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Localization of Kinetoplast DNA Maxicircle Transcripts in Bloodstream and Procyclic Form *Trypanosoma brucei*

KENNETH D. STUART[1]* AND STANTON B. GELVIN[2]†

Issaquah Health Research Institute, Issaquah, Washington 98027,[1] and Department of Microbiology and Immunology, University of Washington, Seattle, Washington 98195[2]

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Over 80% of the maxicircle and numerous minicircles of *Trypanosoma brucei* kinetoplast DNA have been cloned. The uncloned maxicircle segment contains few restriction endonuclease cleavage sites, varies in size among strains, and may be unstable in conventional cloning systems. cDNA prepared to bloodstream or procyclic trypomastigote RNA hybridized to all but one maxicircle segment, but did not hybridize to minicircles. Fourteen maxicircle transcripts were detected in RNA from both bloodstream and procyclic trypomastigotes. The coding sequences for these transcripts were localized and account for most of the maxicircle. One region of the maxicircle, which borders the variable region, was not found to be transcribed. We conclude that the maxicircle is largely but not completely transcribed in both bloodstream and procyclic trypomastigotes, whereas minicircle transcription is minimal or absent in these stages. Qualitative transcriptional differences which could account for mitochondrial respiratory differences between the bloodstream and procyclic trypomastigotes were not observed.

Members of the kinetoplastida contain within their single large mitochondrion an unusual DNA, called kinetoplast DNA (kDNA), which is a distinguishing feature of this protozoan order. kDNA exists as a complex catenated association of two classes of circular molecules. In *Trypanosoma brucei* the kDNA contains approximately 45 copies of maxicircles which are 21 kilobase pairs (kbp) in size and 5,500 1-kbp circles called minicircles (23).

Genetically, the maxicircle has been equated with other mitochondrial DNAs. This is suggested by the following observations: (i) the size and sequence complexity of maxicircle DNA resembles that of other mitochondrial DNAs (6); (ii) the maxicircle is transcribed and the major transcription products appear to be the mitochondrial rRNAs (16, 21, 24); (iii) mitochondrial gene sequences from other organisms hybridize to maxicircle DNA (J. B. Johnson, G. C. Hill, T. D. Fox, and K. Stuart, Mol. Biochem. Parasitol., in press; L. Simpson, A. Simpson, T. Spithill, and L. Livington, Cold Spring Harbor Meeting, abstr. no. 117, 1981); and (iv) mutation or deletion of kDNA eliminates the ability of the cells to grow under conditions normally requiring cytochrome-mediated ATP generation (22).

† Current address: Department of Biological Sciences, Lilly Hall of Life Sciences, Purdue University, W. Lafayette, IN 47907.

T. brucei grown in the mammalian host (bloodstream trypomastigotes) generate energy by glycolysis. They lack cytochromes and the Krebs cycle enzymes. These cells transform into procyclic trypomastigotes upon transfer into the insect host (the tsetse fly) or into culture at 28°C. Procyclic trypomastigotes have a fully developed mitochondrial respiratory system, contain a full complement of cytochromes, have a complete Krebs cycle, and metabolize glucose to CO_2 (P. Englund, S. Hajduk, and J. Marini, Annu. Rev. Biochem., in press). Little is known about the genetic regulatory mechanisms which control the switch from the bloodstream to the procyclic trypomastigotes.

We have analyzed the transcription of the kDNA of *T. brucei* and have found that, except for one segment, all regions of the maxicircle are transcribed. We detected no transcription of minicircles. Bloodstream and procyclic forms showed no qualitative differences in kDNA transcription; however, procyclic forms appeared to contain increased levels of all RNA species detected.

MATERIALS AND METHODS

Strains and culture conditions. The organism used in this study was *T. brucei* subsp. *brucei* clone IsTat 1 of stock EATRO 164. The history and origin of this clone have been described elsewhere (1). Bloodstream forms were grown in rats and isolated by using DEAE columns as previously described (17). Procyclic forms

were grown in a rich medium containing serum and blood cell lysate (14). Isolated washed cells were stored as frozen pellets at −20°C.

Nucleic acid isolation. kDNA was isolated by published procedures (23). Briefly, cells were dissolved in buffered Sarkosyl containing disodium EDTA at 60°C and then treated with RNase A and proteinase K. The kDNA was sedimented from solution by centrifugation, washed, and purified by cesium chloride equilibrium centrifugation.

RNA was isolated by a modification of the procedure of Rowe et al. (19). The RNA used was that precipitated by 2 M LiCl.

Preparation of cloned kDNA maxicircle sequences. Several strategies were used to clone the maxicircle restriction endonuclease fragments. kDNA was either digested with *Hin*dIII and ligated with *Hin*dIII-cleaved pBR322 or digested with *Eco*RI and ligated with *Eco*RI-cleaved pBR325 (3). These DNAs were transformed into *Escherichia coli* HB101 by the method of Cohen et al. (9). Clones pTKH38, pTKH128, pTKR9, and pTKR10 were constructed in this manner. pTKH, pTKR, and pTKHR refer to plasmids (p) containing trypanosome (T) kDNA (K) inserts having *Hin*dIII (H), *Eco*RI (R), or *Hin*dIII and *Eco*RI (HR) termini. To isolate smaller cloned segments, the maxicircle DNA was enriched from total kDNA. To do this, kDNA was digested with *Bam*HI, which cleaves the maxicircle at a single site and cleaves few minicircles. The digested DNA was then centrifuged for 20 min in an Eppendorf microcentrifuge to sediment the bulk of the minicircles in the form of the catenated network. The supernatant solution containing cleaved maxicircles was then further digested with *Hin*dIII and *Eco*RI and ligated into pBR322 or pBR325 cleaved with the same combination of enzymes. The clone pTKHR67 was isolated after *Hin*dIII and *Eco*RI digestion of the cloned segment pTKH38 and inserted into pBR322. The clone pTKHR34 was similarly subcloned from pTKR10. Clones pTKHR40 and pTKHR117 are inserted into pBR322, whereas pTKHR34, pTKHR38, pTKHR42, and pTKHR67 are inserted into pBR325.

It was not possible to clone one segment of the maxicircle. This was the segment R4-R1 (see Fig. 2). This segment was preparatively purified from isolated kDNA which had been digested with *Bam*HI and centrifuged to enrich for maxicircles. The enriched maxicircles were digested with *Eco*RI, and the digest was loaded onto a preparative 0.7% agarose gel. The section of the gel containing the R4-R1 fragment was excised, and the DNA was electroeluted onto dialysis tubing. The DNA was washed from the dialysis tubing and precipitated by the addition of sodium acetate to 0.3 M and 2.5 volumes of 95% ethanol. The precipitated DNA was collected by centrifugation in an Eppendorf microcentrifuge, washed twice with 70% ethanol, and dissolved in water.

Other nucleic acid manipulations. Restriction endonuclease digestions were according to the supplier's recommendations. Restriction endonuclease fragments were subjected to electrophoresis on agarose gels by the method of Helling et al. (15).

The DNA was radiolabeled by nick translation (18), and radiolabeled cDNA to RNA was made by using avian myeloblastosis virus reverse transcriptase and random priming with calf thymus DNA (12).

Transfer of and hybridization to restriction endonuclease fragments of kDNA were done by the method of Thomashow et al. (26). RNA was subjected to electrophoresis in 1.5% agarose gels containing 6% formaldehyde (25) and transferred to nitrocellulose by the method of Gelvin et al. (12a). Hybridization of nick-translated DNA to RNA blots was performed by the method of Thomashow et al. (26).

The RNA sizing standards used were yeast and *E. coli* RNAs and cowpea chlorotic mottle virus RNA. The sizes adopted for these standards were those used by Hoeijmakers et al. (16), except that the yeast small subunit rRNA was taken to be 1,789 nucleotides (20), and the cowpea chlorotic mottle virus RNA was taken to be 876 nucleotides (10).

RESULTS

Identification of cloned maxicircle segments. The identities of the cloned maxicircle DNA segments were established by hybridization to restriction endonuclease fragments of kDNA. pTKHR42 hybridized only to the 1.35-kbp R1-H1 fragment (Fig. 1A, lanes 1 and 2). (Refer to maxicircle map [Fig. 2] for fragment designations.) pTKHR67 hybridized to the 2.4-kbp R1-B and 1.10-kbp B-R2 fragments in *Eco*RI-*Bam*HI double digests (Fig. 1A, lanes 3 and 5) and to the 2.05-kbp H1-B and the 4.15-kbp B-H2 fragments in *Hin*dIII-*Bam*HI double digests (Fig. 1A, lanes 4 and 6). Since this probe did not hybridize to the 6.0-kbp R2-R3 fragment in the above *Eco*RI-*Bam*HI double digests, the hybridization was only to the H1-R2 segment. This could not be demonstrated directly in *Hin*dIII-*Eco*RI double digests since several fragments had similar electrophoretic mobilities. For the same reason, the identity of pTKHR40 was determined in a similar fashion. pTKHR40 hybridized to the 6.0-kbp R2-R3 fragment in *Eco*RI-*Bam*HI double digests (Fig. 1A, lanes 5 and 7) and to the 4.15-kbp B-H2 fragment in *Hin*dIII-*Bam*HI double digests (Fig. 1A, lanes 6 and 8). Since pTKHR40 did not hybridize to the 1.10-kbp B-R2 fragment in *Eco*RI-*Bam*HI double digests or the 6.45-kbp H2-H3 fragment in *Hin*dIII-*Bam*HI digests, the hybridization of pTKHR40 was only to the R2-H2 segment.

pTKHR117 hybridized to the 1.45-kbp H2-P and the 1.50-kbp P-R3 segments (Fig. 1A, lanes 9 and 10), indicating that it only hybridized to the H2-R3 segment. pTKHR38 hybridized only to the 3.50-kbp R3-H3 segment (Fig. 1A, lanes 10 and 11), and pTKHR34 hybridized only to the H3-R4 segment (Fig. 1A, lanes 12 and 13).

pTKR9 hybridized only to the 4.5-kbp R1-R2 segment (Fig. 1B, lanes 1 and 2). pTKR10 hybridized only to the 6.20-kbp R3-R4 fragment (Fig. 1B, lanes 3 and 4), whereas pTKH128 hybridized only to the 6.25-kbp H2-H3 fragment (Fig. 1B, lanes 5 and 6). The faint hybridization observed in some cases always corresponded to

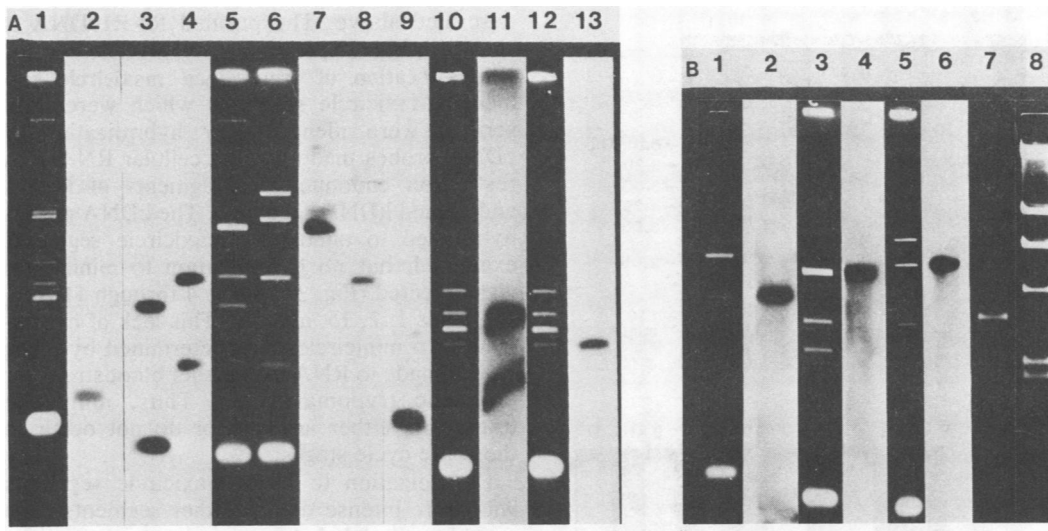

FIG. 1. Hybridization of radiolabeled, cloned maxicircle segments to restriction endonuclease fragments of kDNA bound to nitrocellulose after electrophoretic separation. (A) kDNA digested with *Hin*dIII, *Eco*RI, and *Pst*I (lane 1) and corresponding autoradiogram with pTKHR42 as probe (lane 2); kDNA digested with *Eco*RI and *Bam*HI (lane 5) or *Hin*dIII and *Bam*HI (lane 6) and corresponding autoradiograms with pTKHR67 (lanes 3 and 4) or pTKH40 (lanes 7 and 8) as probe; kDNA digested with *Hin*dIII, *Eco*RI, and *Pst*I (lanes 10 and 12) and corresponding autoradiograms with pTKHR117 (lane 9), pTKH38 (lane 11), or pTKHR34 (lane 13) as probe. Fragment sizes are given in the text. (B) kDNA digested with *Eco*RI (lane 1) and corresponding autoradiogram with pTKR9 as probe (lane 2); kDNA digested with *Eco*RI and *Pst*I (lane 3) and corresponding autoradiogram with pTKR10 as probe (lane 4); kDNA digested with *Hin*dIII and *Bam*HI (lane 5) and corresponding autoradiogram with pTKH128 as probe (lane 6); R4-R1 fragment isolated from kDNA (lane 7); lambda DNA digested with *Hin*dIII (lane 8).

incomplete digestion fragments containing the fragment with which the hybridization signal was most intense.

Figure 2 summarizes the identities of the cloned maxicircle segments. pTKH38 has been previously identified (24). The restriction endonuclease sites of the cloned segments were identical to those of the corresponding segments of native maxicircle DNA (data not shown). Thus, the maxicircle segments appear to have been cloned intact and replicated faithfully.

Several attempts have been made to clone the R4-R1 segment of the maxicircle in both pBR322 and pBR325. These attempts employed total kDNA, purified maxicircles, and purified R4-R1 fragments. All attempts were unsuccessful. R4-R1 DNA used as probes in experiments described below was thus preparatively isolated as

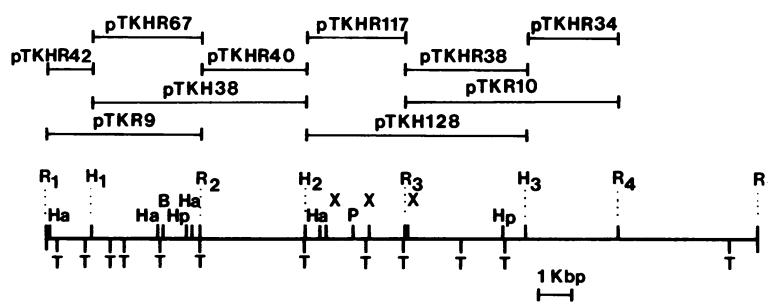

FIG. 2. Diagram summarizing the identity of recombinant plasmids containing maxicircle DNA segments. The circular map has been linearized for clarity. The region that was not cloned and which varies in size among *T. brucei* strains is on the extreme right. Abbreviations: B, *Bam*HI; H, *Hin*dIII; Ha, *Hae*III; Hp, *Hpa*II; P, *Pst*I; R, *Eco*RI; T, *Taq*I; X, *Xba*I.

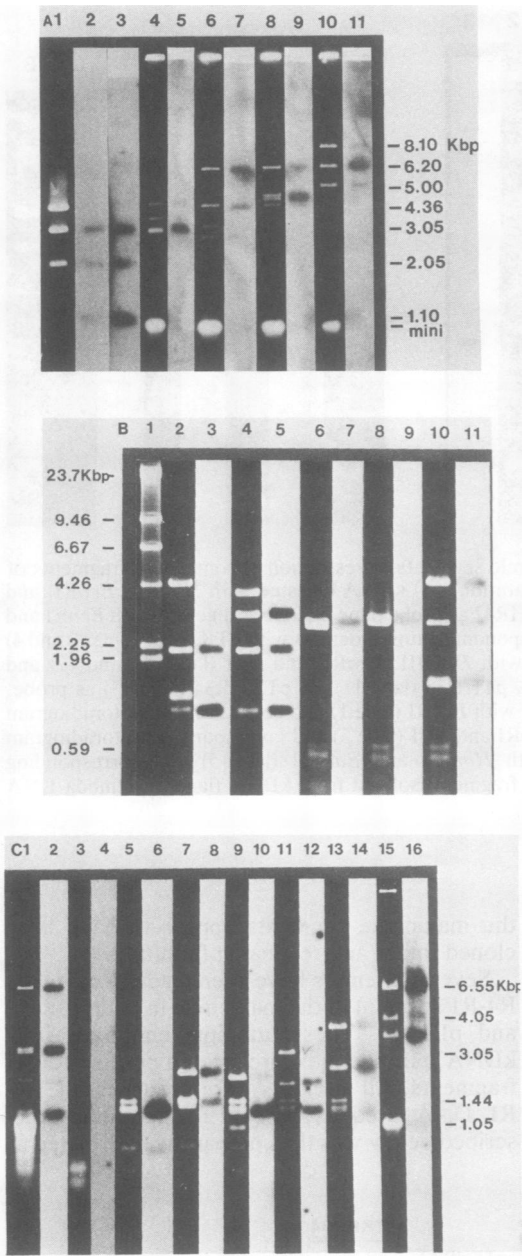

FIG. 3. Hybridization of radiolabeled cDNA made to *T. brucei* RNA with restriction endonuclease fragments of kDNA bound to nitrocellulose filters after electrophoretic separation. All RNA is from procyclic forms except in lane 2 of panel A. (A) pTKH38 digested with *Eco*RI, *Hin*dIII, and *Bam*HI (lane 1); autoradiogram when hybridized with bloodstream form cDNA (lane 2) or procyclic form cDNA (lane 3); kDNA digested with *Eco*RI and *Hin*dIII (lane 4), autoradiogram (lane 5); kDNA digested with *Eco*RI and *Hpa*II (lane 6), autoradiogram (lane 7); kDNA digested with *Eco*RI and *Pst*I (lane 8), autoradiogram (lane 9); kDNA digested wtih *Hin*dIII and *Pst*I (lane 10), autoradiogram (lane 11). (B) Lambda DNA digested with *Hin*dIII (lane 1); pTKR9 digested with *Eco*RI, *Hin*dIII, and *Bam*HI (lane 2), autoradiogram (lane 3); pTKH38 digested with *Eco*RI, *Hin*dIII, and *Bam*HI (lane 4), autoradiogram (lane 5); pTKR10 digested with *Hin*dIII, *Eco*RI, and *Hpa*II (lane 6), autoradiogram (lane 7); pTKH128 digested with *Hin*dIII, *Eco*RI, and *Hpa*II (lane 8), autoradiogram (lane 9); pTKH128 digested with *Hin*dIII, *Pst*I, and *Hpa*II (lane 10), autoradiogram (lane 11). (C) kDNA digested with *Taq*I (lane 1), autoradiogram (lane 2); pTKHR42 digested with *Taq*I and *Hpa*II (lane 3), autoradiogram (lane 4); pTKHR67 digested with *Taq*I (lane 5), autoradiogram (lane 6); pTKHR117 digested with *Taq*I (lane 7), autoradiogram (lane 8); pTKHR38 digested with *Taq*I (lane 9), autoradiogram (lane 10); pTKH128 digested with *Taq*I (lane 11), autoradiogram (lane 12); pTKR10 digested with *Taq*I (lane 13), autoradiogram (lane 14); kDNA digested with *Eco*RI and *Bam*HI (lane 15), autoradiogram (lane 16).

described above. The purified R4-R1 DNA is shown in Fig. 1B, lane 7.

Identification of transcribed maxicircle segments. Maxicircle segments which were transcribed were identified by hybridization of cDNA probes made to total cellular RNA with restriction endonuclease fragments of kDNA and cloned kDNA segments. The cDNA probes hybridized to almost all maxicircle segments examined, but no hybridization to minicircles was detected (Fig. 3A, lanes 4 through 11; Fig. 3C, lanes 1, 2, 15, and 16). This lack of hybridization to minicircles was determined by using cDNA made to RNA from either bloodstream or procyclic trypomastigotes. Thus, minicircle transcripts either are rare or do not occur in these life cycle stages.

Hybridization to some maxicircle segments was more intense than to other segments. The most heavily labeled maxicircle region was the H1-H2 segment. In kDNA digested with both *Hin*dIII and *Pst*I, the H1-H2 fragment was heavily labeled (Fig. 3A, lanes 10 and 11), whereas the other maxicircle fragments were less intensely labeled. Similarly, when kDNA was digested with both *Eco*RI and *Hin*dIII, the comigrating H1-R3 and R2-H2 subfragments of the H1-H2 fragment were heavily labeled (Fig. 3A, lanes 4 and 5). The H2-R3 fragment also comigrates with these fragments, but since H2-P and P-H3 fragments were not heavily labeled in the *Hin*dIII-*Pst*I double digest, this fragment was not heavily labeled. Intense hybridization to the R2-R3 fragment and the R1-Hp1 fragment, which comigrates with the R4-R1 fragment (Fig. 3A, lanes 6 and 7) and the heavy labeling of the R1-R2 and R2-P fragments (Fig. 3A, lanes 8 and 9) confirmed this observation.

The differential in labeling of maxicircle segments was further illustrated by using cloned

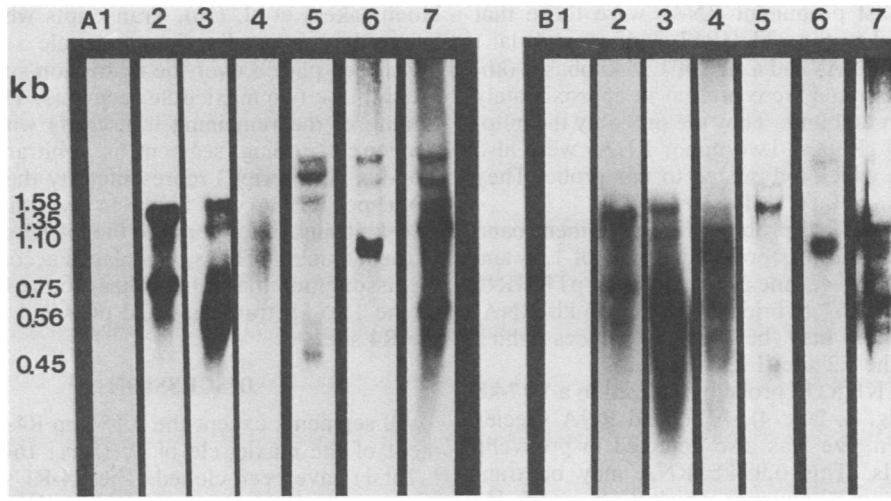

FIG. 4. Hybridization of radiolabeled maxicircle segments to procyclic form (A) and bloodstream form (B) RNA bound to nitrocellulose filters after electrophoretic separation. Probes: pTKHR42 (lane 1), pTKHR67 (lane 2), pTKHR40 (lane 3), pTKHR117 (lane 4), pTKHR38 (lane 5), pTKHR34 (lane 6), purified R4-R1 restriction endonuclease fragment (lane 7).

maxicircle fragments. The use of the maxicircle clones eliminated many of the problems of comigration. In Fig. 3B the H1-B, B-R2, and R2-H2 fragments were heavily labeled (lanes 2 through 5), whereas the other cloned maxicircle fragments were less intensely labeled (lanes 7 through 11). None of the cloning vehicle restriction endonuclease fragments was labeled. The fragments in Fig. 3B represent the entire maxicircle except for the R4-R1 fragment.

In addition to the heavy labeling of the H1-H2 segment of the maxicircle, all but one of the other maxicircle fragments examined were transcribed. Restriction endonuclease digestions of total kDNA, such as kDNA digested with *Taq*I (Fig. 3C, lanes 1 and 2), kDNA digested with both *Eco*RI and *Bam*HI (Fig. 3C, lanes 15 and 16), and kDNA digested with both *Hin*dIII and *Pst*I (Fig. 3A, lanes 10 and 11), showed hybridization to each of the visible maxicircle fragments. The hybridizations with cloned maxicircle segments allowed for a more detailed analysis which was not obscured by comigration of bands and the presence of minicircles.

All combinations of *Hin*dIII, *Eco*RI, *Bam*HI, *Hpa*II, and *Hae*III digestion revealed transcription of the H1-H2 segment, suggesting that this region is completely transcribed. This was also true for the H2-R3 region. The H2-P segment (Fig. 3B, lanes 10 and 11) and the two subfragments resulting from *Taq*I digestion (Fig. 3C, lanes 7 and 8) were transcribed. Both *Taq*I subfragments of the R3-H3 segment were transcribed (Fig. 3C, lanes 9 and 10). All three subfragments of the R3-R4 segment were transcribed (Fig. 3C, lanes 13 and 14). The R4-R1 segment was also transcribed (Fig. 3A, lanes 8 and 9). Hence, with the exception of the R1-H1 segment, all segments of the maxicircle encoded detectable transcription products.

The cDNA used for the analysis described above was made to procyclic form total RNA. The same results were obtained, however, when cDNA made to bloodstream form total RNA was used as a probe (Fig. 3A, lane 2, and data not shown). Hybridization to blots with bloodstream form cDNA was consistently less intense than the hybridization to the corresponding blots with procyclic form cDNA.

Analysis of the RNA. Each segment of the maxicircle hybridized to a unique set of RNA molecules (Fig. 4), except for the R1-H1 maxicircle segment, which did not hybridize to any RNAs (Fig. 4, lane 1). The sizes of the observed maxicircle transcripts are summarized in Table 1.

TABLE 1. Maxicircle transcripts and recombinant plasmids to which they hybridized

Probe	Maxicircle segment	RNA molecules hybridized (kb)
pTKHR42	R1-H1	
pTKHR67	H1-R2	1.35, 1.10, 0.75, 0.56
pTKHR40	R2-H2	1.58, 1.30, 0.56
pTKHR117	H2-R3	1.30, 1.17, 0.98[a]
pTKHR38	R3-H3	1.90, 1.58, 0.45
pTKHR34	H3-R4	1.05, (0.55)[b]
—	R4-R1	1.30, 1.05, 0.68

[a] Not detected in bloodstream forms.

[b] cDNA clone pcTKP436, which has a 550-bp insert.

The most prominent RNAs were those that hybridized to the pTKHR67 clone (Fig. 4, lane 2). These RNAs had a size of 1.35 kilobases (kb) and 0.75 kb and were present in approximately equimolar amounts. They are probably the mitochondrial rRNAs. Two minor RNAs were also observed which hybridized to this probe. They were 1.1 and 0.56 kb in size.

The pTKHR40 probe gave a prominent band of 0.56 kb and less prominent ones of 1.58 and 1.30 kb (Fig. 4, lane 3). Since both pTKHR40 and pTKHR67 hybridized to a 0.56-kb RNA, transcription may be from sequences which overlap the R2 site (Fig. 4, lane 2).

The pTKHR117 probe hybridized to a 1.17-kb RNA (Fig. 4, lane 4). A second RNA species 0.98 kb in size was also detected in procyclic form cells. This 0.98-kb RNA may be transcribed from a sequence which overlaps the H2 site. The probe pTKHR38 hybridized to a 0.45-kb RNA and a 1.58-kb RNA (Fig. 4, lane 5). Probe pTKHR34 hybridized to a 1.05-kb RNA (Fig. 4, lane 6), whereas the R4-R1 segment hybridized most prominently to 1.30- and 0.68-kb transcripts (Fig. 4, lane 7). Weak hybridization of pTKHR34 to the 0.68-kb transcript suggests that it may be transcribed from a sequence that overlaps the R4 site. In addition, the R4-R1 sequence hybridized to several RNAs of lower intensity, the most prominent of which was 1.05 kb.

There was no specific hybridization to cytoplasmic RNAs. Apparent hybridization to the three cytoplasmic rRNAs was observed, but this is interpreted to be nonspecific binding which reflects the large abundance of this RNA since it was observed with all of the cloned probes.

RNase treatment of the nucleic acid preparation before electrophoresis resulted in the absence of all hybridization except that near the origin, which is due to contaminating kDNA.

The maxicircle transcripts observed and their relative positions on the maxicircle are summarized in Fig. 5. The placement and order of the putative mitochondrial rRNAs are based on Hoeijmakers et al. (16). Transcripts which hybridized to two adjacent maxicircle segments have been placed over the restriction site separating these two maxicircle segments. The positioning of the remaining transcripts within the maxicircle coding segment is arbitrary. The 0.55-kb "transcript" represented by the cDNA clone pcTKP436 was found to hybridize to a 1.05-kb transcript, but not to the R4-R1 segment of the maxicircle. Thus, it is placed according to the assumption that it hybridizes to the left side of the 1.05-kb transcript and does not overlap the R4 site.

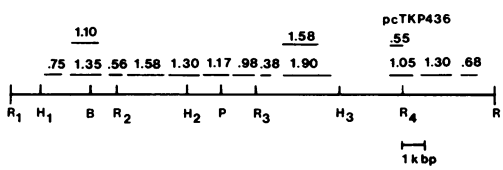

FIG. 5. Diagram summarizing maxicircle transcript sizes and the approximate location of their coding sequences on the maxicircle. The coding sequences are positioned as described in the text. pcTKP436 is a cDNA clone containing a 550-base-pair insert. It hybridized to a 1.05-kb transcript and the H3-R4 maxicircle segment, but not to the R4-R1 segment.

DISCUSSION

All segments except the 4.05-kbp R4-R1 segment of the maxicircle of *T. brucei* 164 (clone IsTat 1) have been cloned. The R4-R1 segment may not be stable in conventional cloning systems; other investigators have also attempted to clone this segment without success (7, 21). Maxicircle segments from EATRO 427 which correspond to the R1-R2 and R2-R3 segments and from STIB 366D which correspond to the H1-H2 and H2-H3 segments have been cloned in lambda and plasmid vectors (7, 16, 21). The maxicircles of different *T. brucei* strains have nearly identical restriction endonuclease maps, except in this segment, where both size and restriction site variations occur (4). For example, *T. brucei* strain 164 has about 300 fewer nucleotides in the R4-R1 segment than does strain 366D (24). Strain 164 also has an *Eco*RI site (site R4) which is absent in strain 427 and has about 1,000 nucleotides more than strain 1,125 within the R3-R1 segment. This segment of the maxicircle seems to have evolved more rapidly than the maxicircle as a whole. As suggested by Borst and Hoeijmakers (6), this segment may be analogous to the A plus T rich segments in *Drosophila* mitochondrial DNA and similarly serve as the site for initiation of transcription and DNA replication.

With the exception of the R4-R1 segment, the other cloned maxicircle segments are stable based on a comparison of their molecular sizes and the conservation of restriction endonuclease sites with the corresponding kDNA restriction endonuclease fragments.

Most regions of the maxicircle are transcribed. cDNA made to cellular RNA hybridized to all but one restriction endonuclease fragment of the maxicircle—the R1-H1 segment. Similarly, RNA blots revealed transcripts for all maxicircle segments with the exception of the R1-H1 segment. The inability to detect R1-H1 transcripts indicates that either they are present in low abundance or they are not transcribed at all in bloodstream and procyclic trypomastigotes. Alternatively, the RNAs may not be

present in the LiCl-precipitated RNA samples (as would be the case for tRNAs).

The R1-H1 segment borders the R4-R1 maxicircle segment which varies in size among species and which has few restriction endonuclease recognition sites. Although three transcripts have been observed for the R4-R1 segment, their coding sequences have not been localized, and thus a portion of this segment may resemble the R1-H1 segment in transcription properties. Similarly, the H3-R4 segment was not saturated with transcripts.

In addition to the mitochondrial rRNAs, several other maxicircle transcripts have been observed. The 1.10-kb transcript appears to correspond to the R3a and R3b transcripts which were resolved by Hoeijmakers et al. (16) into two transcripts. Similarly, the 0.56-kb transcript appears to correspond to their R3c and R3d transcripts. These transcripts were mapped by Hoeijmakers et al. to the B-R2 fragment (our designation). The 1.30-kb transcript appears to correspond to the R2a transcript of Hoeijmakers et al. (16), which mapped to the R2-H2 region (our designation). They also reported a R2b transcript which corresponds to our 0.56-kb transcript in size and by virtue of its abundance. We have interpreted the 0.56-kb transcript, which is heavily labeled by pTKHR40, to be the same transcript as that labeled more faintly by pTKHR67. This assumes that its coding sequence overlaps the R2 site, but is primarily localized in the R2-H2 segment. Hoeijmakers et al. did not observe this transcript with their H1-R2 (our designation) probe. This leaves open the possibility there may be two distinct transcripts rather than one overlapping the R2 site.

A plasmid pZME1, which contains the *Zea mays* mitochondrial DNA sequence for cytochrome oxidase subunit II (11), hybridizes to the R2-H2 segment of the maxicircle (Johnson et al., in press). Thus, the 1.58-, 1.30-, and 0.56-kb transcripts are candidates for the cytochrome oxidase II transcript. The cytochrome oxidase subunit II transcript is abundant in yeast, implying that the 0.56-kb transcript is the most likely candidate. Cytochrome oxidase subunit II polypeptide is about 26,000 daltons in other species (2, 5) with about 700-base-pair transcripts, which are significantly larger than the 560-base-pair transcript found in *T. brucei*. The resolution of whether this transcript is that of cytochrome oxidase subunit II will be the object of future study.

We have additionally identified several other maxicircle transcripts which are listed in Table 1 and Fig. 4. Some of these transcripts are encoded by the variable region of the maxicircle. Transcripts for this region were detected in both Southern (Fig. 3) and Northern (Fig. 4) blot experiments. In the Northern blot experiments, R4-R1 DNA prepared from kDNA was used as a probe for the transcripts among size-fractionated cellular RNA. The transcripts observed cannot be due to contamination of the probe with nuclear DNA or other maxicircle fragments since the probe did not label cytoplasmic rRNA (which is by far the most abundant RNA in the sample) and did not label mitochondrial rRNA encoded by the most likely maxicircle DNA contaminant, based on proximity in the preparative gel.

Studies with *Drosophila* mitochondrial DNA indicate that the promoter is located in the variable A+T-rich segment (6, 13). By analogy, this could place the promoter, if there were a single promoter, in the vicinity of the R4-R1 segment. The lack of detection of a transcript for the H1-R1 segment may indicate that this region may reside near the polymerase recognition site or DNA replication initiation site or both.

The main conclusions from the studies reported here are that the maxicircle is almost completely transcribed in both bloodstream and procyclic trypomastigotes. Since only a small difference in the amounts of specific transcripts was observed between these two forms, it is unlikely that the differences in mitochondrial respiratory activity can be explained by differential transcription. Whether all of the proteins encoded by the structural genes and resultant transcripts are produced in bloodstream and procyclic trypanosomes remains to be seen.

We have not been able to demonstrate transcription of the minicircle DNA. It is possible that this transcription occurs, but that the steady state levels of these RNAs are too low to detect by the blotting procedure depicted in Fig. 3. Certainly, any representation of minicircle transcripts does not compare with the abundant representation of minicircles in the total kDNA population. Cloned minicircle sequences were hybridized with RNA transferred to nitrocellulose after electrophoretic separation (data not shown). An intense band was observed at 1.05 kb. This was probably the result of hybridization to minicircle DNA contaminating the RNA preparation. It is difficult to eliminate such contamination completely, but from experiments in which cDNA to cellular RNA was hybridized to kDNA, it is clear that minicircle transcripts are not abundant in bloodstream and procyclic trypomastigotes.

In addition to the lack of evidence for minicircle transcription in bloodstream and procyclic *T. brucei*, the finding of numerous translation termination codons in minicircle sequences (8) adds to the doubt that minicircles have a conventional protein-coding function. Only short segments of potential coding sequence have

been reported. Thus, the function of minicircles remains a mystery.

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