Clonal variation of chromosome size derived from the rDNA cluster region in *Candida albicans*

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Of the eight *Candida albicans* chromosomes, chromosome 2, assigned by the MGL1 probe, is more variable in size than the other chromosomes among strains. We found that the clonal variation of chromosome 2, which carries a rDNA gene, occurred at a frequency of up to 10% of the progeny clones. After total chromosomal digestion with *Xho*I, which has no recognition sites within the rDNA repeat unit, the fragments containing the rDNA cluster were detected by Southern hybridization. The difference in fragment sizes corresponded to the clonal size variation of chromosome 2. The intensity of hybridization with rDNA also correlated with the difference in size. In addition, there was no size change in the non-rDNA region as detected by *Not*I digestion of chromosome 2, and there was no observed change in the individual rDNA basic repeat unit size. From these lines of evidence, we confirmed that the clonal size variation of chromosome 2 which occurs at high frequency is derived from the size change of the rDNA cluster.

Introduction

Candida albicans is responsible for oral and vaginal candidiasis and sometimes causes severe systemic candidiasis in immunocompromized hosts (Odds, 1988). This organism does not have a sexual cycle and naturally exists in the asexual diploid state (Poulter *et al.*, 1981; Riggsby *et al.*, 1982). Accordingly, mutant isolation and genetic analysis in this organism are not trivial. However, recent developments in molecular genetic approaches, including electrophoretic karyotyping using pulsed-field gel electrophoresis (PFGE), have brought new information concerning the genome of *C. albicans* (for a recent review, see Scherer & Magee, 1990). By this method, the haploid number has been determined to be eight (Iwaguchi *et al.*, 1990; Lasker *et al.*, 1989; Wickes *et al.*, 1991). Chromosomal DNA bands separated by PFGE have been assigned using cloned DNAs of *C. albicans* or the homologous DNAs of *Saccharomyces cerevisiae* and a genetic map is being constructed (Scherer & Magee, 1990).

Electrophoretic karyotype polymorphisms have been found between strains within a single species (Iwaguchi *et al.*, 1990; Lott *et al.*, 1987; Merz *et al.*, 1988; Snell *et al.*,

1987). This karyotyping method should be useful in clinical applications for strain identification (Merz, 1990). It has been reported that a change in karyotype appears to be correlated with a change in colony morphology (Rustchenko-Bulgac *et al.*, 1990; Suzuki *et al.*, 1989) although a definitive relationship between phenotype and karyotype has yet to be determined.

In our previous study, the chromosomal DNAs of *C. albicans* stock strains were resolved into 7–12 bands ranging in size from about 0.4 Mb to about 3.0 Mb (Iwaguchi *et al.*, 1990). The separated bands were assigned to eight chromosomes based on hybridization patterns using eight different cloned DNA probes. The size of at least one homologue of seven out of eight chromosomes was found to be fairly well conserved. Thus, the karyotype polymorphism between strains was thought to be mainly derived from large size heterogeneity in one of the homologues (Iwaguchi *et al.*, 1990). On the other hand, chromosome 2, assigned by the MGL1 probe, showed a marked degree of variation in both homologues. The underlying mechanism(s) for variation could be due to (i) deletion, (ii) amplification, (iii) translocation or (iv) unequal crossing-over. It has been suggested that these events occur at a high frequency in subtelomeric regions (Corcoran *et al.*, 1988), rDNA regions (Szostak & Wu, 1980), or transposon regions (Shapiro, 1983).

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Abbreviations: PFGE, pulsed-field gel electrophoresis.

Chromosome 2 is the most variable in size among strains isolated from patients (Iwaguchi *et al.*, 1990). Strains isolated from individual patients after intervals of 1–6 months had different karyotypes, most of which were attributable to changes in chromosome 2 (Asakura *et al.*, 1991). From these results, we assumed that chromosome 2 contains one or more regions responsible for chromosome rearrangement. Similar variable chromosomes have been observed in different groups (Rustchenko-Bulgac, 1991; Wickes *et al.*, 1991). It has been shown that the variable chromosome carries a rDNA gene. It has also been demonstrated that a frequent chromosome size variation in *Candida glabrata* occurs in a chromosome carrying the rDNA gene (Asakura *et al.*, 1991). However, it is not known how prevalent the phenomenon of chromosome size variation is, or whether it is due to some selective aspect of prolonged maintenance in culture. In this study, we first examine the frequency of the clonal size variation of chromosome 2 under vegetative growth, and second demonstrate that the size variation is derived from a size change in the rDNA cluster region of the progeny clones.

Methods

Strains. Isolates of *C. albicans*, TCH297, TCH298 and TCH300 were collected from 3 individual out-patients of the Obstetrics and Gynecology Department in Chita City Hospital, Japan, in 1989. The TCH strains were obtained from vaginas and were identified as *C. albicans* by their assimilation pattern with the API 20 C system (Buesching *et al.*, 1979), by serological testing with Candida check (Shinoda *et al.*, 1981), and by chlamydospore formation. Strain FC18 was used as a reference strain.

Plasmids. The plasmids used in this study as probes in Southern hybridizations are listed in Table 1.

Preparation of yeast chromosome DNA for PFGE. The sample plug containing yeast chromosome DNA for PFGE was prepared by the method described previously (Iwaguchi *et al.*, 1990).

PFGE. PFGE was carried out by the contour-clamped homogeneous electric fields (CHEF) method (Chu *et al.*, 1986) using the Pulsaphor system with a hexagonal electrode array (Pharmacia-LKB). The yeast chromosome DNAs were separated in a 0.8% agarose gel usually with a 300 s switch time at 140 V for 24 h followed by a 1200 s switch time at 80 V for 48 h as described previously (Iwaguchi *et al.*, 1990). *S. cerevisiae* (X2180-1A; Mortimer & Schild, 1985) and *Schizosaccharomyces pombe* (HM422 derived from 972h⁻; Fan *et al.*, 1988) chromosomes were used as the size reference markers. The sizes of the

chromosomes of *Sch. pombe* HM422 were estimated from the construction of a chromosome map; chromosome I is 5.7 Mb, chromosome II is 4.6 Mb and chromosome III is 3.5 Mb (Fan *et al.*, 1988). In our previous report (Iwaguchi *et al.*, 1990), we estimated the chromosome size of *C. albicans* by using a different reference strain. Thus, we have revised the size of the large bands in *C. albicans* as follows: the 2.86 and 2.66 Mb chromosome bands of FC18 have been corrected to 3.5 and 3.18 Mb. λ ladder concatemer DNA (Bio-Rad) and λ DNA digested by *Eco*T14I (Takara) were also used as size markers.

Preparation of chromosome DNA probes for Southern hybridization. Chromosome DNAs of NUM46 were separated by PFGE in a 0.7% agarose gel and stained with 0.5 μ g ethidium bromide ml⁻¹ for 1 h and destained with distilled water overnight. The chromosome 2 band was cut out with a razor blade under 365 nm UV light. The gel slice was transferred to a dialysis bag filled with 0.5 $\times$ TBE [1 $\times$ TBE is (g l⁻¹): boric acid, 5.5; EDTA, 0.74; Tris, 10.8]. The electroelution was performed using field inversion gel electrophoresis (FIGE) in 0.5 $\times$ TBE with a 16 s pulse forward and 8 s backward for 5 h at 6.7 V cm⁻¹, and then for 2 min at 6.7 V cm⁻¹ in the reverse direction to release the DNA from the wall of the dialysis bag. The DNA solution was treated once with an equal volume of phenol and then concentrated to 50 μ l with sec-butanol. The concentrated DNA solution was dialysed with 1 mM-EDTA/10 mM-Tris/HCl (pH 8.0) and used as a probe for Southern hybridization.

Southern hybridization. Southern hybridization was performed using the LYS2 probe, a rDNA probe, and the chromosome DNA probe using the method described previously (Iwaguchi *et al.*, 1990). The intensity of the hybridization signals was measured with an image analyser system (BAS2000; Fuji). The signal intensity of the LYS2 probe was used as a reference to standardize the DNA concentration in each sample.

Restriction digestion of chromosome DNA in PFGE sample plugs. Sample plugs for PFGE were equilibrated in the appropriate reaction buffer (200 μ l) for restriction enzymes *Xho*I or *Not*I for 30 min at room temperature. The plugs were transferred to fresh restriction buffer containing *Xho*I or *Not*I (20 units in 200 μ l) and incubated overnight at 37 °C. The sample plugs were then washed once with 50 mM-EDTA (pH 9.0) and the digested chromosome DNA in the plugs was separated by PFGE.

Restriction digest of chromosome 2 bands separated on PFGE. Chromosomes of TCH297, TCH298 and TCH300 were separated in a 0.7% agarose gel by PFGE, stained with ethidium bromide, and destained with distilled water. The chromosome 2 bands were cut out with a razor blade under 365 nm UV light. Ethidium bromide was immediately extracted with three washes of sec-butanol saturated with 1 M-NaCl, 1 mM-EDTA, and 10 mM-Tris/HCl (pH 8.0), respectively, for 10 min each. The blocks were then equilibrated in 200 μ l of a restriction buffer for *Not*I, transferred into 200 μ l of fresh restriction buffer containing 20 units of *Not*I and incubated overnight at 37 °C. The digested DNA of chromosome 2 was separated by PFGE with a 100 s switch time at 180 V for 15 h followed by a 300 s switch time at 140 V for 20 h.

Table 1. DNA probes for hybridization

Probe	Plasmid	DNA fragment	Source (references)
MGL1	pYSK210	4.5 kb <i>Hind</i> III	J. A. Gorman (Iwaguchi <i>et al.</i> , 1990; Magee <i>et al.</i> , 1988)
LYS2	pTK2-9-1	7.7 kb <i>Eco</i> RI	B. B. Magee (Iwaguchi <i>et al.</i> , 1990; Magee <i>et al.</i> , 1988)
rDNA	pAT68	10.8 kb <i>Sal</i> I	A. Toh-e (Asakura <i>et al.</i> , 1991; Sugihara <i>et al.</i> , 1986)
BEN1	pbenS10	0.9 kb <i>Pst</i> I and <i>Eco</i> RI	J. A. Gorman (Iwaguchi <i>et al.</i> , 1990; Magee <i>et al.</i> , 1988)
pCHR7	pCHR7	2.6 kb of <i>Bam</i> HI and <i>Hind</i> III	B. B. Magee (Iwaguchi <i>et al.</i> , 1990; Magee <i>et al.</i> , 1988)

Results

Frequency of clonal size change in chromosome 2

To examine the frequency of the size variation of chromosome 2, karyotypes were compared in five clones derived from each strain of TCH297, TCH298 or TCH300. Variation between the clones was observed in their karyotypes (Fig. 1 shows a part of the result). Four and three different karyotypes appeared in TCH297 and TCH298, respectively. On the other hand, clonal variation of karyotype was not observed in TCH300. The size difference of the chromosomal band in the karyotype amongst the clones was attributed to one or two large-sized bands. These bands were assigned as chromosome 2 by Southern hybridization using the MGL1 probe (data not shown) and the rDNA probe (Fig. 1b). The size change of chromosome 2 was observed in only one of the two homologues and the 3.1 Mb chromosome 2 band was not changed amongst the clones. In a clone of TCH298-2, which was derived from TCH298, clonal variation was also observed in the size of chromosome 6 assigned by the LYS2 probe, in which the size difference was only 10 kb (Fig. 1, lane 6).

Next, three clones in each of the TCH strains, which are referred to as parental clones, were streaked on a YPD plate and 10 colonies were picked. These are referred to as progeny clones. The karyotype variation of the progeny clones was investigated. Fig. 2 shows the karyotypes of the parental and some of the progeny clones which were different from the parental karyotype. In one or two clones out of ten clones for each progeny group, the sizes of chromosome 2 bands were different from those of the parental clone. The size change of chromosome 2 was observed in 12% of all the progeny clones. Each of the parental colonies contained about 2×10^7 cells which were multiplied by 24.3 generations. It was calculated that the frequency of a detectable size change was about 5×10^{-3} per cell division. In two out of 90 clones examined, TCH297-2-10 (data not shown) and 300-3-2 (Fig. 2, lane 10), the chromosomes which changed in size were not assigned by MGL1 or an rDNA probe but by a BEN1 probe as chromosome 7 and by a pCHR7 probe as chromosome 8, respectively.

The size change of the rDNA cluster region among the clones

Since the size of chromosome 2 changed at high frequency and the rDNA gene is located on chromosome 2, we examined the possibility that the size change of chromosome 2 amongst the strains was due to frequent DNA rearrangement in the rDNA region where the gene is highly repeated.

Fig. 1. Size variation of chromosome 2 bands of parental clones. (a) Electrophoretic karyotypes of the parental clones isolated from three strains, TCH297, TCH298 and TCH300 (lanes 2–10) and *C. albicans* standard strain FC18 (lane 1). Arrows at the right of each lane indicate the bands which hybridized to rDNA in (b). (b) rDNA hybridization profile of the chromosome 2 band(s) of each clone shown in (a). Arrowheads indicate the largest band of FC18. Numbers on the right margin indicate the size of the *S. cerevisiae* and *Sch. pombe* marker DNAs.

For this purpose, we attempted a survey using a restriction enzyme which had no recognition site within the rDNA repeat unit and which cut out the rDNA cluster region. PFGE sample plugs of strain FC18 were treated with 15 restriction enzymes having 5-, 6- or 8-base recognition sequences and the rDNA fragments were detected by hybridization. Amongst them, *Hind*III and *Xho*I cut out the rDNA cluster. However, a *Hind*III site was present in the rDNA repeat unit of some, but not all, strains used. Therefore, *Xho*I was used for digestion. After separation by PFGE and assigning by the rDNA probe, two large *Xho*I fragments were detected in the range of 130–1530 kb in each clone (Fig. 3 and Table 2), and were thought to be derived from each of the homologues. Most of the size differences between the *Xho*I fragments corresponded to those between the intact chromosome 2 bands in each strain (Table 2). Although chromosome 2 of clone TCH297-1 was separated as a

Fig. 2. Size variation of chromosome 2 bands of progeny clones. (a) Electrophoretic karyotypes of progeny clones which had different karyotypes from their parental clones. The clones are defined as follows: for example, 297-1 is a parental clone of TCH297 and both 297-1-1 and 297-1-4 are progeny clones derived from 297-1 (lanes 1–3). Arrows at the right of each lane indicate the bands which hybridized to rDNA in (b). (b) rDNA hybridization profile of the chromosome 2 band(s) of each clone shown in (a). Arrowheads indicate chromosome 2 bands of TCH297-1. Numbers on the right margin indicate the size of the *S. cerevisiae* and *Sch. pombe* marker DNAs.

single band by PFGE, two fragments of 500 and 370 kb were detected as a rDNA cluster in a *XhoI* digest. This suggested that the size of the region, except for the rDNA cluster, was different between homologues in strain TCH297. A part of the non-rDNA region was also demonstrated to be different in size between the homologues (Fig. 4).

Assuming that the intensity of the hybridization signal of the rDNA probe is correlated with the number of rDNA repeat units, the intensity of the rDNA hybridization signal of *XhoI* fragments in Fig. 3 was measured by a BAS2000 image analyser system. To normalize total DNA content in each lane, the intensity of the hybridization signal of the LYS2 probe was measured as a reference. The relative intensity of the *XhoI* fragment assigned by the rDNA probe is shown in Table 2. The rDNA hybridization signal intensity roughly corresponds to the size of the *XhoI* rDNA fragment.

Fig. 3. *XhoI* fragments derived from rDNA cluster regions. PFGE sample plugs of each clone of TCH strains shown in Fig. 2(a) were digested with *XhoI*. After PFGE with a 100 s switch time at 180 V for 15 h followed by a 300 s switch time at 140 V for 20 h Southern hybridization using a rDNA probe was performed. The lanes correspond to those in Fig. 2(a). The estimated size of *XhoI* fragments in each clone is indicated in Table 2. Numbers on the left margin indicate the size of the *S. cerevisiae* marker DNAs.

The size change of the non-rDNA regions

It was suggested that the clonal size variation of chromosome 2 was due to the size change of the rDNA cluster. To confirm this, the size of non-rDNA regions was estimated in chromosome 2. As the *NotI* 8-base recognition sequence is rare, the *NotI* digestion generates large-sized fragments of the chromosome. Fortunately, *NotI* has only one cutting site within the rDNA gene of the repeating units. This makes it possible to detect size changes of the non-rDNA fragments.

A chromosome 2 band was cut out from a PFGE gel and the homologues were named a and b according to their size. After the chromosome 2 band was treated with *NotI* and separated again by PFGE, the fragments were detected by Southern hybridization using a chromosome 2 probe (Fig. 4). The bands between 15 and 160 kb

Table 2. Size of rDNA region on chromosome 2 band(s) in TCH strains

Clones (TCH)*	Intact chromosome size (kb)	Size of <i>Xho</i> I fragments of rDNA cluster (kb)†	Size of <i>Not</i> I fragments of chromosome 2 (kb)		Relative density of rDNA‡	No. of rDNA repeat units per chromosome¶
			Non-rDNA region‡	rDNA cluster§		
297-1	3100	370, 500	2640, 2560	460, 540	0.85, 1.00	25, 33
297-1-1a	3380	740	2640	740	1.09	49
297-1-1b	3080	410	2560	520	0.71	27
297-1-4a	3180	570	2560	620	0.96	38
297-1-4b	2960	210	2640	320	0.47	14
298-1a	4350	1530	2580	1770	1.88	102
298-1b	3080	420	2620	460	0.72	28
298-1-6a	4350	1530	2580	1770	1.99	102
298-1-6b	2880	197	2620	260	0.39	13
298-1-8a	3940	1230	2580	1360	1.44	82
298-1-8b	3080	420	2620	460	0.71	28
300-1a	3500	1000	2540	960	1.70	67
300-1b	3100	480	2650	450	0.89	32
300-1-3a	3280	640	2650	630	1.25	43
300-1-3b	2700	130	2540	160	0.24	9
300-3a	3580	1000	2560	1020	1.71	67
300-3b	3120	500	2660	460	0.82	33
300-3-2a	3750	1200	2560	1190	1.96	80
300-3-2b	2960	240	2660	300	0.47	16

* The larger of the two homologues of chromosome 2 is designated as 'a', the smaller homologue as 'b'.

† The size of the *Xho*I fragments was assigned by a rDNA probe as shown in Fig. 3.

‡ Total size of *Not*I fragments in each chromosome 2 band, except for the rDNA region.

§ The size of the rDNA cluster was calculated by subtracting the total size of the non-rDNA region *Not*I fragments from the intact size of the corresponding chromosome 2.

|| The density of rDNA hybridization to *Xho*I fragments in Fig. 3 was measured using a BAS2000 image analyser. The DNA content in each lane was normalized with the density of LYS2 hybridization.

¶ The number of rDNA repeat units was calculated by dividing the size of the rDNA fragment produced by *Xho*I digestion by the size of the rDNA repeat cluster *Not*I fragment (15 kb).

(indicated by bracket A in Fig. 4) were recognized by the rDNA probe but were not detected reproducibly (Fig. 5). They seem to be partially digested fragments of rDNA. These were not detected after longer incubation with the enzyme, although other fragments did not change in size and hybridized intensively. The 420 kb fragment seen in 300-1a and 300-3a (Fig. 4, lanes 12 and 16) seem to be derived from the chromosome 1 band which comigrated with chromosome 2 on the PFGE gel, because this fragment was also recognized by a chromosome 1 probe (data not shown). Chromosome 1 has some homology to chromosome 2 (unpublished data). Thus, the 420 kb fragments probably contain the region homologous to chromosome 2. The smallest bands had a very strong signal (Fig. 4) and were also found by the rDNA probe (Fig. 5). This suggests that the bands correspond to the repeating unit derived from the rDNA cluster. The size of the repeating unit was shown to be strain-specific; 14.3 and 15 kb for TCH297, 15 kb for TCH298, or 15.2 kb for TCH300 (Fig. 6). However, the size of the rDNA repeat unit was not changed amongst the clones of

each strain. The 260 kb fragment was also recognized by the rDNA probe, although the intensity of the hybridization signal was low, similar to that seen with the MGL1 probe (Fig. 5). This is thought to be caused by the fragments adjacent to the rDNA cluster which contain a part of the rDNA repeat unit.

Four large *Not*I fragments of a non-rDNA region were detected in each chromosome 2 band except for 297-1, whose homologues were not separated on the gel (Fig. 2). The homologues of 297-1 gave five fragments of 160, 260, 790, 870 and 1350 kb, though the 790 and 870 kb fragments were not adequately separated (Fig. 4, lane 1), but either the 790 kb or the 870 kb fragment was found in the separated homologue of chromosome 2 from the progeny clones (Fig. 4, lanes 2–5). The fragments of non-rDNA did not appear to change between the parental and progeny clones in each strain. In addition, we calculated the size of the rDNA cluster in each chromosome 2 band by subtracting the total size of the non-rDNA fragments from the length of intact chromosome 2 estimated by PFGE (Table 2). In all the

Fig. 4. *NotI* fragments of chromosome 2 band(s) detected by Southern hybridization using a chromosome 2 DNA probe. Chromosome 2 band(s) of each clone shown in Fig. 2 were cut out from a PFGE gel, digested with *NotI* and separated again by PFGE under the conditions described in the legend to Fig. 3. The larger homologue was referred to as 'a' and the smaller one as 'b'. The smallest band in each lane corresponded with the rDNA repeat unit. Numbers on the right margin indicate the *NotI* fragment size of the larger chromosome 2 band in TCH300-3 (300-3a). Bracket A indicates bands caused by incomplete digestion (see Results). Numbers on the left margin indicate the size of the *S. cerevisiae* marker DNAs.

Fig. 5. rDNA hybridization profile of *NotI* fragments. PFGE sample plugs of each clone of the TCH strains shown in Fig. 2(a) were digested with *NotI*. After PFGE with a ramping switch time from 20 to 40 s at 200 V for 18 h in 1% agarose gel, Southern hybridization using a rDNA probe was carried out. The lanes correspond to those in Fig. 2(a). Numbers on the left margin indicate the size markers of the λ concatemer ladder and of λ DNA digested with *EcoT14I*. Numbers on the right margin indicate the size of the *NotI* fragments estimated from the size markers.

Fig. 6. rDNA repeat unit. After samples were prepared and the DNA fragments were separated by PFGE as described in the legend to Fig. 5, the gel was stained with ethidium bromide (a), and Southern hybridization using a rDNA probe was carried out (b). Numbers on the left margin indicate the estimated size of the larger band of the rDNA repeat unit of TCH297-1.

chromosome 2 bands, the calculated size was in reasonable agreement with the size of the *XhoI*-digested fragment assigned as the rDNA region.

These data supported the concept that the size change of chromosome 2 occurs in the rDNA cluster and not in the non-rDNA region.

Discussion

The remarkable variation in the size of chromosome 2 (alternatively called chromosome R or chromosome VIII), which carries a rDNA gene, has been observed in stock strains (Iwaguchi *et al.*, 1990; Wickes *et al.*, 1991), clinical isolates (Asakura *et al.*, 1991), and morphological mutants (Rustchenko-Bulgac, 1991). When we analysed

multiple clones derived from a common ancestor, we found that clonal change of electrophoretic karyotype is detectable with a frequency of variation greater than 10% for chromosome 2 of the progeny clones. It has been assumed that the rDNA gene sequence is usually highly repeated in a single region, DNA rearrangement occurs at high frequency in the region, and such rearrangements may be responsible for the change in chromosome size. We confirmed the assumption that the clonal size change of chromosome 2 is derived from the size change of the rDNA cluster from the following lines of evidence. (i) The size change of the rDNA fragment produced by *XhoI*, whose recognition site was not present in the rDNA repeat unit, corresponded to the size change of the intact chromosome 2. (ii) The hybridization intensity of rDNA correlated to the size of the rDNA fragment. (iii) The non-rDNA fragments of chromosome 2 produced by *NotI*, which had a single recognition site in the rDNA repeat unit, did not change in the progeny clones of each strain. (iv) The calculated size of the rDNA cluster of chromosome 2, which was obtained by subtracting the total size of the non-rDNA *NotI* fragments from the intact size of chromosome 2, corresponded to the size of the rDNA *XhoI* fragment. (v) The size of the rDNA repeat unit was not altered in the clones of each strain.

The number of rDNA repeat units for each chromosome 2 can be estimated by dividing the size of the rDNA *XhoI* fragment by the size of the rDNA repeat unit. When we take 15 kb as the rDNA repeat unit size, the number of rDNA repeating units per homologue of chromosome 2 varies from about 9 to about 100 and the number per cell varies from about 50 to about 130 (Table 2). Compared to the parental clone, the number tended to decrease in the progeny clones. It has usually been believed that the rDNA cluster size is relatively stable in vegetative growth. A haploid strain of *S. cerevisiae* has about 140 copies of the rDNA repeat unit (Cramer *et al.*, 1972) and this number is thought to be maintained by unequal crossing-over events during mitotic growth (Stark & Wahl, 1984; Szostak & Wu, 1980). A similar maintenance mechanism for the rDNA number is observed in *Neurospora crassa* (Rodland & Russell, 1982, 1983; Russell & Rodland, 1986).

It has been shown that mitotic recombination in the rDNA cluster of *S. cerevisiae* is suppressed by the combined action of topoisomerase I and topoisomerase II (Christman *et al.*, 1988) or suppressed by the function of a transcriptional silencer gene, SIR2 (Gottlieb & Esposito, 1989). It has been reported that a mitotic recombination hot spot, namely HOT1, is located in the rDNA repeat unit (Keil & Roeder, 1984). Recombination in the rDNA cluster in *C. albicans* may involve homologous factors.

It has been roughly estimated that the rate of marker

loss in the rDNA region of *S. cerevisiae* is 5×10^{-4} per cell division (Szostak & Wu, 1980). Therefore the frequency of unequal crossing-over within the whole rDNA region is 10^{-2} per cell division, based on the value of 140 repeat units of the rDNA cluster of *S. cerevisiae*. The frequency of detectable size change of the rDNA region of *C. albicans* was estimated to be about 5×10^{-3} per cell division. It would appear that the high frequency change observed in *C. albicans* cannot be solely attributable to unequal crossing-over (Petes & Hill, 1988), although it is obviously difficult to compare rates based on different experimental approaches.

The bobbed mutants of *Drosophila melanogaster* with partial deletions of rDNA show abnormal phenotypes, such as shorter bristle and slow development as well as other pleiotropic effects (Hawley & Marcus, 1989; Tartof, 1974). We originally suspected that the size change of the rDNA cluster might result in a phenotypic change such as a change in colony morphology which had been reported to be associated with karyotypic change (Rustchenko-Bulgac *et al.*, 1990; Suzuki *et al.*, 1989). However, no detectable phenotypic change was observed in clones carrying the size change in the rDNA cluster. The minimal number of rDNA units per cell was estimated to be 50 copies in the clones (Table 2). This number may be sufficient to ensure its correct function in *C. albicans*.

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