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Characterization of the Inverted Duplication in the Mitochondrial DNA of *Candida albicans*

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The mitochondrial DNA (mtDNA) of *Candida albicans* contains a large inverted duplication. As is the case with most chloroplast DNAs and one other mtDNA, the nonduplicated regions of the molecule occur in two orientations with respect to each other, indicating that internal recombination occurs. Like some other mtDNAs, the *C. albicans* mtDNA contains a single *SalI* restriction site located near one end of the large rRNA gene. In contrast to other cases, however, the inverted duplication does not appear to contain any sequences coding for rRNA.

The *Candida albicans* mitochondrial genome is a circle of about 40 kilobase pairs (15). The circular molecule contains a large inverted duplication analogous to that found in almost all chloroplast DNAs (14), in some other mitochondrial DNAs (mtDNAs) (7, 16), and in other isolated instances. In nearly all of the cases of inverted duplications examined, the duplication contains sequences coding for rRNA. In the mtDNA of the water mold *Achlya ambisexualis*, homologous recombination occurs between the copies of the duplication, leading to two different relative orientations of the nonduplicated segments (7). This phenomenon is also observed in chloroplast DNA and in the yeast 2 μm circle (1). In this paper, we characterize the *C. albicans* inverted duplication in more detail. We demonstrate that recombination does occur in this mtDNA. We describe experiments which show that, in contrast to most mtDNAs with inverted duplications, the duplication in *C. albicans* does not contain sequences coding for rRNA. Finally, we note that a relatively simple and inexpensive method developed for extraction of DNA from filamentous fungi (6) can be adapted for the extraction of yeast mtDNA suitable for restriction fragment analysis and nick translation.

We have previously reported the cloning of five of the six *Eco*RI restriction fragments by ligating a mixture of pBR322 DNA and whole mtDNA from *C. albicans* H317, both cleaved with *Eco*RI (15). This procedure did not yield a clone containing the largest fragment, E1. Consequently, we cloned this fragment by electroelution of the E1 fragment from an agarose gel (13) and ligation into plasmid pBR322 (4).

Restriction site mapping was carried out as described previously (17), except for the *Ava*I and *Bst*EII sites in fragments E2 and E3. For these sites, DNA fragments consisting of the unique portions of E2 and E3 were isolated (J. A. Shaw, Ph.D. dissertation, University of Tennessee, Knoxville, 1989) and used to probe *Ava*I and *Bst*EII partial digests of E2 and E3. DNA from *C. albicans* was isolated as described previously (17). mtDNA from petite strains of *Saccharomyces cerevisiae* (kindly provided by A. Tzagoloff) was prepared essentially as described by Garber and Yoder (6) for filamentous fungi. This method proved to be relatively quick and inexpensive for the isolation of yeast mtDNA.

Internal rearrangement. We have shown before (17) that the *C. albicans* mtDNA contains a large inverted duplication, as illustrated by the heavy pointed arcs in Fig. 1. Figure 1 also shows the five *Kpn*I restriction sites, all located in the nonduplicated parts of the molecule. Consequently, digestion of whole mtDNA with *Kpn*I should yield five fragments (designated K1 to K5), whose sizes should be 18.5, 8.8, 6.9, 5.0, and 1.8 kilobases (kb), respectively. The results of such

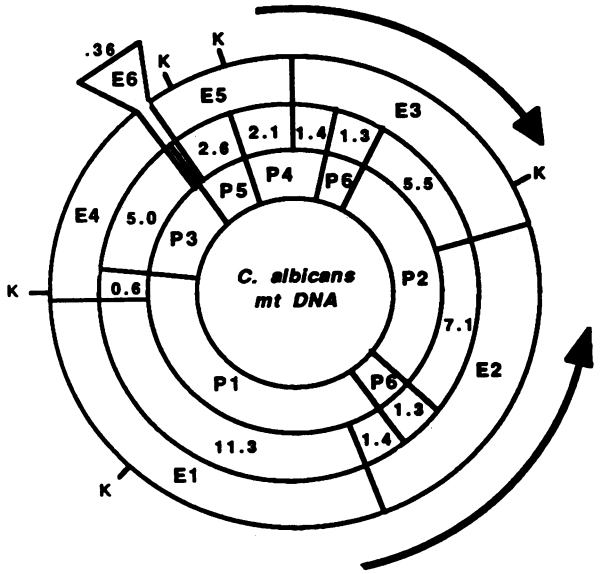

FIG. 1. Circular restriction map of *C. albicans* mtDNA. *Eco*RI (E) and *Pvu*II (P) restriction sites are indicated within the circles. *Kpn*I restriction sites (K) determined from the cloned fragments are indicated on the periphery. The heavy arcs indicate the approximate position of the inverted duplication.

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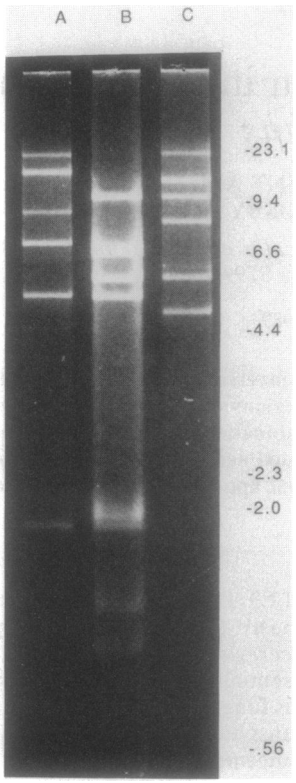

FIG. 2. Restriction fragment analysis of *C. albicans* whole mtDNA digested with *Kpn*I (lane A), *Eco*RI and *Kpn*I (lane B), or incompletely with *Eco*RI (lane C). The sizes of marker fragments (bacteriophage lambda DNA digested with *Hin*dIII) are shown on the right in kilobases.

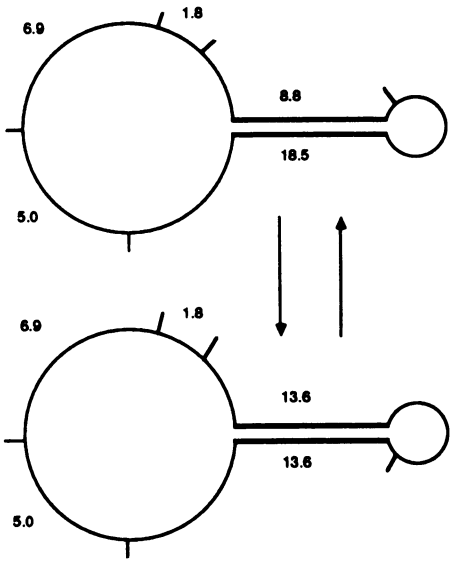

FIG. 3. Generation of unexpected *Kpn*I restriction fragments by internal homologous recombination of *C. albicans* mtDNA. Top, Redrawing of the *Kpn*I map shown in Fig. 1. Here the inverted duplication is indicated by the two horizontal lines and the nonduplicated regions are indicated by circles. *Kpn*I sites are indicated by short lines, and the sizes of the expected fragments are indicated in kilobases. Homologous recombination within the duplication would produce the structure shown at bottom.

a digestion are shown in Fig. 2. There are two unusual aspects to these results. First, in addition to the five expected bands, there is an additional band representing fragments of about 13.6 kb. Second, the intensities of the bands do not decrease with decreasing size as expected. In particular, the novel 13.6-kb fragment has a signal more intense than that of the 18.5-kb fragment.

These results can be explained if the molecule has undergone internal homologous recombination. Our analysis and interpretation of the internal recombination in the inverted duplication follow the strategies of Hudspeth et al. (7) and of Palmer (9), whose Fig. 3 is a model for our Fig. 3. In order to detect this phenomenon, it is necessary to use an enzyme which has cleavage sites only in the nonduplicated part of the molecule. Inspection of the map in Fig. 1 shows that *Kpn*I satisfies this requirement for *C. albicans* mtDNA. Generally, an internal recombination event leads to the production of two unexpected bands. However, the relative positions of the relevant sites may be such that the two new fragments are not resolvable (7); that is the case here, so that we observe a single band of double intensity rather than two separate bands.

Superficially, it might appear that the results obtained with *Kpn*I digestion could be attributed to incomplete digestion of the DNA rather than to the appearance of additional fragments due to internal recombination. On closer analysis, however, it is clear that this cannot be the case. First, the additional fragments generated are smaller than the largest band predicted by the data from the cloned fragments; incomplete digestion would be expected to produce larger rather than smaller fragments. Second, no combination of two adjacent fragments would give a fragment of the observed size; the sum of the lengths of K3 and K4 is 11.9 kb, which is about 2 kb smaller than the unexpected fragment. The sum of the lengths of K3, K4, and K5 is 13.7 kb, which is approximately the size of the unexpected fragment; for this fragment to be the result of incomplete digestion, it would be necessary for three of the five sites to experience incomplete digestion. But even this strained interpretation is ruled out by the relative intensities of the signals. If the unexpected fragment is an incompletely digested fragment including K3, K4, and K5, then its appearance would be at the expense of signals from those three separate fragments. As the data show, however, it is fragments K1 and K2 whose intensities are reduced relative to those of the other bands (Fig. 2), not K3, K4, and K5. Finally, the pattern shown in Fig. 2 is observed when the DNA is digested for 40 h at a 10-fold excess of enzyme (data not shown). We conclude that incomplete digestion cannot account for the unexpected fragment and that internal recombination does occur in this DNA.

In all organelle DNAs that contain an inverted duplication, the size of the duplicated region appears to vary with the size of the entire molecule in a systematic way. Although the size of the complete molecule varies from 27 to 145 kb, the fraction that is duplicated is consistently in the range of 30 to 39% (3, 8, 11). *C. albicans* is no exception to this generalization. This consistency may arise because of a compromise between the supposed stability gained from an inverted duplication and the energy required to replicate the extra DNA. Palmer et al. (10) point out a strong correlation between the presence of an inverted repeat and a stable chloroplast genome. However, they also showed that deletion of an inverted duplication did not always result in an unstable genome. In an analysis of the *Achlya* inverted

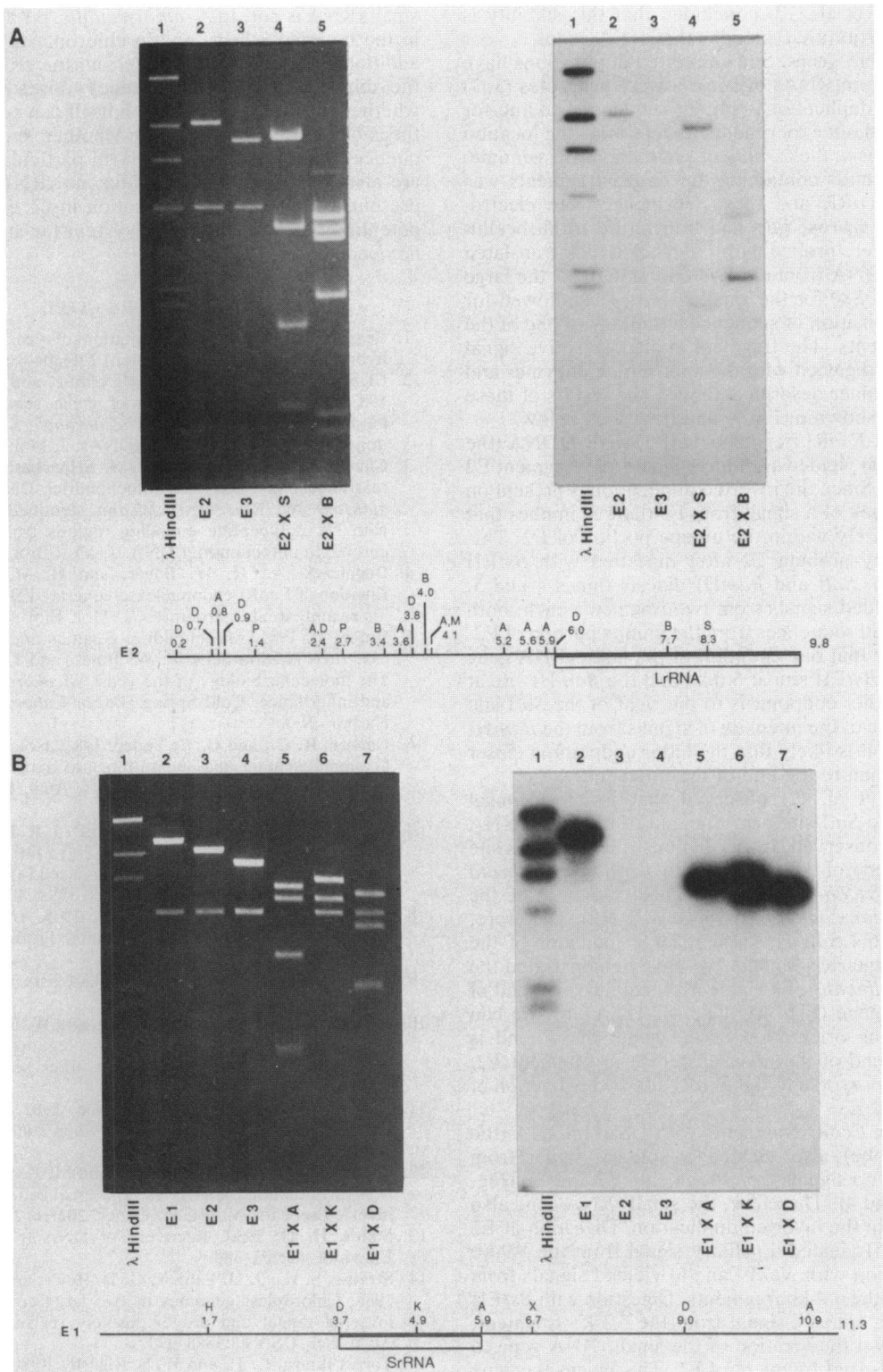

FIG. 4. Location of rRNA genes of *C. albicans* mtDNA. (A) Ethidium bromide-stained gel (left) and autoradiograph (right) of blot probed with ^{32}P-labeled mtDNA from petite strain DS631. Lanes: 1, lambda *Hin*dIII marker; 2, fragment E2, digestion with *Eco*RI; 3, fragment E3, digestion with *Eco*RI; 4, fragment E2, digestion with *Eco*RI and *Sal*I; 5, fragment E2, digestion with *Eco*RI and *Bam*HI. (Fragments E1 and E4 to E6, which gave no signals, are not included here for the sake of clarity.) (B) Ethidium bromide-stained gel (left) and autoradiograph (right) of blot probed with ^{32}P-labeled mtDNA from petite strain DS80. Lanes: 1, lambda *Hin*dIII marker; 2, fragment E1, digestion with *Eco*RI; 3, fragment E2, digestion with *Eco*RI; 4, fragment E3, digestion with *Eco*RI; 5, fragment E1, digestion with *Eco*RI and *Ava*I; 6, fragment E1, digestion with *Eco*RI and *Kpn*I; 7, fragment E1, digestion with *Eco*RI and *Bst*EII. (Fragments E4 to E6, which gave no signals, are not included here for the sake of clarity.) The 4.4-kb fragment which appears in most lanes at the same position as the fourth-largest marker fragment is the pBR322 vector DNA. This fragment is cleaved into smaller fragments by *Ava*I, *Sal*I, and *Kpn*I. LrRNA, Large rRNA; SrRNA, small rRNA.

repeat, Shumard et al. (12) concluded that this stability is limited to the portion that encodes the rRNA genes.

Location of rRNA genes. Since inverted duplications have been found in the mtDNAs of some lower eucaryotes (3, 7) and all of these duplications contain sequences coding for rRNA, we carried out experiments to determine the location of the rRNA genes in the *C. albicans* mitochondrial genome. Each of the plasmids containing the *Eco*RI fragments was digested with *Eco*RI, and these fragments were electrophoresed on 1% agarose gels and transferred to nitrocellulose. The blots were probed with ^{32}P-labeled nick-translated *S. cerevisiae* mtDNA from either strain DS631 for the large rRNA or strain DS80 for the small rRNA. This allowed for the primary localization of sequence homology to one of the six *Eco*RI fragments. The fragment giving a positive signal was then further digested with the appropriate enzymes and probed in the manner described above. The results of these experiments are shown in Fig. 4 and described below.

Probing the six *Eco*RI fragments with DS631 mtDNA (the large rRNA probe) yielded a solitary signal from fragment E2 (Fig. 4A, lane 2). Since the inverted duplication is present on E2 and E3, the lack of a signal from E3 (lane 3) implies that the gene lies entirely within the unique portion of E2. This was confirmed by probing E2 after digestion with *Bst*EII (data not shown). *Sal*I and *Bam*HI digests (lanes 4 and 5, respectively) yielded signals from two fragments each, both of which contained sequences from the unique portion of E2. We can conclude that one endpoint of the large rRNA gene lies between the *Bst*EII site at 6.0 kb and the *Bam*HI site at 7.7 kb and the other endpoint is to the right of the *Sal*I site at 8.3 kb. Because of the intensity of signals from the *Bam*HI and *Sal*I digests, it is likely that the latter endpoint is closer to the *Sal*I site than to the end of the fragment.

Clark-Walker et al. (2) observed that in most fungal mtDNAs, a single *Sal*I site near one end of the large rRNA gene is strongly conserved. It was demonstrated that this site was near the 3′ end of the gene in both *Torulopsis glabrata* and *Kloeckera africana* (3). This was also shown to be the case in *S. cerevisiae* (5) and *A. ambisexualis* (12). Therefore, it appears that this *Sal*I site is a reliable indicator of the polarity of the large rRNA gene. We have demonstrated the existence in *C. albicans* of a single *Sal*I site near one end of the large rRNA gene (Fig. 4A, lane 4). Therefore, we can infer the following orientation of the gene: the 5′ end is proximal to the end of the inverted repeat in fragment E2, and the 3′ end is proximal to the *Eco*RI site at the junction of fragments E2 and E3.

Probing the six *Eco*RI fragments with DS80 mtDNA (the small rRNA probe) also yielded a solitary signal from fragment E1 and no signals from fragments E2 and E3 (Fig. 4B, lanes 2, 3, and 4). Therefore, the small rRNA gene also does not lie within the inverted duplication. Digestion of E1 with *Ava*I (lane 5) yielded a solitary signal from the 5.9-kb fragment. Digestion with *Kpn*I (lane 6) yielded signals from both the 4.9- and the 6.4-kb fragments. Digestion with *Bst*EII (lane 7) yielded a solitary signal from the 5.3-kb fragment. This narrows down the location of the small rRNA gene to the 2.2-kb *Bst*EII-*Ava*I fragment of E1. This places the gene at least 5 kb from the end of the inverted duplication.

Until now, all organelle DNAs with inverted duplications were observed to carry all or part of the ribosomal sequences within the duplicated region. In *K. africana*, only a portion of the large rRNA gene is in the duplication; in *Tetrahymena* spp., the entire large rRNA is in the duplication while the small rRNA is not. In *A. ambisexualis*, both rRNA genes are in the inverted repeat, and in chloroplasts (which carry an additional, smaller rRNA gene), all the rRNA genes are in the duplication. This consistency raises the question of whether an inverted duplication itself can confer stability on the molecule containing it or whether specific coding sequences within the duplication (in particular, rRNA genes) are also necessary. The fact that no rRNA sequences are present in the inverted duplication in *C. albicans* makes it potentially useful as a model system for studying this phenomenon.

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