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Chromosome Loss Followed by Duplication Is the Major Mechanism of Spontaneous Mating-Type Locus Homozygosis in *Candida albicans*

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ABSTRACT

Candida albicans, which is diploid, possesses a single mating-type (*MTL*) locus on chromosome 5, which is normally heterozygous (**a**/α). To mate, *C. albicans* must undergo *MTL* homozygosis to **a**/**a** or α/α. Three possible mechanisms may be used in this process, mitotic recombination, gene conversion, or loss of one chromosome 5 homolog, followed by duplication of the retained homolog. To distinguish among these mechanisms, 16 spontaneous **a**/**a** and α/α derivatives were cloned from four natural **a**/α strains, P37037, P37039, P75063, and P34048, grown on nutrient agar. Eighteen polymorphic (heterozygous) markers were identified on chromosome 5, 6 to the left and 12 to the right of the *MTL* locus. These markers were then analyzed in *MTL*-homozygous derivatives of the four natural **a**/α strains to distinguish among the three mechanisms of homozygosis. An analysis of polymorphisms on chromosomes 1, 2, and R excluded meiosis as a mechanism of *MTL* homozygosis. The results demonstrate that while mitotic recombination was the mechanism for homozygosis in one offspring, loss of one chromosome 5 homolog followed by duplication of the retained homolog was the mechanism in the remaining 15 offspring, indicating that the latter mechanism is the most common in the spontaneous generation of *MTL* homozygotes in natural strains of *C. albicans* in culture.

IN haploid *Saccharomyces cerevisiae*, mating type is regulated by a cassette system consisting of two loci, *HML* and *HMR*, which contain unexpressed copies of the *MAT***a** and *MAT*α locus, respectively, and a third, *MAT*, which contains one expressed copy of either the *MAT***a** or *MAT*α locus (Herskowitz and Oshima 1981; Haber 1998). The *MAT***a** locus contains the mating-type gene *MAT***a**1, and the *MAT*α locus contains the mating-type genes *MAT*α1 and *MAT*α2. Mating type is dictated by the *MAT* locus genotype. Haploid *S. cerevisiae* can change its mating type by site-specific recombination at the *MAT* locus with a copy of the alternative silent locus (Butler *et al.* 2004). This represents a conserved system, since no mating-type information is lost when cells switch mating type. Although the yeast pathogen *Candida albicans* possesses mating-type genes similar to those in *S. cerevisiae*, *C. albicans*, which is diploid, possesses a single mating-type (*MTL*) locus situated on chromosome 5 (Hull and Johnson 1999), which is normally heterozygous (**a**/α) in natural strains (Lockhart *et al.* 2002). The *MTL* locus of one chromosome 5 homolog contains the genes *MTL***a**1 and *MTL***a**2, while the *MTL* locus on the other homolog contains the genes *MTL*α1 and *MTL*α2 (Hull and Johnson 1999; Tsong *et al.* 2003). *MTL***a**2 plays a unique role as a positive regulator of **a**-specific genes in *C. albicans* (Tsong *et al.* 2003). For *C. albicans* to express a mating type, the *MTL* heterozygote must undergo homozygosis to either **a**/**a** or α/α (Hull *et al.* 2000; Magee and Magee 2000; Lockhart *et al.* 2003). Hence, in contrast to *S. cerevisiae*, *C. albicans* loses the alternative mating-type information to be mating competent. An analysis of the *MTL* genotypes of a collection of 220 natural *C. albicans* strains revealed that ~97% were *MTL* heterozygous, while only 3% were *MTL* homozygous (Lockhart *et al.* 2002). Of the *MTL*-heterozygous strains, ~4% underwent spontaneous homozygosis in culture (Lockhart *et al.* 2002; Pujol *et al.* 2003). *MTL* zygosity has been shown to regulate not only mating competency, but also white-opaque switching. Cells can switch from white to opaque, a requirement for mating, only when they have undergone homozygosis (Lockhart *et al.* 2002; Miller and Johnson 2002). Hence, understanding how **a**/α cells become **a**/**a** or α/α is important not only for understanding mating, but also in understanding phenotypic switching.

C. albicans might employ three possible mechanisms to achieve homozygosis at the *MTL* locus. First, mitotic recombination (Whelan and Soll 1982) may occur between the chromosome 5 homologs at a site between the *MTL* locus and centromere, as depicted in Figure 1A, resulting in cosegregation of two *MTL***a** or two *MTL*α loci. Second, one *MTL* locus could undergo precise gene conversion to the alternative mating type (*i.e.*, **a** to α or α to **a**), as occurs at the *MAT* locus in *S. cerevisiae* (Butler *et al.* 2004), resulting in cosegregation of two *MTL***a** or two *MTL*α loci, as depicted in Figure 1B. Third,

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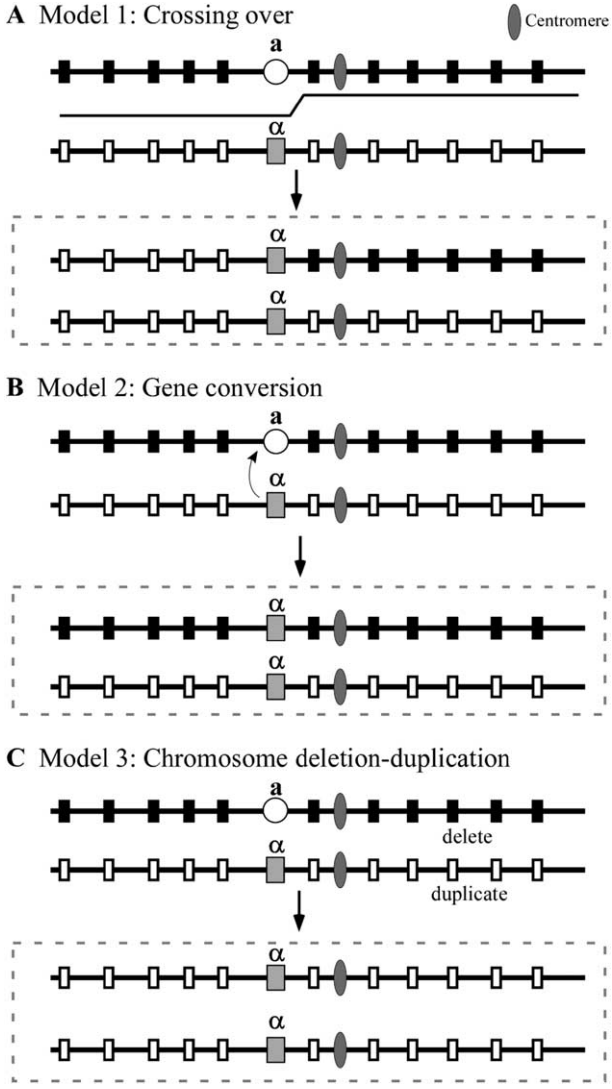

FIGURE 1.—Models for the three possible mechanisms of mating-type locus (*MTL*) homozygosis in *Candida albicans*, mitotic recombination (A), gene conversion (B), and deletion of one homolog of chromosome 5 followed by duplication of the retained homolog (C). Hypothetical polymorphic alleles on the chromosome 5 homolog harboring *MTL***a** (**a**) are represented as solid boxes and those on the chromosome 5 homolog harboring *MTL*α (α) are represented as open boxes. The centromere is represented as a solid ellipsoid. Dashed boxes represent outcome of homozygosis for each possible mechanism. The step in the line between the **a** and α homologs in A is the position of a crossover. The small curved arrow from α to **a** in B represents a gene conversion. The **a** homolog is deleted and the α homolog duplicates in C.

one chromosome 5 homolog may be lost and the retained homolog duplicated, as depicted in Figure 1C. This last mechanism (Figure 1C) has been demonstrated to occur when *C. albicans* is grown in medium in which sorbose is the sole carbon source (JANBON *et al.* 1998; MAGEE and MAGEE 2000).

To test which mechanism(s) is responsible for spontaneous *MTL* homozygosis in natural strains of *C. albicans* in culture, we first identified polymorphic (heterozygous) sequences on either side of the *MTL* locus on chromosome 5 in four *C. albicans* **a**/α strains that were previously demonstrated to undergo spontaneous *MTL* homozygosis in culture (LOCKHART *et al.* 2002; PUJOL *et al.* 2003; our unpublished observations). We then analyzed the zygosity of these markers in spontaneous *MTL*-homozygous derivatives of these strains. If *MTL* homozygosis results from mitotic recombination, then the *MTL*-homozygous derivative would contain a combination of chromosome segments containing homozygous and heterozygous markers, and the *MTL* locus would be harbored in a segment containing other homozygous markers, as in Figure 1A. If *MTL* homozygosis results from precise gene conversion, as in *S. cerevisiae*, all markers in the *MTL*-homozygous derivative should be heterozygous except for the *MTL* locus, as in Figure 1B. Finally, if *MTL* homozygosis results from chromosome deletion, all markers in the *MTL*-homozygous derivatives should be homozygous, including the *MTL* locus, as in Figure 1C. In this scenario, polymorphisms on chromosomes other than chromosome 5 should remain polymorphic. The results we have obtained demonstrate that spontaneous *MTL* homozygosis in *C. albicans* is achieved both by mitotic recombination along chromosome 5 and by deletion of one chromosome 5 homolog followed by duplication of the retained homolog. The latter proved to be by far the most frequent mechanism.

MATERIALS AND METHODS

Strain origin, maintenance, and growth: The four *C. albicans* *MTL*-heterozygous (**a**/α) strains P37037, P37039, P75063, and P34048, selected for analysis, have been demonstrated in earlier studies to undergo spontaneous *MTL* homozygosis in culture (LOCKHART *et al.* 2002; PUJOL *et al.* 2003; unpublished observations). To obtain *MTL*-homozygous derivatives, cells were plated at low density (~50 colony-forming units per 10-cm-diameter petri dish) on nutrient agar medium (LEE *et al.* 1975) supplemented with zinc and arginine ("modified Lee's medium"), according to BEDELL and SOLL (1979), and 5 μg/ml of phloxine-B, which differentially stains opaque phase sectors and colonies red (ANDERSON and SOLL 1987). Since only *MTL*-homozygous cells switch from white to opaque (LOCKHART *et al.* 2002; MILLER and JOHNSON 2002), *MTL*-homozygous derivatives were readily obtained by cloning cells from red sectors or colonies and verifying *MTL* homozygosity by polymerase chain reaction or Southern analysis (LOCKHART *et al.* 2002; see later section). Six *MTL*-homozygous derivatives were obtained from **a**/α strain P37037 (α/α-1, α/α-2, α/α-3, α/α-4, α/α-5, and **a**/**a**-1), one from **a**/α strain P37039 (α/α-1), two from **a**/α strain P75063 (**a**/**a**-1, **a**/**a**-2), and seven from **a**/α strain P34048 (α/α-1, α/α-2, α/α-3, α/α-4, α/α-5, α/α-6, and **a**/**a**-1). The minority of *MTL*-homozygous offspring were obtained independently from colonies generated by different clones of the **a**/α parent strains. The majority were from sectors. Hence, all *MTL*-homozygous offspring originated from independent homozygosis events. All original **a**/α and derivative **a**/**a** or α/α strains were stored in 25% glycerol at −80°. For experimental purposes, stored cells were plated and grown on modified Lee's medium agar.

Polymorphisms identified by sequence analysis: Fifty intergenic regions 500–600 bp in length along chromosome 5 were randomly selected from a contig map created from data

TABLE 1

Oligonucleotides used in this study and their positions in contigs

Primer	Target regions[a]	Oligonucleotide sequences
Contig19-10080		
10080A-F	1845–1862	5′-CAAAAGTAGACCCCTTCCTC-3′
10080A-R	2379–2397	5′-CCTGCACATTGTCAATCTCG-3′
Contig19-10137		
10137A-F	5130–5151	5′-GCAGGCATCGATACATATTCTC-3′
10137A-R	5682–5701	5′-ACCAATGCAGCCAAACCAAG-3′
Contig19-10170		
10170B-F	1–21	5′-CATGCCAAGTCTGTACACGCT-3′
10170B-R	650–670	5′-GTTGTGGTAGCCATAGTGTGG-3′
1990A-F	5212–5232	5′-TGGAGACATTTAGAGAACTTA-3′
1990A-R	5829–5849	5′-CCGGTGACAACTCCACTCAAC-3′
1990C-F	6792–6802	5′-GTCATCATCATTTCTTCTGAA-3′
1990C-R	7515–7535	5′-CGGCAAATATTGTATGTTAAT-3′
1854A-F	25288–25306	5′-TGACTACTTTTAGCTAGT-3′
1854A-R	26737–26755	5′-CCACTCACCTTCACGGTC-3′
STI1-F	61258–61278	5′-GGATTACCCGAAGCAGTG-3′
STI1-R	60689–60719	5′-CAGGAGTTCACCCTTCAA-3′
YAP3-F	62976–62996	5′-CCTGTGAATATACTCAATTGG-3′
YAP3-R	63584–63604	5′-TCCGAGAACGTAAAGAATCTA-3′
GAP1-F	69153–69173	5′-CAGCATAGTTCCCCTTGG-3′
GAP1-R	69657–69677	5′-GAGTCTATATGCACATAC-3′
CLN1-F	92354–92374	5′-GAAGCAAATAGCCAACCTCAA-3′
CLN1-R	92954–92974	5′-GGATCCATTAGTTGATATTGACT-3′
10170D-F	96001–96021	5′-CATCAATACTATAATTGATTT-3′
10170D-R	96675–96695	5′-ATATTGAAGATGGTAAGAAC-3′
10170A-F	97726–97746	5′-ATGCATCTACATGTAGTATTT-3′
10170A-R	98400–98420	5′-ACG ATTAATCTATATTGTTAT-3′
Contig19-10202		
2466C-F	11481–11501	5′-GAAGATGATCAACAACTCGCG-3′
2466C-R	10810–10830	5′-TCGTTCATGACGCTATCAACA-3′
2466A-F	64543–64523	5′-CTAAGTACCCATTCAAACTAT-3′
2466A-R	63889–63869	5′-CCTACTCAGTATTAAGAAGCA-3′
Contig19-10155		
2284B-F	23818–23798	5′-GTGATAACCCATCACCATCAC-3′
2284B-R	23274–23254	5′-GTACAGAGTTGTCTCAGTGCG-3′
Contig19-10194		
2222C-F	84429–84449	5′-TCAGATTGTTTAACAACCACT-3′
2222C-R	84388–84408	5′-TCAACTGGTTTAGTACATCGA-3′
2222A-F	70577–70597	5′-AGCTCGTGAAATGGTCAGGTT-3′
2222A-R	71176–71195	5′-TTCAACCCATATAGATTCTCT-3′
YIR12-F	238693–238713	5′-AGATATAACAGACTCTTTATG-3′
YIR12-R	238308–238328	5′-TTTGGAAGGTTCTTCCCAGAT-3′
Contig19-10205		
10205A-F	8016–8036	5′-TGCTTGTCTCTTCAATTGTAA-3′
10205A-R	8662–8642	5′-CCCAACTTTAATTCCTTCCTT-3′
Contig19-10196		
10196A-F	82083–82103	5′-AAGGAGCTTCATTTGTTAACA-3′
10196A-R	82603–82583	5′-TAGTACCATTAGTGGTGCTGT-3′
Contig19-10057		
10057A-F	83015–83035	5′-TGCTTGTCTCTTCAATTGTAA-3′
10057A-R	83498–83478	5′-CCCAACTTTAATTCCTTCCTTT-3′

[a] Position of the primer on the contig sequences obtained from the Stanford Genome Technology Center *Candida albicans* sequencing project. Contig19-10205, contig19-10196, and contig19-10057 are located on chromosomes 1, 2, and R, respectively.

provided by the Stanford Genome Technology Center *C. albicans* sequencing project (http://www-sequence.stanford.edu/group/candida/) and sequenced in the four *MTL*-heterozygous test strains. Of these, 13 intergenic regions on chromosome 5 were found to be polymorphic in one or more of the four **a**/α strains. The sites on chromosome 5 are listed as "target regions" for primers in Table 1 and diagrammed in Figure 2. The placement of polymorphic sequences on chro-

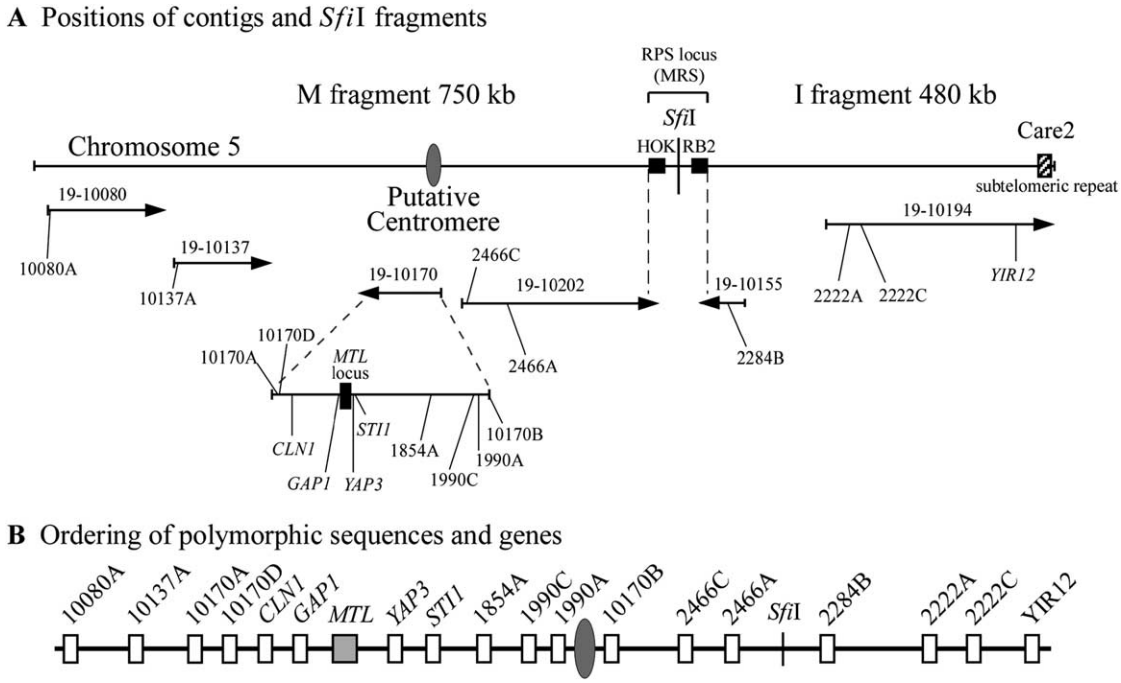

FIGURE 2.—Development of a partial contig map for chromosome 5 and placement of the polymorphic genes and intergenic sequences used in this study in their respective contigs. (A) the placements of contigs with associated genes along chromosome 5 were interpreted from data obtained from the Stanford Genome Technology Center *C. albicans* sequencing project database (http://www-sequence.stanford.edu/group/candida/), the partial physical map posted by P. T. Magee and colleagues (http://alces.med.umn.edu/Candida.html; FORCHE *et al.* 2004) and Whiteway and colleagues (http://cbr-rbc.nrc-cnrc.gc.ca/biovis/candida/). An explanation of the logic behind the map is presented in RESULTS. The 5′-3′ orientations of the contigs are indicated by arrow direction. The positions of the putative centromere (SANYAL *et al.* 2004), the RPS locus, and the CARE2 locus are noted. (B) Tentative ordering of polymorphic genes and intergenic sequences along chromosome 5. Note that the orientation of the markers 1990C and 1990A could be either on the right or left of the centromere, but have been arbitrarily ordered as in the sequence of contig 19-10170 for simplicity. A change in orientation changes none of the interpretations or conclusions in this article. The distances between markers are arbitrary.

mosome 5 in relation to the *MTL* locus, the putative centromeric region (SANYAL *et al.* 2004), the *Sfi*I restriction sites (CHU *et al.* 1993; CHIBANA *et al.* 1998), and the subtelomeric Care2 site on chromosome 5 is presented in Figure 2A. Their placement was in agreement with the partial physical map of P. T. Magee and colleagues (http://alces.med.umn.edu/Candida.html; FORCHE *et al.* 2004) and Whiteway and colleagues (http://cbr-rbc.nrc-cnrc.gc.ca/biovis/candida/). Intergenic regions along chromosomes 1, 2, and R were similarly selected and sequenced. One polymorphic region was selected on each chromosome from contigs 19-10205, 19-10196, and 19-10057, respectively (Table 1).

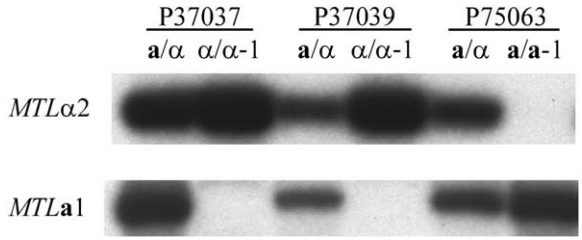

FIGURE 3.—Southern blot analysis of *MTL***a**1 and *MTL*α2 for three of the *MTL*-heterozygous strains and one *MTL*-homozygous derivative from each. Note that the intensity of *MTL***a**1 and *MTL*α2 bands in the *MTL*-heterozygous strains is roughly half that of the derivative *MTL*-homozygous strain. Similar results were obtained for the remaining *MTL*-heterozygous strain and additional *MTL*-homozygous derivatives not shown.

The polymorphic regions were then sequenced in *MTL*-homozygous derivatives of the four original *MTL* heterozygous strains. For sequencing, primers were designed from the genome sequences (Table 1). These regions were then amplified as follows. One nanogram of genomic DNA isolated by methods previously described (SOLL *et al.* 1996) was used in each 50-μl amplification reaction. Amplification with Taq DNA polymerase was performed as recommended by the manufacturer (Invitrogen, Carlsbad, CA). Polymerase chain reaction (PCR) mixtures were denatured by incubating them for 10 min at 94°. PCR reactions were then run through 40 cycles for 1 min at 94°, for 1 min at 47°, and for 1 min at 68° and then elongated for 8 min at 68°. Sequencing was performed in both directions with an ABI sequencing apparatus (PE-ABI, Foster City, CA), using the same primers as those used for PCR amplification.

Polymorphisms identified by Southern analysis: Primers were generated from the open reading frame sequences of 20 genes along chromosome 5 obtained from the Stanford Genome Technology Center *C. albicans* sequencing project database and the sequences were synthesized by PCR. The PCR products were labeled and used as probes in Southern analysis of the four *MTL*-heterozygous strains. Southern analy-

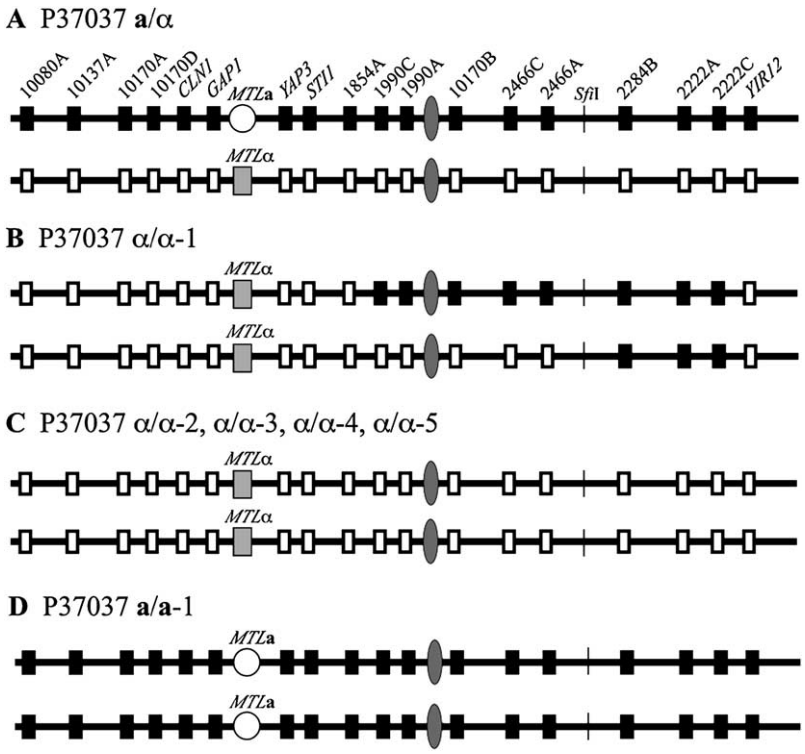

FIGURE 4.—*MTL* homozygosis in the **a**/α strain P37037 generating six *MTL*-homozygous derivatives occurred by either mitotic recombination along chromosome 5 in one case or by loss of one chromosome 5 homolog followed by duplication of the retained homolog in five cases. (A) Diagram of polymorphic sites along the homologs of chromosome 5 in the original **a**/α strain. The solid and open boxes represent gene and intergenic sequence polymorphisms. Diagrams of sites along the homologs of chromosome 5 are presented for the α/α-1 derivative (B); the α/α-2, α/α-3, α/α-4, and α/α-5 derivatives (C); and the **a**/**a**-1 derivative (D). The interpretive mechanisms of *MTL* homozygosis for the derivatives are mitotic recombination for α/α-1 and loss of one chromosome 5 homolog followed by duplication of the retained homolog for α/α-2, α/α-3, α/α-4, α/α-5, and **a**/**a**-1.

sis was performed according to methods previously described (SRIKANTHA *et al.* 2000, 2001). Five of the genes (*CLN1*, *GAP1*, *YAP3*, *STI1*, and *YIR12*) proved polymorphic in one or more of the **a**/α strains. The primers used to synthesize these gene sequences are presented in Table 1. The amplified probes were then used to assess polymorphisms in the *MTL*-homozygous derivatives primarily by Southern analysis or sequence analysis of the PCR products.

DNA fingerprinting: To verify that *MTL*-homozygous strains were indeed derivatives of the four *MTL*-heterozygous strains, cells were DNA fingerprinted with the complex DNA fingerprinting probe Ca3 by methods previously described (SCHMID *et al.* 1990; SOLL 2000). All derivatives were genetically similar to the presumed strains of origin.

RESULTS

Isolation of *MTL*-homozygous derivatives: Because switching occurs only in *MTL*-homozygous cells (LOCKHART *et al.* 2002; MILLER and JOHNSON 2002), *MTL*-homozygous derivatives of the four original *MTL*-heterozygous strains (P37037, P37039, P75063, and P34048) were isolated by screening for opaque phase sectors and colonies, which are selectively stained red by phloxine B in the supporting agar (ANDERSON and SOLL 1987). To be sure that each *MTL*-homozygous derivative was the result of an independent homozygosis event, selected derivatives were from colony sectors or colonies from different clones of the **a**/α strain. *MTL* homozygosity of the selected offspring was verified by Southern blot hybridization with labeled *MTL***a**1 and *MTL*α2 probes (HULL and JOHNSON 1999). While the original strain in each case possessed copies of both *MTL***a**1 and *MTL*α2, all derivative clones contained copies of either *MTL***a**1 or *MTL*α2, but not both (Figure 3). In each case, the hybridization signal of *MTL***a**1 or *MTL*α2 in the *MTL*-homozygous derivative was approximately twice that of either *MTL***a**1 or *MTL*α2 of the *MTL*-heterozygous strain from which it was derived (Figure 3), indicating that two copies of either *MTL***a**1 or *MTL*α2 were in the derivative clone. DNA fingerprinting with the complex probe Ca3 (SCHMID *et al.* 1990; SOLL 2000) verified that all of the identified *MTL*-homozygous strains were true derivatives of the *MTL*-heterozygous strain of origin (data not shown).

Identification of polymorphic sequences along chromosome 5: To assess the mechanism of *MTL* homozygosis (Figure 1), polymorphic sequences were identified in the four *MTL*-heterozygous strains along chromosome 5 by sequence analysis of 50 random intergenic regions and 5 genes. In addition, polymorphic genes were identified in three strains (P37037, P37039, and P75063) by Southern analysis of 20 genes distributed throughout the chromosome. A combined 13 intergenic polymorphisms and 5 gene polymorphisms were identified (Figure 2). Six polymorphic genes and intergenic sequences were identified to the left of the *MTL* locus and 12 to the right (Figure 2).

Development of the contig map for chromosome 5: The sequences of the contigs were obtained from assembly 19 of the Stanford Genome Technology Center *C. albicans* sequencing project database (http://www-sequence.stanford.edu/group/candida/). A single RPS locus, also known as MRS (major repeat sequence), on

TABLE 2

Sequence analysis of chromosome 5 intergenic regions and the genes *YAP3* and *YIR12* for strain P37037 a/α and the *MTL*-homozygous derivatives α/α-1, α/α-2, α/α-3, α/α-4, α/α-5, and a/a-1

Marker	Strain		Polymorphic sites													
		bp:	43	158	170	180	200	213	217	223	228	234	236	238	248	342
10080A	**a**/α		C/T	A/G	A/G	A/G	C/T	G/T	C/T	C/T	G/T	A/C	A/T	A/T	C/T	A/G
	α/α-1		C/C	A/A	A/A	G/G	T/T	G/G	C/C	T/T	G/G	C/C	T/T	A/A	C/C	A/A
	α/α-2, -3, -4, -5		C/C	A/A	A/A	G/G	T/T	G/G	C/C	T/T	G/G	C/C	T/T	A/A	C/C	A/A
	a/**a**-1		T/T	G/G	G/G	A/A	C/C	T/T	T/T	C/C	T/T	A/A	A/A	T/T	T/T	G/G
		bp:	218	234	255	324	340	377								
10137A	**a**/α		C/T	A/G	A/G	C/T	C/T	C/T								
	α/α-1		C/C	A/A	A/A	T/T	C/C	T/T								
	α/α-2, -3, -4, -5		C/C	A/A	A/A	T/T	C/C	T/T								
	a/**a**-1		T/T	G/G	G/G	C/C	T/T	C/C								
		bp:	75													
10170A	**a**/α		C/T													
	α/α-1		T/T													
	α/α-2, -3, -4, -5		T/T													
	a/**a**-1		C/C													
		bp:	137	265												
10170D	**a**/α		A/T	C/T												
	α/α-1		A/A	T/T												
	α/α-2, -3, -4, -5		A/A	T/T												
	a/**a**-1		T/T	C/C												
		bp:	130	214	268	442	539	555								
YAP3	**a**/α		A/T	A/G	A/T	A/G	C/T	A/G								
	α/α-1		A/A	G/G	T/T	G/G	T/T	A/A								
	α/α-2, -3, -4, -5		A/A	G/G	T/T	G/G	T/T	A/A								
	a/**a**-1		T/T	A/A	A/A	A/A	C/C	G/G								
		bp:	196													
1854A	**a**/α		T/−													
	α/α-1		−/−													
	α/α-2, -3, -4, -5		−/−													
	a/**a**-1		T/T													
		bp:	112	177	221	431										
1990C	**a**/α		A/G	C/T	A/G	C/T										
	α/α-1		A/G	C/T	A/G	C/T										
	α/α-2, -3, -4, -5		G/G	C/C	G/G	C/C										
	a/**a**-1		A/A	T/T	A/A	T/T										
		bp:	255	460												
1990A	**a**/α		A/T	A/G												
	α/α-1		A/T	A/G												
	α/α-2, -3, -4, -5		T/T	G/G												
	a/**a**-1		A/A	A/A												
		bp:	74	125	190	326	370	375	393	403	426	435				
10170B	**a**/α		A/G	C/G	A/G	C/T	C/G	C/T	C/T	C/T	C/T	C/T				
	α/α-1		A/G	C/G	A/G	C/T	C/G	C/T	C/T	C/T	C/T	C/T				
	α/α-2, -3, -4, -5		G/G	G/G	A/A	C/C	C/C	T/T	C/C	C/C	C/C	C/C				
	a/**a**-1		A/A	C/C	G/G	T/T	G/G	C/C	T/T	T/T	T/T	T/T				

(*continued*)

TABLE 2

(Continued)

Marker	Strain		Polymorphic sites													
		bp:	138	144	423	448	449	567	579	603						
2466C	**a**/α		A/G	A/T	A/G	C/T	A/T	C/T	A/G	A/G						
	α/α-1		A/G	A/T	A/G	C/T	A/T	C/T	A/G	A/G						
	α/α-2, -3, -4, -5		G/G	A/A	G/G	T/T	T/T	T/T	G/G	G/G						
	a/**a**-1		A/A	T/T	A/A	C/C	A/A	C/C	A/A	A/A						
		bp:	380	436												
2466A	**a**/α		C/T	C/T												
	α/α-1		C/T	C/T												
	α/α-2, -3, -4, -5		T/T	T/T												
	a/**a**-1		C/C	C/C												
		bp:	152	349	391	406	438	443								
2284B	**a**/α		C/T	A/G	A/C	C/T	A/G	C/T								
	α/α-1		T/T	A/A	A/A	T/T	A/A	T/T								
	α/α-2, -3, -4, -5		C/C	G/G	C/C	C/C	G/G	C/C								
	a/**a**-1		T/T	A/A	A/A	T/T	A/A	T/T								
		bp:	59	60	74	93	95	114	135	139	214	220	223	232	335	544
2222A	**a**/α		A/G	C/G	A/C	A/G	G/T	A/C	C/T	C/T	A/G	C/T	C/T	C/G	C/G	A/G
	α/α-1		A/A	G/G	C/C	G/G	T/T	C/C	T/T	G/G	G/G	C/C	C/C	C/C	C/C	A/A
	α/α-2, -3, -4, -5		G/G	C/C	A/A	A/A	G/G	A/A	C/C	C/C	A/A	T/T	T/T	G/G	G/G	G/G
	a/**a**-1		A/A	G/G	C/C	G/G	T/T	C/C	T/T	G/G	G/G	C/C	C/C	C/C	C/C	A/A
		bp:	290	300	307	321	334	336	347	360	372					
2222C	**a**/α		A/C	A/G	C/T	A/C	C/−	A/G	A/T	C/T	A/G					
	α/α-1		C/C	A/A	T/T	C/C	−/−	G/G	A/A	T/T	G/G					
	α/α-2, -3, -4, -5		A/A	G/G	C/C	A/A	C/C	A/A	T/T	C/C	A/A					
	a/**a**-1		C/C	A/A	T/T	C/C	−/−	G/G	A/A	T/T	G/G					
		bp:	312													
YIR12	**a**/α		A/G													
	α/α-1		G/G													
	α/α-2, -3, -4, -5		G/G													
	a/**a**-1		A/A													

Consensus sequences were generated between the sequences obtained for P37037, P37039, P75063, P34048, and their derivatives. Base pair positions are given for the polymorphic sites as a function of the consensus sequences. Position 1 corresponds to the 5′ end of the forward primers described in Table 1, except for the sequences of genes *YAP3* and *YIR12*, where position 1 corresponds to the 5′ end of the reverse primer due to the presence of a 6-bp insertion/deletion near the beginning of the sequence obtained with the forward primer. Dashes in the sequence indicate single-base-pair deletions.

chromosome 5 contained the repeat sequence HOK at one end and the tandemly repeated RPS sequences and the repeat sequence RB2 at the opposite end (Iwaguchi *et al.* 1992; Anderson *et al.* 1993; Lockhart *et al.* 1995; Chindamporn *et al.* 1998; Pujol *et al.* 1999). The RPS locus contains the *Sfi*I sites that separate chromosome 5 into fragments I and M (Figure 2). The orientation of contig 19-10202 was based on the fact that it contained the HOK sequence at one end. The orientation of contig 19-10155 was based on the position of the RB2 sequence at one end. Their locations on the chromosome were in agreement with the partial physical map generated by P. T. Magee and colleagues (http://alces.med.umn.edu/Candida.html; Forche *et al.* 2004). Contigs 19-10194 and 19-10170 were assigned to fragments I and M, respectively, according to the partial physical map and Forche *et al.* (2004). The orientation of contig 19-10194 was based upon the fact that it contains CARE2-homologous sequences at one end. CARE2 has been shown to be subtelomeric on chromosome 7 and has been suggested to be subtelomeric on other chromosomes (Chibana *et al.* 1998). This orientation is in agreement with that in Forche *et al.* (2004). The orientation of contig 19-10170, 19-10080, and 19-10137 follows that of Forche *et al.* (2004). The location of a putative centromere on chromosome 5 was based on

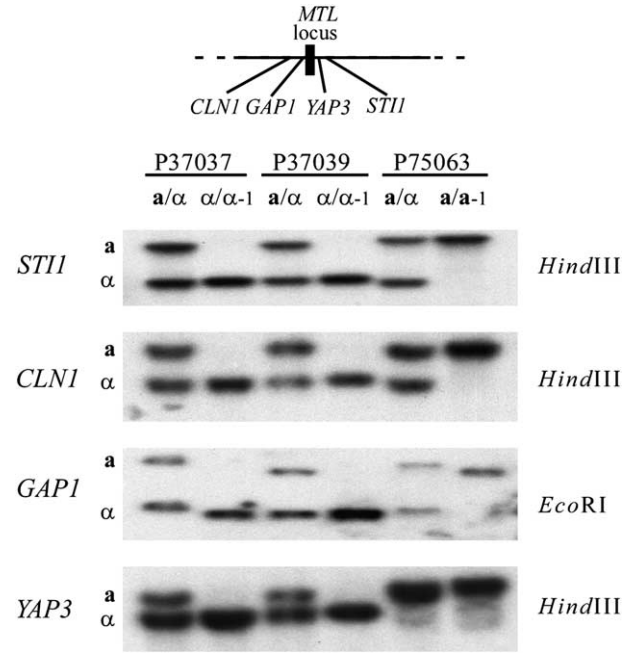

FIGURE 5.—Southern blot hybridization analysis of four polymorphic genes positioned within a 30-kb region harboring the mating-type locus (*MTL*). The relative positions of the genes are shown in the schematic above the Southern blots. Restriction enzymes used to show the polymorphism for each gene are indicated to the right. The primers used to generate the open reading frame of each gene are presented in Table 1.

Cse4p binding studies recently reported by SANYAL *et al.* (2004). This sequence is located in contig 19-10170 between positions 1 kb and 3.2 kb. This latter region is between two inverted repeat sequences (Figure 2). The intergenic marker 10170B is located ~1 kb downstream of the putative centromeric region (Figure 2). The markers 1990A and 1990C are located ~2 and 3.6 kb, respectively, from the putative centromeric region, but because of the inverted repeat sequences surrounding the putative centromeric region, the orientation (to the left or right) has not yet been resolved. We have placed these markers to the left, as in the latest assembly (assembly 19) of the *C. albicans* genome (http://www-sequence.stanford.edu/group/candida/).

The analysis of homozygous derivatives of strains P37037, P37039, P37063, and P34048 was made under the assumption that the integrity of chromosome 5 was not disrupted by chromosomal rearrangements, specifically at the RPS locus (CHU *et al.* 1993; PUJOL *et al.* 1999; CHIBANA *et al.* 2000; JOLY *et al.* 2002). The integrity of chromosome 5 was verified in the majority of derivative strains by Southern blot hybridization of contour-clamped homogeneous electric field (CHEF) gels with the markers 1854A and 2466C on fragment M and markers 2284B and *YIR12* to the right of the RPS locus on fragment I (data not shown).

MTL* homozygosis in strain P37037:** In the *MTL*-heterozygous (**a**/α) strain P37037, 6 markers to the left and 12 to the right of the *MTL* locus were heterozygous (Figure 4A). The nucleotide polymorphisms in **a**/α P37037 of the intergenic sites, and genes *YAP3* and *YIR12*, are presented in Table 2, and the polymorphisms identified by Southern analysis for the genes *STI1*, *CLN1*, *GAP1*, and *YAP3* are presented in Figure 5. Of the six *MTL*-homozygous derivatives of **a**/α P37037 identified in the screen for white-opaque switching, five were α/α derivatives (α/α-1, α/α-2, α/α-3, α/α-4, and α/α-5) and one an **a**/**a** derivative (**a**/**a**-1). In derivative α/α-1 markers in the portion of chromosome 5 beginning between markers 1854A and 1990C and ending between markers 2466A and 2284B remained heterozygous (Figure 4B). This region included the putative centromeric sequence. All markers to the left of this heterozygous region, however, were homozygous, containing the polymorphisms associated with the original *MTL*α homolog of chromosome 5. Three markers to the right of the heterozygous region, beginning between markers 2466A and 2284 B and ending between markers 2222C and *YIR12*, were homozygous, containing the polymorphisms associated with the original *MTLa** homolog of chromosome 5, and the last marker to the right, *YIR12*, was homozygous for the polymorphism associated with the original *MTL*α homolog of chromosome 5. These results indicate that at least three crossovers occurred in the genesis of α/α-1, one between markers 1854A and 1990C, one between markers 2466A and 2284B, and one between markers 2222C and *YIR12* (Figure 4, A and B). The region between markers 2644A and 2284B harbors the RPS locus (Figure 2). These results indicate

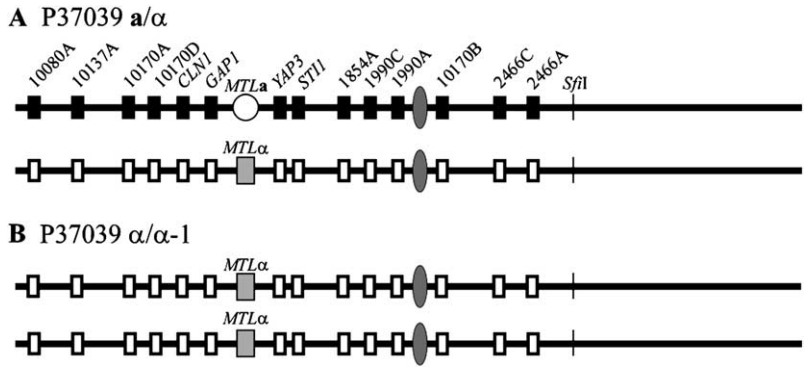

FIGURE 6.—*MTL* homozygosis in the **a**/α strain P37039 generating an *MTL*-homozygous derivative occurred by loss of one chromosome 5 homolog followed by duplication of the retained homolog. (A) Diagram of polymorphic sites along the homologs of chromosome 5 in the original **a**/α strain. The solid and open boxes represent the gene and intergenic sequence polymorphisms. (B) Diagram of sites is presented for the α/α-1 derivative.

TABLE 3

Sequence analysis of chromosome 5 intergenic regions and the gene *YAP3* for strain a/α p37039 and the *MTL*-homozygous derivative α/α-1

Marker	Strain		Polymorphic sites													
		bp:	43	158	170	180	200	213	217	223	228	234	236	238	248	342
10080A	**a**/α		C/T	A/G	A/G	A/G	C/T	G/T	C/T	C/T	G/T	A/C	A/T	A/T	C/T	A/G
	α/α-1		C/C	A/A	A/A	G/G	T/T	G/G	C/C	T/T	G/G	C/C	T/T	A/A	C/C	A/A
		bp:	218	234	255	324	340	377								
10137A	**a**/α		C/T	A/G	A/G	C/T	C/T	C/T								
	α/α-1		C/C	A/A	A/A	T/T	C/C	T/T								
		bp:	75													
10170A	**a**/α		C/T													
	α/α-1		C/C													
		bp:	137	265												
10170D	**a**/α		A/T	C/T												
	α/α-1		A/A	T/T												
			130	214	268	442	539	555								
YAP3	**a**/α		A/T	A/G	A/T	A/G	C/T	A/G								
	α/α-1		A/A	G/G	T/T	G/G	T/T	A/A								
		bp:	196													
1854A	**a**/α		T/−													
	α/α-1		−/−													
		bp:	112	177	221	431										
1990C	**a**/α		A/G	C/T	A/G	C/T										
	α/α-1		G/G	C/C	G/G	C/C										
		bp:	255	460												
1990A	**a**/α		A/T	A/G												
	α/α-1		T/T	G/G												
		bp:	74	125	190	326	370	375	393	403	426	435				
10170B	**a**/α		A/G	C/G	A/G	C/T	C/G	C/T	C/T	C/T	C/T	C/T				
	α/α-1		G/G	G/G	A/A	C/C	C/C	T/T	C/C	C/C	C/C	C/C				
		bp:	138	144	423	448	449	567	579	603						
2466C	**a**/α		A/G	A/T	A/G	C/T	A/T	C/T	A/G	A/G						
	α/α-1		G/G	A/A	G/G	T/T	T/T	T/T	G/G	G/G						
		bp:	380													
2466A	**a**/α		C/T													
	α/α-1		T/T													

See Table 2 footnote.

that homozygosity in α/α-1 arose by mitotic recombination and exclude a mechanism of precise gene conversion at the *MTL* locus, as occurs in *S. cerevisiae*, or a mechanism of chromosome deletion.

In the *MTL*-homozygous derivatives α/α-2, α/α-3, α/α-4, and α/α-5, all of the markers, including 6 to the left and 12 to the right of the *MTL* locus, were homozygous (Figure 4C). Most notably, the markers 1990A, 1990C, and 10170B, located close to the putative centromeric region, were homozygous. The nucleotide sequences of intergenic markers and the genes *YAP3* and *YIR12* are presented in Table 2. These results indicate that the chromosome 5 homolog harboring the *MTL***a** locus was deleted in the genesis of the α/α-2, α/α-3, α/α-4, and α/α-5 derivatives of P37037. Similarly, in the *MTL*-homozygous derivative **a**/**a**-1, the 6 markers to the left and the 12 markers to the right of the *MTL* locus were homozygous (Figure 4D). The nucleotide sequences of intergenic markers and genes *YAP3* and *YIR12* are presented in Table 2. These results indicate

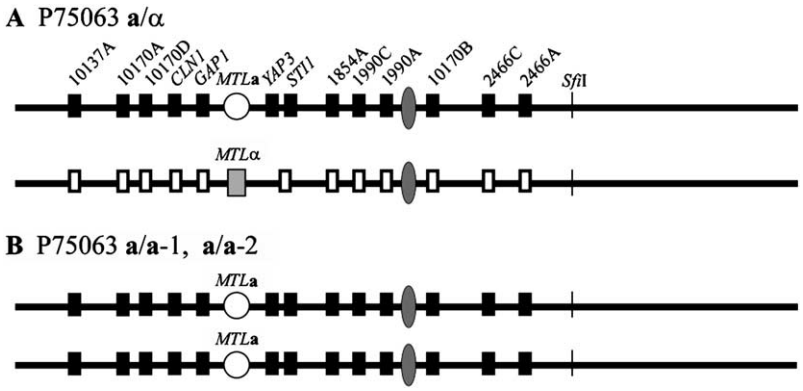

FIGURE 7.—*MTL* homozygosis in the **a**/α strain P75063 generating two independent *MTL*-homozygous derivatives occurred by loss of one chromosome 5 homolog followed by duplication of the retained homolog. (A) Diagram of the polymorphic sites along the homologs of chromosome 5 in the original **a**/α strain. The solid and open boxes represent the gene and intergenic sequence polymorphisms. (B) Diagrams of the tested sites along the homologs of chromosome 5 are presented for the P75063 derivatives **a**/**a**-1 and **a**/**a**-2.

that the chromosome 5 homolog harboring the *MTL*α locus was lost, and the retained chromosome 5 homolog duplicated in the genesis of the **a**/**a**-1 derivative of P37037.

MTL* homozygosis in strain P37039:** In the *MTL*-heterozygous (**a**/α) strain P37039, six markers to the left and eight to the right were polymorphic (Figure 6A). The polymorphic nucleotide sequences of the intergenic sites and the gene *YAP3* are presented for **a**/α P37039 in Table 3, and the polymorphisms identified by Southern analysis of *STI1*, *CLN1*, *GAP1*, and *YAP3* are presented in Figure 5. In the *MTL*-homozygous derivative α/α-1 of strain P37039, all markers were homozygous, including those in close proximity to the putative centromeric region (Figure 6B). A comparison of the polymorphic sequences between the original **a**/α and α/α-1 derivative of strain P37039 is presented in Table 3, and Southern analysis of the polymorphic genes is shown in Figure 5. These results indicate that the chromosome 5 homolog harboring the *MTLa** locus was deleted in the genesis of the α/α-1 derivative of P37039. The intensity of the *MTL*α2 band in the Southern blot of the P37039 α/α-1 derivative was approximately twice that of either the **a** or α band of the parent *MTL*-heterozygous strain (Figure 3), suggesting that loss of the chromosome 5 homolog harboring the *MTL***a** locus was followed by duplication of the homolog harboring the *MTL*α locus. Similar results were obtained in Southern analyses of the genes *CLN1*, *GAP1*, *STI1*, and *YAP3* (Figure 5), supporting this scenario of homolog duplication after loss.

MTL* homozygosis in strain P75063:** In the *MTL*-heterozygous (**a**/α) strain P75063, five markers to the left and eight to the right of the *MTL* locus were heterozygous (Figure 7A). In each of the two independently isolated *MTL*-homozygous derivatives **a**/**a**-1 and **a**/**a**-2, all markers were also homozygous, including those in close proximity to the putative centromere (Figure 7B). A comparison of **a**/**a**-1 and **a**/**a**-2 revealed that all homozygous alleles were identical, suggesting that no additional recombination event had occurred. A comparison of the nucleotide sequences of polymorphic intergenic and *YAP3* sequences between the original **a**/α strain, **a**/**a**-1 derivative, and **a**/**a**-2 derivative is presented in Table 4, and Southern analysis of the polymorphic genes *STI1*, *CLN1*, and *GAP1* in the **a**/α P75063 strain, and the **a**/**a**-1 derivative, is presented in Figure 5. *YAP3* exhibited no polymorphisms in Southern blots. These results indicate that the chromosome 5 homolog harboring the *MTL*α locus in strain P75063 was deleted in the independent genesis of both the **a**/**a**-1 and **a**/**a**-2 derivatives of P75063. In addition, the intensity of the *MTLa**1 band in the Southern blot of the P75063 **a**/**a**-1 derivative was approximately twice that of either the **a** or α bands of the parent *MTL* heterozygote (Figure 3), again suggesting that the loss of the homolog of chromosome 5 harboring the *MTL*α locus was followed by duplication of the homolog that was retained. Similar results were obtained in Southern analyses of the genes *STI1*, *CLN1*, and *GAP1* (Figure 5), further suggesting that duplication followed chromosome loss.

***MTL* homozygosis in strain P34048:** In the *MTL*-heterozygous (**a**/α) strain P34048, two markers to the left and nine markers to the right of the *MTL* locus were heterozygous (Figure 8A). The nucleotide sequences are presented in Table 5. In the *MTL*-homozygous derivatives α/α-1, α/α-2, α/α-3, α/α-4, α/α-5, and α/α-6, all markers were homozygous (Figure 8B; Table 5). In the *MTL*-homozygous derivative **a**/**a**-1, all markers were also homozygous (Figure 8C; Table 5). Hence, all *MTL*-homozygous derivatives of P34048**a**/α were generated by loss of a chromosome 5 homolog.

Chromosome 5 homolog-specific polymorphisms: In comparing the polymorphic sites along chromosome 5 in *MTL*-homozygous offspring from the different **a**/α strains, we noted similar linkage of specific polymorphisms associated with either *MTL***a** or *MTL*α. Hence, when **a**/α strains shared the base polymorphism X/Y at a particular site, X segregated with *MTL***a** and Y segregated with *MTL*α in the majority of cases for unrelated strains (Table 6).

Ruling out meiosis: Although we proposed three possible mechanisms for homozygosis that could be discriminated by analyzing polymorphic markers along

TABLE 4

Sequence analysis of chromosome 5 intergenic regions and the gene *YAP3* for a/α strain P75063 and the *MTL*-homozygous derivatives a/a-1 and a/a-2

Marker	Strain	Polymorphic sites							
		bp: 545							
10137A	**a**/α	C/T							
	a/**a**-1, **a**/**a**-2	C/C							
		bp: 70	80	161	173				
10170A	**a**/α	G/T	C/T	C/T	A/G				
	a/**a**-1, **a**/**a**-2	G/G	C/C	T/T	A/A				
		bp: 587							
10170D	**a**/α	A/G							
	a/**a**-1, **a**/**a**-2	G/G							
		bp: 540	555	559					
YAP3	**a**/α	C/T	A/G	A/G					
	a/**a**-1, **a**/**a**-2	T/T	A/A	A/A					
		bp: 67	116	196					
1854A	**a**/α	A/G	A/G	T/−					
	a/**a**-1, **a**/**a**-2	A/A	G/G	T/T					
		bp: 112	167	170	177				
1990C	**a**/α	A/G	C/T	A/C	C/T				
	a/**a**-1, **a**/**a**-2	A/A	T/T	C/C	T/T				
		bp: 160	253	255	316	460			
1990A	**a**/α	A/G	C/T	A/T	C/T	A/G			
	a/**a**-1	A/A	T/T	A/A	C/C	A/A			
		bp: 72	190	252	326	375	426	435	455
10170B	**a**/α	C/T	A/G	C/T	C/T	C/T	C/T	C/T	C/T
	a/**a**-1, **a**/**a**-2	T/T	G/G	C/C	T/T	C/C	T/T	T/T	C/C
		bp: 96	99	546	567				
2466C	**a**/α	A/G	A/T	C/T	C/T				
	a/**a**-1, **a**/**a**-2	G/G	T/T	C/C	C/C				
		bp: 122	130	133	231	345	459	462	602
2466A	**a**/α	A/G	A/G	C/T	A/G	A/G	A/G	A/T	A/G
	a/**a**-1, **a**/**a**-2	G/G	A/A	C/C	G/G	G/G	A/A	A/A	G/G

See Table 2 footnote.

chromosome 5, there was also a fourth improbable mechanism that would give the same chromosome 5 results as chromosome loss followed by duplication, namely meiosis. If meiosis occurred, all genes in the genome would become monomorphic. To test this possibility, we identified polymorphic intergenic sequences on contig 19-10205 of chromosome 1, 19-10196 of chromosome 2, and 19-10057 of chromosome R and analyzed these sequences in all of the *MTL*-homozygous offspring of strain **a**/α P37037, **a**/α P37039, **a**/α P75063, and **a**/α P34048 (Table 7). Polymorphisms on chromosomes other than chromosome 5 were maintained in all 16 *MTL*-homozygous offspring (Table 7). In Table 2, P37037α/α-2 and P37037α/α-4, loss of heterozygosity occurred on the chromosome 1 marker, but polymorphisms were conserved on chromosomes 2 and R (Table 6), suggesting that these offspring, which had become α/α through independent homozygosis events, had both undergone parallel homozygosis at the chromosome 1 marker analyzed, but not meiosis. Together, these results support the conclusion that meiosis can be excluded as the mechanism for *MTL* homozygosis in all 16 *MTL*-homozygous offspring analyzed in this study.

DISCUSSION

MTL zygosity regulates not only mating (Hull *et al.* 2000; Magee and Magee 2000; Lockhart *et al.* 2003) but also phenotypic switching (Lockhart *et al.* 2002;

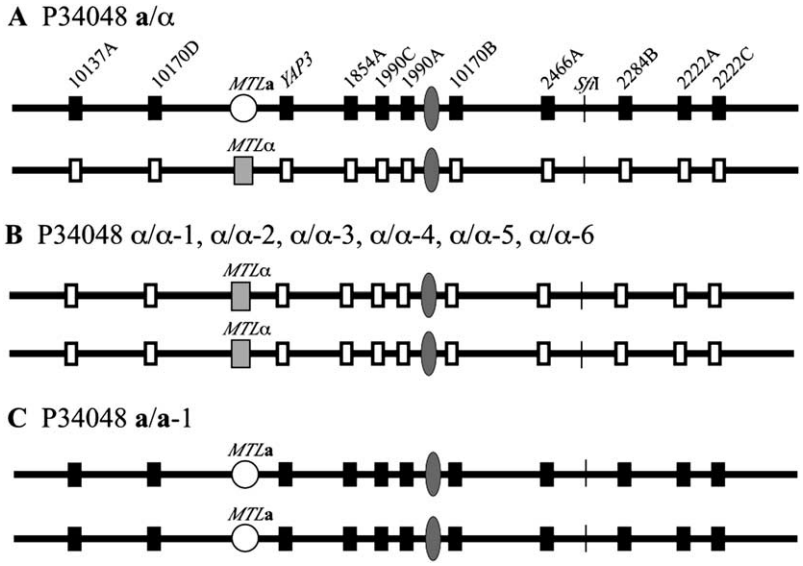

FIGURE 8.—*MTL* homozygosis in the **a**/α strain P34048 generating seven *MTL*-homozygous derivatives occurred by loss of one chromosome 5 homolog followed by duplication of the retained homolog in all cases. (A) Diagram of polymorphic sites along the homologs of chromosome 5 in the original **a**/α strain. The solid and open boxes represent gene and intergenic sequence polymorphisms. Diagrams of sites along the homologs of chromosome 5 are presented for the α/α-1, α/α-2, α/α-3, α/α-4, α/α-5, and α/α-6 derivatives (B) and the **a**/**a**-1 derivative (C).

MILLER and JOHNSON 2002). Here, we have examined the mechanism of spontaneous *MTL* homozygosis in natural strains of *C. albicans* grown in culture. Sixteen spontaneous *MTL*-homozygous offspring, both **a**/**a** and α/α, that were derived from four natural **a**/α strains were obtained by screening colonies for the opaque phenotype. Care was taken to be sure that each *MTL*-homozygous derivative was the product of an independent homozygosis event. By analyzing markers distributed along the entire length of chromosome 5, which harbors the *MTL* locus, we were able to deduce the mechanism of homozygosis for each of the 16 *MTL*-homozygous derivatives. We proposed three possible mechanisms for homozygosis, mitotic recombination, precise gene conversion, as in *S. cerevisiae*, and loss of a chromosome 5 homolog followed by duplication of the retained homolog. We found that only 1 of the 16 derivatives arose through mitotic recombination, while 15 derivatives arose by loss of one chromosome 5 homolog followed by duplication of the retained homolog. We found no evidence for precise gene conversion, although it cannot be ruled out as a minor mechanism, such as mitotic recombination. However, there was also the unlikely possibility that homozygosis could arise by meiosis. An analysis of polymorphic markers on chromosomes 1, 2, and R, however, revealed that all 16 *MTL*-homozygous derivatives maintained heterozygosity in chromosomes other than chromosome 5 homologs, excluding meiosis as a mechanism of *MTL* homozygosis *in vitro*. Two independent derivatives of P37037, P37037α/α-2 and P37037α/α-4, appeared to have similarly undergone the loss of one chromosome 1 homolog as well as one chromosome 5 homolog. Both exhibited polymorphisms on chromosomes 2 and R, indicating that they had undergone chromosome loss, not meiosis.

In the one documented case of mitotic recombination (P37037α/α-1), the parent strain was observed to undergo minor but frequent changes in its DNA fingerprint patterns identified by Southern blot hybridization with the complex DNA fingerprinting probe Ca3 (data not shown). Changes in the Ca3 fingerprinting pattern can be due to unequal crossing over at RPS loci (PUJOL *et al.* 1999; JOLY *et al.* 2002). P37037 was also the only strain that exhibited homozygosis in a second chromosome, in this case in chromosome 1, consistent with the conclusion that this **a**/α strain is genetically less stable than the other three that were analyzed.

In two of the four **a**/α strains analyzed (P37037 and P34048), we isolated both **a**/**a** and α/α offspring that arose through loss of one chromosome 5 homolog followed by duplication of the retained homolog. In a previous study of mitotic recombination, the presence of recessive lethal alleles was demonstrated (WHELAN and SOLL 1982). Since offspring from the same strains possessing duplicate copies of either homolog of chromosome 5 were viable, we can conclude that chromosome 5 does not harbor recessive lethal alleles. However, it is curious that in these same strains (P37037 and P34048), from which we isolated the most *MTL*-homozygous offspring, the majority of offspring were α/α, 5 out of 6 in the case of P37037 and 6 out of 7 in the case of P34048. Indeed, of the 16 *MTL*-homozygous offspring isolated from the four natural **a**/α strains in this study, 12 (75%) were α/α. The probability of this happening by chance is 0.02, suggesting that there may be a bias toward the loss of the chromosome 5 homolog harboring the *MTL***a** locus. This bias may be the result of the culture conditions used in our study, since it has been demonstrated that the sugar source can affect loss of a chromosome 5 homolog (JANBON *et al.* 1998; MAGEE and MAGEE 2000). Hence, it is possible that the amino acid-rich medium used in our study may have biased

TABLE 5

Sequence analysis of chromosome 5 intergenic regions and the gene *YAP3* for strain P34048 a/α and the *MTL*-homozygous derivatives α/α-1, α/α-2, α/α-3, α/α-4, α/α-5, α/α-6, and a/a-1

Marker	Strain		Polymorphic sites								
		bp:	96	251	327	490					
10137A	**a**/α		C/T	G/T	A/T	A/G					
	α/α-1, -2, -3, -4, -5, -6		T/T	G/G	A/A	G/G					
	a/**a**-1		C/C	T/T	T/T	A/A					
		bp:	126	180	275	321	372				
10170D	**a**/α		C/T	C/T	C/T	C/T	A/G				
	α/α-1, -2, -3, -4, -5, -6		C/C	T/T	T/T	T/T	A/A				
	a/**a**-1		T/T	C/C	C/C	C/C	G/G				
		bp:	292	540	559						
YAP3	**a**/α		A/G	C/T	A/G						
	α/α-1, -2, -3, -4, -5, -6		A/A	T/T	A/A						
	a/**a**-1		G/G	C/C	G/G						
		bp:	541	608	609						
1854A	**a**/α		A/G	A/C	A/G						
	α/α-1, -2, -3, -4, -5, -6		A/A	C/C	A/A						
	a/**a**-1		G/G	A/A	G/G						
		bp:	125	193	221						
1990C	**a**/α		A/C	C/T	A/G						
	α/α-1, -2, -3, -4, -5, -6		C/C	T/T	A/A						
	a/**a**-1		A/A	C/C	G/G						
		bp:	590								
1990A	**a**/α		A/G								
	α/α-1, -2, -3, -4, -5, -6		A/A								
	a/**a**-1		G/G								
		bp:	192	240	297	385	405				
10170B	**a**/α		A/G	C/T	C/T	A/G	C/T				
	α/α-1, -2, -3, -4, -5, -6		G/G	T/T	C/C	A/A	T/T				
	a/**a**-1		A/A	C/C	T/T	G/G	C/C				
		bp:	122	130	133	151					
2466A	**a**/α		A/G	A/G	C/T	C/T					
	α/α-1, -2, -3, -4, -5, -6		A/A	G/G	T/T	C/C					
	a/**a**-1		G/G	A/A	C/C	T/T					
		bp:	176	180	349	406					
2284B	**a**/α		G/T	C/T	A/G	C/T					
	α/α-1, -2, -3, -4, -5, -6		T/T	T/T	G/G	C/C					
	a/**a**-1		G/G	C/C	A/A	T/T					
		bp:	59	300							
2222A	**a**/α		A/G	C/T							
	α/α-1, -2, -3, -4, -5, -6		G/G	C/C							
	a/**a**-1		A/A	T/T							
		bp:	290	300	307	321	334	336	347	360	372
2222C	**a**/α		A/C	A/G	C/T	A/C	C/−	A/G	A/T	C/T	A/G
	α/α-1, -2, -3, -4, -5, -6		A/A	G/G	C/C	A/A	C/C	A/A	T/T	C/C	A/A
	a/**a**-1		C/C	A/A	T/T	C/C	−/−	G/G	A/A	T/T	G/G

See Table 2 footnote.

TABLE 6

Linkage of specific polymorphisms with *MTL*a and *MTL*α in chromosome 5 homologs

Marker	Strain	Polymorphic sites[a]																													
		bp:	43		158		170		180		200		213		217		223		228		234		236		238		248		342	
10080A			**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α
	P37037		T	C	G	A	G	A	A	G	C	T	T	G	T	C	C	T	T	G	A	C	A	T	T	A	T	C	G	A
	P37039			C		A		A		G		T		G		C		T		G		C		T		A		C		A
		bp:	218		234		255		324		340		377																	
10137A			**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α																
	P37037		T	C	G	A	G	A	C	T	T	C	C	T																
	P37039			C		A		A		T		C		T																
		bp:	75																											
10170A			**a**	α																										
	P37037		C	T																										
	P37039			C																										
		bp:	137		265																									
10170D			**a**	α	**a**	α																								
	P37037		T	A	C	T																								
	P37039			A		T																								
		bp:	130		214		268		442		539		540		555		559													
YAP3			**a**	**α**	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α												
	P37037		T	A	A	G	A	T	A	G	C	T	NP[b]		G	A	NP[b]													
	P37039			A		G		T		G		T	NP[b]			A	NP[b]													
	P75063		NP[b]		NP[b]		NP[b]		NP[b]		NP[b]		T		A		A													
	P34048		NP[b]		NP[b]		NP[b]		NP[b]		NP[b]		C	T	NP[b]		G	A												
		bp:	196																											
1854A			**a**	α																										
	P37037		T	—																										
	P37039			—																										
	P75063		T																											
		bp:	112		177		221		431																					
1990C			**a**	α	**a**	α	**a**	α	**a**	α																				
	P37037		A	G	T	C	A	G	T	C																				
	P37039			G		C		G		C																				
	P75063		A		T		NP[b]		NP[b]																					
	P34048		NP[b]		NP[b]		G	A	NP[b]																					
		bp:	255		460																									
1990A			**a**	α	**a**	α																								
	P37037		A	T	A	G																								
	P37039			T		G																								
	P75063		A		A																									

(*continued*)

TABLE 6

(Continued)

Marker	Strain	Polymorphic sites[a]																	
	bp:	74		125		190		326		370		375		393		403		426		435	
10170B		**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α
	P37037	A	G	C	G	G	A	T	C	G	C	C	T	T	C	T	C	T	C	T	C
	P37039		G		G		A		C		C		T		C		C		C		C
	P75063	NP[b]		NP[b]		G		T		NP[b]		C		NP[b]		NP[b]		T		T	
	bp:	138		144		423		448		449		567		579		603					
2466C		**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α				
	P37037	A	G	T	A	A	G	C	T	A	T	C	T	A	G	A	G				
	P37039		G		A		G		T		T		T		G		G				
	P75063	NP[b]		NP[b]		NP[b]		NP[b]		NP[b]		C		NP[b]		NP[b]					
	bp:	122		130		133		380													
2466A		**a**	α	**a**	α	**a**	α	**a**	α												
	P37037	NP[b]		NP[b]		NP[b]		C	T												
	P37039	NP[b]		NP[b]		NP[b]		T													
	P75063	G		A		C		NP[b]													
	P34048	G	A	A	G	C	T	NP[b]													
	bp:	349		406																	
2284B		**a**	α	**a**	α																
	P37037	A	G	T	C																
	P34048	A	G	T	C																
	bp:	59																			
2222A		**a**	α																		
	P37037	A	G																		
	P34048	A	G																		
	bp:	290		300		307		321		334		336		347		360		372			
2222C		**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α	**a**	α		
	P37037	C	A	A	G	T	C	C	A	—	C	G	A	A	T	T	C	G	A		
	P34048	C	A	A	G	T	C	C	A	—	C	G	A	A	T	T	C	G	A		

In addition to these shared polymorphisms obtained through sequencing, polymorphisms obtained by Southern analyses in strains P37037, P37039, and P75063 and their derivatives showed additional polymorphisms specific to *MTL***a** and *MTL*α.

[a] Polymorphic sites that were shared by at least two of the four **a**/α strains used in this study are represented for each marker. The nucleotides associated to *MTL***a** (**a**) and (or) *MTL*α (α) are represented for each strain. In strains P37037 and P34048 for which *MTL***a** and *MTL*α derivatives were obtained, nucleotides are given for both. In strain P37039, nucleotides are given for the lone *MTL*α derivative obtained. In strain P75063, nucleotides are given for the *MTL***a** derivatives obtained.

[b] No polymorphism was found for these strains at these positions.

TABLE 7

Heterozygosity of independent genetic markers eliminates meiosis as a mechanism of *MTL* homozygosis

Marker	Strains	Polymorphic sites												
	bp:	59	60	135	147	150	165	241	383	483	543	561	567	576
10205A on chromosome 1	P37037 **a**/α, α/α-1, α/α-3, α/α-5, **a**/**a**-1	**A/C**	**A/T**	**C/T**	**A/C**	**C/T**	**A/G**	**A/C**	**C/T**	**C/T**	**A/G**	**A/G**	**A/G**	**A/G**
	P37037 α/α-2, α/α-4	C/C	T/T	C/C	C/C	T/T	G/G	A/A	C/C	T/T	G/G	G/G	A/A	G/G
	P37039 **a**/α, α/α-1	**A/C**	**A/T**	**C/T**	**A/C**	**C/T**	**A/G**	**A/C**	**C/T**	**C/T**	**A/G**	**A/G**	**A/G**	**A/G**
	P75063 **a**/α, **a**/**a**-1, **a**/**a**-2	C/C	T/T	**C/T**	**A/C**	**C/T**	**A/G**	C/C	T/T	T/T	A/A	A/A	G/G	A/A
	P34048 **a**/α, α/α-1, α/α-2, α/α-3, α/α-4, α/α-5, α/α-6, **a**/**a**-1	A/A	A/A	T/T	A/A	C/C	**A/G**	**A/C**	**C/T**	**C/T**	**A/G**	**A/G**	**A/G**	**A/G**
	bp:	72	140	266	336	343								
10196A on chromosome 2	P37037 **a**/α, α/α-1, α/α-2, α/α-3, α/α-4, α/α-5, **a**/**a**-1	**C/T**	**C/T**	**C/T**	**G/T**	**A/G**								
	P37039 **a**/α, α/α-1	**C/T**	**C/T**	**C/T**	**G/T**	**A/G**								
	P75063 **a**/α, **a**/**a**-1, **a**/**a**-2	C/C	C/C	C/C	**G/T**	**A/G**								
	P34048 **a**/α, α/α-1, α/α-2, α/α-3, α/α-4, α/α-5, α/α-6, **a**/**a**-1	C/C	C/C	C/C	G/G	A/A								
	bp:	78	119[b]											
10057A on chromosome R	P37037 **a**/α, α/α- 1, α/α-2, α/α-3, α/α-4, α/α-5, **a**/**a**-1	T/T	**A**/−											
	P37039 **a**/α, α/α-1	**C/T**	**A**/−											
	P75063 **a**/α, **a**/**a**-1, **a**/**a**-2	C/C	**A**/−											
	P34048 **a**/α, α/α-1, α/α-2, α/α-3, α/α-4, α/α-5, α/α-6, **a**/**a**-1	C/C	**A**/−											

[a] Heterozygous nucleotide positions are noted in boldface type.
[b] Heterozygosity due to a single-base-pair insertion/deletion (dashes denote a deletion).

loss of the homolog harboring the *MTL***a** locus. Alternatively, the bias may be due to deleterious, but not necessarily lethal, alleles.

Our results also indicate that the chromosome 5 homolog that harbors the *MTL***a** locus also harbors alleles of other genes that are specific to that homolog and that the homolog harboring *MTL*α also harbors specific alleles. This held true for shared polymorphic sites between the four unrelated **a**/α strains, which represented three clades (Soll and Pujol 2003), suggesting that there is strong selection pressure for these allelic linkages on the chromosome 5 homologs.

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LITERATURE CITED

Anderson, J. M., and D. R. Soll, 1987 Unique phenotype of opaque cells in the white-opaque transition of *Candida albicans*. J. Bacteriol. **169:** 5579–5588.

Anderson, J., T. Srikantha, B. Morrow, S. H. Miyasaki, T. C. White *et al.*, 1993 Characterization and partial sequence of the DNA fingerprinting probe Ca3 of *Candida albicans*. J. Clin. Microbiol. **31:** 1472–1480.

Bedell, G., and D. R. Soll, 1979 The effects of low concentrations of zinc on the growth and dimorphism of *Candida albicans*: evidence for zinc resistant and zinc sensitive pathways for mycelium formation. Infect. Immun. **26:** 348–354.

Butler, G., C. Kenny, A. Fagan, C. Kurischko, C. Gaillardin *et al.*, 2004 Evolution of the *MAT* locus and its Ho endonuclease in yeast species. Proc. Natl. Acad. Sci. USA **101:** 1632–1637.

Chibana, H., B. B. Magee, S. Griddle, Y. Ran, S. Scherer *et al.*, 1998 A physical map of chromosome 7 of *Candida albicans*. Genetics **149:** 1739–1752.

Chibana, H., J. L. Beckerman and P. T. Magee, 2000 Fine resolution physical mapping of genomic diversity in *Candida albicans*. Genome Res. **10:** 1865–1877.

Chindamporn, A., Y. Nakagawa, I. Nizuguchi, H. Chibana, M. Doi *et al.*, 1998 Repetitive sequences (RPSs) in the chromosomes of *Candida albicans* are sandwiched between two novel stretches, HOK and RB2, common to each chromosome. Microbiology **144:** 849–857.

Chu, W.-S., B. B. Magee and P. T. Magee, 1993 Construction of an *Sfi*I macrorestriction map of the *Candida albicans* genome. J. Bacteriol. **175:** 6637–6651.

Forche, A., P. T. Magee, B. B. Magee and G. May, 2004 Genome-wide single-nucleotide polymorphism map for *Candida albicans*. Eukaryot. Cell **3:** 705–714.

Haber, J., 1998 Mating-type gene switching in *Saccharomyces cerevisiae*. Annu. Rev. Genet. **32:** 561–599.

Herskowitz, I., and Y. Oshima, 1981 Control of cell type in *Saccharomyces cerevisiae*: mating type and mating-type interconversion, pp. 181–210 in *The Molecular Biology of the Yeast Saccharomyces*: *Life*

Cycle and Inheritance, edited by J. N. Strathern, E. W. Jones and J. R. Broach. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

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.

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.

Iwaguchi, S. I., M. Homma, H. Chibana and K. Tanaka, 1992 Isolation and characterization of a repeated sequence (RPS1) of *Candida albicans*. J. Gen. Microbiol. **138:** 1893–1900.

Janbon, G., F. Sherman and E. Rustchenko, 1998 Monosomy of a specific chromosome determines L-sorbose utilization: a novel regulatory mechanism in *Candida albicans*. Proc. Natl. Acad. Sci. USA **95:** 5150–5155.

Joly, S., C. Pujol and D. R. Soll, 2002 Microevolutionary changes and chromosomal translocations are more frequent at RPS loci in *Candida dubliniensis* than in *Candida albicans*. Infect. Genet. Evol. **2:** 19–37.

Lee, K. L., H. R. Buckley and H. R. Campbell, 1975 An amino acid liquid synthetic medium for development of mycelia and yeast forms of *Candida albicans*. Sabouraudia **13:** 148–153.

Lockhart, S. R., J. J. Fritch, A. S. Meier, K. Schröppel, T. Srikantha *et al.*, 1995 Colonizing populations of *Candida albicans* are clonal in origin but undergo microevolution through C1 fragment reorganization as demonstrated by DNA fingerprinting and C1 sequencing. J. Clin. Microbiol. **33:** 1501–1509.

Lockhart, S. R., C. Pujol, K. Daniels, M. Miller, A. Johnson *et al.*, 2002 In *Candida albicans*, white-opaque switchers are homozygous for mating type. Genetics **162:** 737–745.

Lockhart, S. R., K. J. Daniels, R. Zhao, D. Wessels and D. R. Soll, 2003 Cell biology of mating in *Candida albicans*. Eukaryot. Cell **2:** 49–61.

Magee, B. B., and P. T. Magee, 2000 Induction of mating in *Candida albicans* by construction of *MTL***a** and *MTL*α strains. Science **289:** 310–313.

Miller, M. G., and A. D. Johnson, 2002 White-opaque switching in *Candida albicans* is controlled by the mating type (*MTL*) locus and allows efficient mating. Cell **110:** 293–302.

Pujol, C., S. Joly, B. Nolan, T. Srikantha and D. R. Soll, 1999 Microevolutionary changes in *Candida albicans* identified by the complex Ca3 fingerprinting probe involve insertions and deletions of the full-length repetitive sequence RPS at specific genomic sites. Microbiology **145:** 2635–2646.

Pujol, C., S. A. Messer, M. Pfaller and D. R. Soll, 2003 Drug resistance is not directly affected by mating type locus zygosity in *Candida albicans*. Antimicrob. Agents Chemother. **47:** 1207–1212.

Sanyal, K., M. Baum and J. Carbon, 2004 Centromeric DNA sequences in the pathogenic yeast *Candida albicans* are all different and unique. Proc. Natl. Acad. Sci. USA **101:** 11374–11379.

Schmid, J., E. Voss and D. R. Soll, 1990 Computer-assisted methods for assessing strain relatedness in *Candida albicans* by fingerprinting with the moderately repetitive sequence Ca3. J. Clin. Microbiol. **28:** 1236–1243.

Soll, D. R., 2000 The ins and outs of DNA fingerprinting the infectious fungi. Clin. Microbiol. Rev. **13:** 332–370.

Soll, D. R., and C. Pujol, 2003 DNA fingerprinting *Candida albicans* clades. FEMS Immunol. Med. Microbiol. **39:** 1–7.

Soll, D. R., T. Srikantha and S. Lockhart, 1996 Cloning and characterizing developmentally regulated genes in *Candida albicans*, pp. 17–37 in *Microbial Genome Methods*, edited by K. W. Adolph. CRC Press, Boca Raton, FL.

Srikantha, T., L. 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.

Srikantha, T., L. K. Tsai, A. Klar and D. R. Soll, 2001 The histone deacetylase *HDA1* and *RPD3* play distinct roles in the regulation of high-frequency phenotypic switching in *Candida albicans*. J. Bacteriol. **183:** 4614–4625.

Tsong, A. E., M. G. Miller, R. M. Raisner and A. D. Johnson, 2003 Evolution of a combinatorial transcriptional circuit: a case study in yeasts. Cell **115:** 389–399.

Whelan, W. L., and D. R. Soll, 1982 Mitotic recombination in *Candida albicans*: recessive lethal alleles linked to a gene required for methionine biosynthesis. Mol. Gen. Genet. **187:** 477–485.

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