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# Sequence Organization of Maxicircle Kinetoplast DNA from *Leishmania tarentolae*

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The kinetoplast DNA of the hemoflagellate protozoa (Simpson 1972) consists of two molecular species, the minicircles and the maxicircles, catenated together to form a giant network of DNA situated within the single mitochondrion of the cell (Borst and Hoeijmakers 1979a; Englund 1980). The maxicircle appears to represent the homolog of the informational mtDNA found in other cells (Simpson and Simpson 1978); the role of the minicircle is unknown. The major RNA species present in a purified kinetoplast fraction from *Leishmania tarentolae* are the 9S and 12S RNAs, which were shown to be transcribed from the maxicircle DNA in an ethidium-bromide-sensitive process (Simpson and Simpson 1978). Similar RNAs have also been isolated from purified kinetoplast fractions of *Phytomonas davidae* (Cheng and Simpson 1978), *Trypanosoma brucei* (Simpson and Simpson 1980), and *Crithidia fasciculata* (unpubl.). A high level of sequence conservation of 9S and 12S RNAs has been demonstrated for several hemoflagellate species by hybridization techniques (Simpson and Simpson 1978; Borst and Hoeijmakers 1979b; Simpson and Simpson 1980). In this paper we describe several additional maxicircle transcripts and present a general method to identify mitochondrial structural genes by the use of heterologous DNA probes.

## MAXICIRCLE TRANSCRIPTS: NORTHERN BLOT ANALYSIS

Total kinetoplast RNA and poly(A)$^+$ kinetoplast RNA were isolated from Renografin-purified kinetoplast fractions of *L. tarentolae* (Simpson and Simpson 1978), separated by electrophoresis in formaldehyde-agarose gels, and blotted onto nitrocellulose filters. The filters were probed with nick-translated maxicircle DNAs, including the 6.6-kb cloned *Eco*RI/*Bam*HI fragment that contains the 9S RNA and 12S RNA genes (pLt120) (Masuda et al. 1979), the total *Eco*RI-linearized maxicircle DNA isolated

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by Hoechst-dye–CsCl gradient centrifugation (RI Maxi DNA) (Simpson 1979), several M13 *Sau*3A subclones of the 120 fragment, and several gel-isolated restriction fragments of maxicircle DNA. At least seven RNAs map to the 120 region (Simpson et al. 1980) and at least ten additional RNAs map to the remainder of the maxicircle. Several of the RNAs appear to be polyadenylated. A preliminary transcription map of the maxicircle was derived from these hybridizations. Hoeijmakers et al. (1981) have also found several polyadenylated maxicircle transcripts in *T. brucei*.

## DIRECTION OF TRANSCRIPTION

The 9S and 12S RNAs were shown to be transcribed from the same strand in the direction 12S → 9S. This was demonstrated by strand separation in agarose of the *Eco*RI/*Msp*I fragment of the 120 insert that contains the entire 12S RNA gene and much of the 9S RNA gene. Hybridization of in-vitro-labeled 9S and 12S RNAs to blots of this gel occurred only with the slow strand. The polarity of the strand was determined by single 5′-end-labeling this fragment at the *Eco*RI site prior to strand separation in agarose. The 5′-end label was in the fast strand, implying a 3′ → 5′ orientation of the slow strand in the *Eco*RI → *Bam*HI direction and a 12S → 9S direction of transcription. Hoeijmakers et al. (1981) have also shown a 12S → 9S direction of transcription in *T. brucei*. Direct Gilbert-Maxam sequence analysis of the 3′-terminal portion of the 9S RNA gene demonstrated an abundance of termination codons and a definite but low-level sequence homology with the 3′ ends of several known small subunit rRNAs. The identification of the 9S and 12S RNAs as mitochondrial rRNAs is strongly indicated by these data. This identification was difficult to substantiate previously owing to the unusually small size of the RNAs and to the difficulty in isolating bona fide mitochondrial ribosomes from hemoflagellates. Another unusual characteristic of these mitochondrial mini-rRNAs is, clearly, the direction of transcription, from large rRNA to small rRNA.

## AT-RICH REGIONS IN THE MAXICIRCLE

The maxicircle DNA of *L. tarentolae* has an overall base ratio of 79% AT, and the 9S and 12S RNAs reflect this overall base ratio with AU contents of 80% (Simpson and Simpson 1978). However, the distribution of AT-rich regions in the maxicircle is clearly heterogeneous, as shown previously by analytical CsCl analysis (Simpson 1979) and in this report by partial denaturation mapping of molecules spread from 70% formamide. The existence of three asymmetrically localized denaturation loops in the cloned 120 fragment allowed the localization of the 120 side of the *Eco*RI linearized maxicircle. The maxicircle map is characterized by two small

AT-rich loops corresponding to the 9S and 12S genes, several large AT-rich regions near the center of the molecule, and six or seven short closely spaced AT-rich regions at the other side of the molecule.

### LOCALIZATION OF SPECIFIC MITOCHONDRIAL STRUCTURAL GENES

Localization of specific mitochondrial structural genes was attempted by low-stringency hybridization of labeled DNA probes isolated from the mtDNA of several yeast petite mutants that contain the genes, or portions of the genes, for cytochrome oxidase subunits I (COI), II (COII), and III (COIII); ATPase subunits 6 and 9; and cytochrome *b* (Table 1). The DNA fragments were isolated from agarose and labeled by nick-translation. RI Maxi DNA was digested with several enzymes and the fragments were separated on agarose and blotted onto nitrocellulose filters, which were hybridized with the yeast probes under low-stringency conditions (corresponding to $T_m$ − 49°C to $T_m$ − 42°C) (Howley et al. 1979). Hybrids were washed under increasingly stringent conditions to determine the melting temperatures and approximate percentage of mismatch.

Hybridization to maxicircle fragments was observed for the yeast COI, COII, and COIII, ATPase-6, and cytochrome-*b* probes. No hybridization was observed with the yeast ATPase-9 probe, nor was hybridization of any of the yeast probes observed under these conditions with $\lambda$ or $\phi$XRF fragments. Usually, at a low stringency, several maxicircle fragments showed varying degrees of hybridization, but as the stringency was increased the hybridization became limited to one or two adjacent fragments. The COI, COII, and cytochrome-*b* hybridizing regions could be established fairly precisely. The COIII and ATPase-6 regions could not be as precisely defined but extended over several maxicircle fragments. The thermal stabilities of the heterologous hybrids were in the order

*Table 1* Yeast petite probes for mitochondrial structural genes

| Strain | Gene | Digest | Fragments isolated[a] (bp) |
|---|---|---|---|
| DS6/A407[b] | COI (exon A5) | *Hin*fI/*Taq*I | ~1000, ~840, [280], 240, 210 |
| DS302[c] | COII | *Pvu*I/*Taq*I | ~2800, [272], [168] |
| DS40[d] | COIII | *HpH*I/*Pvu*II | 1948, 1580, [478], [170] |
| DS14[e] | ATPase 6 | *Mbo*I/*Eco*RI | 1747, 1205, [405], [254] |
| DS400/A3[f] | ATPase 9 | *Alu*I/*Hpa*II | 611, 388, 359, 222, [84], [75] |
| DS400/M8[g] | cytochrome *b* (exon B1) | *Hae*III/*Hin*fI | 807, [477], [233] |

[a]Fragments in boxes were isolated and labeled.
REFERENCES: [b]Bonitz et al (1980); [c]Coruzzi and Tzagoloff (1979); [d]Thalenfeld and Tzagoloff (1980); [e]Macino and Tzagoloff (1980); [f]Macino and Tzagoloff (1979); [g]Nobrega and Tzagoloff (1980).

(lowest to highest): COIII → COI, COII → ATPase 6, cytochrome *b*. We realize that the hybridization results provide only tentative gene localizations, and definitive identification of the hybridizing regions as specific structural genes must await direct sequence analysis. However, from the tentative localizations of these structural genes and from our preliminary maxicircle transcription map, we have several possible candidates for RNA transcripts of these structural genes. For example, a 1400-nucleotide RNA localized outside the 120 region is a candidate for the COI transcript and a 1200-nucleotide RNA localized within the 120 region is a candidate for the cytochrome-*b* transcript.

## DISCUSSION

The conservation of mitochondrial gene sequences first noted in the comparisons of yeast, bovine, and human sequences (Fox 1979; Macino et al. 1979; Anderson et al. 1981) appears to be sufficient to allow the localization of mitochondrial genes by hybridization with heterologous DNA probes. The yeast petite mutants characterized extensively by Tzagoloff (Table 1) represent especially favorable material to obtain specific mitochondrial gene probes. Macino et al. (1980) used the yeast probes to localize specific mtDNA of the related fungus *Aspergillus nidulans*. We have used the yeast probes to localize specific structural genes in the maxicircle DNA of a hemoflagellate protozoan. It appears that this may be a general method to localize mitochondrial structural genes even in distantly related organisms.

The transcription of the maxicircle DNA of *L. tarentolae* involves the production of two mini-rRNAs and several polyadenylated presumptive mRNAs ranging in size from 1.8 kb to 0.2 kb. The number, sizes, and localizations of the polyadenylated transcripts are adequate to account for the tentatively identified and assumed mitochondrial genes present in maxicircle DNA.

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