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# A *Candida albicans* dispersed, repeated gene family and its epidemiologic applications

(gene conversion/mobile element/orthogonal field alternating gel electrophoresis/DNA polymorphism)

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ABSTRACT **_Candida albicans_ causes a wide variety of infections but can readily be isolated from the skin and mucosa of healthy individuals. To enable high-resolution epidemiologic studies on this common pathogen, a species-specific DNA probe has been isolated from its genome. There are ≈10 copies of the sequence dispersed among the chromosome-sized DNA molecules resolved by pulsed-field electrophoresis. New DNA polymorphisms in this gene family arise at high rates. As a consequence, this probe will readily distinguish strains from different patients in the same hospital and from various sites in individual patients. The DNA polymorphisms detected by using this probe are largely due to internal changes in members of the family rather than movement to new genomic locations. This suggests recombination or gene conversion rather than transposition as the mechanism producing the observed variation.**

Epidemiologic studies of microorganisms rely on precise and reproducible methods for their identification. Many of the tests in current use measure phenotypes with the assumption that the underlying genetic differences that produce the phenotypic changes are the same in different isolates. With the advent of recombinant DNA technology it has become possible to replace many of these phenotypic tests with ones that directly examine the genomes of the organisms of interest (1–4). The highest resolution in studies of this type using molecular probes can be achieved by examining segments of the genome that are changing the most rapidly.

We have taken this approach in studies of the ubiquitous eukaryotic pathogen *Candida albicans*. The spectrum of disease caused by *Candida* ranges from superficial infections of the skin and mucosa to severe systemic disease in individuals with impaired immunity (5, 6). Recently, *Candida* has emerged as a common source of hospital-acquired infections. Because *Candida* can be readily isolated from the skin and mucosa of healthy individuals, the availability of precise methods for typing of strains is especially important in studies of transmission (7–9).

An initial step in DNA-based methods for typing *Candida* species and strains is direct examination of the fluorescence pattern obtained after restriction digests of total genomic DNA and electrophoresis. These DNA fingerprints distinguish *Candida* species and many *C. albicans* subtypes (10, 11). One drawback of this approach is that only a limited number of sites in the genome of a given species can be scored for differences, primarily the highly repeated ribosomal DNA and the mitochondrial DNA (10, 12).

To examine many sites in the genome simultaneously, a dispersed, repeated DNA segment from the *C. albicans* genome has been isolated. This sequence family produces new DNA polymorphisms at rates that can be measured in the laboratory. Therefore, it should be possible not only to resolve closely related strains but to approximate the number of generations that clinical isolates have been separated. In addition, the availability of DNA probes for highly polymorphic loci dispersed throughout the genome should facilitate the development of a combined physical and genetic map of the organism.

High frequencies of DNA polymorphisms in populations are due to a number of mechanisms. Often, the polymorphisms are associated with movement of transposable elements (13–15). The gene family described here has many of the features common to a variety of eukaryotic transposable elements. These include moderate repetition and dispersion throughout the genome, production of new DNA polymorphisms at high rates, and species specificity. However, the DNA polymorphisms detected by this probe are generally due to changes of internal sequences in copies of the element. Mechanisms capable of producing the observed changes are presented in the *Discussion*.

## MATERIALS AND METHODS

*C. albicans* strains 609 and 616 (lanes A and B of Fig. 1) and *Candida tropicalis* strain 615 (lane C of Fig. 1) are recent clinical isolates from the University of California Los Angeles Medical Center. Strain 609 is a blood isolate, strain 616 derives from an abdominal abscess, and strain 615 was cultured from urine. All cloned DNA segments described in this work are derived from strain 616. Strains 609 and 615 were used as the DNA probes to isolate the species-specific clones.

Growth of *Candida* strains and small-scale DNA isolations have been described (11). Larger-scale *Candida* DNA preparations suitable for molecular cloning were made as follows. Exponentially growing cells in rich medium were washed with 1 M sorbitol. Protoplasts were prepared by resuspending the cells 10-fold concentrated in 1 M sorbitol/50 mM potassium phosphate, pH 7.5/14 mM 2-mercaptoethanol with 100 μg of zymolyase 100T per ml (Miles) and incubating at 30°C for 30 min. The protoplasts were pelleted and resuspended in 50 mM $Na_3EDTA$/0.2% sodium dodecyl sulfate ($NaDodSO_4$) with 100 μg of proteinase K per ml. This was incubated at 50°C for 3 hr. The DNA was extracted three times with phenol/chloroform (1:1) and precipitated with 2 vol of ethanol. The DNA was resuspended in 10 mM Tris·HCl, pH 7.5/1 mM $Na_3EDTA$ (TE buffer) with 10 μg of RNase A per ml. After overnight incubation at 4°C the DNA was precipitated with 2 vol of 2-propanol and resuspended in TE buffer.

Agarose gel electrophoresis and Southern transfers were performed by standard procedures (12). After transfer to nitrocellulose, hybridization was done in 50% formamide/0.9 M NaCl/50 mM sodium phosphate, pH 7.4/5 mM

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EDTA/0.3% $NaDodSO_4$/100 μg of denatured salmon DNA per ml with $10^6$ dpm of nick-translated probe per ml (16). Pulsed-field electrophoresis was done in an orthogonal field alternating gel electrophoresis apparatus (17) with the following parameters: 125 V, 5-min pulse time, 11°C buffer, 72-hr run time. The buffer was 50 mM Tris borate/2.5 mM $Na_3EDTA$.

Libraries of *Eco*RI fragments from strain 616 in λ vector 1149 (18) were prepared as described by Davis *et al.* (16). The phage were packaged *in vitro* and plated on an *hsdR hflA Escherichia coli* strain to select recombinant phage. Plate lysates were prepared that represent a pool of ≈750,000 independent recombinants. Approximately 85% of the phage, including the species-specific clones 23A, 24A, and 27A (Fig. 1), contained a single *Eco*RI insert.

A library of *Sau*3A partial-digestion products [15–20 kilobases (kb)] from strain 616 was prepared by using vector EMBL3 (18). Products of the *in vitro* packaging reaction were screened directly with $^{32}P$-labeled plasmid 701 (an internal *Eco*RI–*Hin*dIII fragment of clone 27A in pBR322). Among the isolates recovered from this screen were two phage that contained an *Eco*RI fragment identical to that in clone 27A.

## RESULTS

**Isolation of Species-Specific DNA Probes.** One approach for the isolation of mobile DNA segments is the examination of closely related species for differences in their genomes (19). Given the small size of the *Candida* genome, it is possible to screen cloned segments in phage λ directly by using total genomic DNA labeled by nick-translation. The vector NM1149 was selected because its vigorous growth would result in enhanced signals in plaque filter hybridization and recombinant phage can be selected on an *hflA E. coli* strain to remove rejoined vector (18). By using this vector, a library of about 100 genome equivalents of *C. albicans* *Eco*RI fragments was constructed. Approximately 3000 recombinant phage from this library were plated at a density such that individual plaques were clearly resolved and duplicate plaque filters were prepared (8). DNA from *C. albicans* and *C. tropicalis* were labeled by nick-translation to $2 \times 10^8$ dpm/μg and hybridized to the replica filters overnight at 37°C (see *Materials and Methods*).

After washing and autoradiography, all plaques were found to label with the *C. albicans* probe. Only plaques that labeled strongly with the *C. albicans* labeled with the *C. tropicalis* probe. Five plaques were identified that labeled strongly with the *C. albicans* (self) probe and weakly or not at all with the *C. tropicalis* probe. Phage from these plaques were plaque-purified, rescreened, plaque-purified again, and grown to prepare DNA. Labeled DNA from each phage was hybridized to identical Southern transfers of *Eco*RI digests of DNA from two unrelated *C. albicans* and one *C. tropicalis* isolates (Fig. 1). Two of these clones had been identified previously (unpublished results). They are likely to be a spacer segment of the rRNA-encoding DNA (rDNA) repeat that is not present in *C. tropicalis* DNA. A third clone (24A) was repeated highly in *C. albicans*, gave similar patterns with the two *C. albicans* strains, and was present at reduced copy number in *C. tropicalis*. The final two clones differed greatly in their hybridization patterns with the two *C. albicans* strains and did not hybridize at all with the *C. tropicalis* DNA.

One of this final pair (27A) was chosen for further study. Labeled DNA of this clone showed no hybridization to a variety of *C. tropicalis* and *C. parapsilosis* strains and distinguishable hybridization patterns to *Eco*RI digests of 30 *C. albicans* strains representing various phenotypic groups and subgroups (not shown).

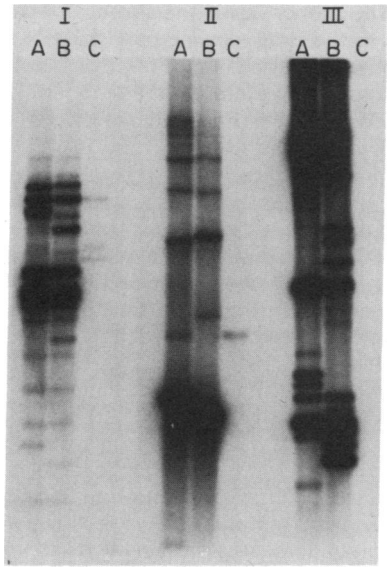


FIG. 1. Hybridization of cloned segments to *C. albicans* and *C. tropicalis* DNA. Clones representative of the three types described in the text are shown. Each filter has *Eco*RI digests of two *C. albicans* DNAs (lanes A and B) and one *C. tropicalis* DNA (lane C). Hybridization was overnight at 37°C. The first panel shows a dispersed, repeated clone (24A) present at reduced copy number in *C. tropicalis*. In panel II, a clone (23A) likely to be rRNA-encoding DNA (rDNA) spacer is shown. Weak hybridization to other rDNA fragments and to partial-digestion products from the highly repeated rDNA cluster is seen. The final panel is the species-specific probe (27A) used in the epidemiologic studies.

To demonstrate that the hybridizing fragments are dispersed throughout the *C. albicans* genome, a subclone of 27A in pBR322 (701 in *Materials and Methods*) was hybridized to the chromosome-sized DNA molecules of *C. albicans* (Fig. 2) separated by pulsed-field electrophoresis (17). All but one of the molecules resolved by this technique were

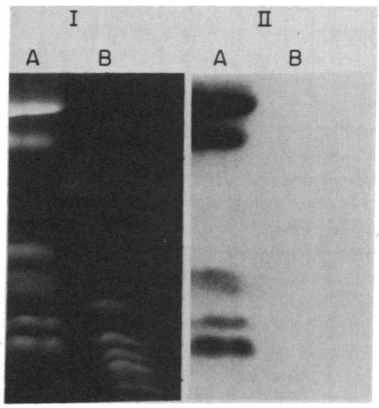


FIG. 2. Sequences homologous to the mobile DNA probe are found on the molecules resolved by pulsed-field electrophoresis in *C. albicans*. *Candida* DNA was prepared by the method used for *Saccharomyces* DNA (20, 21). The fluorescence pattern is shown at the left in panel I with *Saccharomyces cerevisiae* size standards. The intensity of the fluorescence of the bands does not appear to be proportional to molecular weight. Splitting of homologue pairs and unresolved chromosomes are the likely explanations of this result. In the hybridization pattern in panel II, all size classes of *Candida* DNA molecules but one are labeled. None of the *Saccharomyces* chromosomes that remain on the gel label with the probe. No smaller *Candida* molecules have been seen in shorter runs. The smallest *Candida* chromosomes are estimated to be 1000 kb. The largest chromosomes are significantly larger than *Saccharomyces* chromosome XII.

labeled with the probe. Given the absence of labeling of one of the bands in the fluorescence pattern, it is unlikely that this family of repeated sequences is one involved in chromosome structure (for example, the telomere repeats).

**Clone 27A Will Detect DNA Polymorphisms That Arise in the Laboratory.** To estimate the rate at which the DNA polymorphisms associated with this family of sequences arise, two experiments were done. One was to serially passage four different *C. albicans* strains 30 times on rich medium (≈300 generations) and examine 6 single colonies obtained from the final passage of each strain. DNA from 1 of these 24 colonies showed a change in the Southern pattern. Other eukaryotic mobile elements produce DNA sequence polymorphisms at detectable rates (14, 15).

In a second experiment, 10 spontaneous *C. albicans* 5-fluorocytosine-resistant mutants (22) were examined in similar fashion. One of these mutants showed a change in its hybridization pattern with this probe (Fig. 3). It is unknown whether the changes in hybridization pattern of this family are the cause of the mutation recovered by selection; however, the result underscores the sensitivity this type of probe can give to the detection of strain differences. Similar changes are seen when spontaneous cadmium-resistant mutants are examined by using this probe (S. Grindle and S.S., unpublished observations). The loss of a hybridizing fragment need not be due to a transposition event. Resistance to 5-fluorocytosine is generally due to a recessive mutation (23). The loss could arise from the elimination (by mitotic recombination) of a heterozygous copy of the element linked to the locus conferring drug resistance when the new mutation was made homozygous.

**Use of Probe 27A to Resolve Epidemiologically Related Strains.** Isolates from different body sites from several patients were examined by using the mobile DNA probe. In five of six individuals, some differences could be detected in the different body-site isolates. One of the five patients appeared to be infected by two unrelated *C. albicans*, as judged by DNA fingerprints (11). The other four carried a set of closely related strains. Examples are shown in Fig. 4. It will be interesting to determine if these differences arise during growth within the patients or represent preexisting variation in the infecting clones of cells.

Finally, we examined a group of *C. albicans* strains, obtained in the presence of *Candida* infection, from patients in the same hospital. Examination of the fluorescence patterns of this set of strains divided it into six groups. By using the mobile DNA probe to provide a more sensitive test, a great variety of strains was shown to be present and identical isolates were in some instances recovered from different individuals (Fig. 5). Results obtained with both DNA typing techniques do not agree with those from phenotyping meth-

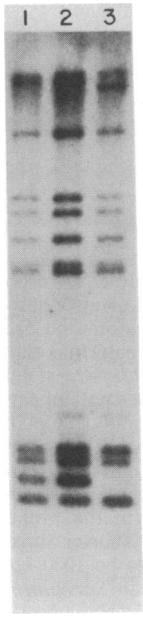


FIG. 3. DNA polymorphisms arise in the laboratory. Lane 1 is the parent of the mutants shown. Independent mutants were derived by selection on solid minimal medium containing 5-fluorocytosine at 10 μg/ml after overnight growth in rich medium. Mutants were isolated as single colonies on selective medium before DNA preparation. Nine of 10 mutants tested were like the one shown in lane 2, identical in hybridization pattern to the parent by using probe 27A. The mutant analyzed in lane 3 shows two differences, the change in mobility of a high molecular weight fragment and the loss of a smaller one.

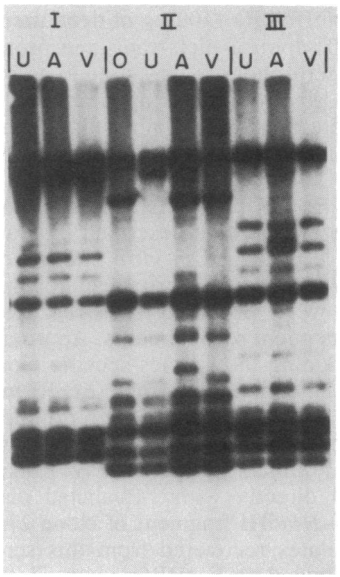


FIG. 4. Isolates from multiple body sites from three patients. Samples were prepared and then digested with *Eco*RI. Hybridizations at 42°C were done (lanes from the same gel). Body sites were vaginal (V), anal (A), oral (O), and urine (U). Samples in panel I appear identical. Note the differences in the isolates in panels II and III. For all lanes in each panel, the DNA fingerprints were identical.

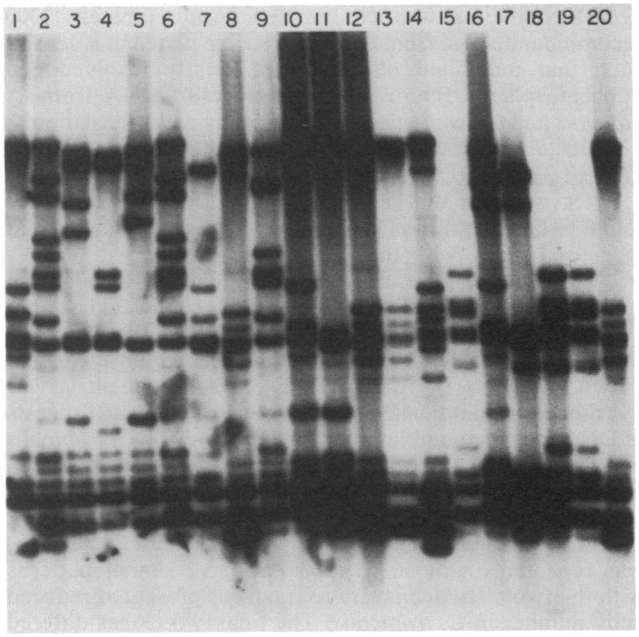


FIG. 5. *C. albicans* strains in a hospital. Samples were prepared as described in the legend to Fig. 4. At least six distinguishable fluorescence patterns (not shown) were seen: lanes 8, 12, 13, and 20; lanes 3–5; lanes 2, 6, 7, 9, 10, and 16; lanes 11, 15, 18, and 19; lanes 1 and 14; lane 17. All strains that have identical patterns when hybridized with probe 27A also have indistinguishable staining patterns; however, the converse is clearly not true. The difference in hybridization pattern is variable in the group of six strains with similar staining patterns.

ods (7, 8). Some similarity in hybridization pattern is seen among strains with clearly different fingerprint patterns.

**Characterization of the DNA Sequence Polymorphisms.** To determine the nature of the DNA polymorphisms revealed in the experiments described above, additional physical characterization of the gene family was performed. A library of *Sau*3A partial-digestion products of *C. albicans* cloned in vector EMBL3 was screened with a plasmid subclone of 27A. Among the positive clones, two overlapping clones were identified that contained an *Eco*RI fragment identical in size and restriction map to 27A (not shown). Fig. 6 shows a map of this region of the genome. Hybridization of radioactive total genomic DNA to Southern blots of restriction fragments of these clones revealed that most of the cloned region was repeated DNA. Additional copies of the element were obtained in the same screen. The terminal fragments from the cloned region surrounding 27A did not label a different clone recovered from the EMBL3 library that extends a corresponding distance from the portions of the clone homologous to 27A. This suggests that the ends of the repeated segment lie within the cloned region shown in Fig. 6.

Additional mapping studies (not shown) indicate that one end of the repeated region lies within the boundaries of probe 1. The *Eco*RI fragments to the left of probe 1 label single bands in Southern blots to *Eco*RI-digested genomic DNA. A *Hin*dIII fragment spanning the left end of probe 1 labels many bands in the same digest, indicating that the repeated region ends near the left end of probe 1. Similar mapping studies place probe 3 near the right end but within the repeated segment. The size of the repeated region is at least 12 kb. The terminal probes did not label other restriction fragments from the cloned region, indicating an absence of long terminal repeats in the cloned region.

Clone 24A (Fig. 1) is strongly homologous to probe 1 of Fig. 6. It has a different restriction map than clones 137 and 146 and probably derives from another copy of the element. Hybridization of clone 24A to other *Candida* species might derive from the DNA adjacent to the element in that probe.

Hybridization of probes from terminal regions of the element to DNA from epidemiologically related strains is not effective in revealing DNA polymorphisms. Three pairs of isolates, each pair having indistinguishable DNA fingerprints, were examined. Southern blots of *Eco*RI-digested DNA from all six strains were hybridized with each of the three probes shown in Fig. 6. Strains with different DNA fingerprints often will be distinguished by using probes 1 and 3. Strains that cannot be resolved by either DNA fingerprinting or the end probes will often reveal many differences with probe 2. Results obtained with one pair are shown in Fig. 7. Differences in band intensity might be due to changes in the

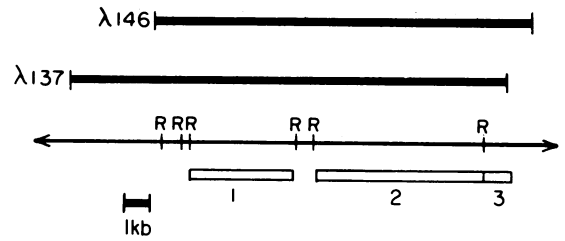


FIG. 6. Genomic clones overlapping 27A. The relative positions of two overlapping *Sau*3A partial-digest clones (137 and 146) are shown above a map of the *Eco*RI sites (R) of the region. Three probes are indicated as open boxes below the composite restriction map. Probe 2 is identical to probe 27A. Probe 1 is an *Eco*RI fragment strongly homologous to clone 24A (Fig. 1). Probe 3 is the terminal region of clone 137 (from the *Eco*RI site shown to the *Sal* I site in the polylinker of EMBL3). The repeated region extends from near the left end of probe 1 to near the right end of probe 3.

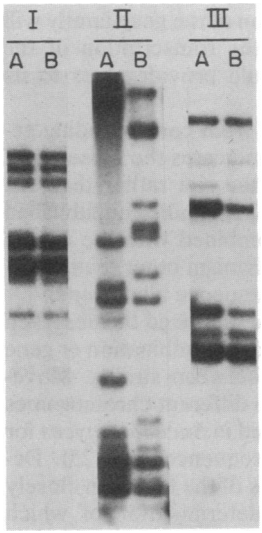


FIG. 7. Hybridization of different segments of the mobile element to epidemiologically related strains. The three probes used are shown in Fig. 6. The three panels (from different gels) are *Eco*RI digests of the strains in lanes 9 and 10 of Fig. 5 (here lanes B and A, respectively). The relative position and number of sites make *Eco*RI well suited for this type of comparison. Note how the flanking probes (probes 1 and 3) label sets of identically sized fragments in the pair of isolates and the large number of differences detected with central probe 2. If the element were moving to new genomic locations, the reverse result would be predicted: the production of different-sized fragments detected with the end probes and no change of internal fragments.

number of copies at particular loci on homologous chromosomes or substitution of sequences with less homology to the probe. Larger copies of the element than those described here might exist and substitute larger regions of nonhomologous DNA. If the changes in band intensity in panel III of Fig. 7 were due to deletions, it would imply a hot spot for DNA rearrangements internal to the element. If all copies of the element are similar in size and organization, only the largest one or two labeled fragments shown in Figs. 1 and 3–5 could extend into the flanking low-copy DNA.

## DISCUSSION

We have isolated a repeated DNA segment that provides a species-specific probe for *C. albicans* and will distinguish all but the most closely related strains. DNA polymorphisms associated with the gene family arise at rates that can be measured in the laboratory. The polymorphisms are primarily internal changes in the gene family and not due to movement to new genomic locations.

The great sensitivity that highly mobile DNA probes can give to epidemiologic studies may necessitate some change in the notion of the identity of strains. Clearly, the strains examined in this study are changing at measurable rates. A more useful measure may be an estimate for the number of generations that separate two strains. It should be possible to examine the spread of *Candida* infections within an individual by techniques analogous to those used to study development with genetic mosaics. The rates of change observed by using probe 27A are sufficiently low that reversion to prior configurations would be unlikely. A number of questions in *Candida* epidemiology will be open to more detailed investigation by this approach. These include recurrent vaginitis and the association of particular types of infections with particular strains. Comparison must be made of the rates at which DNA polymorphisms arise in animals and the rates at which they arise in laboratory media. Clearly, different probes vary in their efficacy in identifying polymorphisms in the genome.

The techniques used here to isolate the mobile gene family can be extended with little modification to screen for *C. albicans* single-copy DNA segments that are absent in other *C. albicans* strains. Additional copies of the gene family described here might exist that are not detected with internal probes such as 27A. As it is not yet clear how much of the observed polymorphism is due to heterozygosity, it is uncertain whether the end probes are detecting the same number of copies of the element as the central probe.

Continuing structural characterization of the gene family will resolve these issues. Analysis of the transcription of the family and its DNA sequence should provide clues to its biological role.

Examination of cloned fragments from corresponding genomic locations in different strains indicates the presence of different copies of the element at the site rather than the existence of empty sites (S.S. and S. Grindle, unpublished observations). This observation, combined with the results presented here, points to some mechanism other than transposition as the origin of the rapid sequence alterations.

Other mechanisms that might have produced the observed changes include mutational hot spots, recombination or gene conversion, and genetic exchange between strains. Movement of repeated DNA sequences to different chromosomes by gene conversion has been observed in *Saccharomyces* for transposable and nontransposable sequences (24, 25). Detailed examination of all of the copies of the family in closely related strains should permit the determination of which mechanisms are operating. If a gene conversion mechanism is at work, it should be possible to identify the donor sequences for the recombinational events.

We have detected high frequencies of new DNA polymorphisms with unselected passage and with spontaneous mutations. The frequency at which polymorphisms are seen when single colonies of spontaneous mutants are examined is much greater than that seen in unselected serial passage. It is not yet known which of these two frequencies more closely reflects the *in vivo* rate. As much of the polymorphism observed with this gene family is due to internal changes and the same frequency is seen with two different drug selections, it is unlikely that the polymorphisms are in fact the selected mutations. More likely would be that the selective agents are inducers of recombination or that the observed changes are linked to the newly selected mutations.

*C. albicans* is known to have at least two morphologic switching systems that result in high frequencies of spontaneous phenotypic changes (26, 27). No correlation between changes in this mobile gene family and the known phenotypic systems has been seen. The association of spontaneous mutations with either movement of this gene family to new sites or the production of linked DNA polymorphisms would provide a major tool in the development of the *Candida* genetic system.

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1. Schaberg, D. R., Tompkins, L. S. & Falkow, S. (1981) *J. Clin. Microbiol.* **13,** 1105–1108.
2. Tenover, F. C. (1985) *Clin. Lab. Med.* **5,** 413–436.
3. Tompkins, L. S. (1985) *Clin. Lab. Med.* **5,** 99–107.
4. Tompkins, L. S., Plorde, J. J. & Falkow, S. (1980) *J. Infect. Dis.* **141,** 625–636.
5. Edwards, J. E. (1978) *Ann. Intern. Med.* **89,** 91–106.
6. Lerner, C. W. & Tapper, M. L. (1984) *Medicine* **63,** 155–164.
7. Odds, F. C., Abbott, A. B., Stiller, R. L., Scholer, H. J., Polak, A. & Stevens, D. A. (1983) *J. Clin. Microbiol.* **18,** 849–857.
8. Stiller, R. L., Bennett, J. E., Scholer, H. J., Wall, M., Polak, A. & Stevens, D. A. (1982) *Antimicrob. Agents Chemother.* **22(3),** 482–487.
9. Warnock, D. W. (1984) *J. Hosp. Infect.* **5,** 244–252.
10. Magee, B. B., D'Souza, T. M. & Magee, P. T. (1987) *J. Bacteriol.* **169,** 1639–1643.
11. Scherer, S. & Stevens, D. A. (1986) *J. Clin. Microbiol.* **25,** 675–679.
12. Wills, J. W., Lasker, B. A., Sirotkin, K. & Riggsby, W. S. (1984) *J. Bacteriol.* **157,** 918–924.
13. Calos, M. P. & Miller, J. H. (1980) *Cell* **20,** 579–595.
14. Cameron, J. R., Loh, E. Y. & Davis, R. W. (1979) *Cell* **16,** 739–751.
15. Strobel, E., Dunsmuir, P. & Rubin, G. (1979) *Cell* **17,** 429–439.
16. Davis, R. W., Botstein, D. & Roth, J. R. (1980) *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
17. Carle, F. G. & Olsen, M. V. (1984) *Nucleic Acids Res.* **12,** 5647–5663.
18. Murray, N. (1984) in *Lambda II* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 123–144.
19. Wickman, H. A., Potter, S. S. & Pine, D. S. (1985) *Nature (London)* **317,** 77–80.
20. Schwartz, D. C. & Cantor, C. (1984) *Cell* **37,** 67–75.
21. Snell, R. G. & Wilkins, R. J. (1986) *Nucleic Acids Res.* **14,** 4401–4406.
22. Whelan, W. L. & Kerridge, D. (1984) *Antimicrob. Agents Chemother.* **26,** 570–574.
23. Whelan, W., Markie, D. & Kwon-Chung, K. J. (1986) *Antimicrob. Agents Chemother.* **29,** 726–729.
24. Roeder, S. & Fink, G. R. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5621–5625.
25. Scherer, S. & Davis, R. W. (1980) *Science* **209,** 1380–1384.
26. Slutsky, B., Buffo, J. & Soll, D. R. (1985) *Science* **230,** 666–669.
27. Slutsky, B., Staebell, M., Anderson, J., Risen, L., Pfaller, M. & Soll, D. R. (1987) *J. Bacteriol.* **189,** 189–197.