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Transcription of telomere repeats in protozoa

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The telomerically located variant cell surface glycoprotein (VSG) gene expression sites of the protozoan parasite *Trypanosoma brucei* are transcribed by an unusual α-amanitin resistant RNA polymerase. We show that the telomere GGGTTA repeats located at the chromosome ends of *T.brucei* and the related protozoan *T.equiperdum* are also transcribed by α-amanitin resistant RNA polymerases. This transcription predominantly proceeds unidirectionally towards the end of the chromosome, in both bloodstream and insect form trypanosomes and results in the generation of heterogeneously sized steady state RNA. We postulate that telomere repeat transcription results from readthrough downstream of telomeric genes. Telomere repeat transcription was found in all seven protozoan species tested, but was α-amanitin resistant only in trypanosome species which exhibited antigenic variation. The data indicate that in some trypanosome species a subset of telomeres is transcribed by a different type of RNA polymerase.

Key words: α-amanitin/telomere transcription/*Trypanosoma brucei*/variant cell surface glycoprotein

Introduction

The protozoan parasite *Trypanosoma brucei*, causative agent of sleeping sickness, proliferates in the mammalian bloodstream and is transmitted between hosts by an insect vector, the tsetse fly. Insect form (procyclic) and bloodstream form trypanosomes differ in numerous biochemical characteristics. These include the presence in bloodstream form trypanosomes of a variant cell surface glycoprotein (VSG) coat, and the expression in insect forms of an abundant cell surface protein called the procyclic acidic repetitive protein (PARP or Procyclin; Roditi *et al.*, 1987; Mowatt and Clayton, 1987).

Trypanosoma brucei survives in the mammalian bloodstream because it can escape immuno-destruction by undergoing antigenic variation. New antigenic variants result from the periodic generation of a few parasites which express an antigenically distinct VSG coat, made possible by a switch to the expression of a single new VSG gene (Vickerman, 1969; Cross, 1975; for reviews see Borst, 1986; Borst and Greaves, 1987; Van der Ploeg, 1987). A peculiarity of the active VSG gene is that it is invariably located at one of several telomeric VSG gene expression sites. The transcription of the VSG gene expression site is resistant to the drug α-amanitin at concentrations up to 1 mg/ml (Kooter and Borst, 1984). This level of resistance is characteristic of transcription by RNA polymerase I (RNA pol I), which in other eukaryotes exclusively transcribes ribosomal RNA (rRNA) genes. All other protein coding genes identified thus far in trypanosomes and other eukaryotes are transcribed by the α-amanitin sensitive (5 μg/ml) RNA polymerase II (RNA pol II) (Evers *et al.*, 1989; Smith *et al.*, 1989). It has not been determined whether the α-amanitin resistant, polycistronic transcription of the VSG gene expression sites is mediated by RNA pol I, or alternatively by a modified RNA pol II (Evers *et al.*, 1989).

The significance of a telomeric location for transcribed VSG genes is unclear. Trypanosomes have >100 mini chromosomes of 50−150 kb, many of these with telomeric VSG genes (Van der Ploeg *et al.*, 1984a). At least 20 larger chromosomes exist (Van der Ploeg *et al.*, 1989). Presumably the ends of all trypanosome chromosomes share the repeat sequence GGGTTA at the tip of the telomere, flanked by different subtelomeric, more chromosome internally located repeats, which share AT rich sequences and GC rich repeats (M.D.Weiden and L.H.T.Van der Ploeg, unpublished). Telomerically located transcription units other than VSG gene expression sites have not yet been identified in *T.brucei*. However, many other examples of telomeric genes that are located within a few kb of the end of the chromosome exist; among these are the VSG genes of the related protozoan *Trypanosoma equiperdum* and an unrelated telomeric gene family in *Trypanosoma cruzi* (Peterson *et al.*, 1986). The α-amanitin sensitivity of transcription of most of these genes has not been investigated.

Knowing that the actively transcribed VSG gene expression sites of bloodstream form trypanosomes are located at telomeres, we used telomeric sequences as a 'tag' to search for the presence of α-amanitin resistant transcription units in other protozoa belonging to the order of the Kinetoplastida and in *Plasmodium falciparum*. The assumption in these experiments was that transcriptional readthrough occurs from telomerically located genes into the telomeric $(GGGTTA)_n$ repeats located downstream of telomeric genes (De Lange *et al.*, 1983; Van der Ploeg *et al.*, 1984b; Blackburn and Challoner, 1984).

We show that in all protozoan species tested telomere repeat transcription occurs. Transcription is mainly unidirectional, proceeding from a chromosome internal position towards the chromosome end. Only in trypanosome species which exhibit antigenic variation is this transcription resistant to α-amanitin.

Results

Transcription of the telomere $(GGGTTA)_n$ repeats in T.brucei

At a high post-hybridization stringency (65°C, 0.1 × SSC, 0.1% SDS), all GGGTTA repeats that hybridize in Southern blots are sensitive to the exonuclease *Bal*31 (Van der Ploeg

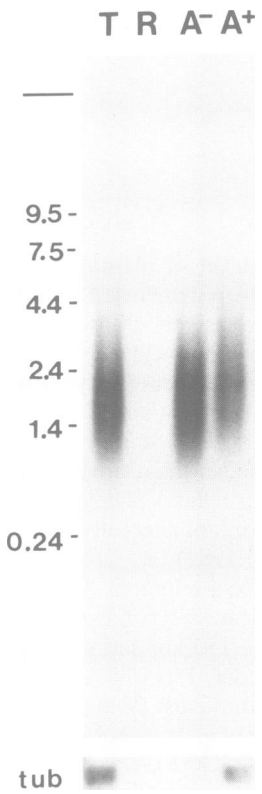

Fig. 1. Northern analysis of *T.brucei* RNA. RNA from insect form trypanosomes was size separated in an agarose gel, blotted onto a nitrocellulose filter and hybridized with probe TT6, which contains 300 nt of the telomere GGGTTA repeat. Post-hybridization washes were performed at 65°C and 0.1 × SSC. The same filter was rehybridized with an $\alpha\beta$ tubulin control probe (see insert at the bottom of the figure) in order to check the quality of the poly(A)$^+$ fractionation. The different lanes contain: T, 10 μg total RNA; R, 10 μg of RNA after treatment with RNase A; A$^-$, 9 μg poly(A)$^-$ RNA and A$^+$, 1 μg poly(A)$^+$ RNA. The sizes of an RNA size marker are indicated on the side.

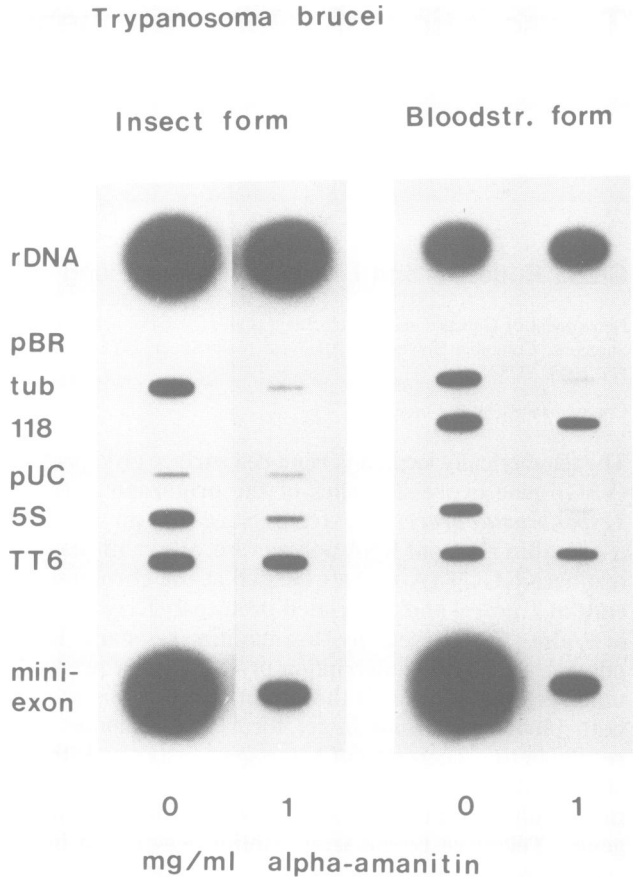

Fig. 2. Comparison of hybridization signals with nascent RNA from insect and bloodstream form trypanosomes. Nuclear run-on experiments were performed as described in Materials and methods. The slot blots contain 2 μg plasmid DNA:rDNA (an entire *T.brucei* ribosomal DNA repeat), pBR (plasmid pBR322), tub (an $\alpha\beta$ tubulin genomic clone), 118 (a VSG 118 cDNA), pUC (plasmid pUC18), 5S (a *T.brucei* 5S rRNA repeat), TT6 (the telomere clone pTT6 containing a 300 nt GGGTTA repeat) and a mini-exon repeat clone (see Materials and methods for a detailed description of the different clones). The filters were hybridized with nuclear run-on RNA made in the absence of α-amanitin (0) or in the presence of 1 mg/ml α-amanitin (1).

Table I. *T.brucei* procyclic nuclear run-on (% transcription)

RNA pol		UTP			GTP
Pol I	rDNA	53	71	75	56
Pol II	tub	7	5	9	6
	mini-exon	5	6	12	5
	CRAM	ND	11	ND	4
Pol III	5S	5	8	0	9
Pol ?	TT6	53	69	68	57

Quantitation of hybridization signals with nascent RNA labeled with UTP (three experiments with insect form nuclei) or with GTP (one experiment) as a radioactive marker were performed as described in Materials and methods. The relative rates of transcription for each clone after addition of α-amanitin are presented as percentages of the incubations without α-amanitin. Abbreviations are as in Figures 2 and 3. CRAM is an insect and bloodstream form expressed pol II transcribed control gene. ND, not determined.

et al., 1984b). On the basis of this criterion, it has previously been determined that the telomere $(GGGTTA)_n$ repeats are exclusively located at chromosome ends (Van der Ploeg *et al.*, 1984b; Blackburn and Challoner, 1984).

In a Northern blot of size-separated total insect form trypanosome RNA, a smear of hybridization between 800 and 4000 nt could be detected when the telomere repeat $(GGGTTA)_n$ was used as a probe (Figure 1, lane T). The same result was obtained with bloodstream form RNA (results not shown). This hybridization signal was sensitive to pre-treatment of the RNA with the ribonuclease RNase A (lane R) and therefore represented heterogeneously sized transcripts and not DNA which might have contaminated our RNA preparations. A proportion of these transcripts could be retained by oligo(dT) fractionation (Figure 1, lane A$^+$). An almost equal hybridization signal is, however, detected in the poly(A)$^-$ flow through fraction (Figure 1, lane A$^-$) while hybridization of the same Northern filter with a control $\alpha\beta$ tubulin gene probe (Figure 1, panel tub) showed that the poly(A)$^-$ fraction had been depleted of the poly(A)$^+$ mRNAs. We concluded that the RNA which hybridizes with the telomere repeats constitutes a heterogeneous population (Figure 1, panel tub).

We next determined whether transcription of the telomere repeats is resistant to α-amanitin. Finding α-amanitin resistant telomere transcription in insect form trypanosomes would provide evidence for additional α-amanitin resistant non-VSG gene transcription units. We did not expect to find readthrough into the telomeric repeats from the tandem

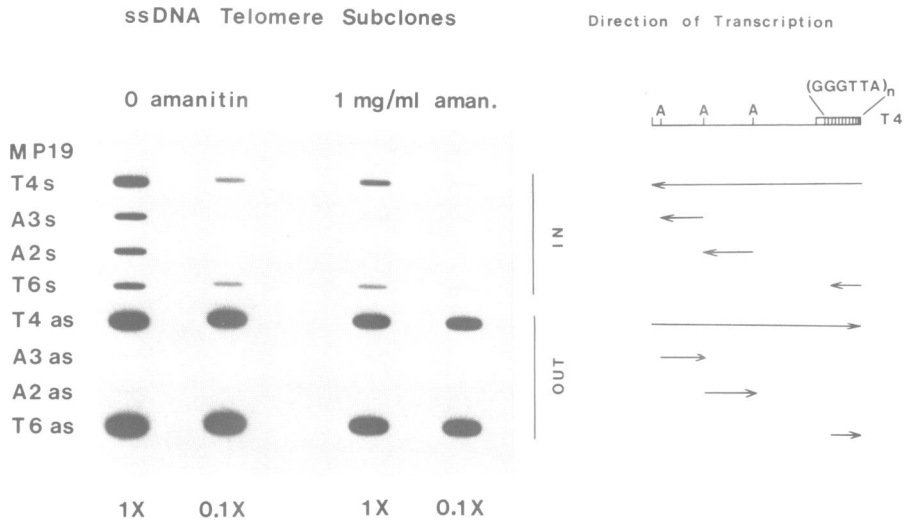

Fig. 3. Directionality of telomere transcription. Telomere clone pT4 (indicated in the physical map on the right hand side) was digested with the restriction enzyme *Alu*I (A) and subcloned into the M13 phage vector mp18. Single strand phage DNA of the different subclones of clone pT4 was assayed for relative rates of transcription. The origin of the single stranded M13 mp18 clones is schematically indicated on the right hand side together with the direction of transcription that can be identified by hybridization to the sense (s) DNA strand and anti-sense (as) DNA strands. Two different panels are shown which were washed at different post-hybridization stringencies of 65°C 1 × SSC and 0.1 × SSC respectively. On the right hand side of the figure, 'in' refers to transcription proceeding towards a chromosome internal position; 'out' refers to transcription proceeding from a chromosome internal position towards the chromosome end.

arrays of α-amanitin resistantly transcribed rRNA genes, as they had previously been determined to consist of discrete transcription units flanked by non-transcribed spacers (White *et al.*, 1986). We performed nuclear run-on assays by generating ^{32}P-labeled nascent RNA, which was hybridized to clones of different trypanosome genes. The nuclear run-on assays were performed without α-amanitin or with 1 mg α-amanitin/ml [Figure 2, panels labeled 0 (0) and 1 (1) mg/ml α-amanitin respectively]. As expected, the hybridization to the rRNA genes and the bloodstream form specifically expressed VSG 118 gene is minimally affected by the addition of α-amanitin. In contrast, RNA pol II transcription of αβ tubulin, the mini-exon donor RNA (medRNA) genes and the RNA pol III transcription of 5S rRNA genes is severely inhibited (Figure 2; see Table I for quantitation). The transcription of the telomere clone TT6, containing 300 bp of the $(GGGTTA)_n$ repeats is minimally inhibited by the drug, as is the case for rRNA genes and the VSG 118 gene. (Table I provides values of the quantitation). The use of [^{32}P]GTP instead of [^{32}P]UTP as a radioactive tracer in the nuclear run-on reaction did not affect these results.

A comparison of nuclear run-on transcription in insect form (Figure 2, left hand panel) and bloodstream form trypanosomes (Figure 2, right hand panel) shows that α-amanitin resistant transcription of the telomeric $(GGGTTA)_n$ repeat is present in both life-cycle stages. Since the insect form of the parasite does not transcribe its VSG gene expression sites, the α-amanitin resistant GGGTTA telomere transcription cannot be derived from readthrough from telomeric VSG genes into the telomeric repeats. Rather, the transcription of $(GGGTTA)_n$ repeats in the insect forms must originate from transcription unit(s) that are regulated independently of the VSG gene expression sites.

Directionality of transcription

Telomere repeat transcription occurring from readthrough at chromosome ends should occur unidirectionally, proceeding from a chromosome internal position towards the chromosome end. Transcription of the other strand, proceeding towards a chromosome internal position would indicate non-specific transcription initiation at free chromosome ends. In order to determine the direction of transcription, nascent RNA was hybridized to different strands of the telomere fragment cloned into the ssDNA phage M13. In Figure 3 the different strands of telomere clone pT4 are defined as sense (s) and anti-sense (as); the sense DNA strand proceeds towards the chromosome end and the anti-sense strand towards a chromosome internal position. In Figure 3 we show that the majority of α-amanitin resistant transcripts hybridize to the anti-sense strand of the telomere clones. Transcription thus proceeds from a chromosome internal position towards the chromosome end (as schematically indicated by the arrows below the physical map of the telomere clones in Figure 3). This is illustrated by the large difference in the level of hybridization with clones T4as and T6as versus T4s and T6s at a post-hybridization washing stringency of 0.1 × SSC at 65°C.

The AT-rich subtelomere regions of clone pT4 (clones A3 and A2) do not appear to be transcribed by α-amanitin resistant RNA polymerases when the filters are washed at a high post-hybridization stringency (note the signals of clones A3s, A2s, A3as and A2as at 0.1 × SSC). The hybridization signal at a more relaxed stringency (1 × SSC) in the presence of α-amanitin is most probably due to cross-hybridization with the other subtelomeric repeats. The lack of hybridization to subtelomeric repeats at a high stringency of hybridization presumably reflects the fact that our clone pT4 is not representative of the subtelomeric repeat sequences of the transcribed telomeres.

In contrast to the overall unidirectional character of GGGTTA transcription, the transcription of subtelomere repeats proceeds towards a chromosome internal position as well (see Figure 3, note signals at A3s and A2s). This transcription can only be detected at a relaxed stringency of hybridization (1 × SSC, 65°C) and occurs mainly by

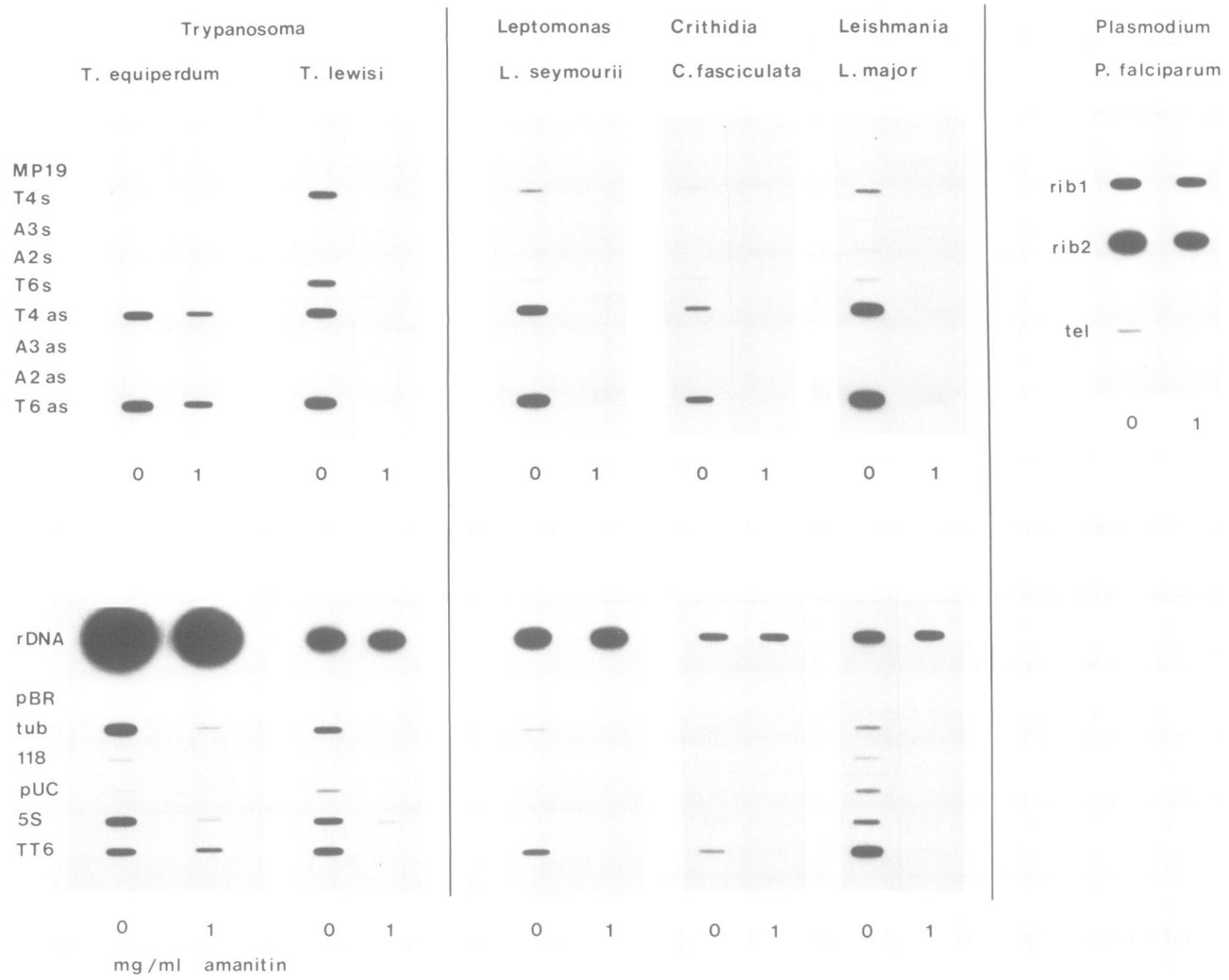

Fig. 4. Comparison of telomere transcription in different protozoa. The top panel shows telomere transcription and the bottom panel transcription of the control genes in the same nuclear run-on experiment. Abbreviations are as indicated in Figures 2 and 3. We tested telomere transcription in the Salivarian species: *T.equiperdum* (belonging to the same subgenus as *T.brucei*); and the Stercorarian species: *T.lewisi* (belonging to a different subgenus from *T.brucei* but classified in the same genus *Trypanosoma*); and members of different genera: *L.seymourii, C.fasciculata* and *L.major*. We hybridized the nascent RNA from these various kinetoplastids with the conserved DNA clones obtained from *T.brucei*. Post-hybridization washes were performed at a stringency of 0.3 × SSC and 65°C to favor hybridization of the related but not identical control, rRNA, 5S rRNA, $\alpha\beta$ tubulin and medRNA genes. Variation in the intensity of the signal at the control genes results from the variable extent of cross-hybridization of the *T.brucei* genes with nascent RNA from each species. In addition, we tested *P.falciparum*, belonging to a different subphylum. Post-hybridization washes with *P.falciparum* ^{32}P-labeled nascent RNA were performed at 65°C and 0.1 × SSC. rib1 and rib2 are *P.falciparum* rRNA gene clones. 'tel' is a *P.falciparum* telomere clone. 0 and 1 refer to nuclear run-on RNA made without α-amanitin (0) or with 1 mg α-amanitin/ml (1).

α-amanitin sensitive RNA polymerases. Since some subtelomere repeat sequences are resistant to the exonuclease *Bal*31 (Van der Ploeg *et al.*, 1984b) we assume that this transcription is partially derived from diverged subtelomeric repeat sequences located in other non-telomeric transcription units.

The overall unidirectional α-amanitin resistant GGGTTA telomere transcription shows that transcription initiation at free chromosome ends does not occur. A plausible explanation for the transcription is that it results from readthrough downstream of telomerically located genes.

Telomere transcription in other protozoa

The predominantly unidirectional telomere transcription in *T.brucei* led us to determine whether α-amanitin resistant transcription of telomeres is a common feature of species belonging to the order of the Kinetoplastida or is restricted to those kinetoplastid species that exhibit antigenic variation.

Table II. Species comparison nuclear run-on (% transcription)

RNA pol		*T.equiperdum*	*T.lewisi*	*P.falciparum*
Pol I	rDNA	41	105	rib1 69
				rib2 77
Pol II	tub	8	3	
	mini-exon	11	ND	
Pol III	5S	18	28	
Pol ?	TT6	40	5	tel 0

See footnote of Table I for explanation.

We used the *T.brucei* telomere repeats to study transcription in other protozoan species of the order Kinetoplastida, since Blackburn and Challoner (1984) had previously shown that the telomere repeat of *T.brucei* was present in a highly conserved or identical form in a number of other kinetoplastids, including *Leptomonas, Crithidia* and *Leishmania*.

We hybridized nascent RNA from various kinetoplastids with conserved DNA clones obtained from *T.brucei* (Figure 4). We tested telomere transcription in the species *Trypanosoma equiperdum* (bloodstream form), *Trypanosoma lewisi* (bloodstream form), *Leptomonas seymourii* (insect form), *Crithidia fasciculata* (insect form) and *Leishmania major* (insect form). In all these species the telomere repeats were transcribed. Transcription proceeded mainly, as in *T.brucei*, unidirectionally towards the chromosome end, except in *T.lewisi*, where at least 40% of the transcription proceeded on the other strand of the telomere clone (Figure 4, compare the signals at T6as and T6s). *Trypanosoma lewisi* appeared to be the only protozoan with transcription of both strands. One explanation may be that the telomere repeats in *T.lewisi* are not exclusively located at chromosome ends, allowing the repeat to be transcribed bidirectionally. Only in *T.equiperdum*, a Salivarian species closely related to *T.brucei*, is the telomere transcription resistant to the drug α-amanitin (Figure 4, far left panel; see Table II for quantitation). This is of interest as *T.equiperdum* also transcribes its telomerically located VSG genes in an α-amanitin resistant manner (H.Eisen and L.H.T.Van der Ploeg, unpublished). In contrast, in *Leptomonas, Crithidia, Leishmania* and in *T.lewisi* transcription of the telomeres was completely inhibited by the addition of α-amanitin (1 mg/ml). Finally, we tested a phylogenetically distant protozoan, *Plasmodium falciparum*, using cloned DNA sequences derived from *Plasmodium*. The direction of transcription was not determined. As can be seen in Figure 3 the transcription of two ribosomal DNA clones (rib1 and rib2) was hardly inhibited by the addition of α-amanitin, as is to be expected for transcription by RNA pol I (see Table II for quantitation). Telomere transcription occurred, though it was almost completely inhibited by the addition of α-amanitin.

Unidirectional transcription of telomeric repeats thus occurs in a variety of different eukaryotes, though with different sensitivities to α-amanitin. This transcription may result from readthrough downstream of telomerically located genes. Telomere transcription is resistant to α-amanitin in the subclass of Salivarian trypanosomes, which exhibit antigenic variation.

Discussion

Unidirectional, α-amanitin resistant transcription of the telomere GGGTTA repeats occurred in both insect and in bloodstream form *T.brucei* and in the related protozoan *T. equiperdum*. In contrast, in all other kinetoplastid species tested, the predominantly unidirectional transcription of telomere repeats was sensitive to α-amanitin. The data thus indicate that a subset of telomeres is transcribed by an α-amanitin resistant RNA polymerase exclusively in trypanosome species which exhibit antigenic variation. We cannot exclude that the α-amanitin resistant transcription of telomeres in *T.brucei* and in *T.equiperdum* results from readthrough downstream of a telomerically located truncated rRNA gene lacking the usual terminator. However, we consider it unlikely that a similar truncation event would occur independently in two species.

We postulate that the transcription results from readthrough downstream of telomeric genes. Telomere transcription could also result from transcription initiation within the telomere itself and have a different role, for instance, in telomere replication. The latter possibility does not, however, explain why telomeres appear to be transcribed by different RNA polymerases in various kinetoplastid species. Given the telomeric location of VSG gene expression sites and the unidirectional α-amanitin resistant telomere transcription, it is tempting to speculate that transcription results from readthrough and that the unusual RNA polymerase transcribes telomeric genes in insect form trypanosomes as well.

Since GGGTTA transcription could be detected in all the protozoan species tested, the generation of GGGTTA-containing RNA appears to be significant. In higher eukaryotes, as in humans, RNA containing the GGGTTA telomere repeat sequences has also been detected (T. De Lange, personal communication). Telomere repeat transcription therefore appears to be common to many different eukaryotes.

The fact that α-amanitin resistant transcription can be found in insect form *T.brucei* indicates that the use of the α-amanitin resistant RNA polymerase is not unique to the bloodstream form or to the process of antigenic variation. This is further substantiated by our finding that the PARP genes of insect form trypanosomes are also transcribed by an α-amanitin resistant RNA polymerase (G.Rudenko, D.Bishop, K.Gottesdiener and L.H.T.Van der Ploeg, submitted). α-Amanitin resistant telomere transcription was only found in the species *T.brucei* and *T.equiperdum*, providing indirect evidence that the unusual RNA polymerase may be restricted to Salivarian trypanosomes exhibiting antigenic variation.

Materials and methods

Protozoan strains and culture conditions

Trypanosoma brucei strain 427 bloodstream form variant 118d were used for the experiments (Cross, 1975; Michels *et al.*, 1983). The *T.brucei* 427 procyclic trypanosomes were initially obtained from Dr R.Brun and have been maintained in SDM79 medium at 24°C as described by Brun and Schonenberger (1979). The *T.equiperdum* strain used was Botat 1/78 (Longacre *et al.*, 1983). *Trypanosoma lewisi* was grown in rats for 7 days to peak parasitemia as described (Giannini and D'Alesandro, 1984). *Leptomonas seymourii* (strain ATCC 30220) was cultured according to Bone and Steinert (1956). *Crithidia fasciculata* (wild-type, originally derived from Dr G.Hill) was cultured according to Le Trant *et al.* (1983). *Leishmania major* (strain WR300; Giannini *et al.*, 1986) was cultured in Schneider's medium (Gibco) with the addition of 10% fetal calf serum. *Plasmodium falciparum*, isolate NF-54 of the Dutch airport strain, was cultured essentially as described by Ponnudurai *et al.* (1982) with the exception that antibiotics were omitted from the medium. The medium was changed manually every day and cultures were maintained in 75 mm^2 tissue culture flasks.

Description of plasmid constructs

The *T.brucei* telomere clone pT4 is as described previously by Van der Ploeg *et al.* (1984b). The TT6 *T.brucei* telomere subclone is the 330 bp pT6 insert, containing only $(GGGTTA)_n$ repeats, as described in Van der Ploeg *et al.* (1984b) cloned into the plasmid T3/T7lac (Boehringer Mannheim). The *T.brucei* subtelomere clones A3 and A2 are the 440 bp and 287 bp *Alu*I restriction enzyme fragments of plasmid pT4 cloned into the phage vector M13 mp18. The *T.brucei* ribosomal clone pR4 is as described in Kooter and Borst (1984), the 5S clone is as described in Lenardo *et al.* (1985), the mini-exon repeat clone pCL103 is described in Laird *et al.* (1985), the VSG 118 cDNA clone TcV118-2 in Bernards *et al.* (1981), and the $\alpha\beta$ tubulin genomic clone pTb $\alpha\beta$ T-1 in Thomashow *et al.* (1983). The *P.falciparum* ribosomal clones rib1 and rib2 are described in Langsley *et al.* (1983) and the telomere clone in Ponzi *et al.* (1985). The CRAM clone is a 3350 bp cDNA encoding a *T.brucei* receptor-like protein cloned into the plasmid pUC18 (M.Lee, B.Bihain, R.J.Decklebaum and L.H.T.Van der Ploeg, submitted).

Northern blot analysis

Total trypanosome RNA was isolated by lysis of cells in guanidium isothiocyanate followed by RNA isolation by centrifugation over a cesium chloride cushion (MacDonald *et al.*, 1987). Oligo(dT) selection of poly(A)$^+$ RNA and size separation of RNA on 1% formaldehyde agarose gels was performed essentially as described by Maniatis *et al.* (1982). The RNA was transferred to Hybond-N membranes (Amersham), and the filters incubated at 42°C in a hybridization mixture containing 50% formamide, 100 μg/ml tRNA, according to the manufacturer's specifications. DNA probes were labeled by random priming (Feinberg and Vogelstein, 1983). After the hybridization the filters were washed at a post-hybridization stringency of 0.1 × SSC at 65°C.

Run-on transcription analysis

Slot blots were made by transferring 2 μg of plasmid DNA or 3 μg of single stranded (ss) M13 phage DNA onto nitrocellulose filters (pore diameter 0.45 μm) using the Schleicher & Schuell Minifold II Slot-blotter according to Kafatos *et al.* (1979). Essentially, the DNA was denatured in 0.3 M NaOH at 65°C for 30 min, cooled on ice, and neutralized with an equal volume of 2 M ammonium acetate. After binding of DNA the filters were washed with 4 × SSC and baked at 80°C for 2 h.

Nuclei were isolated from trypanosomes using a Stansted cell disrupter according to the method of Kooter and Borst (1984) and Kooter *et al.* (1984). Briefly, bloodstream form trypanosomes were grown to a high parasitemia in rats, harvested by cardiac puncture, and immediately passed through the cell disrupter. The disrupted cells were collected in 2 vol of cold Pipes buffer and pelleted at 4°C by centrifugation for 10 min at 3000 r.p.m. in a Sorvall table top centrifuge. The pellet was washed several times by centrifugation followed by suspension in Pipes buffer. Nuclei were stored at −140°C in 100 μl aliquots in nuclei storage buffer at a concentration of $5-10 \times 10^9$ nuclei/ml. Essentially the same procedures were used for the generation of nuclei preparations of insect form trypanosomes and other protozoan described, which were grown to low log densities in culture medium. *Plasmodium falciparum* was grown to an estimated concentration of 5×10^7 parasites/ml infected blood (15% parasitemia; total vol of the cultures was 500 ml). The cells were sedimented and nuclei prepared as described by passing through the cell disrupter.

Nascent RNA elongation was performed according to Kooter *et al.* (1987). Incubations were performed for 5 min at 37°C for all nuclei preparations prepared from bloodstream form trypanosomes. Nuclei from insect form trypanosomes and protozoa cultured at room temperature were incubated at 22°C. Nuclear run-on reactions which were performed in the presence of α-amanitin (Serva Fine Biochem. Inc.) occurred with nuclei that had been pre-incubated for 15 min on ice in storage buffer in the presence of α-amanitin (α-amanitin concentrations are as indicated for each of the specific experiments). Nuclei were next pelleted and resuspended in the incubation buffer in the presence of α-amanitin. The incubation buffer was as described in Kooter *et al.* (1987) except 25% glycerol was used. 200 μCi of [α-^{32}P]UTP or [α-^{32}P]GTP (3000 Ci/mmol) was used per reaction.

Nascent RNA was hybridized to the nitrocellulose filters for 48 h at 42°C in a hybridization buffer containing 50% formamide, 5 × SSC, 5 × Denhardt's, 0.1% SDS, 1 mM sodium pyrophosphate, 20 mM sodium phosphate, pH 6.8, and 100 μg/ml tRNA. Post-hybridization washes of the filters were performed for 30 min each with solutions of 3 × SSC, 1 × SSC and 0.3 or 0.1 × SSC and 0.1% SDS at 65°C.

Quantitation of the nuclear run-on signal was performed by dissolving the nitrocellulose filter strips in Econofluor (Dupont) and by counting in a liquid scintillation counter. The background hybridization to the plasmid control was subtracted.

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