MOLECULAR AND CELLULAR BIOLOGY, Nov. 1988, p. 4721–4726
0270-7306/88/114721-06$02.00/0

Vol. 8, No. 11

Assignment of Cloned Genes to the Seven Electrophoretically Separated *Candida albicans* Chromosomes

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By using orthogonal-field alternating gel electrophoresis (OFAGE), field-inversion gel electrophoresis (FIGE), and contour-clamped homogeneous field gel electrophoresis (CHEF), we have clearly resolved 11 chromosomal bands from various *Candida albicans* strains. OFAGE resolves the smaller chromosomes better, while FIGE, which under our conditions causes the chromosomes to run in the reverse order of OFAGE, is more effective in separating the larger chromosomes. CHEF separates all chromosomes under some conditions, but these conditions do not often resolve homologs. The strains examined are highly polymorphic for chromosome size. Fourteen cloned *Candida* genes, isolated on the basis of conferral of new properties to or complementation of auxotrophic deficiencies in *Saccharomyces cerevisiae*, and three sequences of unknown function have been hybridized to Southern transfers of CHEF, FIGE, and OFAGE gels. Four sets of resolvable bands have been shown to be homologous chromosomes. On the basis of these data, we suggest that *C. albicans* has seven chromosomes. Genes have been assigned to the seven chromosomes. Two chromosomes identified genetically have been located on the electrophoretic karyotype.

The genetics of the pathogenic dimorphic yeast *Candida albicans* has been the subject of considerable work in the past 6 years. Auxotrophic mutants were isolated and partially characterized in the 1970s in several laboratories, but no mapping and recombinational studies were done until the 1980s. Once the organism as commonly isolated was shown definitely to be diploid (18, 26), several laboratories began to use parasexual genetics and mitotic recombination to determine complementation groups, linkage relationships, and dominance and recessivity (7, 8, 16, 18, 20). Several linkage groups were identified, including one with four relatively closely linked markers (6–8). Although mitotic recombination can be used to map genes relative to a centromere, individual centromeres (and thus chromosomes) can only be distinguished from one another by showing that two centromere-linked genes are unlinked to each other. This can be accomplished by heat-induced chromosome loss in heterozygous nuclei formed after protoplast fusion (6). As there is a limited number of mapped genetic markers in *C. albicans*, there is not a good genetic estimate of the number of chromosomes in this organism.

The advent of a transformation system for *C. albicans* (9–11) has stimulated efforts to clone as many of its genes as possible. The use of libraries of *Candida* DNA for complementation of auxotrophic mutations or other characters in *Saccharomyces cerevisiae* has led to the identification of a significant number of DNA sequences (5, 19). These sequences are generally assumed to be the cognate genes in *C. albicans*, although the definitive test, disruption of the cloned sequence in the wild type with the resultant production of the predicted auxotroph, has been reported only once, for *URA3* (9). Nevertheless, it seems most likely that these sequences represent the *C. albicans* structural genes corresponding to the *S. cerevisiae* mutations they complement.

Concomitant with the achievement of transformation in *C. albicans* has come the development of the pulsed-field techniques of orthogonal-field alternating gel electrophoresis (OFAGE) (2, 21), field-inversion gel electrophoresis (FIGE) (1), and contour-clamped homogeneous field electrophoresis (CHEF) (3). Using the first technique, we have recently presented evidence for nine separable bands in *C. albicans*, although in no one strain were all nine resolvable (14). Similar results were obtained by Snell et al. (24, 25). Since blotting of these electrophoretic karyotypes is possible (2), one can assign cloned genes to particular bands and thus, in principle, identify the electrophoretic band corresponding to a genetically determined linkage group. One can also determine which of the bands in the electrophoretic karyotype of this diploid yeast correspond to homologous chromosomes. We report here that under some conditions 11 bands can be resolved. We also report the assignment of 17 sequences to electrophoretic bands, show linkage of several genes, and present evidence that four sets of resolvable bands are homologous chromosomes, leading to a haploid chromosome number of 7.

MATERIALS AND METHODS

Table 1 shows the *C. albicans* and *S. cerevisiae* strains used in this study. OFAGE was carried out as described (14). FIGE was performed according to Carle et al. (1), using a 0.9% agarose gel in 0.5 TBE buffer. The gel was 14 by 11 by 0.35 cm and was run at 7.5 V/cm with a 16-s forward–8-s reverse switching cycle for 24 h. CHEF was performed using a 10-cm-square 1% agarose gel run at 100 V at a 2.5-min switch for 19 h, followed by a 5-min switch for 24 h, followed by a 20-min switch at 80 V for 24 h or as mentioned in figure legends. Transfer from any of the chromosome separation techniques was as described for OFAGE (2).

The isolation of *C. albicans* genes *HIS3* and *TRP1* has been described (19). *GAL1* was isolated in a similar fashion, except that strain JWG2-11D was the *S. cerevisiae* recipient. *LYS2* was isolated analogously in B107, and *ADE1* was isolated by complementation in B93. (This *ADE1* clone does

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TABLE 1. List of strains used in this study

Organism	Strain	Genotype	Source (reference)
C. albicans	FC18		This laboratory (26)
	SC5314		A. Gillum (5)
	B792		J. Kwon-Chung (13)
	WO-1		D. Soll (22)
	C9		D. Ahearn (12)
S. cerevisiae	DBY746	a *leu2-3,112 his3-1 trp1-289 ura3-52 gal2*	D. Botstein
	JWG2-2C	a *leu2-3,112*	J. W. Gorman
	JWG2-11D	a *leu2-3,112 trp1-289 his3 gal1A152*	J. W. Gorman
	B107	α *ade2 can1-100 his4-519, his7-2 lys2-1 leu2-3,112*	B. DiDomenico
	B93	α *ade1 his1 leu2-3,112 lys7 met3 trp5 ura3*	B. DiDomenico

not complement all *ADE1* alleles in *Saccharomyces cerevisiae*.) *SOR2* and *SOR9* were isolated based on their ability to confer on JWG2-2C the ability to grow on medium containing YNB (Difco) and 4% sorbitol. (*C. albicans* grows well on sorbitol.) Ben^R was obtained on the basis of its conferral of resistance to 50 μg of Benomyl per ml on JWG2-2C, *MGL1* was isolated by its ability to allow JWG2-2C growth on α-methyl-D-glucoside, and Mtx^R was isolated by its conferral of methotrexate resistance to JWG2-11D. *TUB2* was isolated by colony hybridization of the YEp13 library described by Rosenbluh et al. (19), using as probe a fragment of the plasmid pRB128 containing the *S. cerevisiae* beta-tubulin gene (23). *URA3* (5) and *ADE2* (10) were a generous gift from D. Kirsch (Squibb). Note that only these two have been shown to be the cognate gene in *C. albicans*. Clones pB9, pCHR4, and pCHR7 were from a library prepared in the plasmid pEMBLY23 by partial *Bam*HI-*Hin*dIII digestion of *C. albicans* WO-1. They were identified by colony hybridization using nick-translated bands cut from an OFAGE separation. These clones were then used as probes to look for separation of homologs by hybridization back to pulsed-field electrophoresis separations as described in the text. Plasmids or fragments containing the cloned DNA were labeled by nick translation or by using a random oligonucleotide primer. The *S. cerevisiae* rDNA clone was obtained from L. Hereford (15). All of the clones were used unmodified from the original library. All gave Southern blot restriction patterns with genomic DNA cut with six-base-pair-specific restriction enzymes characteristic of single-copy DNA.

RESULTS

Use of FIGE, OFAGE, and CHEF for maximum chromosome resolution in *C. albicans*. We recently reported the resolution of seven to nine chromosomal bands in *C. albicans* by OFAGE (14). Although the smaller five bands were well separated, the larger two to four were not resolved. The FIGE technique can be used for larger DNA molecules, if the switching cycle is properly adjusted (1), as can CHEF (3). Since *C. albicans* chromosomes vary from 1,100 to greater than 2,200 kilobases, combinations of the three techniques seemed likely to provide the maximum resolution throughout the size range. As we have shown previously, there is a great deal of polymorphism in the electrophoretic karyotypes of different isolates of this organism, although any one strain gives reproducible patterns even after many transfers under laboratory conditions. The polymorphisms which appear on OFAGE also occur with the other two systems, indicating that they are not artifacts of the former process. Using the combination of these techniques and looking at several strains, we can now identify 11 electrophoretically resolvable bands from *C. albicans*. As will be shown later, four sets of these bands appear to be homologous chromosomes. We are therefore numbering them (from the top down in CHEF) 1a, 1b, 2, 3, 4, 5a, 5b, 6a, 6b, 7a, and 7b (see Fig. 2). (The rationale for this numbering is described below.) These bands all can be observed by comparing Fig. 1, 2, and 5. In OFAGE, chromosomes 7a and 7b and 5a and 5b of strain C9 are resolved, while 6a and b, 4, 3, 2, and 1 do not resolve well in this strain. Under our FIGE conditions (16-s forward–8-s reverse, 0.9% agarose) the order of migration of the chromosomes is the reverse of that on OFAGE. An OFAGE run was made under standard conditions, then loaded horizontally across the top of a gel and subjected to FIGE (Fig. 1). A FIGE run of strain C9 is shown at the left for comparison. It is clear that the chromosomes at the top of the OFAGE gel migrate most rapidly on FIGE. Note that 5a and b, which are clearly resolved on OFAGE, run as one band on FIGE. Figure 2 shows strain C9 subjected to CHEF, along with another *C. albicans* strain, FC18, and two other yeasts, *S. cerevisiae* and *Schizosaccharomyces pombe*. In this figure, resolution of chromosome 7 is apparent and one can count nine separate bands. The *S. pombe* and *S. cerevisiae* karyotypes provide markers which permit estimation of the size of the *C. albicans* chromosomes. These range in size from 0.9 to 3.8 megabases (Fig. 2). In this run, the chromosomes 1 of FC18 and C9 appear to have been resolved into homologs which differ greatly in electrophoretic mobility. This separation is not always apparent, but when it is, both bands hybridize to rDNA, indicating that both contain chromosome 1.

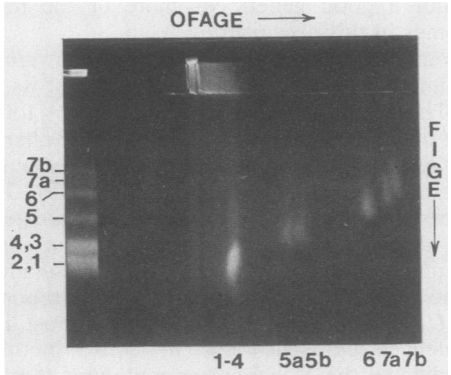

FIG. 1. Comparison of OFAGE and FIGE separation of *C. albicans* chromosomes. C9 cells were prepared and subjected to OFAGE as described in Materials and Methods. Subsequently, the OFAGE lane was turned 90° and run on FIGE for 22 h. The lane at the left shows strain C9 run on FIGE under standard conditions.

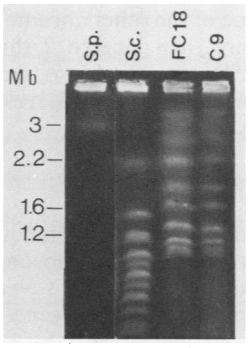

FIG. 2. CHEF separation of chromosomes from *S. pombe* (S.p.), *S. cerevisiae* DCO4 (S.c.), and *C. albicans* strains FC18 and C9. The 10-cm-square 1% agarose gel was run with a 2.0-min switch at 110 V (5.5 V/cm) for 18 h, then a 5-min switch at 100 V for 24 h, and then a 20-min switch at 80 V for 24 h. Mb, Megabases.

The existence of several bands which resolve under some conditions and not under others does not demonstrate which are homologous. Since all of these gels can be blotted and probed with labeled DNA, the best way to ascertain this relationship is to show that the same genes blot to the two resolved bands, although this method is not infallible (see below). Figure 3 shows a series of blots in which a gel of C9 similar to that shown in Fig. 2 was probed with nine separate clones. Five of the clones (*LYS2*, *pCHR4*, *URA3*, *TUB2*, and rDNA) hybridized to one band only. Clone *pCHR7* hybridized to both of the two smallest bands, indicating that these are homologous. pB9 hybridizes to chromosomes 3 and 6; for reasons discussed below, we do not believe that these are homologs. *SOR9* and *MGL1* hybridize to both chromosomes 1 and 2. Except for *MGL1*, the intensity of hybridization was approximately equal for each of the clones, although background makes the bands seem light in some cases. As discussed below, we feel that this probably indicates that *SOR9* and *MGL1* are repeated, in analogy to the *SUC* and *MAL* genes in *S. cerevisiae*.

Identification of resolved homologs. As pointed out previously (14), the fact that *C. albicans* is a diploid means that the number of resolvable bands on pulsed-field gel electrophoresis is not necessarily the number of genetically independent chromosomes. Figures 2, 3, 4, and 5 show the resolution of homologous chromosomes in some strains. The

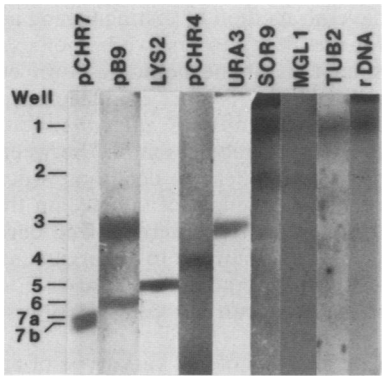

FIG. 3. Strips of a nitrocellulose Southern transfer of the strain C9 chromosomes from a CHEF gel similar to that shown in Fig. 2, probed with known genes or random plasmids from a *C. albicans* clone bank. pB9 hybridizes to two different chromosomes which can be shown with other genes not to be homologous (see Fig. 4A).

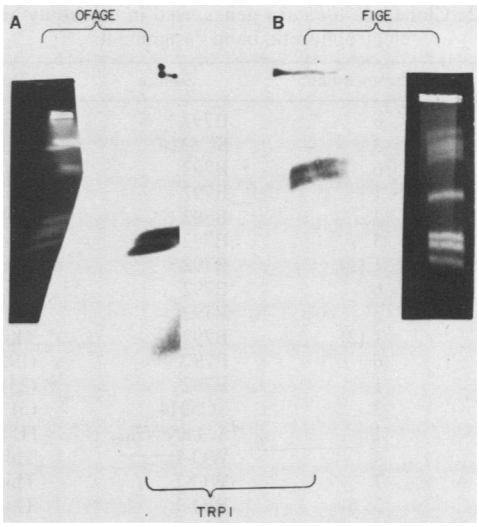

FIG. 4. Resolution of chromosome 6 in strain SC5314. The probe was *TRP1*. (A) Stained gel and radioautograph of Southern transfer from OFAGE. (B) Southern transfer and stained gel from FIGE.

use of cloned genes as probes is required for the identification of homologs. In four cases where an electrophoretic band which was single in some strains appeared to be double in others, the double bands turned out to be homologs, since they hybridized to an identical set of genes. *TRP1* hybridizes to bands 6a and b of SC5314 when they are resolved either by OFAGE or FIGE (Fig. 4). Figure 5 shows similar data for chromosomes 5a and 5b. In this case, the gene used as probe was *LYS2* and the strain was C9. We have also been able to resolve chromosome 7 by CHEF (Fig. 3, lane 1). In experiments not shown, we have found that rDNA hybridizes to both large bands in strain FC18 (Fig. 2). Thus, by choosing appropriate strains and conditions we can resolve four of the seven chromosomes into homologs.

Assignment of cloned genes to electrophoretically separated bands. To assign genetic functions to the electrophoretic karyotype, we used a variety of cloned genes to probe OFAGE, FIGE, and CHEF transfers to explore the genetic content of the various bands. These experiments have led to

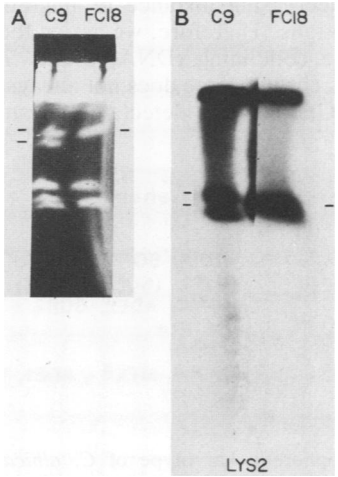

FIG. 5. Chromosomes 5a and 5b can be resolved in C9 but not in FC18. An OFAGE separation (A) of the two strains was blotted and probed with the plasmid containing the *LYS2* gene (B).

TABLE 2. Cloned *C. albicans* genes used in this study and their electrophoretic band assignments

Gene	Chromosome[a]	Source	Reference
ADE1	6	B792	This study
ADE2	3	SC5314	(10)
Ben^R	6	B792	This study
GAL1	1	B792	This study
HIS3	2	B792	(19)
LYS2	5	B792	This study
MGL1	1,(2)	B792	This study
Mtx^R	6	B792	This study
SOR2	3	B792	This study
SOR9	1,(2)	B792	This study
TRP1	6	B792	(19)
TUB2	1	B792	(23)
URA3	3	SC5314	(5)
rDNA	1	*S. cerevisiae*	(15)
pCHR4[b]	4	WO-1	This study
pCHR7[b]	7	WO-1	This study
pB9[b]	3, 6	WO-1	This study

[a] Bands are assigned as numbered in Fig. 6.
[b] These are sequences, not genes, since they were isolated by hybridization.

the assignment of a total of 14 genes and three cloned sequences to various chromosomes (Table 2). Figure 6 shows the electrophoretic karyotype (using CHEF) of FC18 with the associated genes.

Relationship of genetically and electrophoretically identified chromosomes. Although several linkage groups have been identified by parasexual genetic techniques in *C. albicans* (7, 8, 16, 17), only Hilton et al. (6) have been able to identify chromosomes, through heat-shock chromosome-loss techniques. They have numbered the chromosomes so identified 1, 2, and 3. We can associate two of our electrophoretic bands with their data, since we have sequences which complement *ade1* and *ade2* mutations in *S. cerevisiae*, and these markers each characterized one of the chromosomes defined by Hilton et al. It was recently agreed among workers in the field (at the ASM Conference on the Biology and Pathogenicity of *Candida albicans*, 14–16 May 1987, at the Annenberg Center for Health Sciences, Palm Springs, Calif.) that the most logical chromosome numbering system would be to call the largest band chromosome 1 and number the rest with decreasing distance of migration on FIGE under our conditions. Therefore, we assign the number 1 to the chromosome containing rDNA, *GAL1*, *TUB2*, *SOR9*, and *MGL1*. This chromosome does not always migrate most slowly on OFAGE, but it is detectable because it tends to migrate out of the line of the other chromosomes. The rest of the chromosomes are then labeled 2 through 7. Chromosomes 1 and 3 of Hilton et al. (6) are chromosomes 6 (tentatively; see Discussion) and 3, respectively, on the electrophoretic karyotype.

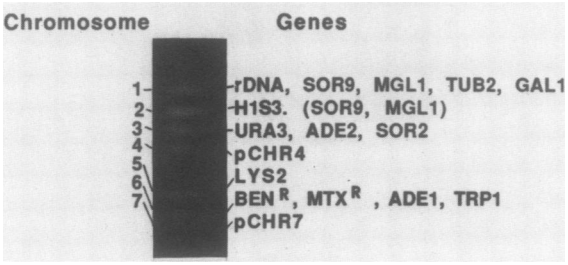

FIG. 6. Electrophoretic karyotype of *C. albicans* FC18. The chromosomes of strain FC18 were separated by CHEF, using a 2-min switch at 110 V for 18.5 h, then a 5-min switch at 100 V for 22.25 h, and then a 20-min switch at 80 V for 24 h. Gene assignments are described in the text.

DISCUSSION

By applying modifications to techniques developed by others (1–3, 21) to separate chromosome-sized pieces of DNA, we have been able to identify seven chromosomes in *C. albicans*. We investigated the genetic content of the electrophoretically separable bands by hybridizing labeled clones of various genes to Southern transfers of FIGE, CHEF, and OFAGE separations. The genes were identified on the basis of their ability to complement known genetic lesions in *S. cerevisiae* (*HIS3*, *ADE1*, *ADE2*, *URA3*, *TRP1*, *LYS2*), to confer new properties on *S. cerevisiae* (Ben^R, *SOR2*, *SOR9*, Mtx^R, *MGL1*), or to hybridize to *S. cerevisiae* genes (*TUB2*, rDNA). Two of these clones, *URA3* and *ADE2*, have been shown to contain the cognate genes from *C. albicans* (9, 10). On the basis of these results, we have established physical linkage of three groups of genes. Since we do not have clones of any two genes determined to be linked on the basis of parasexual genetic analysis, we have not been able to show congruence between the two methods. However, we have confirmed that *ADE1* and *ADE2* are on separate chromosomes, in agreement with Hilton et al. (6).

Snell and co-workers (24, 25) have used pulsed-field electrophoresis to separate *C. albicans* chromosomes. They conclude that *ADE2* is on the largest resolvable chromosome, indicating they did not resolve chromosome 1, 2, and 3. They count six chromosomes by assuming their largest band contains two sets of homologs. From our results, it must contain three.

C. albicans is highly polymorphic for chromosome migration in these three gel systems, and several strains show more than seven bands, depending upon both the separation system used and the conditions of separation. We have shown, in four cases, that two separable bands are homologous chromosomes, since they contain the same genetic material. The molecular basis for the different migration rates is not clear. Although size is an important factor in the migration rates of large DNA molecules in pulsed-field electrophoresis or FIGE, other factors, such as the amount (and possibly the distribution) of repeated DNA and the overall configuration of the molecule, may play a role. At the moment, we have no method of distinguishing among these explanations for the ability to resolve homologs. It is interesting to consider that, if the homologs turn out to be of greatly differing sizes, this is more evidence against meiosis having an important role in the *C. albicans* life cycle, since one might imagine that recombination between different-sized chromosomes could lead to deletions which would be lethal. This argument depends, of course, on the structure and gene arrangement of the different-sized chromosomes; in any case, it lends some support to the argument of Whelan and Soll, based on the frequency of recessive lethal mutations (27), that the organism has completely lost its sexual cycle.

For a complete analysis of the karyotype of any strain of *C. albicans*, it is probably necessary to use two of the three pulsed-field methods presently available. The reason is that conditions which most effectively separate large chromosomes (e.g., our CHEF conditions) fail to separate smaller ones effectively, often not resolving homologs (cf. chromo-

some 5 from C9 in Fig. 3 [CHEF] and in Fig. 5B [OFAGE]). FIGE also fails to resolve these homologs (Fig. 1). We do not know whether this is an area of low resolution under our FIGE conditions, but this is the most likely possibility. While conditions can be found to resolve several of the homologs in CHEF, they tend to be time-consuming and awkward. It is easier to use two systems.

In our experiments, the assignment of genes to chromosomal bands is ambiguous for three clones. One of these, pB9, is a cloned sequence of unknown function. It was isolated on the basis of its hybridization to chromosome 3, but when used as a probe on CHEF it lights up both chromosomes 3 and 6. This could be due to these bands being homologs, but that explanation is unlikely for two reasons. The first is that in strain SC5314, two closely running homologs of chromosome 6 are resolved and chromosome 3 is present as well. The second argument is that of the four other genes that hybridize to chromosome 6, none hybridizes to chromosome 3. The *SOR9* and *MGL1* clones also hybridize to two chromosomes, 1 and 2, in strain C9 but not in several other strains (e.g., FC18), and for reasons analogous to those for 3 and 6, these do not appear to be homologs. Four genes hybridize to 1 and not to 2.

What then is the explanation for this hybridization behavior? By analogy with *S. cerevisiae*, we might expect genes involved in carbohydrate degradation to be present in more than one copy. Thus, we may have isolated two nonunique genes. For *MGL1*, which hybridizes relatively weakly, an alternative explanation might be that there is repeated DNA which lights up large chromosomes more strongly than smaller ones. The nonunique explanation might hold for pB9, since we have no idea of its function. A second possibility is that pB9 is composed of pieces from both chromosomes 3 and 6 which were inadvertently ligated together during preparation of the library. In turn, this explanation is also possible for *MGL1* and *SOR9*, but the fact that two clones show the same behavior renders it most unlikely.

As more *C. albicans* genes are cloned, the map we present here can be rapidly expanded. It is important that the chromosomes identified genetically be located on the electrophoretic karyotype. We have done this with two of those described by Hilton et al. (6). Chromosome 1 (containing *ADE1*) is probably our chromosome 6; however, the fact that the sequence which complements *ade1* in *S. cerevisiae* has not been shown to complement *ade1* in *C. albicans* means that this can only be a tentative assignment. If the *ADE1* clone turns out to be a suppressor, our assignment could be wrong. Chromosome 3 containing *ADE2* (6) is our chromosome 3. Since we do not have a *LYS* gene which has been shown to complement the *lys1* of Hilton et al., we cannot assign their chromosome 2 to any of our bands.

It is interesting that chromosome 6, with a large number of genetic markers (17), is one of the smallest physically. One explanation is that the number of mutations isolated is so small, and the chromosomes are so large, that there is no significance to this correlation. A second explanation is that the larger chromosomes have a greater probability of carrying recessive lethal mutations, and since the demonstration of linkage requires homozygosis for a part at least of the chromosome, it is harder to map genes on the larger chromosomes. More genetic analysis will be required to decide between these alternatives.

Taken together, the evidence in this paper strongly supports the chromosome number of seven for *C. albicans*. OFAGE, CHEF, and FIGE (when the latter technique is run under the conditions described here) give similar bands for any given strain, although the order of migration is reversed for FIGE. We have shown that all the bands contain unique genetic information. The only possibility of additional chromosomes is a band (or bands) unresolved by our present techniques. The major problem at the moment is the paucity of genes identified by mutation which can be assigned to the electrophoretic karyotype. By analyzing the karyotype of auxotrophs derived from fusion products by heat shock-induced chromosome loss (6), one may soon be able to begin to remedy this deficiency. Such correlations would greatly enhance the usefulness of both techniques.

There are several possibilities for refinement of the experiments described here. One can demonstrate closer linkage by digesting chromosomes with enzymes like *NotI*, which cut infrequently, and showing that two genes hybridize to the same band (4). One can also use the chromosome loss technique (6) to correlate bands with genetic markers in fusion hybrids of strains which show polymorphisms in chromosome size. Such experiments are under way in our laboratory.

ACKNOWLEDGMENTS

This work was partially supported by Public Health Serivce grant AI16567 from the National Institutes of Health.

We are grateful to Erik Rikkerink for helpful discussions.

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