Molecular and Biochemical Parasitology, 37 (1989) 257–262
Elsevier

MOLBIO 01235

Genomic organisation of nuclear tRNA^{Gly} and tRNA^{Leu} genes in *Trypanosoma brucei*

David A. Campbell[1], Yoshitaka Suyama[3] and Larry Simpson[2]

[1]*Department of Microbiology and Immunology, UCLA School of Medicine, and* [2]*Department of Biology, University of California, Los Angeles, CA, U.S.A.; and* [3]*Department of Biology, University of Pennsylvania, Philadelphia, PA, U.S.A.*

(Received 22 May 1989; accepted 5 July 1989)

We have isolated a 0.3-kb *Hae*III restriction fragment from *Trypanosoma brucei* which contains two tRNA genes. Secondary structure models predict that the two genes identified encode tRNA molecules which specify glycine (anticodon UCC) and leucine (anticodon CAG). The two genes are separated by 86 nucleotides, transcribed in the same direction and contain features of conventional RNA polymerase III transcription units. Southern blot analysis indicates the presence of multicopy tRNA gene families in *T. brucei*.

Key words: Transfer RNA gene; *Trypanosoma brucei*; RNA polymerase III promoter

Introduction

Cytoplasmic tRNA molecules possess a number of invariant and semi-invariant features (for a review, see ref. 1). A combination of these conserved features allows tRNA sequences to be inferred from primary DNA sequence data. For example, cytoplasmic tRNA molecules are folded, by Watson-Crick intramolecular base pairing, into 'cloverleaf' secondary structures possessing four stems and three loops. The 5′ and 3′ ends of the tRNA form a 7-bp stem, which may determine amino acid specificity. Additionally, the nucleotides in the D-loop and dihydrouridine-loop are subject to constraints since they contain elements of the RNA polymerase III promoter. While the predicted secondary structure of tRNAs is well defined, the tertiary structure of a tRNA has been experimentally determined in only a few cases [2,3].

The primary transcript from an eukaryotic nuclear tRNA gene is processed by the removal of 5′-leader sequences by RNAse P followed by 3′ processing [4], the 3′-terminal addition of a non-template-encoded, aminoacyl-accepting CCA. Subsequently specific nucleotides are modified and the tRNA is transported to the cytoplasm where it is amino-acylated.

An unusual feature of the trypanosome mitochondrial genome is the apparent absence of identifiable tRNA coding regions. Although the partial RNA sequence of the *Crithidia oncopelti* mitochondrial valine tRNA has been reported [5], no bona fide tRNA genes have been described in the trypanosomatid protozoa. The putative tRNA sequence found in the *Trypanosoma brucei* mitochondrial maxicircle [6] does not conform to a generalised tRNA structure. To address the question of tRNA structure in the nucleus and mitochondrion of *T. brucei*, we have identified the genes encoding the nuclear tRNA^{Gly} and tRNA^{Leu} species.

Correspondence address: David A. Campbell, Dept. of Microbiology and Immunology, UCLA School of Medicine, University of California, Los Angeles, CA 90024, U.S.A.

Note: Nucleotide sequence data reported in this paper have been submitted to the EMBL database under the accession number X13750.

Abbreviations: DHU, dihydrouridine; pCp, cytidine 3′,5′-bisphosphate; SDS, sodium dodecyl sulphate; SSC, saline sodium citrate; TψC, thymidine pseudouridine cytidine.

Materials and Methods

DNA manipulation. Bloodstream form *T. brucei* (strain 427) was purified away from blood elements by DEAE chromatography [7]. *T. brucei* high-molecular-weight DNA was obtained as described [8]. The cosmid library was constructed in the vector c2XB [9] and propagated in *Escherichia coli* strain 490A. Approximately 5000 cosmid-containing colonies were immobilised on Whatman 541 paper [10] and hybridised with size-fractionated, pCp-labelled [11] tRNA. Subclones were made from cosmid DNA by ligation into the *Sma*I site of pBluescriptKS+ (Stratagene). Southern blots [12] were hybridised in Blotto [13] at 65°C. Washes were performed in 0.1 × SSC at 55°C.

DNA sequencing was performed by the dideoxy chain-termination method [14] (Sequenase™; US Biochemicals) on collapsed-supercoil plasmid DNA [15]. DNA sequence analysis was performed on the DSAS package (J. Neigel, University of Southwestern Louisiana). Predicted secondary structures were derived either by eye or by the computer package of Staden [16].

RNA manipulation. RNA was obtained from purified bloodstream form *T. brucei* by hot phenol extraction [17].

After electrophoresis of total *T. brucei* RNA on high-resolution, denaturing, 10% acrylamide gels (the first dimension gel of ref. 18), the low-molecular-weight region was transferred to Nytran membranes by electroblotting. After immobilisation of the RNA by UV irradiation, blots were hybridised in buffer containing 5 × SSC, 0.2% SDS, 1 × Denhardt's solution and 50% formamide at 37°C. Washes were performed in 1% SDS, 0.1 × SSC at 37°C.

Results

Isolation of T. brucei glycine and leucine tRNA genes. Screening of the *T. brucei* cosmid library with pCp-labelled *T. brucei* tRNA yielded five strongly hybridising colonies which were purified by a second round of colony hybridisation. These putative tRNA-gene containing cosmids were isolated and subjected to restriction analysis, which showed four of them to possess similar restriction patterns. The four overlapping cosmid clones were subsequently found to contain the tandemly repeated ribosomal RNA genes, which had been selected due to the presence, in the tRNA fraction, of the small ribosomal RNA 6 [19,20]. A Southern blot of the unique cosmid clone (cTtRNA2) probed with pCp-labelled tRNA revealed hybridisation to *Hae*III fragments of 0.8, 0.5 and 0.3 kb and *Alu*I fragments of 1.9, 1.2 and 0.45 kb. The 0.3-kb tRNA-containing *Hae*III fragment was subcloned into pKS+ (pTtRNA1.1).

Sequence of T. brucei glycine and leucine tRNA genes. Sanger sequencing of both strands of pTtRNA1.1 showed the insert to be 335 nucleotides long (Fig. 1A) and to contain two tRNA genes, which are encoded on the same strand of the DNA and are separated by 86 nucleotides. The inferred tRNA secondary structures (Fig. 1B) show all the invariant and semi-invariant features expected of a tRNA molecule. The glycine tRNA (anticodon UCC) is 72 bases in length and shows sequence identity with the heterologous glycine tRNAs (Fig. 2A) from both eukaryotes (e.g., rat; 72 bases, 72.2%) and prokaryotes (e.g. *E. coli*; 72 bases, 63.9%). The leucine tRNA (anticodon CAG) is 82 bases in length, while all other heterologous leucine tRNAs are larger due to additional bases in the extra-arm (Fig. 2B). The exception is *Xenopus*, to which the highest base identity (78.8%) with *T. brucei* was found.

As with all other eukaryotic tRNA genes, the *T. brucei* glycine and leucine tRNA genes do not encode the 3′-CCA found on the mature tRNA. The primary sequence also demonstrates the presence of Box A (TGGCnnAGTGG) and Box B (GTTCRAnnCC) RNA polymerase III promoter elements [21] (see Fig. 1A), which are typical of eukaryote tRNA genes. Additionally, a run of at least five T residues is found at the 3′-terminus of both tRNA genes. The poly-T signal is associated with the termination of RNA polymerase III transcription in eukaryotes and also transcription termination in prokaryotes [22].

Confirmation that the glycine and leucine tRNA genes identified do indeed encode tRNA molecules is provided by Northern blot analysis of the

```
          HaeIII          [tRNAGly]--->
    1  GGCCGCAGCG gcaatcgtgg tccaacggtt aagatttccg ccttccaagc gggagacccg
                                 BoxA
   61  ggttcgactc ccggcgattg caCATTTTTT CGTTTTTATC ATTCGGCATT ATGTTATGCT
         Box B
                 HindIII(AluI)                        HaeII          [tRNALeu]-->
  121  CTTGAAAAGC TTTGACACAA CATTGCAACG TTAACTCAGG TGGCGGTGTg gcaagatggt
                                                                      
  181  cgagtggtct aagacgtcac gttcaggtcg tcatctctcc ggaggcgtgg gttcaaaccc
       Box A                      Sau3A                          Box B
  241  cactcttgtc aATATTTTTG TCCGATCATA TCGGACTCAG CGGTTTCAAG TGTGGTCTCA
                                                  HaeIII
  301  ACCGTCGCCG AAGTGTCAAT AGGCGCGAAA ACAAAGGCC   339
```

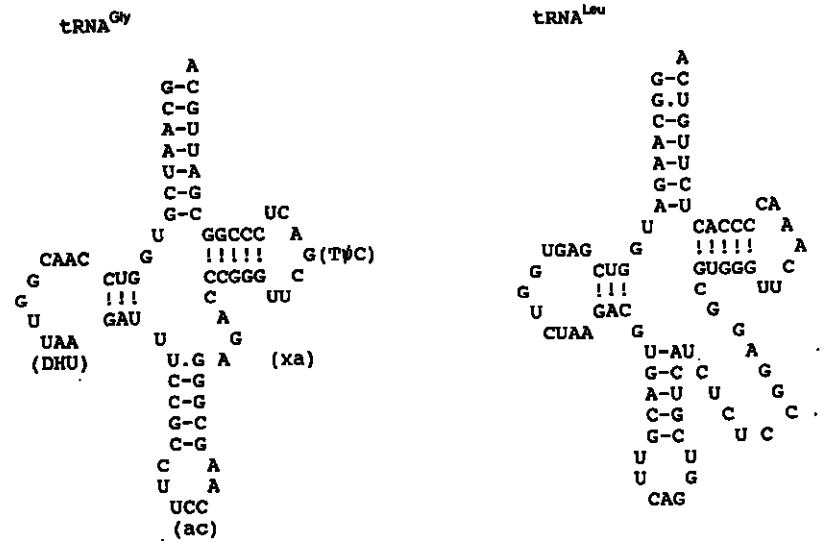

Fig. 1. (A) DNA sequence of pTtRNA1.1 (EMBL database accession No. X13750). DNA sequence was obtained on both strands. Inferred tRNA sequences are indicated by lower case lettering. Consensus RNA polymerase III Box A and Box B promoter elements are indicated by underlining. The glycine and leucine tRNA-specific probes used in blotting experiments comprise nucleotides 1–129 and 130–339, respectively. (B) Predicted secondary structure models of *T. brucei* glycine and leucine tRNAs. The dihydrouridine (DHU), anticodon (ac), variable (xa) and TψC (TψC) loops are indicated next to the glycine tRNA.

tRNA fraction of *T. brucei* RNA (Fig. 3). In the absence of external size markers, we can conclude that each probe detects a transcript in the tRNA fraction, and that the larger size of the leucine tRNA is consistent with the primary sequence data. Interestingly, the glycine tRNA probe also detects a minor transcript of smaller size which is still within the tRNA region.

Genomic organisation of glycine and leucine tRNA genes. Hybridisation of tRNA-specific probes to Southern blots of *T. brucei* genomic DNA indicates that both tRNA genes are members of multigene families (Fig. 4). A glycine-tRNA-specific probe reveals two major bands of hybridisation and at least two minor bands (lanes 1–4). A leucine-tRNA-specific probe reveals three bands of approximately equivalent hybridisation with one stronger and one weaker band suggesting the possibility of six different genes (lanes 5–8). Furthermore, both probes hybridise to DNA from *Crithidia fasciculata* and *Leishmania tarentolae* (data not shown) indicating that both genes are highly conserved across kinetoplastid species.

Discussion

By analysing two nuclear tRNA genes, we show that *T. brucei* possesses conventional tRNA genes whose transcripts may form typical cloverleaf structures. Both $tRNA^{Gly}$ and $tRNA^{Leu}$ genes appear to be transcribed and possess the signals re-

A. tRNA^{gly} (TCC)

```
      aas      ds    dl    ds   acs   acl   acs  xa  tψs   tψl   tψs   aas     #nt  %Identity
       / /    /     /    //    /     /     /   /    /     /     /    /
Tb  GCAAUCGTGGTCCAACGGTTAAGATTTCCGCCTTCCAAGCGGGAGACCCGGGTTCGACTCCCGGCGATTGCA    72   -
Ec  **GGG*A*C**AT**T**C**TT*CC**A**********T*AT**TG*********T*****CT*CCC**T    72  63.9
Hv  ***CCG******T**T***.****CA*TG**********CAATT*T*T*********T****A**CGG****   71  69.9
SM  AT*GATA*AAGTT**TT*G***AC*GGAT*T********A*ATTGA*TG**A******T**T**CTATC*AT*   72  45.8
Bm  **GT*G*****GT**T***C*GC**AG*T**********A*TT**T*********T*******C*AC***    72  72.2
Rt  **GT*G*****AT*GT***G*GC**AG*T**********A*TT**********T*******C*AC***      72  72.2
```

B. tRNA^{leu} (CAG)

```
      aas       ds    dl       ds   acs   acl   acs       xa        tψs   tψl    tψs  aas     #nt  %Identity
       / /     /     /       //    /     /     /                 /     /     /     /
Tb  GGCAAGATGGTCGAGT.GGTCTAAGACGTGACGTTCAGGTCGTCATCTCTCC.GGAG.G.CGTGGGTTCAAACCCCACTCTTGTCA    82    -
Ec  *CG***G***CG**A*T***.AG*CG**CT*GC********GT*AG*G**CTTAC*GAC*.T*G*********GT***C*C*C*CG**   84   56.6
An  *CGG*AC***CG**A*T***.AG*CG**CTAGA********TC*AG*GGT*T*ACGACTG.TCC*********GT***GGGT*CCG**   84   47.0
Xl  *T**G*****C****C.********G**CTG**********CAG****C**T****.*.*********G**T*******TC**A**   83   78.8
Rt  *T**G*****C****C.********GCGCTG**********CAG****C**TA***A*G*********G**T*******C**A**   85   75.5
```

Fig. 2. Comparison of *T. brucei* glycine tRNA (A) and leucine tRNA (B) sequences with homologous tRNAs from other eukaryotes and prokaryotes. Tb, *Trypanosoma brucei*; Ec, *Escherichia coli*; Hv, *Halobacterium volcani*; SM, *Saccharomyces* mitochondrion; Bm, *Bombyx mori*; Rt, Rat; An, *Anacystis nidulans*; Xl, *Xenopus laevis*; #nt, number of nucleotides; *, bases matching the Tb sequence; •, nucleotide absent; aas, amino acyl stem; ds, DHU stem; dl, DHU loop; acs, anticodon stem; acl, anticodon loop; xa, extra arm; tψs, TψC stem; tψl, TψC loop.% Identity is expressed as [number of matching nt/(number of species 1 (Tb RNA) + number of nt of species 2)/2] × 100. Heterologous tRNA sequences were extracted from the compilation of Sprinzl et al. [34].

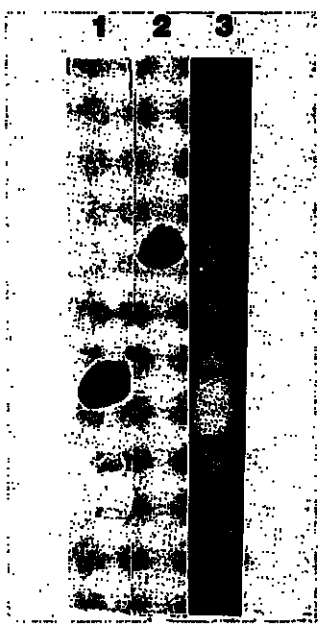

Fig. 3. Northern blot analysis of *T. brucei* tRNAs. Total *T. brucei* RNA resolved on a 10% denaturing acrylamide gel was transferred to a nylon membrane and hybridised with a glycine tRNA-specific probe (lane 1) or a leucine tRNA-specific probe (lane 2). Lane 3 shows the ethidium bromide staining pattern of the RNA before transfer.

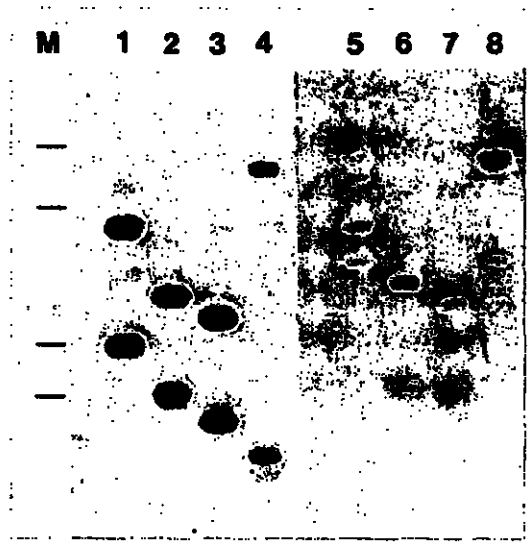

Fig. 4. Genomic organisation of the *T. brucei* glycine and leucine tRNA genes. *T. brucei* genomic DNA digested with *Alu*I (lanes 1 and 5), *Hae*III (lanes 2 and 6), *Hin*PI (lanes 3 and 7) and *Rsa*I (lanes 4 and 8) probed with glycine tRNA-specific probe (lanes 1–4) or leucine tRNA-specific probe (lanes 5–8). The size markers (lane M; phage λ DNA digested with *Pst*I) are in descending order: 1.7 kb, 1.1 kb, 0.5 kb and 0.3 kb.

quired for transcription by a conventional RNA polymerase III. Thus with the identification of a gene encoding the largest subunit of RNA polymerase III in *T. brucei* [23,24] and the presence of Box A and Box C [25] promoter elements in the *T. brucei* 5S RNA gene [26,27] and an intermediate level of α-amanitin resistance for 5S transcription [28], it appears that trypanosomes possess both conventional RNA polymerase III enzyme and templates.

The presence of multiple bands on genomic Southern blots is indicative of a family of closely related genes for each isoacceptor tRNA class. Since all six leucine codons are used in *T. brucei* [29], all the leucine tRNA genes may be accounted for by the bands of hybridisation in Fig. 4. The lack of hybridisation of the leucine-tRNA probe to a band at 1.9 kb in the *Alu*I digest of genomic DNA may be explained by an *Alu*I site in the genomic DNA, which is not accessible in the cloned DNA (see Fig. 1A).

The differential hybridisation seen with the glycine-tRNA-specific probe may be explained by either of two possibilities given that all four glycine codons are used in *T. brucei* [29]. Firstly the strong and weak bands of hybridisation may represent two distantly related families encoding glycine tRNA. Secondly, the presence of two $tRNA^{Gly}$ genes closely linked on the same restriction fragments would result in the same pattern, in which case a second glycine tRNA gene should be encoded on cTtRNA2. The weaker signals on the Southern blot may thus represent tRNA genes with lower sequence identity and possibly a different amino acid specificity. Although the $tRNA^{Gly}$ and $tRNA^{Leu}$ genes are closely linked on clone pTtRNA1.1, the lack of hybridisation to other common bands in the genomic Southern blot (Fig. 4) suggests that other $tRNA^{Gly}$ genes are not closely linked to other $tRNA^{Leu}$ genes.

The faint smaller band seen on the Northern blot with the glycine tRNA-specific probe is apparently a very small tRNA and may represent a transcript from these distantly related genes. A precedent for the existence of a very small tRNA has been set. The mammalian mitochondrial $tRNA^{Ser}$ lacks the dihydrouridine loop and arm [30]. Furthermore, it has been inferred that all nematode mitochondrial tRNAs are missing the TψC arm and loop structure, with the exception of $tRNA^{Ser}$ which, instead, lacks the DHU stem and loop [31].

Our results show that the *T. brucei* nuclear genome contains two tRNA genes which are conventional by all the criteria that we have applied to them. Since the *T. brucei* nuclear $tRNA^{Leu}$ probe hybridizes to a mitochondrial tRNA fraction from *Leishmania tarentolae* [32], the clone described here may be used to address the origin of mitochondrial tRNAs in kinetoplastid protozoa. Alternative hypotheses for the origin of kinetoplastid mitochondrial tRNAs include: import of nuclear tRNAs (as has been described for *Tetrahymena* [18] and *Phaseolus* [33]), transcription from the minicircle component of the kinetoplast DNA network, or that primary mitochondrial transcripts are edited by extensive uridine addition or deletion.

Acknowledgements

We thank Sandy Wong for technical assistance, Dallas Hughes for *Crithidia* DNA, Agda Simpson for *Leishmania* DNA and pCp-labelled tRNA, and Patricia Johnson for critical reading of the manuscript. This work was supported by Basic Sciences Research Grant RR5354 (D.A.C.). Y.S. was supported by the Burroughs Wellcome Foundation (W880309) and PHS AI09102 (L.S.).

References

1 Dirheimer, G., Keith, G., Sibler, A.-P. and Martin, R.P. (1979) The primary structure of tRNAs and their rare nucleosides. In: Transfer RNA: Structure, Properties, and Recognition (Schimmel, P.R., Soll, D. and Abelson, J.N., eds.), pp. 19–41, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

2 Ladner, J.E., Jack, A., Robertus, J.D., Brown, R.S., Rhodes, D., Clark, B.C. and Klug, A. (1975) Structure of yeast phenylalanine transfer RNA at 2 angstrom resolution. Proc. Natl. Acad. Sci. USA 72, 4414–4418.

3 De Bruijn, M.H.L. and Klug, A. (1983) A model for the tertiary structure of mammalian mitochondrial transfer RNAs lacking the entire 'dihydrouridine' loop and stem. EMBO J. 2, 1309–1321.

4 Frendewey, D., Dingermann, T., Cooley, L. and Soll, D. (1985) Processing of precursor tRNAs in *Drosophila*: processing of the 3' end involves an endonucleolytic cleavage and occurs after 5' end maturation. J. Biol. Chem. 260, 449–454.

5 Entilis, N.T., Maslov, D.A., Bolshakova, E.B. and Zaitsva, G.N. (1987) Primary structure of an unusual valine tRNA species from the mitochondrion of *Crithidia oncopelti*. Dokl. Akad. Nauk. SSSR 297, 1498–1501.

6 Stuart, K. (1983) Mitochondrial DNA of an African trypanosome. J. Cell. Biochem. 23, 13–26.

7 Lanham, S.H. and Godfrey, D.G. (1970) Isolation of salivarian trypanosomes from man and other animals using DEAE cellulose. Exp. Parasitol. 28, 521–534.

8 Bernards, A., Van der Ploeg, L.H.T., Frasch, A.C.C., Borst, P., Boothroyd, J.C., Coleman, S. and Cross, G.A.M. (1981) Activation of trypanosome surface glycoprotein genes involves a duplication-transposition leading to an altered 3' end. Cell 27, 497–505.

9 Bates, P.F. and Swift, R.A. (1983) Double *cos* site vectors: simplified cosmid cloning. Gene 26, 137–146.

10 Gergen, J.P., Stern, R.H. and Wensink, P.C. (1979) Filter replicas and permanent collections of recombinant DNA plasmids. Nucleic Acids Res. 7, 2115–2122.

11 England, T.E. and Uhlenbeck, O.C. (1978) Enzymatic oligoribonucleotide synthesis with T4 RNA ligase. Biochemistry 17, 2069–2076.

12 Southern, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98, 503–517.

13 Johnson, D.A., Gautsch, J.W., Sportsman, J.R. and Elder, J.H. (1984) Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene Anal. Techn. 1, 3–8.

14 Sanger, F., Nicklen, S. and Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74, 5463–5467.

15 Chen, E.Y. and Seeburg, P.H. (1985) Supercoil sequencing: a fast and simple method for sequencing plasmid DNA. DNA 4, 165–170.

16 Staden, R. (1986) The current status and portability of our sequencing handling software. Nucleic Acids Res. 14, 217–231.

17 Boothroyd, J.C. and Cross, G.A.M. (1982) Transcripts coding for variant surface glycoproteins of *Trypanosoma brucei* have a short, identical exon at their 5' end. Gene 20, 281–289.

18 Suyama, Y. (1986) Two dimensional polyacrylamide gel electrophoresis analysis of *Tetrahymena* mitochondrial tRNA. Curr. Genet. 10, 411–420.

19 Hasan, G., Turner, M.J. and Cordingley, J.S. (1984) Ribosomal RNA genes of *Trypanosoma brucei*: mapping the regions specifying the six small ribosomal RNAs. Gene 27, 75–86.

20 White, T.C., Rudenko, G. and Borst, P. (1986) Three small RNAs within the 10-kb trypanosome rRNA transcription unit are analogous to domain VII of other eukaryotic 28S rRNAs. Nucleic Acids Res. 14, 9471–9489.

21 Galli, G., Hofstetter, H. and Birnstiel, M.L. (1981) Two conserved sequence blocks within eukaryotic tRNA genes are major promoter elements. Nature 294, 626–631.

22 Tomizawa, J.-I. and Masukata, H. (1987) Factor independent termination of transcription in a stretch of deoxyadenosine residues in the template DNA. Cell 51, 623–630.

23 Kock, J., Evers, R. and Cornelissen, A.W.C.A. (1988) Structure and sequence of the gene for the largest subunit of trypanosomal RNA polymerase III. Nucleic Acids Res. 16, 8753–8772.

24 Smith, J.L., Levin, J.R., Ingles, C.J. and Agabian, N. (1989) In trypanosomes the homolog of the largest subunit of RNA polymerase II is encoded by two genes and has a highly unusual C-terminal domain structure. Cell 56, 815–827.

25 Picler, T., Hamm, J. and Roeder, R.G. (1987) The 5S gene internal control region is composed of three distinct sequence elements, organised as two functional domains with variable spacing. Cell 48, 91–100.

26 Cordingley, J.S. (1985) Nucleotide sequence of the 5S ribosomal RNA gene repeat of *Trypanosoma brucei*. Mol. Biochem. Parasitol. 17, 321–330.

27 Lenardo, M.J., Dorfman, D.M. and Donelson, J.E. (1985) The spliced leader sequence of *Trypanosoma brucei* has a potential role as a cap donor structure. Mol. Cell. Biol. 9, 2487–2490.

28 Laird, P.W., Kooter, J.M., Loosbroek, N. and Borst, P. (1985) Mature mRNAs of *Trypanosoma brucei* possess a 5' cap acquired by discontinuous RNA synthesis. Nucleic Acids Res. 13, 4253–4266.

29 Michels, P.A.M. (1986) Evolutionary aspects of trypanosomes: Analysis of genes. J. Mol. Evol. 24, 45–52.

30 De Bruijn, M.H.L., Schrier, P.H., Eperon, I.C., Barrell, B.G., Chen, E.Y., Armstrong, P.W., Wong, J.F.H. and Roe, B.A. (1980) A mammalian mitochondrial serine transfer RNA lacking the 'dihydrouridine' loop and stem. Nucleic Acids Res. 8, 5213–5222.

31 Wolstenholme, D.R., Macfarlane, J.L., Okimoto, R., Clary, D.O. and Wahleithner, J.A. (1987) Bizarre tRNAs inferred from DNA sequences of mitochondrial genomes of nematode worms. Proc. Natl. Acad. Sci. USA 84, 1324–1328.

32 Simpson, A.M., Suyama, Y., Dewes, H., Campbell, D.A. and Simpson, L. (1989) Kinetoplastid mitochondria contain functional tRNAs which are encoded in nuclear DNA and also contain small minicircle and maxicircle transcripts of unknown function. Nucleic Acids Res. 17, 5427–5445.

33 Marechal-Drouard, L., Weil, J.-H. and Guillemaut, P. (1988) Import of several tRNAs from the cytoplasm into the mitochondria in bean *Phaseolus vulgaris*. Nucleic Acids Res. 16, 4777–4788.

34 Sprinzl, M., Hartmann, T., Meissner, F., Moll, J. and Vorderwulbecke, T. (1987) Compilation of tRNA sequences and sequences of tRNA genes. Nucleic Acids Res. 15, r53–r188.