Comparison of the separation of *Candida albicans* chromosome-sized DNA by pulsed-field gel electrophoresis techniques

B.A.Lasker*, G.F.Carle+, G.S.Kobayashi and G.Medoff

Divisions of Infectious Diseases, of Dermatology, and of Laboratory Medicine, Department of Medicine and Department of Genetics, Washington University School of Medicine, St Louis, MO 63110, USA

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ABSTRACT

Pulsed-field gel electrophoresis techniques were used to study chromosome-sized DNA molecules of *C. albicans*. Chromosome-sized DNA of two strains of *Candida albicans* has been resolved into 8 bands by orthogonal-field-alternation gel electrophoresis (OFAGE). Six bands were observed in chromosomal preparations of *C. albicans* using field-inversion gel electrophoresis (FIGE). Differences in the electrophoretic mobilities of bands of the strains of *C. albicans* examined suggests that chromosome-length polymorphisms exist and make it difficult to correlate the banding patterns among strains. These correlations were facilitated, however, by assignment of *C. albicans* chromosomes by hybridization using a collection of cloned DNA probes specific for each of the 8 observed bands. Southern blotting showed that the 6 FIGE bands consisted of 4 singlets and 2 comigrating doublets, accounting for the 8 bands observed by OFAGE analysis. The agreement between OFAGE and FIGE analysis suggests that the *C. albicans* haploid genome contains a minimum of 8 chromosomes.

INTRODUCTION

Pulsed-field electrophoretic separations of chromosome-sized DNA molecules and the assignment of specific gene probes to chromosomes has been used to determine the karyotypes of organisms refractory to genetic analysis (1,2,3). *Candida albicans* has attracted particular attention because: 1) It is an important human pathogen and information about its karyotype is of potential diagnostic use; 2) Genetic analysis has been difficult because the organism is naturally diploid and lacks a known sexual cycle; and 3) The successful application of orthogonal-field-alternation gel electrophoresis (OFAGE) and field-inversion gel electrophoresis (FIGE) to *C. albicans* chromosomes would provide better standards of comparison for DNA molecules which fall in the size range between chromosome XIII of *Saccharomyces cerevisiae* (950 kilobases) and the chromosomes of *Schizosaccharomyces pombe* (3.5−5.7 megabase pairs).

In this study, we separated 8 chromosome-sized DNA molecules of *C. albicans* by FIGE and OFAGE. Corresponding chromosomes separated by FIGE and OFAGE were identified using cloned *C. albicans* DNA probes specific for single chromosomes. When different clinical isolates of *C. albicans* were compared, chromosome-length polymorphisms were observed.

METHODS

Strains, Maintenance and Cell Growth

Saccharomyces cerevisiae AB972 was obtained from Dr. Maynard Olson (Washington University School of Medicine, St. Louis, MO). Characterization of the chromosomes of this strain has been described previously (4).

Schizosaccharomyces pombe AB4660 (972h$^-$) was obtained from Jack Szostak (Massachusetts General Hospital, Boston, MA).

Candida albicans H317 which was obtained from Dr. Stuart Riggsby (University of Tennessee, Knoxville, TN), been described previously (5). *Candida albicans* 1012A and B311 (ATCC 32354) were obtained from stock cultures at the Barnes Hospital Diagnostic Laboratory (St. Louis, MO). The identity of all the strains of *C. albicans* were confirmed by API 20C assimilation tests, germ tube formation in serum and chlamydospore formation on cornmeal agar.

Yeast stocks were maintained on Sabouraud Dextrose Agar (Difco) and stored at 4°C with monthly transfers to fresh agar slants. For long term storage, cell suspensions were mixed with 8% dimethylsulfoxide and frozen at −80°C as described by Scherer and Stevens (6).

Sample Preparation

Cells were grown for 24 to 48 hours at 25°C on a rotatory shaker set at 125 rpm in 200 ml of YPD (1% yeast extract, 2% bacto-peptone and 1% glucose) in 1 liter flasks. Cells were harvested by centrifugation in 50 ml conical centrifuge tubes at 4,000×g for 5 minutes at 4°C.

Chromosomal DNA of *S. cerevisiae* and *C.albicans* was prepared by a modification of the embedded lysis procedure (4,7) using zymolyase 20T (Seikagaku Kogyo Co, Tokyo, Japan) instead of zymolyase 60,000. The modifications included using 250 μg/ml of proteinase K (Boehringer Mannheim) instead of 1 mg/ml in the lysis solution and an incubation temperature of 45°C instead of 50°C. The lysing solution was removed by suction and replaced with 5 ml of 0.5 M EDTA, pH 9.0. Petri plates were stored at 4°C for up to 3 weeks. Chromosomal DNA of *S.pombe* was prepared by a modification of the method described by Smith et al (8). Our modifications involved using zymolyase-20T instead of zymolyase-100T, reducing the proteinase K concentration from 1mg/ml to 250 μg/ml and adding 100 μg/ml of zymolyase-20T to the suspension of spheroplasts before mixing with low-gelling-temperature agarose (Bethesda Research Laboratories, Gaithersburg, MD).

FIGE and OFAGE

FIGE was performed as described by Carle, Frank and Olson (9) using a Model H4 horizontal gel apparatus (Bethesda Research Laboratories).

Separation of chromosomal DNA molecules was performed on a modified OFAGE apparatus (10) with nearly homogeneous electric fields intersecting at an angle of 115 degrees.

Southern Transfers and Hybridizations

Chromosomal-sized DNA was subjected to acid depurination as described by Carle and Olson (4) and transferred to nitrocellulose filters (type BA85, Schleicher and Schuell, Keen, NH) or Nitroplus 2000 (Micron Separations Company, Westboro, MA) using 1.0 M ammonium acetate/20 mM NaOH as the neutralization and transfer buffer (11). Filters were baked *in-vacuo* for 2 hours at 80°C, and placed in a sealed plastic bag in 10−15 ml of prehybridization buffer as described by Wills, Troutman and Riggsby (12). Filters were then incubated overnight in a waterbath at 60°C.

DNA fragments were separated by agarose gel electrophoresis and the desired fragments were isolated by electroelution as described by Smith (13). DNA fragments were labeled with [α-(32)P]-deoxycytosine triphosphate (3000 Ci/mmol) purchased from Amersham (Arlington Heights, IL) by random priming as described by Feinberg and Vogelstein (14) or by a nick translation kit (Amersham). Hybridizations were carried out for 18 to 24

hours at 60°C as described by Wills, Troutman and Riggsby (12). Filters were washed once at room temperature for 15 minutes with 0.5×SSC (1×SSC=0.15 M sodium chloride and 0.015 M trisodium citrate, pH 7.0)/0.1% SDS (sodium dodecyl sulfate), then washed twice for 30 minutes each with 0.1×SSC/0.1% SDS at 60°C. Filters were blotted dry on Whatman 3 MM paper and autoradiography was performed at −80°C. The same nitrocellulose filters were reused for several hybridizations after stripping the bound probe with 500 ml of boiling TE buffer (20 mM Tris, pH 7.8, and 1 mM EDTA) and allowing the buffer to cool for 30 minutes before a second wash.

The hybridization probes included the *C. albicans* clones for orotidine-5′-phosphate decarboxylase (URA3) in plasmid pET39 and the 25S rRNA gene in plasmid pET2, generously provided by Dr. Donald R. Kirsch, (Squibb Institute for Medical Research, Princeton, NJ) (15). Plasmids pCaAct1 containing the *C. albicans* actin gene and plasmid pE2 containing a 10 kbp *Eco* RI fragment of the *C. albicans* mitochondrial genome (12) were kindly provided by Dr. Stuart Riggsby. The *C. albicans* genes for TRP1 in plasmid pAR84-2 and HIS3 in plasmid pAR84-3 were kindly supplied by Dr. Jessica Gorman (Smith Kline and French Laboratories, Philadelphia, PA) (16). The clone for the *C. albicans* beta-tubulin gene in plasmid pTUB6 was graciously provided by Dr. Herb Smith (Smith Kline and French Laboratories) (17).

Preparation of a Random Probe Library

C. albicans H317 whole cell DNA was partially cleaved with *Sau* 3A (18), and the DNA fragments were separated according to size on a sucrose gradient (19). Plasmid pUC18 (New England Biolabs, Beverly, MA), which had been digested with Bam HI and treated with bacterial alkaline phosphatase (BRL) was mixed with an equal amount (0.5 μg) of pooled *Sau* 3A fragments ranging from 2 to 5 Kbp. The conditions for ligation and transformation of recombinant plasmids have been described previously (12), except we transformed into *Escherichia coli* DH5α (Bethesda Research Laboratories, Inc). Plasmid DNA was isolated from white colonies growing on L-broth agar supplemented with ampicillin (100 μg/ml) and X-gal/IPTG (20) by the method described by Ish-Horowicz and Burke (21). Plasmid DNA was analyzed on 0.8% agarose gels (12) following digestion with *Bam* HI.

Preparation of Chromosome-Specific Clones

C. albicans chromosomal bands were separated by preparative FIGE using electrophoretic conditions described in Figure 3. Following destaining of the gel, the appropriate bands were visualized under an ultraviolet transilluminator and excised from the gel. DNA in agarose blocks was digested with *Eco* RI as described by Wellems et al (22). After *Eco* RI digestion the DNA was recovered from the agarose plugs by electroelution (13) or by the freeze-squeeze method of Thuring, Sanders and Borst (23). DNA fragments were extracted with an equal volume of buffer saturated with phenol, concentrated by ethanol precipitation and resuspended in 10 μl of sterile TE buffer as described by Maniatis (24).

Plasmid pUC18 was digested with *Eco* RI and treated with bacterial alkaline phosphatase and mixed with an equal quantity of chromosome-specific *Eco* RI fragments (0.1 μg). Ligation transformation and isolation of plasmid DNA were carried out as described above. Plasmid DNA was analyzed on 0.8% agarose gels following *Eco* RI digestion.

RESULTS

Comparison of the Chromosomal DNA-Patterns of S. cerevisiae, C. albicans and S. pombe Following OFAGE

Eight distinct bands were observed in the chromosomal DNA preparation from *C. albicans*

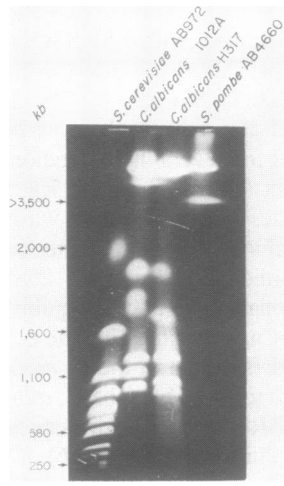

Figure 1. Separation of *C. albicans* chromosome-sized DNA molecules by OFAGE. Agarose plugs containing chromosomal DNA of *S. cerevisiae* AB972, *C. albicans* 1012A, *C. albicans* H317 and *S. pombe* AB4660 were loaded into a slot of a 0.7% agarose gel and electophoresed under the following conditions: 5 minutes switching, 3 V/cm, for 84 hours at 14°C. Size calibrations, based on the size of the *S. cerevisiae* AB972 and *S. pombe* 4660 chromosomes, are displayed on the left.

1012A and six were present in the *C. albicans* H317 preparation (Figure 1). The mobilities of the chromosomal bands of *C. albicans* fell between *S. cerevisiae* AB972 chromosome XIII and *S. pombe* 4660 chromosomes. The comigration of *C. albicans* H317 and 1012A bands with two unresolved *S. pombe* chromosomes (8), both of which are greater than 3.5 megabase pairs, suggests that the *C. albicans* bands may not be completely resolved in this region of the gel under the conditions employed (as described in Figure 1). The different patterns of bands in the two strains of *C. albicans* make it difficult to estimate accurately the karyotype of the yeast and to correlate the banding pattern among strains without performing hybridization studies. The differences in electrophoretic migration of the chromosome bands of the two strains suggest that chromosome-length polymorphisms exist.

Assignment of Cloned Probes by Hybridization to Chromosomal DNA of C. albicans Separated by OFAGE

Chromosome-specific hybridization probes were used to establish a consistent nomenclature for the bands that could be easily resolved in strain 1012A. Figure 2A displays an OFAGE gel, run under conditions similar to those in Figure 1, but on which the largest chromosomes are better resolved (25). The 8 bands visible in Figure 2A were designated as letters A−H on the basis of hybridization to a cloned genomic DNA probe specific to a given band. A chromosome nomenclature based on hybridization with standard probes should provide a better standard than a system based on mobility that can vary with strain, electrophoretic conditions or technique (26). Figures 2 (B−D) show the results of Southern blot hybridizations with several probes. The mitochondrial DNA probe hybridized to the material in the well but not to any bands, suggesting that all bands are due to nuclear DNA (data not shown). The actin gene hybridized to band H in strain 1012A, but to band G/H in strain H317 (Figure 2B). The TRP1 and Beta-tubulin genes also hybridized to band H in strain 1012A, but to band G/H in strain H317 (data not shown). The 25 S rRNA gene

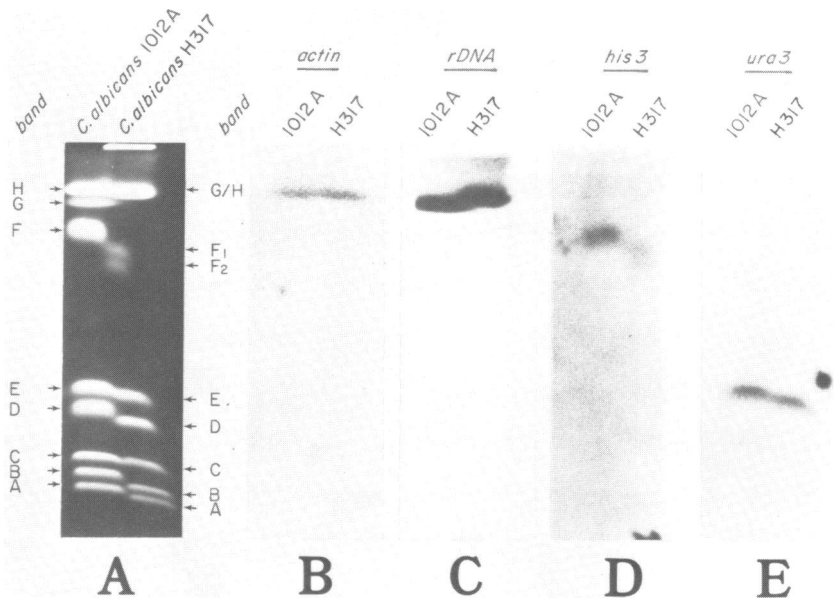

Figure 2. Band assignments using OFAGE and cloned gene probes. Agarose plugs containing chromosomal DNA of *C. albicans* 1012A and *C. albicans* H317 were loaded into adjacent slots of a 0.7% agarose gel and electrophoresed under the same conditions as described for Figure 1. The ethidium-bromide-staining pattern is shown in Panel A. Bands were labeled A to H. DNA from the gel in Panel A was transferred to nitrocellulose and sequentially hybridized to DNA probes specific for the actin (Panel B), rDNA (Panel C), HIS3 (Panel D) and URA3 (Panel E) genes of *C. albicans*.

hybridized to band G in strain 1012A, but to band G/H in strain H317 (Figure 2C). The HIS3 gene hybridized to band F in strain 1012A, but to two bands, F(1) and F(2), in strain H317 (Figure 2D). The URA3 gene hybridized to band E in strains 1012A and H317 (Figure 2E). Hybridization analysis for the remaining bands with chromosome specific probes (Table 1) showed that bands D, C, B and A of *C. albicans* 1012A corresponded to bands D, C, B and A respectively in *C. albicans* H317 (data not shown). This analysis suggests that band G/H of strain H317 is equivalent to bands G and H in strain 1012A, and bands F(1) and F(2) of strain H317 are equivalent to band F in strain 1012A. The latter might represent separation of chromosomal homologs in *C. albicans* as suggested by Magee and Magee (27). The lack of smearing below the hybridization signals indicates that the chromosome sized DNA molecules were intact and bands had undergone little or no degradation.

FIGE Banding Patterns of C. albicans and S. cerevisiae Chromosomal DNA

The *C. albicans* genome was examined by FIGE (9,25) to compare the resolution of large DNA molecules by FIGE and OFAGE. Six bands were observed in the chromosomal preparations from three strains of *C. albicans*, and several of these bands displayed a lower mobility than any of the *S. cerevisiae* chromosomes (Figure 3A). The six bands of the three strains of *C. albicans* were designated letters A−D, E/H, and F/G based on hybridization analysis with chromosome- specific probes used in the OFAGE analysis. Differences in band migration between strains H317 and 1012A confirmed the presence of chromosome-length polymorphisms, as observed by OFAGE. The banding pattern of

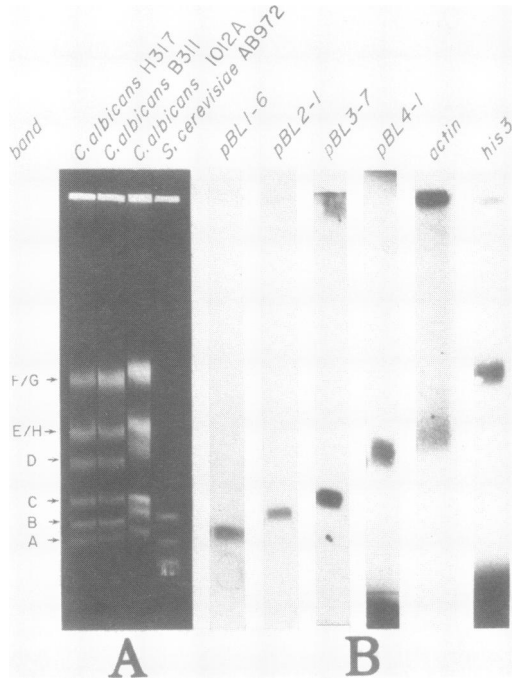

Figure 3. Separation of chromosome-sized DNA molecules from *C. albicans* by FIGE. Agarose plugs containing *S. cerevisiae* AB972 and *C. albicans* chromosomal DNA were loaded into adjacent wells of a 0.3% agarose gel and separated by FIGE using a switching time of 100 seconds forward and 25 seconds backwards at a voltage gradient of 6 volts/cm at 14°C for 24 hours. Panel A shows the ethidium-bromide-staining pattern; bands A to H were identified by hybridization analysis. DNA from the gel in panel A was transferred to Nitroplus 2000 and hybridized to DNA probes that recognize single chromosomal bands (Panel B).

strain B311 was identical to that of H317.

Southern blots were done for the three strains of *C. albicans*, but only the hybridization signals of strain 1012A are shown in Figures 3B. Figure 3B shows the hybridization of the HIS3 gene to band F/G. The rRNA gene also hybridized to band F/G (data not shown). The actin gene hybridized to band E/H (Figure 3B). Genes for Beta-tubulin, TRP1 and URA3 also hybridized to band E/H (data not shown). Hybridization analysis using DNA probes specific for bands D, C, B and A are shown in Figure 3B. Plasmid pE2 containing mitochondrial DNA hybridized only to the material in the wells (data not shown). A summary of all of the probe assignments to *C. albicans* 1012A FIGE and OFAGE bands is listed in Table 1.

Comparison of the Hybridization Patterns of C. albicans 1012A Obtained from FIGE with the Pattern Obtained from OFAGE.

We next tried to correlate the banding pattern obtained with FIGE for *C. albicans* 1012A, to the banding pattern resulting from OFAGE analysis. Hybridization analysis shows that OFAGE bands D, C, B and A of *C. albicans* 1012A corresponded to FIGE bands D, C, B and A respectively (Table 1).

Comparison of the hybridization data of the four largest chromosomes separated by FIGE and OFAGE was more complex. Hybridization analysis of OFAGE filters identified band

Table 1. Summary of probe assignments to OFAGE and FIGE separated chromosomal DNA of *Candida albicans* 1012A

OFAGE Bands (Fig. 2)	Probe	FIGE Bands (Fig. 3)	Probe	Size (Megabases)
well	mitoch	well	mitoch	---
H	TRP1 actin, Beta-tubulin	F/G	HIS3 rDNA	---
G	rDNA	E/H	Beta-tubulin, actin, trp-1, ura-3	---
F	HIS3			---
E	URA3			---
D	pBL 4-1	D	pBL 4-1	1.90
C	pBL 3-7	C	pBL 3-7	1.30
B	pBL 2-1	B	pBL 2-1	1.12
A	pBL 1-6	A	pBL 1-6	1.00

G by hybridization to the rRNA gene (Figure 2C) and band F was identified by hybridization to the HIS3 gene (Figure 2D). However, hybridization analysis of FIGE filters showed the hybridization of both the HIS3 gene (Figure 3B) and the rRNA gene (data not shown) to band F/G of Figure 3A. Hybridization analysis of OFAGE filters identified band H by hybridization to the actin gene (Figure 2B) and band E was identified by hybridization of the URA3 gene (Figure 2E). However, hybridization analysis of FIGE filters showed the hybridization of both the actin gene (Figure 3B) and the URA3 gene (data not shown) to band E/H of Figure 3A. These results suggested that band F/G of Figure 3 was a doublet composed of both bands F and G, and band E/H of Figure 3 was a doublet composed of both bands E and H.

To test if bands F/G and E/H of Figure 3 were doublets, we attempted to separate by FIGE the chromosome components in the doublets by increasing the switching interval from 100 seconds forward and 25 seconds backwards to 125 seconds forward and 31.3 seconds backwards. Six ethidium bromide staining bands were detected (Figure 4A) and the bands were designated by letter, A−F, G/H, based on hybridization analysis with chromosome specific probes (data below). Southern blot analysis of the bands in Figure 4 showed that increasing the switching interval decreased the mobility of band H, and increased the mobility of band E causing it to migrate further into the gel (see Figure 5). This resolved band E/H of Figure 3A into two bands: band H was identified by hybridization to the actin gene (Figure 4B) and the band for chromosome E was identified by hybridization to the URA3 gene (Figure 4C). Figure 4 shows that the new switching interval also caused band F to migrate further into the gel while band G remained at the same mobility (see Figure 5). Band F/G of Figure 3A was therefore resolved into its two chromosome components: band G was identified by hybridization to the rRNA gene (Figure 4D) and band F was identified by hybridization to the HIS3 gene (Figure 4E). Smearing of the hybridization signals by the rRNA and actin probes suggest that the largest *C. albicans* chromosomes, corresponding to bands G and H respectively, does not enter the gel efficiently under the electrophoretic conditions used.

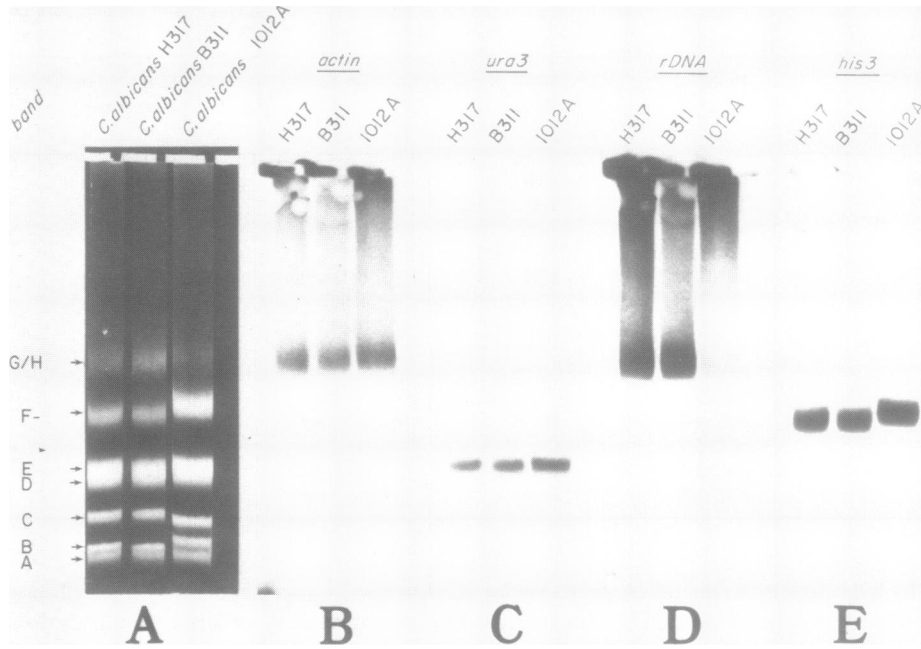

Figure 4. Resolution of bands F/G and E/H using FIGE. Agarose plugs of *C. albicans* H317, *C. albicans* B311 and *C. albicans* 1012A chromosomal DNA were loaded into the slot of a 0.3% agarose gel and electrophoresed under the following conditions: a switching time of 125 seconds forward and 31.3 seconds backwards at a voltage gradient of 6 V/cm, for 24 hours at 14°C. Panel A shows the ethidium-bromide-staining pattern and the corresponding band designations based on hybridization experimetns. DNA from the gel in Panel A was transferred to a sheet of nitrocellulose and separately hybridized to DNA probes specific for actin (Panel B), URA-3 (panel C), rDNA (Panel D) and HIS-3 (Panel E).

DISCUSSION

Two different pulsed-field electrophoresis techniques were used to analyze the set of chromosome-sized DNA molecules present in two strains of *C. albicans*. Hybridizations with a collection of cloned, genomic DNA probes was necessary to identify corresponding bands in the two strains because migration patterns did not always reflect chromosome identity due to chromosome-length polymorphisms. In addition, hybridizations of the bands with our collection of cloned DNA probes allowed us to identify each of the chromosomes in the different strains under a variety of electrophoretic conditions. By varying the electrophoretic conditions to open up the appropriate window of resolution, it was possible to identify a minimum of 8 chromosomes in both strains of *C. albicans* by OFAGE.

Each of the genomic probes hybridized to only a single band and did not cross hybridize with the other 7 bands except for HIS3. Hybridization with the HIS3 gene showed that bands F(1) and F(2) of *C. albicans* H317 were equivalent to band F in *C. albicans* 1012A (Figure 2D). Since *C. albicans* is a naturally occurring diploid (5), two possible interpretations could account for this observation. First, *C. albicans* H317 could have two closely migrating chromosomes, F(1) and F(2), both of which contain sequences homologous to the probe. More likely, bands F(1) and F(2) may be a homolog pair. The data (see Figure 2D) are consistent with the resolution of a homolog pair since the two

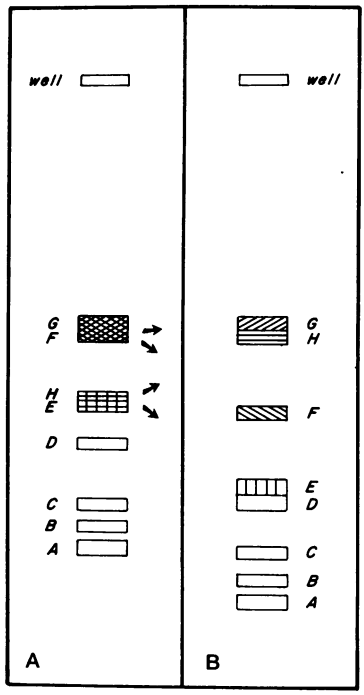

Figure 5. A schematic presentation of the mobility of *C. albicans* 1012A chromosomes subjected to FIGE. *C. albicans* 1012A chromosomes were separated by FIGE using the conditions described for Figure 3 (Panel A) and Figure 4 (Panel B). Band mobility was directly measured from gels and drawn to scale. The arrows show the direction of the changes in mobilities observed when the switching intervals were lengthened. The symbols for the chromosomes are: chromosome B (▨), chromosome F (▩), chromosome H (▤), and Chromosome E (▥).

bands stain with approximately half the intensity displayed by neighboring bands that appear to represent single chromosomes (27). Polymorphic homolog pairs in *C. albicans* appear to be more common than predicted. Currently, 5 of 8 *C. albicans* chromosomes have been resolved into homolog pairs (26 and this study). The ability to resolve a homolog pair by OFAGE may be due to a high resolution window for a given size range. The extent and nature of homolog-pair polymorphisms in the *C. albicans* genome is presently unknown.

When FIGE was used to analyze the set of chromosome-sized DNA molecules present in 3 strains of *C. albicans*, 6 bands were identified. Chromosome-sized polymorphisms between the same two strains analyzed by OFAGE were again observed with FIGE.

When hybridizations with our genomic probes were carried out, we discovered that the differing numbers of bands observed by OFAGE (9) and FIGE (6) was due to comigration of bands in FIGE. FIGE band E/H in Figure 3A corresponded to OFAGE bands H and E in Figure 2A and FIGE band F/G in Figure 3A corresponded to OFAGE bands F and G in Figure 2A. When hybridizations were carried out on chromosomal DNA subjected to longer FIGE switching intervals, bands F and E migrated a greater distance into the gel than bands G and H, allowing the resolution of the doublet pair (Figure 5). The simplest interpretation of these results is that the size order increases in the sequence A to H. In FIGE at shorter switching intervals (Figure 3), the largest molecules (i.e., those in band

H have mobilities that are directly proportional to size, while at longer switching times, the more typical inverse relationship between size and mobilities is observed. Similar effects have been described earlier for FIGE separations of smaller molecules using shorter switching intervals (9).

We did not observe chromosomes smaller than 1.0 megabase pairs under a variety of electrophoretic conditions. Moreover, when strips of *C. albicans* chromosomes from a preparative FIGE gel were hybridized with randomly selected DNA probes from a *Sau* 3A genomic library of *C. albicans*, each of more than 50 tested probes hybridized to one or more of the bands visualized by OFAGE and FIGE (data not shown). Thus, it is unlikely that chromosomes went undetected because they were too large or too small to form visible bands under the electrophoretic conditions that we employed.

Mertz, Connelly and Hieter (28) reported a variable karyotype of *C. albicans* consisting of 7 to 9 bands for 34 clinical isolates analyzed by OFAGE. FIGE analysis of the *C. albicans* genome by Lott, Boiron and Reiss (24) detected 5 major mobility groups while FIGE analysis by Snell, et al (29) detected 5 to 7 bands for several strains of *C. albicans*. Magee and Magee (27) found the genome of *C. albicans* to be highly polymorphic and originally estimated a karyotype of 9 to 10 chromosomes as determined by OFAGE. More recently, these same workers have used OFAGE, FIGE, contour-clamped homogeneous field (CHEF) gel electrophoresis and assignment of cloned genes to electrophoretically separate bands by Southern hybridization to determine that *C. albicans* has seven chromosomes (25). It is difficult to compare our results with those of Magee et al (26) because of differences in experimental conditions, and also differences in the categorization of the chromosomes. In regard to the latter Magee et al (26) used a chromosome numbering system based on designating the largest band chromosome 1 and numbering the rest sequentially with increasing distance of migration on CHEF gel electrophoresis. The chromosomes in our study were designated as letters A−H on the basis of hybridization to cloned genomic DNA probes specific to a given band. We believe that this nomenclature provides a better standard than the former system because it is not based on mobility which can vary with different strains of the same organism and different electrophoretic conditions. Only five of the gene probes were the same in our study and that of Magee et al (26). However, TUB2 or Beta-tubulin and rDNA were assigned to the same chromosome in their study using CHEF gel electrophoresis (chromosome 1; their terminology) whereas in our OFAGE gels they could be separated into two distinct chromosomes (rDNA on G, TUB2 on H; our terminology). This separation is the most likely explanation for the additional chromosome (eight) identified in our study compared to the seven chromosomes reported by them.

In summary, we have utilized pulsed-field electrophoresis separation of chromosome-sized DNA molecules and the assignment of specific gene probes to chromosomes to determine that the haploid genome of *C. albicans* has a minimum of eight. The variability in the number of chromosomes reported for *C. albicans* by different groups, including our own, is probably due to the lack of a reference strain among investigators, variation in band migration due to chromosome size polymorphisms, and differing experimental conditions. It should be possible to resolve these differences by the use of the same strains under the same experimental conditions, particularly in conjunction with hybridization analyses using chromosome-specific probes to identify specific chromosomes rather than their migration characteristics.

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Present addresses: *BAL, Mycology Section, Center for Disease Control, Atlanta, GA 30333, USA and +GFC, Centre de Biochimie, Université de Nice, 06034 Nice Cedex, France

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