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

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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 Candida albicans (Iwaguchi et al., 1990; Lott et al., 1987; Metz 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

Keywords: Candida albicans, spheroplast fusion, chromosome rearrangement, chromosome transfer
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⁺ spheroplast obtained after UV irradiation of C. albicans NUM961 (Suzuki et al., 1989). Another C. albicans strain 1006, carrying Arg⁺ Lys⁻ Ser⁺ Ura⁺ MPA⁺ (mycophenolic-acid-resistant) arg genes 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 PstI 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 x 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 pre-warmed mixture containing 20% (v/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 x 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% (v/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 diaphyamine according to Riggby 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 x 10⁻⁸ 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 x 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 I, Nippon-gene) were cut and applied to 0.9% (w/v) agarose gels (15 x 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 (95-7-h; Vollrath et al., 1988) were used as molecular size reference markers.

Restription 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 HindIII (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, 0.25 M EDTA, pH 7.5) at 70 mA for approximately 3 h. HindIII 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-
Chromosome rearrangement during *C. albicans* fusion

**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 NARAZ (lanes 3 in a and c). The karyotypes of prototrophic fusion derivatives obtained following fusion between A5153 and NARAZ (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 NARAZ 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 Biodyne Transfer 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).

![Figure 1: 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.](image)

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, were absent or present in smaller amounts.

<table>
<thead>
<tr>
<th>Parent DNA</th>
<th>Fusant DNA</th>
</tr>
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<tbody>
<tr>
<td>Mb</td>
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</tr>
<tr>
<td>3.0</td>
<td>AN2-1</td>
</tr>
<tr>
<td>2.5</td>
<td>AN2-2</td>
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<tr>
<td>2.0</td>
<td>AN2-3</td>
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<td>AN2-5</td>
</tr>
<tr>
<td>0.5</td>
<td>AN2-6</td>
</tr>
</tbody>
</table>

**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.
Chromosome rearrangement during C. albicans fusion

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

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

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 regeneration 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 subculturing 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 (±so) of C. albicans parent strains and prototrophic derivatives obtained following spheroplast fusion

<table>
<thead>
<tr>
<th>Strain/derivative</th>
<th>DNA content per cell (fg)</th>
<th>Strain/derivative</th>
<th>DNA content per cell (fg)</th>
<th>Strain/derivative</th>
<th>DNA content per cell (fg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A5153</td>
<td>39.0±0.9</td>
<td>NARA2</td>
<td>38.1±1.3</td>
<td>1006</td>
<td>40.1±4.2</td>
</tr>
<tr>
<td>(A5153 × NARA2)</td>
<td></td>
<td>(NARA2 × 1006)</td>
<td></td>
<td>(A5153 × 1006)</td>
<td></td>
</tr>
<tr>
<td>AN2-1</td>
<td>59.0±0.6</td>
<td>N26-1</td>
<td>74.3±1.9</td>
<td>A6-1</td>
<td>66.7±4.9</td>
</tr>
<tr>
<td>AN2-2</td>
<td>53.7±1.2</td>
<td>N26-2</td>
<td>74.3±4.9</td>
<td>A6-2</td>
<td>71.7±2.0</td>
</tr>
<tr>
<td>AN2-3</td>
<td>71.3±0.9</td>
<td>N26-3</td>
<td>72.6±3.5</td>
<td>A6-3</td>
<td>73.7±2.3</td>
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<tr>
<td>AN2-4</td>
<td>40.0±3.1</td>
<td>N26-4</td>
<td>73.3±1.8</td>
<td>A6-4</td>
<td>65.0±2.0</td>
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<tr>
<td>AN2-5</td>
<td>66.7±2.4</td>
<td>N26-5</td>
<td>73.7±1.5</td>
<td>A6-5</td>
<td>67.7±3.2</td>
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<td>43.0±1.7</td>
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<td>59.0±2.1</td>
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<tr>
<td>AN2-7</td>
<td>35.5±1.3</td>
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<td>35.3±1.2</td>
<td>A6-7</td>
<td>68.3±4.2</td>
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</tbody>
</table>
Fig. 3. (a) Gel electrophoresis of strain-specific HindIII fragments of the C. albicans parent strains. 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 HindIII 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 HindIII, followed by electrophoresis (lanes 1 and 3). The HindIII bands were identified by using the 4.8 kb HindIII 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 HindIII-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-.

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Parents</th>
<th>rDNA type</th>
<th>Total colonies examined</th>
<th>Segregants</th>
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<tbody>
<tr>
<td>N26-7</td>
<td>1006 x NARA2</td>
<td>NARA2</td>
<td>4999</td>
<td>Lys-, 39</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ser-, 1</td>
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<td></td>
<td>His-, 1</td>
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<tr>
<td></td>
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<td>Trp-, 1</td>
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<td>Lys-, Trp-, 1</td>
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<td>Met-, Pro-, 1</td>
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<td></td>
<td>Pro-, 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lys-, 1</td>
</tr>
<tr>
<td>AN2-6</td>
<td>A5153 x NARA2</td>
<td>A5153</td>
<td>7441</td>
<td>Met-, Pro-, 83</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Met-, Pro-, His-, Trp-, 1</td>
</tr>
</tbody>
</table>

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 HinfI, 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 HinfI. 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 HinfI. The digests were separated on a gel and analysed by Southern hybridization analysis using the 4.8 kb HinfI 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 HinfI 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 HinfI digests from each fusion derivative were examined. Fusion derivatives usually contained rDNA-associated HinfI fragments similar in size to the rDNA-associated HinfI 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 HinfI 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 cosegregation 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-associating Hinfl 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 A5153 and C. albicans NARA2, 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 Hinf1 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.

ACKNOWLEDGEMENTS

This work was supported in part by Grant-in-aid (#1480018 and #4640641) for Scientific Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES


Received 29 March 1994; revised 4 July 1994; accepted 1 August 1994.