MOLECULAR AND CELLULAR BIOLOGY, Jan. 1991, p. 338–343
0270-7306/91/010338-06$02.00/0

Vol. 11, No. 1

Transient Activity Assays of the *Trypanosoma brucei* Variant Surface Glycoprotein Gene Promoter: Control of Gene Expression at the Posttranscriptional Level

DAVID JEFFERIES, PATRICIA TEBABI, AND ETIENNE PAYS*

Department of Molecular Biology, University of Brussels, 67, rue des Chevaux, B1640 Rhode-Saint-Genèse, Belgium

Received 21 August 1990/Accepted 15 October 1990

The putative promoter of the variant surface glycoprotein (VSG) gene of *Trypanosoma brucei* was cloned into a plasmid containing the chloramphenicol acetyltransferase (CAT) gene. After electroporation into trypanosomes, this construct directed the expression of the CAT reporter gene. The essential region for promoter activity was found to reside within 88 bp upstream of the putative transcription start site. Transcription of the CAT construct occurred at approximately the same level in both bloodstream and procyclic forms and was resistant to α-amanitin. However, CAT expression appeared to be modulated in the two forms of the parasite. Sequences 3′ to the gene seemed to be important in this respect, as CAT activity in bloodstream forms was readily detectable only when the 3′ region of a VSG cDNA was placed downstream of the CAT gene. Two separate VSG gene promoter sequences, both cloned from *T. brucei* AnTat 1.3A, were equally able to direct CAT expression, which suggests that there are a number of potential VSG gene promoters in the genome, although usually only one expression site is fully active at any one time.

African trypanosomes evade the immune responses of their vertebrate hosts by the process of antigenic variation, whereby the surface coat of the parasite, the variant surface glycoprotein (VSG), is replaced by a new coat of different antigenic specificity. This antigenic switch, which has been most intensively studied in *Trypanosoma brucei*, is brought about by the semirandom sequential expression of different VSG genes from a large repertoire (26, 40). Only one VSG gene is expressed at any one time, and transcription can only occur in telomeres (11). There are a large number of trypanosome telomeres, but from hybridization studies, only around 5 to 20 potential expression sites (ESs) (9, 20, 30). The ES usually contains a number of additional genes (ES-associated genes [ESAGs]) which are coordinately expressed with the VSG gene. Switching from one VSG to another has been demonstrated to occur through either in situ activation or DNA recombination (for reviews, see references 5 and 29). Despite intensive study, the mechanism(s) underlying these molecular events remains unknown. The VSG gene is expressed only in bloodstream forms; on uptake by the vector, the VSG coat is lost and is replaced by procyclin, the major cell surface protein of the procyclic insect stage (32, 33).

The polycistronic nature of transcription in trypanosomes (8, 15, 17, 23, 39) and the phenomenon of transsplicing, by which a 39-nucleotide miniexon or spliced leader is added to the 5′ end of all mRNAs (25, 38), has hampered the analysis of DNA control elements in these organisms. However, the entire *T. brucei* AnTat 1.3A ES has recently been cloned (30), and the promoter region has been approximately defined (28). From run-on transcription and cDNA analysis, we have demonstrated that initiation of transcription of the VSG ES occurs at similar levels in bloodstream and procyclic forms and that the differential expression of the VSG ES during the parasite life cycle is due to variations in RNA elongation and stability (27, 28). This study describes the successful identification of the trypanosome VSG gene promoter by transient activity assays and shows that the expression of trypanosome genes can be extensively modulated at the posttranscriptional level.

* Corresponding author.

MATERIALS AND METHODS

Trypanosomes. Bloodstream trypanosomes of *T. brucei brucei* variant AnTat 1.3A were isolated from rat blood at the peak of parasitemia by low-speed centrifugation or by DEAE-cellulose chromatography (21). After electroporation, bloodstream forms were maintained at 37°C in Baltz medium (with 2 mM pyruvate, 0.2 mM 2-mercaptoethanol, 0.1 mM hypoxanthine, and 0.016 mM thymidine) (2) supplemented with 15 to 20% inactivated horse serum and 10 μg of gentamicin per ml. Procyclic forms (EATRO 1125) were grown at 25 to 27°C in SDM-79 (6) or Cunningham medium (10) supplemented with 15 to 20% inactivated fetal calf serum and 10 μg of gentamicin per ml.

Plasmids. Plasmids were prepared by the alkaline lysis method and purified by cesium chloride density gradient centrifugation (22). The original chloramphenicol acetyltransferase (CAT) vector, pGCATA, consisted of a 1.6-kb CAT-coding *Hin*dIII-*Bam*HI restriction fragment, containing downstream simian virus 40 (SV40) sequences, cloned into plasmid pGEM4 (13). Construction of the VSG gene promoter-dependent CAT vector is described in the text. Deletion plasmids prepared by restriction digests were digested with the appropriate enzymes before end filling and religation. Plasmids pDE2, pDE4, and pDE6 were prepared by 5′–3′ unidirectional deletion with exonuclease III (14). Briefly, pD1 was digested with *Pst*I and *Sph*I, followed by exonuclease III at 15°C. Aliquots were removed from the reaction mixture at 2, 4, and 6 min, and the DNA was end filled and religated. Candidate plasmids were chosen by screening for insert sizes, and the remaining promoter regions were checked by DNA sequencing (35). Finally, plasmid pD1ES was constructed by digesting pDE6, which

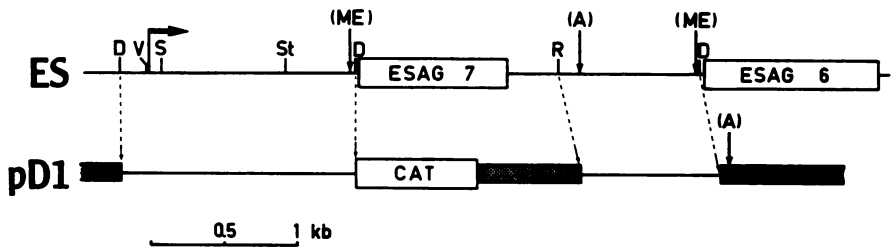

FIG. 1. Construction of the CAT expression plasmid. The map at the top shows the beginning of the AnTat 1.3A VSG ES (or its presumptive allelic locus [AL], which is 98% homologous), with boxes for the open reading frames of the first two genes (ESAGs 7 and 6). The horizontal arrow corresponds to the putative transcription start, and the vertical arrows correspond to sites where ES transcripts have been found to be 3′ spliced (ME) and polyadenylated (A) (30). The second map shows the structure of plasmid pD1. This plasmid was generated by introducing a 1.6-kb *DraI-DraI* fragment from AL and a 0.95-kb *RsaI-DraI* fragment from ES upstream and downstream, respectively, of the CAT open reading frame (as indicated by the dashed arrows between the two maps). The shaded boxes represent the SV40 sequence present in pGCATA downstream of the CAT gene, which has been interrupted by the cloning of the fragment from ES. The SV40 poly(A) consensus site is indicated by a vertical arrow. The black boxes represent the plasmid vector sequence (pGEM4). The pD1 construct was approximately 7.0 kb in size. Abbreviations for restriction endonuclease sites: D, *DraI*; R, *RsaI*; S, *SalI*; St, *StuI*; V, *Eco*RV.

no longer contained a functional promoter region, with *Eco*RV and *SalI* before ligation with the *DraI-SalI* ES promoter fragment (28).

Electroporation. Trypanosomes were centrifuged and resuspended at a concentration of 6.25×10^7/ml in a modified form of PSG 6:4 (21), termed PSGSA, which contained: NaH_2PO_4 (0.47 g/liter), Na_2HPO_4 (8.1 g/liter), NaCl (2.55 g/liter), glucose (1.98 g/liter), sucrose (27.4 g/liter), and adenosine (53.4 mg/liter), adjusted to pH 7.4. Plasmid DNA (50 μg in 50 μl of Tris buffer) was added to cuvettes containing 800 μl of trypanosome suspension (5×10^7 cells) and subjected to two pulses of 300 V at a capacitance of 960 μF with a Bio-Rad gene pulser, which caused 40 to 60% cell death (all of these procedures were carried out at room temperature). After 5 to 10 min of incubation, the contents of the cuvettes were transferred directly to culture flasks containing the appropriate medium. After 18 to 24 h, trypanosomes were counted, centrifuged, washed with PSGSA, and resuspended at a concentration of 5×10^7/50 μl in 250 mM Tris (pH 8.0) containing 20 μg of leupeptin per ml. Cells were lysed by three cycles of freeze-thaw, centrifuged, incubated at 68°C for 10 min, recentrifuged, and placed on ice until assayed.

The CAT assay was modified from Bellofatto and Cross (3). Extracts from 5×10^7 cells were incubated at 37°C for 3 h in a final volume of 100 μl after addition of 0.5 μCi of [ring-3,5-^{3}H]chloramphenicol with a specific activity of 43.1 Ci/mmol (NEN Dupont) and 0.25 mM butyryl coenzyme A (Sigma) as the substrate (36). Labeled compounds were extracted twice with 800 μl of ethyl acetate, evaporated to dryness, and spotted onto silica gel plates in 20 μl of ethyl acetate. Products were separated by ascending thin-layer chromatography, using chloroform-methanol (95:5) as the solvent. Thin-layer chromatography plates were sprayed with an autoradiographic enhancer (DuPont) and exposed for 24 to 72 h at −70°C. Levels of CAT activity were determined by redissolving butyrylated CAT assay products in ethyl acetate and counting in Insta-gel II (Packard). Bacterial CAT enzyme (Sigma) was used as a positive control in each experiment to ensure the reproducibility of results. In addition, plasmid pD1 was included in each experiment, and the CAT activities of all other constructs were expressed as percentages of pD1 activity, usually from three separate experiments.

Run-on transcription assays. Between 1.5×10^9 and 2×10^9 trypanosomes were electroporated in aliquots of 5×10^7 cells as described above, and run-on transcription assays were performed the following day as previously described (24).

RESULTS

Construction of a VSG gene promoter-dependent CAT vector. A 1.6-kb *DraI* fragment from the presumptive allelic locus (AL) of the AnTat 1.3A ES (29), containing a putative VSG gene promoter and virtually the entire noncoding region upstream of the first AL gene (the equivalent of ESAG 7; 28, 30), was cloned into the polylinker region of a vector containing the CAT gene (pGCATA; 13). In addition, the intergenic region between ESAGs 7 and 6 was inserted into an *HpaI* site downstream to provide a trypanosome poly(A) addition site (8). The map of this construct (pD1) is shown in Fig. 1.

Transient CAT gene transcription in bloodstream and procyclic forms. After introduction of pD1 by electroporation, low but easily detectable levels of CAT were present in cell extracts from procyclic-form trypanosomes (0.005 U per 5×10^7 cells) (Fig. 2A, lane g). In contrast, cells electroporated with plasmids containing the CAT gene alone or with 1.1 kb of the ESAG 7 5′ noncoding region inserted upstream did not express the reporter gene (data not shown). CAT activity decreased markedly after 24 h and 4 days postelectroporation was reduced to background levels (Fig. 2B). In bloodstream forms, CAT activity was barely detectable, even if the quantity of electroporated DNA was doubled (Fig. 2A, lanes c and d). Experiments in which the activity of purified bacterial CAT enzyme was compared in cell extracts of procyclic and bloodstream forms showed that in the absence of protease inhibitors, CAT activity was about 50% of that in procyclic forms (data not shown). Even in the presence of inhibitors, the figure was still only 70% (compare lanes a and e in Fig. 2A). However, while it was clear that bloodstream forms contained higher levels of protease activity, this was not sufficient to account for the low level of functional CAT enzyme. To determine whether the CAT gene was transcribed but not expressed in these forms, nuclear run-on assays were performed after electroporation of bloodstream- and procyclic-form trypanosomes. The run-on transcripts were hybridized with pGCATA (digested to separate the CAT gene from the plasmid vector) and with plasmids containing procyclin, actin, and VSG ES-specific sequences as controls for the stage specificity and α-amanitin sensitiv-

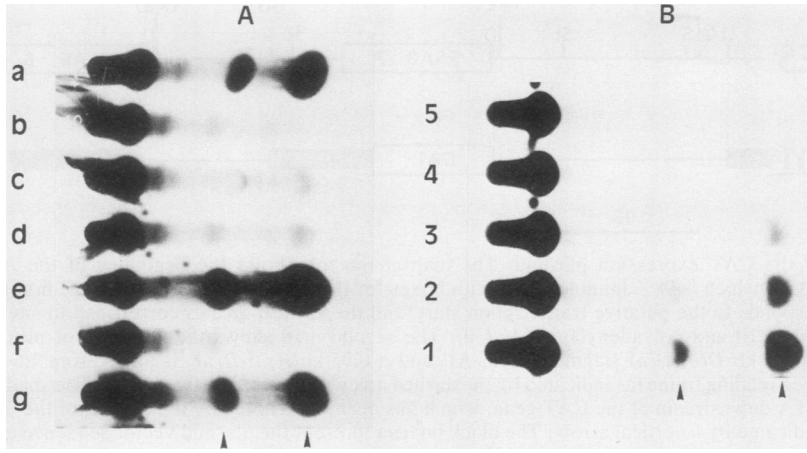

FIG. 2. Transient CAT activity in bloodstream and procyclic forms of *T. brucei*. (A) CAT activity in bloodstream (lanes a to d) and procyclic (lanes e to g) trypanosome cell extracts (5×10^7 cells). Lanes: a and e, 5×10^{-2} U of bacterial CAT; b and f, no DNA added; c and g, 50 μg of pD1; d, 100 μg of pD1. The autoradiograph was overexposed to show residual activity in pD1 electroporated bloodstream forms. (B) Time course of CAT expression in procyclic forms. CAT activity was determined 24 (lane 1), 48 (lane 2), 72 (lane 3), 96 (lane 4), and 120 (lane 5) h after electroporation with pD1. Arrowheads indicate positions of CAT assay products.

ity of the transcription assays. It appeared that transcription of pD1 occurred at low but comparable levels in both life cycle stages and that the whole plasmid construct was transcribed (Fig. 3). As expected for the VSG gene promoter (19), this polymerase activity was found to be resistant to high levels of α-amanitin.

Delineation of the VSG gene promoter. A number of deletion plasmids were generated from pD1 by using convenient restriction sites or by 5′–3′ unidirectional digestion with exonuclease III. The results obtained by using these plasmids are summarized in Fig. 4. Removal of the region 5′ of the *Eco*RV site, at position −10 with respect to the

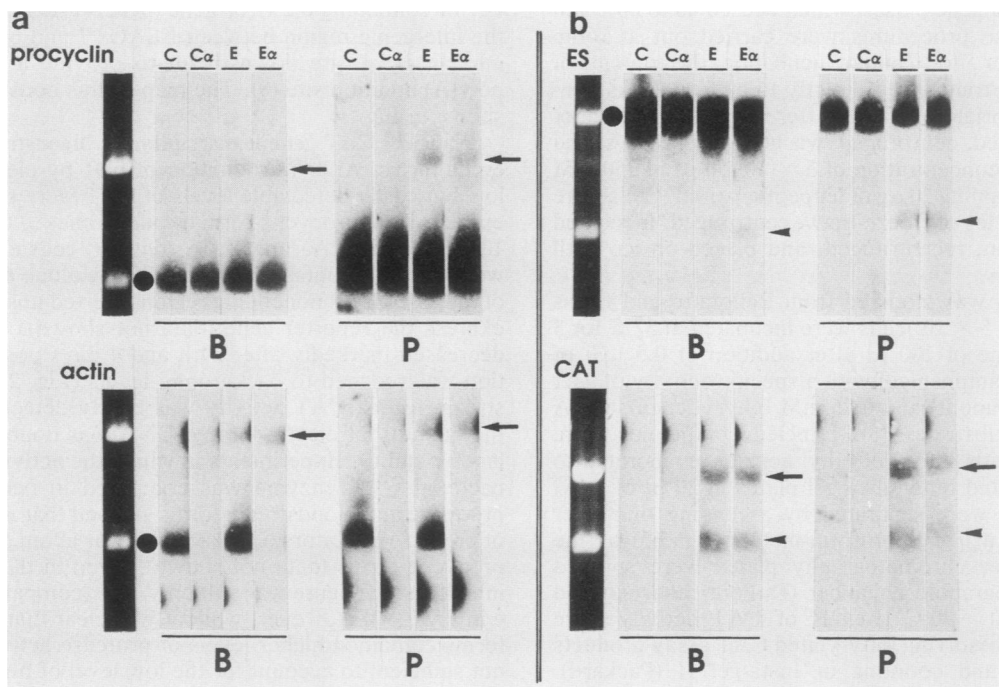

FIG. 3. Transcription of pD1 in nuclei from bloodstream and procyclic forms. Nuclei were extracted from either bloodstream (B) or procyclic (P) forms, electroporated with either pGCATA (C) or pD1 (E). Run-on transcription was conducted in the presence of α-amanitin (1 mg/ml) where indicated (α). Run-on transcripts were hybridized to restriction digests from different plasmids. The plasmids in panel a contain a *T. brucei* procyclin cDNA (32) and an actin gene (4), as indicated. They were digested with *Eco*RI and *Sal*I, respectively. In panel b, ES and CAT refer to plasmids pD5 and pGCATA, respectively; both plasmids were digested with *Sac*I and *Hpa*I. In all cases, the first lane shows ethidium bromide staining of the digests, with subsequent lanes representing the hybridization patterns with run-on transcripts. The autoradiographs were overexposed to show the transcription of plasmid and CAT gene sequences. Dots indicate the fragments containing trypanosome sequences; arrows and arrowheads point to plasmid and CAT gene-containing fragments, respectively.

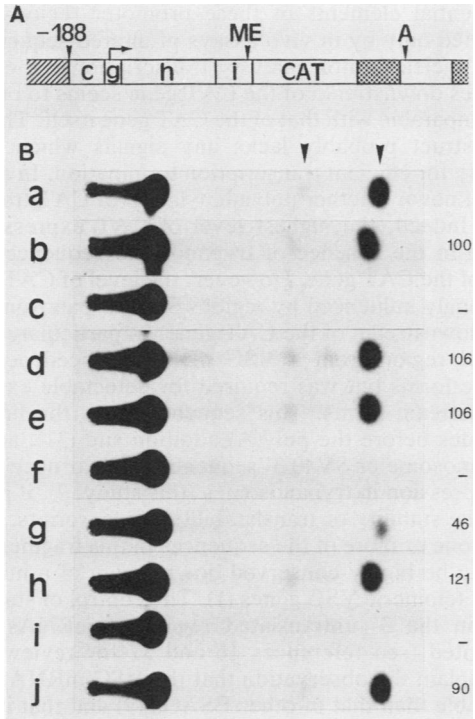

FIG. 4. CAT activities of deletion plasmids generated from pD1. (A) Schematic representation of the pD1 construct. −188 indicates the 5′ extremity, in base pairs, of the *Dra*I insert with respect to the putative transcription start site; ME and A show the positions of the miniexon acceptor site and the ESAG 7 poly(A) addition site, respectively; letters indicate regions deleted in different plasmids. (B) CAT assays. Lanes (restriction endonucleases in parentheses are those used for the deletions [the sites not present in the 1.6-kb *Dra*I insert shown in Fig. 1 are unique sites within the plasmid polylinker region], and numbers in parentheses represent the 5′ extremity of the insert remaining after exonuclease III digestion, with respect to the putative transcription start site): a, 5×10^{-3} U of bacterial CAT; b, pD1; c, pD4 (*Hin*dIII-*Eco*RV); d, pDE2 (−122); e, pDE4 (−88); f, pDE6 (−57); g, pD3 (*Eco*RV-*Sal*I); h, pD6 (*Sal*I-*Stu*I); i, pD7 (*Stu*I-*Kpn*I); j, pD1 ES. Numbers on the right represent the CAT activity of each construct compared with that of pD1 (activity = 100%). Arrowheads indicate positions of CAT assay products.

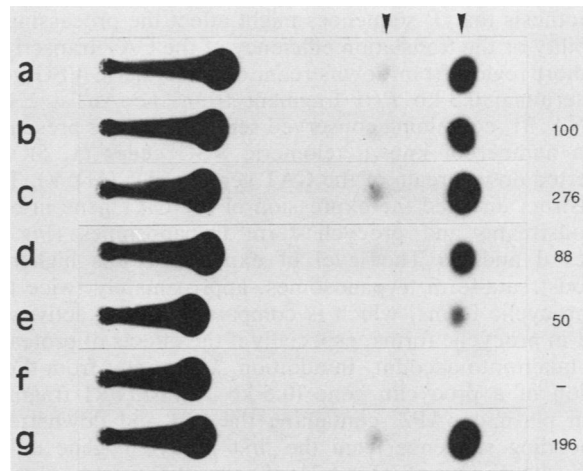

FIG. 6. CAT activities of plasmids containing different sequences downstream of the CAT gene (see text for details). Lanes: a, 5×10^{-3} U of bacterial CAT; b, pD1; c, pD5; d, pD5V (bloodstream forms); e, pD5V (procyclic forms); f, pD5P (bloodstream forms); g, pD5P (procyclic forms). Numbers on the right represent the CAT activity of each construct compared with that of pD1 (activity = 100%). Arrowheads indicate positions of CAT assay products.

putative transcription start site, eliminated CAT activity (Fig. 4B, lane c). Use of plasmids pDE2, pDE4, and pDE6, produced by unidirectional deletion, revealed that promoter activity resided within approximately 90 bp of the putative start site (lanes d to f). The nucleotide sequence of this region (Fig. 5) bears no obvious similarity to sequences of known promoters and is characterized only by the presence of three TATTAC direct repeats. Removal of a 90-bp region spanning the putative start site (*Eco*RV-*Sal*I fragment) reduced CAT activity (lane g). However, the CAT gene was still expressed, which suggests either that some nonspecific initiation of transcription may have been occurring or that there are other, minor transcription start sites outside of the deleted region. Removal of the central 0.9 kb (*Sal*I-*Stu*I fragment) of the noncoding region upstream from ESAG 7 did not significantly affect CAT activity (lane h), whereas the absence of the 0.4 kb (*Stu*I-*Kpn*I) immediately upstream of the CAT gene, which contains a 3′ splice site, reduced CAT expression to background levels (lane i). Finally, the promoter region from AL was replaced by the corresponding region from the AnTat1.3A ES, which is 98% homologous to AL in this region (28). This construct (pD1ES) showed approximately the same level of activity as did plasmid pD1 (lane j).

Modulation of CAT activity by 3′ sequences. When the trypanosome sequences upstream from the CAT gene in pD1 were cloned into the original CAT vector (pGCATA), which created a construct lacking trypanosome sequences downstream of the CAT gene (plasmid pD5), the level of CAT activity in procyclic forms was increased two- to threefold (Fig. 6, lane c). This observation prompted us to test the

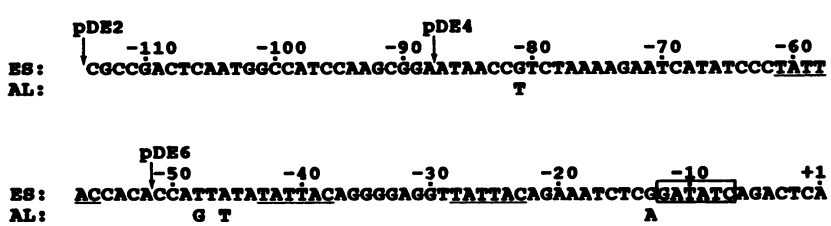

FIG. 5. Nucleotide sequence of the VSG gene promoter region in ES and AL. Mapping of the putative transcription start (+1) has been described previously (28). The 5′ limits of the promoter sequence in different deletion plasmids (pDE2, pDE4, and pDE6) are indicated by arrows. Direct repeats are underlined, and an *Eco*RV site is boxed. Only the differences with respect to ES are indicated in AL.

hypothesis that 3′ sequences might affect the processing or stability or the translation efficiency of the CAT transcripts. A short region from downstream of a telomeric VSG gene (3′-terminal 0.3-kb *Pst*I fragment from the AnTat 1.10A cDNA; 31) containing conserved sequence motifs present 3′ of a number of known telomeric VSG genes (1, 5) was inserted downstream of the CAT gene in pD5 (pD5V). This construct directed the expression of the CAT gene in both bloodstream- and procyclic-form trypanosomes (Fig. 6, lanes d and e). The level of expression was higher in bloodstream-form trypanosomes, approximately twice that in procyclic forms, which is comparable to the activity of pD1 in procyclic forms, especially if the effects of proteases are taken into account. In addition, a fragment from the 3′ region of a procyclin gene (0.5-kb *Nde*I-*Eco*RI fragment from plasmid pAP2, containing the end and downstream noncoding sequence from the first procyclin gene of the procyclin A locus of IlTat 1.21; 18) placed downstream of the CAT gene (pD5P) gave a level of CAT activity between that of plasmids pD1 and pD5 in procyclic forms but showed only background levels in bloodstream forms (Fig. 6, lanes g and f).

DISCUSSION

We report here the functional characterization of the *T. brucei* VSG gene promoter by transient activity assays of the CAT gene, incorporated into trypanosomes by electroporation. A sequence of approximately 90 bp appears to be sufficient to allow α-amanitin-resistant transcription of the bacterial gene, but successful expression of the encoded enzyme probably requires that the transcripts be trans-spliced. No 3′ trypanosome sequences are necessary for CAT expression, but 3′ regions from different trypanosome genes inserted downstream of the CAT gene strongly affect the level of CAT activity, depending on the life cycle stage. These data confirm and extend previous observations (27, 28) that post-transcriptional controls are primarily responsible for the differential expression of the two major cell surface proteins, VSG and procyclin, of the bloodstream and procyclic forms, respectively, during the life cycle of *T. brucei*.

Bellofatto and Cross (3) were the first to develop a transformation system for a kinetoplastid protozoan, identifying the sequences flanking the miniexon gene of *Leptomonas seymouri* necessary for CAT expression. Recently the applicability of the CAT assay to the analysis of trypanosome promoter regions has been confirmed by the functional characterization of the *T. brucei* procyclin gene promoter (7, 34). Here, as well as identifying the VSG gene promoter, we have shown that this technique can be used to identify 3′ sequences which may be important in RNA processing in trypanosomes.

VSG gene promoter activity appears to reside within the first 88 bp upstream of the putative transcription start site. This region seems to contain all of the sequence elements necessary for initiation of transcription, as it was sufficient for CAT expression. It does not appear to share any extensive homology with known promoters, notably those of the *T. brucei* ribosomal DNA (41) and procyclin genes (7, 28, 34), which, like the VSG gene, are transcribed by an RNA polymerase resistant to α-amanitin. The only noteworthy feature within this sequence is the presence of three TAT TAC direct repeats 23 to 61 bp upstream of the putative transcription start site. Rudenko et al. (34) draw attention to the presence of four short repeats within the promoter region of a PARP (procyclin) gene, but these repeats bear no resemblance to those present in the VSG gene promoter. The essential elements of these promoter regions can be determined only by in vivo assays of altered sequences.

From nuclear run-on analysis, transcription of the plasmid sequences downstream of the CAT gene seems to occur at a level comparable with that of the CAT gene itself. Therefore, the construct probably lacks any signals which may be necessary for efficient transcription termination. In addition, it is not known whether polyadenylation of CAT transcripts occurs. Indeed, the highest level of CAT expression was obtained in the absence of trypanosome sequences downstream of the CAT gene. However, the level of CAT activity was strongly influenced by regions 3′ of trypanosome genes placed downstream of the CAT gene. In particular, addition of the 3′ region from a VSG cDNA reduced activity in procyclic forms but was required for detectable expression in bloodstream forms. This sequence lacks the final eight nucleotides before the poly(A) addition site (31), and since no trypanosome or SV40 3′ sequences appear necessary for CAT expression in trypanosomes (this study; 7), it probably affects the stability or translatability of transcripts, perhaps through one or more of the sequences in this fragment which appear to be highly conserved downstream of a number of different telomeric VSG genes (1). The control of stability by signals in the 3′ untranslated region of mRNAs is well documented (see references 16 and 37 for reviews). This could explain the observation that the VSG mRNA is much more stable than that of other ESAGs (9) and that its decay is strongly accelerated during differentiation into procyclic forms (12). Similarly, the levels of CAT activity resulting from the presence of the 3′ untranslated regions from ESAG 7 and procyclin downstream of the CAT gene may reflect the relative stability of their respective mRNAs. However, it is not altogether clear why CAT activity in procyclic forms is higher in the absence of 3′ trypanosome sequences. At this stage, other explanations of these effects cannot be discounted. The interpretation of these results is further complicated by the fact that the procyclin and ESAG 7 3′ untranslated regions are intergenic regions, which form part of polycistronic transcription units, while the VSG cDNA 3′ region is from the end of such a unit. Further work is required to determine the exact role of these sequences in RNA processing.

Transcription of the CAT gene occurred in both bloodstream and procyclic forms, although VSG gene promoter activity would be expected to result exclusively in the expression of bloodstream-specific genes. This observation adds further support to evidence already presented (27, 28) that this promoter is constitutively active during the life cycle of *T. brucei*. In addition, we found that promoters from both active and silent expression sites are capable of directing transcription at similar levels. This is in keeping with the high sequence homology (98%) between these promoters and suggests that there are a number of independent VSG gene promoters in the *T. brucei* genome (one per telomeric expression site?), although only one ES is fully transcribed at any one time.

ACKNOWLEDGMENTS

We thank T. Frebourg, Villejuif (France), for the CAT plasmid and M. F. Ben Amar (Free University, Brussels) and I. Roditi (Karlsruhe Federal Republic of Germany), for cloned *T. brucei* DNA.

This work was supported by the Convention ARC 89/94-134, by the Belgian Fonds de la Recherche Scientifique Médicale, and by research contracts between the University of Brussels and Solvay & Cie (Brussels) and between the University and the Commission of the European Communities (TDS-M-023B). D.J. is the holder of a Wellcome Trust training fellowship.

REFERENCES

1. **Aline, R. F., Jr., and K. D. Stuart.** 1989. *Trypanosoma brucei*: conserved sequence organization 3′ to telomeric variant surface glycoprotein genes. Exp. Parasitol. **68**:57–66.
2. **Baltz, T., D. Baltz, C. Giroud, and J. Crockett.** 1985. Cultivation in a semi-defined medium of animal infective forms of *Trypanosoma brucei*, *T. equiperdum*, *T. evansi*, *T. rhodesiense* and *T. gambiense*. EMBO J. **4**:1273–1277.
3. **Bellofatto, V., and G. A. M. Cross.** 1989. Expression of a bacterial gene in a trypanosomatid protozoan. Science **244**: 1167–1169.
4. **Ben Amar, M. F., A. Pays, P. Tebabi, B. Dero, T. Seebeck, M. Steinert, and E. Pays.** 1988. Structure and transcription of the actin genes of *Trypanosoma brucei*. Mol. Cell. Biol. **8**:2166–2176.
5. **Borst, P.** 1986. Discontinuous transcription and antigenic variation in trypanosomes. Annu. Rev. Biochem. **55**:701–732.
6. **Brun, R., and M. Schoenenberger.** 1979. Cultivation and in vitro cloning of procyclic culture forms of *Trypanosoma brucei* in a semi-defined medium. Acta Trop. **36**:289–292.
7. **Clayton, C. E., J. P. Fueri, J. E. Itzakhi, V. Bellofatto, D. R. Sherman, G. S. Wisdom, S. Vijayasarathy, and M. R. Mowatt.** 1990. Transcription of the procyclic acidic repetitive protein genes of *Trypanosoma brucei*. Mol. Cell. Biol. **10**:3036–3047.
8. **Coquelet, H., P. Tebabi, A. Pays, M. Steinert, and E. Pays.** 1989. *Trypanosoma brucei*: enrichment by UV of intergenic transcripts from the variable surface glycoprotein gene expression site. Mol. Cell. Biol. **9**:4022–4025.
9. **Cully, D. F., H. S. Ip, and G. A. M. Cross.** 1985. Coordinate transcription of variant surface glycoprotein genes and an expression site-associated gene family in *Trypanosoma brucei*. Cell **42**:173–182.
10. **Cunningham, I.** 1977. New culture medium for maintenance of tsetse tissues and growth of trypanosomatids. J. Protozool. **24**:325–329.
11. **De Lange, T., and P. Borst.** 1982. Genomic environment of the expression-linked extra copies of genes for surface antigens of *Trypanosoma brucei* resembles the end of a chromosome. Nature (London) **299**:451–453.
12. **Ehlers, B., J. Czichos, and P. Overath.** 1987. RNA turnover in *Trypanosoma brucei*. Mol. Cell. Biol. **7**:1242–1249.
13. **Frebourg, T., and O. Brison.** 1988. Plasmid vectors with multiple cloning sites and CAT-reporter gene for promoter cloning and analysis in animal cells. Gene **65**:315–318.
14. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**:351–359.
15. **Imboden, M. A., P. W. Laird, M. Affolter, and T. Seebeck.** 1987. Transcription of the intergenic regions of the tubulin gene cluster of *Trypanosoma brucei*: evidence for a polycistronic transcription unit in a eukaryote. Nucleic Acids Res. **15**:7357–7368.
16. **Jackson, R. J., and N. Standart.** 1990. Do the poly(A) tail and 3′ untranslated region control mRNA translation? Cell **62**:15–24.
17. **Johnson, P. J., J. M. Kooter, and P. Borst.** 1987. Inactivation of transcription by UV irradiation of *T. brucei* provides evidence for a multicistronic transcription unit including a VSG gene. Cell **51**:273–281.
18. **Koenig, E., H. Delius, M. Carrington, R. O. Williams, and I. Roditi.** 1989. Duplication and transcription of procyclin genes in *Trypanosoma brucei*. Nucleic Acids Res. **17**:8727–8739.
19. **Kooter, J. M., and P. Borst.** 1984. Alpha-amanitin insensitive transcription of variant surface glycoprotein genes provides further evidence for discontinuous transcription in trypanosomes. Nucleic Acids Res. **12**:9457–9472.
20. **Kooter, J. M., H. J. Van der Spek, R. Wagter, C. E. d'Oliveira, F. Van der Hoeven, and P. Borst.** 1987. The anatomy and transcription of a telomeric expression site for variant-specific surface antigens in *T. brucei*. Cell **51**:261–272.
21. **Lanham, S. M., and D. G. Godfrey.** 1970. Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. Exp. Parasitol. **28**:521–534.
22. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Muhich, M. L., and J. C. Boothroyd.** 1988. Polycistronic transcripts in trypanosomes and their accumulation during heat shock: evidence for a precursor role in messenger RNA synthesis. Mol. Cell. Biol. **8**:3837–3846.
24. **Murphy, N. B., A. Pays, P. Tebabi, H. Coquelet, M. Guyaux, M. Steinert, and E. Pays.** 1987. *Trypanosoma brucei* repeated element with unusual structural and transcriptional properties. J. Mol. Biol. **195**:855–871.
25. **Murphy, W. J., K. P. Watkins, and N. Agabian.** 1986. Identification of a novel Y branch structure as an intermediate in trypanosome mRNA processing: evidence for trans-splicing. Cell **47**:517–525.
26. **Pays, E.** 1989. Pseudogenes, chimaeric genes and the timing of antigen variation in African trypanosomes. Trends Genet. **5**:389–391.
27. **Pays, E., H. Coquelet, A. Pays, P. Tebabi, and M. Steinert.** 1989. *Trypanosoma brucei*: posttranscriptional control of the variable surface glycoprotein gene expression site. Mol. Cell. Biol. **9**:4018–4021.
28. **Pays, E., H. Coquelet, P. Tebabi, A. Pays, D. Jefferies, M. Steinert, E. Koenig, R. O. Williams, and I. Roditi.** 1990. *Trypanosoma brucei*: constitutive activity of the VSG and procyclin gene promoters. EMBO J. **9**:3145–3151.
29. **Pays, E., and M. Steinert.** 1988. Control of antigen gene expression in African trypanosomes. Annu. Rev. Genet. **22**:107–126.
30. **Pays, E., P. Tebabi, A. Pays, H. Coquelet, P. Revelard, D. Salmon, and M. Steinert.** 1989. The genes and transcripts of an antigen gene expression site from *T. brucei*. Cell **57**:835–845.
31. **Pays, E., S. Van Assel, M. Laurent, M. Darville, T. Vervoort, N. Van Meirvenne, and M. Steinert.** 1983. Gene conversion as a mechanism for antigenic variation in trypanosomes. Cell **34**: 371–381.
32. **Roditi, I., M. Carrington, and M. Turner.** 1987. Expression of a polypeptide containing a dipeptide repeat is confined to the insect stage of *Trypanosoma brucei*. Nature (London) **325**:272–274.
33. **Roditi, I., H. Schwartz, T. W. Pearson, R. P. Beecroft, M. K. Liu, J. P. Richardson, H. Büring, J. Pleiss, R. Bülow, R. O. Williams, and P. Overath.** 1989. Procyclin gene expression and loss of the variant surface glycoprotein during differentiation of *Trypanosoma brucei*. J. Cell Biol. **108**:737–746.
34. **Rudenko, G., S. Le Blancq, J. Smith, M. G. S. Lee, A. Rattray, and L. H. T. Van der Ploeg.** 1990. Procyclic acidic repetitive protein (PARP) genes located in an unusually small α-amanitin-resistant transcription unit: PARP promoter activity assayed by transient DNA transfection of *Trypanosoma brucei*. Mol. Cell. Biol. **10**:3492–3504.
35. **Sanger, F., S. Nicklen, and A. R. Coulsen.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
36. **Seed, B., and J.-Y. Sheen.** 1988. A simple phase-extraction assay for chloramphenicol acetyl transferase activity. Gene **67**:271–277.
37. **Shapiro, D. J., J. E. Blume, and D. A. Nielsen.** 1987. Regulation of messenger RNA stability in eukaryotic cells. BioEssays **6**:221–226.
38. **Sutton, R. E., and J. C. Boothroyd.** 1986. Evidence for trans-splicing in trypanosomes. Cell **47**:527–535.
39. **Tschudi, C., and E. Ullu.** 1988. Polygene transcripts are precursors to calmodulin mRNAs in trypanosomes. EMBO J. **7**:455–463.
40. **Van der Ploeg, L. H. T., D. Valerio, T. De Lange, A. Bernards, P. Borst, and F. G. Grosveld.** 1982. An analysis of cosmid clones of nuclear DNA from *Trypanosoma brucei* shows that the genes for variant surface glycoproteins are clustered in the genome. Nucleic Acids Res. **10**:5905–5923.
41. **White, T. C., G. Rudenko, and P. Borst.** 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.