

Effect of the Major Repeat Sequence on Chromosome Loss in *Candida albicans*

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Received 28 January 2005/Accepted 7 February 2005

The major repeat sequence (MRS) is found at least once on all but one chromosome in *Candida albicans*, but as yet it has no known relation to the phenotype. The MRS affects karyotypic variation by serving as a hot spot for chromosome translocation and by expanding and contracting internal repeats, thereby changing chromosome length. Thus, MRSs on different chromosomes and those on chromosome homologues can differ in size. We proposed that the MRS's unique repeat structure and, more specifically, the size of the MRS could also affect karyotypic variation by altering the frequency of mitotic nondisjunction. Subsequent analysis shows that both natural and artificially induced differences in the size of the chromosome 5 MRS can affect chromosome segregation. Strains with chromosome 5 homologues that differ in the size of the naturally occurring MRSs show a preferential loss of the homologue with the larger MRS on sorbose, indicating that a larger MRS leads to a higher risk of mitotic nondisjunction for that homologue. While deletion of an MRS has no deleterious effect on the deletion chromosome under normal growth conditions and leads to no obvious phenotype, strains that have the MRS deleted from one chromosome 5 homologue preferentially lose the homologue with the MRS remaining. This effect on chromosome segregation is the first demonstration of a phenotype associated with the MRS.

Candida albicans, a diploid yeast, is the most frequently isolated fungal pathogen in humans, accounting for 8% of hematogenously disseminated infections, primarily in immunocompromised patients (1). It is also a commensal of humans, inhabiting 50 to 70% of the population. It seems likely that a frequent scenario for disease is a breakdown in host defenses followed by an efflorescence and pathogenesis of the commensal population. Whether the fungus undergoes any irreversible changes during this process is unknown, although a number of factors, such as extracellular hydrolytic enzymes and the ability to switch among yeast, pseudohyphal, and hyphal growth forms, have been shown to be essential for virulence (25, 35).

Permanent changes of the pathogenic population are inferred from genomic studies, which have shown that many clinical isolates have karyotypic variations (2, 24, 28) from the standard arrangement of eight pairs of chromosome homologues found in strain FC18. Lephart et al. found that a recurring infection of a graft was caused by a strain with a nonstandard karyotype and that this strain persisted in situ despite antifungal treatment (20). Legrand et al. examined a series of six isolates taken over a period of 4 years from an AIDS patient with recurrent candidiasis. The last three of these showed independent karyotypic variations concomitant with increased clinical fluconazole resistance (19). Variant karyotypes are identified by the appearance of new chromosome bands, and/or the loss of existing chromosome bands compared to the standard karyotype. Despite the preponderance of variant karyotypes in the clinical population, no cause-and-effect rela-

tionship has been shown between these variations and increased virulence or drug resistance.

A special feature of the *C. albicans* genome is the major repeat sequence (MRS). Intact copies of this intermediate repeat appear one or more times on all but one chromosome (chromosome 3). In 1998, Chindamporn et al. determined the complete sequence of the highly conserved MRS (7), showing that it consists of a repeat unit called the RPS flanked by two novel conserved regions, HOK and RB2 (Fig. 1). RPS is a ≈2-kb conserved sequence that contains four SfiI sites and by Southern blot analysis is found on all chromosomes except chromosome 3 (4, 6, 14). HOK is a ≈8-kb conserved sequence that is also found on all chromosomes except chromosome 3 (7). RB2 is a ≈6-kb conserved sequence that is found on every chromosome, including chromosome 3, where it apparently exists without an RPS or HOK unit (lone RB2 subunits have been found on chromosomes R and 5 as well) (7). Consequently, Chindamporn et al. named this novel set of repeats, consisting of one or more RPS subunits flanked by HOK and RB2, the MRS.

The MRS occurs at least 10 times in the haploid genome of *C. albicans*, amounting to about 3% of the total genomic DNA content, yet there is no phenotype associated with this predominant genomic feature and its role in genome dynamics is unknown. However, there are tentative links between the variable karyotype of *C. albicans* and the MRS. Early efforts to discover the root of the karyotypic variability in *C. albicans* showed that in two strains with different karyotypes (strains 1006 and WO-1), the endonuclease SfiI produced similar restriction fragment length polymorphism (RFLP) patterns (8). Chu et al. deduced that in order for the SfiI digest patterns of strains 1006 and WO-1 to be similar despite their different karyotypes, the translocations that produced the variant karyo-

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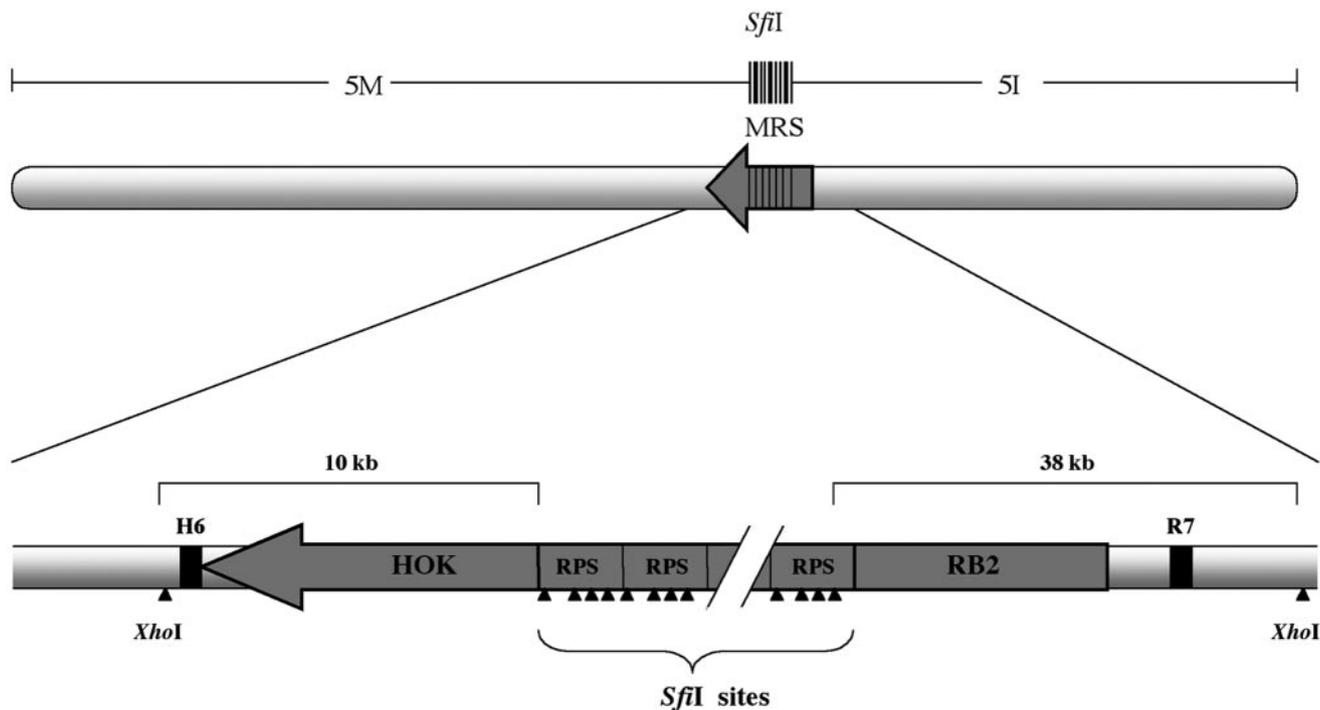


FIG. 1. Structure of the MRS; schematic representation of the MRS on chromosome 5. The locations of the *XhoI* and *SfiI* restriction sites are indicated by solid arrowheads. *XhoI* is not located within the MRS, and there are multiple *SfiI* sites within each RPS subunit. The locations of probes H6 and R7 are indicated by black boxes proximal to the HOK and RB2 subunits, respectively. The size and location of the *XhoI* and *SfiI* invariant fragments are indicated above the representation of the MRS.

type of WO-1 must have occurred at or near the *SfiI* sites in the MRS, implicating the MRS in reciprocal chromosomal translocation. Furthermore, several laboratories have shown that alteration in the number of RPS repeats within the MRS can alter chromosomal size, leading to homologues which are separable by pulse-field gel electrophoresis (3, 16, 29).

Although it is not known how chromosomal translocation or RPS repeat expansion and contraction occur, karyotypic diversity is not generated solely by translocations and chromosome size polymorphism. Chromosome nondisjunction also plays a role and, unlike translocation and size polymorphism, has been shown to affect the phenotype of a cell directly. Nondisjunction of specific chromosomes has been shown to be the most frequent route for *C. albicans* to acquire the ability to grow on sorbose medium (17, 18, 30) and can also lead to enhanced resistance to fluconazole (27). We report here that the frequency with which a homologue of chromosome 5 is lost is directly related to the size of the MRS on that homologue. Thus, not only does the MRS appear to be involved in all aspects of karyotype variation in *C. albicans*, it also is the first time a phenotype has been demonstrated to be MRS dependent.

MATERIALS AND METHODS

Strains. The strains used in this study are listed in Table 1. DNA cassettes used to replace the MRS (or lone RB2) were created by PCR with oligonucleotides that contained ≈ 60 bp of homology to unique sequences on either side of the MRS (or lone RB2) and ≈ 20 bp of *URA3* sequence to drive a PCR across the *URA3* gene (Table 2). These primers were then used with their appropriate

partner in a PCR with the *URA3* open reading frame as the template to create the MRS deletion cassette (or lone RB2 deletion); 10 to 20 μ g of DNA was produced by PCR for each transformation tube. Strains were transformed with the deletion cassette DNA according to protocols established previously (37). The plates were incubated at 30°C for 3 days and transformant colonies were picked and patched for colony PCR verification.

Media. Minimal sorbose medium was prepared from 0.67% (wt/vol) yeast nitrogen base without amino acids (YNB) with 1.5% (wt/vol) agar. The YNB agar solution was autoclaved, and a 20% L-sorbose filter-sterilized solution was added to a final concentration of 2% L-sorbose. YEPD contains 1% yeast extract, 1% Bacto-peptone, and 2% glucose. Uridine was routinely added at 20 mg/liter. YEPD plates contained 1.5% agar. Synthetic medium for transformations was prepared according to Wilson et al. (37).

Pulsed-field gel electrophoresis and restriction fragment length polymor-

TABLE 1. Strains used in this work

Strain	Relevant genotype	Reference
NUM46	Wild type	This work
FC18	Wild type	34
3153A	Wild type	26
CAI4 (parent of strains below)	<i>ura3::imm434/ura3::imm434</i>	10
3 RB2 Δ -1	3RB2:: <i>URA3</i> /3RB2	This work
3 RB2 Δ -2	3RB2:: <i>URA3</i> /3RB2	This work
5 α MRS Δ -2	5 α MRS:: <i>URA3</i> /5 α MRS	This work
5 α MRS Δ -39	5 α MRS:: <i>URA3</i> /5 α MRS	This work
5 α MRS Δ -79	5 α MRS:: <i>URA3</i> /5 α MRS	This work
5 α MRS Δ -4	5 α MRS:: <i>URA3</i> /5 α MRS	This work
5 α MRS Δ -14	5 α MRS:: <i>URA3</i> /5 α MRS	This work
5 α MRS Δ -22	5 α MRS:: <i>URA3</i> /5 α MRS	This work
5 α MRS Δ -2	5 α MRS:: <i>URA3</i> /5 α MRS:: <i>URA3</i>	This work
5 α MRS Δ -4	5 α MRS:: <i>URA3</i> /5 α MRS:: <i>URA3</i>	This work

TABLE 2. Oligonucleotides

Name	Sequence	Reference
<i>MTLa</i> F	TTGAAGCGTGAGAGGCAGGAG	23
<i>MTLa</i> R	GTTTGGGTTCCCTTCTTCTCATT	23
<i>MTLα</i> F	TTCGAGTACATTCTGGTCGCG	23
<i>MTLα</i> R	TGTAAACATCCTCAATTGTACCCGA	23
5 MRS RB2 \checkmark / 5 MRS HOK \checkmark	CTATTACAAATTACACAAGCGCCTGAG GACAGAATCATCCCACCCTGACT	This work This work
3RB2 \checkmark F 3RB2 \checkmark R	CACTACGGATTCTAATAACCCAGCAC GACATACCCAAGACATTTAGGACGC	This work This work
URA3 DETECT R URA3 DETECT F	TCACCAGTAGCACAACGAT TCCAACCTCCAGGTGTCAT	This work This work
5RB2 MRS Δ	TTTTTAATTAACCTGTACATGATTTGGAAGTGGGGCAACAGTTGTCCCTTCTCCCTGTCTAGA AGGACCACCTTTGATTG	This work
5HOK MRS Δ	ACCACAAATTATTAGTGGTTGTTTGTCTTCATCACAACCTGCATGCTTAATCCTCGCTAATGCTG GTAAAGATTCTGAACGAC	This work
3' Chr3RB2 Δ	GGCGAATTCAAATGGATATGTTATTATTCCTTCAACTGCTCCTGCAGTTCCACCAGCTCTAG AAGGACCACCTTTGATTG	This work
5' Chr3RB2 Δ	CAAATCGGAAACTTCACAGGGTTATGGTTGTTGCAACCTAACGTATTTTTTGCCTAATGCTG GTAAAGATTCTGAACGAC	This work

phism analysis. Plugs for pulsed-field gel electrophoresis containing yeast chromosomal DNA were prepared as described previously (3). Chromosome separation was carried out at 15°C in 0.5X TBE (44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA) on a Bio-Rad contour-clamped homologous electric field (CHEF) DRIII instrument. The conditions for separation were as follows: 1% agarose gel, 60 s to 120 s, 6 V/cm, 120° included angle for 36 h, and then 120 s to 300 s, 4.5 V/cm, 120° included angle for 12 h.

The restriction enzymes XhoI and XhoI with SfiI were used to digest the yeast chromosomal DNA for the restriction fragment length polymorphism analysis. The plugs containing the yeast chromosomal DNA were incubated with the enzyme(s) at 37°C overnight. Separation of fragments was carried out as above. The conditions for separation were as follows: 1.1% agarose gel, 3 s to 8 s, 6 V/cm, 120° included angle for 14 h, and then 8 s to 30 s, 5 V/cm, 120° included angle for 6 h.

Southern blots and hybridization. Probes to determine the sizes of the MRS on chromosome 5 in various strains were constructed by PCR with the primers described in Table 2. Chromosome 5-specific MRS flanking probes H6 and R7 (corresponding to the HOK side and RB2 side, respectively) were used to probe Southern blots of the XhoI digests of the genomic DNA of these strains. Figure 1 shows the locations of the MRS flanking probes and the XhoI restriction sites outside the MRS. The sequences for the R7 and H6 primers were derived from *C. albicans* sequence assembly 6 (<http://www-sequence.stanford.edu/group/Candida/search.html>).

Probes were generated in 100- μ l PCRs containing 200 mM deoxynucleoside triphosphates (Fisher Scientific); 1 unit of *Taq* polymerase (Sigma, St. Louis, Mo.); 1X PCR buffer; and 3.75 mM MgCl₂. The PCR conditions were as follows: 93°C for 5 min; 29 cycles of 93°C for 45 s, 68°C ramped down to 55°C at 0.2°/s, 55°C for 30 s, and 68° for 1 min; 68°C for 10 min; and hold at 4°C.

CHEF gels were transferred onto Hybond-N+ membrane (Amersham) and hybridized with the random-primer-labeled ([³²P]dCTP) probe overnight at 65°C in hybridization buffer (1 mM EDTA, 0.25 M sodium phosphate, pH 7.2, 7% sodium dodecyl sulfate) (Amersham). The membrane was washed twice for 10 min each at room temperature in 2 \times (1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate)SSC-0.1% sodium dodecyl sulfate (SDS); twice at 37°C in 1 \times SSC-0.1% SDS; and once for 15 min at 65°C in 1 \times SSC-0.1% SDS. The membrane was then exposed to X-Omat AR film (Eastman Kodak, Rochester, N.Y.) for up to 2 days at -80°C.

Colony PCR. A 20- μ l Pipetman tip was used to pick a small amount of cells from a colony and resuspend them in 20 μ l of colony PCR incubation solution (1.2 M sorbitol, 100 mM sodium phosphate, pH 7.4, and 2.5 mg of yeast lytic enzyme [ICN Biochemicals Inc., Aurora, Ohio] per ml). This solution was then

incubated at room temperature for 15 min, and 1 μ l was used as the template for the PCR; 20- μ l PCRs used deoxynucleoside triphosphates (Fisher) at 200 mM; 0.4 units of *Taq* (Sigma); 1 \times PCR buffer; and MgCl₂ at 3.75 mM. Thermocycler (MJ Research PTC-200, Waltham, Mass.) conditions for PCR were as follows: 93°C for 5 min; 29 cycles of 93°C for 45 s, 68°C ramped down to 55°C at 0.2°/s, 55°C for 30 s, and 68° for 1 min; 68°C for 10 min; and hold at 4°C.

The PCR primers used to amplify the *MTL* loci and the primers used to verify the deletion of the MRS are given in Table 2. The primers used to amplify the *MTLa* locus were *MTLa* F and *MTLa* R, while the primers used to amplify the *MTL α* locus were *MTL α* F and *MTL α* R. The primers to check the deletion of the MRS on the 5M (HOK) side of the deletion were 5 MRS HOK \checkmark (unique 5 M sequence) and URA3 DETECT F (internal *ura3* sequence). The primers to check the deletion of the MRS on the 5I (RB2) side of the deletion were 5 MRS RB2 \checkmark (unique 5I sequence) and URA3 DETECT R (internal *ura3* sequence). The URA3 DETECT F and URA3 DETECT R primers were also used in conjunction with 3RB2 \checkmark R and 3RB2 \checkmark F, respectively, to verify the deletion of the lone RB2 on chromosome 3.

RESULTS

MRS size determination. There are often significant differences in the size of MRSs between pairs of homologues. In order to determine if the size of the MRS has an effect on chromosome maintenance, we first identified strains with significant differences in the MRS length on the chromosome 5 homologues by CHEF gel. In strains CAI-4 and FC18, only one band was observed in the predicted chromosome 5 position, suggesting no large size differences between these chromosome 5 homologues (Fig. 2A, lanes 3 and 4). In strains 3153A and NUM46, two bands were observed in the predicted position of chromosome 5, suggesting a significant size difference between the chromosome 5 homologues (Fig. 2A, lanes 7 and 10).

To determine if the size differences between the chromosome 5 homologues observed in 3153A and NUM46 were due to MRS size variation, genomic DNA was digested with XhoI

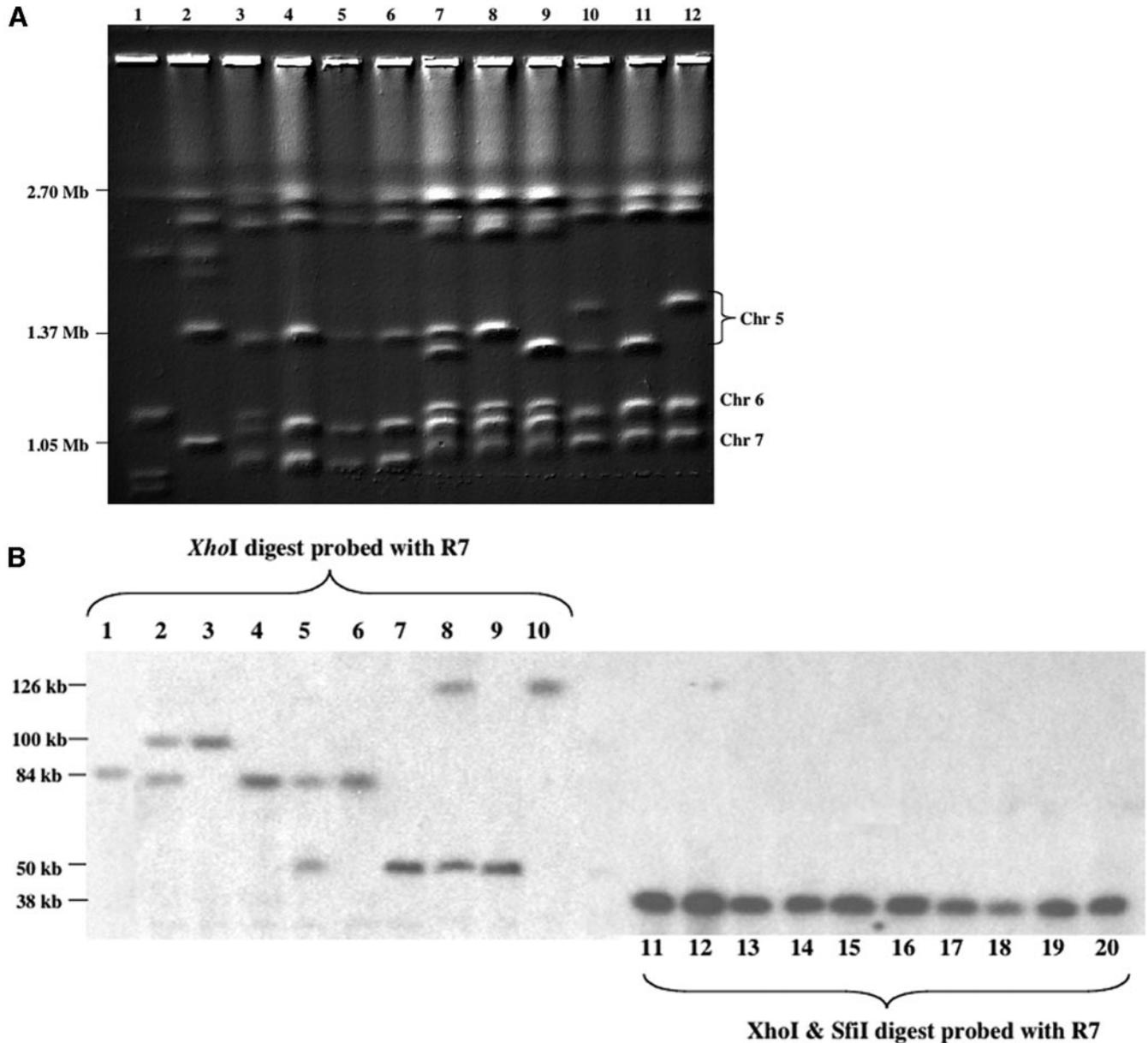


FIG. 2. (A) CHEF gel of various strains run under conditions to enhance separation of chromosome 5 homologues. Lane 3, strain CAI4 *MTLa/α*; lane 4, FC18 *MTLa/α*; lane 5, FC18 *MTLa*; lane 6, FC18 *MTLα*; lane 7, 3153A *MTLa/α*; lane 8, 3153A *MTLa*; lane 9, 3153A *MTLα*; lane 10, NUM46 *MTLa/α*; lane 11, NUM46 *MTLa*; lane 12, NUM46 *MTLα*. The locations of the various chromosome 5 homologues are indicated on the right. Karyotypes of *Saccharomyces cerevisiae* (lane 1) and *Hansenula wingeii* (lane 2) are included as size standards, with relevant sizes shown on the left. (B) *XhoI* and *XhoI/SfiI* Southern blot analysis. Southern blot hybridization zprobe R7 of *XhoI* (lanes 1 to 10) and *XhoI/SfiI* (lanes 11 to 20) restriction enzyme digests of genomic DNA from strains CAI4 *MTLa/a* (lanes 1 and 11), FC18 *MTLa/a* (lanes 2 and 12), FC18 *MTLa* (lanes 3 and 13), FC18 *MTLα* (lanes 4 and 14), 3153A *MTLa/a* (lanes 5 and 15), 3153A *MTLa* (lanes 6 and 16), 3153A *MTLα* (lanes 7 and 17), NUM46 *MTLa/α* (lanes 8 and 18), NUM46 *MTLa* (lanes 9 and 19), and NUM46 *MTLα* (lanes 10 and 20). The figure is a composite image from three experiments.

and probed with chromosome 5-specific MRS flanking probes R7 and H6 (see Fig. 1). If the size difference between the chromosome 5 homologues is due to the MRSs, then Southern blot hybridizations with the R7 or H6 probe of *XhoI*-digested genomic DNA will yield two bands, one for each homologue. Furthermore, the size difference between these two bands is an estimate of the size difference between the two MRSs and

should be comparable to the size difference deduced from the CHEF gel.

Strain CAI-4 had one band of ≈ 86 kb following Southern blot hybridization with probe R7, suggesting that the MRSs of the chromosome 5 homologues are of similar size (Fig. 2b, lane 1). Strains 3153A and NUM46 both revealed two bands (84 kb and 50 kb; 126 kb and 50 kb, respectively), suggesting that the

MRSs of the chromosome 5 homologues in these two strains are of different sizes (Fig. 2b, lanes 5 and 8). Similar results were observed with the H6 probe (data not shown). The size difference between the MRS alleles in strain 3153A was ≈ 34 kb and in strain NUM46 was ≈ 76 kb. These results are similar to the size differences inferred from the CHEF gel (≈ 40 kb for strain 3153A and ≈ 80 kb for strain NUM46), suggesting that the size difference between the chromosome 5 homologues is primarily due to size variation in the MRS.

Finally, strain FC18, which had one chromosome 5 band by CHEF gel (Fig. 2a, lane 4), revealed two bands (100 kb and 84 kb) after hybridization with probe R7 (Fig. 2b, lane 2). The size difference between the MRS alleles in strain FC18 was ≈ 16 kb. It is possible that this size difference cannot be readily resolved by CHEF gel under the conditions used; however, since we could readily detect a ≈ 36 -kb size difference between chromosome 5 homologues, it is possible that other variations occur within the chromosome 5 homologues that compensate for the difference at the MRS in strain FC18. Thus, we have identified three *C. albicans* strains whose chromosome 5 homologues have different-sized MRSs.

We next asked if the size difference between the MRS alleles of chromosome 5 is due to variation in the number of RPS subunits. To address this possibility, genomic DNA was digested with XhoI, which cuts at sites flanking the MRS, and SfiI, which cuts within the RPS subunit, and analyzed it by Southern blot hybridization with the R7 or H6 probe. If the size difference between MRS alleles is due to variation in the number of RPS subunits, then XhoI and SfiI digestion should yield identical bands when probed with R7 or H6 DNA. However, if the size difference is due to variation between the RB2 and/or HOK sequences or in flanking sequences between the MRS and the XhoI cut site, then XhoI and SfiI digestion should yield two distinct bands when probed with R7 and/or H6 DNA. In strains CAI-4, 3153A, NUM46, and FC18, Southern blot hybridization of XhoI- and SfiI-digested genomic DNA probed with R7 revealed a single band of ≈ 38 kb (Fig. 2b, lanes 11 to 20). Similar results were observed with the H6 probe and revealed an invariant band of ≈ 10 kb (data not shown). These results demonstrate that the size difference observed between XhoI fragments in strains 3153A, NUM46, and FC18 is due to variability in the number of RPS subunits within the MRS.

An individual RPS subunit is ≈ 2 kb, and the invariant XhoI fragments containing RB2 and HOK are a total of ≈ 48 kb (Fig. 1). Thus, we can estimate the number of RPS subunits and infer the approximate size of the MRS. For example, in strain CAI-4, XhoI digestion yields a band of ≈ 86 kb. Since the invariant fragments total ≈ 48 kb, the size of the RPS region is 38 kb; thus, there are (38 kb/2 kb) 19 RPS repeats. Since HOK is 8 kb and RB2 is 6 kb, the size of the MRS in CAI-4 is 52 kb. We used this approach to estimate the sizes of the chromosome 5 MRSs in strains CAI-4, 3153A, NUM46, and FC18 (Table 3).

MRS sequences are not essential for chromosome stability. Since all but one chromosome contains an intact MRS sequence and the remaining chromosome contains a portion of the MRS, we predicted that MRS sequences have an essential function in *C. albicans* chromosome biology. To test this hypothesis, we attempted to delete the MRS from chromosome

5 (Fig. 3a) and the lone RB2 from chromosome 3. The chromosome 5 MRS and chromosome 3 RB2 were deleted in strain CAI-4 with the PCR product-targeted gene disruption approach (37). Correct integration at the MRS locus was determined by PCR, with primers that bind within the *URA3* gene and that bind unique sequences outside the site of interest (Fig. 3a, lanes 3 to 10). Furthermore, CHEF gel analysis of the MRS Δ deletion strains revealed a wild-type copy of chromosome 5 and a new band below chromosome 5 of ≈ 1.32 Mb, the predicted size for a chromosome 5 lacking a ≈ 50 -kb MRS (Fig. 3b). Correct integration at the RB2 locus was determined via the PCR, with primers that flank the RB2 locus, which allows both the wild-type and deleted alleles to be assessed simultaneously (Fig. 3a, lanes 12 and 13). Thus, the MRS of chromosome 5 and the lone RB2 sequence of chromosome 3 could be deleted, suggesting that these elements are not essential for the propagation of a given chromosome.

While the presence of MRS sequences on every chromosome is not essential, we considered the possibility that the MRS is only required on one of the two homologues. Thus, in the heterozygous MRS Δ and RB2 Δ strains, the presence of a wild-type sequence on the sister homologue is sufficient for chromosome maintenance. To test this idea, we plated the chromosome 5 MRS Δ heterozygous strains on sorbose medium plus uridine to select for loss of one homologue of chromosome 5 (18). We were able to recover sorbose-positive Ura⁺ isolates that had lost the MRS-containing homologue from independent MRS Δ heterozygous strains, suggesting that an MRS is not essential for chromosome 5 maintenance.

The heteroallelic *MTL* locus is located on chromosome 5 ≈ 350 kb from the MRS. We used this property to identify sorbose-positive isolates that had lost all or part of one homologue of chromosome 5. Sorbose-positive colonies were screened with *MTL* α and *MTL* β -specific primers to determine if these isolates had lost one allele of the *MTL*. In most cases, sorbose-positive Ura⁺ colonies had a single *MTL* allele. The remaining sorbose-positive isolates retained their heteroallelic *MTL* and had acquired the ability to utilize sorbose by some other means, perhaps by the loss of chromosome 1 (33). CHEF gel analysis revealed that tested homoallelic-*MTL* sorbose-positive colonies had in fact lost one homologue of chromosome 5 (Fig. 3b, lanes 8 and 9, for example). Furthermore, from independent MRS Δ heterozygotes, we identified isolates in which the MRS Δ was linked to the *MTL* α locus (strains 5a MRS Δ -2, 5a MRS Δ -39, and 5a MRS Δ -79) and isolates in which the MRS Δ was linked to the *MTL* β locus (strains 5 α MRS Δ -4, 5 α MRS Δ -14, and 5 α MRS Δ -22). These results demonstrate that the MRS Δ sorbose-positive isolates have lost much if not all of a chromosome 5 homologue.

We next allowed these MRS Δ sorbose-positive strains to regenerate a diploid chromosome 5. Strains 5a MRS Δ -2 and 5 α MRS Δ -4 were plated on YEPD, which promotes reduplication of the chromosome 5 homologue (19) and resulted in strains 5a MRS Δ/Δ -2 and 5 α MRS Δ/Δ -4 (Table 1). Neither the heterozygous (MRS Δ /MRS) nor the homozygous (MRS Δ /MRS Δ) MRS Δ strain showed obvious phenotypic differences from the parent strain CAI-4, including colony size, morphology, filamentation, and opaque development (data not shown). These results strongly suggest that the MRS or MRS se-

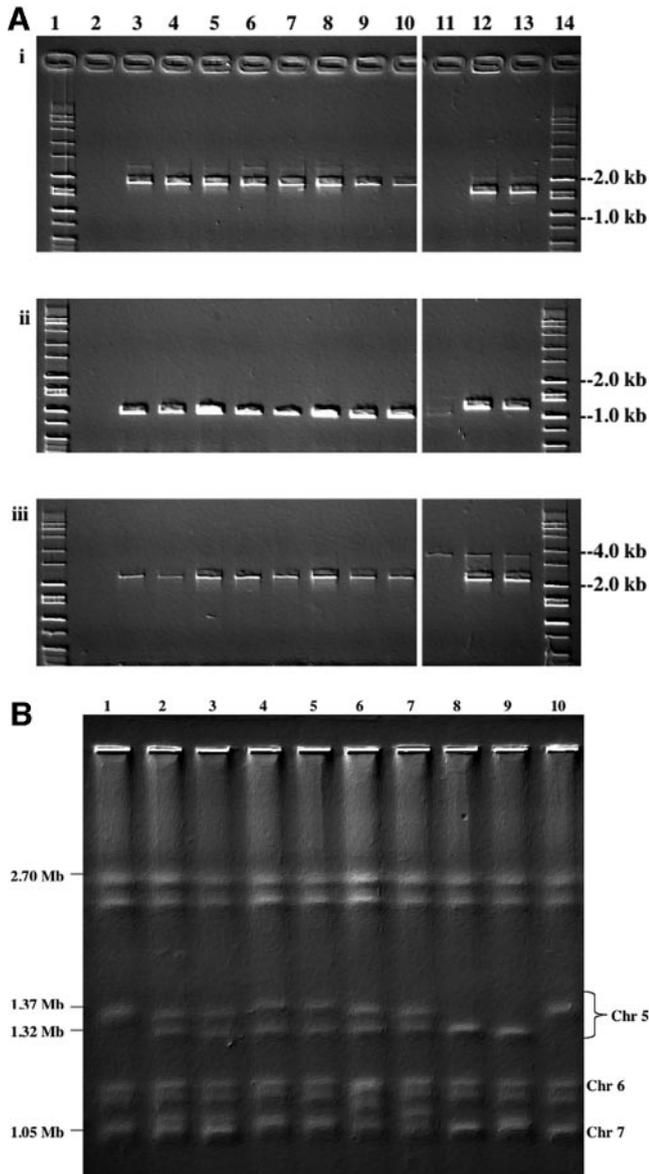


FIG. 3. (A) MRS and lone RB2 deletion verification by PCR. PCR verification of the deletion of the MRS from chromosome 5 (lanes 3 to 10) and the lone RB2 from chromosome 3 (lanes 12 to 13). Lanes 2 and 11 were run with DNA from CAI4 and serve as a negative control. i. Lanes 2 to 10 show the PCR with primers 5 MRS HOK \checkmark and URA3 DETECT F. Lanes 11 to 13 show the reaction with 3RB2 \checkmark F and URA3 DETECT F. The presence of a 1.9-kb band (lanes 2 to 10) or a 1.7-kb band (11 to 13) indicates proper integration of the deletion cassette on one side. PCR was run with DNA from strains 5 α MRS Δ -2 (lane 3), 5 α MRS Δ -4 (lane 4), 5 α MRS Δ -14 (lane 5), 5 α MRS Δ -22 (lane 6), 5 α MRS Δ -39 (lane 7), 5 α MRS Δ -79 (lane 8), 5 α MRS Δ / Δ -2 (lane 9), 5 α MRS Δ / Δ -4 (lane 10), 3 RB2 Δ -1 (lane 12), and 3 RB2 Δ -2 (lane 13). Lanes 1 and 14 contained size markers, and the sizes of relevant bands are shown at the right. ii. PCR with primers 5 MRS RB2 \checkmark (lanes 2 to 10), 3RB2 \checkmark R (lanes 11 to 13), and URA3 DETECT R (lanes 2 to 13). The presence of a 1.0-kb (lanes 2 to 10) or a 1.2-kb (11 to 13) band indicates proper integration of the deletion cassette on the opposite side. iii. PCR with primers 5 MRS HOK \checkmark and 5 MRS RB2 \checkmark (lanes 2 to 10) or 3RB2 \checkmark F and 3RB2 \checkmark R (11 to 14). The presence of a 2.2-kb band indicates proper integration of the deletion cassette on both sides. Due to the small size of the lone RB2 on chromosome 3, the flanking deletion detection primers also produce a 3.6-kb product across the intact lone RB2, and this band

TABLE 3. MRS effect on segregation bias

Allele	% Frequency of loss (MRS size, kb)			
	CAI4	FC18	3153A	NUM46
<i>MTL</i> α	51 (52)	74 (66)	64 (50)	20 (16)
<i>MTL</i> α	49 (52)	26 (50)	36 (16)	80 (92)
No. of colonies screened	81	87	64	104
<i>P</i>	0.92	<1.00E-04	2.00E-02	<1.00E-04

quences do not play an essential role in the replication or segregation of chromosomes.

Effect of MRS size difference on chromosome loss. To determine if the size of the MRS affects chromosome loss, strains CAI4, FC18, 3153A and NUM46 were grown overnight in YEPD with uridine liquid cultures and plated on medium containing sorbose as the sole carbon source to select for the loss of one chromosome 5 homologue. Each experiment is the result of plating from several different precultures, and each preculture gave the same overall result. Thus, although each sorbose-resistant colony is not from an individual experiment, they are from several different precultures and thus many must be independent.

As with the MRS Δ sorbose-positive colonies, most sorbose-positive colonies from these strains had a single *MTL* allele by PCR analysis. CHEF gel analysis revealed that tested homoallelic-*MTL* sorbose-positive colonies had in fact lost one homologue of chromosome 5 in strains where chromosome 5 homologues could be differentiated (Fig. 2a, lanes 7 to 12, for example). These results demonstrate that in strains 3153A and NUM46, sorbose utilization occurs primarily by loss of chromosome 5. In strain FC18, in which the chromosome 5 homologues could not be differentiated on a CHEF gel, the coincident loss of an *MTL* locus and loss of one MRS-containing XhoI band (Fig. 2b, lanes 2 to 4) demonstrated that in this strain, sorbose utilization also occurs primarily by loss of a chromosome 5 homologue.

We were then able to determine which *MTL* allele (α or α) was linked to the MRS allele found in the XhoI digest. In strain CAI4, which has indistinguishable chromosome 5 MRS alleles, sorbose-positive colonies that were either *MTL* α or *MTL* α were recovered at the same frequency ($P = 0.92$, Table 3). In strains FC18, 3153A, and NUM46, which have differently sized chromosome 5 MRS alleles, sorbose-positive colonies showed a bias towards either *MTL* α or *MTL* α ($P \leq 0.02$, Table

shows up in both CAI4 and the three RB2 heterozygous deletion strains where one chromosome still contains an intact lone RB2. (B) MRS deletion verification by CHEF gel; CHEF gel of the chromosome 5 MRS deletion. Strains CAI4 (lanes 1 and 10), 5 α MRS Δ -2 (lane 2), 5 α MRS Δ -4 (lane 3), 5 α MRS Δ -14 (lane 4), 5 α MRS Δ -22 (lane 5), 5 α MRS Δ -39 (lane 6), 5 α MRS Δ -79 (lane 7), 5 α MRS Δ / Δ -2 (lane 8), and 5 α MRS Δ / Δ -4 (lane 9) are shown. In each of the heterozygous MRS deletion strains (lanes 2 to 7), one chromosome 5 homologue has decreased in size equivalent to the size of the deleted MRS. The homozygous MRS deletion strains (lanes 8 and 9) show the same decrease in both chromosome 5 homologues. Chromosomes 5, 6, and 7 are indicated on the right, with relevant sizes indicated on the left.

TABLE 4. MRS deletion effect on segregation bias

Allele	% Frequency of loss (MRS size, kb)								
	CAI4	5 MRSa Δ 4	5 MRSa Δ 14	5 MRSa Δ 22	Summary for 5 MRSa Δ	5 MRS α Δ 2	5 MRS α Δ 39	5 MRS α Δ 79	Summary for 5 MRS α Δ
<i>MTLa</i>	49 (52)	33 (0)	37 (0)	42 (0)	40 (0)	63 (52)	67 (52)	57 (52)	61 (52)
<i>MTLα</i>	51 (52)	67 (52)	63 (52)	58 (52)	60 (52)	37 (0)	33 (0)	43 (0)	39 (0)
No. of colonies screened	81	89	104	259	452	158	81	229	468
<i>P</i>	0.92	6.00E-03	1.00E-02	1.00E-02	1.40E-05	8.00E-04	3.00E-03	4.00E-02	1.70E-05

3). Furthermore, in all three strains, the chromosome 5 homologue with the smaller MRS allele was preferentially retained.

In strain FC18, the MRS on the *MTLa* homologue is \approx 16 kb larger than the MRS on the *MTL α* homologue, and the larger homologue was lost in 74% of the sorbose positive colonies. In strain 3153A, the MRS on the *MTLa* homologue is \approx 36 kb larger than the MRS on the *MTL α* homologue, and the larger homologue was lost in 64% of the sorbose-positive colonies. This chromosome loss bias does not appear to be due to the *MTL* allele, because in strain NUM46, the MRS on the *MTL α* homologue is \approx 66 kb larger than the MRS on the *MTLa* homologue and the larger homologue was lost in 80% of the sorbose-positive colonies. Thus, the chromosome 5 homologue with the smaller MRS appeared more frequently in the sorbose-positive progeny than the homologue with the larger MRS. This result implies that chromosomes that contain a larger MRS than their homologue are lost at a higher rate.

We considered the possibility that the MRS is not contributing to this chromosome loss bias. For example, another heterozygous locus could exist on chromosome 5, and one allele of this locus could contribute to the chromosome loss bias independent of the MRS. In strains FC18, 3153A, and NUM46, this allele could fortuitously be on the homologue with the larger MRS (a 1 in 8 chance for all three strains). To address this possibility, we analyzed our CAI-4 heterozygous MRS Δ strains for a chromosome loss bias. We have isolates which contain a deletion of the MRS on the *MTLa* homologue and isogenic isolates which contain a deletion of the MRS on the *MTL α* homologue. Since we did not observe a chromosome loss bias in CAI-4 (Table 3), this hypothetical locus should be homozygous in this strain. Then, if the chromosome loss bias observed in FC18, 3153A, and NUM46 is due to a heteroallelic locus, we do not expect to observe a chromosome loss bias in the various MRS Δ isolates derived from CAI4.

The heterozygous MRS Δ strains were plated on sorbose, and colonies were assayed via PCR to determine which *MTL* locus was retained (Table 4). In every strain, the chromosome 5 homologue which still contained an MRS was lost more often ($P < 0.04$). These results could be due to selection for aneuploid strains carrying the *URA3* marker on the MRS Δ homologue if the chromosome 5 loss event is occurring in the initial YPD culture prior to sorbose plating. However, the majority of colonies that arise on sorbose are thought to undergo the chromosome 5 loss event after some time on the plate (17) and thus would not be subject to this bias. Therefore, these MRS Δ results, taken together with the results of the analysis of natural isolates with MRS size differences, support the idea that the size of the MRS plays a large role in the frequency with which a chromosome homologue is likely to be lost.

DISCUSSION

The MRS is an intriguing feature of the *C. albicans* genome, in part because it has no known homologues in the eukaryotic world. In this study, we have found that the MRS plays a role (whose mechanism is presently unknown) in the proper maintenance of chromosomes. This effect on chromosome stability is dependent on the size of an MRS allele; the larger the MRS allele, the higher the likelihood of loss of that homologue. The analysis of naturally occurring disparities in the size of the MRS on chromosome homologues showed a preferential appearance of the smaller MRS in the monosomic progeny selected on sorbose. Although the MRS on chromosome 5 in CAI-4 is approximately 52 kb, we were still able to delete it with our standard methods.

This is the first report of deletion of a sequence of that size by oligonucleotide-mediated gene disruption. When one MRS was eliminated entirely by gene replacement, the segregation ratio continued to be in favor of the appearance of the homologue bearing the smaller (or absent) MRS. The ratio of monosomic strains in all the experiments did not appear to be a function of the size difference between the two MRSs, but it did seem to be associated with the absolute size of the remaining MRS (Tables 3 and 4). Thus, the 52-kb MRS of the deletants was lost at about the same frequency (60 to 61%) as the 50-kb MRS of 3153A (64%). In the first case, the homologue preferentially appearing had an MRS of 0 kb, while in the second it was 16 kb. Similarly, the 66-kb MRS of FC18 was lost 74% of the time, while the 92-kb MRS of NUM46 was lost 80% of the time.

There are several possible explanations for the association of MRS size with chromosome loss. The MRS could be a preferred site for cohesin binding, making sister chromatids with larger sequences late in separating, leading to nondisjunction. Cohesin appears not to have a sequence preference for binding, but cohesin sites appear to be incompatible with transcription (12). This would predict that the areas of the chromosomes including the MRS would lag during normal mitosis. In the only study done looking at the MRS in mitosis, Chibana and Tanaka (5), using fluorescence in situ hybridization to the repeated RPS subunit, found that the MRS appeared to separate in an asynchronous manner throughout anaphase. Thus, while the separation of MRSs occurs both early and late, it is possible that the larger MRSs may be the late-separating ones, accounting for the variability seen in the times of separation in the fluorescence in situ hybridization experiment.

The general (but not absolute) sequence conservation of the MRS (4, 7) and its high frequency of appearance within the genome speak to the importance of the MRS, yet its absence

from chromosome 3 and the results of the deletion experiments in this paper clearly demonstrate that it is not an essential part of *C. albicans* chromosomes. The concept of genetic drift implies that random base pair changes should become fixed in DNA that is not performing a function and should result in a loss of conservation of that genomic locus over time. *C. albicans* is thought to exist mainly as a clonal population (13, 21, 22), in which unselected random changes should accumulate rapidly, and divergence of nonfunctional DNA should be seen in different lineages of the species. However, such divergence does not seem to be occurring in the MRS, as it is conserved not only within the genome of a single strain but also among the different strains of *C. albicans* that we have analyzed.

What important function, then, could the MRS be fulfilling for *C. albicans*? The nonessential nature of the lone RB2 subunit on chromosome 3 and the complete MRS on chromosome 5 seems to negate the possibility that the MRS has a centromeric role, and indeed, the centromeres have been shown to be localized in unique regions of the chromosomes (32). However, here we show evidence for a possible role of the MRS in adaptation of *C. albicans* to its many growth niches. Chromosome nondisjunction leading to aneuploidy can affect cell phenotype, as shown by selection for L-sorbose utilization, D-arabinose utilization, or fluconazole resistance (17, 18, 27, 30, 31), and we have shown that a large MRS increases the frequency of loss of a chromosome homologue after growth on sorbose. Perhaps strains that have large MRS alleles on homologous chromosomes are more readily able to undergo beneficial mitotic nondisjunction events and adapt more rapidly to a new environment. Strains that have smaller MRS alleles or nonfunctional MRS alleles may then be at a disadvantage, as they would not be able to adapt as quickly as the large-MRS strain and may be at a competitive disadvantage in that niche.

Previous research has shown that the MRS is a preferred site for translocations between heterologous chromosomes (3, 8) and that translocations can alter the phenotype of the resultant cell (30, 36). The control of the translocation process is not understood, and most translocations seem to occur either during growth in the host or when induced by chromosome damage in vitro (15). If the MRS is the location for translocations between heterologous chromosomes, as the data would suggest, it may also play a role in translocations between homologous chromosomes via mitotic recombination. Although heterologous translocations are difficult to detect in a high-throughput manner, it is possible to study the mitotic recombination rate across the MRS under a variety of conditions. Research on the effect of the MRS on mitotic recombination is ongoing.

If the translocations occur exactly within the MRS, then there is no clear explanation as to how this can affect the resultant phenotype of the cell unless the MRS exerts transcriptional control on genes located near it. A role in silencing of proximal genes would not be unusual for a genetic structure such as the MRS. Large repeat regions in eukaryotes are often heterochromatic in nature, and heterochromatin frequently exerts an inhibitory transcriptional effect on adjacent genes (9). Interestingly, while there is no obvious homologue of the MRS in eukaryotes, limited structural but not sequence homology to the MRS is found in the human D4Z4 repeat system, which is

known to have an inhibitory effect on transcription (11). The D4Z4 repeat consists of 3.3-kb subunits with 11 to 150 repeats per structure, compared to \approx 2-kb RPS subunits with 1 to 50 repeats per MRS. The base subunit of the D4Z4 repeat also contains a highly conserved 27-bp sequence that is a binding site for a transcriptional repression complex, while similarly, each RPS subunit of an MRS contains four to five highly conserved 29-bp sequences that have no known function. Thus, the function of the D4Z4 repeat is the inhibition of transcription of genes that are up to 3 Mb away from the repeat, with the extent of the inhibition being dependent on the number of repeats. The MRS may have a similar effect on nearby genes, and this effect may be dependent on MRS size (RPS number) as well. Experiments are under way to study the expression of genes located up to 50 kb proximal to the MRS in strains CAI4 and in the MRS deletion strains created in this study.

The MRS remains a cryptic structure within the genome of *C. albicans*; it has tantalizing connections to essential functions and chromosome dynamics, yet is clearly not essential to the chromosome. We have shown in this paper that while neither the MRS nor the lone RB2 is necessary for chromosome maintenance and function in vitro, the MRS does have an effect on chromosome mitotic nondisjunction. Further work will be required to determine how this effect is mediated and whether the MRS has any other phenotypic effects.

ACKNOWLEDGMENTS

This work was supported by NIH grants AI16567 and AI46351 and contract AI05406 awarded to P.T.M. from NIAID. P.L. was supported by training grant T32 GM08347. Sequencing of *Candida albicans* was accomplished with the support of the NIDR and the Burroughs Wellcome Fund.

Sequence data for *Candida albicans* were obtained from the Stanford Genome Technology Center website at <http://www-sequence.stanford.edu/group/Candida>. We are grateful to Bebe Magee, the members of the Magee lab, and Judith Berman, David Kirkpatrick, and Dana Davis for helpful discussions. We also thank Shin-Ichi Iwaguchi for sharing unpublished information.

REFERENCES

1. **Abi-Said, D., E. Anaissie, O. Uzun, I. Raad, H. Pinzcowski, and S. Vartivarian.** 1997. The epidemiology of hematogenous candidiasis caused by different *Candida* species. *Clin. Infect. Dis.* **24**:1122–1128.
2. **Asakura, K., S. Iwaguchi, M. Homma, T. Sukai, K. Higashide, and K. Tanaka.** 1991. Electrophoretic karyotypes of clinically isolated yeasts of *Candida albicans* and *C. glabrata*. *J. Gen. Microbiol.* **137**:2531–2538.
3. **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.
4. **Chibana, H., S. Iwaguchi, M. Homma, A. Chindamporn, Y. Nakagawa, and K. Tanaka.** 1994. Diversity of tandemly repetitive sequences due to short periodic repetitions in the chromosomes of *Candida albicans*. *J. Bacteriol.* **176**:3851–3858.
5. **Chibana, H., and K. Tanaka.** 1996. Analysis of the cell cycle in the budding yeast *Candida albicans* by positioning of chromosomes by fluorescence in situ hybridization (FISH) with repetitive sequences. *Genes Cells* **1**:727–740.
6. **Chindamporn, A., Y. Nakagawa, M. Homma, H. Chibana, M. Doi, and K. Tanaka.** 1995. Analysis of the chromosomal localization of the repetitive sequences (RPSs) in *Candida albicans*. *Microbiology* **141**:469–476.
7. **Chindamporn, A., Y. Nakagawa, I. Mizuguchi, H. Chibana, M. Doi, and K. Tanaka.** 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.
8. **Chu, W. S., B. B. Magee, and P. T. Magee.** 1993. Construction of an SfiI macrorestriction map of the *Candida albicans* genome. *J. Bacteriol.* **175**:6637–6651.
9. **Elgin, S. C., and S. I. Grewal.** 2003. Heterochromatin: silence is golden. *Curr. Biol.* **13**:R895–898.
10. **Fonzi, W. A., and M. Y. Irwin.** 1993. Isogenic strain construction and gene mapping in *Candida albicans*. *Genetics* **134**:717–728.

11. **Gabellini, D., M. R. Green, and R. Tupler.** 2002. Inappropriate gene activation in FSHD: a repressor complex binds a chromosomal repeat deleted in dystrophic muscle. *Cell* **110**:339–348.
12. **Glynn, E. F., P. C. Megee, H. G. Yu, C. Mistrot, E. Unal, D. E. Koshland, J. L. DeRisi, and J. L. Gerton.** 2004. Genome-wide mapping of the cohesin complex in the yeast *Saccharomyces cerevisiae*. *PLoS Biol.* **2**:E259.
13. **Graser, Y., M. Volovsek, J. Arrington, G. Schonian, W. Presber, T. G. Mitchell, and R. Vilgalys.** 1996. Molecular markers reveal that population structure of the human pathogen *Candida albicans* exhibits both clonality and recombination. *Proc. Natl. Acad. Sci. USA* **93**:12473–12477.
14. **Iwaguchi, S., 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.
15. **Iwaguchi, S., M. Suzuki, N. Sakai, Y. Nakagawa, P. T. Magee, and T. Suzuki.** 2004. Chromosome translocation induced by the insertion of the URA blaster into the major repeat sequence (MRS) in *Candida albicans*. *Yeast* **21**:619–634.
16. **Iwaguchi, S. I., M. Sato, B. B. Magee, P. T. Magee, K. Makimura, and T. Suzuki.** 2001. Extensive chromosome translocation in a clinical isolate showing the distinctive carbohydrate assimilation profile from a candidiasis patient. *Yeast* **18**:1035–1046.
17. **Janbon, G., F. Sherman, and E. Rustchenko.** 1999. Appearance and properties of L-sorbose-utilizing mutants of *Candida albicans* obtained on a selective plate. *Genetics* **153**:653–664.
18. **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.
19. **Legrand, M., P. Lephart, A. Forche, F. M. Mueller, T. Walsh, P. T. Magee, and B. B. Magee.** 2004. Homozygosity at the MTL locus in clinical strains of *Candida albicans*: karyotypic rearrangements and tetraploid formation. *Mol. Microbiol.* **52**:1451–1462.
20. **Lephart, P., P. Ferrieri, and J. A. van Burik.** 2004. Reservoir of *Candida albicans* infection in a vascular bypass graft demonstrates a stable karyotype over six months. *Med. Mycol.* **42**:255–260.
21. **Lockhart, S. R., J. J. Fritch, A. S. Meier, K. Schroppel, T. Srikantha, R. Galask, and D. R. Soll.** 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.
22. **Lott, T. J., B. P. Holloway, D. A. Logan, R. Fundyga, and J. Arnold.** 1999. Towards understanding the evolution of the human commensal yeast *Candida albicans*. *Microbiology* **145**:1137–1143.
23. **Magee, B. B., and P. T. Magee.** 2000. Induction of mating in *Candida albicans* by construction of MTL α and MTL α strains. *Science* **289**:310–313.
24. **Merz, W. G., C. Connelly, and P. Hieter.** 1988. Variation of electrophoretic karyotypes among clinical isolates of *Candida albicans*. *J. Clin. Microbiol.* **26**:842–845.
25. **Naglik, J. R., S. J. Challacombe, and B. Hube.** 2003. *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol. Mol. Biol. Rev.* **67**:400–428.
26. **Olaia, A. F., J. R. Steed, and S. J. Sogin.** 1980. Deoxyribonucleic acid-deficient strains of *Candida albicans*. *J. Bacteriol.* **141**:1284–1290.
27. **Perepnikhatka, V., F. J. Fischer, M. Niimi, R. A. Baker, R. D. Cannon, Y. K. Wang, F. Sherman, and E. Rustchenko.** 1999. Specific chromosome alterations in fluconazole-resistant mutants of *Candida albicans*. *J. Bacteriol.* **181**:4041–4049.
28. **Pfaller, M. A., J. Rhine-Chalberg, S. W. Redding, J. Smith, G. Farinacci, A. W. Fothergill, and M. G. Rinaldi.** 1994. Variations in fluconazole susceptibility and electrophoretic karyotype among oral isolates of *Candida albicans* from patients with AIDS and oral candidiasis. *J. Clin. Microbiol.* **32**:59–64.
29. **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.
30. **Rustchenko, E. P., D. H. Howard, and F. Sherman.** 1994. Chromosomal alterations of *Candida albicans* are associated with the gain and loss of assimilating functions. *J. Bacteriol.* **176**:3231–3241.
31. **Rustchenko, E. P., D. H. Howard, and F. Sherman.** 1997. Variation in assimilating functions occurs in spontaneous *Candida albicans* mutants having chromosomal alterations. *Microbiology* **143**:1765–1778.
32. **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.
33. **Wang, Y. K., B. Das, D. H. Huber, M. Wellington, M. A. Kabir, F. Sherman, and E. Rustchenko.** 2004. Role of the 14-3-3 protein in carbon metabolism of the pathogenic yeast *Candida albicans*. *Yeast* **21**:685–702.
34. **Whelan, W. L., R. M. Partridge, and P. T. Magee.** 1980. Heterozygosity and segregation in *Candida albicans*. *Mol. Gen. Genet.* **180**:107–113.
35. **Whiteway, M., and U. Oberholzer.** 2004. *Candida* morphogenesis and host-pathogen interactions. *Curr. Opin. Microbiol.* **7**:350–357.
36. **Wickes, B. L., J. E. Golin, and K. J. Kwon-Chung.** 1991. Chromosomal rearrangement in *Candida stellatoidea* results in a positive effect on phenotype. *Infect. Immun.* **59**:1762–1771.
37. **Wilson, R. B., D. Davis, and A. P. Mitchell.** 1999. Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions. *J. Bacteriol.* **181**:1868–1874.