Occurrence of chromosome rearrangements during the fusion process in the imperfect yeast *Candida albicans*

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Auxotrophic derivatives of three strains of the pathogenic yeast *Candida albicans* of different origins, including 1006 derived from CBS5736, A5153 derived from FC18 and NARA2 derived from NUM961, were used in spheroplast fusion experiments. The DNA content of the prototrophic fusion product obtained following fusion between strains 1006 and A5153 approximated to the sum of those of the parents, but was variable when NARA2 was used as the parent for fusion. Chromosome-sized DNA molecules of the fusion derivatives were separated by pulsed-field gel electrophoresis to examine whether either or both of the chromosome-sized DNA molecules of each parent were transferred into the fusion derivatives. In the fusion derivatives obtained following fusion between strains 1006 and A5153, nearly the full complement of chromosomes was shown to be transferred, but partial transfer of chromosomes occurred in the fusion derivatives that were obtained following fusion between strains NARA2 and A5153. Results indicated that chromosome loss also occurred when these two strains were fused. Variations in the size of R chromosomes, the rDNA-containing chromosomes, were observed in all fusion derivatives tested, indicating high-frequency recombination between R chromosomes during the fusion process.

Keywords: *Candida albicans*, spheroplast fusion, chromosome rearrangement, chromosome transfer

INTRODUCTION

Isolates of *Candida albicans* are the most important fungal pathogens of humans and animals (Odds, 1988). The absence of a known sexual cycle and the generally diploid nature of this organism (Whelan *et al.*, 1980; Riggsby *et al.*, 1982) have limited the development of genetic systems in this organism. A parasexual genetic system has been developed for this species that employs spheroplast fusion for complementation testing (Kakar *et al.*, 1983; Poulter *et al.*, 1981), mitotic recombination (Whelan *et al.*, 1980; Whelan & Soll, 1982) and chromosome loss for linkage determination (Hilton *et al.*, 1985).

Naturally occurring strains of this organism have been found to be heterozygous for a limited number of nutritional markers and additional heterozygosity can be induced by treatment with a mutagen or with UV irradiation (Kakar *et al.*, 1983). Spheroplast fusion followed by regeneration on selective media revealed complementation among auxotrophic mutants. However,

stable fusion products are relatively rare (Scherer & Magee, 1990; Whelan *et al.*, 1985). Spheroplast fusion does not appear to be a one-to-one process but involves the initial formation of large syncytia, which can apparently either undergo nuclear fusion or become metastable heterokaryons (Sarachek & Rhoads, 1983; Sarachek & Weber, 1984). These heterokaryons can then undergo either nuclear fusion or transfer of a limited amount of genetic material from one nucleus to the other and segregate recombinants which largely resemble one parent (Goshorn & Scherer, 1989).

With the advent of pulsed-field gel electrophoresis (PFGE), electrophoretic karyotyping has provided information concerning the structure of the genome of this organism (Scherer & Magee, 1990). Furthermore, electrophoretic karyotype polymorphisms have been found among unrelated strains of *C. albicans* (Iwaguchi *et al.*, 1990; Lott *et al.*, 1987; Merz *et al.*, 1988; Snell *et al.*, 1987), and karyotyping has been found to be useful for strain differentiation (Merz, 1990). We show here, using three

auxotrophic strains of *C. albicans* of different origins, the electrophoretic karyotypes of fusion products generated with each pairwise combination. One strain, NARA2, was a derivative of NUM961. One variant from NUM961 was shown to have chromosome rearrangements which could be correlated with a change in colony morphology (Suzuki *et al.*, 1989). The karyotypes of fusion products obtained with strain NARA2 and one of the other two parents indicated that, during the process of spheroplast fusion, chromosome loss or transfer of a limited number of chromosomes occurred. In contrast, the rDNA-containing chromosomes were shown to undergo molecular size variations in all pairwise combinations of the parents during the fusion process or following regeneration.

METHODS

Strains and plasmids. *C. albicans* strain A5153 was a His⁻ Trp⁻ Lys⁻ auxotrophic derivative of FC18 (Whelan *et al.*, 1980; Suzuki *et al.*, 1986). *C. albicans* strain NARA2 was isolated as a Met⁻ Pro⁻ auxotroph obtained after UV irradiation of *C. albicans* NUM961 (Suzuki *et al.*, 1989). Another *C. albicans* strain 1006, carrying Arg⁻ Lys⁻ Ser⁻ Ura⁻ MPA^r (mycophenolic-acid-resistant) markers and derived from CBS5736, was kindly given to us by S. Scherer (Department of Microbiology, University of Minnesota School of Medicine, Minneapolis, MN, USA) (Suzuki *et al.*, 1986; Goshorn & Scherer, 1989).

The plasmid pCR4 was the ligation product of pBR322 and the 10.2 kb *Pst*I fragment that contained a repeating unit of the rRNA genes of *C. tropicalis* (Kamiryo *et al.*, 1991). That cloned insert was used as a probe in Southern hybridization. This plasmid was propagated using *Escherichia coli* HB101 as a host strain, according to the procedure of Sambrook *et al.* (1989).

Media. Yeast cells were grown in YPD medium [1% (w/v) yeast extract, 2% (w/v) peptone and 2% (w/v) glucose] or in MIN medium [0.67% (w/v) yeast nitrogen base without amino acids (Difco) and 2% (w/v) glucose]. Amino acids and uridine were added to MIN medium to a final concentration of 50 µg ml⁻¹, when necessary. Regeneration media for making fusion derivatives consisted of MIN or YPD media supplemented with 1 M sorbitol. Both MIN and YPD plates were made by adding 2% (w/v) agar (Nacalai Tesque).

Spheroplast formation and fusion. *C. albicans* cells were converted to spheroplasts according to the procedure of Kakar & Magee (1982). Approximately 1.5 × 10⁷ spheroplasts from each of two parent strains were mixed in micro-centrifuge tubes and left at room temperature for 10 min. These were then centrifuged at 10000 *g* for 10 s and the pellet was gently resuspended in 1 ml of fusion mix containing 20% (w/v) polyethylene glycol (average molecular mass 3000 Da) and 10 mM CaCl₂ in 10 mM Tris/HCl (pH 7.5). After 10 min at room temperature, spheroplasts were again pelleted and resuspended in 1 ml SOS buffer (1 M sorbitol, 15 mM CaCl₂ in 0.3 × YPD).

For regeneration, 0.1 ml aliquots of the appropriate dilutions of the fusion suspensions were each mixed with 5 ml of top layer medium containing 1 M sorbitol, 2% (w/v) molten agar (45–50 °C) and either YPD or minimal medium supplemented with the appropriate amino acids and tetracycline (10 µg ml⁻¹).

The mixture was then immediately poured onto the plates of the corresponding regeneration medium and incubated at 28 °C. Similarly treated unmixed protoplasts, and control protoplasts without polyethylene glycol treatment, were plated to check for reversion of parental strains.

Colonies resulting from regenerated spheroplasts were detected after 4–5 d and were picked onto YPD master plates for replica plating onto appropriate media to determine their auxotrophic requirements.

To examine reproducibility, fusion experiments were done in triplicate on each pairwise combination of the three parent strains.

Nuclear staining. Ethanol-fixed yeast cells were stained with propidium iodide in the presence of RNase A, as described elsewhere (Suzuki *et al.*, 1989).

DNA content determination. The DNA content of yeast cells was determined using diphenylamine according to Riggsby *et al.* (1982). Highly polymerized calf thymus DNA (type V, Sigma) was used as a reference for DNA content determination.

Mitotic segregation analysis. Spontaneous mitotic segregation of auxotrophs was assayed by sub-culturing in YPD medium followed by replica-plating onto fully supplemented MIN medium and MIN medium lacking appropriate nutritional factors. UV-induced mitotic segregation was achieved by irradiating cells, plated on YPD plates, with 6 × 10⁻⁵ J mm⁻² UV light using a Toshiba GL 15 W germicidal lamp to give 60–80% survival. The auxotrophy of survival colonies was checked by replica-plating onto supplemented MIN medium and MIN medium without appropriate nutritional factors.

Preparation of yeast chromosome-sized DNA molecules and PFGE. Intact yeast chromosome-sized DNA molecules were prepared by the method of Schwartz & Cantor (1984) and separated using the Pulsaphor system with a hexagonal electrode array (Pharmacia-LKB). The electrophoresis buffer used was 1 × TBE, usually containing boric acid at 5.5 g l⁻¹, EDTA at 0.74 g l⁻¹ and Tris at 10.8 g l⁻¹, and the buffer temperature was maintained at 9 °C. DNA samples embedded in low-melting-point agarose (Agarose L, Nippon-gene) were cut and applied to 0.9% (w/v) agarose gels (15 × 15 cm) which were formed by pouring 130 ml molten agarose (Agarose 1600, Wako Chemicals) in electrophoretic buffer. The electrophoresis conditions used are described in Results. Gels were stained with ethidium bromide (1 µg ml⁻¹) for 40 min, destained in distilled water for 1–2 h and DNA bands viewed under UV light (302 nm). The gels were then used for Southern hybridization analysis. Chromosomal DNA preparations from *Saccharomyces cerevisiae* (X2180-1A; Mortimer & Schild, 1985) and *Schizosaccharomyces pombe* (975-h⁺; Vollrath *et al.*, 1988) were used as molecular size reference markers.

Restriction digestion of total cellular DNA. Total cellular DNA was prepared from *C. albicans* strains by the method of Magee *et al.* (1987). DNA (10 µg) was digested with the restriction endonuclease *Hinf*I (2 units of enzyme per µg of DNA) (Toyobo) for 18 h at 37 °C. To ensure complete digestion, an additional 1 unit of the enzyme was added (per µg of DNA) to the reaction mixture and further incubated at 37 °C for 18 h. DNA fragments were separated electrophoretically on 1% (w/v) agarose gels in Tris/acetate buffer (40 mM Tris, 20 mM sodium acetate, 2.0 mM EDTA, pH 7.5) at 70 mA for approximately 3 h. *Hind*III digests of λ DNA (Takara Shuzo) were used as molecular mass standard markers. The gels were stained with ethidium bromide (0.5 µg ml⁻¹) for 15 min, de-

Fig. 1. Electrophoretic karyotypes of *C. albicans* parental strains and prototrophic fusion derivatives. Each stained gel (left) was examined by hybridization analysis, using the rDNA insert of the plasmid pCR4 as the probe (right). Chromosome bands from *C. albicans* FC18 (lanes 1 in a and b) and *Schiz. pombe* (lane 1 in c) were used as molecular size reference standards. The parent *C. albicans* strains karyotypes are shown as follows: A5153 (lanes 2 in a and b), 1006 (lane 3 in b and lane 2 in c) and NARA2 (lanes 3 in a and c). The karyotypes of prototrophic fusion derivatives obtained following fusion between A5153 and NARA2 (AN2-1 to AN2-7, lanes 4 to 10 in a, respectively), A5153 and 1006 (A6-1 to A6-7, lanes 4 to 10 in b, respectively), and NARA2 and 1006 (N26-1 to N26-7, lanes 4 to 10 in c, respectively) are shown.

stained in distilled water for 30 min and then used for Southern hybridization analysis.

Recovery and purification of DNA separated on agarose gels.

Chromosome bands or restriction fragments, after staining and destaining following agarose gel electrophoresis, were excised with a razor blade under 365 nm UV light. DNA was recovered and purified from the agarose block using a Sephaglas BandPrep Kit purchased from Pharmacia, according to the manufacturer's instructions.

Southern transfer and hybridization. Agarose gels containing separated DNA molecules were prepared for Southern transfer according to the procedure described by Sambrook *et al.* (1989). The DNA molecules were transferred to nylon membrane filters (Pall Biotransfer Membranes; Pall BioSupport Division) by the vacuum transfer system (Hybaid). Transferred DNA was fixed to the membranes by baking at 80 °C for 2 h.

DNA probes were labelled by random primed DNA labelling, using a non-radioactive DNA labelling kit (DIG-ELISA) purchased from Boehringer Mannheim, and used according to the manufacturer's instructions.

The procedure of hybridization and detection of hybridized DNA bands were performed using a DNA detection kit purchased from Boehringer Mannheim according to the manufacturer's instructions.

RESULTS

Resolution of *C. albicans* chromosome-sized DNA by PFGE

C. albicans chromosome-sized DNAs have been characterized to consist of DNA bands ranging from 1 Mb to less than 3 Mb in size (Magee *et al.*, 1988; Iwaguchi *et al.*, 1990). We have tried to separate all the *C. albicans* chromosome-sized DNA molecules of the three parent strains used for protoplast fusion experiments. Since the resolution of chromosome-sized DNAs according to size is highly dependent on factors such as the switching intervals, voltage and duration, samples were electrophoresed under various conditions. We finally decided to combine several conditions stepwise as follows; 2 min switching time for 20 h at 155 V, followed by 5 min switching time for 22 h at 140 V, followed by 1000 s switching time for 40 h at 80 V, and finally a 20 min switching time for 48 h at 80 V.

Nine chromosome-sized DNA bands from *C. albicans* FC18, 9 bands from *C. albicans* A5153, 12 bands from *C. albicans* NARA2 and 8 bands from *C. albicans* 1006 were observed (Figs 1 and 2). *Sacch. cerevisiae* and *Schiz. pombe* chromosome-sized DNAs were used as molecular size reference markers. The estimated sizes of the separated bands of *C. albicans* FC18 DNA were 3.0, 2.86, 2.66, 2.20, 1.90, 1.70, 1.36, 1.18 and 1.08 Mb, respectively. The sum of the estimated sizes of all the chromosomes in *C. albicans* FC18 was 18 Mb which is comparable to the haploid genome size estimated as between 15 and 18 Mb by Scherer & Magee (1990).

In comparison with the banding pattern of *C. albicans* FC18, its auxotrophic derivative, A5153, showed dif-

ferences in the pattern of chromosome-sized DNA bands: the largest band (3.0 Mb) in *C. albicans* FC18 was not detected in A5153 and, instead, an extra band of 2.30 Mb was observed in the latter. Individual bands of A5153 were designated A1–A9 (Table 1). The karyotype of *C. albicans* NARA2 consisted of 12 chromosome-sized DNA bands designated N1–N12 (Table 1). The absence of any detectable bands between 2.0 and 2.4 Mb in size was characteristic of this strain. The karyotype of *C. albicans* 1006 consisted of eight bands designated C1–C8 (Table 1).

From these results, the electrophoretic karyotype of each of the three parent strains was distinguishable, although two bands, A7 of strain A5153 and N9 of strain NARA2,

Fig. 2. Schematic representation of the electrophoretic karyotypes of *C. albicans* parent strains and their prototrophic derivatives obtained following fusion. The ethidium-bromide-stained chromosome bands are represented as solid bars, among which bands that hybridized with the rDNA probe are depicted as hatched bars.

Table 1. Designation and size (Mb) of chromosome bands in *C. albicans* parent strains

A5153	NARA2	1006
A1 2.86	N1 3.00	C1 2.90
A2 2.66	N2 2.86	C2 2.57
A3 2.30	N3 2.70	C3 2.30
A4 2.20	N4 2.54	C4 1.95
A5 1.90	N5 2.45	C5 1.70
A6 1.70	N6 1.95	C6 1.38
A7 1.36	N7 1.65	C7 1.27
A8 1.18	N8 1.40	C8 1.18
A9 1.08	N9 1.36	
	N10 1.20	
	N11 1.15	
	N12 0.90	

and another two, A6 of strain A5153 and C5 of strain 1006, were indistinguishable from each other, in terms of molecular size.

Composition of chromosome-sized DNA in the fusion derivatives

Screening of fusion derivatives obtained following re-generation from spheroplasts was performed with all of the pairwise combinations of the three parental strains. Seven randomly chosen prototrophic fusion derivatives were isolated from each fusion experiment and their DNA content per cell and their electrophoretic karyotype examined (Table 2, Figs 1 and 2). If entire chromosomes of the two parent strains in each combination could be transferred intact to their fusion derivatives, the electrophoretic karyotype of the derivatives would be a mixture of both of the parents. However, actual electrophoretic karyotypes of individual fusion derivatives were not simply mixed ones: some of the parental bands were not detected in the fusion derivatives and in other cases, extra

bands were observed, to which one could not find any equivalent in size in either of its parents. After sub-culturing each derivative several times, its banding pattern did not show any detectable change.

In the case of fusion derivatives obtained with A5153 and NARA2, bands corresponding in size to A1 and A2 of A5153, or bands N1, N2 and N3 of NARA2, were not clearly discriminated from each other in the fusion derivatives (Fig. 1a). However, bands lower in molecular size than these five bands could be distinguished from each other in the fusion derivatives. Bands equivalent in size to A3, A4, A5, A6, A7, A8 or A9 of the *C. albicans* A5153 chromosome were not always detected in the fusion derivatives. Similarly, bands corresponding in size to N3, N4, N5, N6, N7, N8, N10, N11 or N12 of strain NARA2 were not always detected in the fusion derivatives (Figs 1a and 2). In the derivative AN2-2, one band smaller than N12 was detected, which did not correspond in size to any of the bands detectable in either of its parents. In AN2-6, one band, whose molecular size was between those of A7 and N8, was found. If these variations in electrophoretic karyotypes of fusion derivatives reflects the occurrence of chromosome rearrangement during or after protoplast fusion, the occurrence of such a presumed rearrangement could be checked by measuring DNA content of the fusion derivatives. The DNA content per cell of a fusion derivative was compared to the sum of the DNA content of its parents. In the case of fusion derivatives obtained from A5153 and NARA2, the sum of their DNA contents approximated to 78 fg per cell. Individual fusion derivatives from this combination gave variable DNA content per cell, from 40 to 72 fg (Table 2). The three fusion derivatives, AN2-4, AN2-6 and AN2-7, had DNA contents close to that of their parents. Since each fusion derivative gave a consistent DNA content per cell, as well as a stable electrophoretic karyotype pattern, even after subculturing several times on YPD plates (data not shown), such chromosomal rearrangements seemed to have occurred during the fusion process. Another two fusion experiments gave 14 randomly chosen fusion derivatives whose DNA content per cell was 45–70 fg

Table 2. DNA content ($\pm$ sd) of *C. albicans* parent strains and prototrophic derivatives obtained following spheroplast fusion

Strain/ derivative	DNA content per cell (fg)	Strain/ derivative	DNA content per cell (fg)	Strain/ derivative	DNA content per cell (fg)
A5153	39.0 $\pm$ 0.9	NARA2	38.1 $\pm$ 1.3	1006	40.1 $\pm$ 4.2
(A5153 $\times$ NARA2)		(NARA2 $\times$ 1006)		(A5153 $\times$ 1006)	
AN2-1	59.0 $\pm$ 0.6	N26-1	74.3 $\pm$ 1.9	A6-1	66.7 $\pm$ 4.9
AN2-2	53.7 $\pm$ 1.2	N26-2	74.3 $\pm$ 4.9	A6-2	71.7 $\pm$ 2.0
AN2-3	71.3 $\pm$ 0.9	N26-3	72.6 $\pm$ 3.5	A6-3	73.7 $\pm$ 2.3
AN2-4	40.0 $\pm$ 3.1	N26-4	73.3 $\pm$ 1.8	A6-4	65.0 $\pm$ 2.0
AN2-5	66.7 $\pm$ 2.4	N26-5	73.7 $\pm$ 1.5	A6-5	67.7 $\pm$ 3.2
AN2-6	43.0 $\pm$ 1.7	N26-6	69.5 $\pm$ 2.0	A6-6	59.0 $\pm$ 2.1
AN2-7	35.5 $\pm$ 1.3	N26-7	35.3 $\pm$ 1.2	A6-7	68.3 $\pm$ 4.2

Fig. 3. (a) Gel electrophoresis of strain-specific *Hinfi* fragments of the *C. albicans* parent strains. λ *HindIII* fragments were used as molecular size reference markers (lane 1). A5153 (a 4.8 kb band; lane 2), NARA2 (two bands of 4.3 kb and 5.0 kb; lane 3) and 1006 (a 5.7 kb band; lane 4). (b) Gel electrophoresis of *Hinfi* digests of total cellular DNA from NARA2 (lane 2) showing two bands (the molecular mass of the upper band was 5.0 kb and of the lower band 4.3 kb). The two chromosome R bands of NARA2, separated on PFGE, were excised from a PFGE gel and digested with *Hinfi*, followed by electrophoresis (lanes 1 and 3). The *Hinfi* bands were identified by using the 4.8 kb *Hinfi* band from A5153 as the hybridization probe.

(data not shown) and similar variations were observed in electrophoretic karyotypes of these derivatives.

In the case of fusion derivatives obtained from the combination of strains A5153 and 1006, or NARA2 and 1006, the pronounced size variation of chromosome-sized DNA molecules was limited to sizes between 2.5 and 2.8 Mb, and variations in electrophoretic karyotypes of bands smaller than 2.5 Mb appeared less frequently than those from the combination of strains A5153 and NARA2. In the fusion derivatives obtained with A5153 and 1006, only a band equivalent to C8 was not detected in the derivative A6-6. In the fusion derivatives with NARA2 and 1006, the C4-equivalent was not detected either in derivative N26-4 or in derivative N26-6. C6- and C8-equivalents were not found in derivative N26-7. The DNA content per cell of these fusion derivatives was close to the sum of those of the parents, except for derivative N26-7, whose DNA content was half the value of the other fusion derivatives. Another two fusion experiments gave similar results of DNA content per cell and

Fig. 4. Profile of fragments obtained following electrophoretic separation of *Hinfi*-generated fragments of total cellular DNA from parental *C. albicans* strains and their prototrophic fusion derivatives. Parental strains A5153 (lane 2 in a and b), NARA2 (lane 3 in a and lane 2 in c), and 1006 (lanes 3 in b and c), and their fusants AN2-1 to AN2-7 (lanes 4 to 10 in a, respectively), A6-1 to A6-7 (lanes 4 to 10 in b, respectively) and N26-1 to N26-7 (lanes 4 to 10 in c, respectively). *HindIII* fragments of λ DNA were used as molecular size reference markers (lanes 1).

Table 3. Auxotroph segregation by *C. albicans* prototrophic fusion derivatives

C. albicans 1006 is Arg⁻, Lys⁻, Ser⁻, Ura⁻; *C. albicans* NARA2 is Met⁻, Pro⁻; *C. albicans* A5153 is His⁻, Lys⁻, Trp⁻.

Derivative	Parents	rDNA type	Total colonies examined	Segregants
N26-7	1006 × NARA2	NARA2	4999	Lys ⁻ , 39 Ser ⁻ , 1 Ura ⁻ , 1
AN2-4	A5153 × NARA2	NARA2	12652	His ⁻ , 1 Trp ⁻ , 1 Lys ⁻ Trp ⁻ , 1 Met ⁻ Pro ⁻ , 1
AN2-6	A5153 × NARA2	A5153	7441	Pro ⁻ , 4 Lys ⁻ , 1 Met ⁻ Pro ⁻ , 83 Met ⁻ Pro ⁻ His ⁻ Trp ⁻ , 1

electrophoretic karyotypes of fusion derivatives obtained with A5153 and 1006 or with NARA2 and 1006.

Occurrence of chromosome rearrangement in rDNA-containing chromosomes

If the size of a given chromosome changes at high frequency, one could assume that the change in size of the chromosome is due to frequent DNA recombination or deletion of repeated regions. Since yeast rDNA genes have been reported as candidates for such repeated regions (Iwaguchi *et al.*, 1992; Petes & Hill, 1988), we examined the possibility that rDNA may participate in the change in size by Southern hybridization, using *C. tropicalis* rDNA as a probe. Figs 1 and 2 show the rDNA probe assigned to some of the chromosome-sized DNA bands between 2.5 and 2.8 Mb in size in the fusion derivatives. These rDNA-containing chromosomes have been called chromosome R according to the proposal of Wickes *et al.* (1991). A single chromosomal band was identified following hybridization analysis as chromosome R in both *C. albicans* A5153 and 1006, but two bands were determined in *C. albicans* NARA2. Compared to the size of chromosome R of the respective parents, each fusion derivative had R chromosomes of variable size. Some of the fusion derivatives showed three separable chromosomes with homology to the rDNA probe (Fig. 1a, lanes 5 and 6; 1b, lanes 5, 6 and 7; 1c, lanes 5, 6, 7 and 8).

After subculturing of each fusion derivative several times, their respective electrophoretic karyotypes did not show any detectable change and the molecular size of chromosome R bands appeared to be consistent.

Heterozygosity for parental rDNA

Restriction fragment length polymorphisms have been easily detected in *C. albicans* by digesting its total DNA

with *Hinf*I, which cuts tandem repeats of the rDNA and produces the strain-specific pattern of fragments, especially 4–6 kb fragment(s), including the 5S RNA gene and an untranscribed region contiguous to it (Magee *et al.*, 1987; Goshorn & Scherer, 1989). Fig. 3 shows total cellular DNA from the three parent strains following digestion with the restriction endonuclease *Hinf*I. Strain A5153 yielded a band of 4.8 kb, strain 1006 a band of 5.7 kb and strain NARA2 two bands of 4.3 and 5.0 kb. Here arose a question as to whether two types of rDNA repeats in NARA2 were separately located on different homologues of R chromosomes or coexisting on each one. Each of the two chromosome R bands of NARA2 was excised from a PFGE gel and digested with *Hinf*I. The digests were separated on a gel and analysed by Southern hybridization analysis using the 4.8 kb *Hinf*I fragment of strain A5153 as a probe (Fig. 3b, lanes 1 and 3). Since the 4.8 kb fragment was excised from a gel and used as the probe, unspecific multiple bands were also detected (Fig. 3b, lane 2). Each chromosome R of NARA2 contained two *Hinf*I fragments, the same size in each case, which hybridized with the probe, suggesting the coexistence of two types of rDNA on each homologue. To examine whether rDNA species of individual fusion derivatives were derived from either or both of their parents, the *Hinf*I digests from each fusion derivative were examined. Fusion derivatives usually contained rDNA-associated *Hinf*I fragments similar in size to the rDNA-associated *Hinf*I fragments from both parents, indicating that they were heterozygous for chromosome R (Fig. 4). However, in the case of fusion derivatives obtained from the fusion of A5153 and NARA2, derivatives AN2-4 and AN2-7 contained the NARA2 type of rDNA-associated *Hinf*I fragments and derivative AN2-6 the strain A5153 type. One fusion derivative, N26-7, obtained following the fusion of strains 1006 and NARA2, had only the NARA2-type rDNA. Furthermore, for these fusion derivatives homozygous for chromosome R, their

DNA content per cell was close to that of their parents (Table 2). All of the derivatives showed altered lengths of chromosome R when compared to the chromosome R of their parents. This indicated that a DNA rearrangement had occurred even on the chromosome R in the homozygous state during the fusion process.

Heterozygosity of auxotrophic markers in the fusion derivatives

Heterozygosity for auxotrophic markers was determined by segregation of parental markers from the fusion derivatives. Mitotic recombination was induced by UV irradiation. Segregants were scored by replica plating of colonies from YPD master plates onto MIN plates. In the fusion derivatives with heterozygous rDNA which had a DNA content per cell comparable to the sum of both parents, segregation of parental auxotrophic markers occurred. A representative of one such fusion derivative obtained from the combination of strains A5153 and NARA2 is shown in Table 3. In A5153, the *Lys*⁻ and *Trp*⁻ markers are linked (Kakar *et al.*, 1983), and the co-segregation of these two markers by the fusion derivatives indicated the conservation of linkage between the two markers in the fusion derivative. Among fusion derivatives with homozygous rDNA (i.e. ones having either the parental type of rDNA-associated *Hinf*I fragments), the segregation frequency of *Met*⁻ *Pro*⁻ by AN2-6 was 80 times higher than that of *His*⁻ *Trp*⁻. According to the definition of Goshorn & Scherer (1989), this fusion derivative can be classified as exceptional progeny since it does not segregate all parental markers. Table 3 shows the results of segregation of parental markers by the other fusion derivatives falling under the definition of exceptional progeny. The rDNA type of these exceptional progeny was homozygous; N26-7 and AN2-4 were both of the NARA2 type. No direct correlation was apparent between the type of rDNA and the pattern of segregated parental markers.

DISCUSSION

In the sexually active yeast, *Sacch. cerevisiae*, nuclear fusion occurs during conjugation. Yeast nuclear fusion requires the interconnection between the two parental nuclei, which is followed by nuclear congression and then by fusion of spindle pole bodies and nuclear envelopes (Rose, 1991). Heterokaryons have been constructed using the *kar1-1* mutation, which prevents nuclear fusion during conjugation (Conde & Fink, 1976). They occasionally segregated progeny having a recombinant genotype, and this phenomenon was called internuclear transfer of genetic information or chromosome transfer (Dutcher, 1981). In the case of *C. albicans* spheroplast fusion, the nuclear fusion is not a process of conjugation but is an artificially induced state. How or whether any interconnection works in the cell fusion of *C. albicans* remains unknown. As reviewed by Scherer & Magee (1990), the initial formation of large syncytia would be involved after spheroplast fusion. Such syncytia can apparently either

undergo immediate nuclear fusion or become metastable heterokaryons (Sarachek & Rhoads, 1983; Sarachek & Weber, 1984). In the present study, only the fusion derivatives which had undergone such immediate nuclear fusion were examined. In the fusion between *C. albicans* NARA2 and *C. albicans* 1006, or between *C. albicans* 1006 and *C. albicans* A5153, the DNA content of fusion derivatives approximated to the sum of those of both parents, with a few notable exceptions. In contrast, the fusion between *C. albicans* A5153 and *C. albicans* NARA2, resulted in diminished DNA content of the hybrid derivatives. This seemed to depend on the strains used as parents. The combination of A5153 and NARA2 has resulted in instability in their fusion derivatives. Alternatively, the parental *C. albicans* strain 1006 may have some ability to confer stability on fusion products derived from it.

Sometimes fusion of *C. albicans* has been documented to result in the production of hybrids by unidirectional transfer of genetic material leading to aneuploids (Goshorn & Scherer, 1989; Kakar *et al.*, 1983; Sarachek & Weber, 1984). This phenomenon was typically seen when one parent was heterozygous for the partially dominant 5-fluorouracil resistance allele (Whelan *et al.*, 1985). However, in the case of fusion between A5153 and NARA2, fusion derivatives were isolated according to auxotrophic complementation and the auxotrophic markers used here were recessive alleles. As has been shown in *C. albicans* fusion by Goshorn *et al.* (1992), some chromosomes may be stable once transferred to fusion nuclei, however, the other may be unstable and spontaneously lost. A similar phenomenon may have occurred in the fusion products obtained following fusion between spheroplasts of A5153 and NARA2, resulting in frequent chromosome loss or the transfer of a partial set of chromosomes from the donor nucleus to the recipient nucleus prior to karyogamy. Furthermore, in the present study, the other type of chromosome loss observed in hybrids, probably due to chromosome breakage, seems to have occurred in the fusion derivative AN2-2. An extra chromosomal DNA band of the fusant AN2-2 (the smallest band less than 1 Mb in Figs 1 and 2) did not hybridize with the rDNA probe. Further analyses including the use of various DNA probes for hybridization will be needed to determine from which chromosomes it was derived, or where such chromosome breakage occurred in the given chromosomes during the fusion process.

The remarkable variations in the size of chromosome R (alternatively called chromosome 2 or chromosome VIII), which carries a rDNA gene, have been observed in stock strains (Iwaguchi *et al.*, 1990; Wickes *et al.*, 1991), clinical isolates (Asakura *et al.*, 1991) and morphological mutants (Rustchenko-Bulgac *et al.*, 1993). Iwaguchi *et al.* (1992) showed that the clonal size variation of chromosome R is derived from the size change of the rDNA cluster. Clonal size variation of the rDNA cluster region was also found in *S. cerevisiae* (Chindamporn *et al.*, 1993) and recombination in the rDNA cluster has been shown to be affected by several factors, including topoisomerase I and II (Christman *et al.*, 1988), a transcriptional silencer gene

SIR2 (Gottlieb & Esposito, 1989) and a mitotic recombination hotspot (Stewart & Roeder, 1989). The present study showed that, during the process of cell fusion, the size change of the rDNA cluster seemed to have occurred at a very high frequency. However, the fusion derivatives were stable in that their electrophoretic karyotype patterns did not change after several rounds of subculturing in minimal medium as well as in YPD (data not shown), suggesting that clonal size variation of the rDNA cluster region appeared to be suppressed in the derivatives. It is necessary to clarify if the factors described above or other general recombination factors cause the apparent suppression of clonal size variation of the rDNA cluster in the fusion derivatives.

NARA2 (or its parent NUM961) was shown here to possess two types of rDNA repeat units as detected by *HinfI* digestion of genomic DNA. These two types of repeat units were distributed in each homologue of chromosome R. Iwaguchi *et al.* (1992) showed there was no observed change in the individual rDNA basic repeat unit size in the clonal variation of chromosome size in *C. albicans*. We did not observe significant changes in the individual rDNA repeat unit size in the fusion derivatives or in its progeny (data not shown). This supports the explanation that the size variation of the rDNA cluster is due to changes in unit number and not to changes in rDNA unit size. The two classes of rDNA unit of different sizes were distributed on each of the homologous chromosomes of different lengths, which may indicate that recombinations had occurred in the rDNA cluster as well as between the clusters of different chromosomes.

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