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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 *MTLa**1/*MTL***a**2 configuration or the *MTL*α1/*MTL*α2 configuration in different strains. All but one of the 38 tested *C. glabrata* strains were either aaα or aαα. One test strain was ααα. Based on the mating type genotype, the *MTL* genes at the *MTL1* or *MTL2* loci, and the size of the *Xba*I 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 *MTL***a** and *MTL*α (21) and that homozygous *MTL***a** and homozygous *MTL*α 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 *MTL***a**1-*MTL***a**2 or an *MTL*α1-*MTL*α2 configuration in different strains, and that based on expression patterns, *C. glabrata* strains can be distinguished as either **a** mating type or α 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.

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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 Cg12 (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 $CuSO_4$ (26).

Isolation and sequence analysis of *MTL* loci. Based on homology comparisons with the MATα2p or MTLα2p from the species *S. cerevisiae*, *Kluyveromyces lactis*, and *C. albicans* by using the multiple-alignment 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 domains were identified (1, 2, 21, 29). These sequences, WFAKKNIENPY and WVSNRRRKEK, were used to design the forward primer MP2F2 and the reverse primer MP2R1 (Table 2), respectively. These two degenerate primers were used to amplify similar homeobox-containing regions with *Taq* polymerase (Life Tech/Invitrogen Inc., Gaithersburg, Md.) by PCR amplification of *C. glabrata* genomic DNA. PCR products were purified by using the Wizard PCR Clean-up kit (Promega Corp, Madison, Wis.) and cloned into pGEM-T Easy (Promega Corp.). Plasmids pE30.8, pE30.9, and pE30.10, containing 440-, 125-, and 240-bp inserts, respectively, were sequenced in both directions with an ABI model 373A automatic sequencing apparatus and fluorescent Big Dye terminator chemistry (PE-ABI Inc., Foster City, Calif.). Protein-coding regions were determined by the Wu-BLAST/BEAUTY Search algorithm-based program (49).

Sequence analysis and strain comparison. To obtain the full-length *MTL1* locus and its flanking sequences, 10^5 plaques of a *C. glabrata* genomic library of strain 7549 (30) were screened with the 125-bp DNA fragment, which spanned the MATα2p homeodomain. Plaque lysates from 55 positive primary clones were used as a template for PCR, using MP2F1 and MP2R1 as primers (Table 2). Five

TABLE 1. Strains used in this study[a]

Strain	Geographic locale	Body location
26B0	Iowa City, Iowa	Oral
25T1	Iowa City, Iowa	Oral
75P1	Iowa City, Iowa	Oral
29P1	Iowa City, Iowa	Oral
26B9	Iowa City, Iowa	Oral
86B1	Iowa City, Iowa	Oral
7549	Iowa City, Iowa	Oral
65T1	Iowa City, Iowa	Oral
35B11	Iowa City, Iowa	Oral
LAI89	Detroit, Mich.	Vaginal
PB921	Detroit, Mich.	Vaginal
R313	Detroit, Mich.	Vaginal
LP21	Detroit, Mich.	Vaginal
PB656	Detroit, Mich.	Vaginal
NB783	Detroit, Mich.	Vaginal
PB09	Detroit, Mich.	Vaginal
CD457	Detroit, Mich.	Vaginal
1480.41	Richmond, Va.	Oral
1480.42	Richmond, Va.	Oral
1480.44	Richmond, Va.	Oral
1480.46	Richmond, Va.	Oral
1480.50	Richmond, Va.	Oral
1480.49	Richmond, Va.	Oral
1480.47	Richmond, Va.	Oral
1480.43	Richmond, Va.	Oral
J932405	Belgium	Vaginal
J942007	Belgium	Vaginal
J932436	Belgium	Vaginal
J932597	Belgium	Vaginal
J932387	Belgium	Vaginal
J932474	Belgium	Vaginal
J932285	Germany	Oral
J932405	Germany	Oral
9932258	Germany	Oral
J931010	Germany	Oral
J932273	Germany	Oral
J932283	Germany	Oral
J941814	The Netherlands	Blood

[a] All isolates used in this study were confirmed as *C. glabrata* by DNA fingerprinting with the probe, *Cg*12 (22). Each isolate was recovered from a separate human subject.

lambda clones, λMP1, λMP2, λMP5, λMP16, and λMP18, which generated 125-bp PCR products, were chosen for secondary screening. λMP1.1, which contained a 3.4-kb insert, was used to characterize the sequence of the *MTL1* region and flanking sequences by lambda clone walking, using custom primers and the ABI sequencing apparatus. Similarly, λMP16.1 was used to characterize the *MTL2* locus, and λMP5.1 and λMP18.1 were used to characterize the *MTL3* locus. Based on the DNA sequence of *MTL1*, two primers, MLFLF2 and MLFLR2 (Table 2), spanning the flanking region of the *MTL1* open reading frames (ORFs) were designed and used in PCRs to generate homologous DNA regions from two additional strains, 1480.47 and PB921. The derived PCR products were cloned into pGEM-T Easy and sequenced. Comparisons and alignments of protein sequences were performed by using the multiple alignment editor of Clustal W/Jalview software (www.embi.kun.nl.bioinf/tools/clustalw.shtml). Promoter analysis was performed with the Matrix method for identifying putative regulatory protein binding sites in the *S. cerevisiae* Promoter Database (http://ctsigma.cshl.org/jiar/).

Southern and Northern analyses. Southern and Northern analyses were performed according to procedures previously described (26, 45, 46). In Southern analyses in which more than one probe was used, the originally hybridized blots were stripped at 75°C according to the protocols of Church and Gilbert (7). For Southern analysis, the hybridization probes for *MTL***a**2/α2, *MTL***a**1, and *MTL*α1 contained ORF regions derived from the primer pairs P1RACE1-CGPF1, A1RACE1-CGAR1, and CGPIF1-CGPIR2, respectively (Table 2). For Southern analysis involving oligonucleotide-based hybridization to distinguish *MTL***a**2 and *MTL*α2 sequences among three *MTL* loci, 500 ng of FuncP2 (Table 2) was end labeled with [^{32}P]ATP by using T4 polynucleotide kinase as described by Conner et al. (9). Southern blotting was done with the nylon membrane Hybond N^+. The protocols for hybridization and washing of Southern blots were those of Landsman et al. (28). In Northern analyses, the hybridization probes for *MTL*α1 and *MTL***a**1 contained ORF regions derived from the same primer pairs.

RESULTS

Isolation of a conserved domain of *C. glabrata MAT*a2/*MAT*α2. Based on homology comparisons between MATα2p and MAT**a**2p 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 *MAT*α2 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 MATα2p 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 *MAT*α2. 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 MATα2p or MTLα2p of *S. cerevisiae*, *K. lactis*, and *C. albicans*, respectively.

Isolation and characterization of the *MTL1*a and *MTL2*a 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 *MAT*α2-like insert by PCR. One of these clones, λMP1.1, was used to determine the sequence of a 2,587-bp insert by customized primer walking in both directions (Fig. 1A and 2A). A BLAST-type search of the sequence identified a 312-bp *S. cerevisiae* MAT**a**1p-like protein-coding sequence and a 510-bp *S. cerevisiae* MAT**a**2p/MATα2p-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 MAT***a**1-like coding sequence in *MTL1*, which we will refer to as *MTL1***a**1, was 126 bp shorter than the *S. cerevisiae MAT***a**1 coding region. The deduced 104-amino-acid sequence had 28% overall similarity with *S. cerevisiae* MAT**a**1p (Fig. 3A). The-carboxy terminal 57 amino acids showed a high level of similarity with the carboxy-terminal regions of *C. albicans* MTL**a**1p (21), *K. lactis* MAT**a**1p (1), and *S. cerevisiae* MAT**a**1p (2) (Fig. 3A). The *S. cerevisiae MAT***a**2/*MAT*α2-like coding sequence in *MTL1* was 150 bp longer than the *S. cerevisiae MAT***a**2 coding region. The deduced 170-amino-acid sequence had an overall identity of 26% with both *S. cerevisiae* MAT**a**2p and *S. cerevisiae* MATα2p. The carboxy-terminal 123 amino acids exhibited 31% identity with the carboxy-terminal 119 amino acids of both *S. cerevisiae* MAT**a**2p and *S. cerevisiae* MATα2p (Fig. 3B). The carboxy-terminal two-thirds of MATα2p is identical to the full-length MAT**a**2p in *S. cerevisiae* (2). Align-

TABLE 2. Primers used in this study

Primer	Sequence
MP2F2	5′-TGGTTTGCAAAGAAYANNGAGAAYCCNTA-3′
MP2R1	5′-TTTTTCTTTTCTTCTTCTATTCGANACCCA-3′
PIRACE1	5′-GCCATCAAGGTAGGTCTGAAT-3′
CGPF1	5′-TAACCAACTTATATCATTGTGTTCCA-3′
AIRACE1	5′-GAACTTGATTGGTGGTGATCCCA-3′
CGAR1	5′-CAACACGGTAGGTTTACGATA-3′
CGPIF1	5′-ATGAAGTATACTGCCACAAAA-3′
CGPIR2	5′-CTGAGAGAATGACGGAGAGTGTA-3′
MLFLR2	5′-TATGTCTTGCGCGTCCAATTGCT-3′
MLFLF2	5′-ATAAGCAATCAGTATGTGTA-3′
FLR2	5′-ACATCACCAACATACGCACCGCT-3′
12.36F1	5′-ATGTCAGTCTGAACTAGTGAATA-3′
MLFLF3	5′-AGGGACATCGCTGAGGCCAGA-3′
MLFLR3	5′-ATTGACCCAAGAAGTGGTGAGA-3′
MTLOCR1	5′-GCTTGCAATCAAAGTGTTCTG-3′
MTLOCF1	5′-GTGCTGATCACTATCGAATGC-3′
FuncP2	5′-TATTGGATTAAGTAGATGCGTAATACTAATT CTTGATTTCTTTGACAT-3′

ment of MTL1**a**2p with *S. cerevisiae* MAT**a**2p 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 (WVXNRRR) (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 MAT**a**1p in *S. cerevisiae* (29). The two protein-coding regions in *MTL1* of *C. glabrata* strain 7549 will be referred to as *MTL1***a**1 and *MTL1***a**2, respectively (Fig. 1A).

No potential methionine initiation amino acid could be identified in frame in either MTL1**a**1p or MTL1**a**2p. Sequencing of three independent 5′ rapid amplification of cDNA ends (5′-RACE)-derived PCR products of *MTL***a**1 mRNA revealed an additional 21-bp sequence upstream of the first isoleucine codon (Fig. 2A). 5′-RACE-derived PCR products of *MTL1***a**2 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 *MTL1***a**1 or *MTL1*α2 transcripts are translationally functional. The *MTL1***a**1 and *MTL1***a**2 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 *MTL1***a**1 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 *MTL1***a**2 was flanked by the undefined sequence FLR (Fig. 1A). In *S. cerevisiae*, the functional *MAT***a** locus is flanked by *BUD5* at the 3′ end of *MAT***a**1 and by *TAF2* at the 3′ end of *MAT***a**2 (Fig. 1A), while the silent *HMR* and *HML* loci are flanked by YCRWDDta12/YCR097W-a and YCL068C/HCl065W, respectively (http://genome-www.stanford.edu/saccharomyces/). The intergenic promoter region between *MTL1***a**1 and *MTL1***a**2 was similar in size to that for *MAT***a** in *S. cerevisiae* (Fig. 1), but the sequence of the *MTL1***a**1-*MTL1***a**2 intergenic region was dissimilar to that of *S. cerevisiae MAT***a**1-*MAT***a**2, except for the presence of two putative binding sites for *TBP1*, one at the 3′ end of each of the presumed overlapping promoters for *MTL1***a**1 and *MTL1***a**2 (Fig. 1A).

To identify a DNA fragment containing a second *MTL***a** locus in strain 7549, a negative PCR selection strategy, involving two primer pairs for the *MTL1* 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 *MTL1***a**1 and *MTL1***a**2, respectively (Table 2). Lambda clone λ16.1 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 *MTL***a**1 ORF and an *MTL***a**2 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 *MTL2***a**1 ORF, which included 472 nucleotides flanking the 3′ end, was identical to *MTL1***a**1. A stretch of 347 nucleotides at the 3′ end of *MTL2***a**1 represented a unique flanking sequence of *MTL2***a**. The full-length ORFs of both *MTL1***a**2 and *MTL2***a**2 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, MTL2**a**2p is 9 amino acids longer than MTL1**a**2p. The *MTL2***a** flanking region, which spans the 3′ end of *MTL2***a**1, contained a short sequence homologous to *S. cerevisiae* and *C. albicans* Nep1p

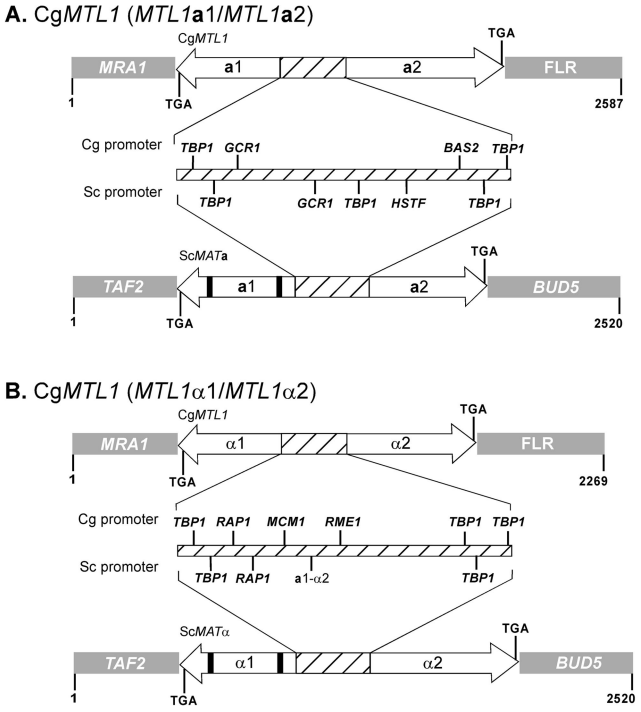

FIG. 1. Configurations of the *C. glabrata MTL1* locus (A) The *MTL1* locus containing *MTL1***a**1 and *MTL1***a**2 in strain 7549. (B) The *MTL1* locus containing *MTL1*α1 and *MTL1*α2 in strains PB921 and 1480.47. In both cases comparisons are made to comparable *S. cerevisiae* loci *MAT***a** and *MAT*α. *MRA1*, 3′ flanking region of *MTL1***a**1; *FLR*, 3′ flanking region of *MTL1***a**2; *TAF2*, 5′ flanking region of the *MAT***a**1/α1 locus; *BUD*5, 3′ flanking region of *MAT***a**2/α2. a1 and a2, *MTL1***a**1 and *MTL1***a**2, respectively, or *MAT***a**1 or *MAT***a**2, respectively; α1 and α2, *MTL1*α1 and *MTL1*α2, respectively, or *MAT*α1 or *MAT*α2, respectively. The thick black bars in *MAT***a**1 and *MAT*α1 represent introns. Hatched portions of loci represent intergenic promoter regions. Arrow directions reflect orientation of transcription on complementary Crick and Watson DNA strands.

A. Nucleotide sequence of the *MTL1***a** locus

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AGGGACATCGCTGAGGCCAGACCTGATATTACGCACCAGTGTTTGCTGACGTTGCTGGACTCCCCGATAAATAAGGCCGG   80
TCTGTTACAAGTCTACATCCTGACCAAGAAGAACGTGCTGATCGAGGTGAATCCATCTGTGAGAATACCCAGAACGTTCA  160
AGAGATTCTCCGGTCTGATGGTGCAATTGTTACACAAGCTATCTATCAGGTCTATGGAGAGTTCGAACACACATTTGCTG  240
AGAGTGGTGAAGAACCCAGTTACGAAATACCTGCCTGCCGACTGCCGGAAGGTCACTCTTTCTTTTGACGCTGAGGTGAT  320
GAGGCCGCAGGAGTACCTTGGGGATAAGCAATCAGTATGTGTATTTGTGGGGGCGATGGCGAGAGGCCACGACTCCTTTG  400
CTGATGAATATGTAGATGACAAAATAGCTATATCTAACTACCCGCTCTCCGCCTCCGTCGCCTGCTCCAAGTTCTGTCAT  480
GGCGCTGAGGACGCATGGGCCATTATATAATTTACAAACCCACACCGAGGACTCGCAAAATTAAACTGTTGCGAAAAGCA  560
GATAGTTTGGATTGCAAAGTAAGCGAAATTTAAAGAATTGTTTTGTATTGAATTGTACGGGGAGAAAACTATAAAAGAGT  640
AAGGAGGAACTATAGCTAGTCACGATTGTTTAGATCTTTCGATCTCTTGCGCCTATTTGCAAACTGTATGTTCTAAGTTA  720
GTATTTACTACAGAGGTTGAATTGGTTTCACTCAATTTAGAAAACATACCCAAATTTGAACTTGATTGGTGGTGATCCCA  800
CATTGAACCGCCAACTGATGTTTTTCACATCTAGTCAGGTACCGTTTCTTGGAAAATGTTTCAACTAATTGTAGCTTGGC  880
CTTTGCTGGTAGAGGCTTCCTAGTTGTATATGTTTTATTTTTTCTTTGACAATACAGCAGGTATTGAGAAGCACTTCTGC  960
GAAGTGCTTCTCTAGTCTGTTTGGGTAAATCTATGTTGTTGATCAACTTATTGTCGGTGTTAGTAGTGCTTATCTCAATA 1040
TTTGAAATGATAATATTATCGTAAACTTACCGTGTTGTAATCCCATGTTTCTTCTTCCAATAGAGTATTAGAATTAACCG 1120
ATTGCAATATCTGTAAACAGATATTGCGTAGATCTTGTATTGGGTCTACTGTCATCATATTATTGATTTGTCTAAAGATT 1200
TTGAATATTAATGACACAAGTTATAACAAGAAGAAAATTTAAAGGAAAAATTGCAAGAGATTAATAACCAACTTATATCA 1280
TTGTGTTCCAGTTTACCCAAAAGACAGTCGCTCCCGGGGCCGTCGTCCGATATCCTAAGGTTCCTATCGAGAAACAACTT 1360
GGACCCACAAGAGATAGGGTTGATCAAGACAACCTATCGACTGTCTACTCTTCTGTCAAAACTACGGGAGCATGAGATCG 1440
TATTCAATGTGGTTACCAAAGACCATCTGCTTAAAAAAGGCGTCCCCAATCACTATGCCGCTTCCTATCGAGGCCACCGC 1520
TTCACAAGGGAAAACGTACAGATACTAGAGACCTGGTACCGCAACCATATCGACAACCCATACCTCGACCACAACAGCCA 1600
GCAATATCTAGCACAGAAGACAAACCTATCAAAAATACAGATCAAAAACTGGGTGGCAAACAGAAGAAGAAAACAAAAGT 1680
CAATTTACATCTCACTATTCAGACCTACCTTGATGGCTCAAAACAGCCTCGACACTTGACTCAACATATACATTTCTCTC 1760
TGTCTTCTGCCACTACCTTATTTTTCTACCCTGGAATATCTTGAAATGGGAAACAATTTCAAGACACCACGCACCGACTG 1840
ACCACACAAGAGATCACCACCTTCGAAATAAACTTTCAGTCAAAATGATAACCATTCTTATACATTTTTAGAACCCGATT 1920
CTAGGTTCATCACTTCTTATGTAAATTACCCAAAAAACACCAAAAAATTTCGGAACCTGCTACAGTAAATTGTCACGCGA 2000
CTGGAACCTTTGCCAAGAACTCCCACATCACCAACATACGCACCGCTCGACACCATATTGACTACAATTAGCTCCCTTTA 2080
TACACAGCTATATACTTTACCCAAATACCATTAAACTCCAATAAATCCAAATCTTTTTTAAAATCGTTGCTAAGTCCCGA 2160
GGTCTCGACTACGCAAGACCAACGCTGGCAAAAATAAACTCTAAAATTAGACCACTACTGCAACTTACATCATAATCACA 2240
GGAAAGACGCCTAATCACACATCAATAATTAATACAACTGTCAATGGATTCTCCGACAAGCCAGTATGATAATGGCGCAT 2320
CGCCGACTTTACCCCTCGACACGTCTTTCCAGACCGCGTCCTTCCACACTGCAACCTCTAGTCCTACACCATCGCAGCAA 2400
TTGGACGCGCAAGACATATCAAAAGAGATAGAGCAACTGAGGCTTTACCAAGATAATAAACGTATTTCCTTCACAACGCC 2480
TGTCGTGAATACATCCCCACTACACCTGCCGCCAGACATAACACCAAGAGCCGACCAAGTTCAGCCGGAAAACCAACAAG 2560
GATTTGTATCTCACGGCCTCGGTATAT 2587
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B. Nucleotide sequence of *MTL1*α locus

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ATAAGCAATCAGTATGTGTATTTGTGGGGGCGATGGCGAGAGGCCACGACTCCTTTGCTGATGAATATGTAGATGACAAA   80
ATAGCTATATCTAACTACCCGCTCTCCGCCTCCGTCGCCTGCTCCAAGTTCTGTCATGGCGCTGAGGACGCATGGGCCAT  160
TATATAATTTACAAACCCACACCGAGGACTCGCAAAATTAAACTGCTGCGCGAATATGTCCCAAATATTTTGATCCGTTT  240
CTGGGGCGTTCCAAGCTTCTGAGAGAATGACGGAGAGTGTATTTTGTTTGAGGCCGGTGCCTAATTGGGCATAGTATGTA  320
CGAAATGCCATAAAAGCGTTCATAGGTCTTTTCTTACTGCTGGATTTCGATTTTAAAACATTTCCGATGGAAGTATTGAA  400
GTGATGAGATATGAACTTATCTATCTCCCATGATGTTTCAATATCGATGATATTGATACCTGACTCAAATGAGCTCGTCA  480
GTTTCATCCTTTCTTCTCTAATTCGCTTTAGTAACACAGGGTGAGGGGGCGGGATCTGAAACTTGTTCGGTTTAGTTAAC  560
AGCATGTTAAGACCAAAGGAGCTCTTTGTGGAACTACAGCCCAGAAAATCGGAGTGTTTTTCAGGATATTTTTGGCTTCT  640
GAGTCTTTTATTTGTTCTAACCCTGAATTTTGTGGCAGTATACTTCATAGTCAGTGTTTCAGTTAACATTAGTAGAGTGA  720
CTTCAGATATTGTACAACTGACATAAAGGAATGGTTAATTTCTGCTCATTAATCATGTTAACATGGCAATTTTTTGGGTT  800
TCTTTTATATTAATTCTCTTGGATATGAGACTTGTGTAAGATTAATATAGTATTTACAGTATTTTACCATATTAGGTAAA  880
TATGATAGACAGGGAGTGTAGTTTTATTAAAATTGCAATTTTTAGGCGGTTGAACCTCAATAAATTTATATAAAAGGAAC  960
GGAATGAAATTCAGTCTGAACTAGTGAATATATATGTGGTTTAAAAATACCAAAGTACATTATGTCAAAGAAATCAAGAA 1040
TTAGTATTACGCATCTACTTAATCCAATACAAGAAGAAAATTTAAAAGAAAAATTGCAAGAGATTAATAACCAACTTATA 1120
TCATTGTGTTCCAGTTTACCCAAAAGACAGTCGCTCCCGGGGCCGTCGTCCGATATCCTAAGGTTCCTATCGAGAAACAA 1200
CTTGGACCCACAAGAGATAGGGTTGATCAAGACAACCTATCGACTGTCTACTCTTCTGTCAAAACTACGGGAGCATGAGA 1280
TCGTATTCAATGTGGTTACCAAAGACCATCTGCTTAAAAAAGGCGTCCCCAATCACTATGCCGCTTCCTATCGAGGCCAC 1360
CGCTTCACAAGGGAAAACGTACAGATACTAGAGACCTGGTACCGCAACCATATCGACAACCCATACCTCGACCACAACAG 1440
CCAGCAATATCTAGCACAGAAGACAAACCCCTCAAAAATACAGATCAAAAACTGGGTAGCAAACAGAAGAAGAAAACAAA 1520
AGTCAATTTACATCTCACCATTCAGACCTACCTTGATGGCTCAAAACAGCCTCGACACTTGACTCAAACATATACATTTC 1600
TCTTTGTCTTCTGCCACTACCTTATTTTTCTAACCTGGAATTATCTTGAAATTGTTTCACAATTTCAAGAACCACACACG 1680
CACCGACTGACCCACACAAGAGATCACCACCTTCGAAATAAACTTTCAGTCAAAATGATAACCATTCTTATACATTTTTA 1760
GAACCCGATTCTAGGTTCATCACTTCTTATGTAAATTACCCAAAAAACACCAAAAAATTTCGGAACCTGCTACAGTAAAT 1840
TGTCACGCGACTGGAACCTTTGCCAAGAACTCCCACATCACCAACATACGCACCGCTCGACACCATATTGACTACAATTA 1920
GCTCCCTTTATACACAGCTATATACTTTACCCAAATACCATTAAACTCCAATAAATCCAAATCTTTTTTAAAATCGTTGC 2000
TAAGTCCCGAGGTCTCGACTACGCAAGACCAACGCTGGCAAAAATAAACTCTAAAATTAGACCACTACTGCAACTTACAT 2080
CATAATCACAGGAAAGACGCCTAAATCACACATCAATAATTAATACAACTGTCAATGGATTCTCCGACAAGCCAGTATGA 2160
TAATGGCGCATCGCCGACTTTACCCCTCGACACGTCTTTCCAGACCGCGTCCTTCCACACTGCAACCTCTAGTCCTACAC 2240
CATCGCAGCAATTGGACGCGCAAGACATA 2269
```

FIG. 2. Nucleotide sequences of the *MTL1***a** locus derived from strain 7549 and the *MTL1*α locus derived from strain PB621. (A) The ORFs for *MTL1***a**1 and *MTL1***a**2 are shown in boldface. The primer pairs for *MTL1***a**1 (AIRACE1 and CGAR1) are shown as black boxes with boldface white print, and those for *MTL1***a**2 (CGPF1 and PIRACE1) are shown as grey boxes with boldface white print. The primer pairs which flank the 3′ end of the *MTL1***a**1 ORF and the 3′ end of the *MTL1***a**2 ORF are underlined. (B) The ORFs for *MTL1*α1 and *MTL1*α2 are shown in boldface print. The primer pairs for *MTL1*α1 (CGPIR1 and CGPIF1) are shown as black boxes with white print, and those for *MTL1*α2 are shown as grey boxes with white print. The primers used to link *MTL1*α1 with *MTL1*α2 are underlined. Forty-eight nucleotides at the 5′ end of the *MTL1*α2 ORF that are common to *MTL1*α2 and *MTL3*α2 are show by dashed lines. The rest of *MTL*α2, excluding the open-box sequence, is common to *MTL*a2. The nucleotide sequence unique to the 3′ end of the *MTL1*α2 ORF is shown in an open box.

A. CgMTL1**a**1p

```
CgMTL1a1p --------------------ISNIEIST--------TNTDNKLINNIDLPKQTREA-------------------------  28
ScMATa1p  --------------------MDDICSMA--------ENINRTLFNILGTEIDEIN-------------------------  27
CaMTLa1p  -MNSEIESSLTLLKSVEKLVQATSVYK--------NEDNEEIFLQLKRERQENSN---LHEETETFFHESLDRLMKLSSI  68
KlMATa1p  MCDNDMADIQSKLSSFCEEIRALALKEGYNLEGDKSPSSKPYFMSWPKEIDVNHPNFAFFTKLQFQYDKSLETILNSCYL  80
                          :                 ..  :       :

CgMTL1a1p ---------LRRSASQYLLYCQR------------KNK---------------------------------TYTTRK  51
ScMATa1p  --------LNTNNLYNFIMESNL--TKVEQHTLHKNIS-------NNRLEIYHHIKKEK---------------SPKGKS  75
CaMTLa1p  AGSKVQYELSLNNLYNLLLRTRK--QEEEEAFIFPTVSTEEFALEQITFEVSDQMDDDKDSEDDILIKGEEIKKSKKKRQ 146
KlMATa1p  QELQLDPTRIIETLQQHFNNSILRYADIENISDVKDESPMSFDTEHECTDTSEDISDKSEISSTNSDNPIQNCYPTYKRS 160
                    ..  : :                 .                                        .   :.

CgMTL1a1p ----------------------------------TYTTRKPLPAKAKLQLVETFSKKRYLTRCEKHQLAVQCGITTNQVQ  97
ScMATa1p  -----NNRLEIYHHIKKEK---------------SPKGKSSISPQARAFLEQVFRRKQSLNSKEKEEVAKKCGITPLQVR 135
CaMTLa1p  EFALEQITFEVSDQMDDDKDSEDDILIKGEEIKKSKKKRQRLDNSTKEFLEKVFEKNKQPNRRERELIAEKHGVSLSQIR 226
KlMATa1p  SFDTEHECTDTSEDISDKSEISSTNSDNPIQNCYPTYKRSFIKHESRGILEKIFKVKQCPNTSERLYIAQKLDLTPSQVR 240
                                            .   :. :  .::  * : *  ::  .  *:  :* : .::  *::

CgMTL1a1p IWVCFLN---------------------- 104
ScMATa1p  VWFINKRMRSK----------------- 146
CaMTLa1p  VWFTNKRMRKKEPKLKAKSSSNST---- 250
KlMATa1p  IWFTNKRMRAKKHTTGKGTKRKSKKYPS 268
          :*.   .
```

B. CgMTL1**a**2p

```
CgMTL1a2p -------------QEENLKEKLQEINNQLISLCSSLPKR-QSLPGPSS----DILRFLSRNNLDP---------------  47
ScMATa2p  ------------------------------------------------------------------------------  -
ScMATα2p  MNKIPIKDLLNPQITDEFKSSILDINKKLFSICCNLPKLPESVTTEEEVELRDILLFLSRANKNRKISDEEKKLLQTTSQ  80

CgMTL1a2p ---------QEIGLIKTTYRSSTLSSKLR-EHEIVFNVVTKDHSLKKGVPNHYAASYRGHRFTRENVQILETWYRN-HID 116
ScMATa2p  -----------MRSIENDRSNYQLTQKNKSADGLVFNVVTQDMINKSTKP------YRGHRFTKENVRILESWFAK-NIE  62
ScMATα2p  LTTTITVLLKEMRSIENDRSNYQLTQKNKSADGLVFNVVTQDMINKSTKP------YRGHRFTKENVRILESWFAKKNIE 154
                    :  *:.   .  *:.* :  . :******:*   *.  *      *******:***:***:*: : :*:

CgMTL1a2p NPYLDHNSQQYLAQKTNLSKIQIKNWVANRRRKQKSIYISLFRPTLMAQNSLDT--- 170
ScMATa2p  NPYLDTKGLENLMKNTSLSRIQIKNWVSNRRRKEKTITIAPELADLLSGEPLAKKKE 119
ScMATα2p  NPYLDTKGLENLMKNTSLSRIQIKNWVSNRRRKEKTITIAPELADLLSGEPLAKKKE 211
          ***** :. : * ::*.**:*******:*****:*:* *:   . *:: :.* .
```

FIG. 3. Sequence comparison of MTL1**a**1p and MTL1**a**2p of *C. glabrata* strain 7549 with MAT**a**1ps and MAT**a**2ps of other yeast species. (A) Aligned sequences of *C. glabrata* (Cg) MTL1**a**1p and MAT**a**1ps or MTLa1ps of *S. cerevisiae* (Sc), *C. albicans* (Ca), and *K. lactis* (Kl). B. Aligned sequences of *C. glabrata* MTL1**a**2p and *S. cerevisiae* MAT**a**2p and MATα2p. The shaded sequences in both panels represent the highly conserved amino acid residues (boldface) involved in the interaction between *S. cerevisiae* MAT**a**1p and *S. cerevisiae* MATα2p in the formation of the **a**1-α2 repressor complex. Note that *S. cerevisiae* MAT**a**2p and MATα2p share identical amino acid residues in this region, but the physiological role of *S. cerevisiae* MAT**a**2p 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 secondary structure. The accession numbers for sequences other than those of *C. glabrata* are as follows: ScMAT**a**1p, PO1366; CaMAT**a**1p, AAD51404.1; KlMAT**a**1p, AAG21094.1; ScMAT**a**2p, CAA246201.1; and ScMATα2p, AAA34762.1. The accession numbers for *C. glabrata MTL1***a** and *MTL2***a** are AY191461 and AY191464, respectively.

(14). The 3′ end of *MTL2***a**2 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 *MTL*α 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 *MTL* locus in two additional strains of *C. glabrata*, PB921 and 1480.47. The primers MLFLF2 and MLFLR2 (Table 2), which represented the 3′ ends of the flanking regions of *MTL1***a**1 and *MTL1***a**2, 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 MAT*α1-like coding sequence and an *S. cerevisiae MAT***a**2/*MAT*α2-like coding sequence (23). Because the *MAT*α1-like and *MAT*α2-like coding sequences were similar to those of *S. cerevisiae MAT*α1 and *MAT*α2, respectively, the paired coding regions were designated *MTL1*α1 and *MTL1*α2. The flanking sequences of the *MTL1*α locus in strains PB921 and 1480.47 were identical to those of *MTL1***a** in reference strain 7549. Both *MTL1*α2 and *MTL1*α1 contained uninterrupted ORFs beginning with codons for the initiation amino acid methionine. As in the case of the *MTL***a**2-*MTL***a**1 configuration of the *MTL1* and *MTL2* loci in strain 7549, *MTL1*α2 and *MTL1*α1 were positioned on complementary Crick and Watson DNA strands, with an intergenic promoter (Fig. 1B and 2B). The *MTL1*α promoter encompassed 333 bp, which was 70 bp longer than the *MAT*α promoter of *S. cerevisiae*. The *MTL*α promoter contained three TATA box binding protein sites and a putative binding site for the repressor-activator protein Rap1p, both found in the *MAT*α2 promoter of *S. cerevisiae* (Fig. 1B). The *MTL*α promoter of *C. glabrata* also contained putative Mcm1p and Rme1p binding sites, neither of which was present in the *MAT*α promoter of *S. cerevisiae* (Fig. 1B). There was no recognizable binding site for the MAT**a**1p-MATα2p heterodimer (**a**1-α2) (Fig. 1B and 2B) found in the promoter of *S. cerevisiae* (Fig. 1B).

The *MTL1*α1 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

FIG. 4. Sequence comparison of MTL1α1p and MTL1α2p of *Candida glabrata* strains PB921 (CgMTL1α1pa and CgMTL1α2pa) and 1480.47 (CgMTL1α1pb and CgMTL1α2pb) with MATα1ps or MTLα2ps and MATα2ps or MTLα2ps of other yeast species. (A) Aligned sequences of *C. glabrata* MTLα1pa and MTLα1pb with MATα1p of *S. cerevisiae* (Sc), MATα1p of *K. lactis* (Kl), and MTL**a**1p of *C. albicans* (Ca). (B) Aligned sequences of *C. glabrata* MTL1α2pa with MATα2p of *S. cerevisiae*, MATα2p of *K. lactis*, and MTL1α2p 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, AAA34763.1; KlMATα1p, AAG21092.1 (note that the carboxy-terminal 42 amino acids of this *K. lactis* sequence are not shown); CaMATα1p, AAD51411.1; ScMATα2p, AAA34762.1; KlMATα2p, AAG21091.1; and CaMATα2p, AAD51408.1. The accession numbers for *C. glabrata MTL1*α**a**, *MTL1*α**b**, and *MTL3*α are AY191462, AY191463, and AY207368, respectively.

amino acids showed 50% identity with MATα1p (Fig. 4A). The *MTL1*α2 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). MTL1α2p was identical to MTL1**a**2p in *C. glabrata* strain 7549, except that the former was 16 amino acids longer at the amino-terminal end and contained an initial methionine. Since the primers used to clone the *MTL1* loci in strains PB921 and 1480.47 represented the flanking regions of *MTL1* in strain 7549, these results indicate that the same *MTL* locus, *MTL1*, harbors alternative *MTL1***a** or *MTL1*α genes in different strains.

A preliminary Southern analysis of *Xba*I-digested DNA of strain 7549 hybridized with *MTL1***a**2-α2 revealed three bands, but the same Southern blot probed with *MTL1***a**1 revealed only two of these bands, suggesting a third locus containing an *MTL*α ORF. To identify this third locus in strain 7549, a positive PCR selection strategy employing the *MTL1*α1 ORF-specific primer pair CGP1F1-CGP1R2 (Table 2), was used to screen 55 primary lambda clones. Two clones, λMP5.1 and λMP18.1, containing the *MTL*α1 ORF were selected. The sequence of 2,169 nucleotides was determined by a primer walking strategy. This locus was designated *MTL3*α. Sequence analysis revealed that this locus harbored divergently transcribed *MTL3*α1 and *MTL3*α2 ORFs, both organized similarly to the *MTL1*α locus (see Fig. 6B). The sequence of the *MTL3*α1 ORF was identical to that of the *MTL1*α1 ORF, except that the alanine codon at bp 152 was replaced by a serine codon. Although both the *MTL1*α2 and *MTL3*α2 ORFs contained identical stretches of 164 amino acids at their amino-terminal ends, their carboxy termini were dissimilar. MTL3α2p contained a unique stretch of 22 amino acids at the carboxy terminus that was not shared by MTL1α2p (see Fig. 6B). The flanking regions of *MTL3* 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 *Bam*HI, *Eco*RI, *Pst*I, or *Xba*I and probed with the full-length ORF of *MTL1a**1, *MTL1***a**2, or *MTL1*α1. 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 *MTL1*α2 and *MTL1***a**2 (referred to as *MTL***a**2-α2) included two or three bands, depending on the restriction enzyme (Fig. 5A and D). The *Xba*I patterns of the four test isolates probed with *MTL***a**2-α2 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 *MTL1***a**2 was identical to the 414 bp of the 3′ end of *MTL1*α*2*, Southern blot hybridization with *MTL***a**2-α2 could not distinguish between bands containing *MTL***a**2 or *MTL*α2 sequences.

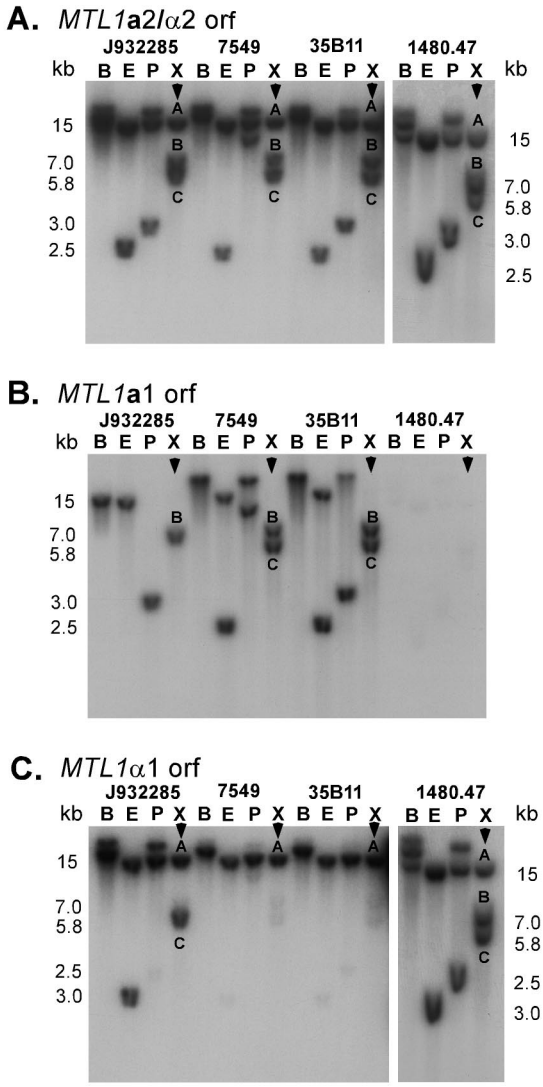
In order to distinguish between *MTL***a**2 and *MTL*α2 sequences, Southern blots of *Xba*I-digested genomic DNAs of 38 strains were probed with the end-labeled antisense oligonucleotide primer FuncP2 (Table 2; Fig. 5D), which is unique to the 5′ ends of *MTL*α2 ORFs. The hybridization identified among the strains three fragments (A, B, and C) containing *MTL*α2 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 *MTL*α2 ORF (Table 3).

The Southern blot hybridization patterns of *Xba*I-digested DNAs of the four test strains probed with *MTL***a**1 (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 *MTL***a**1 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, *MTL***a**1 did not hybridize to any band (Fig. 5B), although the *MTL*α2-specific primer hybridized to three bands (Fig. 5C). *MTL***a**1 did not hybridize to band A in any of the 38 tested strains, in contrast to the *MTL*α2-specific primer (Table 3).

The Southern blot hybridization patterns of *Xba*I-digested DNAs of the four test strains probed with *MTL*α1 (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, *MTL*α1 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 *MTL*α1 ORF always pairs with the *MTL*α2 ORF. The Southern blot hybridization patterns of all 38 test strains included band A (Fig. 4C; Table 3). In 58% of strains, *MTL*α1 hybridized only to band A, the same group of strains in which *MTL***a**1 hybridized to bands B and C. We designate strains in this group class I (Table 3). In 18% of strains, *MTL*α1 hybridized to both A and B; this is the same group of strains in which *MTL***a**1 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, *MTL*α1 hybridized to both A and C; this is the same group of strains in which *MTL***a**1 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 *MTL1*α1 and *MTL1*α2, while in class III isolates, *MTL2* contains this configuration. In class IV, there was no hybridization to *MTL***a**1; *MTL*α1 hybridized to bands A, B, and C (Fig. 5C). If we distinguish loci as **a** or α by whether they harbor *MTL***a**1 or *MTL*α1, respectively, then these results suggest that class I strains possess two *MTL***a** loci and one *MTL*α locus (**aa**α), class II strains possess two *MTL*α loci and one *MTL***a** locus (**a**αα), class III strains possess two *MTL*α loci and one *MTL***a** locus (**a**αα), and class IV strains possess three *MTL*α loci (ααα) (Tables 3 and 4).

To test the relationship between the bands containing *MTL* ORFs and the three *MTL* loci, *Xba*I-digested DNAs of the 38 test strains were probed sequentially with the DNA fragments flanking the 3′ ends of *MTL1***a**1 and *MTL1***a**2 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 *MTL***a**2 in *MTL1*, and primer pair MLFLR3 and MLFLF3 (Table 2) generated a 246-bp PCR product that spanned the 3′ flanking region of *MTL***a**1 in *MTL1* (Fig. 1A). Southern blots of *Xba*I-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 *MTL***a**1 and only band A when probed with either *MTL*α1 or FuncP2 (class I) exhibited only the 7-kb band B when probed with the 3′ flanking regions (Table 3), demonstrating that in these strains only one of the two *MTL***a**1 genes is located in the *MTL1* locus. A majority of strains that exhibited bands A and B when probed with *MTL*α1 exhibited hybridization with only the 5.2- or 7-kb band B (class II) when probed with the 3′ flanking regions (Table 3), demonstrating that in these strains only one of the two *MTL*α1 genes is located in the *MTL1* locus. Surprisingly, two strains that exhibited bands A and C when probed with *MTL*α1 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 *MTL*α1 genes are located at the *MTL2* locus. Southern blots of *Xba*I-digested DNAs from the 38 test strains probed with the 280-bp PCR product flanking the 3′ end of the *MTL2***a**1 ORF, which was generated with the primer pair MTLOCF1-MTLOCR1 (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



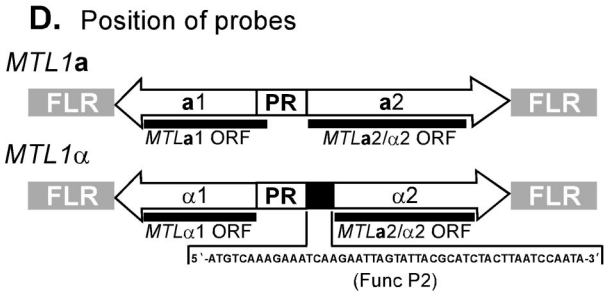


FIG. 5. Southern blot analysis of the *MTL* loci of *C. glabrata*. Approximately 2.5 μg of total genomic DNA from strains J932285, 7549, 35B11, and 1480.47 were individually digested with the four restriction enzymes *Bam*HI (B), *Eco*RI (E), *Pst*I (P), and *Xba*I (X). Digested DNA was resolved in an agarose gel and transferred to a Hybond N nylon membrane. Triplicate Southern blots were individually hybridized with the ORFs for *MTL1* genes. (A) Hybridization with the 450-bp PCR fragment (primers CGPF1 and P1RACE1) containing sequences identical to both *MTL1***a**2 and *MTL1*α2. (B) Hybridization with the 300-bp PCR fragment (primers CGAR1 and A1RACE1) containing the *MTL1***a**1 ORF. (C) Hybridization with the 550-bp PCR fragment (primers CGPIF1 and CGPIR1) containing the *MTL1*α1 ORF. Three distinct hybridizable fragments in *Xba*I-digested genomic DNA are designated A (15 kb), B (7 kb) and C (5.8 kb). (D) Schematic representation of *MTL1***a** and *MTL1*α loci, showing the positions of probes. The primer pairs used to generate the *MTL1* ORF probes are described in Fig. 2. The sequence of the *MTL*α2-specific oligonucleotide FuncP2 is also shown. The molecular sizes of the expected fragments are shown to the right and left of panels A and C and to the left of panel B.

demonstrate that the *MTL* genes were similar in size between strains and between loci. A PCR-based approach was used to investigate this question. To test for any variability in the sequence common to *MTL1***a**2 and *MTL1*α2 at the *MTL1* locus, the primer pair CGPF1 and P1RACE1 (Table 2) was used to generate a single PCR product of 450 bp (Fig. 6B). This 450-bp PCR product was obtained in each of 10 test strains (Fig. 6A), which included five class I strains, four class II strains, and one class IV strain, and in each of the additional 28 isolates in the test collection (data not shown). These results demonstrate that the region common to *MTL***a**2 and *MTL*α2 is highly conserved in *C. glabrata*. Since the nucleotide sequences encompassing PIRACE1 is present in *MTL1***a**2and *MTL1*α2 but not in the *MTL3* (band A) or *MTL2* (band C) locus, the results clearly suggest that the 450-bp PCR product must have been generated from *MTL1* in all classes. To discriminate between (i) *MTL***a**2 and *MTL*α2 and (ii) *MTL1*α2 and *MTL3*α2, the 5′ primer 12.36F1, which is unique to the full-length *MTL*α2 ORFs of either *MTL1*α or *MTL3*α loci, and the 3′ primer P1RACE1, which is common to *MTL1***a**2 and *MTL1*α2 but not to *MTL3*α2 (Fig. 6B), were employed. This primer pair generated a 560-bp PCR product for the four class II strains and one class IV strain but produced no product for the five class I strains (Fig. 6A). These results therefore suggest that class II and IV strains contain an *MTL1*α2 gene at the *MTL1* locus, while class I strains contain no *MTL1*α2 gene. Southern analysis revealed that class I isolates contained *MTL*α1 and *MTL*α2 solely at the *MTL3* locus in the A fragment (Tables 3 and 4). These PCR results (Fig. 6A) confirm that in class I isolates, *MTL3*α1 is paired with an *MTL3*α2-type sequence and not an *MTL1*α2-type sequence in the A fragment.

To investigate further the linkage between *MTL1*α1 and *MTL1*α2, we employed the primer pair CGPIR2, which is common to both *MTL3*α1 and *MTL1*α1 (and probably *MTL2*α1) and PIRACE1, which is unique only to the 3′ end of *MTL1*α2 (Fig. 6B) but is absent in *MTL3*α2 and probably in *MTL2*α2. These primers generated a 1,300-bp PCR fragment which included full-length *MTL1*α1 and *MTL1*α2 ORFs for the four class II strains and one class IV strain but not for any of the class I strains (Fig. 6A). These results demonstrate that in class II and IV strains, *MTL1*α1 paired with *MTL1*α2 at the *MTL1* locus. For two strains, PB921 and 1480.47, sequencing data confirmed that *MTL1*α1 was paired with *MTL1*α2 in the B fragment (Fig. 1B and 2B). These results also confirm that *MTL3*α1 is paired with *MTL3*α2 and not with an *MTL1*α2-type ORF in band B in class II strains.

To investigate whether the *MTL***a**1 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***a**1 (Fig. 6B), was em-

TABLE 3. Classification of 38 *C. glabrata* strains based on the distribution of *MTL***a**1, *MTL***a**2 (or *MTL*α2), and *MTL*α1 revealed by Southern analysis

Class	No. of isolates	% of isolates	Band pattern with probe:				
			*MTL1***a**1	*MTL***a**2-α2[a]	FuncP2[b]	*MTL1*α1	*MTL*1 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			A, B, C	A, B, C	A, B, C	B
V	NA[c]	NA	A, B, C	A, B, C			B

[a] This probe does not distinguish between *MTL***a**2 and *MTL*α2.
[b] This probe distinguishes between *MTL***a**2 and *MTL*α2.
[c] NA, not applicable.

ployed. 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 *MTL***a**1. These primers also generated a 300-bp PCR product in the 28 additional isolates in the collection. Finally, to test whether *MTL*α1 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 *MTL*α1 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 *MTL*α1. Since the sequences of *MTL1*α1 and *MTL3*α1 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 *MAT*α or *MAT***a**, depending upon the genotype of the *MAT* locus. 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 *MTL***a**1 and *MTL*α1, 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 *MTL*α1, no class I isolates expressed it (Fig. 7). Conversely, while all tested isolates in class I expressed *MTL***a**1, 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 **a**-expressors. Predicated on the cassette model in *S. cerevisiae*, predictions of the *MTL* expression locus can be made based on the duplication of either *MTL*α1 or *MTL***a**1 in two of the three *MTL* loci. While all class II and IV isolates possibly carry identical *MTL*α1 ORFs at both the *MTL1* and *MTL3* loci, class III isolates possibly carry identical *MTL*α1 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 *MTL***a**1 ORFs at both the *MTL1* and *MTL2* loci. Therefore, in class I isolates, either *MTL1* or *MTL2* may represent the expression locus.

TABLE 4. Models for the six classes based on the distribution of *MTL***a**1, *MTL***a**2 (or *MTL*α2), and *MTL*α1 revealed by Southern analysis

Class	Genes in band:		
	A	B	C
I (**aa** α)	*MTL*α*MTL3*	*MTL***a***MTL1*	*MTL***a***MTL2*
II (**a** αα)	*MTL*α*MTL3*	*MTL*α*MTL1*	*MTL***a***MTL2*
III (**a**αα)	*MTL*α*MTL3*	*MTL***a***MTL1*	*MTL*α*MTL1*
IV (ααα) (1480.47)	*MTL*α*MTL3*	*MTL*α*MTL1*	*MTL*α*MTL2*
V (**aaa**)	*MTL***a***MTL3*	*MTL***a***MTL1*	*MTL***a***MTL2*

Transcription of *MTL* genes during phenotypic switching. Since the differential expression of *MAT***a** and *MAT*α 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 *MTL*α1 and *MTL***a**1 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 $CuSO_4$: 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 $CuSO_4$, were clonally plated, and Wh, LB, and DB colonies were analyzed. *MTL*α1 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 *MTL***a**1 (Fig. 8). Cells of *C. glabrata* strain 35B11, an **a**-expressor exhibiting the LB phenotype on agar containing $CuSO_4$, were clonally plated, and LB, DB, and IWr colonies were analyzed. Neither LB, DB, nor IWr cells expressed *MTL*α1, but all three expressed *MTL***a**1 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 *MTL*α1 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),

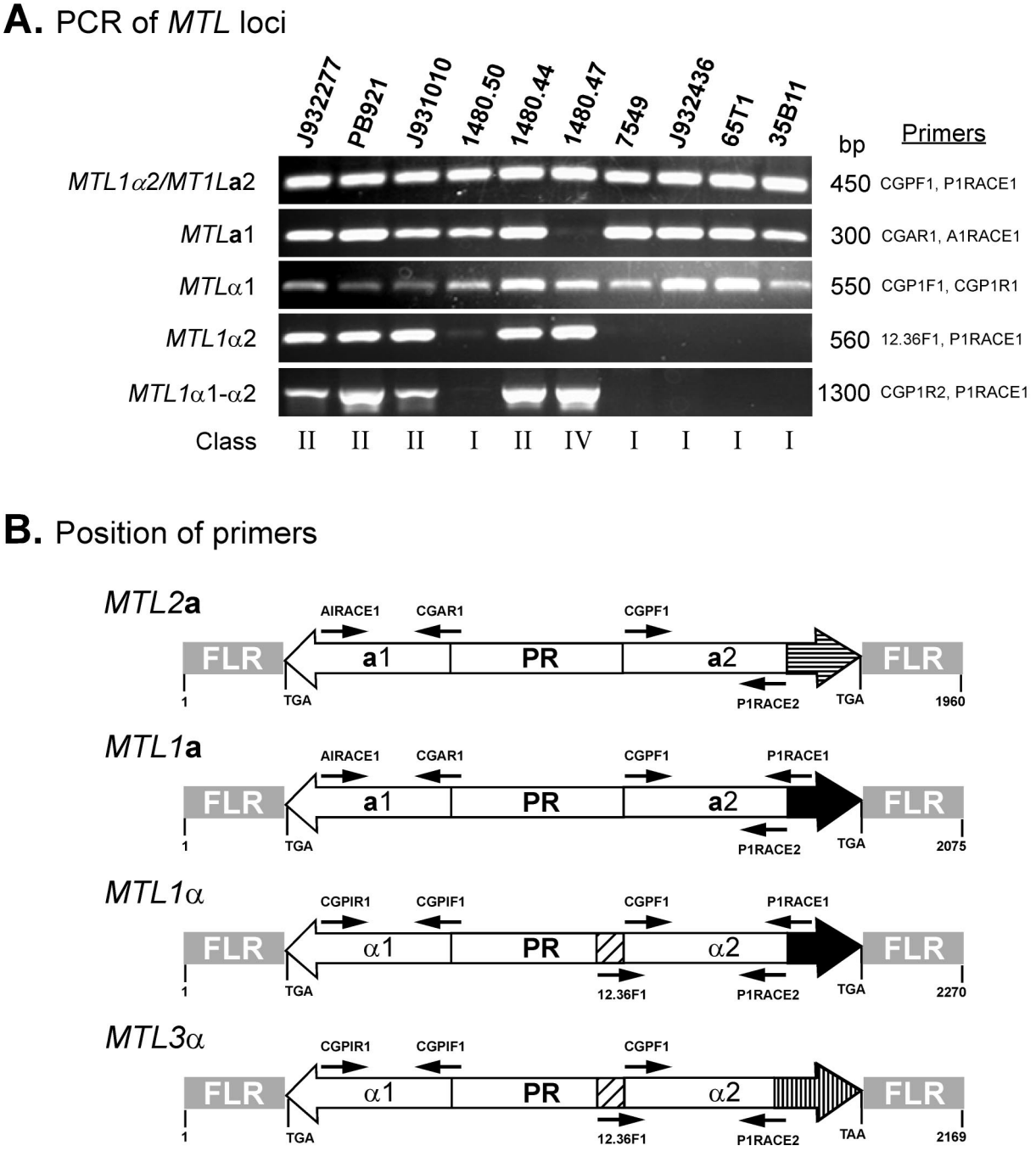


FIG. 6. PCR analysis of the *MTL* loci of *C. glabrata.* (A) PCR assays were performed with genomic DNAs of 10 *C. glabrata* strains. Five separate PCRs were performed with each strain to test the presence or absence of the full-length ORFs. Note that the primers CGPF1 and P1RACE1 do not discriminate between *MTL***a**2 and *MTL*α2, and the primers CGPIR2 and PIRACE1 generate a fragment containing *MTL*α1, *MTL*α2 and the intergenic promoter region. (B) Schematic representation of *MTL2***a**, *MTL1***a**, and *MTL3*α from strain 7549 and *MTL1*α from strains PB621 and 1480.47. Positions of key primer pairs used in the PCR analysis are shown.

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 α genes, suggesting that a cassette system similar to the one basic to *S. cerevisiae* mating may exist in *C. glabrata* (18).

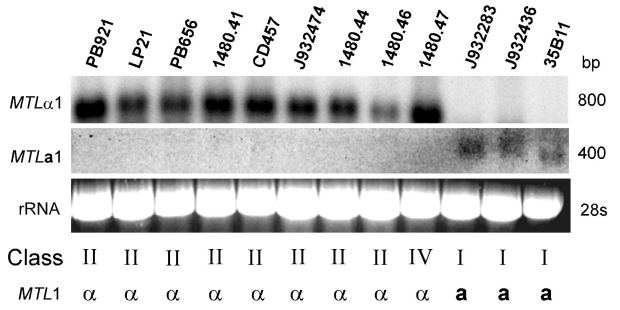


FIG. 7. Northern analysis of the expression of *MTL*α1 and *MTL***a**1 in 12 strains of *C. glabrata*. Approximately 20 μg of total cellular RNA was applied to each lane. Duplicate Northern blots were probed with either the 300-bp PCR fragment (primers CGAR1 and A1RACE1) containing the *MTL1***a**1 ORF or the 550-bp PCR fragment (primers CGPIF1 and CGPIR2) containing the *MTL1*α1 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.

Configuration of the three *MTL* loci. *C. glabrata* possesses 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 *MTL***a**1 ORF and an *MTL***a**2 ORF on complementary Crick and Watson DNA strands. Although in-frame AUG codons were not evident in either *MTL***a**1 or *MTL***a**2, one out-of-frame AUG codon was present 39 and 15 nucleotides upstream of an isoleucine or glutamine codon in *MTL***a**1 and *MTL***a**2, respectively. It is not clear whether the hybridizable *MTL***a**1 transcript identified in Northern blots or the 5′-RACE product represents the translatable mRNA. Interestingly, *MAT***a**1, the homolog of *MTL***a**1, has no known function in haploid **a** cells of *S. cerevisiae* (32).

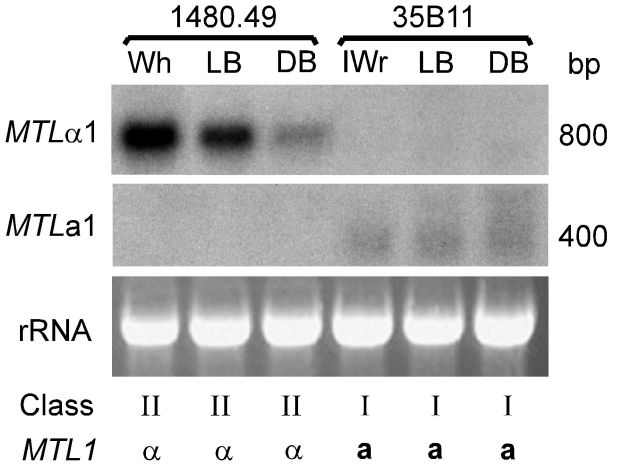


FIG. 8. Northern analysis of the expression of *MTL*α1 and *MTL***a**1 in the switch phenotypes of an *MTL*α-expressing strain (1480.49) and an *MTL***a**-expressing strain (35B11). Cells of the two strains were plated on agar containing 1 mM $CuSO_4$, which discriminates between the switch phenotype 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 CGPIR1) containing the *MTL1*α1 ORF or the 300-bp PCR fragment (primers CGAR1 and A1RACE1) containing the *MTL1***a**1 ORF. To assess loading, ethidium bromide-stained 28S rRNA patterns are included. The class and *MTL1* expression pattern of each strain are noted.

*MTL***a**1 and *MTL***a**2 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 MAT***a**1 and *MAT***a**2 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 *MTL*α1 ORF and the *MTL*α2 ORF resided in the *MTL1* locus on cDNA strands in a configuration similar to that of *MTL***a**1 and *MTL***a**2. Again, while the *MTL*α1-*MTL*α2 configuration included an intergenic promoter, like the one separating and regulating *MAT*α1 and *MAT*α2 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 **a**1-α2 binding site present in the *S. cerevisiae* promoter (24). The latter observation suggests that in **a** cells of *C. glabrata*, transcription of *MTL*α1 may not be repressed by an MTL**a**1p-MTLα2p 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 *MTL*α1 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 *MTL*α1 expression in α-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 α for a number of *C. glabrata* strains based on the presence of *MTL***a**1-*MTL***a**2 or *MTL*α1-*MTL*α2 pairs, which could be discriminated unambiguously at each of the three loci. Our results first demonstrate that the great majority of strains are either **a**αα or **aa**α. Only one of the 38 isolates tested, 1480.47, deviated from the two patterns, exhibiting an ααα genotype. Based on these studies, the 38 tested strains (97%) separated into four classes based on genotype and the size of the *Xba*I fragment harboring *MTL1* or *MTL2* (i.e., fragments B or C) (Fig. 9; Table 4). Class I strains were α**aa** and contained the combination of *MTL***a**1 and *MTL***a**2 at the *MTL1* and *MTL2* loci in bands B and C, respectively (Fig. 9). Class I strains also harbored the combination *MTL*α1-*MTL*α2 at the *MTL3* locus in band A (Fig. 9). Class II strains were αα**a** and contained the combinations of *MTL1*α1 and *MTL1*α2 at the *MTL1* locus in band B, *MTL2***a**1 and *MTL2***a**2 at the *MTL2* locus in band C, and *MTL3*α1 and *MTL3*α2 at the *MTL3* locus in band A (Fig. 9). Class III strains were also αα**a** but contained *MTL2*α1 and *MTL2*α2 at the *MTL2* locus in band C rather than in band B (Fig. 9). Class III strains also harbored the combination *MTL3*α1-*MTL3*α2 at the *MTL3* locus in band A, like the other two classes, and *MTL2***a**1 and *MTL2***a**2 in band B (Fig. 9). Based on the *MTL* genotypes in fragment B or

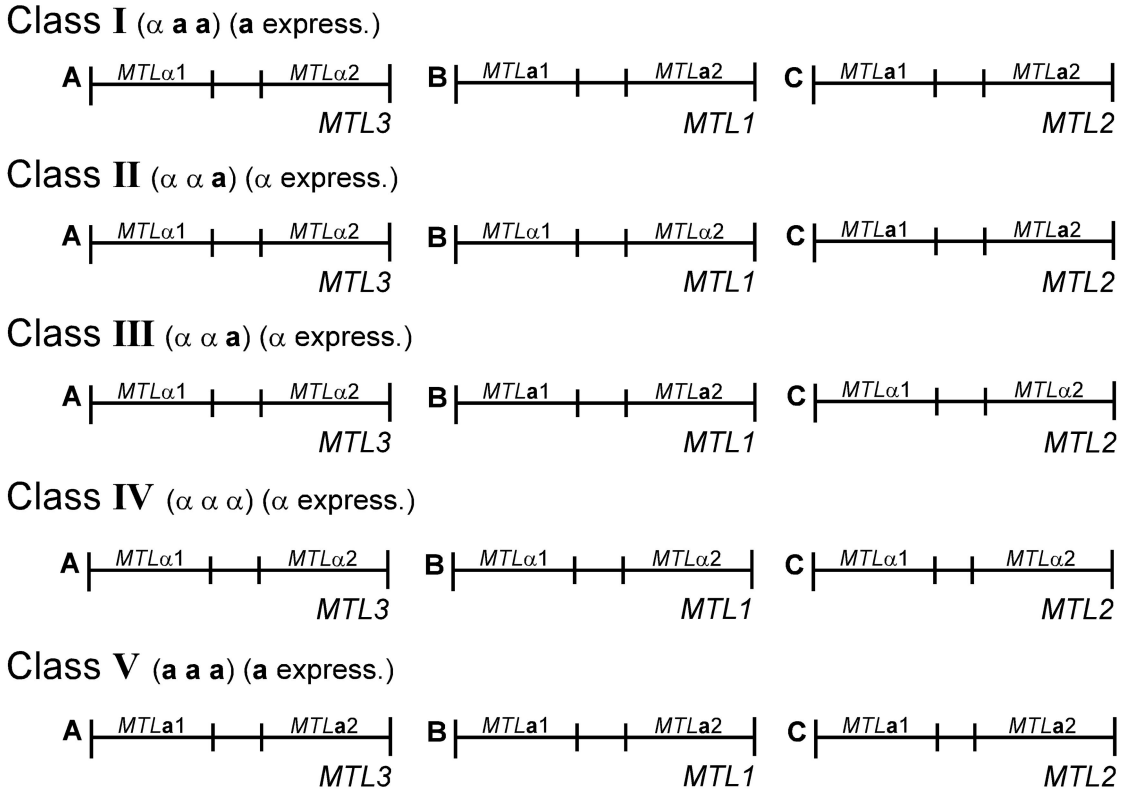


FIG. 9. A model of the *MTL* loci in the four classes of *C. glabrata* strains. The three *MTL* loci were distinguished as *Xba*I fragments A, B, and C in Southern analyses (see Fig. 5). The genotypes of the loci in the different classes were interpreted from the combined results of Southern, PCR, and sequencing data. The strains are distinguished as α**aa** (class I), αα**a** (classes II and III), or ααα (class IV). A hypothesized class V is **aaa**. The expression patterns are distinguished as either **a** expression or α expression, as determined by Northern analysis.

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 *MTL***a**1 or *MTL*α1, never both, just as haploid *S. cerevisiae* expresses *MAT***a**1 or *MAT*α1 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 *MTL*α1, class I strains expressed *MTL***a**1. The former classes of isolates possessed two *MTL*α loci, while the latter class possessed two *MTL***a** loci. Northern analysis demonstrated a direct correlation between the expression of *MTL***a**1 or *MTL*α1 and the presence of two corresponding ORFs at two *MTL***a** and two *MTL*α 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 *MTL*α1 exclusively in all three tested phenotypes in the core switching system (Wh, LB, and DB), while the **a**-expressing strain 35B11 expressed *MTL***a**1 exclusively in both core switch phenotypes (LB and DB) and the IWr phenotype. However, in the α-expressing strain, the levels of *MTL*α1 transcript differed between the three core switch phenotypes according to the hierarchy Wh > LB > DB, suggesting that *MTL*α1 expression is modulated in a graded fashion by the core switching system, just like transcription of the methalothionine gene *MTII*, for pigmentation and phloxine B staining (26, 27). Interestingly, the intergenic promoter that controls transcription of *MTL*α1 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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REFERENCES

1. **Astrom, S. U., A. Kegel, J. O. Sjostrand, and J. Rine.** 2000. *Kluyveromyces lactis* Sir2p regulates cation sensitivity and maintains a specialized chromatin structure at the cryptic alpha-locus. Genetics **156:**81–91.
2. **Astell, C. R., L. Ahlstrom-Jonasson, M. Smith, K. Tatchell, K. A. Nasmyth, and B. D. Hall.** 1981. The sequence of the DNAs coding for the mating-type loci of *Saccharomyces cerevisiae*. Cell **27:**15–23.
3. **Barns, S. M., D. J. Lane, M. L. Sogin, C. Bibeau, and W. G. Weisburg.** 1991. Evolutionary relationships among pathogenic *Candida* species and relatives. J. Bacteriol. **173:**2250–2255.
4. **Blaschke-Hellmessen, R.** 1996. Fluconazole and itraconazole susceptibility testing with clinical yeast isolates and algae of the genus *Prototheca* by means of the Etest. Mycoses **39**(Suppl. 2)**:**39–43.
5. **Blumental-Perry, A., W. Li, G. Simchen, and A. P. Mitchell.** 2002. Repression and activation domains of RME1p structurally overlap, but differ in genetic requirements. Mol. Biol. Cell **13:**1709–1721.
6. **Carninci, P., Y. Shibata, N. Hayatsu, Y. Sugahara, K. Shibata, M. Itoh, H. Konno, Y. Okazaki, M. Muramatsu, and Y. Hayashizaki.** 2000. Normalization and subtraction of cap-trapper-selected cDNAs to prepare full-length cDNA libraries for rapid discovery of new genes. Genome Res. **10:**1617–1630.
7. **Church, G. M., and W. Gilbert.** 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA **81:**1991–1995.
8. **Claret, F. X., M. Hibi, S. Dhut, T. Toda, and M. Karin.** 1996. A new group of conserved coactivators that increase the specificity of AP-1 transcription factors. Nature **383:**453–457.
9. **Conner, B. J., A. A. Reyes, C. Morin, K. Itakura, R. L. Teplitz, and R. B. Wallace.** 1983. Detection of sickle cell beta S-globin allele by hybridization with synthetic oligonucleotides. Proc. Natl. Acad. Sci. USA **80:**278–282.
10. **Covitz, P. A., I. Herskowitz, and A. P. Mitchell.** 1991. The yeast *RME1* gene encodes a putative zinc finger protein that is directly repressed by a1-alpha 2. Genes Dev. **5:**1982–1999.
11. **Covitz, P. A., and A. P. Mitchell.** 1993. Repression by the yeast meiotic inhibitor *RME1*. Genes Dev. **7:**1598–1608.
12. **Csank, C., and K. Haynes.** 2000. *Candida glabrata* displays pseudohyphal growth. FEMS Microbiol. Lett. **189:**115–120.
13. **Eberstadt, M., B. Huang, Z. Chen, R. P. Meadows, S. C. Ng, L. Zheng, M. J. Lenardo, and S. W. Fesik.** 1998. NMR structure and mutagenesis of the FADD (Mort1) death-effector domain. Nature **392:**941–945.
14. **Eschrich, D., M. Buchhaupt, P. Kotter, and K. D. Entian.** 2002. Nep1p (Emg1p), a novel protein conserved in eukaryotes and archaea, is involved in ribosome biogenesis. Curr. Genet. **40:**326–338.
15. **Fidel, P. L. Jr., J. A. Vazquez, and J. D. Sobel.** 1999. *Candida glabrata*: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. Clin. Microbiol. Rev. **1:**80–96.
16. **Fortun, J., A. Lopez-San Roman, J. J. Velasco, A. Sanchez-Sousa, E. de Vicente, J. Nuno, C. Quereda, R. Barcena, G. Monge, A. Candela, A. Honrubia, and A. Guerrero.** 1997. Selection of *Candida glabrata* strains with reduced susceptibility to azoles in four liver transplant patients with invasive candidiasis. Eur. J. Clin. Microbiol. Infect. Dis. **16:**314–318.
17. **Hazen, K. C.** 1995. New and emerging yeast pathogens. Clin. Microbiol. Rev. **4:**462–478.
18. **Herskowitz, I., J. Rine, and J. N. Strathern.** 1992. Mating-type determination and mating-type interconversion in *Saccharomyces cerevisiae*, p. 583–656. *In* E. W. Jones, J. R. Pringle, and J. R. Broach (ed.), The molecular and cellular biology of the yeast *Saccharomyces*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
19. **Hicks, J., J. N. Strathern, and A. J. Klar.** 1979. Transposable mating type genes in *Saccharomyces cerevisiae*. Nature **282:**478–483.
20. **Hitchcock, C. A., G. W. Pye, P. F. Troke, E. M. Johnson, and D. W. Warnock.** 1993. Fluconazole resistance in *Candida glabrata*. Antimicrob. Agents Chemother. **37:**1962–1965.
21. **Hull, C. M., and A. D. Johnson.** 1999. Identification of a mating type-like locus in the asexual pathogenic yeast *Candida albicans*. Science **285:**1271–1275.
22. **Hull, C. M., R. M. Raisner, and A. D. Johnson.** 2000. Evidence for mating of the "asexual" yeast *Candida albicans* in a mammalian host. Science **289:**307–310.
23. **Jacquet, M., J. M. Buhler, F. Iborra, M. C. Francingues-Gaillard, and C. Soustelle.** 1991. The *MAT* locus revisited within a 9.8 kb fragment of chromosome III containing *BUD5* and two new open reading frames. Yeast **7:**881–888.
24. **Johnson, A. D.** 1995. Molecular mechanisms of cell-type determination in budding yeast. Curr. Opin. Genet. Dev. **5:**552–558.
25. **Klar, A. J., J. McIndoo, J. B. Hicks, and J. N. Strathern.** 1980. Precise mapping of the homothallism genes *HML* and *HMR* in *Saccharomyces cerevisiae*. Genetics **96:**315–320.
26. **Lachke, S. A., T. Srikantha, L. K. Tsai, K. Daniels, and D. R. Soll.** 2000. Phenotypic switching in *Candida glabrata* involves phase-specific regulation of the metallothionein gene *MT-II* and the newly discovered hemolysin gene *HLP*. Infect. Immun. **68:**884–895.
27. **Lachke, S. A., S. Joly, K. Daniels, and D. R. Soll.** 2002. Phenotypic switching and filamentation in *Candida glabrata*. Microbiology **148:**2661–2674.
28. **Landsman, D., O. W. McBride, N. Soares, M. P. Crippa, T. Srikantha, and M. Bustin.** 1989. Chromosomal protein HMG-14. Identification, characterization, and chromosome localization of a functional gene from the large human multigene family. J. Biol. Chem. **264:**3421–3427.
29. **Li, T., M. R. Stark, A. D. Johnson, and C. Wolberger, C.** 1995. Crystal structure of the MATa1/MAT alpha 2 homeodomain heterodimer bound to DNA. Science **270:**262–269.
30. **Lockhart, S. R., S. Joly, C. A. Pujol, J. D. Sobel, M. A. Pfaller, and D. R. Soll.** 1997. Development and verification of fingerprinting probes for *Candida glabrata*. Microbiology **143:**3733–3746.
31. **Lockhart, S. R., M. Nguyen, T. Srikantha, and D. R. Soll.** 1998. A MADS box protein consensus binding site is necessary and sufficient for activation of the opaque-phase-specific gene *OP4* of *Candida albicans*. J. Bacteriol. **180:**6607–6616.
32. **Lockhart, S. R., S. Joly, K. Vargas, J. Swails-Wenger, L. Enger, and D. R. Soll.** 1999. Natural defenses against *Candida* colonization breakdown in the oral cavities of the elderly. J. Dent. Res. **78:**857–868.
33. **Lockhart, S. R., C. A. Pujol, K. Daniels, M. G. Miller, A. D. Johnson, M. A. Pfaller, and D. R. Soll.** 2002. In *Candida albicans*, white-opaque switchers are homozygous for mating type. Genetics **162:**737–745.
34. **Lockhart, S. R., K. Daniels, R. Zhao, D. Wessels, and D. R. Soll.** 2003. The cell biology of mating in *Candida albicans*. Eukaryot. Cell **2:**49–61.
35. **Magee, B. B., and P. T. Magee.** 2000. Induction of mating in Candida albicans by construction of MTLa and MTLalpha strains. Science **289:**310–313.
36. **Marichal, P., H. Vanden Bossche, F. C. Odds, G. Nobels, D. W. Warnock, V. Timmerman, C. Van Broeckhoven, S. Fay, and P. Mose-Larsen.** 1997. Molecular biological characterization of an azole-resistant *Candida glabrata* isolate. Antimicrob. Agents Chemother. **41:**2229–2237.
37. **Miller, M. G., and A. D. Johnson.** 2002. White-opaque switching in *Candida albicans* is controlled by mating-type locus homeodomain proteins and allows efficient mating. Cell **110:**293–302.
38. **Odds, F. C.** 1988. *Candida* and candidiasis. Bailliere. Tendall, London, United Kingdom.
39. **Odds, F. C., M. G. Rinaldi, C. R. Cooper, Jr., A. Fothergill, L. Pasarell, and M. R. McGinniss.** 1997. *Candida* and *Torulopsis*: a blinded evaluation of use of pseudohypha formation as basis for identification of medically important yeasts. J. Clin. Microbiol. **35:**313–316.
40. **Pfaller, M. A.** 1996. Nosocomial candidiasis: emerging species, reservoirs, and modes of transmission. Clin. Infect. Dis. **22**(Suppl. 2)**:**S89–S94.
41. **Pfaller, M. A., R. N. Jones, S. A. Messer, M. B. Edmond, and R. P. Wenzel.** 1998. National surveillance of nosocomial blood stream infection due to *Candida albicans*: frequency of occurrence and antifungal susceptibility in the SCOPE Program. Diagn. Microbiol. Infect. Dis. **31:**327–332.
42. **Santos, M. A., T. Ueda, K. Watanabe, and M. F. Tuite.** 1997. The non-standard genetic code of *Candida* spp.: an evolving genetic code or a novel mechanism for adaptation? Mol. Microbiol. **26:**423–431.
43. **Sobel, J. D.** 1996. *Candida* vulvovaginitis. Semin. Dermatol. **15:**17–28.
44. **Spinillo, A., E. Capuzzo, R. Gulminetti, P. Marone, L. Colonna, and G. Piazzi.** 1997. Prevalence of and risk factors for fungal vaginitis caused by non-albicans species. Am. J. Obstet. Gynecol. **176:**138–141.
45. **Srikantha, T., L. K. Tsai, K. Daniels, and D. R. Soll.** 2000. *EFG1* null mutants of *Candida albicans* switch but cannot express the complete phenotype of white-phase budding cells. J. Bacteriol. **182:**1580–1591.
46. **Srikantha, T., L. Tsai, K. Daniels, A. J. Klar, and D. R. Soll.** 2001. The histone deacetylase genes *HDA1* and *RPD3* play distinct roles in regulation of high-frequency phenotypic switching in *Candida albicans*. J. Bacteriol. **183:**4614–4625.
47. **Strathern, J. N., A. J. Klar, J. B. Hicks, J. A. Abraham, J. M. Ivy, K. A. Nasmyth, and C. McGill.** 1982. Homothallic switching of yeast mating type cassettes is initiated by a double-stranded cut in the *MAT* locus. Cell **31:**183–192.
48. **Wong, S., G. Butler, and K. H. Wolfe.** 2002. Gene order evolution and paleopolyploidy in hemiascomycete yeasts. Proc. Natl. Acad. Sci. USA **99:**9272–9277.
49. **Worley, K. C., B. A. Wiese, and R. F. Smith.** 1995. BEAUTY: an enhanced BLAST-based search tool that integrates multiple biological information resources into sequence similarity search results. Genome Res. **5:**173–184.