The EMBO Journal vol.3 no.10 pp.2387–2392, 1984

Discontinuous synthesis of mRNA in trypanosomes

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Communicated by P.Borst

Many trypanosome mRNAs have the same sequence of 35 nucleotides at their 5′ end, encoded by a mini-exon located in 1.35-kb tandemly linked repeats. We have analysed nascent and steady-state mini-exon transcripts to determine how the mini-exon sequence is joined to the main part of trypanosome mRNAs. We show here that steady-state RNA from *Trypanosoma brucei* contains a transcript of 141 nucleotides that starts at the 5′ border of the mini-exon. Isolated nuclei transcribe the segment corresponding to the 141 nucleotide RNA at a high rate; transcription of other areas of the 1.35-kb mini-exon repeat is ~750-fold lower. We propose that transcription of protein-coding genes in trypanosomes is discontinuous and involves the 141-nucleotide transcript as an intermediate.

Key words: discontinuous transcription/'run-on' assay/trypanosome mini-exon repeat

Introduction

Many, if not all, mRNAs in trypanosomes and other kinetoplastid flagellates start with the same 35 nucleotides, which are not encoded contiguously with the remainder of the gene (Van der Ploeg *et al.*, 1982; Boothroyd and Cross, 1982; Liu *et al.*, 1983; Bernards *et al.*, 1984; De Lange *et al.*, 1984a). This mini-exon sequence is part of a small (0.4– 1.35 kb, depending on the species), repeated DNA segment (De Lange *et al.*, 1983, 1984b; Nelson *et al.*, 1983,1984). As most mini-exon repeats are clustered in a few long tandem arrays, not every protein-coding gene can be directly downstream of a mini-exon repeat (De Lange *et al.*, 1983,1984a). Hence, mRNA synthesis in *T. brucei* must either involve discontinuous transcription or the differential splicing of long polycistronic precursor RNAs containing mini-exons at their 5′ terminus (De Lange *et al.*, 1984b). The long precursor RNA intermediates predicted by the latter mechanism have not been detected in steady-state RNA (De Lange *et al.*, 1984a). If such precursors are rapidly processed, however, they may not be detectable in steady-state RNA. We have, therefore, set up a system to analyse nascent trypanosome RNA. The RNA is labeled by allowing isolated nuclei to elongate nascent RNA in the presence of α-^{32}P-labeled nucleotides. This technique has been developed by Bachenheimer and Darnell (1975), Weber *et al.* (1977) and McKnight and Palmiter (1979) and it has been successfully used to delineate transcription units in other organisms than trypanosomes. With this sytem we have examined the transcription unit in the 1.35-kb mini-exon repeat segment of *T. brucei*. From analyses of both nascent and of steady-state RNA, we infer that mini-exon repeat arrays are not transcribed into long precursor molecules, but instead yield a small RNA of 141 nucleotides. These data indicate that the sequence of most mRNAs in trypanosomatids is derived from two disconnected templates: the repetitive mini-exon gene and a structural gene specifying the remainder of the mRNA.

Results

A small transcript of the mini-exon repeat in steady state mRNA

Figure 1 presents a detailed physical map of the tandemly linked 1.35-kb repeat that contains the mini-exon. The position of sequence elements which are conserved in *T. vivax* and *T. cruzi* is indicated; these are mainly limited to the mini-exon itself and the adjacent sequences (see also Figure 3) (De Lange *et al.*, 1984b). Transcripts containing the mini-exon sequence are displayed in the blot shown in Figure 2. Total RNA from *T. brucei* was size-fractionated, blotted onto DBM paper and hybridized (lane B) with a 5′ end-labeled 22-mer, which is complementary to position 13–34 of the mini-exon sequence (De Lange *et al.*, 1983). This gives a broad smear of hybridizing RNAs representing the numerous mRNAs that have the mini-exon sequence at their 5′ end. In addition, a discrete band of ~140 nucleotides is detected. In Figure 2 (lane C) the same 140-nucleotide transcript is detected under more stringent conditions by a larger probe containing 17 bp of the mini-exon and 140 bp flanking region (indicated as X-S probe in Figure 1). A mini-exon transcript of the same size was also found in RNA denatured by glyoxylation.

To localize the 3′ end of the mini-exon repeat transcripts more precisely, S1-nuclease experiments were done with a 3′ end-labeled DNA fragment covering part of the mini-exon plus 360 bp of the 3′-flanking region (see Figure 1). The S1-protected fragment is 123 ±4 nucleotides (Figure 3), which positions the 3′ terminus of the mini-exon transcript at the beginning of the conserved T-stretch (see Figures 1 and 3). As *Xmn*I cuts the mini-exon sequence 18 bp from the presumed first nucleotide, we infer that the RNA is 141 ±4 nucleotides,

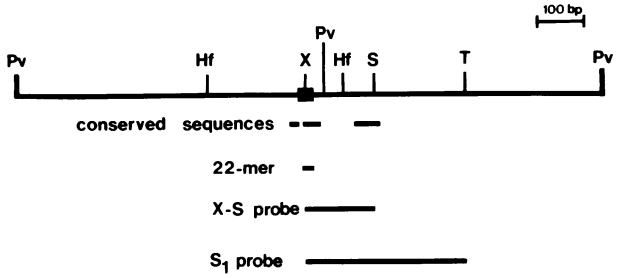

Fig. 1. Physical map of the 1.35-kb mini-exon repeat in *T. brucei* (De Lange *et al.*, 1983,1984b). The black box represents the mini-exon; the direction of transcription is from left to right. The underlined regions show sequence homology with the mini-exon repeats in *T. cruzi* and *T. vivax* (see also Figure 3) (De Lange *et al.*, 1984b). Below the map, the position of the probes used in the experiments in Figures 2 and 3, are shown. Hf, *Hinf*I; Pv, *Pvu*II; S, *Sau*3AI; T, *Taq*I; X, *Xmn*I.

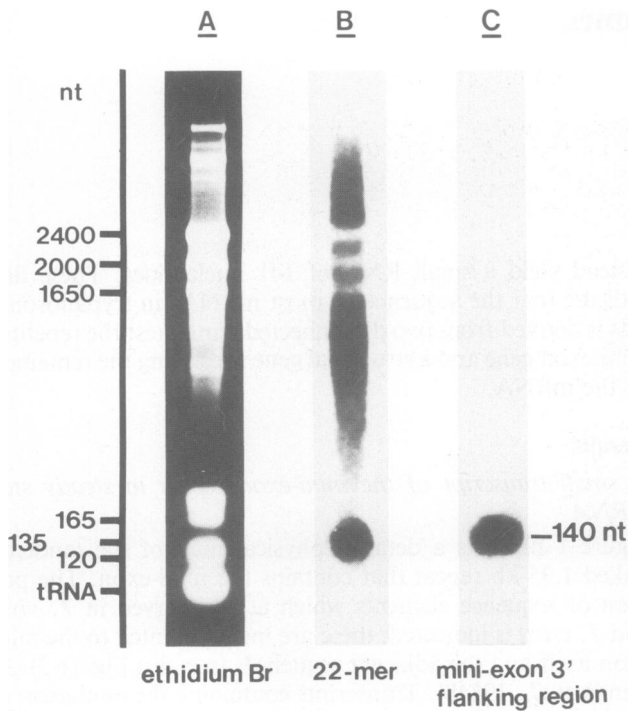

Fig. 2. A 140-nucleotide mini-exon transcript in steady-state RNA. RNA from *T. brucei* (VAT 221a) was size-fractionated on a 2% agarose gel, stained with ethidium-bromide (**A**), transferred to DBM-paper and hybridized to a 22-nucleotides probe for the mini-exon (**B**) or to a 160-bp *Xmn*I-*Sau*3AI fragment from the mini-exon repeat (**C**). See Figure 1 for the position of the probes.

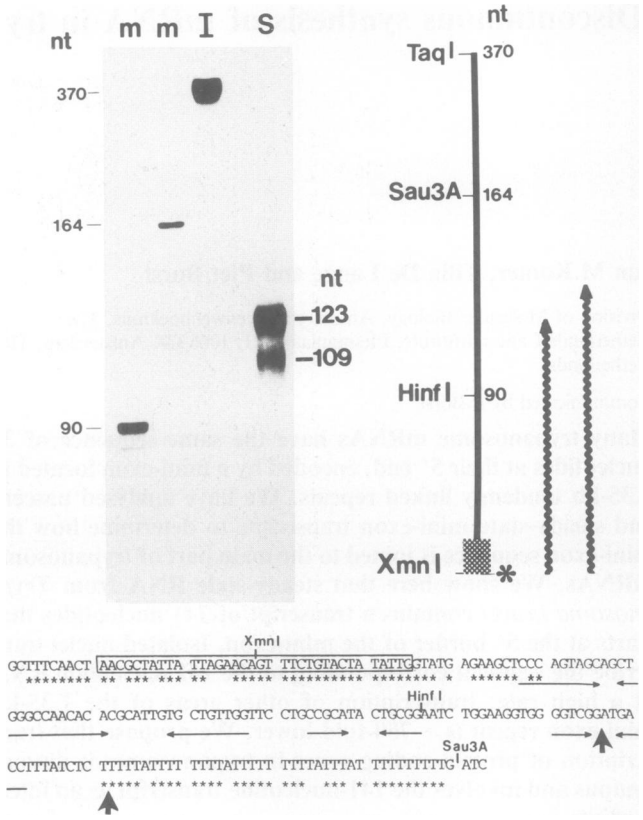

Fig. 3. The 3′ end of the mini-exon repeat transcript determined by S1-nuclease analysis. RNA from *T. brucei* (VAT 221a) was hybridized to a *Xmn*I-*Taq*I fragment (I), derived from the mini-exon repeat (see Figure 1) and labeled at the 3′ end on the *Xmn*I site. The DNA-RNA hybrids were digested with S1-nuclease and size-fractionated on a sequence gel (S). The size of the protected fragments was determined by co-migration with a sequence ladder (not shown). Lanes indicated with (m) contain a 90-bp *Xmn*I-*Hinf*I fragment (left) and a 164-bp *Xmn*I-*Sau*3AI fragment (right). The right hand schematic shows the restriction map of the relevant area and the position of the protected fragments. The lower part of the figure shows the sequence of the mini-exon transcription unit and the position of the ends of the protected fragments (arrowheads); conserved sequences are indicated by asterisks and inverted repeat sequences are indicated with arrows (from De Lange *et al.*, 1984b).

i.e., approximately the length of the RNA detected with various probes on the RNA blots. The size of the S1-protected fragment was not significantly affected by variations in the enzyme concentration or incubation temperature (not shown). In all experiments a minor species of 109 nucleotides was also visible (see Figure 3). Such a transcript is not detected in RNA blots and may have been generated by S1-nuclease digestion of a cruciform generated by the inverted repeats in the 3′ part of the DNA-RNA hybrid.

A small region of the 1.35-kb repeats is transcribed

To test whether the small mini-exon transcripts result from processing of a large precursor RNA, we set up a 'run-on' transcription assay for isolated nuclei from trypanosomes. In this system nascent RNA chains are elongated in the presence of [α-^{32}P]UTP. The labeled RNA was used as hybridization probe on gel-fractionated restriction fragments from a recombinant plasmid containing an array of mini-exon repeat units. Hybridization of labeled nascent transcripts to a mini-exon repeat (Figure 4, panel C, lane I), shows that the major part of the 1.35-kb repeat unit is hardly transcribed. In DNA digested with *Xmn*I and *Sau*3AI only one restriction fragment hybridizes strongly. This fragment (160 bp) extends from the middle of the mini-exon (+19) to position +180, i.e., slightly beyond the 3′ end of the mini-exon transcript mapped by S1-analysis (position +141). The other hybridizing fragment comes from the region adjacent to the mini-exon array present in clone pCL102 which is transcribed. The 1190-bp *Xmn*I-*Sau*3AI fragment containing the remainder of the mini-exon repeat hardly binds nascent transcripts, the region 5′ of the *Xmn*I site is probably too short (18 nucleotides) to form stable hybrids. To quantitate the level of transcription of the entire mini-exon region more precisely, restriction fragments of the mini-exon repeat unit were hybridized to different concentrations of labeled nascent RNA. In Figure 4 (panel C, I−V) only one band in each double digest hybridizes strongly to nascent RNA, apart from the fragments coming from the region adjacent to the mini-exon repeat array in pCL102. With a 100-fold dilution of labeled RNA as probe the 160-bp *Xmn*I-*Sau*3AI fragment is still visible and the hybridization intensity is approximately the same as that of the 1190-bp fragment in lane I. Full-length transcripts of the whole 1.35-kb mini-exon repeat segments are therefore ~100 x 1190/160 = 750-fold less abundant than transcripts from the mini-exon area itself. It is unlikely that this is due to rapid RNA processing, because sequences that are known to be rapidly processed, like transcripts from upstream regions of transcribed VSG genes, are readily detected in nascent RNA (J.M.Kooter, unpublished results).

An analysis of the level of transcription of the mini-exon genes relative to genes encoding α- and β-tubulin and VSG 117 (Figure 5) shows that mini-exon gene transcription is relatively high in isolated nuclei. The amount of coding se-

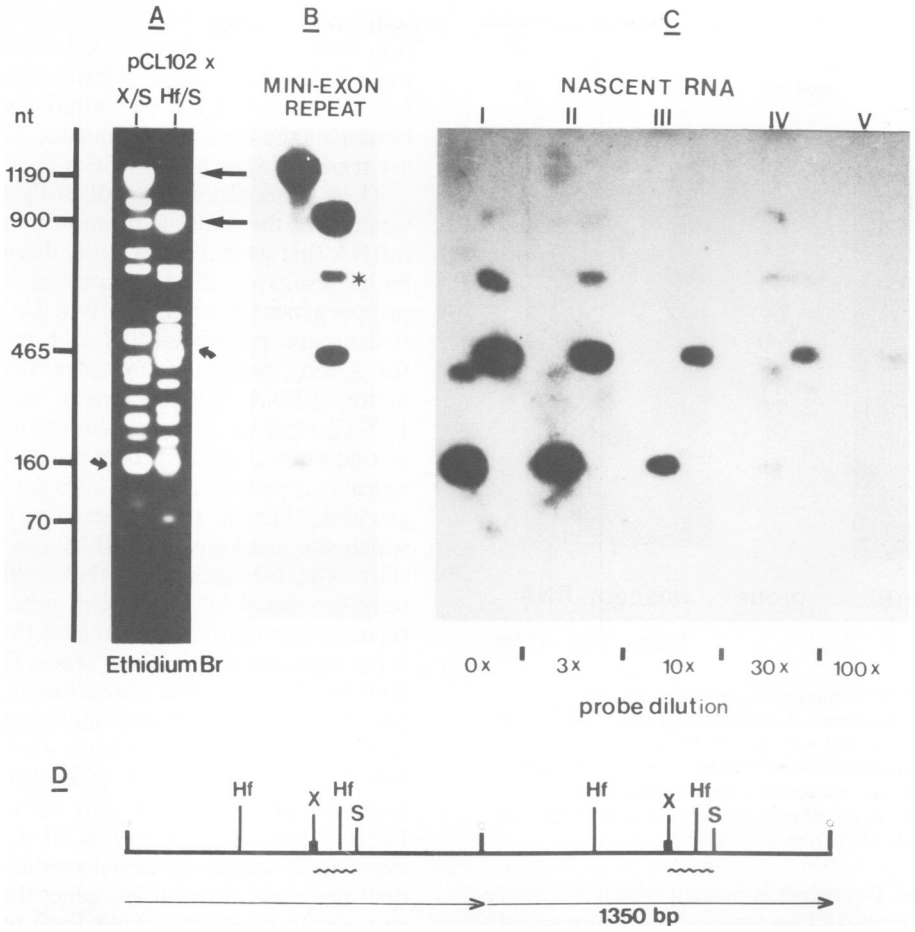

Fig. 4. Only a small part of the mini-exon repeat is transcribed at high rate. A plasmid containing a mini-exon repeat array and flanking non-repeat sequences (pCL102) was digested with *Sau*3AI and either *Xmn*I or *Hinf*I, size-fractionated on a 2% agarose gel, stained with ethidium bromide **(A)** and blotted onto nitrocellulose filter. Fragments derived from the mini-exon repeat unit are identified by hybridization with a 1.35-kb *Xmn*I fragment, which contains a complete mini-exon repeat **(B)**. The repeat-derived fragments are indicated with arrows in **(A)**; the asterisk in **(B)** marks an aberrant fragment that is probably derived from the end of the mini-exon repeat array. Identical nitrocellulose strips containing the DNA shown in **(A)** were hybridized to five different concentrations of labeled nascent RNA from VAT 117b **(C)** as indicated in the figure. The position of mini-exon repeat fragments which hybridize to nascent RNA is underlined in the physical map of two tandemly linked repeats **(D)**.

quence per nucleus is ~28 000 bp for the mini-exon genes [200 copies (De Lange *et al.*, 1983) and 140 bp coding region each (this paper)], ~56 000 bp for the α- and β-tubulin genes [at least 16 copies and 3500 bp coding region each (Seebeck *et al.*, 1983)] and 1600 bp for VSG gene 117 (one copy; Van der Ploeg *et al.*, 1982). This ratio (18:35:1) is not reflected in the hybridization intensities in Figure 5. The mini-exon gene hybridization, for instance, is at least 50-fold higher than the α- and β-tubulin hybridization while the mini-exon coding capacity is ~2-fold less. The high mini-exon transcription is, therefore, not merely due to high copy number. This result is reproducible, with very minor differences, both with freshly prepared and frozen nuclei. The 'run-on' assay is furthermore highly specific because labeled nascent RNA synthesized in VAT 117b nuclei only hybridizes to VSG 117 cDNA (Figure 5) and not to other VSG genes (not shown).

Discussion

Our results show that the mini-exon repeats of *T. brucei* predominantly yield a 141-nucleotide transcript. This small transcript has been independently detected by J.C.Boothroyd and co-workers and N.Agabian and co-workers (personal communications) in steady-state RNA. To determine whether this small RNA is a primary transcript or derived from a larger precursor, we have set up a 'run-on' assay that allows the elongation of nascent transcripts in isolated nuclei. With this assay we find a high transcriptional level for the segment corresponding to the 141-nucleotide mini-exon transcript whereas the remainder of the 1.35-kb mini-exon repeat is transcribed at much lower rate. We have no reason to think that the 'run-on' assay does not faithfully reflect nascent RNA synthesis in the trypanosomes from which the nuclei were derived. Major cellular RNAs – like rRNA, tubulin mRNA or VSG mRNA – were represented in the nascent RNA, and no transcripts of silent VSG genes were detected. We therefore conclude that the 141-nucleotide transcript is most likely the major transcript of the mini-exon repeat array *in vivo*.

The start site for mini-exon RNA synthesis has not been determined precisely but based on the transcript length and the S1-nuclease protection experiments we infer that this is at or just in front of the first nucleotide of the mini-exon sequence. The perfectly conserved sequence of eight nucleotides immediately upstream of the mini-exon sequence might therefore function as a promoter, possibly in collaboration with the conserved mini-exon sequence (De Lange *et al.*, 1984b). Downstream of the mini-exon sequence at position 140 in *T.*

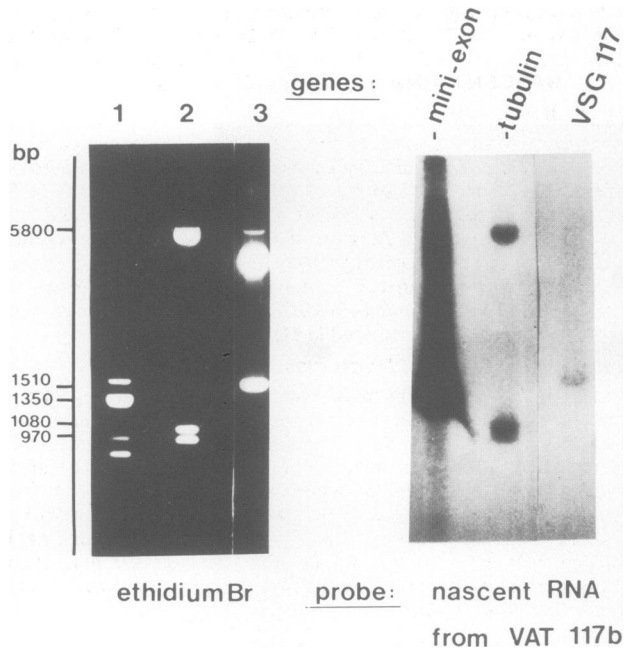

Fig. 5. High level of mini-exon transcription compared with the transcription of some structural genes. Recombinant plasmids containing mini-exon repeats (pCL102) digested with *Sau*3AI **(lane 1)**, α- and β-tubulin genes (pTbαβT-1) digested with *Bgl*II **(lane 2)** and a cDNA of mRNA for VSG 117 (pTcV 117.5) digested with *Pst*I **(lane 3)** were size-fractionated on a 0.7% agarose gel, blotted onto nitrocellulose filters and hybridized to labeled nascent RNA from VAT 117b.

brucei a long stretch of T-residues is present which is conserved in *T. vivax* and *T. cruzi* and we have shown that transcription terminates in this region. Upstream of these T-stretches there are inverted repeat sequences (see Figure 3) which are not conserved in size or nucleotide sequence (De Lange *et al.*, 1984b). It is striking that similar structural features, like a GC-rich inverted repeat sequence followed by a run of consecutive thymidylate residues in the non-template strand mark the end of transcription in *E. coli* (reviewed by Holmes *et al.*, 1983). Termination by eukaryotic polymerases, however, may involve either inverted repeats or runs of T-residues but not both (Birchmeyer *et al.*, 1983; Bogenhagen and Brown, 1981). The sequences at the beginning and end of the 141-nucleotide transcript therefore give no clear clue as to what polymerase is responsible for its synthesis. It is even possible that the potential secondary structures near the 3′ end of the mini-exon transcript only function in linking the 141-nucleotide transcript to other RNA (see below).

Three sets of observations must now be accommodated in a model for mRNA synthesis in trypanosomes: (i) Many, if not all, mRNAs in *T. brucei* start with the same mini-exon sequence (De Lange *et al.*, 1984a). (ii) The 1.35-kb segments in which mini-exons are contained are highly clustered in the genome (De Lange *et al.*, 1983; Nelson *et al.*, 1983). There are ~200 mini-exon repeats per nucleus and from partial restriction digests we have inferred that these are clustered in a few tandem arrays, containing up to 15 tandemly linked repeats (De Lange *et al.*, 1983). In other kinetoplastid flagellates the mRNAs start with an analogous mini-exon sequence and this sequence is also encoded in highly clustered repeats (De Lange *et al.*, 1983,1984a; Nelson *et al.*, 1984). The only part of this repeat that is highly conserved, is the mini-exon itself, eight nucleotides upstream, short blocks of sequences immediately downstream and a T-stretch that marks the end of transcription in *T. brucei* (De Lange *et al.*, 1984b). (iii) The major transcript from mini-exon repeats in steady-state RNA is a 141-nucleotide RNA that initiates approximately at the 5′ border of the mini-exon sequence. Other areas of the repeat are transcribed at a level which is ~750-fold lower.

These observations are not easily reconciled with the early versions of the multiple promoter model for the synthesis of mRNA that we have previously discussed on the basis of our initial results on the transcription of variant surface glycoprotein genes (Borst *et al.*, 1983; De Lange *et al.*, 1983). The first version postulated that each mini-exon acts as the start for a long precursor transcript that is processed to yield mature mRNA. The low level of transcription of most of the 1.35-kb repeat is clearly incompatible with this model. The second version of the model postulated that most mini-exon repeats act merely as entry sites for polymerases in order to assemble them at the farthest downstream mini-exon from which the synthesis of mRNA precursor transcripts would start. The 141-nucleotide RNA could be the abortive transcript predicted by this model; other areas of the mini-exon repeat might not be transcribed if the downstream mini-exon is not adjacent to mini-exon repeat DNA. In view of the very high rate of mini-exon transcription, it is difficult to exclude this model purely on quantitative grounds. What makes it highly unlikely is the enormous waste in nascent RNA that it would imply. Since every precursor RNA would contain a single mini-exon, it could only yield a single mature mRNA. Even if there are as many as 30 separate mini-exon arrays, every array would still have to serve >100 genes, if *T. brucei* does not make do with less genes than *E. coli*. Furthermore, as a single promoter would have to serve many genes, the regulation of gene expression would be highly complicated.

These arguments against huge transcription units are underlined by recent information about tubulin genes in trypanosomes. Thomashow *et al.* (1983) and Seebeck *et al.* (1983) have shown that there are at least eight clusters of linked genes for α- and β-tubulin and these genes are tandemly linked, possibly in a single array. More recently Agabian and co-workers (personal communication) have shown that tubulin mRNAs start with the mini-exon sequence and that this sequence is not present in the tubulin gene area. If such an array of genes were served by a single mini-exon. transcription would not only be wasteful but the multiplicity of tubulin genes would make no sense. We conclude, therefore, that mRNAs in trypanosomes (and presumably in other kinetoplastid flagellates) are made discontinuously and that the 141-nucleotide mini-exon transcript is a precursor in this process.

This conclusion is supported by the analysis of an expression-linked copy of the gene for VSG 1.8 (Van der Ploeg and Cornelissen, 1984; L.H.T.van der Ploeg, unpublished experiments). This copy is located on a 550-kb chromosome, which contains no mini-exon gene, even though the VSG 1.8 mRNA has the mini-exon sequence. This indicates that the mature mRNA is derived from precursor RNAs transcribed from two different chromosomes.

Whether discontinuous transcription involves a jumping polymerase (cf. Campbell *et al.*, 1984), bimolecular splicing (cf. Borst *et al.*, 1983) or the 141-nucleotide transcript as primer for pre-mRNA synthesis cannot be decided from the available data. Why a 141-bp transcription unit should be surrounded by 1210 bp of spacer DNA is also not clear. The

fact that neither the size nor the sequence of this spacer is conserved in evolution (De Lange *et al.*, 1984b), argues against an essential role. Finally it is still obscure why kinetoplastid flagellates synthesize their major mRNAs in such an unusual fashion. The ability to isolate trypanosome nuclei which faithfully transcribe RNA should make it easier to tackle some of these questions, however, and should eventually lead to the reconstruction of discontinuous transcription *in vitro*.

When the mini-exon sequence was first detected at the end of VSG mRNAs (Bernards *et al.*, 1981; Van der Ploeg *et al.*, 1982; Boothroyd and Cross, 1982) and shown to be absent in the DNA segment transposed during the activation of VSG genes by duplicative transposition (Van der Ploeg *et al.*, 1982; Liu *et al.*, 1983; Bernards *et al.*, 1984), it seemed likely that the VSG gene was activated by transposing it downstream of a promoter mini-exon unit in an expression site, i.e., activation by promoter addition. This line of reasoning has lost its basis with the finding of discontinuous transcription. If mini-exons do not have to be present upstream of the gene transcribed to end up in the mature mRNA, the start of VSG gene transcription might be in the transposed segment, transcription being activated by a chromosomal position effect, like in the mating-type switch in yeast (Nasmyth *et al.*, 1981; Klar *et al.*, 1981). We have tested this in the 'run-on' assay described here and find that DNA segments in the expression site upstream of the transposed segment are highly transcribed (T.De Lange and J.M.Kooter, unpublished experiments). Using the 'run-on' assay, it should be possible to locate the promoter for VSG gene transcription precisely and determine how its activity is regulated.

Materials and methods

Trypanosomes

The trypanosomes used in this study belong to strain 427 of *T. brucei brucei*. Variant antigen types 221a and 117b of this strain have been described by Cross (1975) and Michels *et al.* (1983), respectively. Parasites were grown as described by Fairlamb *et al.*, (1978).

Isolation of nuclei

Blood was taken from infected rats by cardiac puncture and immediately passed through the Stansted Cell Disrupter as described by Crumpton and Snary (1974) and Wright *et al.* (1974) to disrupt trypanosomes and blood cells. The homogenate was collected in two volumes of buffer A [20 mM Pipes (pH 7.5), 15 mM NaCl, 60 mM KCl, 14 mM β-mercaptoethanol, 0.5 mM EGTA, 4 mM EDTA, 0.15 mM spermine, 0.5 mM spermidine and 0.125 mM PMSF]. The homogenate was spun for 10 min at 4000 r.p.m. in a Sorvall HB4 rotor at 4°C. The crude nuclei preparation was washed several times in buffer A and finally resuspended in the nuclei storage buffer described by Schibler *et al.* (1983), at a concentration of $2-4 \times 10^9$ nuclei/ml. Nuclei thus prepared were either used directly for *in vitro* elongation of nascent RNA or stored at −70°C.

Elongation of nascent RNA chains in isolated nuclei

The *in vitro* elongation reactions were carried out according to McKnight and Palmiter (1979), Gariglio *et al.* (1981) and Schibler *et al.* (1983) with minor modifications. The incubation mixture contained 100 mM Tris-HCl (pH 7.9), 50 mM NaCl, 4 mM $MnCl_2$, 2 mM $MgCl_2$, 0.25 mM EDTA, 0.06 mM PMSF, 1.2 mM DTT, 10 mM creatine phosphate, 1 mM each of GTP and CTP, 4 mM ATP, 25% glycerol, 125 units RNAsin per ml (Promega biotec), 0.5−1 μM [α-^{32}P]UTP (3000 Ci/mmol, Amersham) and $1-2 \times 10^9$ nuclei/ml. Nuclei were incubated for 4 min at 37°C and the reactions were terminated by the addition of 10 μg DNase I per ml. Labeled RNA was extracted from the reaction mixture according to Groudine *et al.* (1981). Typically, 10^9 nuclei yielded $2-5 \times 10^6$ c.p.m. incorporated in RNase A-digestable nucleic acids.

Analysis of nascent RNA

The nascent transcripts from $1-2 \times 10^9$ nuclei were used as a hybridization probe on Southern blots. The hybridizations were carried out in a mixture containing 10% dextransulphate, 3 x SSC, 50 μg single-stranded salmon sperm DNA per ml, 50−100 μg *E. coli* tRNA per ml, 0.1% SDS, 0.2% PVP, 0.2% BSA and 0.2% Ficoll 400 at 65°C for 24−48 h. The final post-hybridization washes were in 0.3 x SSC at 65°C. Autoradiography was done at −70°C for 1−2 days using Tungsten intensifying screens.

Analysis of steady-state RNA

Trypanosome RNA was isolated with the 'hot-phenol' procedure described by Hyde *et al.* (1982). Contaminating DNA was removed by DNase I treatment as described by Van der Ploeg *et al.* (1982). For blotting analysis, the RNA was size-fractionated on a 2% agarose gel and transferred to DBM paper according to the protocol of Alwine *et al.* (1977). Hybridizations were carried out as described by De Lange *et al.* (1983). S1-analysis was done essentially as described by Berk and Sharp (1977) using restriction fragments labeled at the 3′ end with [α-^{32}P]dCTP and [α-^{32}P]dATP and T4 DNA polymerase (BRL) according to the protocol described in Maniatis *et al.* (1982).

Recombinant plasmids and DNA blotting

Plasmid pCL102 used in this study contains an *Mbo*I partial digestion fragment spanning ~10 mini-exon repeat units plus flanking sequences. This plasmid was isolated from a library described by De Lange *et al.* (1984b). The genomic clone pTb$\alpha\beta$T-1 containing α- and β-tubulin genes was a gift from Dr N.Agabian (Department of Biochemistry, University of Washington, Seattle, WA 98195) and is described by Thomashow *et al.* (1983). The plasmid TcV 117.5 contains a cDNA of mRNA for VSG 117a and has been described by Hoeijmakers *et al.* (1980). DNAs were isolated, restricted, size-fractionated and transferred to nitrocellulose filters using standard procedures (cf. Maniatis *et al.*, 1982).

Acknowledgements

We are grateful to André Bernards, Lex Van Der Ploeg, Dr Albert W.C.A. Cornelissen and Dr Patricia Johnson for helpful discussions and their critical reading of the manuscript, to Henk Hilkmann and Dr Peter F.Lens for their assistance in using the Stansted Cell Disrupter; to Dr N.Agabian (Department of Biochemistry, University of Washington, Seattle, WA 98195) for the gift of pTb$\alpha\beta$T-1. This work was supported in part by a grant from the Foundation for Fundamental Biological Research (BION), which is subsidized by The Netherlands Organization for the Advancement of Pure Research (ZWO).

References

Alwine,J.C., Kemp,D.J. and Stark,G.R. (1977) *Proc. Natl. Acad. Sci. USA*, **77**, 5350-5354.

Bachenheimer,S. and Darnell,J.E. (1975) *Proc. Natl. Acad. Sci. USA*, **72**, 4445-4449.

Berk,A.J. and Sharp,P.A. (1977) *Cell*, **12**, 721-732.

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) *Cell*, **27** 497-505.

Bernards,A., De Lange,T., Michels,P.A.M., Liu,A.Y.C. and Borst,P. (1984) *Cell*, **36**, 163-170.

Birchmeier,C., Folk,W. and Birnstiel,M.L. (1983) *Cell*, **35**, 433-440.

Bogenhagen,D.F. and Brown,D.D. (1981) *Cell*, **24**, 261-270.

Boothroyd,J.C. and Cross,G.A.M. (1982) *Gene*, **20**, 281-289.

Borst,P., Bernards,A., Van der Ploeg,L.H.T., Michels,P.A.M., Liu,A.Y.C., De Lange,T. and Kooter,J.M. (1983) *Eur. J. Biochem.*, **137**, 383-389.

Campbell,D.A., Van Bree,M.P. and Boothroyd,J.C. (1984) *Nucleic Acids Res.*, **12**, 2759-2774.

Cross,G.A.M. (1975) *Parasitology*, **71**, 393-417.

Crumpton,M.J. and Snary,D. (1974) in Ado,G.L. (ed.), *Contemporary Topics in Molecular Immunology*, **3**, Plenum Press, NY, pp. 27-54.

De Lange,T., Liu,A.Y.C., Van der Ploeg,L.H.T., Borst,P., Tromp,M.C. and Van Boom,J.H. (1983) *Cell*, **34**, 891-900.

De Lange,T., Michels,P.A.M., Veerman,H.J.G., Cornelissen,A.W.C.A. and Borst,P. (1984a) *Nucleic Acids Res.*, **12**, 3777-3790.

De Lange,T., Berkvens,T.M., Veerman,H.J.G., Frasch,A.C.C., Barry,J.D. and Borst,P. (1984b) *Nucleic Acids Res.*, **12**, 4431-4443.

Fairlamb,A.H., Weislogel,P.O., Hoeijmakers,J.H.J. and Borst,P. (1978) *J. Cell Biol.*, **76**, 293-309.

Gariglio,P., Bellard,M. and Chambon,P. (1981) *Nucleic Acids Res.*, **9**, 2589-2598.

Groudine,M., Peretz,M. and Weintraub,H. (1981) *Mol. Cell. Biol.*, **1**, 281-288.

Hoeijmakers,J.H.J., Borst,P., Van den Burg,J., Weissman,C. and Cross, G.A.M. (1980) *Gene*, **8**, 391-417.

Holmes,W.M., Platt,T. and Rosenburg,M. (1983) *Cell*, **32**, 1029-1032.

Hyde,J.E., Goman,M., Hall,R., Osland,A., Hope,I.A., Langsley,G., Zolg, J.W. and Scaiff,J.G. (1984) *Mol. Biochem. Parasitol.*, **10**, 269-285.

Klar,A.J.S., Strathern,J.N. and Hicks,J.B. (1981) *Cell*, **25**, 517-524.

Liu,A.Y.C., Van der Ploeg,L.H.T., Rijsewijk,F.A.M. and Borst,P. (1983) *J. Mol. Biol.*, **167**, 57-75.

Maniatis,T., Fritsch,E.F. and Sambrook,J., eds., (1982) *Molecular Cloning: A Laboratory Manual,* published by Cold Spring Harbor Laboratory Press, NY, pp. 117-121.
McKnight,G.S. and Palmiter,R.D. (1979) *J. Biol. Chem.,* **254**, 9050-9058.
Michels,P.A.M., Liu,A.Y.C., Bernards,A., Sloof,P., Van Der Bijl,M.M.W., Schinkel,A.H., Menke,H.H., Borst,P., Veeneman,G.H., Tromp,M.C. and Van Boom,J.H. (1983) *J. Mol. Biol.,* **166**, 537-556.
Nasmyth,K.A., Tatchell,K., Hall,B.D., Astell,C. and Smith,M. (1981) *Nature,* **289**, 244-250.
Nelson,R.G., Parsons,M., Barr,P.J., Stuart,K., Selkirk,M. and Agabian,N. (1983) *Cell,* **34**, 901-909.
Nelson,R.G., Parsons,M., Selkirk,M., Newport,G., Barr,P.J. and Agabian, N. (1984) *Nature,* **308**, 665-667.
Schibler,U., Hagenbüchle,O., Wellauer,P.K. and Pittet,A.C. (1983) *Cell,* **33**, 501-508.
Seebeck,T., Whittaker,P.A., Imboden,M.A., Hardman,N. and Braun,R. (1983) *Proc. Natl. Acad. Sci. USA,* **80**, 4634-4638.
Thomashow,L.S., Milhausen,M., Rutter,W.J. and Agabian,N. (1983) *Cell,* **32**, 35-43.
Van der Ploeg,L.H.T., Liu,A.Y.C., Michels,P.A.M., De Lange,T., Borst, P., Majumber,H.K., Weber,H., Veeneman,G.H. and Van Boom,H. (1982) *Nucleic Acids Res.,* **10**, 3591-3604.
Van der Ploeg,L.H.T. and Cornelissen,A.W.C.A. (1984) *Phil. Trans. R. Soc. Lond. B.,* in press.
Weber,J., Jelinek,W. and Darnell,J.E. (1977) *Cell,* **10**, 611-616.
Wright,B.M., Edwards,A.J. and Jones,V.E. (1974) *J. Immunol. Methods,* **4**, 281-296.

Received on 22 June 1984