JOURNAL OF BACTERIOLOGY, Nov. 1993, p. 7189–7199 Vol. 175, No. 22
0021-9193/93/227189-11$02.00/0
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Variations in the Number of Ribosomal DNA Units in Morphological Mutants and Normal Strains of *Candida albicans* and in Normal Strains of *Saccharomyces cerevisiae*

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Naturally occurring strains of *Candida albicans* are opportunistic pathogens that lack a sexual cycle and that are usually diploids with eight pairs of chromosomes. *C. albicans* spontaneously gives rise to a high frequency of colonial morphology mutants with altered electrophoretic karyotypes, involving one or more of their chromosomes. However, the most frequent changes involve chromosome VIII, which contains the genes coding for ribosomal DNA (rDNA) units. We have used restriction fragment lengths to analyze the number and physical array of the rDNA units on chromosome VIII in four normal clinical strains and seven morphological mutants derived spontaneously from one of the clinical isolates. *Hin*dIII does not cleave the rDNA repeats and liberates the tandem rDNA cluster from each homolog of chromosome VIII as a single fragment, whereas the cleavage at a single site by *Not*I reveals the size of the single rDNA unit. All clinical strains and morphological mutants differed greatly in the number of rDNA units per cluster and per cell. The four clinical isolates differed additionally among themselves by the size of the single rDNA unit. For a total of 25 chromosome VIII homologs in a total of 11 strains considered, the variability of chromosome VIII was exclusively due to the length of rDNA clusters (or the number of rDNA units) in ~92% of the cases, whereas the others involved other rearrangements of chromosome VIII. Only slight variations in the number of rDNA units were observed among 10 random *C. albicans* subclones and 10 random *Saccharomyces cerevisiae* subclones grown for a prolonged time at 22°C. However, when grown faster at optimal temperatures of 37 and 30°C, respectively, both fungi accumulated higher numbers of rDNA units, suggesting that this condition is selected for in rapidly growing cells. The morphological mutants, in comparison with the *C. albicans* subclones, contained a markedly wider distribution of the number of rDNA units, suggesting that a distinct process may be involved in altering the number of rDNA units in these mutants.

Naturally occurring strains of *Candida albicans* are commonly diploid, with eight pairs of chromosomes (11, 19, 20, 26), although independent clinical isolates reveal different electrophoretic karyotypes (1, 7, 13, 14, 18) and aneuploidy (18). We previously demonstrated that standard laboratory strains of *C. albicans* spontaneously give rise to a high frequency of approximately 1.4% of colonial morphology mutants (denoted herein as morphological mutants), which were associated with different single and multiple chromosomal rearrangements (20). The rearrangements constituted changes of chromosome lengths and ploidy and, more rarely, translocations. Furthermore, many of the morphological mutants were unstable, continuously producing additional abnormalities after subcloning (18, 20). We particularly noted that the most frequent spontaneous changes involved both homologs of the long chromosome VIII (also denoted chromosome G [11], chromosome 2 [7], and chromosome R [26]), which carries genes coding for ribosomal DNA (rDNA) units (18); similarly, the electrophoretic position of chromosome VIII is often different among normal laboratory strains and clinical isolates (1, 8, 18, 26).

In this study, we have investigated the physical constitution of chromosome VIII in various *C. albicans* strains, including seven morphological mutants (m3, m7, m16, m17, m20, m500, and m500-3) derived spontaneously from strain 3153A, and including the four commonly used laboratory strains, 3153A (the parental strain), C9, FC18, and WO-1. It was reasonable to assume that the differences in the lengths of chromosome VIII could be due to the differences in the number of rDNA units. To investigate the number of rDNA units, we have taken advantage of the fact that each rDNA cluster is liberated as a single *Hin*dIII fragment because there are no *Hin*dIII sites within the rDNA unit (26). On the other hand, the size of the single rDNA unit can be revealed by cleavage at a single *Not*I site (8). Furthermore, the general paucity of *Not*I sites results in the generation of large chromosome VIII fragments lacking the rDNA units. Therefore, hybridization studies can also reveal the constitution of the remaining chromosome VIII fragments. In addition, we have investigated the variability of rDNA units in random subclones of both *C. albicans* and *Saccharomyces cerevisiae* grown under different physiological conditions.

We have demonstrated that variability of chromosomes carrying rDNA in *C. albicans* and *S. cerevisiae* strains was almost exclusively due to changes in the number of rDNA units and that such changes occurred among subclones after different conditions of growth. On the other hand, larger variations in the number of rDNA units were observed in morphological mutants and different normal laboratory strains of *C. albicans*. We have attributed the variation of rDNA units among normal clinical isolates to the same process that produces variation in morphological mutants. In addition, another source of variabil-

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ity was differences in the size of single rDNA units found in normal laboratory strains.

MATERIALS AND METHODS

Strains, nomenclature, and media. The four *C. albicans* strains, 3153A, WO-1, C9, and FC18, are clinical isolates that have been maintained under laboratory conditions and that have been used in numerous studies (see, for example, references 18 and 20); these are denoted normal laboratory strains in this paper. It should be noted that variants of clinical isolates can arise during laboratory maintenance (see references 18 and 19).

The mutants with various altered colony morphologies, m3, m7, m16, m17, m20, m500, and m500-3, were derived spontaneously from 3153A (18, 20) and are referred to as morphological mutants in this paper.

The random subclones of 3153A are denoted subclones, *C. albicans* subclones, or C.a. subclones. The 40 subclones of *C. albicans* 3153A, denoted C.a.1 to C.a.30, C.a.31F to C.a.35F, and C.a.31S to C.a.35S, were prepared as described below.

D273-10B (ATCC 24657) is a commonly used haploid strain of *S. cerevisiae*. The 20 subclones of *S. cerevisiae* D273-10B, denoted S.c.1 to S.c.20, also were prepared as described below.

The common laboratory strain 924h$^-$ of *Schizosaccharomyces pombe* (kindly provided by P. R. Reynolds, University of Rochester) and strain 867 (also denoted 3482-16-1) of *S. cerevisiae* (25) were used as chromosome size markers.

YPD medium (22) was used to grow liquid cultures of cells as well as to prepare subclones. LBC medium (12) was used to test the colonial morphologies of *C. albicans* subclones and to provide cultures of slowly grown cells.

Maintenance and growth of strains. We previously reported that the clinical isolate 3153A spontaneously produces altered electrokaryotypes at a high frequency of 1.4% (20) as well as highly unstable mutants (18) that often display mixed populations of karyotypes (18, 19). Because of instabilities, attempts were made to use populations of cells that represented a particular strain; strains were preserved in glycerol at −70°C, and the general practice of subcloning was avoided.

Subclones of 3153A, grown under various conditions, were maintained as described below.

S. cerevisiae strains were preserved as frozen stock and subsequently used for chromosomal preparations similarly to *C. albicans.*

Growth of independent subclones of *C. albicans* 3153A. Slow growth of the independent subclones C.a.1 to C.a.10 was achieved by streaking the stock culture of the parent strain 3153A on LBC medium for isolated colonies and incubating the plates for 4 weeks at 22°C and subsequently restreaking single colonies and incubating the plates for an additional 4 weeks. Similarly, after the fast-grown cultures C.a.31F to C.a.35F completed their fast growth regime, slow growth of the independent subclones C.a.31S to C.a.35S was achieved by streaking the fast-grown cultures on LBC medium, as described above for the C.a.1 to C.a.10 subclones.

Fast growth of subclones C.a.21 to C.a.30 was achieved in liquid YPD by incubating the strains at 37°C for 2 weeks. Exponential growth was maintained by reinoculating each culture daily in 15 ml of medium and incubating the tubes on a rotating shaker. Fast growth of C.a.31F to C.a.35F was achieved by incubating the fresh-daily streaks on YPD plates at 40°C for 2 weeks.

Independent subclones C.a.11 to C.a.20 were obtained by streaking the frozen stock culture on a YPD plate for independent colonies. The cultures were grown overnight at 30°C in liquid YPD, and the cells were used to prepare chromosomal DNA.

Growth of independent subclones of *S. cerevisiae* D273-10B. Independent subclones from *S. cerevisiae* were also subjected to slow and fast growth regimes. An independent colony was divided in two and then subjected to two different sets of growth conditions. S.c.1 to S.c.10 of D273-10B were grown in 15 ml of YPD at 22°C for 3 weeks, whereas S.c.11 to S.c.20 of D273-10B were grown in 15 ml of YPD at 30°C for 10 days. Exponential growth was maintained by daily transfers, as described above.

The rates of growths. The following doubling times for the conditions stated were determined by cell counts of cultures grown as described above: *C. albicans* 3135A grown at 22°C in LBC medium, 51 h; grown at 37°C in YPD medium, 1.02 h; and grown at 40°C in YPD medium, 0.90 h; or *S. cerevisiae* D273-10B grown at 22°C in YPD medium, 1.35 h; and grown at 30°C in YPD medium, 1.02 h.

Examination of colonial morphology forms of *C. albicans*. The forms of colonies from 3153A subclones C.a.1 to C.a.30 were examined by using our standard conditions, growth on LBC medium for 4 weeks at 22°C, with approximately 10 colonies per plate (20).

Preparations of chromosomes. For the preparation of DNA from *C. albicans* strains, a heavy inoculum was transferred from the preserved frozen stock to liquid YPD medium and incubated overnight at 30°C. Cells from the YPD cultures were transferred to fresh YPD medium, grown to a titer of not more than 4×10^7 cells per ml at 30°C, harvested, and used to prepare DNA.

Chromosome-size DNA was prepared as described by Carle and Olson (2) by using GIBCO-BRL Life Technologies, Inc., plug molds. Agarose beads containing chromosome-size DNA were prepared as described by Overhauser and Radic (16).

DNA preparation. Total genomic DNAs (minipreparations) were prepared according to the method of Sambrook et al. (21).

Restriction digests. Both *Hin*dIII (Boehringer and Mannheim) and *Not*I (United States Biochemical Corp.) restriction endonucleases were used to digest chromosome-size DNA of *C. albicans* embedded in agarose beads according to the recommendation of Overhauser and Radic (16). *Not*I was also used to digest total genomic DNA by standard procedures.

S. cerevisiae intact chromosomes embedded in agarose beads were digested by *Sal*I and *Mlu*I (United States Biochemical Corp.) by using the same protocol.

Size markers and the measurement of DNA lengths. The top four bands of *S. cerevisiae* 867, which correspond to chromosomes XII, IV, VII plus XV (a doublet), and XIII plus XVI (a doublet) were estimated with a calibration curve of electrophoretic positions by using the top four bands of a commercial strain of *S. cerevisiae* YNN295, provided by Bio-Rad in the form of intact chromosomes embedded in agarose. The remaining bands were estimated directly with a λ ladder, provided by New England BioLabs, which allowed a more precise estimate of the sizes of chromosomes from our reference strain *S. cerevisiae* 867, instead of using the sizes reported by others for similar strains of *S. cerevisiae.*

The longest chromosome of *S. cerevisiae* 867, chromosome XII of 2.2 Mb (this investigation), and the shortest chromosome of *S. pombe* of 3.5 Mb (4) were used as markers for the group of the three largest chromosomes of *C. albicans*, the so-called top group, when separated by pulsed-field gel electrophoresis (PFGE).

The sizes of the chromosome VIII homologs of the parental *C. albicans* 3153 and one of the mutants, m500-3, were

estimated with calibration curves of the electrophoretic positions by using the smallest *S. pombe* chromosome of 3.5 Mb (4) and the top three bands of *S. cerevisiae* 867, with sizes of 2.2, 1.7, and 1.1 Mb (this investigation).

λ-ladder DNAs (Bio-Rad) served as size markers for the clusters of rDNA units of *C. albicans* and ranged from 48.5 to 1,018.5 kb. *S. cerevisiae* 867 chromosome sizes also were used for the same purpose (see Results).

The sizes of the *C. albicans* rDNA units were estimated with a calibration curve of electrophoretic positions by using the following λ DNA restriction fragments: low-range PFGE markers (Bio-Rad) containing 6.56-, 9.42-, 23.10-, and 48.50-kb *Hin*dIII fragments; a 12.43-kb *Stu*I fragment; and a 11.50-kb *Pst*I fragment.

Similarly, the sizes of the rDNA cluster of *S. cerevisiae* D273-10B and its two subclones were estimated by the electrophoretic positions with a calibration curve based on the values of *S. cerevisiae* 867 chromosomes.

Estimates of the relative sizes of *C. albicans* chromosome VIII and *S. cerevisiae* chromosome XII. The relative sizes of chromosome VIII in *C. albicans* subclones and of chromosome XII in *S. cerevisiae* subclones grown under different physiological conditions were estimated from the ratios of the electrophoretic mobilities of the parental strains and their derivatives.

Electrophoretic protocols. A variety of electrophoretic procedures were used to obtain optimal separations of long chromosomes and their restriction fragments for both *C. albicans* and *S. cerevisiae*, and these required the use of the CHEF-DRII (contour-clamped homogeneous electric fields) system (Bio-Rad Laboratories) (3) and conventional gel systems. OFAGE (orthogonal field alternation gel electrophoresis) (2) was used to revise the sizes of chromosomes in *S. cerevisiae* 867.

(i) Separations of *C. albicans* chromosomes. In our earlier work, the eight *C. albicans* chromosomes have been conventionally assigned to top (T), middle (M), and bottom (B) groups; the chromosomes in each group can be optimally separated in OFAGE by different electrophoretic conditions (see, for example, references 18 and 20). In this work, nevertheless, we used CHEF to separate the three longest chromosomes, or so-called top group, because it allowed the examination of as many as 15 samples on one gel. The conditions used were as follows: a constant 2.4 V/cm, approximately 50 mA, a 20-min pulse time, 0.5× Tris-borate-EDTA (TBE) cooling buffer at 10°C (21), 1% agarose, and an 82-h running time.

(ii) Separations of *C. albicans* rDNA clusters. The CHEF conditions to separate the clusters of *C. albicans* rDNA units obtained by *Hin*dIII digestion of DNA embedded in agarose consisted of the following: a constant 4.5 V/cm, approximately 90 mA, a 50- to 100-s ramp pulse time, 0.5× TBE cooling buffer at 14°C, 1% agarose, and a 24-h running time.

(iii) Separations of *C. albicans* rDNA units. The CHEF conditions to separate the *C. albicans* single rDNA units obtained by *Not*I digestion of DNA embedded in agarose consisted of the following: a constant 2.4 V/cm, approximately 40 mA, a 20-min pulse time for the first 60 h of the run and a 5-min pulse time for the next 30 h, 0.5× TBE buffer at 13°C, and 1% agarose. In addition, rDNA units obtained by *Not*I digestion of DNA solutions were also separated with CHEF under the following conditions: a constant 6.0 V/cm, approximately 110 mA, a 1-s pulse time; 0.5× TBE cooling buffer at 10°C, 1% agarose, and a 20-h running time. Care was taken to load appropriate amounts of DNA (approximately 300 ng) so that the bands of interest would not be overloaded. Conventional gel electrophoresis to separate *Not*I-generated rDNA units was performed at 30 V on 0.6% agarose with 0.5× TBE buffer, and a 17-h running time.

(iv) Separations of *S. cerevisiae* chromosomes and rDNA clusters. The CHEF conditions to separate the four longest *S. cerevisiae* chromosomes, including chromosome XII, which contains the rDNA units, and the liberated rDNA clusters, were as follows: a constant 2.4 V/cm, 40 mA, a 20-min pulse time, and 0.5× TBE at 10°C for 48 h in 0.8% Megarose gel, followed by 3.0 V/cm, 70 mA, and a 300-s pulse time for 40 h.

In order to optimally separate the remaining chromosomes, along with λ-ladder size markers, we used OFAGE and applied the following conditions: a constant 9 V/cm, approximately 115 mA, a 70-s pulse time, and 0.5× TBE at 10°C for 38 h in 1.5% agarose gel.

Hybridization probes and protocols. WOL-25 (rDNA) and *SOR9* probes were previously used in our studies (18). The WOL-25 fragment was removed from the Bluescript plasmid by double digestion with *Hin*dIII and *Xho*I. The *ADE1* probe was kindly provided by S. Scherer (University of Minnesota). Two plasmids containing *MGL1* and *CDC10* fragments were kindly provided by B. Magee (University of Minnesota). All six probes mentioned above were used as markers for *C. albicans* chromosome VIII, including WOL-25 (rDNA), which was specifically used to identify the rDNA clusters and units. *S. cerevisiae* rDNA cloned into plasmid pAA3 was used as a marker of chromosome XII as well as of rDNA clusters.

The probes were labeled with either [α-^{32}P]ATP by the random priming method (5) or the Plex luminescent kit (Millipore) by the chemiluminescent detection method. Southern blot hybridizations and autoradiograms were carried out by standard methods (21) or as recommended by the manufacturer.

RESULTS

Revised lengths of chromosomes of the reference strain *S. cerevisiae* 867. The chromosomes of our usual reference strain 867 were separated by both CHEF (3) and OFAGE (2) under conditions which favored the larger sizes. The reference strain YNN295 of *S. cerevisiae*, provided by Bio-Rad with its suggested lengths of chromosomes, was concomitantly run on the same gel on which the four largest chromosomes were well separated (data not shown). The four largest bands of strain 867 were compared, and their lengths were estimated (as described in Materials and Methods) as 2.20 Mb, approximately 1.70 Mb, 1.12 Mb, and 1.05 Mb (Fig. 1). The remaining chromosomes were separated, along with a λ ladder, with OFAGE under different conditions, and their sizes were estimated directly (see Materials and Methods for types of PFGE procedures and the running conditions).

The lengths of rDNA clusters in morphological mutants and laboratory strains of *C. albicans*. *Hin*dIII digestion of agarose beads containing intact chromosomes, as presented in Fig. 2A, liberated long fragments from the seven morphological mutants derived from 3153A (Fig. 2B) and the four normal laboratory strains (Fig. 3A and B). These fragments were shown to constitute the tandem rDNA units by hybridization to the rDNA probe, as shown in Fig. 2C and 3C. Because the rDNA probe hybridized to only the long fragments and not to any of the smaller *Hin*dIII fragments, we can conclude that all rDNA units reside on chromosome VIII in a tandem array, a result that is consistent with previous reports that *Hin*dIII does not cleave within rDNA units (8, 26). On the other hand, hybridization of the same filters with different chromosome VIII probes, *SOR9*, *ADE1*, *CDC10*, and *MGL1*, revealed several short bands at the bottom of the gel but no long

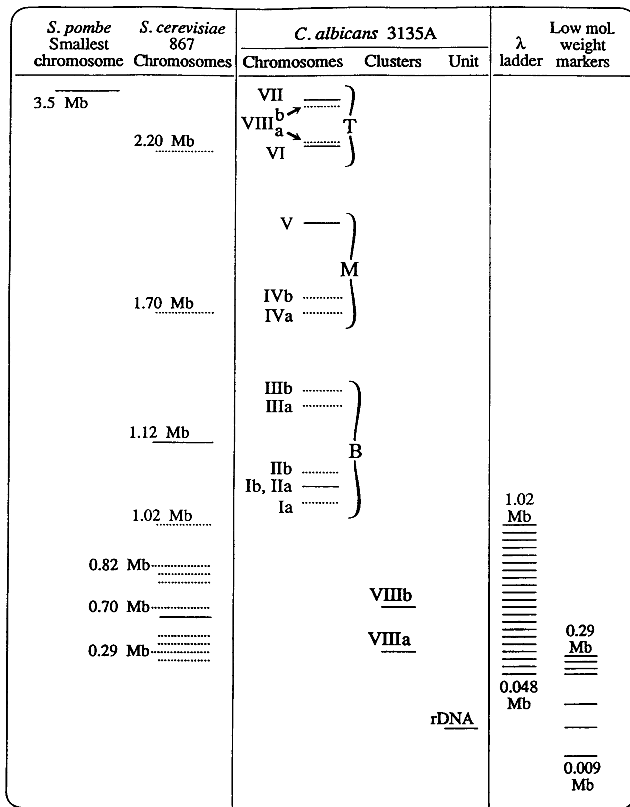

FIG. 1. Schematic representation of the electrophoretic karyotype of *C. albicans* 3153A, tandem rDNA clusters from 3153A, a single rDNA unit, and markers. The three groups of *C. albicans* chromosomes, bottom (B), middle (M), and top (T), represent three groups of sizes which can be resolved by three different conditions of PFGE (see, for example, reference 18). A dotted line denotes a single chromosome, whereas a continuous line denotes the comigration of either a pair of homologous chromosomes or (for the single case of chromosomes Ia and IIb) two nonhomologous chromosomes. Assignments to groups of *C. albicans* chromosomes I to VIII are indicated in the middle column (18, 20). *S. pombe* 924h$^-$, *S. cerevisiae* 867, λ ladders, and low weight markers were used for size markers (see Materials and Methods).

fragments, thus corroborating the notion that the long *Hin*dIII fragment contains only rDNA units (data not shown). The evidence that each cluster was derived from a separate homolog of chromosome VIII is presented in the following section.

The lengths of rDNA clusters from all strains, except for WO-1, were estimated directly by the λ-ladder size markers (see Materials and Methods) as shown in Fig. 2B for the morphological mutants. A very long fragment from WO-1, which was beyond λ-ladder range, was estimated in reference to the longest *S. cerevisiae* 867 chromosome, as shown in Fig. 3B. The sizes of all these rDNA clusters are summarized in Table 1.

Comparisons between the patterns of intact chromosomes VIII of *C. albicans* strains and the rDNA clusters. One of the important questions addressed in this work was what accounts for the differences in the sizes of chromosome VIII observed among the normal laboratory strains and morphological mutants of *C. albicans*. Simple comparisons of the patterns of chromosomes and rDNA clusters shown in Fig. 2A and B and 3A and B revealed that relative sizes of chromosomes VIII from all but one mutant, m500-3, and one normal laboratory strain, C9, accurately corresponded to the relative sizes of rDNA clusters (as schematically presented in Fig. 1) for strain 3153A. Calculations for the parental 3153A strain support these visual estimates. The difference in sizes between two homologs of chromosome VIII, which were estimated as 2,520 kb for the shorter VIIIa homolog and 3,020 kb for the longer VIIIb homolog (see Materials and Methods), consisted of approximately 500 kb. The size difference between the corresponding clusters (728 and 291 kb) is approximately 440 kb, which is in remarkably close agreement, considering inaccuracies of the measurements of the electrophoretic positions and the calibration curve. This result strongly suggests that each homolog of chromosome VIII carries one rDNA cluster and that variation in the length of chromosome VIII is usually defined by variation in the length of its rDNA cluster.

Alterations of non-rDNA sequences of chromosome VIII. The morphological mutant m500-3, which was derived from the unstable morphological mutant m500, may be trisomic for chromosome VIII, as suggested by the three hybridization signals produced with the rDNA probe (18). However, it has not been excluded that, after duplication, the rDNA cluster was transposed or translocated to an other chromosome, resulting in a third rDNA cluster within a chromosome the size of the normal chromosome V.

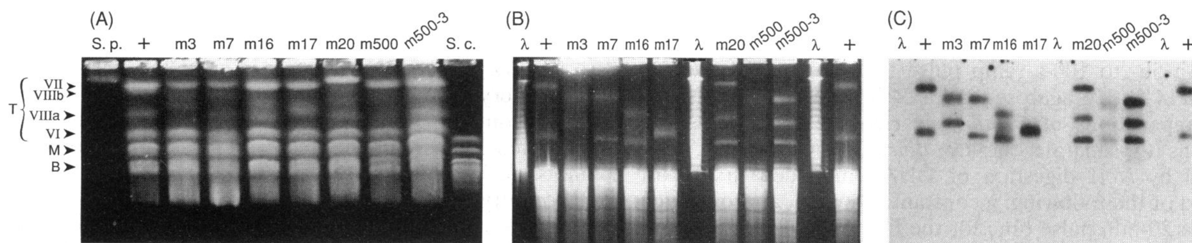

FIG. 2. Variation of the sizes of chromosome VIII and the corresponding sizes of the rDNA clusters in 3153A (+) and seven morphological mutants as revealed with separations by CHEF of DNA preparations in agarose beads. (A) CHEF operating conditions were maximized to reveal the three longest chromosomes VI, VII, and VIIIa and VIIIb (top group [T]) (see Materials and Methods). The remaining chromosomes (middle [M] and bottom [B] groups) were compressed but can be separated well by other operating conditions (18). The longest chromosome of *S. cerevisiae* 867 (S.c.), which is about 2.2 Mb and runs slightly in front of *C. albicans* chromosome VI, and the shortest chromosome of *S. pombe* 924h$^-$ (S.p.), which is about 3 Mb, were used as markers of the top group. (B) *Hin*dIII digests of 3153A and seven mutants, revealing the tandem rDNA clusters, each of which was liberated from a corresponding homolog of chromosome VIII. The bulk of the *Hin*dIII fragments ran in front of the rDNA clusters. λ-ladder DNA (λ) was used as a size reference in three of the lanes. (C) Hybridization of the rDNA probe to the *Hin*dIII fragments shown in B.

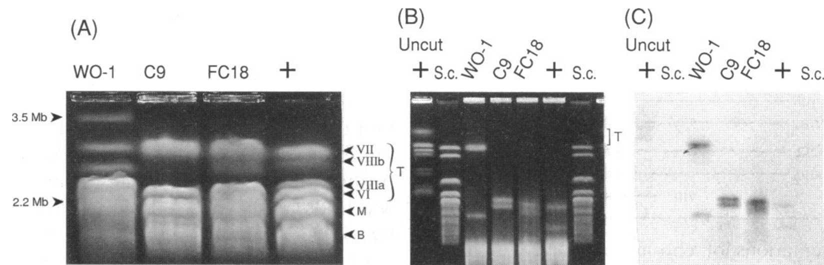

FIG. 3. Variation of sizes of chromosome VIII (A) and rDNA clusters (B and C) from normal laboratory strains 3153A (+), WO-1, C9, and FC18. (A) Separation of the three longest chromosomes VI, VII, and VIIIa and VIIIb (top group [T] described in the legend of Fig. 1A). (B) *Hin*dIII digests of DNA samples embedded in agarose beads, revealing the tandem rDNA clusters. The bulk of *Hin*dIII fragments ran in front of the rDNA clusters. *S. cerevisiae* 867 (S.c.) chromosomes were used as size references for the WO-1 strain. The other strain clusters were estimated by the λ ladder (see Results). The uncut chromosomes of 3153A are shown for comparison. The three largest chromosomes (T) are represented here by only two bands because of inappropriate running conditions. (C) Hybridization of the *Hin*dIII fragments in B with the rDNA probe.

Two homologs of chromosome VIII ran within the usual range (between 2.2 and 3.5 Mb, as defined by the *S. cerevisiae* and *S. pombe* marker chromosomes [Fig. 2A]), whereas the third is greatly reduced in size and comigrates with chromosome V (18), which is approximately 1.95 Mb and which clearly increased the ethidium bromide staining of the corresponding band on the gel. Nevertheless, the three long fragments liberated by *Hin*dIII, which correspond to rDNA clusters from these three homologs, are 242, 412, and 582 kb in length and lie within the usual size range (Fig. 2B and C and Table 1); this result implies that the size of the shortened homolog is not dependent on the number of rDNA units. The rDNA cluster released from the shortest homolog had an intermediate length of 412 kb, as can be deduced by comparing the patterns

TABLE 1. Variations in the number of rDNA units in morphological mutants and laboratory strains of *C. albicans* and in subclones of *S. cerevisiae*

Organism	Strain no.	Size in kb[a]		Approx. no. of rDNA units	
		rDNA unit	Cluster	Per cluster	Total
C. albicans laboratory strains	C9	12.2	728	60	
			873	72	
	FC18	12.2	630	52	
			776	64	
	WO-1[b]	11.5	534	46	222
		12.5	2,200	176	
	3153A	11.6	291	25	87
			728	62	
C. albicans morphological mutants	m3	11.6	388	33	85
			606	52	
	m7	11.6	267	23	75
			606	52	
	m16	11.6	242	21	63
			485	42	
	m17	11.6	291[c]	25[c]	~56
			364[c]	31[c]	
	m20	11.6	242	21	
			461	40	128
			776	67	
	m500	11.6	242	21	
			412	35	106
			582	50	
	m500-3	11.6	242	21	
			412	35	106
			582	50	
S. cerevisiae laboratory strain	D273-10B	9.1[d]	1,595	175	175
S. cerevisiae subclones	S.c.13	9.1[d]	2,110	231	231
	S.c.15	9.1[d]	1,930	212	212

[a] Sizes of *Not*I and *Hin*dIII fragments of *C. albicans*, encompassing, respectively, a single rDNA unit and an rDNA cluster; and size of *Sal*I fragments of *S. cerevisiae* encompassing an rDNA cluster.
[b] The longer rDNA unit (12.5 kb) is arbitrarily assigned to the longer rDNA cluster (2,200 kb).
[c] A heterogeneous population of cells gave rise to a range of sizes of which two extreme values are presented.
[d] The rDNA unit is assumed to be 9.1 kb, the value previously determined for typical *S. cerevisiae* strains (27).

Strain number	Length in kb: Total	Non-rDNA	rDNA	No. of rDNA units	SOR9, ADE1, MGL1, CDC10 / rDNA
3135A	3020	2300	728	62	VIIIb
	2520	2230	291	25	VIIIa
m500-3	2870	2290	582	50	VIIIb
	2520	2280	242	21	VIIIa
	1950	1540	412	35	VIIIc

FIG. 4. Schematic representations of chromosomes VIII in the normal strain 3153A and in the mutant m500-3. The rDNA clusters are denoted by filled boxes. The lengths of chromosome VIII homologs were estimated by calibration curve (see Materials and Methods). The chromosome VIII probes *SOR9*, *ADE1*, *MGL1*, and *CDC10* hybridized to both homologs VIIIa and VIIIb in the normal 3153A strain and to the corresponding two larger homologs in the m500-3 morphological mutant but not to the smallest third chromosome. The third chromosome (VIIIc) could have arisen by a deletion within one duplicated homolog of chromosome VIII or by a transposition or translocation of the rDNA cluster to a duplicated homolog of an other chromosome.

in Fig. 3A and B and as schematically presented in Fig. 4. The hybridization of m500-3 separated chromosomes with the probes *SOR9*, *MGL1*, *CDC10*, and *ADE1* of chromosome VIII (data not shown) demonstrated that this homolog lacks all of these markers, thus revealing a large deletion outside of the cluster, presumably on the other arm of chromosome VIII. By assuming that the chromosome VIII length minus the rDNA cluster is normally 2,260 kb (the average between two homologs of 3135A with the corresponding lengths of the clusters subtracted [see the description above]) the size of the deletion can be roughly estimated as approximately 725 kb, i.e., the difference between the remaining portion outside of the rDNA clusters. The lengths of chromosome VIII homologs from m500-3 and the parent strain 3153A are presented in Fig. 4.

A former clinical isolate, at present the common laboratory strain C9, also probably has a change outside of the cluster similar to that of m500-3 but of a different nature. Despite the two clusters of rDNA having different lengths (as shown in Fig. 3B), both homologs of chromosome VIII ran together, and comigrated with chromosome VII (as seen in Fig. 3A); thus, one of them contains a compensatory change that results in equal sizes. The comigration of homologous chromosomes VIII of C9 could not be attributed to the relatively small difference (approximately 145 kb [Table 1]) between the clusters and, consequently, the chromosomes. Another strain, FC18, has practically the same difference between the clusters (150 kb), although the two homologs are clearly separated and were previously identified by probing (18).

***Not*I fragments of chromosome VIII from *C. albicans* strains.** Each rDNA unit from *C. albicans* strains contains one *Not*I site (8) which is a rare 8-bp recognition site. Intact chromosomes prepared in agarose beads were digested with *Not*I, the fragments were separated by CHEF (Fig. 5A), and the corresponding filter was hybridized to rDNA and other chromosome VIII probes, *SOR9*, *MGL1*, *CDC10*, and *ADE1* (see Materials and Methods). As shown by Fig. 5B, the short *Not*I fragment hybridized only to the rDNA probe, whereas the other larger *Not*I fragments hybridized to the other chromosome VIII probes but not the rDNA probe (see Fig. 5C for hybridization to the *SOR9* probe). In addition, one partial *Not*I fragment from both 3153A and m3 was uncovered, as shown in Fig. 5C, lanes 3 and 4, respectively). This band was approximately 2,100 kb and hybridized to the *ADE1*, *CDC10*, and *SOR9* probes. This result demonstrated that the 1,410- and ~700-kb *Not*I fragments are adjacent to each other.

(i) Sizes of large *Not*I non-rDNA segments of chromosome VIII from *C. albicans*. Hybridization studies with markers of chromosome VIII revealed the following approximate sizes of *Not*I fragments associated with the probes: 1,410 kb with *ADE1* and *CDC10*, ~700 kb with *SOR9*, ~200 kb with *MGL1*, and 12 kb with WOL-25 (rDNA). The sum of these *Not*I fragments is approximately 2.3 Mb, which corresponds to the size of chromosome VIII without the rDNA cluster (see the discussion above and Fig. 4).

(ii) Sizes of single rDNA units of *C. albicans*. In order to more precisely estimate the size of the rDNA unit, total genomic DNAs from all *C. albicans* strains were digested with *Not*I, and the fragments were first separated by conventional gel electrophoresis. Subsequently, specially developed conditions for estimating sizes in the 9.4- to 23.1-kb range with the CHEF system were used to produce a better resolution (see Materials and Methods) (Fig. 6A). The positions of the *Not*I fragments were more precisely estimated by loading relatively small amounts of digested DNA, about 300 ng per well. As expected, the *Not*I fragments from the four normal laboratory strains hybridized to the rDNA probe (Fig. 6B), similarly to the results shown in Fig. 5B. Autoradiograms were made by using either random priming or chemiluminescent methods. By using a calibration curve as described in Materials and Methods, the sizes of the rDNA units were estimated. Strains C9 and FC18 yielded rDNA units equal to 12.2 kb, whereas strain 3153A and all of its spontaneously derived progenies yielded an rDNA unit of 11.6 kb (data not shown). On the other hand, WO-1 had two rDNA units of 11.5 and 12.5 kb. The lengths of the rDNA units and approximate numbers of units in the clusters are presented in Table 1.

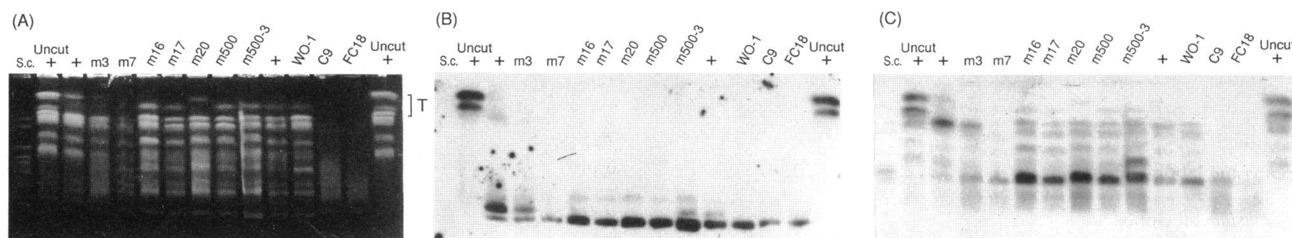

FIG. 5. CHEF separation of *Not*I fragments from all *C. albicans* strains for which DNA samples were prepared in agarose beads. *Not*I digests (A) and hybridization with the rDNA (B) and *SOR9* (C) probes. *Not*I-digested and uncut chromosomes of *C. albicans* 3153A (+) are shown for comparison. The three largest chromosomes (top group [T]) are represented here by only two bands because of inappropriate running conditions. The longest chromosome of *S. cerevisiae* 867 (S.c.) runs slightly in front of T. Partial *Not*I fragments can be seen in lanes 3 (+) and 4 (m3) of panels B and C.

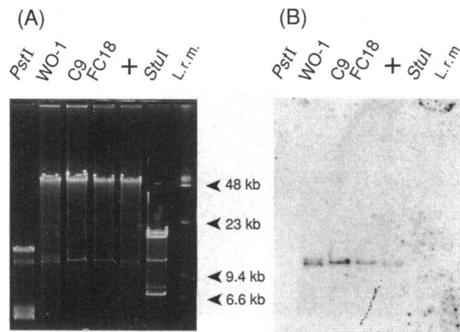

FIG. 6. Variation of sizes of the rDNA unit in natural *C. albicans* WO-1, C9, FC18, and 3153A (+). (A) *Not*I digests of genomic DNA preparations, revealing the size of the single rDNA unit. Size references include λ DNA digested with *Pst*I and *Stu*I as well as long-range PFGE markers (L.r.m.) (Bio-Rad). (B) Hybridization of the rDNA probe to the *Not*I fragments shown in panel A.

The variability of chromosome VIII from *C. albicans* subclones grown under different conditions. Because some of the morphological mutants have diminished growth rates, we have investigated the possible relationship between the number of rDNA units and the physiological states of the cells. The origins of and relationships between subclones used here are presented in Fig. 7. Two groups, each consisting of 10 independent subclones from 3153A, were subjected to two different sets of growth conditions. Slow and prolonged growth was achieved by growing the subclones C.a.1 to C.a.10 on solid LBC medium at 22°C for 2 months, conditions that were originally used in detecting the morphological mutants (20). Faster growth was achieved by growing C.a.21 to C.a.30 at the optimum temperature of 37°C in liquid YPD medium for a relatively long period of 2 weeks. The sizes of chromosome

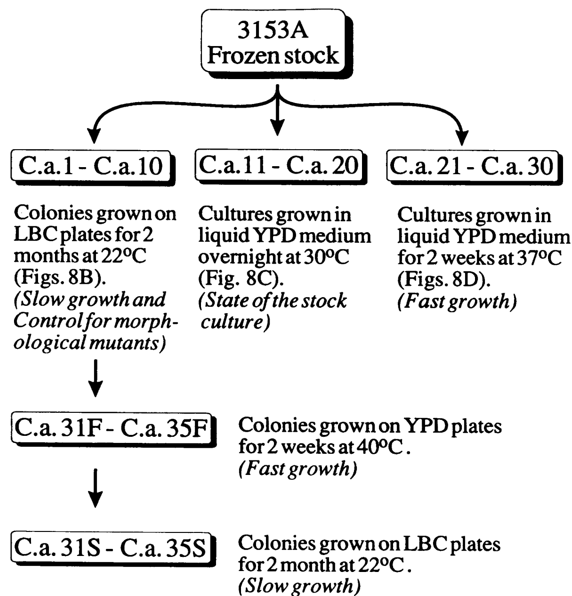

FIG. 7. Schematic representation of origins of and relationships between *C. albicans* 3153A subclones. The frozen stock of *C. albicans* 3153A was used to prepare the various subclones, as described in detail in Methods and Materials.

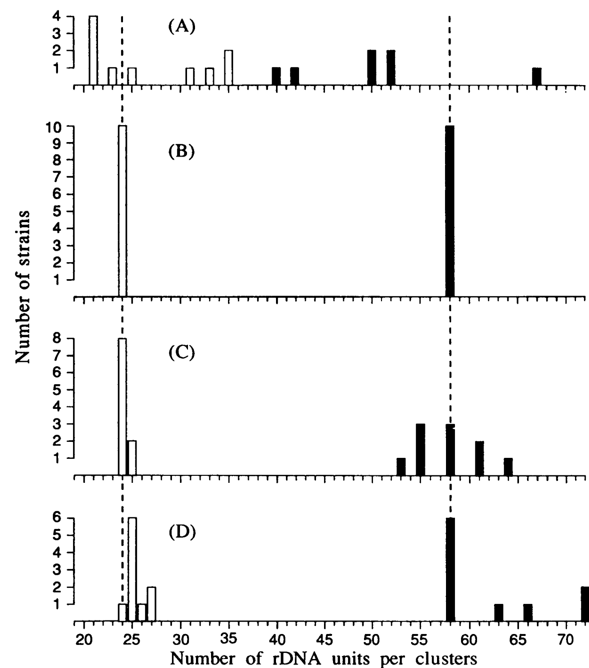

FIG. 8. Schematic representation of the variability of the number of rDNA units in different strains derived from *C. albicans* 3153A. (A) morphological mutants m3, m7, m16, m17, m20, m500, and m500-3 after prolonged slow growth at 22°C on LBC medium as previously described (20); (B) random subclones C.a.1 to C.a.10 grown slowly as described for panel A; (C) subclones C.a.11 to C.a.20 grown at 30°C on YPD medium overnight; (D) random subclones C.a.21 to C.a.30 grown at 37°C for a prolonged period (see Materials and Methods). The results shown in A, B, and D were derived from patterns shown in Fig. 2B. The numbers of subclones with the designated number of rDNA units in homolog VIIIa (open bars) and VIIIb (filled bars) are indicated.

VIII homologs, and consequently the number of rDNA units, were roughly determined by the positions of the bands (data not shown). The distributions in the number of rDNA units per cluster of the morphological mutants and slowly grown subclones, which serve as controls (Fig. 7), are presented for comparison in Fig. 8A and B, respectively. These results clearly reveal a wider distribution of rDNA units per cluster in the group of morphological mutants.

Comparisons of both slow-growth C.a.1 to C.a.10 and fast-growth C.a.21 to C.a.30 and of the control group of C.a.11 to C.a.20 (see Materials and Methods) can be made from the results presented in Fig. 8B, D, and C, respectively. As shown on the histogram, each homolog of chromosome VIIIa or VIIIb can vary slightly in a normal population of cells prepared from the frozen stock culture (Fig. 8C). The prolonged fast growth favors the proliferation of cells with the increased amount of rDNA units (Fig. 8D), while prolonged slow growth, rather, maintains certain types of cells, all with equal numbers of rDNA units in their homologs (Fig. 8B).

In order to corroborate that the representative length of rDNA clusters in cell populations can be shifted by changes in the rate of growth, we used slowly pregrown subclones C.a.1 to C.a.10, which contained fairly homogenous chromosome VIII homologs (see the description above and Fig. 7 and 8B). One half of each final colony generated under these slow-growth conditions was taken for the preparation of chromosomes, which served to reveal the electrophoretic karyotypes of slowly

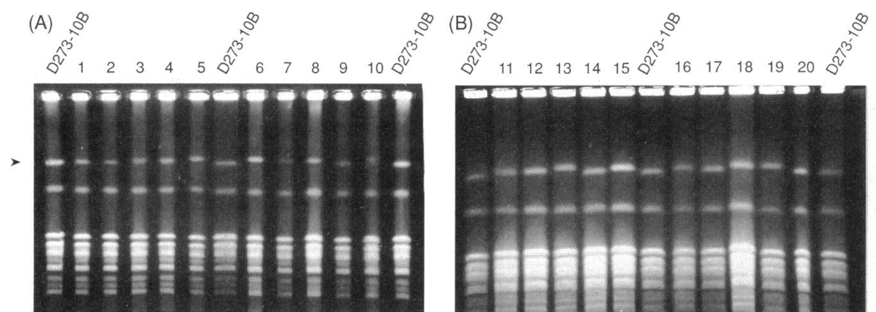

FIG. 9. Variation of the sizes and ploidy of chromosome XII in randomly chosen subclones of *S. cerevisiae* D273-10B grown at 22°C (S.c.1 to S.c.10 [lanes 1 to 10]) (A) and 30°C (S.c.11 to S.c.20 [lanes 11 to 20]) (B), representing, respectively, more slowly and faster-grown cultures (see Materials and Methods). The arrowheads indicate the position of chromosome XII, which contains the rDNA units.

grown cells and as a control for the morphological mutants. The other half was simply streaked onto a YPD plate and grown at elevated temperatures (40°C) for 2 weeks, with daily restreaking on a fresh plate. Thus, the same subclones which previously were grown slowly for a prolonged time were subsequently grown at a faster rate for a prolonged time; these cultures were denoted C.a.31F to C.a.35F (Fig. 7). Subsequently, these cultures were again subjected to the slow-growth conditions on solid LBC medium previously used to generate C.a.1 to C.a.10; these slowly grown strains were denoted C.a.31S to C.a.35S. The growth regimes are also described in Materials and Methods. The karyotypes of C.a.31F to C.a.35F and C.a.31S to C.a.35S (data not shown) revealed that the sizes of the chromosome VIIIa and possible chromosome VIIIb homologs were slightly larger in the faster-grown cells. Although difficult to quantitate, these results established that the number of rDNA units reversibly increases in faster-grown cultures.

Colonial morphology of *C. albicans* subclones. The colonial morphology of all subclones, C.a.1 to C.a.30, used to determine the variability of chromosome VIII after being subjected to various conditions of growth was examined after growth in LBC medium at 22°C by our standard protocol (see Materials and Methods). No obvious differences were observed among C.a.1 to C.a.30 and 3153A strains (data not shown) despite the fact that morphological mutants, with alterations in only chromosome VIII, also had altered colonial forms (18).

Chromosome XII and rDNA clusters of *S. cerevisiae* D273-10B. In order to investigate the generalities of our findings with *C. albicans*, we have examined the variability in the number of rDNA units in *S. cerevisiae*. Two groups of 10 subclones each, S.c.1 to S.c.10 and S.c.11 to S.c.20, respectively, were subjected to slow- and fast-growth conditions for a relatively long time, as described in Materials and Methods. The generation times of slowly and fast-grown cells were approximated at 1.35 and 1.02 h, respectively. At the end of the growth period, each population was subcloned on YPD plates and 10 random subclones from each were chosen for the examination of electrophoretic karyotypes.

The examination of electrophoretic karyotypes of all 20 subclones revealed only a slight variability of chromosome XII among strains grown at 22°C (Fig. 9A). Conversely, 9 of 10 subclones grown at 30°C had variable and increased lengths of chromosome XII (Fig. 9B). One subclone, S.c.10, contained two homologs of XII. The signals after hybridization with the rDNA probe confirmed the positions of chromosome XII and the aneuploidy of S.c.10 (data not shown). The lengths of chromosome XII revealed that all but one subclone grown at the faster rate had higher numbers of rDNA units than the subclones grown at the slower rate.

The restriction endonuclease *Sal*I, which does not cleave within the *S. cerevisiae* rDNA unit (15, 17), was used to liberate the rDNA clusters from chromosomes XII of two chosen subclones, S.c.13 and S.c.15, as shown in Fig. 10A. Signals produced after hybridization with the rDNA probe are shown in Fig. 10B. As discussed above for *C. albicans* rDNA clusters, the rDNA probe hybridized only to the long fragments and not to the other segments, thus establishing that the entire clusters of rDNA repeats were encompassed in the *Sal*I fragment. As shown in Fig. 10, the clusters from both subclones were dramatically increased and were different from each other and from the general population of D273-10B cells. Their lengths were estimated by using a calibration curve (see Materials and Methods) and are presented in Table 1.

DISCUSSION

One major type of genetic instability of *C. albicans* that we have systematically studied (see, for example, references 18 and 19) is the spontaneous occurrence of altered chromosomes (20, 23). We previously attempted the comparative analyses of altered electrophoretic karyotypes in four laboratory strains, 3153A, C9, FC18 and WO-1, and in spontaneous morphological mutants of 3153A (18). The particular interest in chromo-

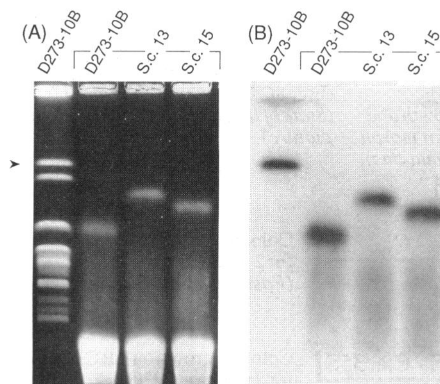

FIG. 10. CHEF separation of tandem rDNA clusters from chosen subclones of *S. cerevisiae* D273-10B. (A) *Sal*I digests of D273-10B and the subclones S.c.13 and S.c.15. (B) Hybridization of the rDNA probe to the clusters shown in A. The arrowhead indicates the position of chromosome XII, which contains the rDNA units.

some VIII, which carries rDNA, initiated from the fact that it varies at least twice as frequently as any other chromosome among spontaneous morphological mutants (18) and that it, along with other chromosomes, consistently varies among natural isolates (1, 18, 26). In addition, a peculiarity of this chromosome is observed when comparing random subclones from the same population of cells (reference 8 and this study). All the subclones had identical electrokaryotypes except for variations in chromosome VIII. Interestingly, in this particular case, colonial morphology of corresponding subclones with altered chromosome VIII did not change, at least when the strains were grown under our standard conditions (reference 8 and this study; data not shown). In contrast, spontaneous morphological mutants have been previously shown to be associated with alterations of various chromosomes, including chromosome VIII (18–20). Because chromosome VIII carries multiple copies of rDNA, the simplest assumption is that its high variability is due to recombinational events within rDNA clusters, as reported for *S. cerevisiae* (24).

Earlier work by Iwaguchi et al. (8) and Wickes et al. (26) with normal laboratory strains of *C. albicans* revealed that the entire rDNA cluster is arranged tandemly with no intervening blocks of non-rDNA and that the clusters can be liberated by restriction enzymes like *Hin*dIII or *Xho*I which do not cleave within the rDNA unit. On the other hand, a restriction enzyme such as *Not*I which cleaves at a single site within rDNA unit, allows the determination of the size of the single unit (8). Similar studies by Pasero and Marilley (17) also demonstrated that restriction enzymes, such as *Xho*I, *Sal*I, *Bam*HI, *Sca*I, and *Pst*I, which do not cleave the rDNA unit of *S. cerevisiae* liberate single fragments of the tandem rDNA cluster, whereas *Mlu*I, for example, cleaves single rDNA units. We have used this approach to compare the lengths of rDNA clusters from normal laboratory strains and spontaneous morphological mutants of *C. albicans* in order to analyze the natural variability of chromosome VIII. We have also analyzed the variability of chromosomes VIII and XII among subclones from normal cultures of *C. albicans* and *S. cerevisiae*, respectively, grown under different conditions in order to understand the dependence between growth rates of cultures and the number of rDNA units per cell.

As summarized in Table 1, morphological mutants and laboratory strains of *C. albicans* varied widely in the lengths of their rDNA clusters and therefore in the number of rDNA units per homolog of chromosome VIII or per cell. Both groups of *C. albicans* strains, the normal clinical isolates and the morphological mutants, were similar in having differences in the total number of rDNAs as high as a factor of 2.5, as can be seen by comparing the morphological mutant m17 with m20 and the natural isolate 3153A with WO-1. As anticipated, the three putative trisomic mutants, m20, m500, and m500-3, contained the highest numbers of rDNA units, from approximately 120 to 150% of the parental value. For simplicity, we can assume that m20, m500, and m500-3 are trisomic for chromosome VIII because these strains have three chromosomes and three *Hin*dIII fragments that hybridize to rDNA. However, the limited number of available chromosomal probes did not permit us to unambiguously determine the nature of the aberrations. It is possible that an entire rDNA cluster was transferred to another duplicated chromosome by a transposition or a translocation. Interestingly, m20 and m500-3 differed in the size of the third chromosome; the third chromosome from m20 remained in the top group, whereas the one from m500-3 comigrated with chromosome V. The lack of hybridization of m500-3 with the *SOR9*, *ADE1*, *CDC10*, and *MGL1* probes is consistent with either an extensive deletion of chromosome VIII or a transposition or translocation.

The wide distribution in the number of rDNA units per cluster or homolog in morphological mutants, as presented in the Fig. 8A histogram (see Fig. 2A and reference 18 for their electrokaryotypes), is in strong contrast to the virtual absence of variability among the control subclones C.a.1 to C.a.10 of the parental strain 3153A (Fig. 8B) grown under the same conditions as the mutants selected (Fig. 7). The variability among morphological mutants is also in contrast to the modest variability that is naturally maintained in a population, as can be seen for C.a.11 to C.a.20 from the Fig. 8C histogram (see also Fig. 7). In these respects, both morphological mutants and laboratory strains are different from subclones and similar to each other. This great variability of chromosome VIII in both groups suggests once again that the natural isolates may have diversified by the same mechanism that generated the morphological mutants, as previously suggested by Rustchenko-Bulgac (18).

One interesting observation presented in Table 1 is that not only can laboratory strains which were originally clinical isolates differ among themselves by the lengths of their rDNA units, but also a single strain can contain two different sizes of rDNA units, as exemplified by WO-1, which contains 11.5- and 12.5-kb units (Fig. 6). We wish to emphasize that the normal strain 3153A and the derived morphological mutants all showed the same size rDNA units (data not shown); in contrast, Iwaguchi et al. (8) reported two different sizes among three subclones of the *C. albicans* strain TCM297. Also, the difference in the ranges of size reported by this group (14.3 to 15.2 kb) and obtained in our work (11.5 to 12.5 kb) was relatively large and could not be easily attributed solely to the differences between strains but may have been due to differences in the methods of measurement; we have attempted to obtain precise measurements by using specially developed optimal conditions for separation and by using size standards in the range of the single rDNA units (see Results).

Several explanations for the variation in the size of rDNA units in *C. albicans* strains can be suggested by analogy with earlier studies of *S. cerevisiae* and other organisms. Jemtland et al. (10) observed length differences due to short deletions and insertions at regular intervals within the nontranscribed spacers of rDNA units in differents strains of *S. cerevisiae*, as well as within one strain. Also, Xiong and Eickbush (28) reported the precise insertion of functional retrotransposons that inactivated a fraction of 28S RNA genes in *Drosophila melanogaster* and *Bombyx mori.*

Laboratory strain WO-1 was previously analyzed by us and found to be a naturally occurring aneuploid with an electrokaryotype markedly different from those of other laboratory strains and with multiple translocations (18, 19). As shown here, since it has a large number of rDNA units and contains two different forms of rDNA unit, WO-1 appears to be more distantly related to the other laboratory strains that were originally clinical isolates. It is tempting to speculate that WO-1 was recently formed by a fusion of two diverse strains: as stressed above, WO-1 is an aneuploid strain, as revealed by bands corresponding to the positions of chromosomes V and IVb (19), and has approximately equal numbers of two different lengths of the rDNA unit (Fig. 6).

Alternatively, WO-1 could be a natural aneuploid, as are many spontaneous morphological mutants (18, 19), with approximately one-half of its rDNA units containing 1-kb insertions or deletions. Such differences in sizes of rDNA units were reported for *D. melanogaster*, which normally has about 50% of

its 28S rDNA genes inactivated by the insertion of retrotransposable elements (9).

A total of 23 of 25 (92%) chromosome VIII homologs of the *C. albicans* strains (Table 1) were altered because of changes in the number of rDNA units, whereas the variability of the remaining two homologs involved other rearrangements. An additional third homolog of m500-3 either had a large deletion (of about 700 kb which included the four genes available as probes, as schematically shown in Fig. 4) or involved a transposition or translocation (as discussed above). In contrast, strain C9 contained a compensatory change of unknown nature which resulted in both chromosome VIII homologs having equal sizes (Fig. 3A), in spite of an obvious size difference of 145 kb between two clusters of rDNA repeats, as shown in Fig. 3C and in Table 1. The portion of chromosome VIII lacking rDNA clusters corresponded to 2.3 Mb, as exemplified for 3153A and m500-3 shown in Fig. 4. Thus, the non-rDNA regions of chromosome VIII undoubtedly are the same size in most strains. This size is remarkably close to the estimate of 2.6 Mb reported by Iwaguchi et al. (8).

This result, together with the analyses of clonal variability of *C. albicans* due to the rDNA shown by Iwaguchi et al. (8), permitted us to use the size of chromosome VIII as an approximate measure of the number of rDNA units per cluster.

In a similar analysis of *S. cerevisiae* chromosome XII, which carries the rDNA repeats, two random subclones of strain D273-10B, S.c.13 and S.c.15, were digested by *Sal*I and shown to vary in the number of rDNA units (Fig. 10) in accordance with the clonal variability in *C. albicans.*

The further analysis of chromosome VIII included the *Not*I fragments of morphological mutants and laboratory strains of *C. albicans* (Fig. 5A). The analysis revealed the following approximate sizes associated with the probes: 1,410 kb with *ADE1* and *CDC10*, ~700 kb with *SOR9* (Fig. 5C), ~200 kb with *MGL1*, and 12 kb with WOL-25 (rDNA) (Fig. 5B). The sum of the three non-rDNA *Not*I fragments was approximately 2.3 Mb, which corresponds to the size of chromosome VIII without the rDNA cluster (see the description above and Fig. 4). This result is in reasonably good agreement with the results obtained by Iwaguchi et al. (8), who reported the following sizes for *Not*I fragments of chromosome VIII from three laboratory strains: 1,350 kb, 870 or 790 kb, 260 kb, and 160 kb. (We uncovered only three non-rDNA fragments with the probes used in our study.) Although no systematic attempt was made to order the *Not*I fragments, the analysis of a partial *Not*I fragment from 3153A demonstrated that the 1,410- and ~700-kb *Not*I fragments are adjacent to each other (see Results).

It is well established that rRNA transcription in *Escherichia coli* is regulated in response to nutritional and environmental conditions (6). The major promoters are regulated so that the net result is the accumulation of rRNAs as ribosomes whose concentration is approximately proportional to the growth rate. In *E. coli*, most ribosomal protein synthesis is controlled posttranslationally by feedback mechanisms that are ultimately dependent on the level of the rRNAs. In the yeast *S. cerevisiae*, transcription of both rRNA and mRNA for ribosomal proteins increases and reaches higher levels within 30 min after being shifted to media allowing faster growth (27). These results and a variety of other experiments reviewed by Woolford and Warner (27) suggest that the relative rate of ribosome biosynthesis in yeasts reflects the rate of growth. Although this regulation is due in part to the rate of rRNA transcription, we suggest that the number of rDNA units also plays a role in controlling the rate of ribosome biosynthesis, especially after prolonged incubation periods.

We suggest that there is normally a distribution in the number of rDNA units per cell in a population because of unequal crossing-over (24) or gene conversion (29) within the rDNA cluster during mitotic growth. Our data on the variability among the subclones from the stock culture (Fig. 8C) reflect this variation. Furthermore, we suggest that there is an optimum number of rDNA units for every growth condition. Because of the heterogeneity in the number of rDNA units per cell, it is reasonable to assume that cells with appropriate numbers of rDNA units would be selected for easily under conditions that favor their particular growth condition. We can speculate, for example, that overproduction of ribosomes for a given physiological state would be wasteful and would be expected to retard growth. For example, the optimum number of rDNA units in cells grown slowly at 22°C on LBC medium would be less than the number in cells grown rapidly at 37°C in YPD medium. Indeed, subclones grown slowly for a prolonged time had 94% of the number of rDNA units of the faster-grown cultures, as schematically presented in Fig. 8B and D, respectively. In addition, slowly grown subclones all were identical, probably representing the predominant optimal type. On the other hand, the distribution among subclones grown faster for a prolonged time was shifted to an increase in the number of rDNA units (Fig. 8D) in comparison with the distribution in the stock culture (Fig. 8C). The small number of cells with the appropriate number of rDNA units would be expected to be enriched after a shift to a different growth conditions. In other words, the representative karyotypes of cell population can be controlled by the conditions of growth. The reversibility in the number of rDNA units per cell was further demonstrated by using the strains C.a.1 to C.a.10 and their fast-grown (C.a.31F to C.a.35F) and slowly grown (C.a.31S to C.a.35S) progenies, as presented in schematics in Fig. 7. The sizes of the shorter homologs of chromosome VIII increased in the faster-grown subclones C.a.31F to C.a.34F in comparison with the parental population of 3153A. One culture, C.a.33F, acquired a dramatically increased number of rDNA units by having two long homologs instead of one long and one short, as revealed by rDNA probing. However, no change was observed in one subclone, C.a.35F. On the other hand, all slowly grown cultures, C.a.31S to C.a.35S, contained reduced numbers of rDNA units in at least their shorter VIIIa homologs. A fast-grown population probably offers a much wider assortment of differently sized clusters in chromosome VIII with the general tendency to have a higher number of rDNA units.

We wish to emphasize that morphological mutants contained a wider distribution of rDNA units per cell than any group of subclones, and this distribution could not be explained by the differences in growth rate. The unusually wide distribution in both morphological mutants and normal laboratory strains suggests to us that there may be a separate mechanism for altering chromosome VIII in morphological mutants, and this same process also may be responsible for producing aberrations of the other chromosomes in these mutants.

ACKNOWLEDGMENTS

We thank S. Scherer (University of Minnesota), M. McEachern (University of California, San Francisco) and B. Magee (University of Minnesota) for providing hybridization probes.

This investigation was supported by Public Health Service research grant R01 AI29433 from the National Institutes of Health.

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