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Trypanosoma brucei: constitutive activity of the VSG and procyclin gene promoters

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The variant surface glycoprotein (VSG) and procyclin are the major surface proteins of the bloodstream and procyclic stages, respectively, of *Trypanosoma brucei*. The promoter regions of the VSG and procyclin gene transcription units could be mapped thanks to the specific enrichment of initial transcripts that occurs following UV irradiation. Whereas the VSG gene is 45 kb distant from its promoter, procyclin genes are located immediately downstream. We show, by run-on assays on isolated nuclei and by cDNA analysis, that transcription occurs from both promoters in bloodstream as well as in procyclic forms. It is inferred that the control of the stage-specific expression of VSG and procyclin genes is not effected at the level of transcription initiation, but most probably by interfering with the elongation and stability of the specific transcripts.

Key words: antigen genes/parasite differentiation/RNA decay/*Trypanosoma brucei*/transcription promoters

Introduction

During their life-cycle, African trypanosomes differentiate into several adaptive forms, the most prominent of which are the bloodstream form in the mammalian host, and the procyclic form in the midgut of the tsetse fly vector. Each of these forms is characterized by the presence of a specific major surface protein, the variant surface glycoprotein (VSG) in the bloodstream form (reviewed in Vickerman, 1985; Vickerman *et al.*, 1988), and procyclin in the procyclic form (Richardson *et al.*, 1988; Roditi *et al.*, 1989). Although trypanosomes possess a large collection of different VSG genes, usually only one of these genes is transcribed at a time (reviewed in Pays and Steinert, 1988). This gene is located in a telomeric expression site, which also contains several other genes named ESAGs, for expression site-associated genes, which are co-ordinately expressed with the VSG gene (Cully *et al.*, 1985; Pays *et al.*, 1989a). Procyclin genes are present at two or three distinguishable loci, in tandem arrays of two or three copies depending on the strain (Roditi *et al.*, 1987; Mowatt and Clayton, 1987, 1988; Koenig *et al.*, 1989). In contrast to VSG genes, procyclin genes do not seem to be telomeric (Roditi *et al.*, 1987) and several copies appear to be expressed simultaneously (Mowatt and Clayton, 1988). VSG or procyclin mRNAs are only detected in those stages of the life-cycle which express the corresponding protein, suggesting transcriptional control of gene expression (Hoeijmakers *et al.*, 1980; Pays *et al.*, 1980; Roditi *et al.*, 1987; Mowatt and Clayton, 1987).

To date, all attempts to characterize transcriptional promoters in trypanosomes and to analyse their activity have failed, for three main reasons: first, transcription seems to occur continuously over very large regions, spanning several genes (Imboden *et al.*, 1987; Johnson *et al.*, 1987; Alexandre *et al.*, 1988; Muhich and Boothroyd, 1988; Tschudi and Ullu, 1988; Coquelet *et al.*, 1989). Second, the maturation of transcripts involves *trans*-splicing events which obscure the mapping of primary RNA start sites (Murphy *et al.*, 1986; Sutton and Boothroyd, 1986). Third, no reliable assay of promoter activity has been developed, although DNA transfection in trypanosomatids appears feasible (Gibson *et al.*, 1987; Bellofatto and Cross, 1989; Laban *et al.*, 1990).

We have taken advantage of the fact that UV irradiation causes an important and specific accumulation of transcripts from the beginning of transcription units to characterize the promoter regions of the VSG and procyclin genes. Using this approach, we have observed that these two promoters are active in both bloodstream and procyclic forms of the parasite. The stage-specific expression of these two genes thus appears to be controlled after the initiation of transcription, and notably involves changes in transcript elongation and stability at different temperatures.

Results

VSG and procyclin gene transcription: evidence for temperature and stage dependent control of elongation, but not of initiation

The transcription of two stage-regulated units, namely the AnTat 1.3A VSG gene expression site (VSG ES) and the procyclic genes was analysed by run-on assays. The actin genes which are constitutively expressed (Ben Amar *et al.*, 1988), were used as a control.

We have shown previously (Pays *et al.*, 1989a) that transcription of the AnTat 1.3A VSG ES starts ~45 kb upstream from the VSG gene (*Sph*I region), since hybridization with run-on transcripts cannot be detected over several kilobases upstream from that region. As can be seen in Figure 1 (panel 2), all the genes in this 45 kb unit are transcribed at 37°C in bloodstream forms. The only apparent exception (the small intergenic fragment 14) has already been commented upon (Pays *et al.*, 1989a). Its lack of hybridization is possibly linked to a high thymidine content (data not shown).

At low temperature (20–27°C), under conditions which induce the differentiation of bloodstream forms to procyclic forms *in vitro*, transcription of the VSG gene is rapidly and selectively switched off (Kooter and Borst, 1984; Ehlers

et al., 1987; Alexandre *et al.*, 1988). Accordingly, we see that, at 20°C, the VSG gene appears completely silent (Figure 1, panel 3, arrowhead). It is clear, however, that most of the VSG ES is still transcribed, although at a reduced rate. As also noted by Kooter *et al.* (1987), the cold shock predominantly inhibits transcription of the 3′ region, which contains the VSG gene, but does not affect transcription at the beginning of the VSG ES (*Sph*I region, fragments 1–6). Apparent exceptions to this rule (low level of inhibition seen for some distal fragments, such as 8, 10, 11 and 17) are due to the existence, elsewhere in the genome, of genes which are related to ESAGs 2, 4 and 5, but which are independently regulated (Pays *et al.*, 1989a; Alexandre *et al.*, 1990).

In procyclic forms, transcription of the VSG ES could not be detected except at its 5′ extremity (Figure 1, panel 6). We compared the relative extent of transcription of this region by hybridization with run-on transcripts from the same number of nuclei from either bloodstream or procyclic forms, taking the actin genes as an internal control (Figures 1 and 2 for independent experiments; similar experiments not shown). In procyclic nuclei, this level is at least 50% of that measured in bloodstream nuclei (fragment 1, panels 5 and 6 in Figure 1; fragment 3 in Figure 2). Note that, in Figure 1, the transcription of the VSG ES was carried out in the presence of α-amanitin, in order to avoid spurious hybridization due to transcripts from sequences related to ESAGs, but located elsewhere (see above). While transcription of the ES is highly resistant to α-amanitin (Kooter *et al.*, 1987; Alexandre *et al.*, 1988; Pays *et al.*, 1989a; compare panels 5 and 2 in Figure 1), ESAG-related transcription outside of the ES is sensitive to the drug, except for a copy of ESAG 2 which seems to be transcribed specifically in procyclic forms (band 17 in panel 6 of Figure 1; E.Pays, unpublished data).

These data show that the beginning of the VSG ES can still be transcribed under conditions where the VSG gene is silenced. They confirm and extend previous observations (Pays *et al.*, 1989b), that the temperature and stage-specific regulation of the VSG ES occurs at the level of transcript elongation, not initiation.

Procyclin is specifically expressed in procyclic forms and, as illustrated in Figure 1 panel 11, specific transcripts are abundant in nuclei of these forms. However, a significant

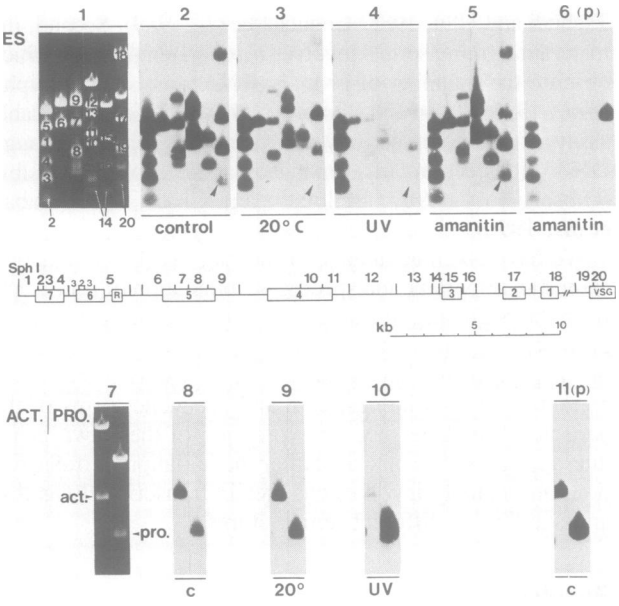

Fig. 1. Transcription of the VSG ES and of the procyclin and actin genes in nuclei isolated from bloodstream and procyclic trypanosomes. Run-on transcription assays were performed on nuclei from either AnTat 1.3A bloodstream forms (panels 2–5 and 8–10) or from procyclic culture forms derived from AnTat 1.1B [panels 6 and 11: (p)]. Unless specified otherwise, bloodstream trypanosomes were incubated *in vitro* at 37°C for 30 min prior to isolation of the nuclei (panels 2, 4, 5, 8 and 10). In panels marked '20°C' trypanosomes were incubated at this temperature in the presence of 3 mM pyruvate and 3 mM citrate, conditions which trigger differentiation into procyclic forms. In panels marked 'UV' trypanosomes were irradiated at 254 nm for 1 min with 1 J/s/m^2, then incubated at 37°C. Where indicated ('amanitin', or 'a'), labelling was performed in the presence of 1 mg/ml α-amanitin. The ^{32}P-labelled transcripts were hybridized to restriction digests of clones from either the VSG ES (top panels, ES) or the actin and procyclin genes (bottom panels). Panels 1 and 7 show the ethidium bromide stained digests, while the other panels show the hybridization to the Southern blots. The interpretation of the digests of the VSG ES is shown in the map, where the boxed numbers are for ESAGs 7 to 1. The DNA fragments from the ES have been numbered from 5′ to 3′ (unnumbered fragments are from the plasmid). Fragments 7 and 9 carry a portion of the vector DNA, hence their size is larger than shown on the map. Arrowheads point to the VSG gene bearing fragment. The plasmids containing sequences from the VSG ES, as well as the actin gene (act.) and procyclin cDNA (pro.), have been described previously (Pays *et al.*, 1989a; Ben Amar *et al.*, 1988; Roditi *et al.*, 1987).

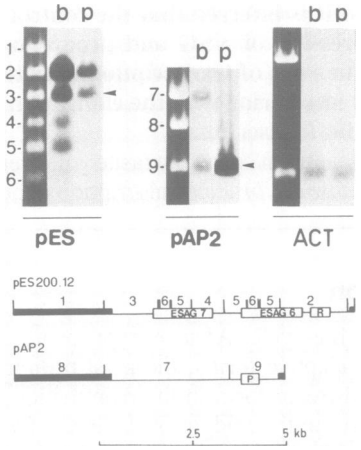

Fig. 2. Transcription at the beginning of the VSG and procyclin gene transcription units in bloodstream and procyclic forms. Run-on transcription assays have been conducted on the same number of nuclei from bloodstream (b) and procyclic (p) forms (final DNA concentration: 500 μg/ml in both cases). The ^{32}P-labelled transcripts were hybridized with stoichiometric amounts of restriction digests from different recombinant plasmids: pES200.12 is specific to the beginning of the VSG expression site, and carries ESAGs 7 and 6 together with a RIME retroposon (R) (Pays *et al.*, 1989a), while pAP2 contains a 4.7 kb *Pvu*II fragment from the procyclin A locus of IlTat 1.21, consisting of 3.7 kb of 5′ flanking sequence, the first of three tandem procyclin genes (P) and 0.3 kb of the 3′ flanking sequence (Koenig *et al.*, 1989). Both plasmids were digested by *Pvu*II + *Ava*III, and the interpretation of the digests is shown in the maps, with black boxes for sequences from the plasmid vectors and open boxes for the genes and RIME. The plasmid in the third panel contains a copy of the *T.brucei* actin gene, in a 1.5 kb *Sal*I fragment (bottom fragment). Arrowheads designate the promoter-proximal fragments. In the first panel, hybridization to fragment 2 is predominantly due to RIME transcripts, which are less abundant in procyclic forms (Murphy *et al.*, 1987).

level of procyclin gene trancription was also demonstrated in bloodstream form nuclei (Figure 1, panel 8), although steady state procyclin mRNA was absent (Roditi *et al.*, 1987, 1989) or at a very low level in these forms (Pays *et al.*, 1989b). We have calculated, from different run-on experiments conducted at either 30°C or 37°C, that the hybridization of transcripts from bloodstream nuclei to the procyclin genes was at least 16% of that of transcripts from procyclic nuclei (Figures 1 and 2). Thus, as for the VSG, the expression of this stage-specific surface protein appears not to be regulated at the level of transcription initiation. Furthermore, the rate of transcription of the procyclin genes is also influenced by a temperature shift, but, as would be expected, in an inverse manner to the effect on the VSG gene. While, in the bloodstream form, the level of transcription of the control actin genes is unaffected at 20°C, that of procyclin genes is increased at this temperature: after 30 min, the increase is ~2-fold in run-on assays (Figure 1, panel 9). Why the procyclin genes are transcribed in the bloodstream form, whereas no VSG-specific transcripts are detected in procyclics, can be explained by their proximity to the transcription promoter, as shown in the next section.

Crude mapping of VSG and procyclin promoters by UV inactivation of transcription

UV irradiation is known to block RNA elongation through generation of pyrimidine dimers in the DNA (Sauerbier and Herculez, 1978). The relative sensitivity of transcription towards UV is proportional to the distance from the transcription start, and may be used to map the promoter. We examined the effects of UV irradiation of trypanosomes on the subsequent transcription in isolated nuclei. After irradiation at 254 nm with 60 J/m^2, overall transcription was inhibited by ~50% (Pays *et al.*, 1989a). Under these conditions, the 5′ region of the VSG ES (encompassing ESAGs 6 and 7) and the procyclin genes were still tran-

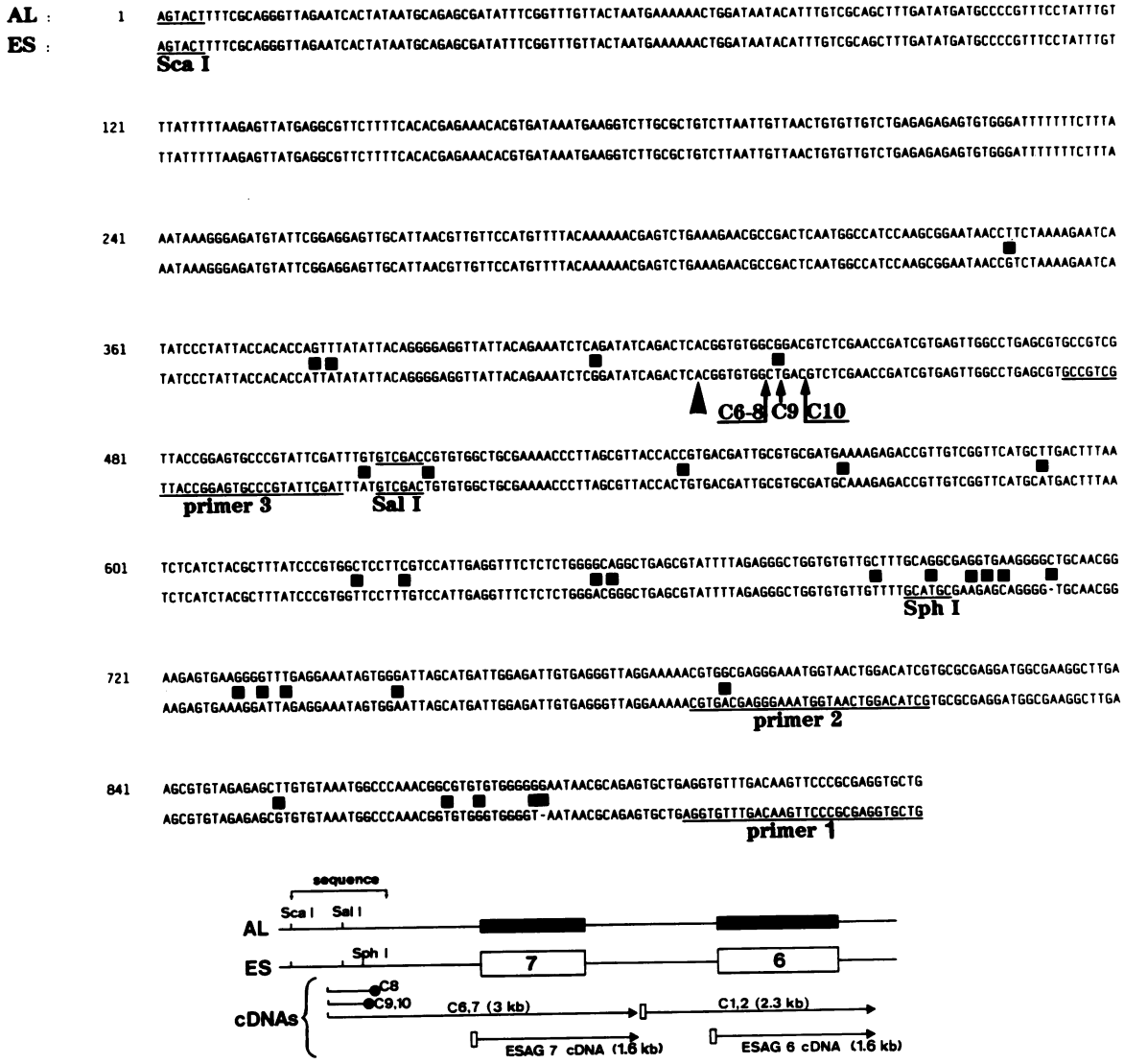

Fig. 3. Nucleotide sequence and map of the VSG promoter region, showing the cDNA clones of initial transcripts. The sequence of the beginning of the VSG ES is aligned with that of its putative allele (AL, see Pays *et al.*, 1989a). The primer sequences used in this work are complementary to the underlined segments. Black squares indicate the differences between 'AL' and 'ES'. The 5′ limits of significant cDNAs (C6–C10, shown below the maps) are indicated by arrows. The arrowhead refers to the discrete stop observed in primer extensions using primer 3 (see Figure 4). The extent of the nucleotide sequence is presented above the maps. Open and black boxes are for ESAG 7 and 6 and their equivalents in 'AL'. In the schematic representations of the cDNAs, black dots are for the primers, while open rectangles and arrowheads are for the mini-exon and poly(A) tail, respectively. The GenBank accession numbers for the AL and ES sequences are respectively M27963 and M27964.

scribed at high levels, while, in contrast, transcription of the rest of the VSG ES and actin genes was strongly inhibited (Figure 1, panels 4 and 10). These results indicate that procyclin genes, in contrast to the VSG and actin genes, are situated close to a promoter.

Two comments should be made at this point. Firstly, considering panel 4, one should note that no simple relation may be expected between relative decrease of hybridization following UV and distance from promoter, because of cross-hybridization of trancripts between different fragments of the 5′ part of the ES, and also because of the presence of a RIME (R) element which is repeated and transcribed elsewhere in the genome. Secondly, as mentioned previously (Pays *et al.*, 1989a), transcription of sequences which are close to the promoter seems to be stimulated after UV irradiation (see panels 4, band 1 and panel 10). In fact this apparent stimulation is due to inhibition of RNA decay (H.Coquelet and E.Pays, unpublished), which, together with inhibition of RNA elongation, leads to specific accumulation of initial transcripts (Coquelet *et al.*, 1989).

Initiation of VSG gene transcription occurs from the same promoter in both trypanosome forms

The cloning and characterization of the VSG ES was achieved up to a 5′ *Sph*I site, thought to be close to the promoter (Pays *et al.*, 1989a). A 0.7 kb *Sca*I–*Sph*I fragment encompassing the putative promoter region was cloned from the isolated 200 kb expressor chromosome. In Figure 3, the nucleotide sequence of the ES promoter region is shown aligned with that of the very closely related sequence AL, cloned from the same chromosome fraction (see Pays *et al.*, 1989a). These sequences were compared with those of cDNAs from promoter-proximal transcripts, stabilized following UV irradiation. To date 12 such cDNAs, totalling 19 kb, have been sequenced (C1–C10, and ESAG7/6 cDNAs: see Figure 3 and Coquelet *et al.*, 1989). All were found to be identical to ES and different from AL. This strongly supports previous observations that the ES sequence is the only one of its family to be transcribed in our AnTat 1.3A trypanosome population.

The most relevant cDNA clones, namely C6–C10, are shown in the lower part of Figure 3. C6 and C7 show the size of the first transcript made from the ES in bloodstream forms. This transcript is 3 kb long and ends at the polyadenylation site of the first gene (ESAG 7). Although detectable in Northern blots (Pays *et al.*, 1989a), it is not abundant and probably unstable. C8 is the largest cloned cDNA synthesized on bloodstream trypanosome RNA using primer 1 (primers 1–3 are shown in Figure 3), and C9 and C10 are the largest cDNAs synthesized on RNA from

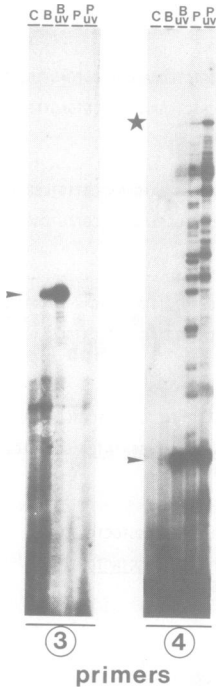

Fig. 4. Mapping of the 5′ limit of major initial transcripts. Poly(A)$^+$ RNA (5 μg) from either untreated or UV-irradiated AnTat 1.3A bloodstream forms (B, Buv) or procyclic forms (P, Puv), was annealed with 150 ng of either ES-specific primer 3 (see Figure 3) or procyclin-specific primer 4 (see Figure 6). As a control, yeast tRNA (5 μg) was processed in the same way (C). These RNAs were then used as templates for the synthesis of cDNA by reverse transcription. Discrete cDNA stops are arrowed (see text). Their counterparts in genomic DNA were determined by sequencing either the *Sca*I–*Sal*I fragment of ES from primer 3, or the plasmid pAP2 from primers 4 and 5. A possible interpretation of the cDNA stop labelled with a star is given in the model of Figure 6.

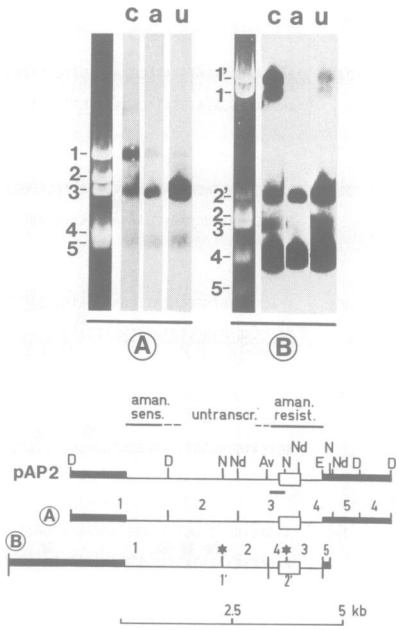

Fig. 5. Nuclear run-on analysis of the regions flanking a procyclin gene. RNA was synthesized in nuclei from either untreated (c) or UV-irradiated (u) procyclic forms. An aliquot of the nuclei from the unirradiated cells was incubated with 1 mg/ml of α-amanitin (a). The ^{32}P-labelled transcripts were hybridized to restriction digests of plasmid pAP2 (see Koenig *et al.*, 1989). The interpretation of the digests is shown in the maps below, where the black and open boxes are for the plasmid DNA and procyclin gene, respectively. The asterisks label *Nar*I sites which could not be completely cleaved, generating extra bands (1′ and 2′). The extent of an apparently untranscribed region, as well as regions transcribed by α-amanitin sensitive or resistant RNA polymerases, is indicated on the restriction map. The reasons for the poor hybridization of the 3′ environment of the procyclin gene (fragment 4 in **A** and 3 in **B**) are not understood, but might be linked to the very high thymidine content; the apparent α-amanitin sensitivity of fragment 3 in B has not been reproducibly observed. The region showing the highest stimulation of hybridization following UV is defined by a bar under the map. The abbreviations for the restriction sites are: Av, *Ava*III; D, *Dra*I; E, *Eco*RI; N, *Nar*I; Nd, *Nde*I.

bloodstream and procyclic forms, respectively, using primer 2. The nucleotide sequences of the bloodstream C9 and procyclic C10 clones were found to be identical. In addition to being the largest found in each experiment, the C6−C10 clones have been judged significant because a sizeable proportion of the other cloned cDNAs were found to be of the same length, while all the rest showed complete scattering: 15 out of 38 cloned cDNAs specific to this region shared the same size as C6 and C7, seven out of 24 cloned cDNAs appeared to be of the same size as C9 and C10, and three out of 16 showed the same size as C8. As indicated by the arrows in the upper part of Figure 3, the 5′ limits of clones 6−10, obtained by sequencing, turned out to be very closely grouped, strongly suggesting that transcription of the VSG ES starts in this region in both trypanosome forms. However, the strategy of cDNA synthesis, involving second strand priming with RNA fragments (Gubler and Hoffman, 1983), precluded the cloning of the 5′ extremity of initial transcripts. In order to refine this mapping, we performed primer extension on RNA from UV-treated cells, using primer 3. As shown in Figure 4, the same discrete stop in the cDNA synthesis was observed in both trypanosome forms, despite an obvious difference in the levels of specific steady-state transcripts. This stop is nine nucleotides upstream from the 5′ limit of the largest cloned cDNAs (C6−C8) (arrowhead in Figure 3). No other stop could be observed upstream, even if primer extension was conducted on total RNA instead of the poly(A)$^+$ RNA fraction (not shown). The cDNAs obtained by primer extension did not hybridize to a mini-exon probe (data not shown), suggesting that the 3 kb primary transcript is not *trans*-spliced.

Initiation of procyclin gene transcription occurs from the same promoter in both trypanosome forms

In the case of procyclin, there is evidence that at least two multigene loci are active simultaneously (Mowatt and Clayton, 1988; Koenig *et al.*, 1989). The regions 5′ of the first procyclin gene in each locus are almost identical for at least 260 bp (E.Koenig and I.Roditi, unpublished). Run-on transcripts were hybridized to plasmid pAP2, which contains a 4.7 kb *Pvu*II fragment from the procyclin A locus of IlTat 1.21, consisting of 3.7 kb of 5′ flanking sequence, the first of three tandem procyclin genes and 0.3 kb of the 3′ flanking sequence (Koenig *et al.*, 1989). This hybridization revealed an untranscribed region at least 2 kb long, mapping to within ~300 bp upstream from the procyclin gene (Figure 5). Unlike that of procyclin genes (Koenig *et al.*, 1989; Rudenko *et al.*, 1989), transcription of sequences upstream from this untranscribed region was weak and sensitive to α-amanitin. In addition, transcription of these sequences was reduced following UV treatment, whereas that of the procyclin gene appeared strongly enhanced (Figure 5). Taken together, these results support the notion that the two regions are part of different transcription units, with the procyclin gene promoter between them, in keeping with our data from UV mapping (see above).

The 5′ limit of the major procyclin initial transcripts in both trypanosome forms was determined by primer extension on RNA from irradiated cells. With a primer from a region conserved upstream from each copy of the procyclin genes (primer 4, see Figure 6), the same major cDNA stop was observed in both forms, 86 nucleotides upstream from the 3′ splice site of the procyclin mRNA (Figure 4, arrowhead in second panel), together with additional minor cDNA stops, the most 5′ of which is 500 nucleotides upstream from the gene (Figure 4, star). When using a primer whose specificity is restricted to the 5′ environment of the first procyclin gene (primer 5, see Figure 6), the major cDNA stop could not be clearly resolved from the background because of a too close proximity to the primer, but the upstream additional minor stops were clearly no longer observed (data not shown). A model, taking these observations and Northern blot data (Pays *et al.*, 1989b) into account, is presented in Figure 6. According to this model, the transcription start

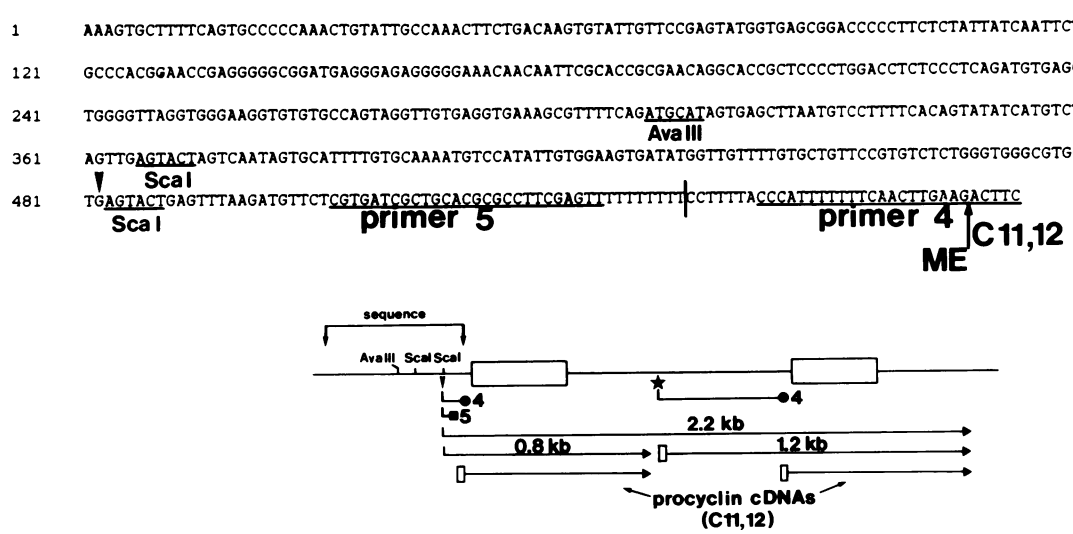

Fig. 6. Nucleotide sequence of a procyclic promoter region. In the nucleotide sequence of plasmid pAP2, the position of the 5′ limit of transcripts, as determined by primer extension (arrowhead; see Figure 4) is shown together with the site at which the mature procyclin mRNA is *trans*-spliced to the mini-exon (ME), as seen in cDNAs from both procyclic forms (C11) and UV-treated bloodstream forms (C12). The sequences complementary to primers 4 and 5, which were used for primer extension and DNA sequencing of pAP2 (Figure 4), are underlined. The vertical bar between the primers shows the site upstream of which the nucleotide sequence is not conserved ahead of each copy of the tandemly linked genes. A model of transcription of the procyclin genes is shown below. In this scheme, the boxes are for the procyclin open reading frame, the black dots and square are for the primers 4 and 5, respectively, and, in the transcripts, the open rectangle and the arrowhead represent the mini-exon and poly(A) tail, respectively. The GenBank accession number for this sequence is M27962.

would be ~100 bp upstream from the first gene of each locus in both trypanosome forms.

Discussion

The enrichment of initial transcripts by UV allowed the approximate mapping of the VSG and procyclin gene promoters. While for procyclin the promoter region is close to the gene, in the case of the VSG gene it is found 45 kb upstream ('*Sph*I region'). Despite its distance from the gene, the latter is most likely the only promoter for the VSG ES, as transcription of all sequences between this region and the VSG gene is inhibited by UV, due to blocking of RNA elongation. Interestingly, the effect of UV to some extent mimics the pattern of transcription seen in procyclic forms or in bloodstream forms triggered to differentiate at 20°C. This suggests that one level of control of transcription from the expression site is exerted at the level of RNA elongation.

The nucleotide sequence of the active VSG gene promoter region is very similar to that of its putative allele (AL), which does not seem to be active in the AnTat 1.3A clone (Pays *et al.*, 1989a). This suggests that different potential VSG promoters are present in the genome, but only one is activated at a time. This hypothesis finds support in our recent observation (D.Jefferies, unpublished) that the 'AL' region corresponding to the VSG promoter can direct expression of a reporter gene in transient activity assays.

Both VSG and procyclin gene promoters are active in bloodstream and procyclic form trypanosomes. The DNA region carrying the VSG gene promoter is repeated at least 5-fold in the genome, so it cannot be ruled out that the transcription detected in procyclics is due to the activation of a promoter silent in bloodstream forms. The data obtained so far do not support this hypothesis however, but rather suggest that the same promoter is active, since cDNAs corresponding to the initial transcripts from both life-cycle stages are identical. In the trypanosome stock analysed here, procyclin genes are present as tandem pairs at two loci per haploid genome (data not shown). As the different loci seem to be transcribed simultaneously in procyclic forms (Mowatt and Clayton, 1988; Koenig *et al.*, 1989), at least one of the promoters should remain active in bloodstream forms.

The activity of the VSG and procyclin gene promoters does not differ widely between the two trypanosome forms. Minimal estimates, from hybridization of run-on transcripts, are at least 50 and 16% of normal activity, respectively, for these two transcription units in non-expressor cells. This permanent promoter activity contrasts with the strictly differential expression of the proteins encoded by the two transcription units. In the case of the VSG gene expression site, premature arrest of RNA elongation prevents the RNA polymerase from reaching the VSG gene (Pays *et al.*, 1989b). The situation is different for the procyclin genes, which are close to their promoter and still at least partially transcribed in bloodstream forms, but transcripts are preferentially degraded, as emphasized by the very low level of steady-state procyclin mRNA in these forms. Such stage dependent control of RNA stability also seems to apply to VSG transcripts. Indeed, VSG mRNA degradation is selectively accelerated when bloodstream forms are induced to transform into procyclics (Ehlers *et al.*, 1987). The controls of RNA elongation and RNA decay therefore seem to be key features of the differential expression of these surface antigens during the trypanosome life-cycle. Whether this reflects a more general control mechanism for stage-specific gene expression in this protozoan is open to conjecture.

Materials and methods

T.brucei bloodstream forms were from the AnTat 1.3A clone, and procyclic forms were derived from the AnTat 1.1B clone (Pays *et al.*, 1989b).

The procedures for DNA and RNA isolation, Southern and Northern blot hybridization, and DNA cloning were as described (Pays *et al.*, 1980; Alexandre *et al.*, 1988). cDNA libraries were constructed in λgt10 according to Gubler and Hoffman (1983), using the Amersham cDNA synthesis and cloning kits. The sequences of DNA fragments, subcloned in bacteriophage M13 derivatives, were determined on both strands by the method of Sanger *et al.* (1980), using a modified T7 DNA polymerase (Sequenase, United States Biochemical Corp.). Run-on transcription assays were conducted as described by Murphy *et al.* (1987). Under our conditions, *in vitro* RNA synthesis is maximal after 60 min at 30°C. UV irradiation was performed under the conditions defined by Johnson *et al.* (1987). Briefly, 125 ml samples of trypanosomes [$1-4 \times 10^7$ cells/ml in Baltz's medium (Baltz *et al.*, 1985)] were irradiated at 254 nm (1 J/s/m^2) in 22 × 22 cm sterile square dishes (Bio-Assay, NUNC), usually for 1 min, and with agitation. The irradiated cells were then transferred to culture flasks and kept in the dark until centrifugation. Incubation of bloodstream forms at 20°C was performed in Baltz's medium, except that mercaptoethanol was replaced by 3 mM pyruvate and 3 mM citrate. Primer extension experiments were conducted as described by Kingston (1987), with ^{32}P-labelled synthetic oligonucleotides as primers.

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