Isolation and characterization of a repeated sequence (RPS1) of *Candida albicans*

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A repeated sequence, named RPS1, approximately 2 kb in size, is found mainly in chromosome 6, the second most variable chromosome among the eight chromosomes of *Candida albicans*. Most of the RPS1 units of chromosome 6 seem to be located within a single region of about 100 kb in strain FC18. In both strains FC18 and NUM812, a part of RPS1 is apparently tandemly repeated. A unit of RPS1 has been cloned and sequenced. It consists of 2114 bp and has a GC content of 40 mol %. The repeat unit contains smaller repeats of about 80–170 bp which are called REP1, REP2, REP3, REP4 and REP5; REP2 is duplicated. The small repeats are classified into two groups by their homology. One comprises REP1, REP2 and REP5, and the other REP3 and REP4. They are termed the REP1 and REP3 families, respectively. The two families both contain a common 29 bp sequence, called COM29. The dispersed repetitive sequence RPS1 may be involved in chromosomal rearrangements and may in part explain chromosome polymorphism in *C. albicans*. The origin of RPS1 was not determined.

Introduction

The imperfect yeast *Candida albicans*, which is an opportunistic pathogen, exists as a diploid in all known strains; the haploid state has not yet been found (Scherer & Magee, 1990). *C. albicans* contains eight pairs of homologous chromosomes (Iwaguchi *et al.*, 1990; Lasker *et al.*, 1989; Wickes *et al.*, 1991). Chromosome size-variation has been detected in this species (Lott *et al.*, 1987; Magee & Magee, 1987; Merz *et al.*, 1988; Snell *et al.*, 1987; Iwaguchi *et al.*, 1990). In our previous study (Iwaguchi *et al.*, 1990), the chromosomal DNAs of *C. albicans* were resolved into 7–12 bands ranging in size from about 0.4 Mb to about 3.0 Mb by pulsed-field gel electrophoresis (PFGE) and no two strains showed an identical electrophoretic karyotype. Of the eight *C. albicans* chromosomes, chromosome 2, assigned by a MGL1 probe, was more variable in size than the other

chromosomes. The karyotypes of the isolates recovered from individual patients after intervals of 1–6 months were virtually identical; however, one or two chromosomes were variable in size (Asakura *et al.*, 1991). In virtually all cases, the chromosome that varied in size was chromosome 2. This suggested that chromosome 2 is too variable to be useful for distinguishing between strains. A similar variable chromosome was also observed by Rustchenko-Bulgac (1991) and Wickes *et al.* (1991) in different strains. More than 10% of clones of our strains exhibit a change in the size of chromosome 2 (Iwaguchi *et al.*, 1992). Chromosome 2 carries an rDNA gene. The rDNA gene sequence is usually highly repeated within a single chromosomal region, in which DNA rearrangement occurs at a high frequency (Szostak & Wu, 1980). We have confirmed the assumption that the clonal size-variation of chromosome 2 is derived from the rDNA cluster size-change by this type of rearrangement (Iwaguchi *et al.*, 1992).

We found that chromosome 6, assigned by a pTK2-9-1 probe which was prepared from a cloned DNA fragment, was the second most variable in size (Iwaguchi *et al.*, 1990). Chromosome 6 did not always strongly hybridize to a probe which was prepared from whole chromosome 6 DNA. This difference between the fragment probe and the whole chromosome was thought to be due to the presence of a repeated sequence in

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Abbreviation: PFGE, pulsed-field gel electrophoresis.

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chromosome 6 which easily translocates and results in a strong hybridization signal. Several repeated sequences have been isolated in *C. albicans* (Scherer & Stevens, 1988; Sadhu *et al.*, 1991; Soll *et al.*, 1987; Cutler *et al.*, 1988; Lasker *et al.*, 1991) and were characterized as being dispersed throughout the whole genome. In the present study we report a repeated sequence which gives a hybridization profile similar to the chromosome 6 probe and which resides primarily in chromosome 6 of the strains tested.

Methods

Strains and plasmids. The strains of *C. albicans* used were FC18, NUM46, NUM1000, NUM1039, NUM215, NUM47, NUM114, NUM961 and NUM812, whose karyotypes have been analysed previously (Iwaguchi *et al.*, 1990).

The plasmids containing *C. albicans* genes used were pHS100 for TUB2 (Iwaguchi *et al.*, 1990), pTK2-9-1 for pTK2-9-1 (Iwaguchi *et al.*, 1990), and SG864 for ERG11 (Kirsch *et al.*, 1988). The 2.2 kb *Hind*III, 7.7 kb *Eco*RI and 6.6 kb *Hind*III fragments from the respective plasmids were separated by agarose gel electrophoresis and used as probes. The probe name corresponds to the gene contained within the fragment except for pTK2-9-1. pTK2-9-1 was previously called LYS2. The gene does not complement *lys2* mutants, whereas a newly isolated DNA fragment does (Scherer & Magee, 1990; Wickes *et al.*, 1991), suggesting that this nomenclature was incorrect. ERG11 is the *C. albicans* gene for cytochrome P450L1A1 (lanosterol 14 α -demethylase).

The *C. albicans* repeat sequences used were 27A (Scherer & Stevens, 1988), Ca3 (Sadhu *et al.*, 1991), Ca7 (Soll *et al.*, 1987) and the *Msp*I fragment (Cutler *et al.*, 1988).

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

Pulsed-field gel electrophoresis (PFGE). PFGE was carried out by the CHEF method (Chu *et al.*, 1986) using the Pulsarphor 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 at 140 V for 24 h followed by a 1200 s switch at 80 V for 48 h as described previously (Iwaguchi *et al.*, 1990). Chromosomes of *Saccharomyces cerevisiae* (X2180-1A; Mortimer & Schild, 1985), chromosomes of *Schizosaccharomyces pombe* (HM422 derived from strain 972h⁻; Fan *et al.*, 1988), λ concatemer ladder DNA (Bio-Rad) and λ DNA digested by *Eco*T14I (TAKARA Co.) were used as the molecular size reference markers.

Preparation of chromosome 6 probe for Southern hybridization. The chromosome 6 probe was prepared as described previously (Iwaguchi *et al.*, 1992) following separation of chromosomal DNAs by PFGE in a 0.7% agarose gel.

Preparation of genomic DNA. Yeast strains were grown at 30 °C in 50 ml YPD broth and early stationary phase cells were collected and washed once with 30 ml 50 mM-sodium EDTA (pH 7.5) containing 0.3 ml 2-mercaptoethanol. After centrifugation, the cell pellets were suspended in 1 ml 50 mM-sodium citrate buffer (pH 5.8) containing zymolyase 20T (5 mg ml⁻¹; Seikagaku Kogyo Co., Japan) and 0.1 ml 2-mercaptoethanol. After 30 min incubation at 37 °C, 0.5 ml proteinase K solution (2 mg ml⁻¹) and 2 ml 10% sodium dodecyl sulphate were mixed with the suspension and the mixture was further incubated for

2 h at 37 °C and then for 30 min at 55 °C. The lysate was extracted twice with an equal volume of phenol/chloroform (1:1, v/v) and once with an equal volume of chloroform/isoamyl alcohol (24:1, v/v); the aqueous phases were mixed with 2 vols 99% (v/v) ethanol. After storage at -20 °C overnight, the precipitates were removed and washed once with 70% ethanol. Dried precipitates were dissolved in 8 ml TE (10 mM-Tris/HCl, 1 mM-EDTA, pH 8.0) and stored at 4 °C.

Southern hybridizations. Southern hybridizations were performed as described previously (Iwaguchi *et al.*, 1990) using *C. albicans* cloned fragments of plasmids and chromosome 6 of strain FC18 prepared by the method described above.

The intensity of hybridization signal with the probes was measured with the BAS2000 image analyser system (Fuji Film Co.). The signal intensity of the TUB2 probe was used as a reference for a single-copy gene.

***Xho*I digestion of total chromosomal DNA in PFGE sample plugs.** Sample plugs for PFGE were equilibrated in restriction buffer (200 μ l) for 30 min at room temperature. The plugs were then transferred to 200 μ l of fresh restriction buffer containing 20 units of *Xho*I and incubated overnight at 37 °C. After the sample plugs had been washed once with 50 mM-EDTA (pH 9.0), the digested chromosomal DNA fragments in the plugs were separated by PFGE.

Restriction analysis of chromosomes separated by PFGE. Chromosomes were separated in a 0.7% agarose gel by PFGE, stained with ethidium bromide, and destained with distilled water. The chromosome bands were cut out with a razor blade under illumination by 365 nm UV light. Ethidium bromide was immediately extracted from the gel slice with three 10 min washes with 2-butanol saturated with 1 M-NaCl, 1 mM-EDTA, 10 mM-Tris/HCl (pH 8.0). The gel slices were then equilibrated in 200 μ l of restriction buffer and transferred into 200 μ l of fresh restriction buffer containing 20 units of *Sma*I or *Xho*I and incubated overnight at 30 °C and 37 °C, respectively.

For partial digestion of chromosome 6 by *Sma*I, the enzyme concentration was serially twofold diluted from 10 units per 200 μ l restriction buffer. The enzyme reaction was carried out for 4 h at 30 °C.

Cloning of the repeated sequence (RPS1). Genomic DNA of strain NUM812 was digested with *Eco*RI, and the DNA fragments were ligated with *Eco*RI-digested plasmid pUC18. The ligated DNA was transformed into *Escherichia coli* JM109. Plasmid DNA from each transformant was digested with *Eco*RI; the size of inserted fragments was checked by agarose gel electrophoresis. A plasmid containing a fragment of 2.1 kb was selected for further analysis.

DNA sequence. The DNA fragment was cloned into M13mp18 in both orientations. Sequence analysis was performed by the dideoxynucleotide method, with the modified T7 DNA polymerase sequence kit (US Biochemical Corp.) and with the *Bca* polymerase sequence kit (TAKARA Co.). A sequential series of deletions of these clones was prepared by means of a Kilo-Sequence deletion kit (TAKARA Co.). Part of the region was sequenced by using special synthesized primers. We sequenced overlapping clones in both orientations.

Sequence data manipulations and analyses were carried out by the software package, DNASIS (TAKARA Co.).

Results

Hybridization profile obtained by using a chromosome 6 probe

The whole of chromosome 6 from strain FC18 was used to probe chromosomal DNA of nine *C. albicans* strains

Fig. 1. Southern hybridization profiles obtained by using the FC18 chromosome 6 DNA probe (a) and the RPS1 probe (b). The chromosomal DNAs were separated using a 300 s switch time at 140 V for 24 h followed by a 1200 s switch time at 80 V for 48 h. The chromosomes were prepared from *C. albicans* FC18 (lane 2), NUM46 (lane 3), NUM1000 (lane 4), NUM1039 (lane 5), NUM215 (lane 6), NUM47 (lane 7), NUM114 (lane 8), NUM961 (lane 9), NUM812 (lane 10) and from *S. cerevisiae* X2180-1A (lane 1). The numbers on the left indicate the size of chromosomal DNAs from *S. cerevisiae*. In (a), the number 6 on the figure indicates the bands assigned by a chromosome 6 marker probe, pTK2-9-1 or ERG11.

(Fig. 1a), whose electrophoretic karyotype had been previously analysed and for which each chromosome band had been assigned by eight cloned probes (Iwaguchi *et al.*, 1990). In three strains (FC18, NUM1000 and NUM1039), the chromosome 6 probe hybridized to the same chromosome band as assigned by pTK2-9-1 and ERG11 probes. These probes are cloned DNAs used as specific marker probes for chromosome 6 (data not shown). In several other strains, the FC18 chromosome 6 probe did not primarily hybridize to the band corresponding to chromosome 6 as determined by using the specific marker probes ERG11 and pTK2-9-1, or it strongly hybridized to other chromosome bands. To

Fig. 2. *Sma*I digestion profiles of chromosomes. Bands containing either chromosome 4, 5 or 6 of FC18, or chromosome 4 and 5, 6a, 6b, or 7 and 8 of strain NUM812 were cut out from a PFGE gel. Each of the chromosome bands was treated with *Sma*I and separated by PFGE using a linear ramping switch time from 20 s to 40 s at 200 V for 18 h in a 1% agarose gel. Lanes 1 to 3 show the digestion profiles of chromosome 4, 5 and 6, respectively, of strain FC18. Lanes 4 and 5 show the profiles of chromosome 6 of NUM812, whose homologues were separated, the larger being referred to as 'a' and the smaller as 'b'. Chromosomes 4 and 5, or 7 and 8, were not separated in NUM812, so the mixed-band profiles are shown in lanes 6 and 7. Numbers on the left indicate the size markers of λ ladder concatemer and λ DNA digested by *Eco*TI4I.

clarify this apparent contradiction, we investigated one case in more detail. Because the FC18 chromosome 6 was recognizing bands corresponding to NUM812 chromosomes 4/5 and 7/8, we isolated these bands and NUM812 chromosome 6 bands and subjected them to restriction digest analysis using *Sma*I in an attempt to identify restriction fragment polymorphisms (Fig. 2). The digestion pattern of chromosome 6 of FC18 was similar, although not identical, to that of chromosome 6 of NUM812, which very weakly hybridized to the FC18 chromosome 6 probe. However, the strongly hybridizing bands (containing chromosome 4 and 5, or chromosome 7 and 8) of NUM812 did not give similar patterns to that

Fig. 3. Hybridization profile of a chromosome 6 probe with genomic DNA of FC18 and NUM812. The FC18 chromosome 6 probe was hybridized to total genomic DNA digested with *Eco*RI (E), *Bam*HI (B), *Hind*III (H), *Pst*I (P) or *Sma*I (S).

of chromosome 6 (Fig. 2, lanes 6 and 7). This suggests that the FC18 chromosome 6 probe recognized other chromosomes whilst the cloned specific marker probes were more useful for chromosome assignment in this case.

The intensity of the FC18 chromosome 6 hybridization signal was very strong and was similar to that obtained when using chromosome 2, which carries a highly repeated rDNA gene cluster, as a probe for PFGE blots (Asakura *et al.*, 1991; Iwaguchi *et al.*, 1992). From the above lines of evidence, it is inferred that chromosome 6 of FC18 contains a sequence which frequently translocates, resulting in strong signals on other chromosomes. This feature suggests the presence of a repeated sequence or a transposable element.

Cloning of a 2.1 kb repeated fragment (RPS1)

When the FC18 chromosome 6 probe was hybridized to total genomic DNA digested by various restriction

Fig. 4. Distribution of RPS1 in chromosome 6. (a) Partial digestion profiles of chromosome 6 by *Sma*I. The FC18 chromosome 6 band was cut out from a PFGE gel and partially digested by *Sma*I. The enzyme concentration was serially diluted twofold from 1 to 10. After PFGE using a linear ramping switch time from 5 s to 25 s at 200 V for 10 h in a 1% agarose gel, Southern hybridization using the RPS1 probe was carried out. (b) *Xho*I fragments containing RPS1. PFGE sample plugs (Total chr.) and excised chromosome 6 band (Chr. 6) were treated with *Xho*I and separated by PFGE using a linear ramping switch time from 10 s to 40 s at 200 V for 15 h in a 1% agarose gel. After PFGE, Southern hybridization using a RPS1 probe was performed. The arrowheads show two fragments (86 and 108 kb) detected in *Xho*I digests of the chromosome 6 band. The numbers on the left of (a) and (b) indicate the size markers of λ concatemer ladder and λ DNA digested by *Eco*T14I or *Hind*III.

enzymes (*Eco*RI, *Bam*HI, *Hind*III, *Pst*I and *Sma*I), it strongly hybridized to a 2.1 kb fragment after *Pst*I and *Sma*I digestion of FC18, and *Eco*RI, *Pst*I and *Sma*I digestion of NUM812 genomic DNA (Fig. 3). This suggests that chromosome 6 of FC18 contains a 2.1 kb repeated sequence.

To clone the 2.1 kb fragment, genomic NUM812 DNA was digested by *Eco*RI and fragments were cloned into the *Eco*RI site of pUC18. The insert sizes were checked by *Eco*RI digestion, and a plasmid, pSI3-12, containing a 2.1 kb fragment was obtained. This fragment probe gave similar hybridization profiles as the FC18 chromosome 6 probe (Fig. 1b). This suggested that the high-intensity signals obtained when using the FC18 chromosome 6 probe were mainly derived from the 2.1 kb repeated fragment. This cloned 2.1 kb fragment was named RPS1.

Although the RPS1 probe strongly hybridized to the chromosome bands usually assigned by pTK2-9-1 or ERG11, it also weakly hybridized to almost all of the other chromosomes (Fig. 1b). In strains NUM215, NUM47, NUM114 and NUM812, some chromosomes other than chromosome 6 strongly hybridized to the RPS1 probe. It is worth noting that only chromosome 4, as assigned by the ADE2 probe, did not hybridize to the RPS1 probe in any of the strains tested.

Distribution of the RPS1 sequence on the chromosome

RPS1 contains unique *Pst*I and *Sma*I sites. When total genomic DNA was digested by either of these two enzymes, a fragment of the same size as RPS1 was detected (Fig. 3). This strongly suggested that some RPS1 sequences were tandemly repeated. To confirm this, chromosome 6 of FC18, which was prepared by cutting out chromosome 6 separated on PFGE, was partially digested by *Sma*I and hybridized to the RPS1 probe (Fig. 4). The RPS1 repeat unit was strongly detected in the completely digested sample (Fig. 4a, lane 1), and the fragments with sizes corresponding to multiples of the RPS1 unit were detected as a ladder of bands in partially digested samples (Fig. 4a, lanes 3–7). The ladder seems to consist of at least ten bands, suggesting that a minimum of 10 RPS1 units constitute a tandemly repeated cluster.

The intensity of the RPS1 hybridizing signal in *Sma*I fragments of total chromosomal DNA in sample plugs of strains FC18 and NUM812 was measured by the BAS2000 image analyser system. The RPS1 signal of FC18 and NUM812 was about 30 and about 40 times, respectively, stronger than that of the TUB2 signal in the same *Sma*I-digested sample. Using the signal of the TUB2 probe, whose GC content is similar to that of the RPS1 probe (see below), as a reference example for a

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1 GAATTCGCGG TGATGTCGGT TGAAGCTGC GCAATGAAA ATAACGCTAC AAAATCAAA
61 CTAGTGGCGA TTTATACCTT TTCTTATGA GTGCTAACCA TGCAGAACT GTTAGAAACG
121 AAATCAACT GCTATCTGTG GAACAAAAA GCGCGTTTTG GCCATAGTA AGGAGCGCG
181 AGCTATGCTC GATCACAAC ACGCGACCAA ATTCAACGCT AAAAAATCA AACTAGTGGC
241 GATTATACC TTGGATTAT GAGTCTATC CCGCAAGAA CTGTTAGAAA CGAATACAA
301 CTGCTATCTG TGAACAAAA AAGCGCGTTT TGCCCATAGT TAAGGAGGCC GCAGCTATGT
361 CTGATCACA CTACGCGACC AAATTCACG CTACAAAAA CAACTAGTG CGATTATTA
421 CTTTGGATT ATGAGTTCTA TCCCTGCAAG AACTGTTAGA AACGAATAC AACTGCTATC
481 TGTGGAACA AAAAGGCGGT TTGGCCATA GTTAAGATC ACTCGTGT TTATTTTGC
541 TACTGCTCGA CCAATAGAGG CGTTACAAG ACCAACTAA TACCGTTTG AAGCCAGAC
601 AAATGTTTAT TATACGACA CGATGCTGGT TGCGTAGGC GAATGATTG AGATGTACA
661 AAAAAAAGG CCGTTTTTTT ATCCCTTTT TCTAACCAA TTTTCAGCA AAAACACCA
721 GATGCTTGC CACCTAATC CGTTGACAG AAAACATCA ATTTGATGC CAGCAAGTC
781 ATTCAAAGAT CAATTGAAG CTACATGTTT GGGGAGAAA CCACTAGATT TTGAACAAA
841 TCGATGGCAC ATTGGAAGCT CATTGCGAT TAAGTGGAT ATTACATAG TGAATTTGG
901 GGGTGTCTG TACAGCAACT TTTGTTAGA CCAACTATG CTTTITTTG ATCAATCTGT
961 AGCCACCGTT TTTAGGTAT ATACCCAGTA ATATGTTGAA TGTAGTGT GTATTGAATG
1021 AACACGCTGT GTTTTATGT TTGTTCAATC GTTTTGAAG AATCGCTACC AATGAGGCA
1081 GAATGTTAG GAAAGTCTA AAATCGTTAA TAATCTGTC AATATAGTG CTAGACTTC
1141 CAAAGTCGG TCACCTTAAA GCGCATTTT GTAGCAACT GTCAATCAA GTTTGTGCA
1201 AGGAAGTCCA ATATTGGTG AGATATTGTC AAAAAACCA TATTTCAAG TTGCATCCA
1261 ACTACATAG TTAAGAAGA AAGTTGAAG GTGAGAGTA GAGGAGGCT ACAGAGCCA
1321 ATCAGGTGCC GATTGTGGC CTACAGTTGT GTTTTTAAA CCGAGAGAA TCGTTAATA
1381 CGGTAATTAG TTGGTGTCTG CAGGACAAA AAGCGCGTTT TGCCATAGT TAAGAGCAC
1441 CTGTAACCC CGTTGCTAA TAGCACACC AATTGAAGT GGTATTGT GGTCTGTGT
1501 TCAATTTATA GCCAACATA AACATTTTCA AATCGTCTA AACCGGTCAA AAGAAGATT
1561 GAGCTTCAT CTCTGGTCA AAAAAGCGG TTTTGGCAT AGTTAAGAC ACCCCCTTT
1621 TGTAGCAAA CCAATTGAAG TTGGTATTTG GTGGCTCTAG TGCCGATTG TAGAGTCAAG
1681 TTATAGTGT TCATCCGAG AGTTTGTATT TATTCAGTGT TGTITTCATT GGTGAGGCG
1741 AAAAGTTTC CATCGAGCG AAAAGGTCGT GCGCGGGGC ATAGTGGATA GACAGCAAT
1801 ACTGATGAC CACATGTGCT ACAAGACCA AACTAGGCC GTTTTGAAG TACATCATG
1861 TAGAGTATTG GGTGTGAAT AGGCATGAT CGATCAGAA TTGTTGAGC TATTGAGAA
1921 AACGTTTTCT CCGTGGAAAT GTGAATTTAA CTCGCCAAG GCTGACACAG TCACCTTCA
1981 TGCTAGAAAG ACCCACTAG TGCCAGTTCA TAGCCATAG ATGTAAGTAC TATGACTGT
2041 AGAGCTGTTA GAACAAGGT TCAATTGCTT TCTGTAGAAC AAAAAAGCC GTTTTGTCCA
2101 TAGTTAAGGA ATTC

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Fig. 5. Nucleotide sequence of RPS1. The sequence of RPS1 is 2114 bp long. The repeat sequences within it, namely REP1 (39–172, 134 bp), REP2 (173–344 and 345–516, 172 bp), REP3 (1407–1511, 105 bp), REP4 (1579–1673, 95 bp), REP5 (2027–2108, 82 bp) and REP6 (662–677, 16 bp), are underlined, with the corresponding number circled.

gene present in a single copy per haploid genome, it was inferred that about 60 and about 80 units of RPS1 were contained in single FC18 and NUM812 cells, respectively.

When chromosome 6 DNA of FC18 was digested by

*Xho*I, RPS1 fragments were detected as 108 kb and 86 kb bands (Fig. 4b, lane 2). Because the FC18 chromosome 6 sample contains two homologues, the two fragments may reside in different homologues, or in two different places within one or both homologues. From the total chromosome digestion data (Fig. 4b, lane 1), many RPS1 units may be dispersed or clustered in a very limited area of chromosome.

Sequence of RPS1

The DNA sequence of RPS1 is shown in Fig. 5. RPS1 consists of 2114 bp and its GC content is 40 mol%. Only one long open reading frame was found, from nucleotides 653 to 973. However, analysis using Fickett's method did not suggest a translated protein.

In the RPS1 sequence, we found smaller repeated sequences which were named REP1 (39–172), REP2 (173–344 and 345–516), REP3 (1407–1511), REP4 (1579–1673), REP5 (2027–2108) and REP6 (662–677). REP2 is tandemly duplicated. The homology between these repeats is compared in Fig. 6(a). REP1 and REP2 are tandemly repeated; however, 36 bases of the 5' region of REP2 are not homologous. The homologous regions are 95% identical. REP5 is also homologous to REP2 but 90 bases of the 5' region are missing, and the rest of the region is less homologous (81% identical) compared to REP1. Assuming RPS1 is tandemly repeated, the 3' end of the RPS1 repeat unit adjoins the next 5' end. Therefore, REP5 and REP1 are directly repeated with about 39 bases of spacer sequence. Accordingly, the four

Fig. 6. Structure of RPS1. (a) Comparison of inner repeat sequences in RPS1. Positions at which the same base occurs as in REP2 or REP3 are indicated with an asterisk. Gaps between sequences are indicated with dashes. The initiation and end point, and the sizes of the REP sequences, are shown in the legend to Fig. 5. The 29 bp common sequence, COM29, is indicated by a line. (b) Schematic structure of the repeated sequence of RPS1. The brackets below the figure indicate the inner repeat regions, REP1, 2, 3, 4, 5 and 6. The REP1 and REP3 families are denoted by stippled and open arrows, respectively. The filled arrowheads indicate the half of the COM29 sequence which corresponds to REP6. The open arrowheads indicate the other half of COM29. The downward-pointing open arrowheads indicate restriction sites: *Eco*RI (E), *Pst*I (P), *Sma*I (S) and *Sfi*I (Sf). The upper scale line shows the length of RPS1 (bp).

direct repeats of REP5, REP1, REP2 and REP2 seem to be adjacent. REP3 and REP4 are directly repeated with 67 bases of spacer sequence although REP4 has a 9 bp gap in the middle region of REP3. REP1, REP2, REP3, REP4 and REP5 all contain a 29 base common sequence, CAAAAAAGGCCGTTTGGCCATAGTTAAG. REP6 has only 16 bases homologous with the first half of the 29 bp common sequence. A schematic location of the repeated sequences for RPS1 is shown in Fig. 6(b).

Discussion

A repeated sequence, RPS1, approximately 2 kb in size, was found primarily on chromosome 6, which is the second most variable of the eight chromosomes of *C. albicans*. The most variable chromosome, chromosome 2, carries rDNA which is only located on chromosome 2, in a tandemly repeated fashion. The rDNA unit size is estimated to be 15 kb. The number of rDNA units varied from about 50 to about 130 per cell among the strains tested in our previous study (Iwaguchi *et al.*, 1992). The number of RPS1 units is about 80 per cell, but the number may be very variable between strains because intensity of the hybridization signal varies between strains; for example it was extremely weak in NUM1000 (Fig. 1b, lane 4). RPS1 or homologous sequences were present not only on chromosome 6 but also on all other chromosomes except chromosome 4.

Most of the chromosome 6 RPS1 units seem to be

located within a very limited region of about 100 kb in strain FC18. At least in strains FC18 and NUM812, a part of RPS1 is inferred to be tandemly repeated from the evidence that the same-sized fragments were detected by digestion with various enzymes (Fig. 3). More direct evidence was obtained by a partial *Sma*I digestion of chromosome 6 from strain FC18. A ladder of bands corresponding to multiple copies of RPS1 was detected by Southern hybridization and the ladder number was at least 10 (Fig. 4). This suggested that more than 10 RPS1 units are tandemly repeated in chromosome 6.

RPS1 contains small repeat units, about 80–170 bp in size, which we have called REP1, REP2, REP3, REP4 and REP5; REP2 is duplicated. The REPs are classified into two groups by their homology (Figs 5 and 6). One, the REP1 family, contains REP1, REP2 and REP5; the other, the REP3 family, contains REP3 and REP4. Both families contain a common 29 bp sequence, called COM29. This 29 bp sequence resembles a specific recombination site such as the λ attachment site and the site of DNA inversion/crossing-over in bacteria (Ehrlich, 1989). For example, the site of bacterial DNA inversion is a 26 bp sequence, composed of a 12 bp imperfect palindrome that consists of a GC-pair-rich core and an AT-pair-rich outer region. The first part of COM29 is composed of a 16 bp imperfect palindrome which resembles the bacterial DNA inversion sequence. The 16 bp palindrome of COM29, REP6, is present away from the other REPs. COM29 might be a candidate for a specific recombination site in *C. albicans*. REP2 might have been duplicated very recently because the copies are identical and duplication has occurred in the sequences flanked by COM29. This may support the notion that COM29 is involved in recombination events.

It is noteworthy that the COM29 region of REP1, REP2 and REP4 contains a cutting site for *Sfi*I, which is an 8-base recognition restriction enzyme and useful for genome analysis. Genome mapping of *C. albicans* is being carried out by using *Sfi*I (P. T. Magee, personal communication).

Dispersed repetitive elements have been characterized in chromosomes of various organisms and are causal agents of chromosomal rearrangements such as deletions, duplications, inversions and translocations (Petes & Hill, 1988; Shapiro, 1983). The *S. cerevisiae* genome contains three repetitive elements, delta, tau and sigma, which are several hundred base pairs long. The delta and sigma elements were identified as the transposon terminal repeats, Ty elements. Tau is thought to be a similar element because of the significant regions of homology with the delta and sigma sequences (Genbaur et al., 1984). In *C. albicans*, transposon-like elements have not been identified yet. Several repeated sequences have been isolated in *C. albicans*: 27A of 15 kb

(Scherer & Stevens, 1988), Ca3 of 12 kb (Sadhu et al., 1991), Ca7 of 9.5 kb (Soll et al., 1987), the *Msp*I fragment of 2.9 kb (Cutler et al., 1988), Care-1 of 0.47 kb (Lasker et al., 1991), and Care-2 of 1.1 kb (GenBank, M74014). Ca7 has been shown to be a telomeric or subtelomeric repeat (Sadhu et al., 1991). Only Care-1 and Care-2 have been sequenced; the sequences are not homologous to the RPS1 sequence or to each other. RPS1 strongly hybridized to 27A and Ca3 but not to the *Msp*I fragment or Ca7 (data not shown). Thus RPS1 seems to contain a sequence of the same repeat family as the sequence contained in 27A and Ca3. A detailed comparison of RPS1 and 27A or Ca3 is in progress.

We failed to find significant homology with any known sequence within the GenBank or EMBL databases. At present, the origin of RPS1 is unknown.

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