Diverse Patterns of Expression of the Cytochrome c Oxidase Subunit I Gene and Unassigned Reading Frames 4 and 5 During the Life Cycle of Trypanosoma brucei

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Transcription of a maxicircle segment from *Trypanosoma brucei* 164 that contains nucleotide (nt) sequences corresponding to cytochrome c oxidase subunit I (COI) and unassigned reading frames (URFs) 4 and 5 of other mitochondrial systems was investigated. Two major transcripts that differ in size by ca. 200 nt map to each of the COI and URF4 genes, while a single major transcript maps to URF5. In total RNA, the larger COI transcript is more abundant in procyclic forms (PFs) than in bloodstream forms (BFs), the smaller COI and both URF4 transcripts have similar abundances in both forms, and the single URF5 transcript is more abundant in BF than PF. These pattterns of expression differ in poly(A)$^+$ RNA as a result of a higher proportion of poly(A)$^+$ mitochondrial transcripts in PFs than in BFs. In addition, small (300- to 500-nt) RNAs that are transcribed from C-rich sequences located between putative protein-coding genes also exhibit diverse patterns of expression between life cycle stages and differences in polyadenylation in PFs compared with BFs. These observations suggest that multiple processes regulate the differential expression of mitochondrial genes in *T. brucei*.

The protozoan parasite *Trypanosoma brucei* possesses an unusual mitochondrial DNA that is known as kinetoplast DNA. This kinetoplast DNA consists of about 50 maxicircles (ca. 22 kilobases in size) and about 5,000 minicircles (ca. 1 kilobase in size) which are concatenated into a single network (12, 31). The function of the minicircles is unknown. Maxicircles have functions similar to mitochondrial DNA from other organisms on the basis of nucleotide and amino acid sequence homology (2, 3, 13, 16, 20, 27).

Profound changes in energy-yielding metabolic pathways accompany differentiation from the BF to the PF of *T. brucei*; BFs depend on glycolysis for energy, whereas PFs utilize tricarboxylic acid cycle- and cytochrome-mediated respiration (12). Mitochondrial gene products are components of the cytochrome-mediated respiratory system, and three such genes, CYb, COI, and COII, have been identified in the *T. brucei* maxicircle (3, 16, 20, 27). The divergent metabolic states suggest that mitochondrial genes may be differentially expressed during the life cycle.

To investigate differential expression of mitochondrial genes, we have identified maxicircle genes (27) and their corresponding transcripts and compared the transcripts present in BFs and PFs (14, 15). These studies revealed that multiple transcripts map to maxicircle genes. In addition, some transcripts differed dramatically in abundance between BFs and PFs. These observations suggest that mitochondrial gene expression in *T. brucei* is regulated during the life cycle by a mechanism which controls transcript abundance. In this report we extend these studies to gene sequences corresponding to mammalian URFs 4 and 5 and COI. URFs 4 and 5 have recently been shown to encode components of respiratory chain NADH dehydrogenase (6). In contrast to other maxicircle gene sequences, a single transcript maps to URF5. In addition, several small transcripts that are encoded by the maxicircle have been identified.

MATERIALS AND METHODS

Abbreviations. BF, bloodstream form; PF, procyclic form; nt, nucleotide(s); R3R4, region of maxicircle between the third and fourth *Eco*RI restriction enzyme sites; COI and COII, cytochrome c oxidase subunits I and II, respectively; CYb, apocytochrome *b*; strand I and strand II, maxicircle sense strands for the COI and COII genes, respectively; URF, unassigned reading frame (homologous to genes from other mitochondria); ORF, open reading frame (no homology to other mitochondrial genes detected).

RNA preparation. BF and PF cells were grown as described previously (33). Total RNA was isolated from BF or PF cells as described by Ross (29) with minor modifications (14, 15). Poly(A)$^+$ RNA was isolated from total RNA with a column containing oligo (dT)-cellulose in 20 mM Tris hydrochloride (pH 7.6)–0.5 M NaCl, 1 mM disodium EDTA–0.1% sodium dodecyl sulfate, followed by elution with 10 mM Tris hydrochloride (pH 7.6)–1 mM disodium EDTA–0.05% sodium dodecyl sulfate. The poly(A)$^+$ RNA was ethanol precipitated, rinsed with 70% ethanol, and redissolved in water, and aliquots were stored at −80°C until use.

RNA analysis. RNA was electrophoresed in 1.5% agarose gels containing 2.2 M formaldehyde (22) and then transferred to nitrocellulose (34) or activated nylon filters (GeneScreen II; New England Nuclear Corp.). The filters were prehybridized in a solution containing 50% deionized formamide, 5× SSPE (0.9 M NaCl, 50 mM NaH$_2$PO$_4$, 5 mM disodium EDTA [pH 7.4]), 0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.1% sodium dodecyl sulfate, 100 μg of denatured salmon sperm DNA, and 20 μg of poly(A) per ml at 42°C for 16 h. Hybridization with the radiolabeled probes was performed under the same conditions for 16 h, followed by a 20-min wash with 2× SSPE–0.1% sodium dodecyl sulfate at room temperature and then at 60°C followed by one wash with 0.2× SSPE at 60°C. DNA probes derived from M13 clones were radiolabeled by using either hybridization primer or sequencing primer (Bethesda Research Laboratories). Restriction enzyme frag-

ments derived from plasmids pTKHR38 and pTKHR34 (33) were isolated from agarose or polyacrylamide gels and subsequently radiolabeled by nick translation (28).

RESULTS

Maxicircle sequences. We determined the nucleotide sequence of the R3R4 segment from the region of the *T. brucei* 164 maxicircle. We confirmed that this region contains sequences with homology to the COI, URF4, and URF5 mitochondrial genes of other organisms (Fig. 1B). This sequence differed at only five positions from that of *T. brucei* 427 (16). Two of the nucleotide changes occurred in URF4; one was silent with respect to amino acid substitution, and the other resulted in a conservative amino acid replacement. The other three were in an intergenic region. In addition to these apparent protein-coding sequences, this segment of maxicircle contained sequences which had a higher G+C content than did identified maxicircle genes or the maxicircle in general and were characterized by G versus C-strand bias (Table 1). The first ATG codon of the COI ORF was separated from that of URF4 by 325 bp, and 396 bp separated the termination codon of URF4 and the first ATG of URF5. The base composition of the putative protein-coding

TABLE 1. Characteristics of putative protein coding sequences and intergenic regions from *T. brucei* 164 maxicircle[a]

Sequence	Length (nt)	% Single-strand base composition				% Amino acid homology[b]
		A	G	C	T	
CYb	1,047	30.5	16.3	6.3	46.8	25
ORFIA	1,336	28.8	7.5	10.9	52.9	
URF1	957	25.3	12.4	13.2	49.1	18
COII	629	33.1	17.1	7.3	42.6	29
ORFIIA	1,041	28.4	11.0	3.7	56.8	
COI	1,647	22.6	17.3	12.2	47.8	38
URF4	1,311	33.7	7.3	12.7	46.3	22
URF5	1,779	26.6	5.6	15.1	52.7	22
Flanking CYb						
5'	625	30.2	**40.5**	7.7	21.6	
3'	402	37.1	**34.3**	5.2	23.4	
Between ORFIA and URF1	87	36.8	**32.2**	4.6	26.4	
Between COI and URF4	325	39.1	4.0	**33.2**	23.7	
Between URFs 4 and 5	396	29.3	18.7	26.0	26.0	
3' URF4	198	25.3	6.1	**39.9**	28.8	
5' URF5	198	33.3	**31.3**	12.1	23.2	

[a] Data are derived from unpublished results and references 3, 16, 20, and 27. See Materials and Methods for abbreviations used. Some numbers are shown in bold type to emphasize the C richness of the transcribed sequence.
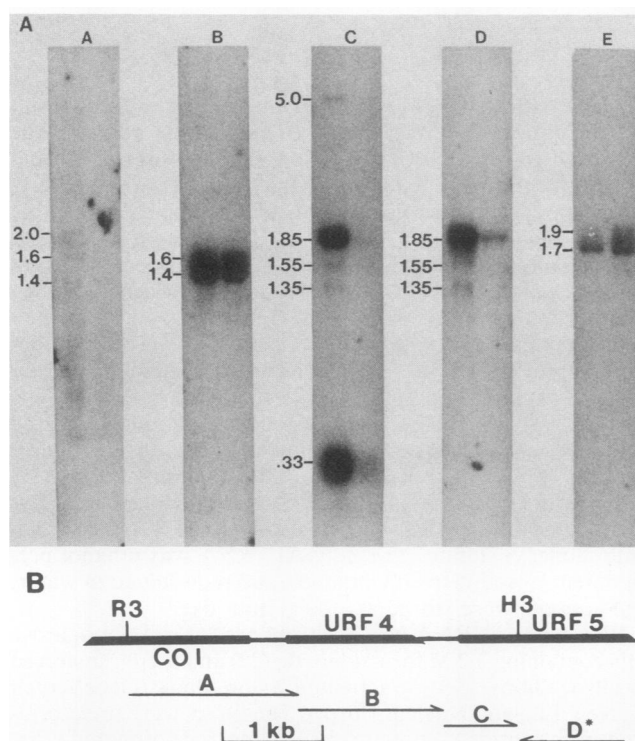[b] Homology to human or bovine mitochondrial genes.

FIG. 1. Identification of transcripts from strand II of the R3R4 segment. (A) Northern blot autoradiograms in which 10 μg of total RNA from the BF (left lane) or PF (right lane) was hybridized to the corresponding probes indicated in panel B. Lane E, the filter in lane C was washed in boiling water and 0.01% sodium dodecyl sulfate and rehybridized to probe E (see Fig. 2B). Single-stranded M13 DNAs containing maxicircle sequences were radiolabeled with hybridization primer or sequencing primer (*). (B) Map indicating the location of the probe sequences in maxicircle. The arrows indicate the length of the insert and its orientation in M13 with respect to direction of sequencing. Transcript sizes were estimated by comparison to denatured pBR322 and lambda restriction fragments and are indicated in kilobases.

sequences ranged from 14.7 to 29.5% G+C, while the intergenic sequences contained 36.8 to 44.7% G+C. The sequence separating COI and URF4 contained 33.2% C and 4% G on strand I (sense strand for COI). The sequence between URF4 and URF5 can be divided into two distinct segments: the first 198 nt of this sequence was composed of 39.9% C and 6.1% G, and the second 198 nt was composed of 31.3% G and 12.1% C on strand I. Thus, the sequence between URF4 and URF5 appeared to be composed of two units with similar sequence characteristics. These intergenic sequences exhibited no sequence homology except for short runs of G or C and T or A.

Transcription. We investigated the transcription of the R3R4 maxicircle segment and compared the abundance of transcripts between BFs and PFs. All of the transcripts that we identified from this segment of maxicircle are summarized in Table 2. None of the transcripts described below are present in dyskinetoplastic mutants that are devoid of kinetoplast DNA (32), indicating that they are all maxicircle transcripts.

Transcripts from the URF4 and URF5 coding strand (strand II) were identified by using single-stranded probes prepared from maxicircle sequences cloned into M13 (Fig. 1). The 1,600- and 1,400-nt transcripts mapped to the URF4 coding sequence (Fig. 1A, panels A and B). These transcripts hybridized strongly to the B probe, which contains 1,195 nt of the URF4 coding sequence and 176 nt of sequence between URF4 and URF5. They also hybridized weakly to the A probe, which contains 122 nt of the URF4 coding sequence. Both of these transcripts were of sufficient length to contain the entire URF4 coding sequence. Moderate hybridization to transcripts intermediate in size between the predominant URF4 transcripts was also observed (Fig. 1A, panel B).

An 1,850-nt transcript hybridized strongly to the C and D probes which contain the coding sequence of URF5 (panels C and D). Thus, unlike the seven other putative maxicircle

TABLE 2. Characteristics of transcripts from the R3R4 segment of *T. brucei* 164 maxicircle[a]

Transcript size (nt)	Hybridization pattern with probe:								Relative abundance	
	A	B	C	D	E	F	G	H	Total RNA	Poly(A)+ RNA
Strand II										
(2,000)	+								=	0
1,600 } URF4	+	+							=	PF
1,400 }	+	+							=	PF
(5,000)			+						BF	0
330[b]			+						BF	=
1,850 URF5			+	+					BF	=
(1,550)			+	+					BF	0
(1,350)			+	+					BF	0
Strand I										
(6,500)					+				=	0
1,900 } COI					+				PF	PF
1,700 }					+				=	PF
(1,400)					+				=	0
(1,850)						+	+	+	=	0
500[b]					+		+		=	BF
390[b]					+		+		=	PF
350[b]						+		+	=	PF

[a] Transcripts were identified by Northern blot hybridization experiments with total or poly(A)+ RNA from BFs or PFs and probes A through H as described in Fig. 1 and 2. +, Hybridization with the indicated probe; BF, transcripts that are more abundant in BFs than PFs; PF, transcripts that are more abundant in PFs than BFs; =, transcripts that are similar in abundance in both forms; 0, not detected. Only major differences in relative abundance of transcripts between life cycle stages are indicated. Brackets indicate transcripts that map to a similar location.

[b] Transcripts from C-rich intergenic strands. Transcripts seen as faintly hybridizing bands are in parentheses. Genes to which transcripts correspond are listed.

genes (14, 15), a single transcript mapped to the URF5 coding sequence. A 330-nt transcript also hybridized strongly to the C probe but not to the B or D probes. Since the C probe is complementary to the 5' 495 nt of the URF5 coding sequence and 224 nt of sequence 5' to the coding sequence, the 330-nt transcript was probably transcribed from the C-rich sequence adjacent to the 5' end of URF5.

Transcripts of 1,900 and 1,700 nt were transcribed from the COI coding sequence on strand I as shown by their hybridization to the E probe (Fig. 2A, panel E) and, as shown previously, to a probe containing 440 nt of the carboxy-terminal end of COI (14). Again, both transcripts were large enough to contain the entire COI coding sequence. Moderate hybridization to transcripts intermediate in size between these two predominant transcripts was also observed.

Small G-rich transcripts of 500 and 390 nt are transcribed from strand I, since they hybridized to the E and G probes (Fig. 2A, panels E and G). The G probe was a double-stranded probe that contained the sequence between COI and URF4 and 223 and 170 nt of these genes, respectively. The relatively strong hybridization to this probe (panel G) and lack of hybridization to probe F (panel F) indicated that the 500- and 390-nt transcripts are complementary to the C-rich intergenic sequence. Similarly, a 350-nt transcript from strand I hybridized to probes F and H (panels F and H), both of which contained the 176-nt intergenic sequence adjacent to URF4. A 367-nt segment derived from probe H which lacks this 176 nt did not hybridize to the 350-nt transcript (data not shown). In addition, a single-stranded probe complementary to strand I between probe H and the H3 restriction site (Fig. 2B) did not hybridize to this transcript (data not shown). Therefore, this 350-nt transcript is transcribed from the C-rich sequence from strand I adjacent to the 3' end of URF4 and is distinct from the 330-nt transcript that appears to be transcribed from the C-rich sequence adjacent to the 5' end of URF5 on strand II. Thus the 500-, 390-, and 350-nt transcripts from strand I and the

330-nt transcript from strand II all appear to be transcribed from C-rich sequences.

Several faint bands of hybridization were detected by using the probes described above. A 2,000-nt RNA was detected with the A probe (Fig. 1A, panel A) but not the B probe, a 5,000-nt RNA was detected with the C probe (Fig. 1A, panel C), and a >6,000-nt RNA was detected with the E probe (Fig. 2A, panel E). These larger transcripts may be processing precursors or products or both. Other probes weakly detected RNAs between about 1,300 and 1,850 nt (Fig. 1A, panels C and D; Fig. 2A, panels E through H); the 1,600- and 1,400-nt transcripts (Fig. 2A, panel F) are the URF4 transcripts from strand II which were detected even though an M13 probe was used. This resulted from preparing this probe with sequencing primer.

The approximate locations of the transcripts listed in Table 2 are diagrammed in Fig. 3. The major transcripts mapped to the putative protein coding genes and the intergenic C-rich sequences. In addition, low-abundance transcripts mapped to most of the remaining sequences, suggesting that this entire segment of maxicircle is transcribed.

To determine which of the major transcripts described above are poly(A)+, poly(A)+ RNA from BFs and PFs was analyzed (Fig. 4). Most of the more abundant transcripts were present in poly(A)+ RNA (Fig. 4A). These results are summarized in Table 2. Some transcripts listed in Table 2 were not detected in poly(A)+ RNA; this may reflect their low abundance rather than lack of 3' poly(A) sequences. Since both COI and both URF4 transcripts are polyadenylated, the size difference between them is not the result of polyadenylation of only one of the two transcripts.

Differential expression. To determine whether the transcripts described above are differentially expressed during the life cycle of *T. brucei*, we compared total RNA from BFs and PFs (Fig. 1A and 2A). The larger (1,900-nt) COI transcript was more abundant in PF than BF RNA, while the 1,700-nt transcript was equally abundant in both stages (Fig.
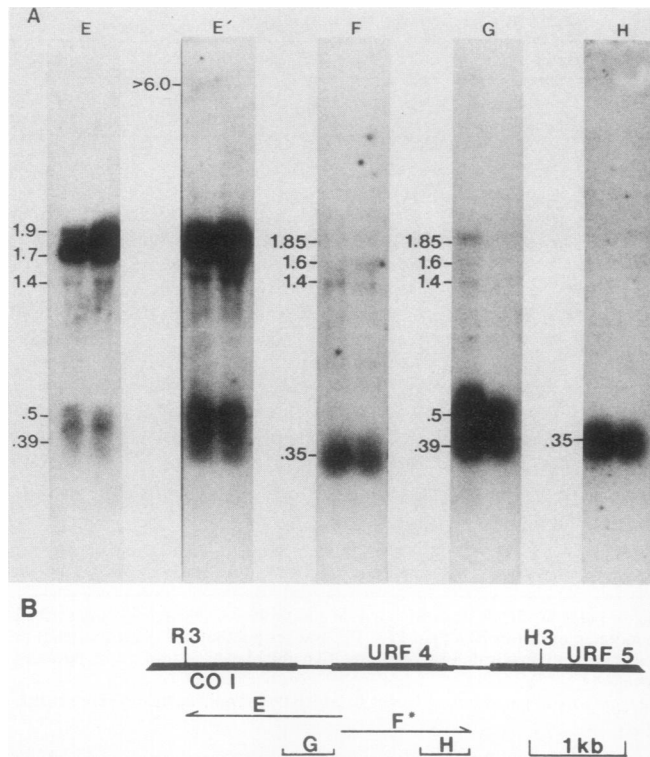
FIG. 2. Identification of transcripts from strand I of the R3R4 segment. (A) Northern blots were prepared as described in the legend to Fig. 1, except that 6 µg of PF RNA was used for lanes G and H; BF and PF RNA are in the left and right lanes, respectively. Lane E' is a longer exposure of lane E. (B) Map indicating the location of probes. Labeling and orientation of the probes is as described for Fig. 1. Probes G and H were nick-translated double-stranded restriction fragments (*Rsa*I and *Rsa*I-*Aha*III, respectively) of pTKHR38 (33). Transcript sizes are given in kilobases.

2A, panel E). The 1,550- and 1,350-nt transcripts that hybridize to the URF5 probes were also more abundant in BFs than in PFs (Fig. 1A, panels C and D), as was the 5,000-nt transcript from strand II (Fig. 1A, panel C). The other transcripts listed in Table 2, including the URF4 transcripts, appeared equally abundant in RNA from these two life cycle stages.

Analyses of BF and PF poly(A)$^+$ RNA revealed that in general a smaller fraction of each maxicircle transcript occurred in BF than PF poly(A)$^+$ RNA (Fig. 4). In addition, the fraction of transcripts in poly(A)$^+$ RNA differed for the various transcripts, adding further complexity to the comparison of transcripts present in BFs versus PFs. Both URF4 transcripts, the 1,700-nt COI transcript, and the 390- and 350-nt transcripts from strand I were all equally abundant in total RNA from BFs and PFs, but were more abundant in PF than in BF poly(A)$^+$ RNA (Table 2; also see Fig. 1A, 2A, and 4A). In contrast, the 500-nt transcript, while equally abundant in total RNA from BFs and PFs, was more abundant in BF poly(A)$^+$ RNA (Fig. 4A, panels E and G). The 1,850-nt URF5 transcript was more abundant in BF than in PF total RNA, but is equally abundant between these two forms in poly(A)$^+$ RNA (Fig. 4A, panel C), again reflecting polyadenylation of a larger proportion of transcripts in PFs than in BFs.

DISCUSSION

The homology of maxicircle sequences to the COI, URF4, and URF5 genes of other mitochondria at the nucleotide and predicted amino acid sequence levels (16; unpublished data) suggests a function for the maxicircle gene products homologous to those of other systems. Our finding that abundant maxicircle transcripts from this segment correspond in location and size to these putative genes further supports the conclusion that these are functional genes. However, the 583-nt ORF (designated URF9 in reference 16) in strains 164 and 427 (16) lacks a corresponding transcript, and an ORF designated URF7 in strain 427 (16) is absent from strain 164 as a result of nucleotide sequence differences and also lacks an appropriately sized transcript, suggesting that these ORFs are not functional genes. Thus, like other mitochondrial DNAs, the maxicircle contains genes for ribosomal RNAs (13), CYb (3, 20), COI and COII (and COIII in *Leishmania tarentolae* [9]), and URFs 1, 4, and 5, as well as other ORFs that are probably functional genes (16, 27).

Three interesting aspects of maxicircle transcription are reported here: (i) two transcripts map to each putative gene, except for URF5, (ii) some of these transcripts are differentially expressed during the life cycle, and (iii) a greater

2A, panel E). Conversely, the 1,850-nt URF5 transcript and the 330-nt transcript from strand II were more abundant in BF than in PF RNA (Fig. 1A, panels C and D). Rehybridization of the filter shown in Fig. 1A, panel C, to probe E (Fig. 1A, panel E) produced a pattern of hybridization similar to that observed for COI in Fig. 2A, panel E. This substantiates the hypothesis that the apparent difference in abundance of the URF5 transcript between BFs and PFs does not result from differential loading. The washing procedure used to detach probe C from the filter apparently resulted in loss of the smaller transcripts from the filter (compare Fig. 1A and
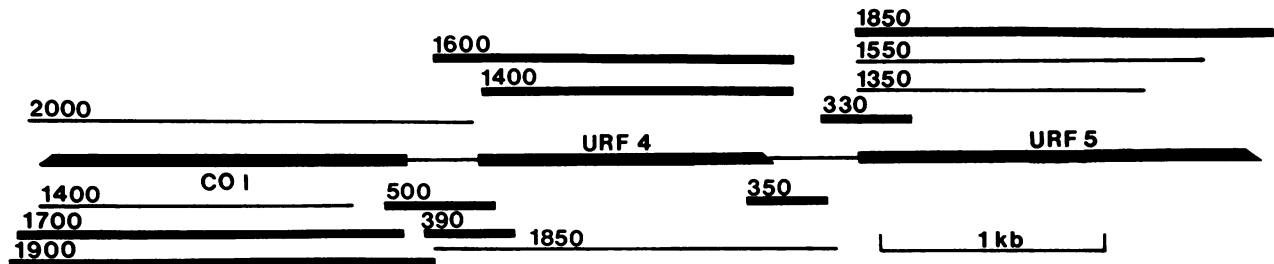

FIG. 3. Transcription map from the R3R4 segment of *T. brucei* maxicircle. Coding sequences for COI, URF4, and URF5 are indicated. The approximate location of transcripts from strands II and I are depicted above and below the map, respectively. Abundant transcripts are shown as heavy lines.
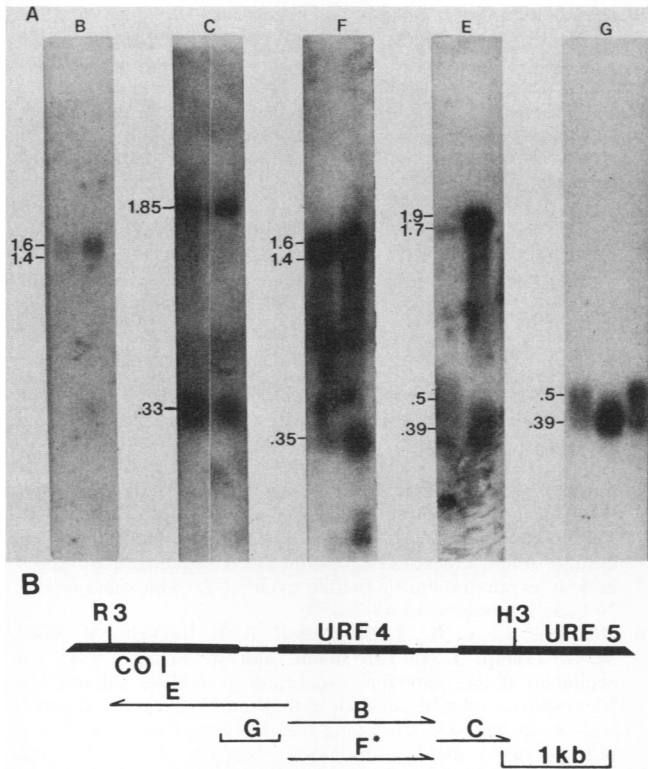
FIG. 4. Polyadenylation of transcripts from the R3R4 segment of *T. brucei* maxicircle. Northern blots for lanes B, C, E, and F were as described for Fig. 1, except that 1 μg of poly(A)$^+$ RNA was loaded per lane. The BF and PF lanes in lane C are nonadjacent lanes from the same autoradiogram. For lane G, 2.3 μg of BF poly(A)$^+$ RNA, 2.3 μg of PF poly(A)$^+$ RNA, and 10 μg of PF total RNA were loaded in the left, middle, and right lanes, respectively. The probes correspond to those used in Figs. 1 and 2. Transcript sizes are shown in kilobases.

fraction of each maxicircle transcript appears to be poly(A)$^+$ in PFs than in BFs.

Two transcripts differing by about 200 nt map to all of the putative protein-coding maxicircle genes (14, 15) except URF5, as shown here. Multiple transcripts which are encoded by the same structural gene but differ at their 5' or 3' ends or internally have been reported for nuclear and chloroplast genes (1, 5, 8, 11, 35). These differences often result in proteins with different functional properties. Nucleotide sequence analyses (2, 3, 16, 20, 27) suggest that maxicircle genes do not have introns. In addition, both transcripts of a set appear to be poly(A)$^+$ (Fig. 4A, panels B and E; reference 15). Thus, intron removal and polyadenylation do not appear to account for the presence of two transcripts. Different sites for initiation or termination of transcripts might account for the two transcripts, but this seems unlikely in view of the tight packing of many maxicircle genes (13, 16, 27) and the presence of a single promoter region in tightly packed mitochondrial genomes (7, 24). Differential processing could account for the two transcripts, which is an interesting possibility in view of the G+C-rich regions flanking the coding sequences that could serve as processing signals. However, transcripts ranging in size between the large and small transcripts also exist; it is unclear whether these result from the same processes that produce the two predominant size classes. An intriguing alternative to explain multiple transcripts is posttranscrip-

tional addition of nucleotides. This could be analogous to spliced leader addition (10, 26) or may involve some novel mechanism. In addition, the preceding possibilities may not be mutually exclusive.

A single transcript corresponds to URF5. If each of the two transcripts from other maxicircle genes encodes protein products with divergent functions, then URF5 may encode only a single protein. Alternatively, if only one transcript of a set is a translatable mRNA, then the expression of URF5 does not require the production of multiple transcripts. Less interesting possibilities are that two transcripts are encoded by URF5 but are sufficiently similar in size that they are not resolved by electrophoresis or that one may be very low in abundance.

Unlike other mitochondrial genomes, maxicircle genes exhibit differential expression in *T. brucei*. In addition, they exhibit various patterns of expression. For instance, one transcript corresponding to each of the respiratory protein genes, COI, CYb (15), and COII (14), is abundant in PF total RNA but is present in reduced or undetectable levels in BF total RNA. Other genes, including URF1 and ORFIIA (14) as well as URF4, are equally abundant in BF and PF RNA. In contrast, the URF5 transcript is more abundant in BF than PF total RNA.

An additional facet of differential expression is the divergent levels of maxicircle transcript polyadenylation in BFs versus PFs. A greater fraction of each maxicircle transcript appears polyadenylated in PFs than in BFs. This is especially evident for the 1,850-nt URF5 transcript (compare Fig. 1A and 4A, panel C), but is also true for other maxicircle transcripts such as the 1,600- and 1,400-nt URF4 transcripts (compare Fig. 1A and 4A, panel B). Consequently, while the transcript abundance pattern between BFs and PFs in poly(A)$^+$ RNA is intensified for the respiratory chain genes, the pattern observed for other genes is altered. For instance, the URF5 transcript is equally abundant in BF and PF poly(A)$^+$ RNA, but is more abundant in BF than PF total RNA. The finding that some maxicircle transcripts are in greater or equivalent abundance in BF than PF RNA is surprising, since dyskinetoplastic mutants lacking kinetoplast DNA survive well as BFs (4, 32). While functional transcripts from protein-coding genes may occur in BFs, the resultant proteins may not be needed for BF survival but may be required for differentiation to subsequent life stages.

For reasons discussed above, it is unlikely that differential expression of maxicircle genes is mediated through multiple promoters. Support for this conclusion comes from the existence of a common putative precursor transcript for both COII and ORFIIA but a different pattern of abundance between BFs and PFs for the final transcripts from these genes (14). Differential expression may be mediated through processes that produce multiple transcripts for a single gene, since the greater abundance of the larger transcript of the respiratory chain protein genes is correlated with expression of the mitochondrial respiratory system (Fig. 2A, panel E) (14, 15). If processing or sequence addition (or both) produces multiple transcripts, then these processes may be differentially regulated during the life cycle. Alternatively, differential degradation may be involved as it is in other genes (18, 19, 21), since sequences with single transcripts are also differentially expressed. These processes may not be mutually exclusive. Furthermore, the pattern of expression varies among maxicircle genes, and thus either the process mediating differential expression can be modulated or multiple control elements are involved.

The abundant small G-rich transcripts may belong to a general class of transcripts, although their function is unknown. It seems unlikely that these sequences encode proteins based on the highly charged nature of the predicted polypeptides (3, 16). These transcripts could represent precursors to tRNAs; the inability to detect tRNA-sized transcripts with maxicircle probes (17, 33) could reflect the inefficient binding of small nucleic acids to nitrocellulose or the exclusion of small RNAs from the RNA preparation. Alternatively, the G-rich sequences may be transcriptional processing signals like tRNAs in mammalian mitochondria (25), or they may have some regulatory function associated with differential expression. None of these possibilities can be excluded at this time.

Transcriptional analysis of the *T. brucei* maxicircle has revealed a novel and complex pattern of expression that differs strikingly from that of other mitochondrial systems. Two predominant transcripts correspond to some but not all protein coding genes; some are differentially expressed during the life cycle. Differentiation to PFs is correlated with a greater abundance of the larger cytochrome gene transcripts and polyadenylation of a larger fraction of maxicircle transcripts. However, these processes appear to be modulated among the various genes. The characteristics distinguishing the two transcripts encoded by individual genes and the significance of differential abundance of transcripts are central questions for future study.

ACKNOWLEDGMENTS

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