

Identification of Sequence Homologies between Maxicircle  
DNA of *Leishmania tarentolae* and Specific Yeast  
Mitochondrial Structural Genes

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INTRODUCTION

The kinetoplast DNA of the hemoflagellate protozoan *Leishmania tarentolae* is composed of two molecular species, minicircles (~0.9 kilobases, kb) and maxicircles (30 kb), catenated together to form a giant network of mitochondrial DNA (mtDNA) (Borst and Hoeijmakers, 1979). The maxicircle DNA appears to represent the equivalent of the informational mtDNA found in other cells. This DNA is transcribed into two abundant RNA species of size 9S (520 b) and 12S (1,020 b) (Simpson and Simpson, 1978) and similar RNAs exhibiting a high degree of sequence conservation have been observed in several kinetoplastid species (Simpson and Simpson, 1980; Simpson *et al.*, 1980) suggesting that these RNAs represent the presumptive ribosomal RNAs in kinetoplastida (Borst and Hoeijmakers, 1979; Simpson *et al.*, 1980). In addition, several less abundant polyadenylated transcripts of maxicircle DNA, ranging in size from 0.1 to 1.8 kb, have been described for *L.tarentolae* (Simpson *et al.*, 1980; Simpson *et al.*, 1982b) and *Trypanosoma brucei*

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(Hoeijmakers *et al.*, 1981) suggesting that maxicircle DNA may also specify information for several mitochondrial structural genes.

An intriguing feature of mitochondrial genomes studied to date has been the retention in mtDNAs of a limited number of structural genes whose amino acid and to a lesser extent nucleotide sequences have been predominantly conserved although the physical arrangement of these structural genes within the genome has varied (Tzagoloff *et al.*, 1979; Anderson *et al.*, 1981; Clark-Walker and Sriprakash, 1981). The conservation of nucleotide sequences in mitochondrial genes has allowed the rapid mapping of genes in heterologous mitochondrial genomes using yeast mtDNA sequences as hybridization probes (Macino *et al.*, 1980).

In an attempt to further define maxicircle DNA structure and function, we have similarly used yeast mitochondrial structural gene fragments as probes to locate homologous sequences in the maxicircle DNA of *L.tarentolae*. Although these results provide tentative gene localizations, they represent the first indication that maxicircle DNA encodes the structural genes for cytochrome *b*, ATPase subunit 6 and cytochrome oxidase subunits I and II (Simpson *et al.*, 1982a,c).

## RESULTS AND DISCUSSION

Maxicircle DNA from *L.tarentolae*, linearized at the unique *EcoRI* site (RIMaxi DNA), was isolated from kinetoplast DNA networks in CsCl gradients (Simpson, 1979). An *EcoRI* - *BamHI* fragment of maxicircle DNA cloned in pBR 322 was isolated as described (Masuda *et al.*, 1979). The purified RIMaxi DNA and cloned insert DNA were digested with several restriction endonucleases, the DNA fragments were size fractionated in agarose gels and blotted onto nitrocellulose. As hybridization probes, we obtained from A. Tzagoloff a series of yeast petite mutants which retained various mitochondrial structural genes, isolated the petite mtDNAs and purified specific restriction fragments bearing DNA sequences contained entirely within the various structural genes (see Simpson *et al.*, 1982a). These gene fragments were labelled by nick translation and hybridized to the maxicircle blots at low stringency (5% Formamide, 1M NaCl, 37°C, 48h). The blots were washed in 6 x SSC at 41°C

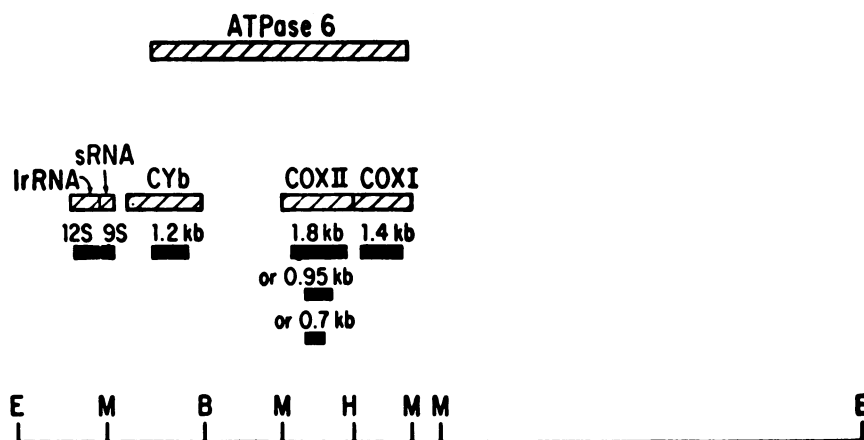


FIGURE 1. Organization of structural genes and tentative RNA transcripts of the genes on the maxicircle DNA of *L. tarentolae*. The maxicircle (30 kb) is linearized at the EcoRI (E) site and the MspI (M), Bam HI (B) and Hha I (H) sites are shown. The hatched bars represent the structural gene localizations and the solid bars represent the positions of RNA transcripts (size in kb) which have been mapped and tentatively assigned to the CYb, COX I and COX II genes (Simpson *et al.*, 1982b). The 9S and 12S RNAs are transcripts of the putative small and large ribosomal RNA genes.

for 2h, exposed to film and then rewashed in 6 x SSC at increasing temperature to determine the thermal stability of the hybrids.

Hybridization to maxicircle DNA was observed with probes representing fragments of the yeast genes for cytochrome b (exon b1) (CYb), ATPase subunit 6, and cytochrome oxidase subunits I (exon A5) and II (COX I, COX II). The probe for ATPase subunit 9 failed to hybridize. As the stringency of the post hybridization washes was increased, it was possible to localize the regions of homology to one or two adjacent maxicircle fragments although the hybridization to the ATPase 6 probe extended over several fragments (Fig. 1).

The thermal stabilities of the hybrids were assessed in order to gain information on the relative degree of mismatch between the various yeast probes and the homologous maxicircle sequences. The order of melting and the approximate melting temperature was: CYb (80°), ATPase 6 (70°), COX I

and II (60°). With an average GC content for a mitochondrial gene of 30% (Nobrega and Tzagoloff, 1980), the  $T_m$  for such a gene under our hybridization conditions is 90°. Assuming a 1° decrease in  $T_m$  per 1.5% base sequence mismatch (Laird et al., 1969), there is approximately a 15-45% sequence mismatch between those yeast and maxicircle sequences sufficiently homologous to form stable hybrids. These results suggest a greater degree of sequence homology between the cytochrome b (exon b1) genes of yeast and Leishmania than any other mitochondrial gene.

The identification of maxicircle DNA sequences which form thermally stable hybrids with defined yeast mitochondrial structural gene sequences implies that the maxicircle of L.tarentolae encodes these structural genes but DNA sequence analysis is needed to prove this. The localizations of the putative structural genes on the maxicircle DNA restriction map are shown in Fig. 1 together with the positions of several maxicircle transcripts which may represent the transcripts of these genes (Simpson et al., 1982b). The CYb (exon b1) gene is mapped within the maxicircle fragment cloned in pBR 322 and this region is currently being sequenced to confirm the presence of this gene. The COX I (exon A5) and COX II genes are positioned on adjacent DNA fragments although the COX I gene may overlap the Hha I site. The ATPase 6 gene cannot be precisely mapped to date since the yeast probe hybridized to several maxicircle fragments in a thermally stable fashion. In each case, candidate transcripts of appropriate size have been mapped to the positions of the genes in the maxicircle.

The question of the presence in maxicircle DNA of genes for ATPase subunit 9 and cytochrome oxidase subunit III is uncertain. The failure to observe hybrids with the ATPase 9 probe suggests either that this gene is absent from maxicircle DNA, as in humans, Neurospora and Aspergillus (Tzagoloff et al., 1979; Macino et al., 1980; Anderson et al., 1981) or that this maxicircle gene has evolved to such an extent that stable hybrids cannot be obtained. With the COX III probe, we observed extensive hybridization to several non-adjacent maxicircle fragments but these hybrids melted at a low temperature (48°) implying extensive mismatch. These results suggest that in Leishmania genes for some mitochondrial proteins may either be found in the nucleus (ATPase 9) or may evolve more rapidly than others (COX III) and this rate of sequence divergence may limit the application of the heterologous gene mapping procedure in evolutionary diverse organisms. Despite these limitations, this procedure has provided the first evidence for the

physical presence and arrangement in maxicircle DNA of mitochondrial structural genes homologous to those found in yeast.

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