Variation in the electrophoretic karyotype analysed by the assignment of DNA probes in *Candida albicans*

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By using pulsed-field gel electrophoresis, we have separated the entire chromosome bands and examined the electrophoretic karyotypes of 27 strains of *Candida albicans*. The electrophoretic karyotype varied widely among these strains. Their chromosomal DNAs were resolved into 7–12 bands ranging in size from 0.42 to 3.0 Mb. Most of the separated chromosomal bands were assigned by eight cloned *C. albicans* DNA probes. These results suggest that the haploid number of *C. albicans* chromosomes is eight. Each of the probes hybridized specifically to one or two bands of similar size in most strains. With the exception of the MGL1 probe, when two bands were detected by one probe, the size of one of them was very conserved whilst the other was of fairly variable size. The sizes of the chromosome bands assigned by the MGL1 probe were much more variable. As *C. albicans* is considered to be a diploid organism, it is inferred that the karyotype polymorphism between strains is mainly derived from wide size heterogeneity in one of the homologous chromosomes. Furthermore, we have confirmed species-specific and strain-specific variation in medically important *Candida* species (*C. stellatoidea*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. guilliermondii*, *C. kefyr* and *C. glabrata*). Electrophoretic karyotype analysis is thus useful for species assignment. The TUB2 probe, encoding *C. albicans* beta-tubulin, hybridized to the chromosomal DNA of all the *Candida* species examined, but four *C. albicans* probes exhibited cross-species hybridization with *C. stellatoidea* only. The karyotype of *C. stellatoidea* seems to be within the range of the intraspecies variation observed in *C. albicans*.

Introduction

The imperfect yeast *Candida albicans*, a commensal in the healthy human body, is an important opportunistic pathogen which infects compromised hosts. It usually exists in a diploid state and a haploid sexual state has not yet been found. Genetical studies are therefore difficult and only a few linkage groups have been identified by parasexual genetics and mitotic recombination (Hilton *et al.*, 1985; Kakar *et al.*, 1983; Kakar & Magee, 1982).

Recent developments in pulsed-field gel electrophoresis (PFGE) have made it possible to separate yeast chromosomal DNAs according to their size as bands in agarose gels, and, in combination with Southern hybridization of various cloned genes, to define the karyotypes of yeast species (Schwartz & Cantor, 1984). Several reports describe the karyotyping of *C. albicans* by different PFGE techniques. OFAGE (orthogonal field

alternation gel electrophoresis) gives fractionated bands which tend to curve outwards, making a comparison of the mobilities of individual bands difficult (Magee & Magee, 1987; Merz *et al.*, 1988; Suzuki *et al.*, 1988). FIGE (field inversion gel electrophoresis) does not give distinct resolution (Lott *et al.*, 1987; Snell *et al.*, 1987). The size of chromosomal DNA molecules resolvable by these methods has been reported to be smaller than 1.6 Mb. The CHEF (contour-clamped homogeneous electric fields) technique is highly effective for comparing chromosome bandings in different lanes, as chromosomal DNAs run straight over the entire size range. Magee *et al.* (1988) separated *C. albicans* chromosomal DNAs into 11 bands by the CHEF method. The 11 bands were assigned to seven chromosomes using 17 cloned probes and four sets of the resolved bands were thought to be homologous chromosomes. Lasker *et al.* (1989) subsequently assigned one of the same probes to a different chromosomal band. Thus, it has been suggested that the haploid chromosome number of *C. albicans* is at least eight.

Abbreviations: CHEF, contour-clamped homogeneous electric fields; PFGE, pulsed-field gel electrophoresis; Mb, megabases.

Several laboratories have shown that electrophoretically separated *C. albicans* chromosomal DNA bands vary in size and number among strains (Lott *et al.*, 1987; Merz *et al.*, 1988; Snell *et al.*, 1987). In a recent study, 14 distinct electrophoretic karyotypes were reported among strains isolated from 17 patients (Merz *et al.*, 1988) and abnormal karyotypes have been observed in morphological mutants derived from one strain (Suzuki *et al.*, 1989; Rustchenko-Bulgac *et al.*, 1990). This karyotypic polymorphism can be assumed to be the result of any of the following events affecting the chromosomes: (i) deletion, (ii) amplification, (iii) translocation, (iv) unequal crossing-over. These events are suggested to occur at a high frequency at subtelomeric regions (Corcoran *et al.*, 1986), rDNA regions (Szostak & Wu, 1980), or transposon regions (Shapiro, 1983). In *C. albicans*, Scherer & Stevens (1988) and Soll *et al.* (1987) have reported that repeated DNA sequences are dispersed throughout the chromosomes. These repeated DNAs might be responsible for genome reorganization in *C. albicans*.

From a practical point of view, electrophoretic karyotype analysis may be superior to other methods, including serotype, resistogram and killer system, for typing clinically isolated strains, and may be useful for aetiological studies of *Candida* infection. Species-specific karyotypes have been reported in medically important *Candida* species (Magee & Magee, 1987; Suzuki *et al.*, 1988).

In this study, we have tried (i) to show the extent of electrophoretic karyotypic variation in *C. albicans* by analysing our many laboratory stock strains, and (ii) to assign separated chromosome bands with a karyotype polymorphism by using various DNA hybridization probes. A similar line of study was extended to other medically important *Candida* species, which gave characteristic karyotypes useful for species assignment.

Methods

Strains and plasmids. In addition to those listed in Table 1, laboratory stock strains of *C. albicans* clinically isolated in our laboratory were used in this study. The other yeast strains used are described in the text. The plasmids containing *C. albicans* genes used as the probes for Southern blotting are listed in Table 2.

Media. TBE, hybridization solution, SSC and Denhardt's solution are described by Maniatis *et al.* (1982).

Preparation of yeast chromosomal DNA. Intact yeast chromosomal DNA was prepared by the method of Schwartz & Cantor (1984) with the following modifications. A yeast strain was grown for 18–24 h at 30 °C in 3 ml YPD broth (1% yeast extract, 1% polypeptone, 2% glucose; all w/v). Early stationary phase cells were collected, washed twice with 50 mM-sodium EDTA (pH 7.5), and suspended in 1 ml 50 mM-Tris/HCl (pH 7.5). A 0.08 ml portion of the suspension was mixed with 0.03 ml Zymolyase 20T solution (5 mg ml⁻¹; Seikagaku

Table 1. Strains of *C. albicans* not isolated in our laboratory

Strain	Source or reference
FC18	} Magee <i>et al.</i> (1988)
C9	
IFO 579	} Institute for Fermentation, Osaka, Japan
NUM1060=IFO 1060	
NUM1061=IFO 1061	
IFO 1385	Shimizu <i>et al.</i> (1987)
NIH-A207	Shibata <i>et al.</i> (1989)
CBS 5736	Suzuki <i>et al.</i> (1986)
NUM1000=Basel A	M. Tokunaga*
NUM1001=Basel B	Shimizu <i>et al.</i> (1987)
NUM1005=ATCC 1011	M. Tokunaga*

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Kogyo Co.) in 0.1 M-sodium citrate buffer (pH 5.8), and then 0.3 ml 1% (w/v) low-melting-point agarose (Agarose L; Nippon-gene Co.) in 0.125 M-sodium EDTA (pH 7.5) was added. After gentle mixing, the solution was poured into a mould chamber (Bio-Rad). The solidified agarose plugs were incubated in 10 mM-Tris/HCl (pH 7.5) containing 25 mM-sodium EDTA and 7.5% (v/v) 2-mercaptoethanol for 18–24 h at 37 °C, and then transferred to 10 mM-Tris/HCl (pH 8.3) containing 25 mM-sodium EDTA, 0.06% sodium dodecyl sulphate and 0.17 mg proteinase K ml⁻¹. After 18–24 h incubation at 50 °C, the plugs were stored in 50 mM-sodium EDTA (pH 9.0) at 4 °C until used.

PFGE. Yeast chromosomal DNA was separated by CHEF (Chu *et al.*, 1986). Electrophoresis was done using the Pulsaphor system, with a hexagonal electrode array (Pharmacia-LKB), or the CHEF-DRII system (Bio-Rad), using 1× TBE and 0.5× TBE, respectively, as running buffer. The buffer temperature was maintained at 10 °C. DNA samples cut in the agarose plugs (see above) were applied to 0.8% agarose gels which were formed by pouring 130 ml molten agarose (Agarose HGS; Nakarai Chemical Co.) in running buffer. The running conditions are described in Results. Gels were stained with ethidium bromide, destained in distilled water and observed under UV light (302 nm).

DNAs from *Saccharomyces cerevisiae* (X2180-1A; Mortimer & Schild, 1985) and *Schizosaccharomyces pombe* (975-h⁺; Vollrath & Davis, 1987) were used as the molecular size reference markers.

Southern hybridization. Chromosome DNA, separated in a PFGE gel, was exposed to UV light (302 nm) for 10 min. The gel was incubated in 0.5 M-NaOH containing 1.5 M-NaCl to denature the DNA and neutralized in 1.0 M-Tris/HCl (pH 7.0) containing 1.5 M-NaCl for 30 min. The DNA was blotted to a nylon membrane (Hybond-N; Amersham) in 10× SSC for 60 min by a vacuum transfer system (Biocraft Co.). The blotted membrane was irradiated with UV light (302 nm) for 5 min to fix the DNA.

The membrane was prehybridized with 30 ml 0.1 M-sodium phosphate buffer (pH 6.7) containing 6× SSC and 2.5× Denhardt's solution at 65 °C for 3 h in a tight box, and then hybridized with a probe, labelled by the Multiprime DNA labelling system (Amersham), in 30 ml hybridization solution at 65 °C overnight. The membrane was washed twice with 50 ml 2× SSC containing 0.1% SDS at 65 °C for 15 min, and then twice more with 50 ml 0.2× SSC containing 0.1% SDS at 65 °C for 30 min. For low-stringency conditions, 1× SSC containing 0.1% SDS was used for the second washing solution.

The membrane-bound labelled probe was detected by autoradiography with an intensifying screen (Lightning-Plus; Dupont).

Table 2. Probe DNAs of *C. albicans* for hybridization

Each plasmid was digested by restriction enzyme(s) and separated in agarose gel. The separated fragment DNA was used as a probe. The probe name corresponds to the gene contained in the fragment (except for pCHR4 and pCHR7).

Probe	Plasmid	DNA fragment	Source (references)
TUB2	pHS100	2.2 kb <i>Hind</i> III	J. A. Gorman (Smith <i>et al.</i> , 1988)
GAL1	pYSK208	5.5 kb <i>Hind</i> III	
MGL1	pYSK210	4.5 kb <i>Hind</i> III	
HIS3	pAR84-3	1.38 kb <i>Hind</i> III	J. A. Gorman (Rosenbluh <i>et al.</i> , 1985)
ADE2	pMK16	2.5 kb <i>Eco</i> RV	
URA3	pMK22	2.2 kb <i>Pst</i> I and <i>Sca</i> I	
pCHR4	pCHR4	2.25 kb <i>Bam</i> HI and <i>Hind</i> III	B. B. Magee (Magee <i>et al.</i> , 1988)
LYS2	pTK2-9-1	7.7 kb <i>Eco</i> RI	
BEN ^r	pbenS10	0.9 kb <i>Pst</i> I and <i>Eco</i> RI	
pCHR7	pCHR7	2.6 kb of <i>Bam</i> HI and <i>Hind</i> III	B. B. Magee (Magee <i>et al.</i> , 1988)

Results

Resolution of the chromosomal DNAs by CHEF

As a first step, we tried to separate all of the *C. albicans* chromosomal DNAs in a CHEF gel. The test strains used were FC18 and C9, which have been previously well characterized (Magee *et al.*, 1988). Since the resolution of chromosomal bands in size order is highly dependent on factors such as the switching interval, voltage and duration, we ran gels under various conditions using different combinations of these parameters. Figs 1(a) and 1(b) are typical of the results of experiments using the CHEF-DRII system, and Figs 1(c) and 1(d) of those with the Pulsaphor system.

Under conditions in which *S. cerevisiae* chromosomal DNAs were resolved into 13 distinct bands (Fig. 1a), the *C. albicans* bands larger than 1.2 Mb were hardly separated. In Fig. 1(b), where samples were run with a longer switching interval and a lower voltage, chromosome bands smaller than 2.2 Mb could be separated with good resolution. Two bands of *C. albicans* C9, which were close to 1.1 Mb, resolved under the conditions used in Fig. 1(a) but did not separate under those used in Fig. 1(b).

When electrophoresis with the Pulsaphor system was performed with a 300 s switch at 140 V for 24 h followed by a 1200 s switch at 80 V for 48 h, *C. albicans* chromosome bands were well separated over the entire size range (Fig. 1c), although chromosomes smaller than 1.6 Mb were less resolvable and the 1.38 Mb and 1.30 Mb bands separated in Fig. 1(b) were not resolved. Under these conditions a virtually identical pattern was obtained for strains C9 and FC18. In order to determine the size of the largest chromosome, the system was run under conditions which permitted the resolution of

S. pombe chromosomal DNAs (Fig. 1d). No bands larger than 3.0 Mb were detected in the two *C. albicans* strains. Eight chromosome bands from FC18 and ten bands from C9 were observed and the calibrated sizes of the chromosomes of FC18 were 2.86, 2.66, 2.20, 1.90, 1.70, 1.36, 1.18 and 1.08 Mb.

Electrophoretic karyotype analysis of the laboratory stock strains

In order to analyse any polymorphism in chromosome patterns of 50 *C. albicans* strains, their chromosomal DNAs were separated in a CHEF gel. Electrophoresis by the CHEF-DRII system under the conditions used in Fig. 1(b) enabled chromosome bands smaller than 2.2 Mb to be resolved into four to seven bands within 24 h in all the strains. Bands of 1.9, 1.7, 1.18 and 1.08 Mb were found in about 40% of the strains. Eighteen strains had bands less than 1.0 Mb, the smallest of which was 0.42 Mb. Even under these conditions, the banding pattern of each strain was distinct (data not shown).

Twenty-seven of the above 50 strains were studied using the Pulsaphor system under the conditions used in Fig. 1(c) (Fig. 2). Each banding pattern was reproducible with at least three independent clones. Chromosomes were resolved into 7–12 bands in the size range 0.42–3.0 Mb; the largest banded between 2.3 and 3.0 Mb, the smallest between 0.42 and 1.08 Mb. Chromosomes larger than 2.0 Mb were separated as two to six bands in all 27 strains. The bands were grouped into three size classes: large (>2.0 Mb), medium (2.0–1.0 Mb) and small (<1.0 Mb). Careful examination of the profiles reproducibly revealed thick bright bands and thin less bright bands. The thick bright bands probably represent multiple chromosomes.

Fig. 1. Separation of chromosomal DNAs by CHEF under various electrophoretic conditions. The switching interval, voltage, and duration time of the gels shown in the different panels were as follows: (a) 60 s for 15 h, then 90 s for 8 h at 200 V; (b) 120 s for 12 h, then 180 s for 12 h at 180 V; (c) 300 s for 24 h at 140 V, then 1200 s for 48 h at 80 V; and (d) 1000 s for 24 h, then 1500 s for 48 h at 80 V. The gels used were 0.8% agarose. (a) and (b) were run on the CHEF-DRII system (Bio-Rad), and (c) and (d) on the Pulsaphor system (Pharmacia-LKB). Samples were prepared from *C. albicans* (C9 and FC18), *Saccharomyces cerevisiae* (S.c.) and from *Schizosaccharomyces pombe* (S.p.). Numbers on the left and right of the photographs indicate the size of *S. cerevisiae* and *S. pombe* chromosomal DNAs, respectively.

Assignment of gene probes to the *C. albicans* chromosome bands

In order to analyse *C. albicans* karyotypic variation in detail, Southern hybridization was performed using cloned probes. The probes, obtained from several laboratories (Table 2), were used to probe chromosomes of strain FC18 after separation under the conditions used in Fig. 1(c) (Fig. 3). With these conditions, the chromosomal DNAs were resolved into eight bands and each probe recognized a different band, except for probe MGL1, which identified two bands, the largest band and a faint one, just below it, which was also recognized by the TUB2 probe.

Southern hybridizations using the 10 probes listed in Table 2 were carried out against the separated chromosome bands of the 27 strains shown in Fig. 2. Profiles obtained using the probe TUB2 are shown in Fig. 4 (data not shown for the other probes). Each probe hybridized to at least one band in all strains. The relative intensities of the hybridized bands usually resembled those of the ethidium bromide stained bands. All except for one band in strains NUM33, NUM65 and NUM1039, or two bands of NUM114, could be assigned by the specific probes. Probes TUB2 and GAL1 and probes ADE2 and URA3 hybridized to the same chromosome bands in all 27 strains. Eight probes are thus sufficient to identify all the chromosomes of *C. albicans*. Generally, in most

strains, each probe hybridized to bands of a similar molecular size, and the relative size order of the bands assigned by the probes was maintained, although there were a few exceptions. We have numbered probes according to the assigned order of the band sizes, from large to small. The scheme of hybridization patterns of 10 representative strains is shown in Fig. 5. The hybridization profiles using 10 probes to probe the chromosome bands of the 27 strains are described below.

(1) The probes TUB2 and GAL1 only hybridized to the largest band in 15 strains or to both the largest and second-largest bands in nine strains. In only three strains (NUM240, NUM114 and NUM961) did they fail to hybridize to the largest band. The size of chromosomes they recognized was in the range 2.3 to 3.0 Mb (Fig. 4).

(2) The probe MGL1 hybridized to a variable number of bands, of variable size. It hybridized to one to three large bands in most strains. In addition to large bands, an extra medium-sized band was recognized in three strains (NUM1000, NUM215 and NUM29). The extra band (1.26 Mb) recognized by MGL1 in NUM1000 was also recognized by the probe BEN^r (Fig. 6).

(3) The probe HIS3 hybridized to a single large band of about 2.2–2.35 Mb in 21 strains. In addition to this band, a large band (2.5–2.8 Mb) was recognized in four strains (NUM65, NUM215, NUM683 and NUM961) and a medium-sized (1.55 and 1.70 Mb) one in two strains (NIH-A207 and NUM29).

Fig. 2. Comparison of chromosome band patterns among 27 *C. albicans* strains. The electrophoretic conditions were the same as for Fig. 1(c). Markers on the left margin indicate the size of *S. cerevisiae* (S.c.) chromosomes. *C. albicans* strains: 1, FC18; 2, C9; 3, NUM46; 4, NUM939; 5, NIH-A207; 6, NUM51; 7, NUM1000; 8, NUM1039; 9, NUM65; 10, NUM978; 11, NUM985; 12, NUM1001; 13, NUM92; 14, NUM215; 15, NUM47; 16, NUM678; 17, NUM63; 18, NUM240; 19, NUM33; 20, NUM58; 21, NUM29; 22, NUM683; 23, NUM114; 24, NUM961; 25, NUM812; 26, NUM963; 27, NUM55.

Fig. 3. Assignment of the various cloned DNA probes to the chromosome bands of *C. albicans* strain FC18. The chromosomal DNAs were separated under the conditions used in Fig. 1(c). The leftmost lane shows a gel stained with ethidium bromide. The other lanes show Southern hybridization profiles using the various DNA probes described in Table 1.

(4) The probes ADE2 and URA3 hybridized to a single medium-sized band ranging from 1.65 to 1.8 Mb in 25 strains. In addition, a large band also hybridized to these probes in two of these strains (NUM1039 and NUM55).

(5) Since the probe pCHR4 gave only a weak signal with our usual conditions, we used low-stringency conditions. The probe hybridized to one or two medium-sized bands of 1.0–1.8 Mb in 25 strains. In addition, a large band (2.1 and 2.3 Mb, respectively) was also recognized in two of these strains (NUM47 and NUM678).

(6) The probe LYS2 hybridized to one or two medium-sized bands of 1.2–1.6 Mb in 21 strains. An additional small band was seen in six strains (NUM51, NUM65, NUM961, NUM812, NUM963 and NUM55).

(7) The probe BEN^r hybridized to a medium-sized band of 1.06–1.22 Mb in 20 strains. An additional one or two bands were seen in seven strains (NIH-A207, NUM1039, NUM65, NUM1001, NUM683, NUM961 and NUM963). For example, there were 1.12, 1.55 and 2.85 Mb bands in NUM961 and 1.22 and 2.3 Mb bands in NUM1039 (Fig. 6c). The 2.3 Mb band in NUM1039 also hybridized to the probe HIS3.

(8) As with the probe pCHR4, low-stringency conditions were used for hybridization with the probe pCHR7. pCHR7 hybridized to one or two bands of 0.72–1.65 Mb in all 27 strains. It recognized the smallest band in 18 of these strains. In the remaining nine strains,

Fig. 4. TUB2 hybridization profiles of the chromosome bands of the 27 *C. albicans* strains shown in Fig. 2. The chromosomal DNA of the strains was separated in the same order and under the same conditions as described for Fig. 2.

one of the three probes LYS2, BEN^r and pCHR4, and not pCHR7, hybridized to the smallest band.

Comparison of karyotypes of different *Candida* species

The electrophoretic karyotypes of the medically important *Candida* species were compared with those of *C. albicans*. Chromosomes from three to five strains of *C. stellatoidea*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. guilliermondii*, *C. kefyr* and *C. glabrata* were run on CHEF with reference to those of FC18 under the conditions described in Figs 1(b) and 1(c). A representative gel of such a CHEF run is shown in Fig. 7(a). With the reservation of minor variations among the strains, the chromosome banding patterns were species-specific.

Southern hybridization of the separated chromosomal DNAs using *C. albicans* gene probes yielded further information. The TUB2 probe hybridized to the band(s) of all *Candida* species tested, the signal intensity varying between the species (Fig. 7b). The probes GAL1, MGL1, LYS2 and BEN^r hybridized only to DNA of *C. stellatoidea* (Fig. 7c shows BEN^r as an example).

Discussion

In order to obtain good resolution of entire *C. albicans* chromosomes, various combinations of switching interval, pulse time, voltage and duration were assessed using the CHEF method (Fig. 1). It is difficult to resolve chromosomes efficiently over the entire size range in one gel. For example, a pair of similarly sized bands, 1.08 and 1.1 Mb, from strain C9, resolved under the conditions of Fig. 1(a), and those of 1.38 and 1.30 Mb resolved with the conditions used in Fig. 1(b), could not be resolved under the conditions used for the separation of large-sized chromosomal DNAs (Fig. 1c). The former and latter pair of bands corresponded to the chromosome bands 7a and 7b, and 5a and 5b, respectively, described by Magee *et al.* (1988). We think that our experimental conditions as used in Fig. 1(c) are sufficient for the separation of all *C. albicans* chromosomes. Our karyotype analyses using the CHEF method showed much variation in chromosomal band number (7 to 12) and size (0.42–3.0 Mb) between the strains maintained in our laboratory; none of the strains had identical patterns.

Our Southern hybridization results with *C. albicans* strain FC18 are essentially as reported by Magee *et al.* (1988). The MGL1 probe gave the only discrepancy between their hybridization and ours. They showed that the MGL1 probe hybridized to the same (largest) band as the TUB2 probe and that the MGL1 probe also hybridized to the second-largest band which hybridized

Fig. 5. Scheme of chromosome band profiles and their hybridization patterns. The chromosome bands were separated using the conditions described for Fig. 1(c). The ethidium bromide stained chromosome bands are represented as bars. Numbers on the left margin indicate the chromosome size of the *S. cerevisiae* marker DNAs. The numbers on the right side of each column indicate the hybridized probes: 1, TUB2 and GAL1; 2, MGL1; 3, HIS3; 4, ADE2 and URA3; 5, pCHR4; 6, LYS2; 7, BEN^r; 8, pCHR7.

Fig. 6. Examples of unusual hybridization profiles in three *C. albicans* strains. The chromosome bands of FC18 and the NUM strains 1000, 1039 and 961 were separated under the same conditions as used in Fig. 1(c) and stained with ethidium bromide (a), or hybridized with the probes MGL1 (b) and BEN^r (c). The black arrowheads indicate the bands from FC18 that hybridized to MGL1 and the open arrowheads indicate the band that hybridized to BEN^r. Arrows indicate the bands from NUM961 that hybridized to BEN^r.

Fig. 7. Karyotypes among *Candida* species. (a) CHEF patterns of *Candida* species using the conditions used in Fig. 1(c). Lanes: 1, *S. cerevisiae*; 2, *C. albicans* strain FC18; 3, *C. stellatoidea* strain IFO 1397; 4, *C. tropicalis* strain NUM37; 5, *C. parapsilosis* strain NUM303; 6, *C. krusei* strain IFO 13; 7, *C. guilliermondii* strain NUM4; 8, *C. kefyr* strain IFO 586; 9, *C. glabrata* strain TIMM1063; 10, *S. pombe*. (b) Southern hybridization using the probe TUB2. The arrow indicates the band in *C. albicans* strain FC18 that hybridized to TUB2. (c) Hybridization with the probe BEN^r. The arrowhead indicates the band recognized by BEN^r in *C. albicans* strain FC18. The faint signals in lane 5 are non-specific hybridization.

to the HIS3 probe. They interpreted these results as indicating that the probes MGL1 and TUB2 assign the same chromosome and that the *MGL1* gene has been translocated to the chromosome assigned by the HIS3 probe. Using the same strain as Magee *et al.* (1988), our

CHEF conditions resolved a faint band just below the largest one assigned by the TUB2 probe. Both bands hybridized with the MGL1 probe and were distinguishable from the third-largest band which hybridized with a HIS3 probe. This can be explained if one of the chromosomes carrying *MGL1* was not separated from the chromosome carrying *TUB2* and *GAL1* in our conditions. Actually, the MGL1 probe hybridized to different chromosome bands from those hybridizing to the TUB2 and GAL1 probes in many strains. We think that the data of Magee *et al.* (1988) were the result of the unfortunate coincidence of chromosome bands assigned by the MGL1, TUB2 and HIS3 probes under their electrophoretic conditions. We thus conclude that *MGL1* is carried on a different chromosome from *TUB2* or *HIS3* in FC18 and other strains.

Although Magee *et al.* (1988) suggested that the haploid chromosome number is seven, on the basis of the result that 17 cloned probes were assigned to seven separated chromosomes, we feel that it should be considered to be at least eight because we and Lasker *et al.* (1989) demonstrated an additional chromosome recognized by both the MGL1 probe and an rDNA probe. Furthermore, the following evidence is conclusive proof that the haploid number is eight in *C. albicans*. The eight kinds of probes we used were able to assign and distinguish almost all of the separated chromosomal bands in 27 different strains, although the size of each chromosome band(s) assigned by a specific probe was variable from strain to strain. This means that the cloned regions of the eight probes are located on eight different chromosomes and that no more chromosomes are present in any of the strains.

In some strains, a few probes hybridized to three bands, suggesting that the sequences used may have translocated to another chromosome. In four strains, one or two chromosome bands could not be assigned by any of the probes used. The regions corresponding to the probe sequences must have been deleted.

We demonstrated that there is wide variation in the electrophoretic karyotype between strains and an individual probe usually hybridized to one or two chromosome bands. With the exception of the MGL1 probe, when a probe assigned a single band, the size of that band relative to others was generally maintained although its absolute value did show variation. When the probe assigned two bands, the size of one was very similar to that of the single assigned band in another strain but the extra band was generally of variable size and the intensity of each band was less than that in strains where a single band was assigned (Fig. 4). As *C. albicans* is considered to be a diploid organism from genetic (Whelan *et al.*, 1980) and biochemical (Riggsby *et al.*, 1982) studies, it is likely that the two bands

recognized by a specific probe are homologues of the same gene. This karyotypic polymorphism between strains is inferred to be derived from the wide size heterogeneity in one of the homologues. We presume that the preservation of one conserved homologue in the genome permits the very changeable nature of the others, even with essential regions.

Karyotype polymorphism has been well studied in the protozoan *Plasmodium falciparum* and shown to be caused by chromosomal rearrangement at a particular sequence (Corcoran *et al.*, 1986, 1988). A surface antigen structural gene is lost during rearrangement. In *C. albicans*, Suzuki *et al.* (1989) and Rustchenko-Bulgac *et al.* (1990) have shown that karyotypic variation among morphological variants (or mutants) derived from a single strain arises spontaneously at frequencies as high as 0.5% and 1.4%, respectively. They suggested that the karyotypic changes are responsible for the morphological changes. By analogy, we postulated that *C. albicans* karyotypic polymorphism might correlate with phenotypic variation. However, no direct correlation was found between the karyotype and clinical symptoms, proteinase secretion, or colony morphology (data not shown).

Electrophoretic karyotype analysis of medically important *Candida* species showed species-specific chromosome banding patterns, as already reported (Magee & Magee, 1987; Suzuki *et al.*, 1988). Moreover, our results show that the intraspecies variation is not so great as to be confused with interspecies variation, meaning that the electrophoretic karyotype can be used for both species and strain identification and will thus greatly contribute to aetiological studies of *Candida* infections.

The identity or dissimilarity of *C. albicans* and *C. stellatoidea* has been discussed (Odds, 1988; Rikkerink *et al.*, 1990). We showed that chromosome DNAs of *C. stellatoidea* but not those of other *Candida* species hybridized to several *C. albicans* probes. However, the karyotype of *C. stellatoidea* is distinguishable from that of *C. albicans* (Kwon-Chung *et al.*, 1989; Suzuki *et al.*, 1988; this study). Given the wide variation of the karyotype between *C. albicans* strains and the fact that one *C. albicans* strain (NUM961) gave a similar Southern blotting pattern to *C. stellatoidea*, it seems likely that the apparently heterogeneous genetic states are derived from a homogeneous one and that *C. stellatoidea* is derived from *C. albicans*. Kwon-Chung *et al.* (1989) classified *C. stellatoidea* into two types with different major genetic characteristics and suggested that it cannot be viewed simply as a mutant derived from *C. albicans*. We think that the two types of *C. stellatoidea* could also have arisen from genomic reorganization and mutation.

Importantly, with the exception of *C. stellatoidea* none of the other *Candida* species have sequences highly homologous to the GAL1, MGL1, LYS2, and BEN^r probes. In other words, these probes can be used as specific probes to distinguish *C. albicans* from other *Candida* species.

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References

- CHU, G., VOLLRATH, D. & DAVIS, R. W. (1986). Separation of large DNA molecules by contour-clamped homogeneous electric fields. *Science* **234**, 1582-1585.
- CORCORAN, L. M., FORSYTH, K. P., BIANCO, A. E., BROWN, G. V. & KEMP, D. J. (1986). Chromosome size polymorphisms in *Plasmodium falciparum* can involve deletions and are frequent in natural parasite populations. *Cell* **44**, 87-95.
- CORCORAN, L. M., THOMPSON, J. K., WALLIKER, D. & KEMP, D. J. (1988). Homologous recombination within subtelomeric repeat sequences generates chromosome size polymorphisms in *P. falciparum*. *Cell* **53**, 807-813.
- GILLUM, A. M., TSAY, E. Y. H. & KIRSCH, D. R. (1984). Isolation of the *Candida albicans* gene for orotidine-5'-phosphate decarboxylase by complementation of *S. cerevisiae* *ura3* and *E. coli* *pyrF* mutations. *Molecular and General Genetics* **198**, 179-182.
- HILTON, C., MARKIE, D., CORNER, B., RIKKERINK, E. & POULTER, R. (1985). Heat shock induces chromosome loss in the yeast *Candida albicans*. *Molecular and General Genetics* **200**, 162-168.
- KAKAR, S. N. & MAGEE, P. T. (1982). Genetic analysis of *Candida albicans*: identification of different isoleucine-valine, methionine, and arginine alleles by complementation. *Journal of Bacteriology* **151**, 1247-1252.
- KAKAR, S. N., PARTRIDGE, R. M. & MAGEE, P. T. (1983). A genetic analysis of *Candida albicans*: isolation of a wide variety of auxotrophs and demonstration of linkage and complementation. *Genetics* **104**, 241-255.
- KURTZ, M. B., CORTELYOU, M. W. & KIRSCH, D. R. (1986). Integrative transformation of *Candida albicans*, using a cloned *Candida ADE2* gene. *Molecular and Cellular Biology* **6**, 142-149.
- KWON-CHUNG, K. J., RIGGSBY, W. S., UPHOFF, R. A., HICKS, J. B., WHELAN, W. L., REISS, E., MAGEE, B. B. & WICKES, B. L. (1989). Genetic differences between Type I and Type II *Candida stellatoidea*. *Infection and Immunity* **57**, 527-532.
- LASKER, B. A., CARLE, G. F., KOBAYASHI, G. S. & MEDOFF, G. (1989). Comparison of the separation of *Candida albicans* chromosome-sized DNA by pulsed-field gel electrophoresis techniques. *Nucleic Acids Research* **17**, 3783-3793.
- LOTT, T. J., BOIRON, P. & REISS, E. (1987). An electrophoretic karyotype for *Candida albicans* reveals large chromosomes in multiples. *Molecular and General Genetics* **209**, 170-174.
- MAGEE, B. B. & MAGEE, P. T. (1987). Electrophoretic karyotypes and chromosome numbers in *Candida* species. *Journal of General Microbiology* **133**, 425-430.
- MAGEE, B. B., KOLTIN, Y., GORMAN, J. A. & MAGEE, P. T. (1988). Assignment of cloned genes to the seven electrophoretically separated *Candida albicans* chromosomes. *Molecular and Cellular Biology* **8**, 4721-4726.
- MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- MERZ, W. G., CONNELLY, C. & HIETER, P. (1988). Variation of electrophoretic karyotypes among clinical isolates of *Candida albicans*. *Journal of Clinical Microbiology* **26**, 842-845.

- MORTIMER, R. K. & SCHILD, D. (1985). Genetic map of *Saccharomyces cerevisiae*, edition 9. *Microbiological Reviews* **49**, 181–212.
- ODDS, F. C. (1988). *Candida and Candidosis*, 2nd edn. London: Baillière Tindall.
- RIGGSBY, W. S., TORRES-BAUZA, L. J., WILLS, J. W. & TOWNES, T. M. (1982). DNA content, kinetic complexity, and the ploidy question in *Candida albicans*. *Molecular and Cellular Biology* **2**, 853–862.
- RIKKERINK, E. H., MAGEE, B. B. & MAGEE, P. T. (1990). Genomic structure of *Candida stellatoidea*: extra chromosomes and gene duplication. *Infection and Immunity* **58**, 949–954.
- ROSENBLUH, A., MEVARECH, M., KOLTIN, K. & GORMAN, J. A. (1985). Isolation of genes from *Candida albicans* by complementation in *Saccharomyces cerevisiae*. *Molecular and General Genetics* **200**, 500–502.
- RUSTCHENKO-BULGAC, E. P., SHERMAN, F. & HICKS, J. B. (1990). Chromosomal rearrangements associated with morphological mutants provide a means for genetic variation of *Candida albicans*. *Journal of Bacteriology* **172**, 1276–1283.
- SCHERER, S. & STEVENS, D. A. (1988). A *Candida albicans* dispersed, repeated gene family and its epidemiologic applications. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 1452–1456.
- SCHWARTZ, D. C. & CANTOR, C. R. (1984). Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* **37**, 67–75.
- SHAPIRO, J. A. (1983). *Mobile Genetic Elements*. New York: Academic Press.
- SHIBATA, N., FUKASAWA, S., KOBAYASHI, H., TOJO, M., YONEZU, T., AMBO, A., OHKUBO, Y. & SUZUKI, S. (1989). Structural analysis of phospho-D-mannan–protein complexes isolated from yeast and mold form cells of *Candida albicans* NIH A-207 serotype A strain. *Carbohydrate Research* **187**, 239–253.
- SHIMIZU, K., KONDOH, Y. & TANAKA, K. (1987). Proteinase production and pathogenicity of *Candida albicans*. *Microbiology and Immunology* **31**, 1045–1060.
- SMITH, H. A., ALLAUDEEN, H. S., WHITMAN, M. H., KOLTIN, Y. & GORMAN, J. A. (1988). Isolation and characterization of beta-tubulin gene from *Candida albicans*. *Gene* **63**, 53–63.
- SNELL, R. G., HERMANS, I. F., WILKINS, R. J. & CORNER, B. E. (1987). Chromosomal variations in *Candida albicans*. *Nucleic Acids Research* **15**, 3625.
- SOLL, D. R., LANGTIMM, C. J., MCDOWELL, J., HICKS, J. & GALASK, R. (1987). High-frequency switching in *Candida* strains isolated from vaginitis patients. *Journal of Clinical Microbiology* **25**, 1611–1622.
- SUZUKI, T., ROGERS, A. L. & MAGEE, P. T. (1986). Inter- and intra-species crosses between *Candida albicans* and *Candida guilliermondii*. *Yeast* **2**, 53–58.
- SUZUKI, T., KOBAYASHI, I., MIZUGUCHI, I., BANNO, I. & TANAKA, K. (1988). Electrophoretic karyotypes in medically important *Candida* species. *Journal of General and Applied Microbiology* **34**, 409–416.
- SUZUKI, T., KOBAYASHI, I., KANBE, T. & TANAKA, K. (1989). High frequency variation of colony morphology and chromosome reorganization in the pathogenic yeast *Candida albicans*. *Journal of General Microbiology* **135**, 425–434.
- SZOSTAK, J. W. & WU, R. (1980). Unequal crossing over in the ribosomal DNA of *Saccharomyces cerevisiae*. *Nature, London* **284**, 426–430.
- VOLLRATH, D. & DAVIS, R. W. (1987). Resolution of DNA molecules greater than 5 megabases by contour-clamped homogeneous electric fields. *Nucleic Acids Research* **15**, 7865–7876.
- WHELAN, W. L., PARTRIDGE, R. M. & MAGEE, P. T. (1980). Heterozygosity and segregation in *Candida albicans*. *Molecular and General Genetics* **180**, 107–113.