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Mitochondrial DNA of an African Trypanosome

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The maxicircles of African trypanosome kDNA are the genetic equivalent of other mitochondrial DNAs, but the function of minicircles is unknown. The maxicircle of Trypanosoma brucei 164 encodes conventional mitochondrial gene products and is largely but not completely transcribed. Nucleotide sequence analysis of a region not found to be transcribed revealed numerous translation termination codons in all three reading frames of both strands and numerous inverted repeats, suggesting that this segment does not have polypeptide-coding function. This segment may encode a t-RNA and has a sequence resembling a consensus sequence found in mitochondrial introns, thus implying that transcript processing occurs in trypanosome mitochondria. While several cloned minicircles had distinct restriction maps reflecting T brucei minicircle heterogeneity, one segment of the minicircle contained a sequence that was conserved by minicircles from other trypanosome strains and species. Of nine mutants unable to grow as the respiring procyclic forms, seven were devoid of kDNA. The other two mutants retained normal amounts of all maxicircle restriction fragments and normal amounts of those minicircle sequences tested. Minicircle alterations probably occur in these mutants, since the kDNA does not stain with Giemsa and bands at an altered density in cesium chloride/ethidium bromide density gradients.

Key words: mitochondrial DNA, maxicircles, rRNA, minicircles, dyskinetoplastic mutants, restriction map, transcripts

The mitochondrial DNA of African trypanosomes shares the general characteristics of other kDNAs. These include the presence of maxicircles and numerous minicircles, all of which are interlocked into a single molecular complex. African trypanosome kDNA is most extensively characterized in Trypanosoma brucei, although important kDNA differences exist within this group (see reviews [1–3]). About 7% of the T brucei cellular DNA is kDNA, which contains about 45 maxicircles and 5,500 minicircles [4].

T brucei kDNA contains the smallest maxicircles among the kinetoplastida [2], and based on restriction endonuclease mapping and renaturation kinetic analyses all

Abbreviations used: kDNA, kinetoplast DNA; Dk, dyskinetoplastic; (k)bp, (kilo)base pair(s); Cox I and II, cytochrome oxidase subunits I and II.

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maxicircles in the kDNA complex are identical [5]. The maxicircle is largely but not completely transcribed [6] and has been shown to encode mitochondrial rRNA [6,7] and possibly cytochrome oxidase subunit II [8].

T brucei minicircles are among the smallest minicircles, but the total complexity of minicircles is the greatest in T brucei. There are at least 300 different minicircles in T brucei kDNA compared to between three and 70 in other kinetoplastids [2]. However, various minicircles differ in their abundance in the kDNA complex and have partial sequence homology with each other [5,9]. Thus, the number of distinct minicircles is underestimated by renaturation kinetic analyses, and it is possible that there are few identical minicircles in T brucei kDNA. T brucei minicircles do not appear to be transcribed and their function is unknown.

In addition to these kDNA differences the mitochondrial respiratory system, whose biogenesis requires the activity of the mitochondrial genetic system, is inactive in bloodstream trypomastigotes of T brucei and active in procyclic trypomastigotes [10]. Bloodstream trypomastigotes lack Krebs cycle and cytochrome enzymes and derive their energy from glycolysis. Procyclic trypomastigotes generate most ATP by oxidative phosphorylation and have fully functional Krebs and cytochrome systems like non-African trypanosome kinetoplastids. With one possible exception, the same maxicircle transcripts are produced by bloodstream and procyclic trypomastigotes [6,7].

Dyskinetoplastic (Dk) mutants of T brucei lack Giemsa-stainable kDNA and are unable to convert to the respiring procyclic forms [11]. They may also lack oligomycin-sensitive ATPase activity and thus resemble petite mutants of yeast [12].

This report presents nucleotide sequence analysis of a maxicircle segment which has not been shown to be transcribed, mapping and nucleotide sequence analysis of minicircles, and characterization of kDNA sequence loss and retention in Dk mutants.

METHODS

The normal T brucei used in this study was strain 164 whose origin, growth, and isolation was previously described [13]. Dk mutants were cloned from a mutagenized population of this strain [11]. Published procedures for kDNA [4] and total cell DNA [14] isolations were used, and the cloning and characterization of the maxicircle and minicircle sequences has been described previously [5,6].

For nucleotide sequence analysis cloned fragments were subcloned into M13 bacteriophage vectors [15], and the nucleotide sequence was determined using the chain termination method [16]. Some of the fragments were produced by restriction endonuclease cleavage and subcloned directly. Other fragments were digested to varying extents with Bal 31 exonuclease to produce a series of fragments that would be convenient for sequence analysis. This was done as follows: 20 μg of plasmid pTKHR42 DNA was linearized by digestion with Hind III. The DNA was then digested with 18 units of Bal 31 in 20 mM Tris, 600 mM NaCl, 12 mM $MgCl_2$, 12 mM $CaCl_2$, 1 mM ethylenediamine tetra-acetic acid (EDTA), pH 8.1, in a final volume of 200 μl at 30 °C. At 40-sec intervals, 6.5-μl samples were withdrawn and pooled into 250 μl 0.25 M EDTA to stop the reaction. Under these conditions DNA was digested at a rate of 307 base pairs (bp)/min/end and the rate of the reaction was linear for at least 8,250 bp (4,125/end). The pooled DNA fragments were extracted with phenol and chloroform and precipitated with ethanol. The DNA was then treated

with 3.5 units Escherichia coli DNA polymerase I in 50 mM Tris, 5 mM $MgCl_2$, 10 mM mercaptoethanol, 60 μM each deoxynucleotide triphosphate (dNTP), pH 7.6, to produce blunt ends. The DNA was ethanol precipitated, then ligated with phosphorylated Hind III linkers in 66 mM Tris, 6.6 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 0.5 mg/ml bovine serum albumin (BSA), 0.6 mM adenosine triphosphate (ATP), pH 7.5, using 1.4 units T4 ligase at 14°C for 20 hr. Three-hundred-pmole linkers had been phosphorylated using 60 units polynucleotide kinase in 70 mM Tris, 10 mM $MgCl_2$, 5 mM DTT, 1 mM spermine, 0.1 mM KCl, 0.25 mg/ml BSA, and 1 mM ATP pH 7.6 at 37 °C for 40 min. The DNA was then digested with 60 units Hind III and 18 units Eco RI for 2 hr at 37°C, extracted with phenol and chloroform, and ethanol-precipitated. One hundred sixty ng of this DNA was ligated with 100 ng M13mp8 vector DNA that had been digested with Hind III and Eco RI under the same ligation conditions used for linker addition, except 0.3 units of ligase was used. The ligated DNA was transformed into JM103 host cells using standard conditions [15].

DNA was digested with restriction endonuclease according to the supplier's recommended conditions. DNA was radiolabeled in vitro by nick translation [17] and hybridization to restriction fragments of DNA that had been electrophoretically separated was performed according to Southern [18]. Isopycnic centrifugation in cesium chloride containing 140 μg/ml ethidium bromide was performed at 48,000 rpm for 16 hr at 15°C using a VTi 65 rotor.

RESULTS

A restriction map of the T brucei 164 maxicircle is shown in Figure 1. Transcripts which have been observed are indicated below the fragment or fragments to which they map. The 1,350- and 750-nucleotide transcripts are the rRNAs which map to the H1R2 fragment [6]. The cytochrome oxidase subunit II gene sequences from yeast and maize mitochondrial DNA hybridize to the R2H2 fragment [8], and the R4R1 fragment varies in size among stocks [5]. When nick-translated clone 18BLTB8107, which contains cytochrome oxidase subunit I sequence from yeast [19], was hybridized to Southern blots of kDNA digested with Hind III, the hybridization

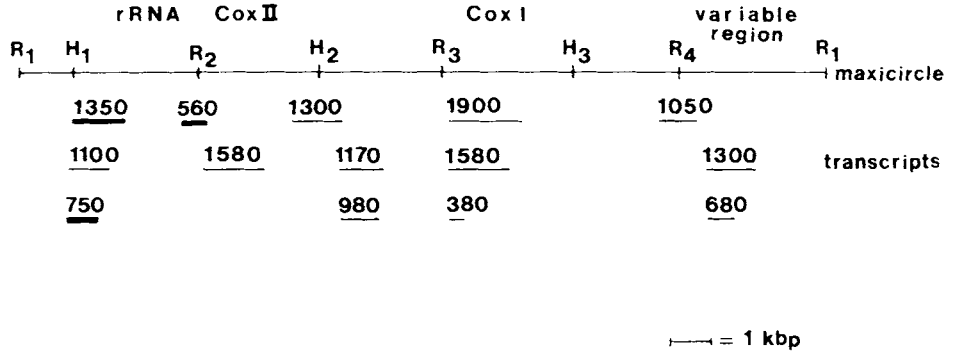

Fig. 1. Simplified linearized restriction endonuclease cleavage map of the T brucei 164 maxicircle. Transcript sizes are in nucleotides and the most abundant transcripts are indicated with a heavier line. Other features are indicated above the map, as detailed in the text. H = Hind III, R = Eco RI.

was restricted to the H2H3 fragment. Hybridization was restricted to the R3R4 fragment when the kDNA was digested with Eco RI and Bam HI or Eco RI and Pst I. Bam HI cleaves the H1R2 fragment, while Pst I cleaves the H2R3 fragment [4]. This hybridization was stable at 60°C in 5 × 0.15M sodium chloride, 0.015 sodium citrate (SSC). Thus the Cox I probe hybridizes to the R3H3 fragment, as indicated in Figure 1.

The maxicircle sequences from the R1H1 fragment were subcloned into M13 vectors, as shown in Figure 2. The subclones indicated with a solid circle were generated by Bal 31 digestion, and the restriction sites are indicated by vertical crosshatches. The arrow indicates the direction of sequencing. The nucleotide sequence of the R1T4 region where overlapping sequence data was obtained is given in Figure 3. This region of the maxicircle is neither A+T or G+C rich compared to the maxicircle as a whole, although partial sequences of the H1R2 region were more G+C rich than the maxicircle as a whole (data not shown). The overall A+T content of the R1T4 region was 75% which is close to that obtained for the maxicircle by isopycnic cesium chloride centrifugation [4]. The R1T4 segment contained numerous mitochondrial translation termination codons, numerous short (6–10 bp) inverted repeats, a sequence resembling a consensus sequence found in mitochondrial introns, two 8-bp G+C rich sequences, and a sequence capable of encoding a t-RNA like molecule.

Translation termination codons occurred frequently in the R1T4 sequence in all three reading frames of both DNA strands, as shown in Figure 4. The largest open reading frame beginning with an initiation codon could encode 59 amino acids, as shown in reading frame 3 reading rightward. Thus, it is unlikely that this region has a polypeptide encoding function.

The numerous short (6–10 bp) inverted repeated sequences were concentrated between nucleotides 105 and 691. They were more abundant in this sequence than in other regions of the maxicircle that we have sequenced and many of these repeats were overlapping. The repeats were so arranged that complex secondary structures could be envisioned which could occur either as cruciform structures in the DNA or stems and loops in the transcript. Figure 5 shows one such secondary structure to

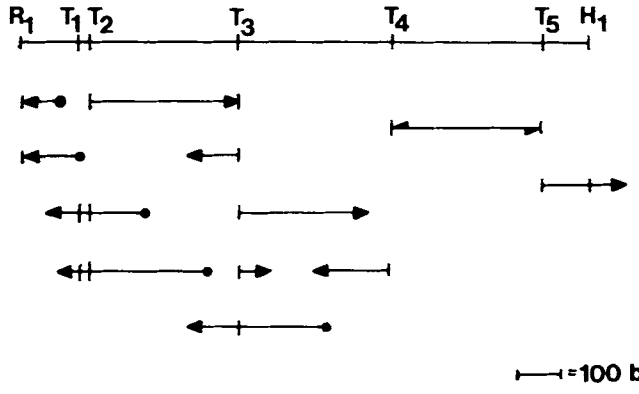

Fig. 2. Fragments of the R1H1 region that were subcloned into M13. Fragments generated by Bal 31 digestion are indicated with a solid circle, restriction sites by vertical crosshatches, and the direction of sequencing by the arrow. R = Eco RI, T = Taq I.

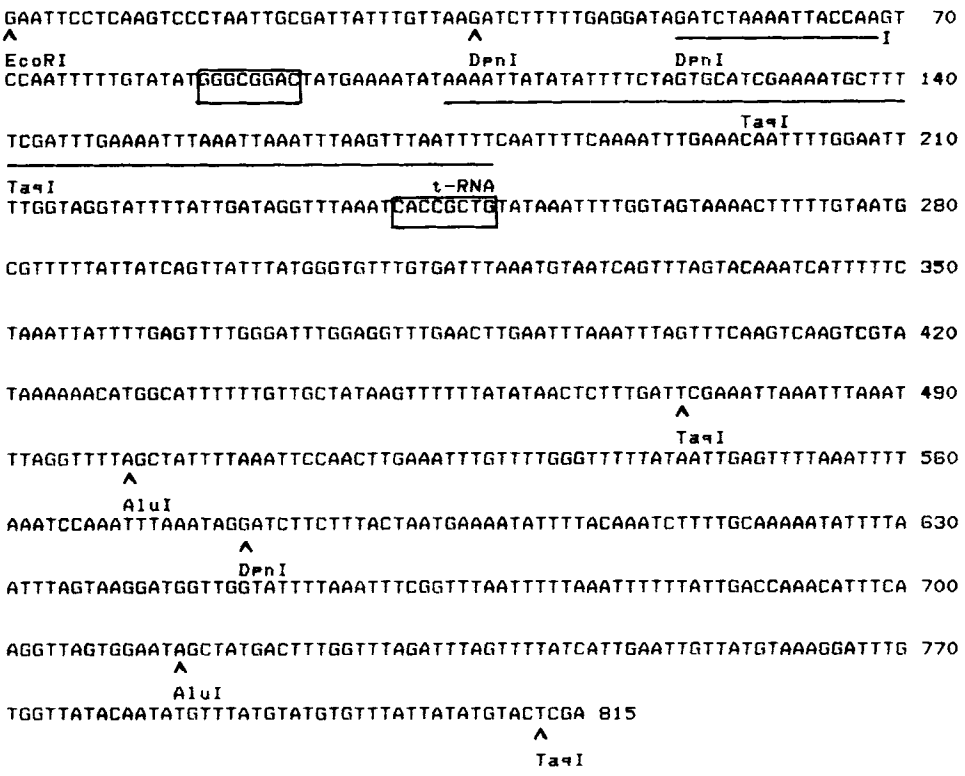

Fig. 3. The nucleotide sequence of the R1T4 segment of the R1H1 maxicircle fragment. The intron consensus-like sequence is indicated by the underline labeled I. The G+C-rich sequences are boxed and flank the possible t-RNA coding sequence indicated by the underline labeled t-RNA. The locations of some restriction sites are indicated.

illustrate the arrangement of the inverted repeats. Another possible secondary structure which resembles t-RNA is described below.

The sequence from nucleotides 52 to 68 has 11 of 16 nucleotides which match a consensus sequence which has been found in mitochondrial introns [20], as shown in Figure 6. The degree of relatedness of this sequence is within the range of divergence that was observed among the several sequences identified in mitochondrial introns. Three other sequences which resemble the intron consensus sequence were found in other regions of the maxicircle. One was found in the H2H3 region which contains the sequence which hybridizes to the Cox 1 probe. Twelve of 16 of the nucleotides in this sequence match those in the consensus sequence. The other two sequences were found in the H1R2 sequence which contains the rRNA coding sequences. Only ten of 16 matched the consensus sequence in one of these, but the matching nucleotides were present in two blocks. In the second, 12 of 16 nucleotides matched the consensus sequence. The presence of these sequences, while not proof, is suggestive of the presence of introns and thus transcript processing in T brucei mitochondria.

The two G+C-rich 8-bp sequences begin at nucleotides 86 and 241 in the R1H1 sequence. These sequences flank a sequence whose transcript could form a secondary

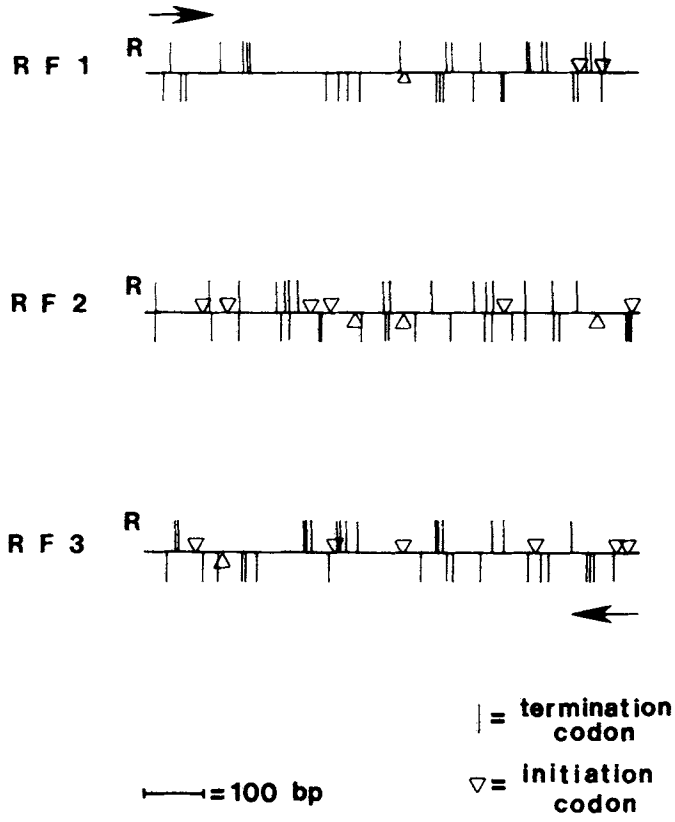

Fig. 4. Initiation (triangle) and termination (vertical line) codon occurance in the R1T4 sequence, assuming conventional mitochondrial codon usage. Each reading frame (RF) is indicated by a separate line. Reading in the 5′ to 3′ direction of the sequence as written in Figure 3 is indicated above the line, while reading the complementary strand in the opposite direction is indicated below the line.

structure which resembles that of a t-RNA (see Fig. 3). This potential secondary structure is illustrated for the DNA sequence from nucleotide 105 to 179 in Figure 7. This structure has several characteristics of a t-RNA, including the potential for nucleotide interactions to form an appropriate tertiary structure. The potential t-RNA is very A+U rich but has G+C or G+T base pairs at strategic locations such as in the anticodon and D-loop stems. Unpaired Gs and Cs occur in the loop, stem, and hinge regions of the molecule and along with other base pairing interactions might contribute to stabilizing the tertiary structure of the molecule. If this sequence were a t-RNA molecule and T brucei employs conventional mitochondrial codons, it would specify alanine and would be the first t-RNA described that was encoded by the maxicircle.

Total minicircle complexity in African trypanosomes is about 300 kbp [2]. This minicircle diversity is illustrated by comparison of the restriction maps of five minicircles which have been cloned from the kDNA of T brucei 164 (Fig. 8). The sizes of these minicircles vary between 950 and 1,040 bp and their restriction maps also differ. The pTKP1 and pTKP2 minicircle clones contain Pst I sites but lack Hind

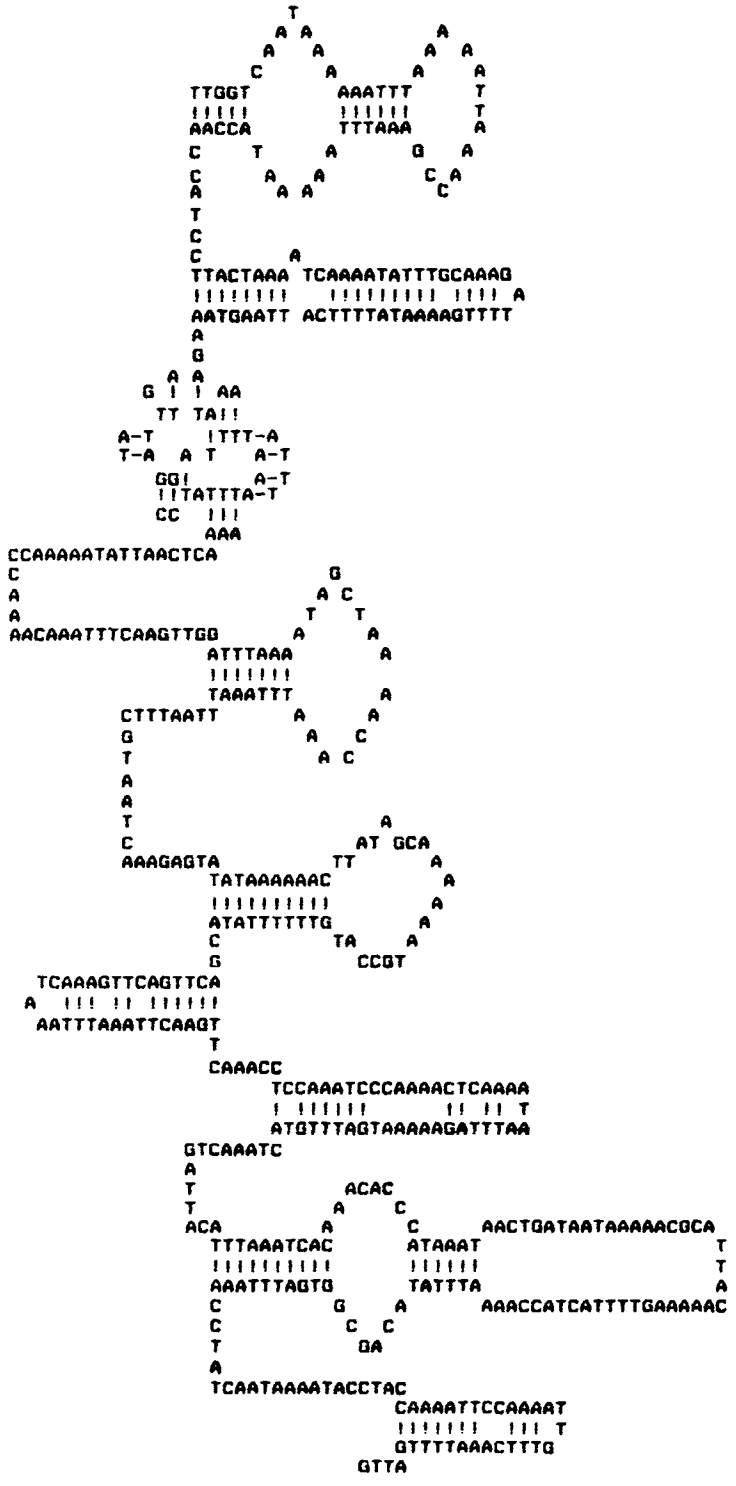

Fig. 5. Potential secondary structure that could be assumed by the sequence given in Figure 3. This illustrates the relative positions of some of the inverted repeated sequences. See text for details.

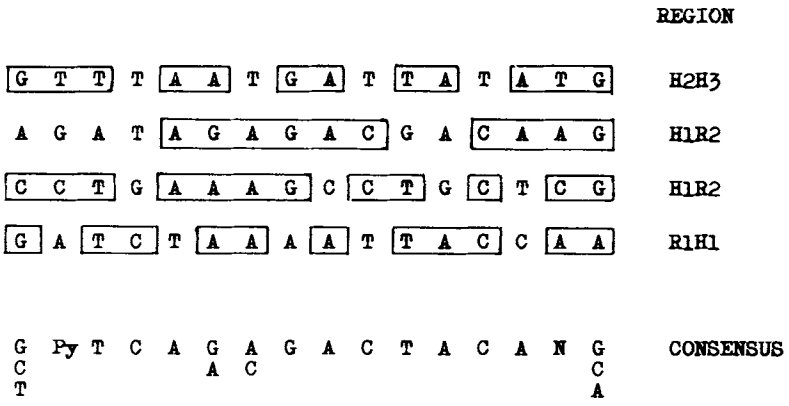

Fig. 6. Nucleotide sequences that occur in the maxicircle of T brucei 164 which resemble the mitochondrial intron consensus sequence which is shown in the bottom line. The nucleotides that match those in the consensus sequences are boxed. See text for details.

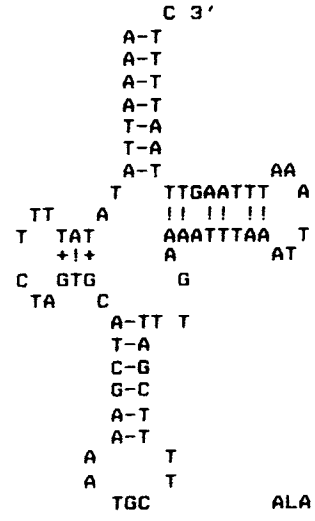

Fig. 7. Secondary structure postulated for a possible t-RNA encoded by the R1T4 sequence. The symbols - and ! indicate Watson-Crick base pairs, while + indicates a G:U base pair.

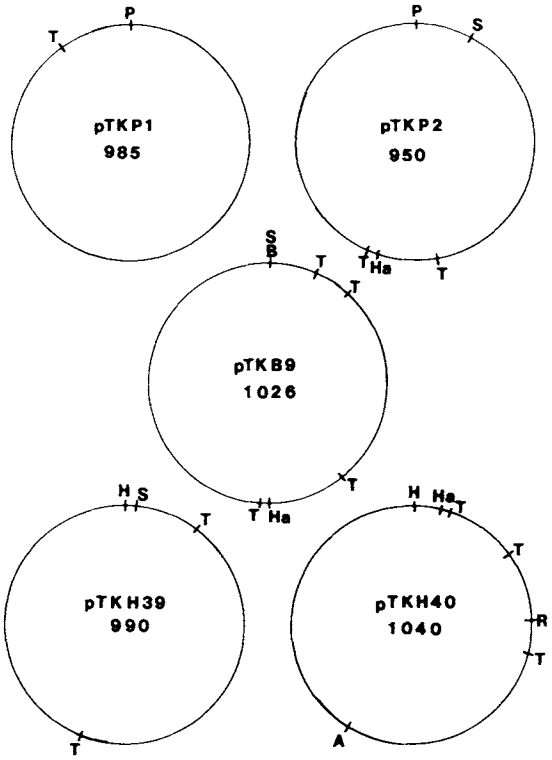

Fig. 8. Restriction endonuclease cleavage maps of five minicircles cloned from T brucei 164 kDNA. The pTKP1 and two clones were cloned from Pst I-digested kDNA. pTKB9 from Bam HI-digested kDNA, and pTKH39 and 40 from Hind III-digested kDNA. The numbers within the circles refer to the minicircle size in base pairs. A = Alu I, B = Bam HI, H = Hind III, Ha = Hae III, P = Pst I, R = Eco RI, S = Sal I, T = Taq I.

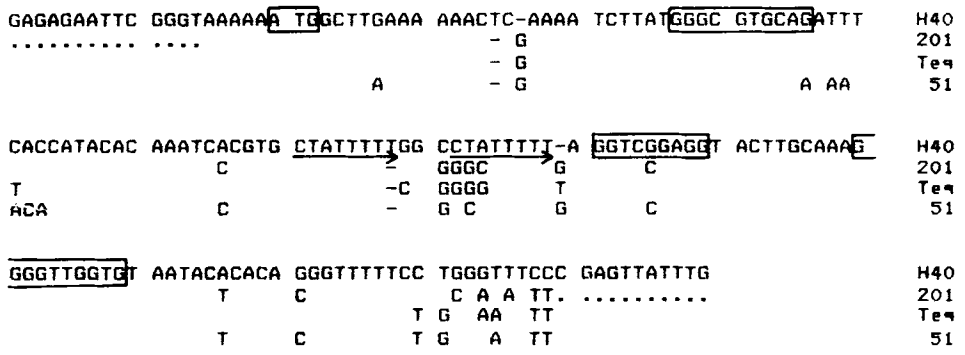

Fig. 9. The nucleotide sequence of the pTKH40 minicircle clone compared to a sequence conserved by other minicircles from African trypanosomes [see 9, 21]. The three published minicircle sequences are identical in this region to the pTKH40 sequence except as indicated by the substituted nucleotides or a nucleotide addition/deletion indicated by –. Homology between pTKH40 and the other minicircles ceases outside the region is indicated by · · · below the appropriate nucleotides. G+C-rich sequences and the ATG initiation codon are boxed, and the direct repeats are indicated by arrows.

III, Eco RI and Bam HI sites. The pTKP1 clone lacks Sau 3a and Hae III sites which are present in pTKP2 and has a single Taq I site rather than two Taq I sites. Thus these two minicircles which contain Pst I sites differ. Both pTKH39 and pTKH40 have Hind III sites, but pTKH40 contains an additional Taq I site plus Eco RI, Hae III, and Alu I sites which are absent in pTKH39, while pTKH39 contains a Sal I site that is absent in pTKH40. Thus, all of these minicircles have distinct restriction maps.

Despite this minicircle sequence diversity minicircles have conserved a part of their sequence. Figure 9 shows the nucleotide sequence of a portion of pTKH40 proceeding counterclockwise from four nucleotides before the Eco RI site. This sequence is compared to minicircle sequences conserved among three other minicircles which have been published [9,21]. The pTKH40 sequence has 93% and 89% homology to sequences from clones 201 and 51, respectively, from another strain of T brucei and 94% homology to the minicircle sequence from T equiperdum. An open reading frame begins just inside the region of homology and could encode an 83 amino acid oligopeptide, but it is not known if such an oligopeptide is actually produced. The region of homology is G+C rich compared to strain 164 kDNA as a whole and compared to the other minicircles. It is 40% G+C compared to 34% G+C for 164 kDNA and 28% G+C for the other T brucei and T equiperdum minicircles. The mismatch among the conserved regions of the minicircles is biased toward G or C substitutions especially if the 3′-most six nucleotide pairs of the conserved region are not considered. Ten of 11 substitutions are G or C in 201, seven of 10 in T equiperdum, and nine of 17 in 51. In addition, a cluster of G or C substitutions occurs near the middle of the region and involves the eight-nucleotide direct repeat CTATTTTT. The conserved region also contains three 9- or 10-nucleotide G+C-rich clusters beginning at nucleotides 47, 101, and 119 in the sequence as written.

Total cellular DNA was extracted from normal and Dk mutant cloned cell lines to avoid selective loss of altered kDNA. When this DNA was examined in cesium chloride density gradients containing ethidium bromide, the kDNA satellite band was not observed in some mutants, but a satellite band was observed at a different position

in the gradient in other mutants. The D4 mutant DNA lacked the satellite, while the D3 mutant DNA contained the satellite band at a lower density position in the gradient. This satellite DNA was kDNA as shown by recovery and hybridization to cloned kDNA sequences in Southern blots (data not shown). This altered banding position could reflect altered density resulting from a change in base composition, altered supercoiling in the kDNA, or a combination of both.

When DNA from nine mutant clones was examined by Southern blots using a combination of restriction enzymes and a mixture of probes, seven of the mutants were found to have less than 1% of the normal amount of kDNA and may be devoid of kDNA. Two mutants, however, retained both maxicircle and minicircle DNA in apparently normal amounts.

Figure 10 shows a Southern blot of cellular DNA from the nine mutant clones and from the normal parental clone which has been digested with Hind III and Bam HI. Two amounts of isolated kDNA were also digested with the same enzyme. The

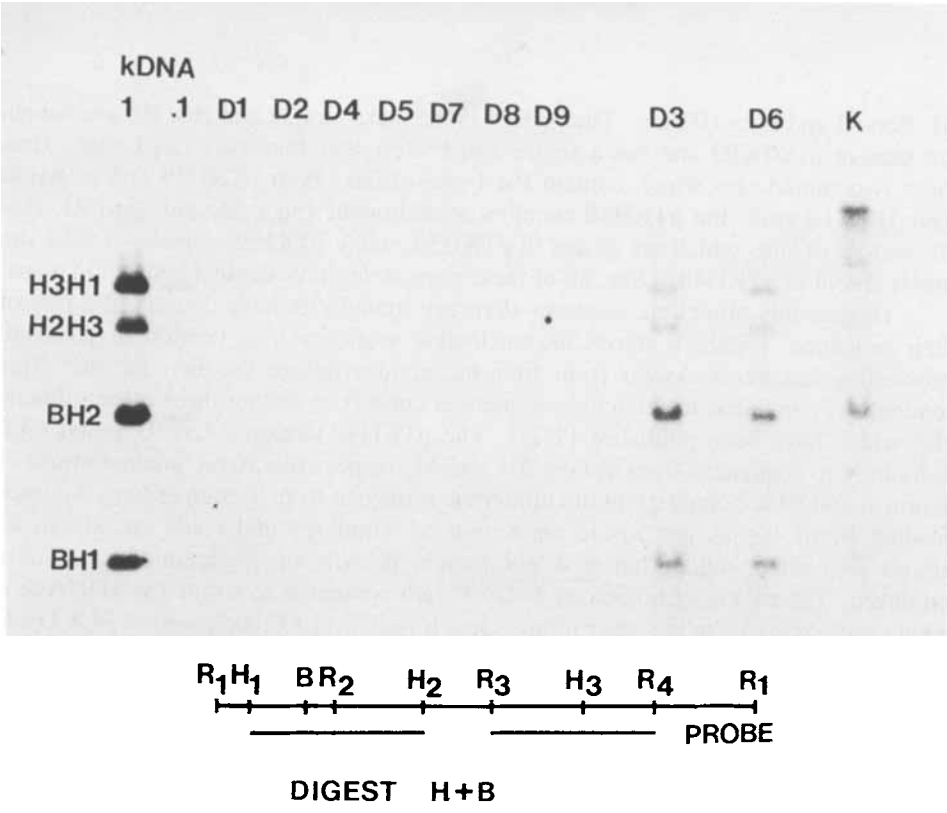

Fig. 10. Autoradiogram of a Southern blot of kDNA or total cell DNA extracted from normal (K) or Dk mutants (D1-D9). The DNAs were digested with Hind III and Bam HI and hybridized with nick-translated cloned probes corresponding to the H1H2 and the R3R4 segments of the maxicircle. Ten micrograms of each cell DNA and 1 or 0.1 μg of purified kDNA was run in the indicated lanes. A restriction map of the maxicircle and those regions represented in the probe are shown below the autoradiogram.

blots were probed with a mixture of cloned maxicircle segments that correspond to the H1H2 and R3R4 regions of the maxicircle. This mixture of probes hybridizes to all maxicircle restriction fragments generated by this digest as seen in the lanes containing isolated kDNA. DNA from mutants D1, D2, D4, D5, D7, D8, and D9 showed no hybridization with the probe even with very long autoradiographic exposures (not shown). The D3 and D6 mutant DNA, however, showed four bands of hybridization which corresponded to all the maxicircle fragments. The mobilities of these fragments was indistinguishable from that of the control kDNA.

The intensity of hybridization was also similar to that in the lane containing DNA from the normal clone which was incompletely digested. It was also within the range expected if the amount of kDNA present in the mutants was similar to the normal complement by comparison to the quantitative kDNA control lanes. Thus,

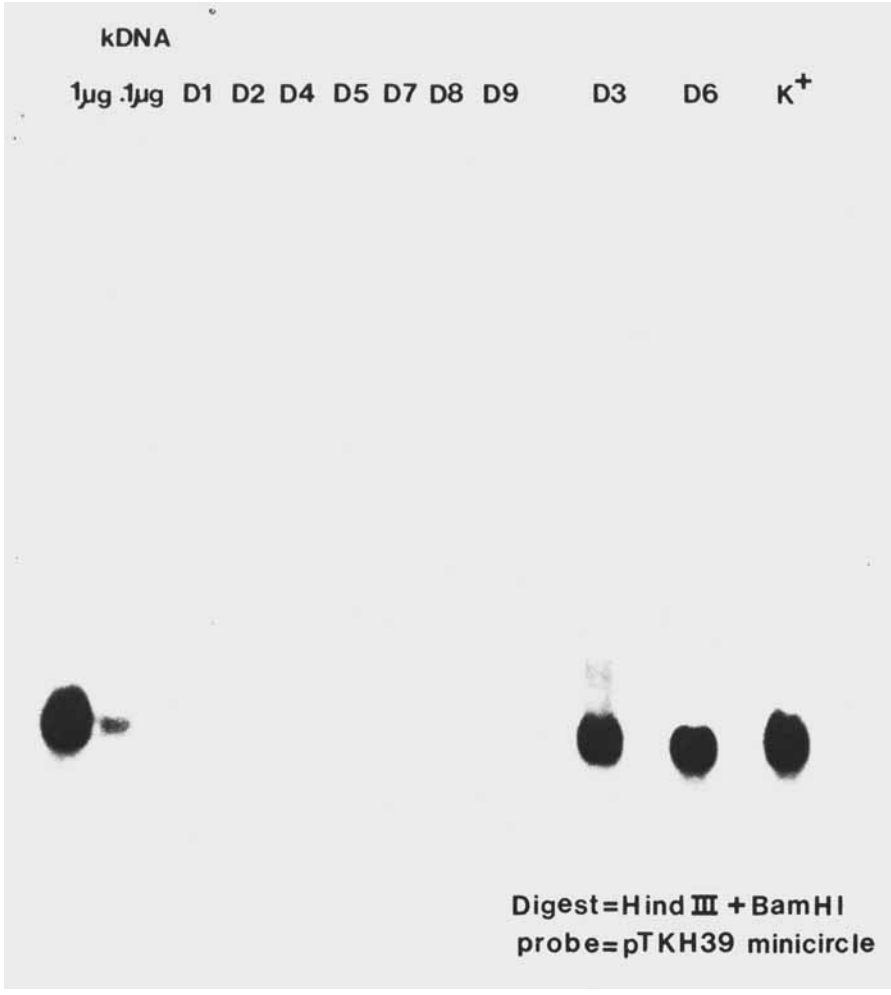

Fig. 11. Autoradiogram of a Southern blot of normal and mutant DNA as described in the legend for Figure 10 except that the cloned minicircle pTKH39 was used as a probe.

while seven mutant clones appeared to be devoid of maxicircle DNA, mutants D3 and D6 contained normal amounts of maxicircle DNA, and all maxicircle restriction fragments were retained without gross alteration in size.

In a similar experiment the DNA from normal and mutant cells was probed with a cloned minicircle sequence (Fig. 11). The pTKH39 minicircle probe has been shown previously to be an abundant minicircle sequence [5]. Like the maxicircle probe, the minicircle probe showed no detectable hybridization to DNA from mutants D1, D2, D4, D5, D7, D8, and D9 but did hybridize to DNA from mutants D3 and D6. Again, the intensity of the hybridization was similar to that observed in the normal DNA control and similar to the normal amount expected for the amount of cellular DNA being examined. The mobility of the hybridizing band in the D3 mutant was indistinguishable from that in the normal control, but the comparable band may have had a slightly greater mobility in the D6 mutant DNA.

DISCUSSION

The maxicircle of T brucei has all the characteristics of other mitochondrial DNAs which include circularity, modest size, and coding functions for mitochondrial gene products which include mitochondrial rRNA and polypeptides of the oxidative phosphorylation system. It should be noted, however, that functional homology of the sequences hybridizing to probes derived from other mitochondria have not yet been demonstrated. In this report we have added the observation that yeast Cox I mitochondrial DNA sequences hybridize to the T brucei maxicircle. We have also found evidence for a potential t-RNA which would be the first report of a t-RNA encoded in a maxicircle DNA.

If the potential t-RNA described in Figure 5 is a bona fide t-RNA, it would be the first t-RNA found to be encoded by a kDNA maxicircle. This t-RNA also would differ substantially from other mitochondrial t-RNAs [see 22]. However, T brucei has the smallest mitochondrial rRNAs described and may contain relatively large putative mitochondrial ribosomes [23]. Hence, the unusual nature of this t-RNA may reflect the peculiar ribosomes of T brucei.

The R1H1 region of the T brucei 164 was not previously found to be transcribed. The experiments, however, would not have demonstrated low molecular weight transcripts such as a t-RNA. They also would not have demonstrated transcripts present in very low abundance, transiently produced, or produced at other stages of the life cycle. The presence of numerous inverted repeated sequences and numerous termination codons in all reading frames of both strands suggests that the R1H1 region does not encode a polypeptide and may not be largely transcribed. The presence of a sequence resembling a mitochondrial intron sequence implies that transcripts of this region may undergo posttranscriptional processing. Perhaps this region encodes additional t-RNAs which are not directly discernible, since they require processing to be activated. Alternatively, this region might have replication origin or promoter functions. Sequences which are not completely transcribed and reside between a region which varies in size among stocks and the rRNA coding sequences contain replication origin and promoter functions in mammalian [22] and Drosophila [24] mitochondrial DNA.

The presence in the maxicircle of sequences resembling a consensus sequence in the introns of other mitochondria implies that posttranscriptional RNA processing

may occur in the mitochondrion of T brucei. The expression of the mitochondrial respiratory system is regulated during the course of the life cycle in T brucei, but differential transcription of maxicircle genes does not appear to mediate this process [6,7]. If differential expression of maxicircle genes is a means by which this process is controlled, perhaps the regulation may occur at the level of posttranscriptional RNA processing.

The significance of minicircle diversity in the African trypanosomes is unknown as is minicircle function. It has been shown that minicircles can supply plasmids with the ability to act as autonomously replicating sequences (ARSs) in yeast [25]. It is not yet known whether this function resides in the conserved segment of the minicircles or if this segment functions as a replication origin in the mitochondrion. The assignment of a replication origin function to the conserved sequence is an assignment by default at this point, since the only known function of minicircles is to replicate. However, the conserved sequence does contain three G + C-rich blocks, as do replication origins in yeast mitochondrial DNA [26], although they share no homology with each other in trypanosomes as they do in yeast.

If all minicircles contained this conserved sequence, it would be an abundant cellular DNA sequence. There would be about 5,500 copies of this sequence per cell. Since kDNA represents about 7% of the cellular DNA, minicircles about 85% of kDNA, and the conserved sequence about 14% of minicircle DNA, the conserved sequence would amount to about 0.8% of the total cellular DNA.

As in the other T brucei minicircles which have been sequenced [9,21], the conserved region in strain 164 contains an open reading frame that could encode an oligopeptide. Although the occurrence of these short open reading frames might be fortuitous it might indicate a minicircle coding function. The T equiperdum minicircle has a termination codon within this sequence. This could either reflect the loss of minicircle function associated with the inability of T equiperdum to infect the insect host or the nonfunctionality of the open reading frame.

kDNA is not essential for survival of the bloodstream trypomastigotes, since several Dk mutants appear to be devoid of kDNA and grow in the bloodstream. kDNA does appear to be essential for conversion to the procyclic forms which have a fully functional respiratory system. This is not surprising, since the development of the mitochondrial respiratory system requires the activity of mitochondrial DNA in other organisms examined and the maxicircle which is absent in these mutants is the genetic equivalent of mitochondrial DNA

The mutants which have retained both maxicircle and minicircle sequences are also unable to grow as procyclic forms. It is possible that point mutations in the maxicircle or mutations in nuclear genes are present in these mutants and thus prevent their conversion to procyclic forms, since a complete set of fully functional mitochondrial gene products is needed for oxidative phosphorylation in procyclic forms. However, while minicircle sequences are present in these mutants they are almost certainly altered. The kDNA no longer stains with Giemsa, although the amounts of maxicircle and at least one abundant minicircle are normal. Thus the kDNA may be structurally altered. The altered banding position in cesium/ethidium gradients may reflect kDNA structural alteration or gross alteration in kDNA base composition. The kDNA alteration, on the other hand, could be rather subtle—such as a change in the supercoiling of the kDNA molecules or alterations in their topological associations. If this is the case, this would imply that these factors could affect the expression of

these genes and that the inability of these mutants to grow as procyclic forms is due to altered maxicircle transcription rather than point mutations in cirtical maxicircle or nuclear genes.

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