

A Genome Sequence Survey Shows that the Pathogenic Yeast *Candida parapsilosis* Has a Defective *MTLa1* Allele at Its Mating Type Locus†

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***Candida parapsilosis* is responsible for ca. 15% of *Candida* infections and is of particular concern in neonates and surgical intensive care patients. The related species *Candida albicans* has recently been shown to possess a functional mating pathway. To analyze the analogous pathway in *C. parapsilosis*, we carried out a genome sequence survey of the type strain. We identified ca. 3,900 genes, with an average amino acid identity of 59% with *C. albicans*. Of these, 23 are predicted to be predominantly involved in mating. We identified a genomic locus homologous to the *MTLa* mating type locus of *C. albicans*, but the *C. parapsilosis* type strain has at least two internal stop codons in the *MTLa1* open reading frame, and two predicted introns are not spliced. These stop codons were present in *MTLa1* of all eight *C. parapsilosis* isolates tested. Furthermore, we found that all isolates of *C. parapsilosis* tested appear to contain only the *MTLa* idiomorph at the presumptive mating locus, unlike *C. albicans* and *C. dubliniensis*. *MTLα* sequences are present but at a different chromosomal location. It is therefore likely that all (or at least the majority) of *C. parapsilosis* isolates have a mating pathway that is either defective or substantially different from that of *C. albicans*.**

Candida species are an important cause of nosocomial infection and rank fourth in bloodstream infections in the United States (8). Invasive candidiasis has an attributable mortality of up to 38%. Although *Candida albicans* has in the past been the most common causative organism, other *Candida* species are becoming increasingly more prevalent. A worldwide study of bloodstream infections from 1997 to 1999 showed that at least 45% of yeast infections were caused by *Candida* species other than *C. albicans* (37). The most common emerging species are *C. glabrata* (16%), *C. parapsilosis* (15%), and *C. tropicalis* (10%) (11, 35, 36). *C. parapsilosis* is now the second most commonly isolated *Candida* species from blood cultures in Europe, Canada, and Latin America and in some European hospitals even outranks *C. albicans* (19, 34, 37). It is responsible for 24% of *Candida* infections in children less than 1 year old (29, 36, 40). Unlike other *Candida* species, *C. parapsilosis* has been found on the hands of health care workers, resulting in subsequent nosocomial infection associated with handling central venous catheters (20, 25, 41). *C. parapsilosis* is a particular problem since it tends to grow as biofilms on implanted medical devices, conferring almost total resistance to antifungal drugs (2). The ability to grow as a biofilm is directly related to clinically significant disease, which is a unique attribute of *C. parapsilosis* (44).

C. parapsilosis strains have historically been categorized as group I, II, or III on the basis of molecular fingerprinting (10, 21, 26). Group I strains (which include the type strain CLIB214, synonymous with ATCC 22019) are predominant in clinical isolates. Analysis of levels of heterozygosity and of mitochondrial genome architecture (39) supports the hypothesis that the three

groups represent three different species, and recently group II and group III isolates have been renamed as the new species *C. orthopsilosis* and *C. metapsilosis* (46). All three species are distantly related to *C. albicans*, relative to *C. dubliniensis* and *C. tropicalis*. Group I isolates form the basis of the present study.

There is some debate regarding the ploidy of *C. parapsilosis* isolates. The type strain, however, is highly likely to be diploid, since it is heterozygous for the *MET* locus (49), and fluorescence-activated cell sorting analysis shows that the DNA content is similar to that of *C. albicans* (7). Heterozygosity levels between *C. parapsilosis* isolates are less than 1 base in 1,000 (10), but analysis of those that exist indicates that other isolates are not haploid; they are either diploid or aneuploid (10, 46).

Until recently, it was assumed that *C. albicans* and related yeasts had no sexual cycle. It is now known that *C. albicans* *MTLa* homozygous strains can mate with *MTLα* homozygotes (14, 31). Mating within *C. dubliniensis* species and between *C. dubliniensis* and *C. albicans* has also been reported (38). Mating is facilitated by white/opaque phenotype switching (27, 32). The distantly related yeast *C. glabrata* also contains many of the genes required for mating and is capable of undergoing mating type switching (3, 5, 45, 51). Mating in *C. albicans* and *C. dubliniensis* is facilitated by growth on skin, suggesting it may play a role in virulence (22, 38). We therefore decided to investigate whether a similar pathway existed in *C. parapsilosis*.

We carried out a genome sequence survey of *C. parapsilosis*, producing >10 million bases and identifying 3,900 genes. Many mating-related genes are present, and we show here that the *MTLa* idiomorph has a similar structure to that of *C. albicans*. However, the *MTLa1* open reading frame (ORF) is defective in all isolates tested.

MATERIALS AND METHODS

Strains and media. The type strain of *C. parapsilosis* (CLIB214) was obtained from the Collection de Levure d'Intérêt Biotechnologique, Thiverval Grignon,

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† Supplemental material for this article may be found at <http://ec.asm.org/>.

TABLE 1. *C. parapsilosis* strains used in this study

Isolate ^a	Geographic origin	Source	<i>MTLa1</i> sequenced ^b
CLIB214 (type strain)	Puerto Rico	Feces	Yes
711701	Aberdeen, United Kingdom	Unknown	Yes
73/107	London, United Kingdom	Mouth	Yes
J931058	Belgium	Nail	Yes
J931845	Japan	Unknown	Yes
81/042	London, United Kingdom	Ear	Yes
81/041	Mayo Clinic, Rochester, Minn.	Vagina	
81/040	London, United Kingdom	Toe space	
73/037	Leeds, United Kingdom	Vagina	
81/253	London, United Kingdom	Nail	Yes
74/046	Leeds, United Kingdom	Aortic valve	
90-137	San Jose, Calif.	Orbital tissue	Yes

^a All isolates, apart from the type strain, were obtained from Frank Odds, Aberdeen, United Kingdom, and are described by Tavanti et al. (47).

^b Where indicated, a 200-bp fragment of the *MTLa1* region was amplified, and the DNA sequence was determined.

France. All other *C. parapsilosis* isolates were obtained from Frank Odds, Aberdeen, United Kingdom (Table 1) (46). The strains were routinely maintained on yeast extract-peptone-dextrose (YPD) medium. The oligonucleotide (oligo) primers used are shown in Table 2.

Genome sequence survey. High-molecular-weight DNA was prepared from *C. parapsilosis* CLIB214 and used to generate a plasmid-based random genomic library with an average insert size of ca. 5 kb (AGOWA [Germany]). Sequences were obtained from both ends of the insert for 8,160 clones. For submission of high-quality reads to GenBank, base calling was carried out by using PHRED (error rate cutoff of 0.05 and a PHRED score of 15) and vector clipping using CROSS_MATCH and a dedicated Perl script. Mitochondrial, rRNA, and tRNA elements were removed by BLASTN searches of a database containing the equivalent sequences from *Saccharomyces cerevisiae* and *C. albicans* and the mitochondrial genome from *C. parapsilosis* (33). Any read with a significant expect value (E-value) of <1e-3 was excluded from subsequent analysis. Contigs were assembled by using PHRAP. Approximately 100 high-quality reads with a significant match (E < 1e-10) to *Pseudomonas* sequences and no or much weaker match to fungal sequences were also removed, since these were assumed to be derived from a low-level contamination of the genomic library. PCR analysis of two of these sequences confirmed that they were not present in the *C. parapsilosis*

TABLE 2. Oligonucleotides used in this study

Oligo	Sequence
CP4.....	5'-CATTTCGTAAGCCTCTCAAAGTAGTCTT TCAAC-3'
CP2.....	5'-CCAAATCTAGCATACGTTGATCGTAAAGG CCGTCC-3'
CP6.....	5'-CAGATTGACACTGTCAACTCTTCACTCACA AATGAC-3'
CP3.....	5'-CGCACACCTTGTACATCATAGGAATAGATT GTGG-3'
3190_1.....	5'-TACTAGGAGTGTGACTGATGAAATACGC GGGACA-3'
CCT7_1.....	5'-CGATGACATTGCAGTTGTGGCACATTTCTC CAATG-3'
MPR1.....	5'-GAGCTTCTTGTAAGAATTTTTCTCGTTA-3'
MTX1.....	5'-ATCCTGTCAATCAATGTAACAC-3'
MTX2.....	5'-GCAACTCAAGGATGTAAGTACT-3'
MTX3.....	5'-ATGAGTTCCGAGTATTATAAAAGC-3'
MTX4.....	5'-TGATATAAGTCCCTTTGTCG-3'
MTX5.....	5'-GGACTCGGTCAATTACCCAAG-3'
MTX6.....	5'-TACCTCTCATTCTTTTGTTCGCG-3'
MAR3.....	5'-GCTTTCGATAAAAACCTGACCAC-3'
MAR4.....	5'-CTGATATTGACACATTGGTTG-3'
MAR5.....	5'-ATTAATTTCTTTTATTGCATTTAGAT-3'
MAR6.....	5'-GTATGATTGTGTATTGTTTC-3'

genome. The sequence reads have been deposited in GenBank with accession numbers from CZ278593 to CZ293207.

Putative identities were assigned to many of the *C. parapsilosis* sequences by using a comparison to a recent annotation of the *S. cerevisiae* genome containing 5,616 ORFs (18). The *S. cerevisiae* protein with the strongest BLASTX score was considered to be the ortholog, providing that the E-value for this hit was <1e-10 and more than 1e10 lower than the E-value for the second-best hit. For each ortholog the cellular function in *S. cerevisiae* was determined by using the "cellular role" categories of the Yeast Proteome Database of the Incyte Bioknowledge Library (12).

Comparisons with *C. albicans* proteins were determined by using a haploid gene set of 7,677 ORFs (including small and overlapping ORFs) predicted from assembly 19 (16). Classification of *C. albicans* proteins into functional categories was performed by using the same criteria as for *C. parapsilosis*. Sequences that did not match the *C. albicans* genome with BLASTX scores of <1e-3 were compared to the nonredundant database.

For the calculation of average sequence identity, only orthologs that are reciprocal best hits were used. This corresponded to 3,547 genes compared between *C. parapsilosis* and *C. albicans* and 5,511 genes compared between *C. dubliniensis* and *C. albicans*. The *C. dubliniensis* gene sequences were obtained from the Sanger Institute (assembly of 1 May 2004 [http://www.sanger.ac.uk/Projects/C_dubliniensis/]).

The *C. tropicalis* *MTL* sequences were obtained from assembly 1 at the MIT sequencing project (http://www.broad.mit.edu). We did not analyze the ORFs in detail since there appear to be several frameshifts and possible sequencing errors in the current assembly.

Isolation of *MTLa1* locus. The sequence of the entire insert from clones cp45f11 (from CpPIKa to Cp19.3202) and cp12c03 (from Cp19.3192 to CpGap1; see Fig. 1) was determined by AGOWA. Oligos CP4 (designed from the *CpPIKa* ortholog) and CP2 (designed from the *CpGAP1* ortholog) were used with Expand Long-Template DNA polymerase (Roche) to amplify a region of 6.3 kb between the two inserts. The PCR product was purified by using a QiaQuick kit (Qiagen) according to the manufacturer's instructions, and the sequence was determined directly by GATC (Germany). The sequence of the PCR product was combined with the overlapping sequence of the cp45f11 and cp12c03 inserts to give a 15.7-kb sequence that includes the entire predicted *CpMTLa1* idiomorph. The sequence has been deposited with GenBank (accession number AY961981). For restriction analysis of the *MTL* locus, fragments of 14 to 21 kb were amplified by PCR by using Expand Long-Template DNA polymerase (Roche) and oligos 3190_1 (designed from within the *Cp19.3190* ORF), CP6 (designed from sequence 900 bp upstream from the start of *CpGAP1*), CP3 (designed from within *CpRCY1*), and CCT7_1 (designed from within the *CpCCT7* ortholog). High-molecular-weight genomic DNA was prepared by using a Genomic-Tip 20/G kit (Qiagen).

To confirm the mutations in *MTLa1*, a 200-bp fragment of the ORF was amplified from eight *C. parapsilosis* isolates by using oligos MPR1 and MTX6. The PCR product was purified as described above, and the sequence was determined directly by GATC.

Southern blot analysis. Digested genomic DNA was separated by electrophoresis on 1% agarose gels, transferred to positively charged nylon membranes (Roche Diagnostics), and treated according to the manufacturer's instructions. Probes for *CaPAPα* and *CaMTLa1* were amplified from *C. albicans* SC5314 genomic DNA by using MAR3/MAR4 and MAR5/MAR6 primer combinations. The *CpMTLa1* probe was amplified from *C. parapsilosis* CLIB214 genomic DNA by using the primers MPR1 and MTX6. All probes were labeled with [³²P]dCTP by using a High Prime DNA Labeling Kit (Roche Diagnostics). For low-stringency conditions, hybridizations were carried out overnight at 60°C in PerfectHyb (Sigma). The blots were washed twice for 30 min in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.1% sodium dodecyl sulfate (SDS) at 60°C (6). High-stringency hybridizations were carried out at 68°C, and the blots were washed once for 5 min at room temperature in 2× SSC-0.1% SDS and twice for 20 min at 68°C in 0.5× SSC-0.1% SDS. The blots were then exposed to X-ray film.

Analysis of RNA splicing. *C. parapsilosis* CLIB214 was cultured overnight in YPD medium, diluted to an *A*₆₀₀ of 0.2 in 50 ml of fresh YPD medium, and incubated at 30°C for 4 h. Total RNA was isolated from 5 ml of culture by using a Ribopure kit (Ambion). DNase I treatment was carried out during the purification process. To generate cDNA, 3 μg of RNA and 291 ng of oligo dT-15 primer in a final volume of 15 μl was incubated at 70°C for 10 min and then chilled on ice. A 45-μl cocktail containing 0.6 mM deoxynucleoside triphosphates, 120 U of RNasin RNase inhibitor (Promega), 600 U of Moloney murine leukemia virus reverse transcriptase (Promega), and 1× Promega reverse transcription-PCR (RT-PCR) buffer was then added. The mixture was incubated at

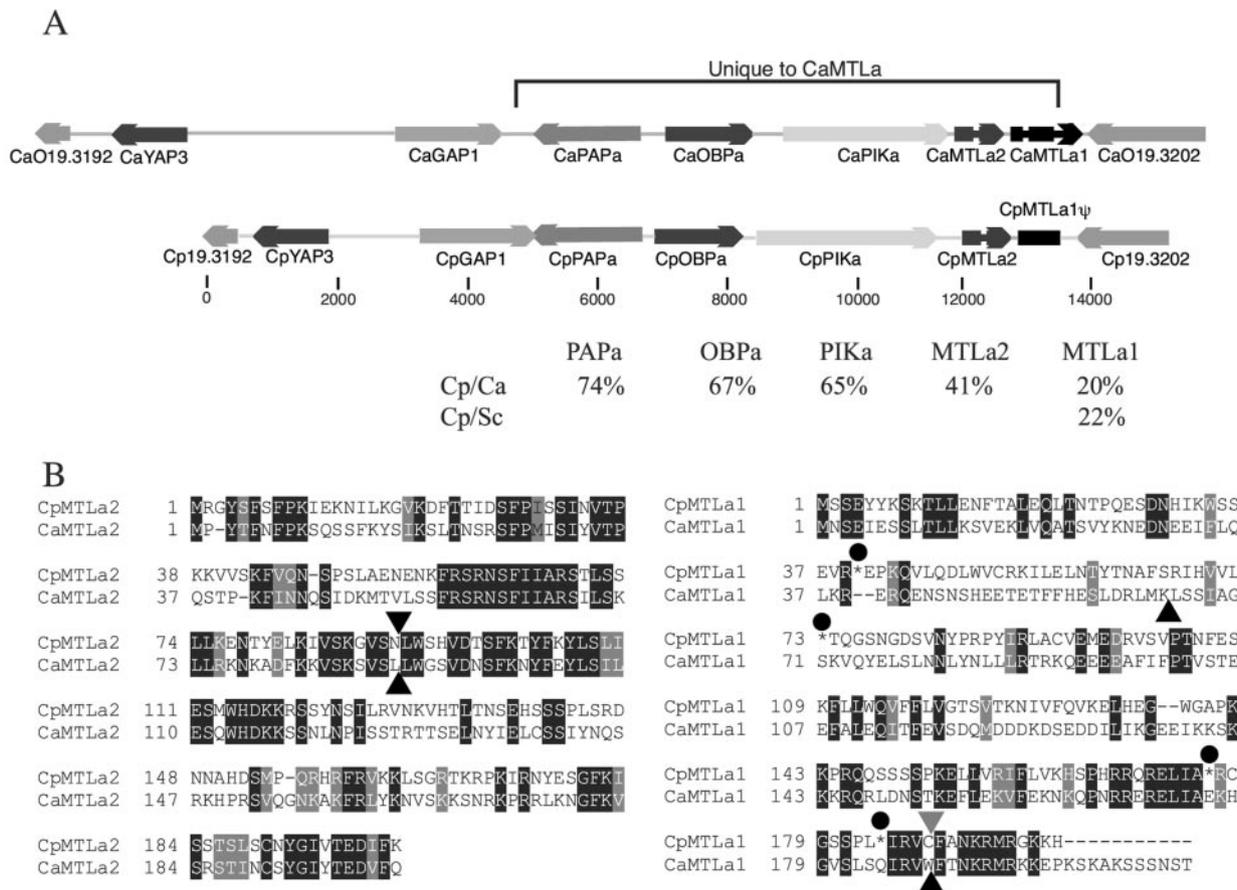


FIG. 1. Identification of an *MTL* idiormorph from *C. parapsilosis*. (A) The top line shows the structure of the *MTLa* sequence and surrounding region from *C. albicans*. The genes are drawn approximately to scale. The region indicated by a bracket is unique to *MTLa*, and the remaining sequences surround both *MTLa* and *MTLa* idiormorphs. The line below illustrates the corresponding structure in the orthologous *CpMTLa* idiormorph that was sequenced in full. The *C. albicans* genome annotation places an ORF (*Orf19.3194*) between *CaYAP3* and *CaGAP1*. However, it is not conserved and is likely to be spurious and so is omitted in this diagram. *Orf19.3192* corresponds to the N terminus of the *Sti1* protein in *S. cerevisiae*. The sequence of the *Cp19.3202* and *Cp19.3192* orthologs at the ends of the fragment is not complete. Distances are shown in base pairs. The percent identity between proteins from *C. parapsilosis* (Cp) and *C. albicans* (Ca) or *S. cerevisiae* (Sc) is shown below. (B) An alignment of the *MTLa2* proteins from *C. parapsilosis* (CpMTLa2) and *C. albicans* (CaMTLa2) was generated by using CLUSTAL W and is shown on the left-hand side. The intron locations are indicated with black triangles. The right-hand side shows an alignment between the *C. albicans* *MTLa1* protein (CaMTLa1) and the hypothetical protein translation of the pseudogene from *C. parapsilosis* (CpMTLa1). Introns in the *C. albicans* sequence are indicated with black triangles, and a putative intron in *C. parapsilosis* is indicated with a gray triangle. Black circles highlight four stop codons in the *C. parapsilosis* sequence.

37°C for 1 h, followed by 95°C for 2 min, and the resulting cDNA was held at 4°C. For each PCR, 5 µl of cDNA was used as a template in a 50-µl volume containing 0.25 mM deoxynucleoside triphosphates, 1× reaction buffer, 1.5 mM MgCl₂, 25 pmol of each primer, and 2.5 U of *Taq* DNA polymerase (Invitrogen). RNA that was not reverse transcribed was used as a control to test for the presence of genomic DNA. The same PCR amplification was also performed with genomic DNA as a template. The *MTLa2* region was amplified by using oligos MTX1 and MTX2, and the *MTLa1* region was amplified by using oligos MTX3, MTX4, MTX5, and MTX6. The MTX1/MTX2 and MTX3/MTX6 RT-PCR products were combined from five reactions, purified as described above, and the DNA sequence was determined directly by GATC.

RESULTS

Genome sequence survey of *C. parapsilosis*. We constructed a plasmid library with random genomic inserts of ca. 5 kb and sequenced both ends of ca. 8,000 clones. After removal of mtDNA and rRNA, 10.1 Mb of high-quality primary sequence data was obtained, corresponding to ca. 0.8× coverage of the

haploid genome size. The sequences were assembled into 5,374 contigs. Putative ORFs were identified by using BLASTX searches of the *C. albicans* assembly 19 ORFs. We identified partial sequence of 3,898 orthologs in *C. parapsilosis*, corresponding to ca. 60% of the 6,419 nonoverlapping ORFs predicted in the haploid *C. albicans* genome (16). We also identified orthologs of seven additional ORFs found in other fungal genomes but which are not present in the *C. albicans* genome sequence (Table 3). The average protein sequence identity between *C. parapsilosis* and *C. albicans* is 59%. In contrast, the average identity between *C. dubliniensis* and *C. albicans* is 82% (based on a comparison of 5,511 *C. albicans* genes).

Since the cellular function of the majority of *C. albicans* genes is not yet determined, we inferred gene function in *C. parapsilosis* from the known functions of the *S. cerevisiae* orthologs. The “cellular role” divisions of the Yeast Proteome

TABLE 3. Some *C. parapsilosis* genes not present in *C. albicans*

Gene name ^a	Organism	GenBank accession no.
DEHA0B13200g (capsid protein, virus L-A)	<i>Debaryomyces hansenii</i>	CAG85523
MG10213.4 (Gentisate 1,2-dioxygenase)	<i>Magnaporthe grisea</i>	EAA48150
DEHA0E11440g (LMBR1-like membrane protein)	<i>Debaryomyces hansenii</i>	XP_459779
FG04776.1 (monooxygenase)	<i>Gibberella zeae</i>	EAA75735
SPAC11D3.03c	<i>Schizosaccharomyces pombe</i>	NP_592800
DEHA0E15411g (esterase)	<i>Debaryomyces hansenii</i>	XP_459950
KLLA0A12045g	<i>Kluyveromyces lactis</i>	XP_451524

^a The name of the closest ortholog is shown and, where possible, a function is inferred from sequences in other organisms and is shown in parentheses.

Database were used to categorize a total of 2,650 *C. parapsilosis* genes with identifiable *S. cerevisiae* orthologs. A similar analysis was performed with 7,677 ORFs from *C. albicans* derived from the haploid gene set (16; see also Table S5 in the supplemental material). We identified orthologs for 47% of the *S. cerevisiae* genes in *C. parapsilosis* and 51% in *C. albicans*. The category “conjugation with cellular fusion” included 94 genes from *C. parapsilosis*, several with known functions in mating. Although many have roles in other cellular processes,

TABLE 4. Some *C. parapsilosis* genes with potential roles in mating

Gene type and name ^a	Function
α-Specific genes	
KEX1	Processing of killer toxin and α -factor
STE13	α -Factor processing
SAG1	Agglutination of α -factor
a-Specific genes	
RAM1	Farnesylation of a-factor
RAM2*	Farnesylation of a-factor
STE23	a-Factor processing
STE6*	Component of a-factor secretory pathway
AXL1	Maturation and cleavage of a-factor
STE2	α -Factor receptor
SST2	GAP protein, desensitisation to α -factor
RBT1*	Predicted glycosylated cell wall protein
Orf19.4688*†	Unknown function
Orf19.2825*†	Unknown function
MTLa2	Regulator of a-specific mating
General mating genes	
FAR1	Cyclin-dependent kinase inhibitor, mediates cell cycle arrest in response to pheromone
AKR1	Negative regulation of pheromone response pathway, required for endocytosis of pheromone receptors
PLP2	Positive regulation of transcription from polymerase II promoter by pheromones
FAR11	G ₁ arrest in response to mating pheromones
LRG1	Rho GTPase, involved in mating and sporulation
GPA1	G protein alpha subunit, coupled to mating factor receptors
STE4	G protein beta subunit, coupled to mating factor receptors
FIG4	Polyphosphoinositide phosphatase, required for efficient mating
KAR4	Karyogamy, functions in mating and meiosis

^a *. Genes described as a-specific in *C. albicans* by Tsong et al. (48); †, original designations were as follows: Orf19.4688 = Orf6.1768 and Orf19.2825 = Orf6.1251.

we extracted a list of 19 that function predominantly in mating (Table 4). These were augmented with orthologs of genes shown to be mating specific in *C. albicans* by Tsong et al. (48). The genes were placed into three classes: a-specific, α -specific, and genes more generally involved in mating. Although some may have several roles (such as Ram1 and Ram2, which are required for farnesylation of a-factor and Ras proteins in *S. cerevisiae*), others (such as Ste2, the α -factor receptor in *S. cerevisiae*) have no known function outside mating.

Identification of a putative *C. parapsilosis* MTL locus. In *C. albicans*, mating type is determined by the genes residing at the MTL locus (13, 48). There are two idiomorphs. MTL_a contains the genes PAPA [poly(A) polymerase], OBP_a (oxysterol-binding protein), PIK_a (phosphatidylinositol kinase), MTL_a2 (a HMG-box protein), and MTL_a1 (a homeodomain protein) (Fig. 1). MTL _{α} contains three genes related to those in the MTL_a locus (PAP _{α} , OBP _{α} , and PIK _{α}), plus MTL _{α} 2 (a homeodomain protein) and MTL _{α} 1 (an α domain protein). The genes surrounding the MTL idiomorphs are the same on both chromosomes. Under certain conditions, cells with two copies of MTL_a (a/a; or hemizygous for MTL_a) can mate with cells with two copies of MTL _{α} (α/α ; or hemizygous for MTL _{α}). While analyzing the data from the sequence survey of *C. parapsilosis*, we noted that several reads matched many of the genes within and surrounding the MTL_a locus. The sequence reads matching MTL_a1, however, contained stop codons in the predicted ORF. To confirm this observation, we determined the sequence of the entire region orthologous to *C. albicans* MTL_a (Fig. 1). This region contains orthologs of all of the genes found in the *C. albicans* MTL_a locus, and the order and transcriptional orientations of the genes are conserved between the two species.

The *C. parapsilosis* MTL_a1 locus is a pseudogene. Although the majority of the genes in the *C. parapsilosis* MTL_a idiomorph are intact, CpMTL_a1 appears to be a pseudogene, because there are four stop codons in the region corresponding

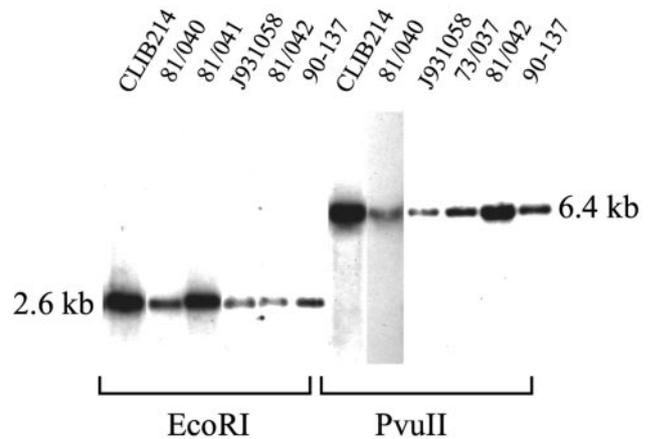


FIG. 2. CpMTL_a1 is detected in a single location in the *C. parapsilosis* genome. Genomic DNA was isolated from the *C. parapsilosis* strains indicated, digested with EcoRI or PvuII, and transferred to nylon membranes. The blots were probed at low stringency with a 200-bp fragment derived from the homeodomain region of CpMtl_a1p. The fragments detected are consistent with those predicted from the sequence of the MTL locus.

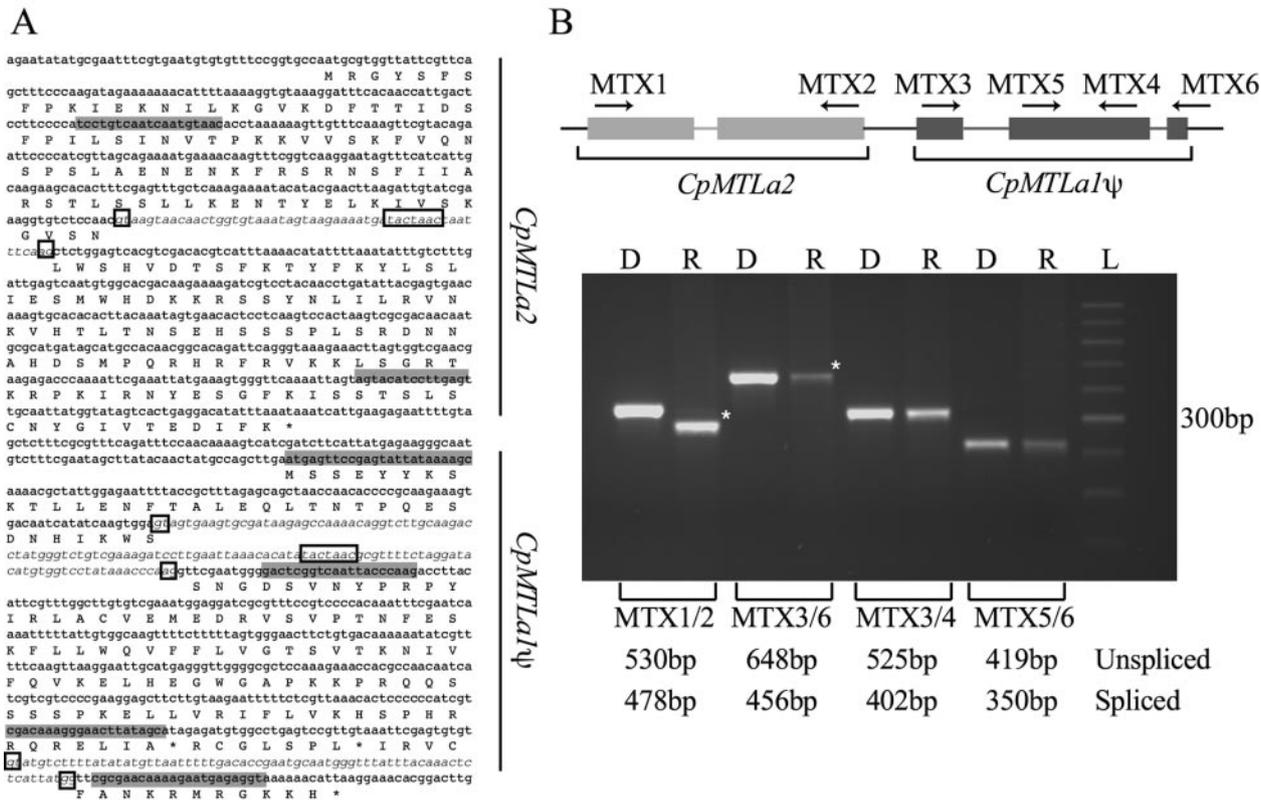


FIG. 3. The *CpMTLa1* sequence is not spliced. (A) Predicted translation of the *CpMTLa2* and *CpMTLa1* region is shown. The positions of putative introns are indicated in italics, and the oligos used in PCR amplifications are shaded in gray. Putative splice sites and TACTAAC sites are boxed. (B) Proposed structure of the *MTLa2* and *MTLa1* region and location of oligos used in PCR. Putative exons are shown as rectangles, and introns as lines. The oligo combinations used in a PCR amplification using genomic DNA (D) and cDNA (R) as templates. The size of predicted spliced and unspliced products are indicated below the gel. Products highlighted with an asterisk were sequenced. L, 100-bp ladder.

to *CaMTLa1* (Fig. 1B). Since *C. parapsilosis* CLIB214 is most likely diploid (7, 49), we considered the possibility that the genome might contain two copies of the *MTLa* idiomorph, one with a defective and one with a functional *MTLa1* sequence. We therefore amplified a portion of the *MTLa1* region from genomic DNA, derived from the conserved homeodomain and encompassing the last two stop codons. The sequence of the PCR product was determined directly. If two alleles are present we should obtain a mixed DNA sequence. However, only one sequence was detected, identical to the *CpMTLa* sequence (Fig. 1). We also amplified this fragment from seven other *C. parapsilosis* isolates (Table 1). All contained the same sequence, with no evidence of heterozygosity.

To confirm there is no copy of *CpMTLa1* elsewhere in the genome, we performed a Southern blot, using the same 200-bp *CpMTLa1* fragment as a probe. As shown in Fig. 2, only one fragment of the predicted size is detected under low-stringency conditions in the genomes of all *C. parapsilosis* isolates tested. Since *C. albicans* probes hybridize to *C. parapsilosis* DNA under these conditions (Fig. 4), it is highly likely that we would detect any related *CpMTLa1* sequence present. Identical results were obtained at high stringencies (not shown).

The structure of *CpMTLa1* as shown in Fig. 1 has one intron at a position equivalent to intron 2 of *CaMTLa1*, although the predicted 5' splice site is GG, and there is no TACTAAC box

(Fig. 3). The gene also has four internal stop codons. We considered another possible structure for this gene, one that incorporated an additional intron at a position similar to, but not identical to, intron 1 of the *C. albicans* gene (Fig. 1 and 3). The possible intron has GT and AG splice sites and a TAC TAAC lariat site and skips over two of the four predicted stop codons, but it also drastically shortens the predicted *C. parapsilosis* protein compared to *C. albicans* and other a1 proteins. To determine whether any splicing occurs, we compared the sizes of products obtained by RT-PCR from an RNA template to those generated by direct amplification of the genomic DNA. Primers were chosen that amplify the entire *MTLa1* region, including the two possible introns (Fig. 3). As a control, we also amplified the *MTLa2* region, which is predicted to contain an intron in both *C. albicans* and *C. parapsilosis*. As shown in Fig. 3, the RT-PCR product from *CpMTLa2* is indeed smaller than the product amplified from genomic DNA. Sequence analysis confirmed that the predicted intron in *MTLa2* is correctly spliced. To our knowledge, this is the first experimental demonstration of splicing of *MTLa2* in *Candida* species. The RT-PCR products from *CpMTLa1*, however, are identical in size to those derived from genomic DNA. The sequence of the largest RT-PCR product was determined, confirming that it is generated from the *CpMTLa1* sequence and that no splicing occurs. We are thus unsure whether *CpMTLa1*

is a pseudogene of a single-intron gene that has gained four stop codons or a pseudogene of a two-intron gene that has gained two stop codons, but in either case it is undoubtedly a pseudogene.

Structure of the *C. parapsilosis* *MTL* locus. Our genome sequencing data yielded several random genomic clones that originated from *MTLa*, but none that matched *MTL α* . To look for *MTL α* sequences in this and other isolates, we first used overlapping clone data to extrapolate the structure of the region surrounding the *CpMTL* locus (Fig. 4). The order of the surrounding genes is identical to the *MTL* loci in *C. albicans* (16). We used four primer combinations to amplify the region surrounding the *MTL* locus from *C. parapsilosis* CLIB214, yielding fragments varying in size from 14.2 to more than 21 kb (Fig. 4). Primer CP6 lies within the region for which the entire sequence was determined (Fig. 1), and the remaining primers were derived from the sequences of overlapping clones. If two idiomorphs are present we should amplify two fragments. However, as shown in Fig. 4B, restriction analysis shows that only one fragment is present. The restriction pattern is also identical for eight isolates amplified by using the primer pair CP6 and CCT7_1 (Fig. 4C), and for 14 isolates amplified by using CP6 and CP3 (not shown), indicating that these PCR products also contain *MTLa* idiomorphs.

To look for evidence of *CpMTL α* sequences elsewhere in the genome, we performed low-stringency Southern blot analysis. We first used an 856-bp fragment derived from *CaPAP α* , which is found in the *MTL α* idiomorph of *C. albicans* (13), *C. dubliniensis* (38), and *C. tropicalis* (<http://www.broad.mit.edu>). We reasoned that at low stringency, this probe should hybridize to the related gene *CpPAP α* , which we have shown is present in the *CpMTLa* idiomorph, and to any ortholog of *PAP α* present. As shown in Fig. 5, *CaPAP α* hybridizes to DNA fragments of 2.7 kb (BglII digest) and 2.9 kb (BamHI/PstI digest). These correspond to the predicted size of *CpPAP α* , which we confirmed in other blots (not shown). However, it hybridizes at higher intensities to a larger fragment in both digests (>8 kb), suggesting the *C. parapsilosis* genome does contain an ortholog of *CaPAP α* . A 145-bp fragment derived from the conserved domain of *CaMTL α 1* also hybridized to sequences in *C. parapsilosis* (Fig. 5B). Both probes detected large fragments in all of the digests tested (not shown), and we were unable to determine whether the sequences are contiguous. It is therefore likely that *C. parapsilosis* does contain *MTL α* sequences, but the PCR results suggest that they are not in the same chromosomal context as *CpMTLa*, even though the order of the genes flanking *CpMTLa* is very similar to those flanking the *MTL* loci of *C. albicans*, *C. dubliniensis*, and *C. tropicalis* (Fig. 4).

DISCUSSION

The results of the genome sequence survey of *C. parapsilosis* yielded partial DNA sequence information for more than 3,900 potential ORFs. The amino acid sequence identity between *C. parapsilosis* proteins and *C. albicans* proteins and between *C. dubliniensis* proteins and *C. albicans* proteins both ranged from ca. 20 to 100%. The most similar sequences included ribosomal proteins, actin, and polyubiquitin (see Table S6 in the supplemental material). Proteins with <25% conservation between

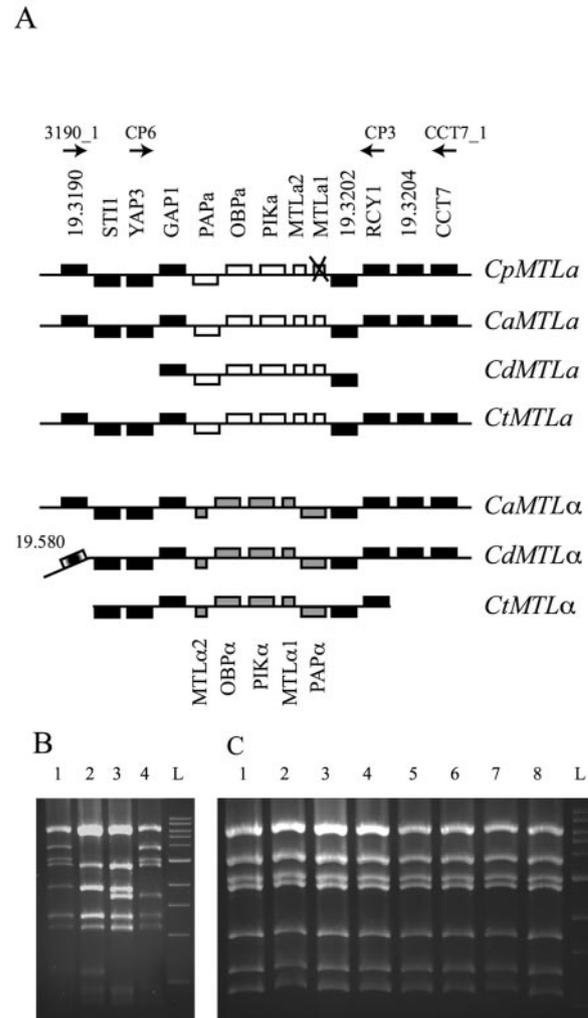


FIG. 4. Amplification of the *C. parapsilosis* *MTL* locus. (A) The structure of the region surrounding *CpMTLa* was extrapolated from overlapping clones and is compared to the gene order around the *MTL* loci in other *Candida* species. The extent of the available contigs is indicated. The unique *MTL α* genes are shown in white, the *MTL α* genes in gray, and the shared surrounding genes in black. The order upstream of the *STII* gene in *C. dubliniensis* *MTL α* differs from the other sequences. The *CpMTLa1* pseudogene is indicated with an "X." The *C. tropicalis* fragments contain many putative sequencing errors that are not marked. The location of the oligo primers used in PCR amplification reactions are shown above. The region is not drawn to scale. (B) The *MTL* region from *C. parapsilosis* CLIB214 was amplified by using oligo primers noted and digested with EcoRI. Lane 1, primers 3190_1 and CCT7_1 (~21 kb); lane 2, primers 3190_1 and CP3 (~16.2 kb); lane 3, primers CP6 and CP3 (~14.5 kb); lane 4, primers CP6 and CCT7_1 (~20 kb); L, 1-kb ladder. (C) The *MTL* locus from eight *C. parapsilosis* isolates was amplified by using primers CP6 and CCT7_1 and digested with EcoRI. Lane 1, CLIB 214; lane 2, 81/041; lane 3, 81/042; lane 4, 81/040; lane 5, 90/137; lane 6, 74/046; lane 7, 73/037; lane 8, J931058. L, 1-kb ladder.

C. parapsilosis and *C. albicans* are mostly uncharacterized, but include *AXL1*, a putative α -pheromone maturase (see Table S7 in the supplemental material) (1). The average sequence identity between *C. parapsilosis* and *C. albicans* proteins, however, is 59%, and between *C. dubliniensis* and *C. albicans* it is 82%.

To identify genes that may be unique to *C. parapsilosis*, we

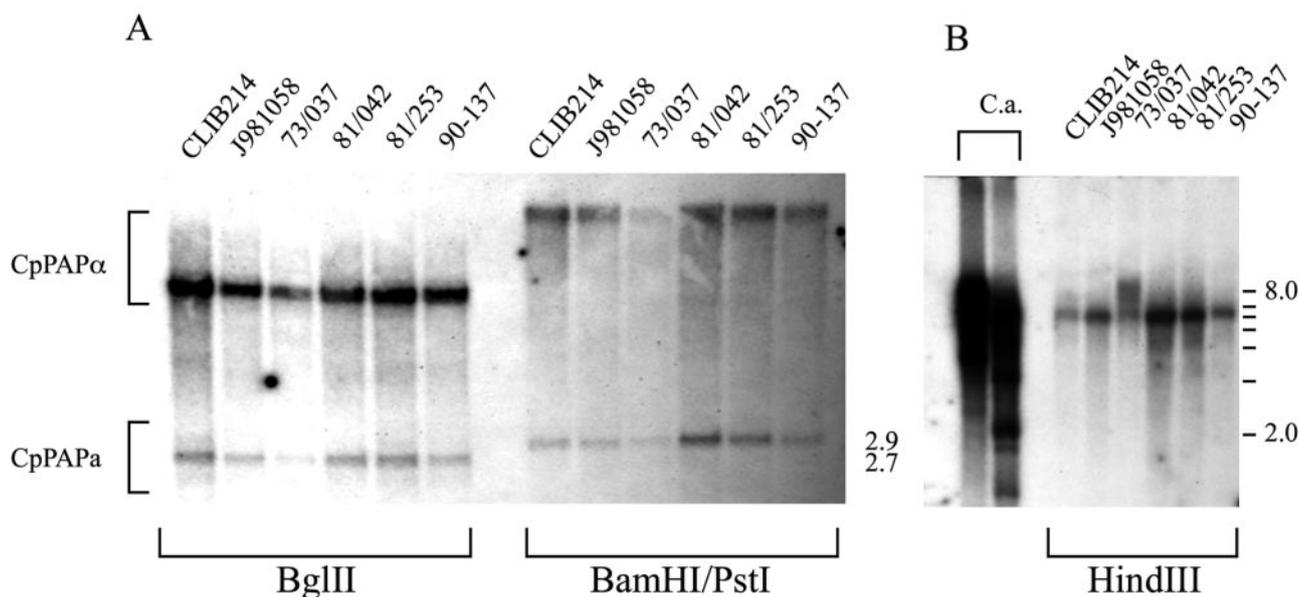


FIG. 5. Identification of *CpMTL* α sequences. Genomic DNA was isolated from the *C. parapsilosis* strains indicated, digested, and transferred to nylon membranes. Hybridizations were carried out at low stringencies. (A) An 865-bp fragment of *CaPAP* α detects two fragments in *C. parapsilosis* genomic DNA. One (2.7 kb with *Bgl*II or 2.9 kb with *Bam*HI/*Pst*I) corresponds to the *CpPAP* α sequence in the *CpMTL* α idiomorph. The other fragment (>8 kb in both digests) most likely corresponds to a *CpPAP* α ortholog. (B) A 145-bp fragment derived from *CaMTL* α hybridizes to a 7-kb *Hin*DIII fragment in genomic DNA from *C. parapsilosis* isolates. C.a., *C. albicans*.

extracted sequences with no similarity to the *C. albicans* genome and looked for orthologs in other species. We identified seven genes all present in other fungal genomes (Table 3). One is strongly similar to the major coat protein of the *S. cerevisiae* L-A virus, suggesting that *C. parapsilosis* may harbor double-stranded RNA viruses or that its genome contains genes derived from such a virus. In other yeasts these viruses encode toxins, some of which have anti-*Candida* activities (42, 43, 50). It will therefore be interesting to look for similar activities in *C. parapsilosis*.

Disruption of the mating locus in *C. parapsilosis*. In *C. albicans*, the ability to mate is closely associated with white-opaque phenotype switching. In heterozygous diploid cells containing both *a* and α idiomorphs, *a*1 and α 2 proteins heterodimerize to generate a negative regulator. As a result, white-opaque switching is repressed and mating-specific functions are switched off (27, 31, 32, 48). White-opaque switching occurs in *C. albicans* cells that have only one functional *MTL* idiomorph because they are homozygous or hemizygous (24, 27). In opaque cells that contain only the *MTL* α idiomorph, the *a*2 protein is a positive regulator of mating functions. Similarly, α 1 positively regulates mating in opaque cells containing only the *MTL* α idiomorph. The closely related species *C. dubliniensis* also undergoes white-opaque switching and mates in a similar fashion (38). Phenotypic switching does occur in *C. parapsilosis* (9, 23, 28), but we have not observed anything directly comparable to white-opaque phenotypes. We have, however, shown that the type strain of *C. parapsilosis* carries a defective *MTL* α 1 allele, with up to four internal stop codons in the presumptive ORF.

The final two stop codons in *CpMTL* α 1 from *C. parapsilosis* CLIB214 are also present in seven other randomly selected isolates, and PCR amplification of genomic DNA and South-

ern blot analysis indicates that there is no functional copy of *MTL* α 1 elsewhere. It is therefore extremely unlikely that *C. parapsilosis* can generate a heterodimeric *a*1/ α 2 protein. The *MTL* α 2 gene, however, is transcribed and spliced, and so should be capable of inducing *a*-specific mating type functions.

If mating occurs in *C. parapsilosis* in a manner that is in any way similar to that in *C. albicans* or *C. dubliniensis*, we would expect to find an *MTL* α idiomorph in an analogous position to *MTL* α . Our sequence survey resulted in 0.8 \times coverage of the genome, with several matches to *MTL* α -specific genes such as *PIK* α , *MTL* α 1, and *MTL* α 2. No similarities to *MTL* α genes were detected. To look for a second idiomorph, we first compared the structures of the *MTL* loci from related *Candida* species. In *C. albicans*, the region unique to *MTL* α is 8,742 bp, and the unique *MTL* α sequence is 8,861 bp (13). The equivalent unique regions in *C. dubliniensis* are 8,728 bp (*CdMTL* α) and 8,845 bp (*CdMTL* α) (http://www.sanger.ac.uk/Projects/C_dubliniensis/), and in *C. tropicalis* they are 8,473 bp (*CtMTL* α) and 10,337 bp (*CtMTL* α) (<http://www.broad.mit.edu>). The gene order within the equivalent idiomorphs is identical (Fig. 4). It is not possible to determine the order of all of the genes surrounding the *MTL* loci, since the assemblies for *C. dubliniensis* and *C. tropicalis* are not complete. However, the synteny is very similar for all of the loci for which sequence is currently available (Fig. 4), although there does appear to be a break upstream from *STI*1 in *C. dubliniensis* *MTL* α . We therefore amplified regions surrounding *CpMTL* by using primers derived from the sequence of the unique genes that lie well outside the borders of the *MTL* locus in related *Candida* species (Fig. 4A). We amplified only one DNA fragment, corresponding to *CpMTL* α , in all isolates tested (Fig. 4B and C). Since there is very little sequence variability in *C. parapsilosis* isolates (46) and we used four primer combinations, it is highly

unlikely that our failure to amplify an *MTL* α -containing fragment was caused by polymorphisms in the primer sequences. It therefore appears that the chromosomal location that corresponds to *MTL* in other *Candida* species contains only *MTLa* in *C. parapsilosis*. However, our Southern blot analysis does suggest that there are *MTL* α -like sequences somewhere in the *C. parapsilosis* genome, since we identified fragments that hybridize to *CaPAP* α and *CaMTL* α 1 (Fig. 5). We conclude that *MTL* α in *C. parapsilosis* has been rearranged somehow, breaking its colinearity with the genomic context of *CpMTLa* and the *MTL* loci in other *Candida* species. It is difficult to envisage how *C. parapsilosis* strains could become mating competent through homozygosity or hemizyosity at the *MTL* genes, when these genes are not at allelic locations in the genome. It is also unlikely that the *MTL* α sequences represent a silent mating cassette involved in mating type switching, since our analysis has shown that this mechanism evolved relatively recently and is restricted to the *Saccharomyces sensu stricto* group and their close relatives (5). The most likely scenario is that the *MTL* α locus has degenerated after rearrangement and has become inactivated, although further sequence analysis is required to support this hypothesis. Despite the overall similarities, however, it is very unlikely that *C. parapsilosis* can mate in the same way as *C. albicans* (15) or *C. dubliniensis* (38).

Despite the defects in the *MTL* sequences, we did identify many genes that are predominantly required for mating in *C. albicans* and *S. cerevisiae* (Table 4). These include orthologs of *STE13*, required for processing of α -factor (17), and *STE2*, the α -factor receptor in *S. cerevisiae* (4). We also identified several genes that are probably *a*-specific, including orthologs of *STE6*, *RAM2*, *Orf19.4688*, *Orf19.2825*, and *RBT1* whose expression in *C. albicans* requires both *MTLa2* and exposure to α -factor (48). *STE6* is known to be required for mating in *C. albicans* (30). The fact that many components of the pathway are apparently intact suggests that *C. parapsilosis* has only recently undergone a rearrangement at the *MTL* locus. However, this contrasts with the observation that the *MTLa1* sequence contains four stop codons, which were presumably acquired in four separate mutational events.

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