
| MFLR2        | 5’-GAACTTGATTGGTGGTGATCCCA-3’                                           |
| MFLF2        | 5’-CAACACGGTAGTGTCTGAATTA-3’                                            |
| A1RACE1      | 5’-CTGAGAGAATGACGGAGAGTGTA-3’                                           |
| MFLR2        | 5’-CAACACGGTAGTGTCTGAATTA-3’                                            |
| FLR2         | 5’-CAACACGGTAGTGTCTGAATTA-3’                                            |
| MFLF2        | 5’-CTGAGAGAATGACGGAGAGTGTA-3’                                           |
| A1RACE1      | 5’-CTGAGAGAATGACGGAGAGTGTA-3’                                           |

### Figure 1. Configurations of the C. glabrata MTL1 locus

(A) The MTL1 locus containing MTL1a1 and MTL1a2 in strain 7549. (B) The MTL1 locus containing MTL1a1 and MTL1a2 in strains PB921 and 1480.47. In both cases comparisons are made to comparable S. cerevisiae loci MATα and MATα2. MRA1, 3’ flanking region of MTL1a1; FLR, 3’ flanking region of MTL1a2. TAF2, 5’ flanking region of the MATα1a1 locus; BUD5, 3’ flanking region of MATα2a2. a1 and a2, MTL1a1 and MTL1a2, respectively, or MATα1 or MATα2, respectively; a1 and a2, MTL1a1 and MTL1a2, respectively, or MATα1 or MATα2, respectively. The thick black bars in MATα1 and MATα2 represent introns. Hatched portions of loci represent intergenic promoter regions. Arrow directions reflect orientation of transcription on complimentary Crick and Watson DNA strands.
A. Nucleotide sequences of the MTL1a locus

AGGGACATTGCTGAGGCACAGTCTGATTGACCAAGAAGCTGTTCACTCTCTGCTGACTTTCCCGCTAATAGCCTGACATCGTTCTCTCCTTCATTTCTG
TTGTCAGTTGAGACAGCTTGATTGCCACAGGAG GTGTGCTGTGTATTATCTGCTTCTCTCTGCTGACTTTCCCGCTAATAGCCTGACATCGTTCTCTCCTTCATTTCTG
TTGTCAGTTGAGACAGCTTGATTGCCACAGGAG GTGTGCTGTGTATTATCTGCTTCTCTCTGCTGACTTTCCCGCTAATAGCCTGACATCGTTCTCTCCTTCATTTCTG
TTGTCAGTTGAGACAGCTTGATTGCCACAGGAG GTGTGCTGTGTATTATCTGCTTCTCTCTGCTGACTTTCCCGCTAATAGCCTGACATCGTTCTCTCCTTCATTTCTG
TTGTCAGTTGAGACAGCTTGATTGCCACAGGAG GTGTGCTGTGTATTATCTGCTTCTCTCTGCTGACTTTCCCGCTAATAGCCTGACATCGTTCTCTCCTTCATTTCTG

FIG. 2. Nucleotide sequences of the MTL1a locus derived from strain 7549 and the MTL1a/H9251 locus derived from strain PB621. (A) The ORFs for MTL1a1 and MTL1a2 are shown in boldface. The primer pairs for MTL1a1 (AIRACE1 and CGAR1) are shown as black boxes with boldface white print, and those for MTL1a2 (CGPF1 and PIRACE1) are shown as grey boxes with boldface white print. The primer pairs which flank the 3' end of the MTL1a1 ORF and the 3' end of the MTL1a2 ORF are underlined. Forty-eight nucleotides at the 5' end of the MTL1a2 ORF that are common to MTL1a2 and MTL3a2 are show by dashed lines. The rest of MTL1a2, excluding the open-box sequence, is common to MTLa2.

B. Nucleotide sequence of MTL1α locus

FIG. 2. Nucleotide sequences of the MTL1a locus derived from strain 7549 and the MTL1a locus derived from strain PB621. (A) The ORFs for MTL1a1 and MTL1a2 are shown in boldface. The primer pairs for MTL1a1 (AIRACE1 and CGAR1) are shown as black boxes with boldface white print, and those for MTL1a2 (CGPF1 and PIRACE1) are shown as grey boxes with boldface white print. The primer pairs which flank the 3' end of the MTL1a1 ORF and the 3' end of the MTL1a2 ORF are underlined. Forty-eight nucleotides at the 5' end of the MTL1a2 ORF that are common to MTL1a2 and MTL3a2 are show by dashed lines. The rest of MTL1a2, excluding the open-box sequence, is common to MTLa2.

The nucleotide sequence unique to the 3' end of the MTL1a2 ORF is shown in an open box.
A. CgMTL1a1p

| CgMTL1a1p | --------------- | ISNEIEIST | TTNDDNLNNIDLPQTREA | 28 |
| CgMTL1a2p | --------------- | MGIDCMMA | ENINTLRMLGRTLEDIN | 27 |
| CgMTL2a1p | --------------- | MNSEISSLVEKCSQLYQTVSVYK | 26 |
| CgLkMTL1 | --------------- | NEHNEFILQLKEQDRDNN | 25 |
| CgMTL1p | --------------- | MCDNMDAQ3KLSFSCIEINAILKEMYLGDSKFS5KPYMSWPEK1EVNHHNFNAFTKLFQYDKSLETILNSCYL | 80 |
| ScMTL1a1p | --------------- | LARSAQYLLYCRQ | 97 |
| ScMTL1a2p | --------------- | LYNWNLNFMESNL | 97 |
| ScMTL2a1p | --------------- | ASHGVYQIEWNLVNLNLRTDR | 97 |
| KIMaMTL1 | --------------- | QELQLDPIRITLQHHNPNNILRYADENSIDVBVDEDPMSPFOTETCITEDDTSDISDKSISITNSDNIPQNYKTRYS | 160 |
| CgMTL1p | --------------- | IVWCFIN | 104 |
| CgMTL1a2p | --------------- | QNEKLKONIQNFLQGCGSLQKPR | 47 |
| ScMTL1a1p | --------------- | WMKIIKDLQLPI7QD8E9S0D10N2M3C4N5P6C7S8T9QS | 90 |
| ScMTL1a2p | --------------- | QIEGLYKITYRRSLSKLR | 97 |
| ScMTL2a1p | --------------- | MSRIENSDRNLQIVQNSQGDDLQVTVQTVQIMKSTK | 97 |
| ScMTL2a2p | --------------- | LITITVLYLKDMSRINSQNYLQIVQNSQGDDLQVTVQTVQIMKSTK | 97 |
| CgMTL1a2p | --------------- | NPYLDHNSQYQQALTQTNKLKSIIKNWVRNNRKRKK | 170 |
| ScMTL1a1p | --------------- | NPYLDTHGKNSWQMLNLSSLR1QFKNRRNQRK | 119 |
| ScMTL1a2p | --------------- | NPYLDTHGKNSWQMLNLSSLR1QFKNRRNQRK | 211 |

B. CgMTL1a2p

| CgMTL1a1p | --------------- | ISNEIEIST | TTNDDNLNNIDLPQTREA | 28 |
| CgMTL1a2p | --------------- | MGIDCMMA | ENINTLRMLGRTLEDIN | 27 |
| CgMTL2a1p | --------------- | MNSEISSLVEKCSQLYQTVSVYK | 26 |
| CgLkMTL1 | --------------- | NEHNEFILQLKEQDRDNN | 25 |
| CgMTL1p | --------------- | MCDNMDAQ3KLSFSCIEINAILKEMYLGDSKFS5KPYMSWPEK1EVNHHNFNAFTKLFQYDKSLETILNSCYL | 80 |
| ScMTL1a1p | --------------- | LARSAQYLLYCRQ | 97 |
| ScMTL1a2p | --------------- | LYNWNLNFMESNL | 97 |
| ScMTL2a1p | --------------- | ASHGVYQIEWNLVNLNLRTDR | 97 |
| KIMaMTL1 | --------------- | QELQLDPIRITLQHHNPNNILRYADENSIDVBVDEDPMSPFOTETCITEDDTSDISDKSISITNSDNIPQNYKTRYS | 160 |
| CgMTL1p | --------------- | IVWCFIN | 104 |
| CgMTL1a2p | --------------- | QNEKLKONIQNFLQGCGSLQKPR | 47 |
| ScMTL1a1p | --------------- | WMKIIKDLQLPI7QD8E9S0D10N2M3C4N5P6C7S8T9QS | 90 |
| ScMTL1a2p | --------------- | QIEGLYKITYRRSLSKLR | 97 |
| ScMTL2a1p | --------------- | MSRIENSDRNLQIVQNSQGDDLQVTVQTVQIMKSTK | 97 |
| ScMTL2a2p | --------------- | LITITVLYLKDMSRINSQNYLQIVQNSQGDDLQVTVQTVQIMKSTK | 97 |
| CgMTL1a2p | --------------- | NPYLDHNSQYQQALTQTNKLKSIIKNWVRNNRKR | 170 |
| ScMTL1a1p | --------------- | NPYLDTHGKNSWQMLNLSSLR1QFKNRRNQRK | 119 |
| ScMTL1a2p | --------------- | NPYLDTHGKNSWQMLNLSSLR1QFKNRRNQRK | 211 |

FIG. 3. Sequence comparison of MTL1a1p and MTL1a2p of C. glabrata strain 7549 with MAT1ps and MAT2ps of other yeast species. (A) Aligned sequences of C. glabrata (Cg) MTL1a1p and MAT1ps or MTL1ps of S. cerevisiae (Sc), C. albicans (Ca), and K. lactis (Kl). Aligned sequences of C. glabrata MTL1a2p and S. cerevisiae MAT2ps and MAT2ps. The shaded sequences in both panels represent the highly conserved amino acid residues (boldface) involved in the interaction between the S. cerevisiae MAT1a1 and S. cerevisiae MAT2a in the formation of the α1-α2 repressor complex. Note that S. cerevisiae MAT2ps and MAT2ps share identical amino acid residues in this region, but the physiological role of S. cerevisiae MAT2a and MAT2a is not known. Asterisks indicate identical residues, two stacked dots indicate conservative substitutions based on similar functional groups, and one dot indicates conservative substitutions based on similar effects on secondary structure. The accession numbers for sequences other than those of C. glabrata are as follows: ScMAT1p, AAD51404.1; KIMaMTL1, AA246201.1; and ScMAT2p, AAA34762.1. The accession numbers for C. glabrata MTL1a and MTL2a are AY191461 and AY191464, respectively.

(14). The 3' end of MTL2a2 was flanked by the threonyl-tRNA and a short sequence homologous to the Fadd death effector domain (13).

Isolation and characterization of an MTL1 locus and an MTL3 locus containing MTLα genes. To test whether the configuration of the MTL1 locus of strain 7549 was common to all C. glabrata strains, we cloned and sequenced an MTL1 locus in two additional strains of C. glabrata, PB921 and 1480.47. The primers MLFLF2 and MLFLR2 (Table 2), which represent the 3' ends of the flanking regions of MTL1a1 and MTL1a2, respectively, of strain 7549, were used to generate PCR products from genomic DNA. While these primers generated a PCR product of 2,075 bp for strain 7549, they generated a PCR product of 2,269 bp from strains PB921 and 1480.47 (Fig. 1B). A BLAST-type search identified an S. cerevisiae MTA1-like coding sequence and an S. cerevisiae MAT2/MAT2-like coding sequence (23). Because the MTA1-like and MAT2-like coding sequences were similar to those of S. cerevisiae MATα1 and MATα2, respectively, the paired coding regions were designated MTL1α1 and MTL1α2. The flanking sequences of the MTL1α locus in strains PB921 and 1480.47 were identical to those of MTL1α in reference strain 7549. Both MTL1α1 and MTL1α2 contained uninterrupted ORFs beginning with codons for the initiation amino acid methionine. As in the case of the MTL2a2-MTL1a1 configuration of the MTL1 and MTL2 loci in strain 7549, MTL1α2 and MTL1α1 were positioned on complementary Crick and Watson DNA strands, with an intergenic promoter (Fig. 1B and 2B). The MTL1α promoter encompassed 333 bp, which was 70 bp longer than the MATα promoter of S. cerevisiae. The MTLα promoter contained three TATA box binding protein sites and a putative binding site for the repressor-activator protein Rap1p, both found in the MATα2 promoter of S. cerevisiae (Fig. 1B). The MTLα promoter of C. glabrata also contained putative Mcm1p and Rme1p binding sites, neither of which was present in the MATα2 promoter of S. cerevisiae (Fig. 1B). There was no recognizable binding site for the MATα1p-MATα2p heterodimer (α1-α2) (Fig. 1B and 2B) found in the promoter of S. cerevisiae (Fig. 1B).

The MTL1α1 ORF encodes a deduced protein of 184 amino acids with an overall identity of 29% and similarity of 51% with S. cerevisiae MATα1p (Fig. 4A). The carboxy-terminal 88
amino acids showed 50% identity with MATα1p (Fig. 4A). The MTLa2 ORF encoded a deduced protein of 187 amino acids with an overall identity of 42% and similarity of 70% with S. cerevisiae MATα2p (Fig. 4B) (18). The highly conserved carboxy-terminal end, spanning 62 amino acids, exhibited 60% identity (Fig. 4B). MTLa1p was identical to MATα1p of S. cerevisiae with an overall identity of 42% and similarity of 70% with C. albicans MATa2p (Fig. 4B) (18). The highly conserved carboxy-terminal end, spanning 62 amino acids, exhibited 60% identity (Fig. 4B).

a. CgMTLα1p

b. CgMTLα2p

FIG. 4. Sequence comparison of MTLα1p and MTLα2p of Candida glabrata strains PB921 (CgMTLα1p and CgMTLα2p) and 1480.47 (CgMTLα1p and CgMTLα2p) with MATα1p or MATα2p and MATα2p or MTLα2p of other yeast species. (A) Aligned sequences of C. glabrata MTLα1p and MTLα1p with MATα1p of S. cerevisiae (Sc), MATα1p of K. lactis (Kl), and MTLα1p of C. albicans (Ca). (B) Aligned sequences of C. glabrata MTLα2p with MATα2p of S. cerevisiae, MATα2p of K. lactis, and MTLα2p of C. albicans. The shaded sequences in panel B and the method used to indicate conserved amino acid sequences are described in the legend to Fig. 3. The accession numbers for the sequences other than those of C. glabrata are as follows: ScMATα1p, AAA34761.1; ScMATα2p, AAA34762.1; CaMATα1p, AY207368; and CaMATα2p, AY191463. The accession numbers for C. glabrata MTLα1a, MTLα1b, and MTLα2a are AY191462, AY191463, and AY207368, respectively.

A preliminary Southern analysis of XbaI-digested DNA of strain 7549 hybridized with MTLα1a-α2 revealed two bands, but the same Southern blot probed with MTLα1a revealed only two of these bands, suggesting a third locus containing an MTLα1 ORF. To identify this third locus in strain 7549, a positive PCR selection strategy employing the MTLα1 ORF-specific primer pair CGP1F1-CGP1R2 (Table 2), was used to screen 55 primary lambda clones. Two clones, MPM5.1 and AMP18.1, containing the MTLα1 ORF were selected. The sequence of 2,169 nucleotides was determined by a primer walking strategy. This locus was designated MTLα1 and contained unique sequences with no significant homology to any sequence in the database.

C. glabrata contains three independent MTL loci. To examine the distribution of the three MTL loci among unrelated strains, Southern blot analyses were initially performed on the genomic DNAs of four independent strains digested with BamHI, EcoRI, PstI, or XbaI and probed with the full-length ORF of MTLα1a, MTLα2a, or MTLα1a. The four test strains
were isolated from different geographic locales and proved to be genetically unrelated when DNA fingerprinted with the complex species-specific probe Cg12 (data not shown). The Southern blot hybridization patterns of the four strains probed with a DNA sequence common to MTL1a2 and MTL1a2 (referred to as MTL1a2-o2) included two or three bands, depending on the restriction enzyme (Fig. 5A and D). The XbaI patterns of the four test isolates probed with MTL1a2-o2 all contained 15-, 7- and 5.8-kb bands, which were designated A, B, and C, respectively (Fig. 5A). The A, B, and C bands represent MTL3, MTL1, and MTL2 loci, respectively. An analysis of 34 additional C. glabrata isolates revealed that all contained three bands either at 15, 7, and 5.8 kb or at 15, 5.8, and 5.2 kb (Table 3). Since MTL1a2 was identical to the 414 bp of the 3′ end of MTL1a2, Southern blot hybridization with MTL1a2-o2 could not distinguish between bands containing MTL1a2 or MTLa2 sequences.

In order to distinguish between MTL1a2 and MTLa2 sequences, Southern blots of XbaI-digested genomic DNAs of 38 strains were probed with the end-labeled antisense oligonucleotide primer FuncP2 (Table 2; Fig. 5D), which is unique to the 5′ ends of MTLa2 ORFs. The hybridization identified among the strains three fragments (A, B, and C) containing MTLa2 ORF sequences. The total number of bands in any one strain was either one or two, with the exception of strain 1480.47, which contained three bands (Table 3). Fifty-eight percent of all strains exhibited only one band, and in those cases, it was always the 15-kb A band. Forty-one percent of strains contained a combination of A plus B or A plus C. Hybridization of the exceptional strain 1480.47 with FuncP2 showed that all three DNA fragments, A, B, and C, contained the MTLa2 ORF (Table 3).

The Southern blot hybridization patterns of XbaI-digested DNAs of the four test strains probed with MTL1a1 (Fig. 5C) contained no bands, one band (band B), or two bands (bands B and C) (Fig. 5B). Southern blots of the remaining 34 isolates of the test collection probed with MTL1a1 revealed patterns of one band (band B or C) or two bands (bands B and C) only (data not shown). In only one unique strain in the entire collection, strain 1480.47, MTL1a1 did not hybridize to any band (Fig. 5B), although the MTLa2-specific primer hybridized to three bands (Fig. 5C). MTL1a1 did not hybridize to band A in any of the 38 tested strains, in contrast to the MTLa2-specific primer (Table 3).

The Southern blot hybridization patterns of XbaI-digested DNAs of the four test strains probed with MTL1a1 (Fig. 5D) contained either one band (band A), two bands (bands A and C), or three bands (bands A, B and C) (Fig. 5C). In the 34 remaining strains in the collection, MTL1a1 hybridized to one band (band A) or two bands (bands A and B or bands A and C) (data not shown), the same pattern observed with the FuncP2 probe, suggesting that the MTL1a1 ORF always pairs with the MTLa2 ORF. The Southern blot hybridization patterns of all 38 test strains included band A (Fig. 4C; Table 3). In 58% of strains, MTL1a1 hybridized only to band A, the same group of strains in which MTL1a1 hybridized to bands B and C. We designate strains in this group class I (Table 3). In 18% of strains, MTL1a1 hybridized to both A and B; this is the same group of strains in which MTL1a1 hybridized only to band C. In this class, the B band exhibited a polymorphism of either 7 or 5.2 kb. Half of the strains in this class contained the 7-kb B band, and the remaining half contained the 5.2-kb B band. We designate strains in this group class II (Table 3). In 5% of strains, MTL1a1 hybridized to both A and C; this is the same group of strains in which MTL1a1 hybridized only to band B. We designate strains in this group class III (Table 3). The distinguishing feature between class II and class III is the composition of MTL1 and MTL2. In class II isolates, MTL1 includes MTL1a1 and MTL1a2, while in class III isolates, MTL2 contains this configuration. In class IV, there was no hybridization to MTL1a1; MTL1a1 hybridized to bands A, B, and C (Fig. 5C). If we distinguish loci as α or β by whether they harbor MTL1a1 or MTLa1, respectively, then these results suggest that class I strains possess two MTLa loci and one MTL1 locus (ααα), class II strains possess two MTLa loci and one MTL1a locus (ααα), class III strains possess two MTLa loci and one MTL1a locus (ααα), and class IV strains possess three MTLa loci (ααα) (Tables 3 and 4).

To test the relationship between the bands containing MTL ORFs and the three MTL loci, XbaI-digested DNAs of the 38 test strains were probed sequentially with the DNA fragments flanking the 3′ ends of MTL1a1 and MTL1a2 of strain 7549. With strain 7549 DNA as template, the primer pair MLFLR2 and FLR2 (Table 2) generated a 395-bp PCR product that spanned the 3′ flanking region of MTL1a2 in MTL1, and primer pair MLFLR3 and MLFLF3 (Table 2) generated a 246-bp PCR product that spanned the 3′ flanking region of MTL1a1 in MTL1 (Fig. 1A). Southern blots of XbaI-digested DNAs from the 38 test strains probed with either the 395- or 246-bp PCR product revealed that both flanking regions of MTL1 were always associated with the B band, either 7 or 5.2 kb, in all strains (Table 3), indicating that each C. glabrata genome contained only one MTL1 locus in the same relative position. All strains that exhibited bands B and C when probed with MTL1a1 and only band A when probed with either MTL1a1 or FuncP2 (class I) exhibited only the 7-kb band when probed with the 3′ flanking regions (Table 3), demonstrating that in these strains only one of the two MTL1a1 genes is located in the MTL1 locus. A majority of strains that exhibited bands A and B when probed with MTL1a1 exhibited hybridization with only the 5.2- or 7-kb band (class II) when probed with the 3′ flanking regions (Table 3), demonstrating that in these strains only one of the two MTL1a1 genes is located in the MTL1 locus. Surprisingly, two strains that exhibited bands A and C when probed with MTL1a1 and FuncP2 (class III) hybridized only to the 7-kb band B when probed with the 3′ flanking regions, demonstrating that in these strains the MTL1a1 genes are located at the MTL2 locus. Southern blots of XbaI-digested DNAs from the 38 test strains probed with the 280-bp PCR product flanking the 3′ end of the MTL2a1 ORF, which was generated with the primer pair MTL1CF1-MTL1CR1 (Table 2), revealed multiple hybridization bands, suggesting that the flanking region harbors a repeat element that is dispersed throughout the genome, in addition to its linkage to the C DNA fragments (data not shown). Interestingly, the complex hybridization pattern was identical among strains in each class, suggesting close genetic relatedness of strains within a class.

**PCR analysis of MTL size and distribution.** Although Southern analysis revealed three MTL loci (MTL1, MTL2, and MTL3) in each strain and discriminated four classes, it did not
...To investigate further the linkage between $MTL1\alpha$ and $MTL1\alpha_2$, we employed the primer pair CGPIR2, which is common to both $MTL3\alpha_2$ and $MTL1\alpha_1$ (and probably $MTL2\alpha_1$) and PIRACE1, which is unique only to the 3' end of $MTL1\alpha_2$ (Fig. 6B) but is absent in $MTL3\alpha_2$ and probably in $MTL2\alpha_2$. These primers generated a 1,300-bp PCR fragment which included full-length $MTL1\alpha_1$ and $MTL1\alpha_2$ ORFs for the four class II strains and one class IV strain but not for any of the class I strains (Fig. 6A). These PCR results (Fig. 6A) confirmed that in class I isolates, $MTL3\alpha_1$ is paired with an $MTL3\alpha_2$-type sequence and not an $MTL1\alpha_2$-type sequence in the A fragment.

To investigate whether the $MTL1\alpha$ ORFs present at the $MTL1$ or $MTL2$ locus are the same size, the primer pair CGAR1 and A1RACE1 (Table 2), which should generate a single 300-bp PCR product of $MTL1\alpha_1$ (Fig. 6B), was em-
employed. This primer pair generated a 300-bp PCR product in the nine test isolates in classes I and II but not in isolates in class IV (Fig. 6A), which was consistent with the results of the Southern analysis, indicating that all strains but 1480.47 contained at least one copy of MTLa1. These primers also generated a 300-bp PCR product in the 28 additional isolates in the collection. Finally, to test whether MTLa1 ORFs present in all three loci are of the same size, the primer pair CGPIF1 and CGPIR1 (Table 2), which generated a single 550-bp PCR product containing the MTLa1 ORF (Fig. 6B), was employed. This primer pair generated a 550-bp PCR product for all 10 test strains (Fig. 6A), as well as the remaining 28 isolates of the collection; these data are consistent with the results of the Southern analysis demonstrating that all strains contain at least one copy of MTLa1. Since the sequences of MTLa1 and MTLa3 were identical, it was not possible to identify the origin of PCR products in class II, III, and IV strains.

**Strain-specific transcription of MTL genes.** In *S. cerevisiae*, mating type genes are present at three loci (MAT, HML, and HMR) but are expressed only at one locus (MAT) (18). *S. cerevisiae* strains can therefore be discriminated phenotypically as *MATa* or *MATα* but are expressed only at one locus (MAT). To test whether similar distinctions can be made for *C. glabrata*, and as a strategy for identifying a possible expression locus, 12 isolates were analyzed by Northern blot hybridization for expression of MTLa1 and MTLa1, using the respective ORFs as probes. Three of the test strains were class I (J932283, J932436, and 35B11), eight were class II (PB921, LP21, PB656, CD457, 1480.46, 1480.41, J932474, and 1480.44), and one was class IV (1480.47). While class II and IV strains expressed MTLa1, no class I isolates expressed it (Fig. 7). Conversely, while all tested isolates in class I expressed MTLa1, no class II or IV isolates expressed it (Fig. 7). Therefore, class II and class IV isolates can be classified as α-expressors, while class I isolates can be classified as α-expressors. Predicated on the cassette model in *S. cerevisiae*, predictions of the MTL expression locus can be made based on the duplication of either MTLα1 or MTLα1 in two of the three MTL loci. While all class II and IV isolates possibly carry identical MTLα1 ORFs at both the MTL1 and MTL3 loci, class III isolates possibly carry identical MTLα1 ORFs at both the MTL2 and MTL3 loci. Therefore, either MTL1 or MTL3 may represent the expression locus in class II, while either MTL2 or MTL3 may represent the expression locus in class III isolates. Class I isolates, on the other hand, carried identical MTLα1 ORFs at both the MTL1 and MTL2 loci. Therefore, in class I isolates, either MTL1 or MTL2 may represent the expression locus.

**Transcription of MTL genes during phenotypic switching.** Since the differential expression of MATα and MATα genes confers cell type specificity in *S. cerevisiae* (2, 18, 23) and since white-opaque switching in *C. albicans* is intimately involved in the mating process (33, 34, 37), we compared expression of MTLa1 and MTLa1 among the phenotypes of the reversible high-frequency core switching system of *C. glabrata* (26, 27). Most strains of *C. glabrata* switch reversibly and at high frequency between the following four core phenotypes, graded in color on agar containing CuSO4: very dark brown, dark brown (DB), light brown (LB), and white (Wh) (26, 27). *C. glabrata* also switches reversibly between core phenotypes and an irregular wrinkle (IWr) phenotype composed primarily of pseudohyphae (27). Cells of *C. glabrata* strain 1480.49, an α-expressor of class II exhibiting the LB phenotype on agar containing CuSO4, were clonally plated, and Wh, LB, and DB colonies were analyzed. MTLα1 was expressed by cells with the tested core phenotypes in a graded fashion that correlated with color gradation (Wh > LB > DB) (Fig. 8). None of cells with the switch phenotypes expressed MTLα1 (Fig. 8). Cells of *C. glabrata* strain 35B11, an α-expressor exhibiting the LB phenotype on agar containing CuSO4, were clonally plated, and LB, DB, and IWr colonies were analyzed. Neither LB, DB, nor IWr cells expressed MTLα1, but all three expressed MTLα1 at similar low levels (Fig. 8). These results demonstrate that while general MTL expression is dictated by the genotype of the MTL1 or MTL2 locus, the core switching system influences the level of MTLα1 expression in a graded fashion.

**DISCUSSION**

Although *C. glabrata* represents the second most prevalent *Candida* species involved in human disease (15, 17, 32, 38, 41),

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**TABLE 3.** Classification of 38 *C. glabrata* strains based on the distribution of MTLa1, MTLa2 (or MTLα2), and MTLα1 revealed by Southern analysis.  

| Class | No. of isolates | % of isolates | Band pattern with probe: |
|-------|----------------|--------------|-------------------------|
|       |                |              | MTLa1                  | MTLα2-α2<sup>a</sup> | FuncP2<sup>b</sup> | MTLα1 | MTL1 3’ and 5’ flanking regions |
| I     | 22             | 58           | A, B, C                 | A, B, C              | A               | A     | B                  |
| II    | 14             | 36           | C                       | A, B, C              | A, B             | A, B   | B                  |
| III   | 2              | 5            | B                       | A, B, C              | A, C             | A, C   | B                  |
| IV    | 1              | NA<sup>c</sup> | A, B, C                 | A, B, C              | A, B             | A, B   | B                  |
| V     | NA             | NA           | A, B, C                 | A, B, C              | A, B             | A, B   | B                  |

<sup>a</sup> This probe does not distinguish between MTLα2 and MTLα2.<br>
<sup>b</sup> This probe distinguishes between MTLα2 and MTLα2.<br>
<sup>c</sup> NA, not applicable.

**TABLE 4.** Models for the six classes based on the distribution of MTLa1, MTLa2 (or MTLα2), and MTLα1 revealed by Southern analysis.  

| Class | Genes in band: |
|-------|----------------|
|       | A   | B   | C   |
| I (aa oo) | MTLαMTL3 | MTLαMTL1 | MTLαMTL2 |
| II (a αo)  | MTLαMTL3 | MTLαMTL1 | MTLαMTL2 |
| III (αo α) | MTLαMTL3 | MTLαMTL1 | MTLαMTL2 |
| IV (oo oo) (1480.47) | MTLαMTL3 | MTLαMTL1 | MTLαMTL2 |
| V (aaa)   | MTLαMTL3 | MTLαMTL1 | MTLαMTL2 |
research into its basic biology has been minimal compared to that for *C. albicans*, primarily because it has been assumed that information gathered for *C. albicans* would be transferable to other *Candida* species. However, genetic comparisons of the major *Candida* species have revealed that *C. glabrata* is far more related to *S. cerevisiae* than it is to *C. albicans* (3, 42, 49). Here we provide evidence for the first time that *C. glabrata* possesses three mating type-like loci with configurations similar to that of *S. cerevisiae*. Expression of mating type genes in *C. glabrata* may be restricted to a single mating type-like locus that can contain either a or $\alpha$ genes, suggesting that a cassette system similar to the one basic to *S. cerevisiae* mating may exist in *C. glabrata* (18).

FIG. 6. PCR analysis of the MTL loci of *C. glabrata*. (A) PCR assays were performed with genomic DNAs of 10 *C. glabrata* strains. Five separate PCRs were performed with each strain to test the presence or absence of the full-length ORFs. Note that the primers CGPF1 and PIRACE1 do not discriminate between $MTL\alpha_2$ and $MTL\alpha_2$, and the primers CGPIR2 and PIRACE1 generate a fragment containing $MTL\alpha_1$, $MTL\alpha_2$ and the intergenic promoter region. (B) Schematic representation of $MTL2\alpha$, $MTL1\alpha$, and $MTL3\alpha$ from strain 7549 and $MTL1\alpha$ from strains PB621 and 1480.47. Positions of key primer pairs used in the PCR analysis are shown.
either strands. Although in-frame AUG codons were not evident in an MTL and MTL2, three independent mating type-like loci (MTL1, MTL2, and MTL3), each containing pairs of mating type genes. All three have been characterized. In our test strain 7549, both MTL1 and MTL2 were demonstrated to contain an MTL1 ORF and an MTL2 ORF on complementary Crick and Watson DNA strands. Although in-frame AUG codons were not evident in either MTL1 or MTL2, one out-of-frame AUG codon was present 39 and 15 nucleotides upstream of an isoleucine or glutamine codon in MTL1 and MTL2, respectively. It is not clear whether the hybridizable MTL1 transcript identified in Northern blots or the 5'-RACE product represents the translatable mRNA. Interestingly, MATA1, the homolog of MTL1, has no known function in haploid a cells of S. cerevisiae (32).

MTL1 and MTL2 were separated by an intergenic promoter region. This configuration is similar to that of the three mating type loci in S. cerevisiae (19, 25, 47), suggesting that regulation may also be similar. However, key regulatory sequences in the intergenic promoter of S. cerevisiae MATA1 and MATA2 were not shared with the comparable promoter of C. glabrata, suggesting differences in regulation. Characterization of the MTL1 locus in two additional strains revealed that in these strains the MTL1 ORF and the MTL2 ORF resided in the MTL1 locus on cDNA strands in a configuration similar to that of MTL1 and MTL2. Again, while the MTL1-MTL2 configuration included an intergenic promoter, like the one separating and regulating MATA1 and MATA2 in S. cerevisiae, the identified regulatory sequences differed. The intergenic promoter region of C. glabrata contained Rap1p, Mcm1p, and Rme1p binding sites (http://cgsigma.cshl.org/jian/), which are not present in the comparable S. cerevisiae promoter, and lacked an a1-a2 binding site present in the S. cerevisiae promoter (24). The latter observation suggests that in a cells of C. glabrata, transcription of MTL1 may not be repressed by an MTL1p-MTL2p heterodimer as in a cells in S. cerevisiae.

Instead, another transacting factor such as Rme1p, a repressor of meiotic gene expression in S. cerevisiae (16, 17), may substitute in the repression of MTL1 transcription in a strains of C. glabrata. Alternatively, Rme1p, which can act as either an activator or a repressor (5, 10, 11), depending on the cis-acting element, may function as an activator of MTL1 expression in a-type strains. Functional characterization of the MTL promoters will be the first step in revealing the unique mechanisms of regulation in C. glabrata.

**Genetic models of the three MTL loci in class I, II, and III isolates.** Using a combination of Southern analysis, PCR analysis, and sequencing, we were able to type each of the three MTL loci as a or a for a number of C. glabrata strains based on the presence of MTL1-MTL2 or MTL1-MTL2 pairs, which could be discriminated unambiguously at each of the three loci. Our results first demonstrate that the great majority of strains are either aaaa or aaaa. Only one of the 38 isolates tested, 1480.47, deviated from the two patterns, exhibiting an aaaa genotype. Based on these studies, the 38 tested strains (97%) separated into four classes based on genotype and the size of the XbaI fragment harboring MTL1 or MTL2 (i.e., fragments B or C) (Fig. 9; Table 4). Class I strains were aaaa and contained the combination of MTL1a and MTL2a at the MTL1 and MTL2 loci in bands B and C, respectively (Fig. 9). Class I strains also harbored the combination MTL1a-MTL2a at the MTL3 locus in band A (Fig. 9). Class II strains were aaaa and contained the combinations of MTL1a1 and MTL2a2 at the MTL1 locus in band B, MTL2a1 and MTL2a2 at the MTL2 locus in band C, and MTL3a1 and MTL3a2 at the MTL3 locus in band A (Fig. 9). Class III strains were also but contained MTL2a1 and MTL2a2 at the MTL2 locus in band C rather than in band B (Fig. 9). Class III strains also harbored the combination MTL3a1-MTL3a2 at the MTL3 locus in band A, like the other two classes, and MTL2a1 and MTL2a2 in band B (Fig. 9). Based on the MTL genotypes in fragment B or

**FIG. 7.** Northern analysis of the expression of MTL1 and MTL1 in 12 strains of C. glabrata. Approximately 20 μg of total cellular RNA was applied to each lane. Duplicate Northern blots were probed with either the 300-bp PCR fragment (primers CGAR1 and A1RACE1) containing the MTL1 ORF or the 550-bp PCR fragment (primers CGPIF1 and CGPIR2) containing the MTL1 ORF. To assess loading, ethidium bromide-stained 28S rRNA patterns are included. The approximate molecular sizes of transcripts are shown to the right of the hybridization patterns. The class and MTL1 expression pattern of each strain are noted.

**FIG. 8.** Northern analysis of the expression of MTLa1 and MTLa1 in the switch phenotypes of an MTLa1-expressing strain (1480.49) and an MTLa1-expressing strain (35B11). Cells of the two strains were plated on agar containing 1 mM CuSO4, which discriminates between the switch phenotype in the core switching system of C. glabrata (26, 27). Cells from Wh, LB, and DB colonies of strain 1480.49 and from IWr, LB, and DB colonies of strain 35B11 were picked and replated before they were analyzed. Duplicate blots were probed with either the 550-bp PCR fragment (primers CGPIF1 and CGPIR2) containing the MTL1 ORF or the 300-bp PCR fragment (primers CGAR1 and A1RACE1) containing the MTL1 ORF. To assess loading, ethidium bromide-stained 28S rRNA patterns are included. The class and MTL1 expression pattern of each strain are noted.
C and the MTL flanking sequence, it is likely that the class IV isolate 1480.47 was derived from either a class II or class III isolate. Since our collection included only a limited number of strains, there is the possibility that additional configurations, such as aaa, exist (Fig. 9).

Expression patterns: suggestion of a cassette system like that in S. cerevisiae. Northern analysis revealed that C. glabrata, like S. cerevisiae, could be categorized based on mating type gene expression. Strains expressed either MTLa1 or MTLα1, never both, just as haploid S. cerevisiae expresses MATa1 or MATα1 but never both. In the case of S. cerevisiae, genes in HMR and HML loci are silent, while genes in the MAT locus are expressed (18). In C. glabrata, the expression pattern suggested a similar scenario. While class II and IV isolates expressed MTLα1, class I strains expressed MTLa1. The former classes of isolates possessed two MTLα loci, while the latter class possessed two MTLa loci. Northern analysis demonstrated a direct correlation between the expression of MTLα1 or MTLα1 and the presence of two corresponding ORFs at two MTLα and two MTLa loci, respectively. However, our data do not distinguish which of the two similar loci in each case is the expression locus.

If only one of the two loci in C. glabrata is the expression locus and a correlate to the MAT locus of S. cerevisiae and the other two MTL loci are correlates to the silent loci HMR and HML, then the three C. glabrata MTL loci may also function in a mobile cassette system similar to that of S. cerevisiae, in which copies of the silent MTL loci recombine with the MTL expression locus to switch mating type. Experiments to test this hypothesis are now in progress.

Expression patterns and phenotypic switching. Our results indicate that MTL expression correlates with the genotype of the MTL1 or MTL2 locus. The α-expression strain 1480.49 expressed MTLα1 exclusively in all three tested phenotypes in the core switching system (Wh, LB, and DB), while the a-expressing strain 35B11 expressed MTLa1 exclusively in both core switch phenotypes (LB and DB) and the IWr phenotype. However, in the a-expressing strain, the levels of MTLa1 transcript differed between the three core switch phenotypes according to the hierarchy Wh > LB > DB, suggesting that MTLα1 expression is modulated in a graded fashion by the core switching system, just like transcription of the methallo-thionine gene MTII, for pigmentation and phloxine B staining (26, 27). Interestingly, the intergenic promoter that controls transcription of MTLα1 contains an Mcm1p binding site, which has been implicated in the regulation of phase-specific gene expression during white-opaque switching in C. albicans (31). The relationship between mating and switching in C. glabrata, therefore, deserves further investigation.

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