Repetitive sequences (RPSs) in the chromosomes of Candida albicans are sandwiched between two novel stretches, HOK and RB2, common to each chromosome

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A novel sequence designated HOK, which is next to the RPS, a repetitive sequence specific to Candida albicans, was cloned and sequenced. HOK hybridized with all of the chromosomes on which the RPSs were located, but did not hybridize with chromosome 3, which does not harbour any RPSs. Sequence determination revealed that a portion of HOK has significant homology with the B and C1 fragments of Ca3, which is used as a molecular epidemiological probe. A homology search of the deduced amino acids of HOK against the protein database showed partial homology with an isocitrate dehydrogenase of Saccharomyces cerevisiae, although an ORF large enough to encode the enzyme was not detected. To verify the existence of other sequences homologous with HOK, a portion of the HOK sequence was amplified using PCR. Sequence determination of the 41 clones from the PCR products resulted in at least six HOK-homologous clones. Another RPS-containing clone, RB2, was isolated from the PstI-digested chromosome R or 1. It was determined that RB2a, one of the subclones from RB2, hybridized with all of the chromosomes, including chromosome 3, with which neither HOK nor RPS hybridized. The hybridization profile also showed that RPS is located between HOK and RB2a on chromosomes other than chromosome 3.

Keywords: repetitive sequence, RPS, physical map, Candida albicans

INTRODUCTION

A characteristic of the yeast Candida albicans is the variability in the size and number of its chromosomes (Magee & Magee, 1987; Merz et al., 1988; Iwaguchi et al., 1990; Doi et al., 1992). This yeast has eight chromosomes per haploid, and lacks a sexual state in its life cycle, proliferating exclusively as a diploid cell (Scherer & Magee, 1990; Iwaguchi et al., 1990; Chu et al., 1993). We have analysed the genetic elements which might be involved in the variations in the chromosome size and in due course have found repetitive sequences, RPSs, which may serve as recombination hot spots generating their variability (Iwaguchi et al., 1992; Chibana et al., 1994; Chindamporn et al., 1995). The RPSs are located on all chromosomes except chromosome 3 (formerly 4 in our previous chromosome numbering system; Nakagawa, 1996) and are tandem-arrayed in some chromosomes (Chibana et al., 1994). These sequences could have some functional significance, considering that they appear on almost all chromosomes. Many of the genetically identified centromeres are known to include repetitive sequences (Takahashi et al., 1992; Centola & Carbon, 1994). A structural analogy between the RPS and human alpha satellite DNA, a possible human centromere, has been suggested (Chibana et al., 1994). However, Chu et al. (1993) suggested, based on SfiI macrorestriction frag-
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Detection of the RPS-positive fragments in a Smal-digested chromosome. Each chromosome from FC18 fractionated by the first PFGE (see Methods) was digested with Smal. The digests were then fractionated by the second PFGE under conditions of 0.8% (w/v) agarose and of 20-100 s with a ramped switching time for 15 h at 180 V. After blotting to the membrane (Hybond-N; Amersham), Southern hybridization was performed using 32P-labelled RPS101 as a probe. Chromosome numbers are indicated on top and the numbers on the left represent the molecular sizes, which were estimated from the DNA standard size markers (see Methods). Chromosomes R and 1 were not separated under the PFGE conditions used here.

Fig. 1. Detection of the RPS-positive fragments in a Smal-digested chromosome. Each chromosome from FC18 fractionated by the first PFGE (see Methods) was digested with Smal. The digests were then fractionated by the second PFGE under conditions of 0.8% (w/v) agarose and of 20-100 s with a ramped switching time for 15 h at 180 V. After blotting to the membrane (Hybond-N; Amersham), Southern hybridization was performed using 32P-labelled RPS101 as a probe. Chromosome numbers are indicated on top and the numbers on the left represent the molecular sizes, which were estimated from the DNA standard size markers (see Methods). Chromosomes R and 1 were not separated under the PFGE conditions used here.

Fig. 2. Detection of the CS-positive fragments in a Smal-digested chromosome. The probe (RPS101) of the blot used in Fig. 1 was detached with a boiling solution of 0.5% SDS and 0.1 x SSC twice. After confirmation that no signals were detected from the membrane, a 32P-labelled CS fragment was applied for Southern hybridization as a probe against the membrane. The numbers indicated are the same as those used in Fig. 1.

METHODOLOGY

Strains and culture conditions. C. albicans strains FC18 and 1006 and eight other clinical isolates of C. albicans (NUM46, 1000, 1039, 215, 47, 114, 961 and 812) were used (Iwaguchi et al., 1990). Cells from the late-exponential or early-stationary phase aerobically cultured in YPD (1% yeast extract, 2% peptone, 2% dextrose) for 16 h at 30 °C were used for DNA preparation.

Preparation of genomic DNA. The sample plug containing yeast chromosomal DNAs for PFGE was prepared by the method described previously (Iwaguchi et al., 1990). PFGE was carried out by the contour-clamped homogeneous electric field method using the Pulsaphor system with a hexagonal electrode array (Pharmacia-LKB Biotechnology) as described previously (Iwaguchi et al., 1990). Sample plugs were also used as a source of genomic DNA for restriction enzyme digestion.

Chromosomes of Saccharomyces cerevisiae (X2180-1A), catenomeric DNA of lambda phage (Bio-Rad), and lambda phage DNA digested by EcoT14I (Takara Shuzo) were used as molecular size markers.

Smal digestion of individual chromosomes. Each chromosome from FC18 was separated by PFGE under conditions of 0.8% (w/v) agarose and of 100 s at 170 V for
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**Fig. 3.** Physical and genomic characterization of the 6 kbp fragment. (a) Physical map of the 6 kbp fragment. The hatched box indicates an RPS region. I, II, III and IV represent the CS subfragments. Subfragments I, II and III were used as probes in (b). i, ii and iii indicate primers 463, 464 and 466, respectively, used in Fig. 6. C, Clal; E, EcoRI; P, PstI; S, SmaI. (b) Hybridization profile of the CS subfragment on each chromosome. The chromosomes of FC18 were fractionated by PFGE (running conditions: 0-8% agarose, 300 s, 140 V, 40 h, and 1200 s, 80 V, 48 h), which was followed by Southern hybridization using the following probes: 1, RPS; 2, fragment I; 3, fragment II; 4, fragment III. The CS subfragments I, II and III are defined in (a).

15 h, 300 s at 130 V for 24 h then 1000 s at 80 V for 48 h. The running temperature was 12 °C throughout the experiment. The individual bands corresponding to each chromosome were cut out from the gel under UV light (365 nm) after staining the gel with ethidium bromide. The samples were subjected to SmaI digestion as described previously (Chindamporn et al., 1995).

**Southern hybridization.** Southern hybridization was performed by using one of the following probes: a PstI digest of pRPS101 for detection of RPSs (Chibana et al., 1994), a Clal-SmaI fragment (CS fragment) of the 6 kbp fragment which did not contain RPSs (for the 6 kbp fragment and the CS fragment, see below and Fig. 3a), and RB2a, which was one of the subclones of the RB2 fragment (for RB2, see below). The probes were prepared by the random primer-labelling method with [32P]dCTP, and the hybridization signals were detected with an image analyser (BAS 2000; Fuji Photo Film).

**Cloning of the 6 kbp fragment.** Genomic DNA of FC18 in a sample plug was digested by SmaI as described above, and the fragments were separated by PFGE. A band of approximately 6 kbp, as estimated by the molecular size marker, was cut out under UV light (365 nm). After phenol/chloroform extraction and ethanol precipitation, this fragment was integrated into the SmaI site of pBluescript II SK(+) (Stratagene) and transformed into *Escherichia coli* XL-1 Blue (Stratagene). The white-coloured recombinants were selected on LB plates (1% tryptone, 0.5% yeast extract, 1% NaCl and 1.5%, w/v, agar) containing 50 μg ampicillin ml⁻¹, 20 μg X-Gal ml⁻¹ and 0.1 mM IPTG. The recombinants were further screened by colony hybridization, using RPS as a probe, because the fragment in question was known to contain a part of the RPS (Chindamporn et al., 1995).

**Cloning of the RB2 and RB3 fragments.** The chromosome band containing R + 1 was excised from the gel after PFGE and digested with PstI. The resulting DNA was recovered by ethanol precipitation and integrated into a PstI-cleaved pUC18 vector. The RPS-positive clones were located by colony hybridization using [32P]-labelled pRPS101 as a probe. Of these, a clone which contained a larger insert size of plasmid than RPS was screened further. By using a similar procedure, the RB3 fragment was isolated from chromosome 2.

**Sequencing.** Subclones of the 6 kbp fragment were constructed by either excision of the 6 kbp fragment with appropriate kinds of restriction enzymes or sequential deletion with exonuclease III and mung bean nuclease (Takara Shuzo). The sequences of all the clones were determined with a DNA Sequencer (model 373A; ABI) using Taq Dye Primer Cycle...
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Fig. 5. Homology search of HOK against the protein database.
(a) Homology of the deduced amino acid sequence from nucleotide positions 2253-2546 of HOK with the amino acid sequence of the isocitrate dehydrogenase of S. cerevisiae (IDH1; Cupp & McAlister-Henn, 1992). The number above the HOK sequence is the amino acid position of a hypothetical ORF of HOK, and the number below the sequence of IDH1 is the amino acid position of Idhlp. Identical amino acids among the six sequences are indicated by bold letters. The alignments of colons and physico-chemically related amino acids are shown by dots and numbers.

Sequencing kits (Perkin Elmer). Analyses of the data were carried out with the software GENETYX-MAC (Software Development).

Amplification and cloning of the HOK-related sequences. The HOK-related sequences were amplified by PCR. The primer sets used were 5'-GAGACCATGTCATATTGC-CCCC-3' (463; forward, position 1870-1891 in the 6 kbp fragment), 5'-CATTTCCGGAGCATATTATGGC-3' (464; forward, 1934-1957) and 5'-CACACCCCCAGTTGG-ATATCCAG-3' (466; reverse, 2134-2156). PCR was performed using Taq polymerase and its accompanying buffer (Takara Shuzo) with the Thermal Cycler (model PJ-2000; Perkin Elmer). Amplification conditions were as follows: 94 °C for 5 min prior to the cycling step; 35 cycles of 94 °C for 20 s, 50 °C for 20 s and 72 °C for 90 s for amplification; followed by 37 °C for 5 min for extension. After amplification, the PCR products were isolated by agarose gel electrophoresis and GeneClean II (Bio101) and digested with EcoRI. The digested products were then introduced into EcoRI-cleaved and alkaline-phosphate-treated pluebriII SK(+)- and transformed into XL-1 Blue.

RESULTS
Detection and cloning of a new conserved sequence
It has been reported that the RPS on each chromosome is located in a limited region, and that on chromosome 5 (chromosome 6 in our previous numbering system) it is flanked by 61 kbp and 130 kbp Smal fragments (Chindamporn et al., 1993). To obtain further information about the RPS-flanking regions on the other chromosomes, each chromosome previously fractionated by PFGE was treated with Smal. The resulting fragments were separated by a second PFGE, followed by Southern hybridization using RPS as a probe (Fig. 1). The bands were categorized into two groups: one was derived from the RPSs themselves, ranging in size from 1.9 kbp to 2.7 kbp (Chibana et al., 1994), and the other was thought to be derived from the boundary regions of the RPS(s) on the respective chromosomes. As shown in Fig. 1, bands of approximately 6 kbp in size were detected in almost all chromosomes, except for chromosome 3, indicating that fragments similar in size to the Smal fragments are present on almost all chromosomes. As these fragments also contain a part of an RPS sequence, it could be interpreted that the Smal recognition site is at a locus which is the same distance from the RPS region on each chromosome. If this is the case, it may be expected that a portion of the sequence in those Smal fragments other than just the RPS region is also conserved, because it is unlikely that it is only by accident that the Smal site is present at the same distance from the RPS region on each chromosome. To verify this assumption, we attempted to clone a new Smal fragment of approximately 6 kbp that could be hybridized with an RPS probe from the genomic DNA of strain FC18.

CS, a conserved fragment
The 6 kbp Smal cloned fragment included a portion of the RPS. Therefore, by digestion of the fragment with ClaI, we constructed a fragment which did not contain a portion of an RPS. This new fragment, designated CS, was then used as a probe for detection of other members of conserved sequences in the genome. After dehybridization of the RPS probe shown in Fig. 1, the same blot was hybridized with a CS probe. As shown in Fig. 2, at the position that corresponds to a size of approximately 6 kbp, distinct signals were detected in chromosomes R+1, 2, 4, 5, 6 and 7. A few extra bands at about 15 kbp and 60 and 80 kbp were detected in chromosomes 2 and 7, respectively. The positions of the signals of about 6 kbp in size which were detected by the CS probe coincided with those detected by the RPS probe. These data indicate that the novel sequence which is distinct from but is neighbouring the RPS is conserved in most of the chromosomes. Physical mapping of the 6 kbp fragment showed that the PstI recognition sites are near the Smal sites at both ends of the fragment. A similar result was obtained by CS probe hybridization against PstI-digested chromosomes (data not shown). This indicates that both Smal and PstI recognition sites are conserved on almost all chromosomes. Because the CS fragment is quite large, approximately 5.4 kbp, we asked which portion(s) of the CS is(are) responsible for the hybridization profile. To answer this question, a CS fragment was divided into four portions: fragment I, 1.5 kbp ClaI-EcoRI; fragment II, 0.6 kbp EcoRI-EcoRI; fragment III, 3 kbp
EcoRI–PstI; and fragment IV, 0.2 kbp PstI–SmaI (shown in Fig. 3a). Each fragment except IV was used as a probe to hybridize the separated chromosomes of FC18. The results are shown in Fig. 3(b). All three probes hybridized to the FC18 chromosomes 4, 5, 6 and 7 with a similar hybridization pattern, suggesting that almost the whole region of the CS fragment is conserved. It was suggested that the 6 kbp fragment cloned here was derived from one of four chromosomes (chromosome 4, 5, 6 or 7), because the intensity of the signal from chromosomes R, 1 and 2 was relatively weak.

The CS-homologous sequence is conserved among different C. albicans strains

To examine whether or not the sequence of the CS fragment was also conserved in other strains, the following experiment was performed. Chromosomal DNA from clinical isolates obtained at the University Hospital and from the standard strain 1006 was digested with SmaI, followed by Southern hybridization using the CS fragment as a probe. As shown in Fig. 4, two to four bands were detected in all strains used. The band corresponding to the 6 kbp fragment was clearly observed with high intensity in all strains examined. We also observed that the CS fragment could hybridize with the C. albicans-related species Candida stellatoidea (data not shown). In addition to the 6 kbp fragment, most of the strains have one to three extra bands corresponding to the CS fragment hybridization. In strain FC18, these signals are derived from the chromosomes R + 1 and 7 (see Fig. 2).

Sequencing analysis and a new conserved sequence, HOK

We determined the base sequence of the 6 kbp SmaI fragment. The fragment is 5782 bp and the G+C content is 35 mol%. One boundary of the 6 kbp fragment included 523 bp of the RPS, and we were able to determine the boundary of the RPS. The sequence of an RPS portion in the 6 kbp fragment did not match with any of the other RPSs already sequenced and with alt, the inner short repetitive sequences in RPS. Thus, we named the 5259 bp fragment, the sequence in which the RPS portion was excluded from the 6 kbp fragment, as HOK. A homology search using the FASTA program revealed a high degree of homology with the B fragment of Ca3, which has frequently been used as a molecular epidemiological probe (Sadhu et al., 1991; Anderson et al., 1993). Most of the B fragment was included in HOK. Recently, Lockhart et al. (1993) sequenced the C1 portion of the C fragment, another EcoRI fragment of Ca3, and reported that C1 contains an RPS element. By comparing the sequence of C1 with that of the 6 kbp fragment, we confirmed that the C1 fragment contains portions of both the RPS and HOK, with a total homology of 95%. Interestingly, the homology between the HOK-related portion of the C1 fragment and HOK was 79% (97 bp in 123 bp), while that between the RPS-related portion of the C1 and the 6 kbp fragment was 99% (851 bp in 859 bp). An ORF search showed three small ORFs within HOK. Surprisingly, the polypeptide sequence determined from one of the ORFs analysed by the FASTA program showed significant homology with part of the isocitrate dehydrogenase protein of the yeast S. cerevisiae, as shown in Fig. 5(a) (Cupp & McAlister-Henn, 1992).

Multiplicity of HOK-related sequences

A CS hybridization experiment (Fig. 2) showed that HOK-related sequences were located on many chromosomes. If there is a multiplicity of HOK-related sequences in the C. albicans genome, it would be possible to obtain different clones when PCR is performed using part of the HOK sequence for amplification. We amplified 1.3 or 1.4 kbp fragments containing the region flanked by EcoRI using primer sets 463–466 and 464–466 (see Fig. 3a; data not shown). After treatment of the PCR products with EcoRI, they were integrated into a pBluescript II SK(+) vector. The 233 clones isolated were classified into 41 groups according to the size of the insert, the digestion profile with HindIII and BamHI, and a Southern blot analysis using the CS fragment as a probe. The sequences of all 41 clones were determined, and they could be categorized into seven kinds of sequence, including the original sequence of HOK (Fig. 6). These results strongly suggest that there are at least seven kinds of HOK-related sequences in the C. albicans genome. Homologies among these sequences ranged from 82% to 97% (data not shown). Of the seven groups, two groups (646620 and 636602) had a 23 bp insertion, and another two clones (646608 and 646611) had an additional EcoRI recognition site.

Another kind of conserved sequence, RB2

From comparing Fig. 1 with Fig. 2, we expected that most signals larger than 6 kbp would be derived from the other boundary of RPS, opposite to HOK. Therefore, we tried to isolate these fragments under conditions which would allow them to hybridize with the RPS probe but not the CS probe and be larger than an RPS (see Methods). As a result, we obtained two clones, named RB2 and RB3. The first of these clones, RB2, was digested using EcoRI and was subcloned into a pBluescript II SK(+) vector. One of the subcloned fragments, designated RB2a and about 4 kbp in size, hybridized with all chromosomes of strain FC18, including chromosome 3 which was not labelled by the RPS or by HOK, as shown in Fig. 7. Considering that the RB2a fragment does not hybridize with HOK, this result strongly suggests that in all chromosomes except chromosome 3, structurally independent, conserved sequences are consecutively arranged in tandem. A partial sequence analysis of the RB2 fragment has shown that the orientation of the sequence of the RPS within RB2 was inverted compared to that within HOK. This result also strongly suggests that RB2 is located on the opposite side of the RPSs to HOK (data not shown).
DISCUSSION

Our studies are summarized in Fig. 8(a), which shows a schematic drawing of the three sequences, HOK, RPS and RB2a, in each chromosome. We have identified new conserved sequences, HOK and RB2, neighbouring the RPS and have determined the base sequence of HOK. RPSs are one of the repetitive sequences in C. albicans which are located on most of the chromosomes. The number of RPS units on each chromosome is estimated from previous results, although an accurate count of the RPS units on chromosome 5 has not been made (Iwaguchi et al., 1992; Chibana et al., 1994; Chandamporn et al., 1995). RPSs are clustered in some chromosomes, but it is still not known whether or not the other new sequences (HOK and RB2a) are located at a single locus. Currently, in the laboratory of P. T. Magee (University of Minnesota, MN, USA), a detailed physical map is being established using a fosmid library. According to the map information, at least on chromosome 7, HOK or its related stretch is located on two loci (P. T. Magee, H. Chibana & S. Scherer, personal communication). This is in accordance with our findings that two bands are observed in chromosome 7 upon hybridization using a CS fragment as a probe (see Fig. 2). Similarly to the case of the RPS, we could not detect a signal from chromosome 3 against the CS fragment hybridization. However, we have shown that the RB2a fragment can hybridize with all chromosomes, including chromosome 3. As shown in Fig. 7, the RB2a hybridization signals from chromosome 3 are relatively weak. Therefore we have concluded that only a portion of the RB2a sequence is homologous in chromosome 3, whereas the overall sequence seems to be homologous with RB2a in other chromosomes. The boundary region between the RPSs and RB2a has not been characterized.

Conservation of the SmaI site on the opposite side of the RPS on each HOK led us to assume that a conserved region would extend beyond the SmaI site. Considering
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The Ca3 molecular epidemiological probe developed by D. R. Soll's group (Sadhu et al., 1991) contains both the RPS- and the HOK-related sequences. We have confirmed that the hybridization patterns of the RPS and of HOK are different from each other (Figs 1 and 2). Therefore, it may be possible to distinguish RPS-positive signals from HOK-positive ones among the Ca3-positive signals. Fig. 8(b) represents the relationship between HOK, RPS and Ca3. Approximately one-half of the 6 kbp fragment (from bp 2831 to 5782) had homology (93%) with the B fragment of Ca3, and another portion (from bp 1 to 645) was homologous with the C1 fragment of Ca3. Lockhart et al. (1995) showed the C1 fragment of Ca3 as a SazI-cleaved subfragment of the C fragment of Ca3. According to the physical map of the C fragment, the C2 fragment is expected to be homologous with a part of HOK. The EcoRI recognition site of the C2 fragment, for which the sequence has not been reported, would then coincide with that of position 2202 of HOK, according to our sequence data. The homology of the 6 kbp fragment with portions of the C1 fragment was 79% in the HOK region and 99% in the RPS region. This low degree of homology for HOK might be due to the different origin of the strains: Ca3 was derived from 3153a (Sadhu et al., 1991) and our 6 kbp fragment was from FC18, which was a clinical isolate from the cervix (Whelan & Magee, 1981). In contrast, the high degree of homology for the RPS between the two independent strains suggests that this sequence may be instrumental in some cellular function(s). We have not yet examined whether HOK or RB2a is homologous with another molecular epidemiological probe, 27A (Scherer & Stevens, 1988); however, it has been shown that many fosmids which can hybridize with the RPS are also labelled with HOK or RB2a (S. Scherer, H. Chibana & P. T. Magee, personal communication).

Sequence analysis of the 6 kbp fragment revealed three short ORFs; one was significantly homologous to a

![Fig. 7. Comparison of the hybridization profiles among three conserved sequences. The chromosomes of FC18 were fractionated by PFGE followed by blotting to the nylon membrane. Hybridization was performed using 32P-labelled probes. (a) RPS101; (b) CS fragment; (c) RB2a fragment. Note that chromosome 3 was labelled with RB2a.](image)

the possible function of HOK (and the other conserved sequences), not only HOK but the region which extends over HOK might also be essential for some proper function.

When a CS fragment is hybridized with the SmaI-digested genomic DNA of several clinical strains, we noticed that a few signals specific to the strain appear in addition to the 6 kbp band common to every strain. Therefore, CS labelling patterns could be used for the delineation of clinical isolates of C. albicans.

![Fig. 8. Schematic representation of HOK and the adjoining sequences. (a) Hypothetical localization of three conserved sequences, HOK, RPS and RB2a. The dashed lines indicate the outside of the three different sequences. (b) Physical map showing the relationship between HOK, RPS and Ca3. The location of the C2 fragment was estimated from the physical map of Lockhart et al. (1995). E, EcoRI; S, SmaI; Sa, SazI.](image)
portion of the isocitrate dehydrogenase gene of *S. cerevisiae*. The conserved region shown in Fig. 5(b) contains the amino acid sequences responsible for binding isocitrate as a substrate as well as the magnesium-ion- and NAD*-binding sites necessary for enzyme reactions (Hurley et al., 1991). Nevertheless, we do not think that this region of HOK functions as an active enzyme for several reasons. First, this ORF is too short to encode a full-size enzyme. It also contains many stop codons but no ATG initiation codon. Finally, it is not likely that this kind of gene is located on many chromosomes. Elzinga et al. (1993) reported that isocitrate dehydrogenase is an RNA-binding protein, although it is not known which region is involved in the actual binding to the RNA.

Another feature of the HOK sequence is the existence of the successive AT-stretch from positions 1098 to 1202 (AAA TAATTTTTCATACAAAAATTTTTTCTCTAGATATTAGTTTTCTCTAGCTGATTAGTTTTGCTTAAATCTATTCTCTTTTACAAGATTTTTTCTCTTTATCTATA) (80% AT content). CDE-II, one of the components of the centromere of the yeast *S. cerevisiae*, is highly AT-rich (> 90%; Clarke, 1990). However, no homologous region corresponding to CDE-I or CDE-III, the centromeric components in *S. cerevisiae*, has been found near the AT-rich region of HOK. Recently, *Candida glabrata*, one of the pathogenic yeasts, has been shown to harbour a *Saccharomyces*-like centromere (Kitada et al., 1996).

It is known that several successive tracts of adenine nucleotide cause a curvature in the secondary structure of a DNA string, resulting in retardation of migration in a gel matrix such as the agarose in gel electrophoresis (Hagerman, 1990). This may be reflected in our experience of estimating the size of the 6 kbp fragment as approximately 6.1 kbp, based on the migration profile in agarose gel electrophoresis, but finding that the actual size by sequence determination is approximately 5.8 kbp.

A curved DNA is also known to be one of the characteristics of kinetochores in *Schizosaccharomyces pombe* (Ueki et al., 1993). Therefore, we tested the possibility that HOK may be one component of the centromere. However, we could not obtain any plasmids containing the RPS and/or HOK which were stable in the *C. albicans* host. The finding that another sequence, RB2a, which is not related to the RPS or to HOK, is conserved among all the chromosomes of strain FC18, along with its boundary region, suggests that RB2a could be a newly discovered component of the machinery for cellular or chromosomal function together with HOK and RPS. Recently, it has been proposed that the name MRS (major repeat sequence) should be given to three successive conserved regions (P. T. Magee, personal communication). To elucidate the cellular and chromosomal function of the MRS, a detailed analysis of the RB2a homologous sequences in the other chromosomes will be necessary.

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