The PARP and rRNA Promoters of *Trypanosoma brucei* Are Composed of Dissimilar Sequence Elements That Are Functionally Interchangeable

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The African trypanosomes express two major surface proteins, the variant surface glycoprotein (VSG) and the procyclic acidic repetitive protein (PARP). The RNA polymerase that transcribes the VSG and PARP genes shares many characteristics with RNA polymerase I. We show that although there is very little similarity in nucleotide sequence, the functional structure of a trypanosome rRNA promoter is almost identical to that of the PARP promoter. Further, domains from the PARP promoter can functionally substitute for the corresponding parts of the rRNA promoter, and vice versa.

The African trypanosomes are parasites of the blood and tissue fluids of mammals, including humans. Among the many peculiarities of the molecular biology of trypanosomes is their habit of making mRNAs as polycistronic precursors. The resulting RNAs are cut into gene-sized units by a 5′ trans splicing reaction and 3′ cleavage and polyadenylation (reviewed in references 8 and 25). Because the 39-nucleotide 5′ "miniexon" is capped, mRNA synthesis is not restricted to RNA polymerase II; for example, functional mRNAs can be made by introduced bacteriophage RNA polymerases (1a, 41a) and by RNA polymerase I (29, 46; reviewed in reference 6). In fact, it is probably RNA polymerase I that transcribes the major surface glycoprotein genes: the genes for the variant surface glycoprotein (VSG) and the procyclic acidic repetitive protein (PARP or procyclin) (6). Although the divergent-cation dependence of the RNA polymerase resembles that of RNA polymerase II (16), the transcription of the surface antigen genes shows the alpha-amatinin and Sarkosyl resistance of trypanosome RNA polymerase I (22, 26, 30). When the PARP promoter is placed upstream of the neomycin phosphotransferase gene and the construct is used to transform trypanosomes to drug resistance, the neomycin phosphotransferase RNAs produced accumulate in, or at the edge of, the nucleolus (29).

The bloodstream trypanosomes that live in the blood and tissue fluids of mammals are covered by a dense coat of VSG. Each trypanosome has been estimated to have about 1,000 VSG genes, but normally only one is expressed at a time. The expressed VSG gene is situated at a telomeric expression site; the corresponding promoter is 40 to 60 kb upstream. Antigenic variation can occur either by genetic rearrangement or by transcriptional control of about 20 possible expression sites (reviewed in references 10 and 12). When the trypanosomes are taken up by a tsetse fly (the disease vector) and transform into procyclic forms, the entire cell surface covering of VSG is replaced by PARP within the space of one or two cell divisions (28, 44). VSG transcripts are no longer detectable, but PARP transcripts are up-regulated at least 104-fold. There is clearly a strong posttranscriptional contribution to this regulation (17, 20), but the nature and extent of transcriptional control (whether of initiation, elongation, or premature termination) are unclear (8, 15, 26, 30). In transient transfection assays using procyclic and bloodstream trypanosomes, the PARP, VSG, and rRNA promoters were found to have similar activities (16a, 17, 48).

Previous in vivo analyses, using deletion and mutagenesis, have revealed two regions that are important for PARP promoter activity: a bipartite core element situated at positions −14 to −43 and −64 to −73 relative to the initiation site and an upstream controlling element from positions −94 to −143 (according to the numbering in Fig. 1) (3, 32). This picture is almost identical to that described for the *Saccharomyces cerevisiae* rRNA promoter (5, 23, 24) and also resembles RNA polymerase I promoters from other organisms (27, 34). However, no trypanosome rRNA promoter had been characterized in detail. In this study, we compared the *Trypanosoma brucei* PARP and rRNA promoters.

**MATERIALS AND METHODS**

**Trypanosomatids and transfection.** Procyclic trypanosome strains Antat 1.1 and MiTat 1 were cultured as described previously (7). Transfections were done by electroporation with 10 to 20 μg of circular plasmid DNA, each DNA sample being transfected in duplicate. Luciferase assays were performed after an incubation period of 15 to 20 h (1b, 32). The average luciferase activity was calculated for each DNA and expressed relative to the value obtained for pHD 50. For most mutants, two apparently identical but independent clones were tested to rule out cloning and PCR artifacts. All transient transfections were performed in duplicate at least three times. Results were expressed as the mean luciferase activity relative to that of pHD 50. Control experiments and extensive experience (including cotransfections with different reporter plasmids) indicated that there was very little variability in transfection efficiency within experiments.

To generate permanent cell lines, hygromycin resistance plasmids were linearized at a unique *ClaI* site in the center of the rRNA nontranscribed spacer region. One hundred micrograms of DNA was transfected into 107 procyclic trypanosomes (derived from Antat 1.1 bloodstream forms), and drug selection (75 μg of hygromycin per ml) was performed after 24 h. Cells were diluted into microtiter wells before selection, but
DNA. DNA for transient transfection experiments was prepared by alkaline lysis, separation on an ion-exchange column (Qiagen), and isopropanol precipitation. Plasmid pHDI was constructed by replacing the chloramphenicol acetyltransferase (CAT) gene of pH54 (32) with the firefly luciferase gene (11). This plasmid contains the PARP promoter, the PARP splice acceptor sequences, the luciferase gene, and the PARP 3'-untranslated region and polyadenylation site cloned into pGEM 4 such that the T7 promoter is upstream of the PARP promoter (17). In subsequent subclonings, this general organization was retained. Each construct was checked by restriction digestion and dideoxy sequencing (Sequenase; U.S. Biochemicals) of mutated regions and junctions according to the manufacturer's instructions. Oligonucleotides were synthesized by the Zentrum für Molekulare Biologie—Heidelberg oligonucleotide synthesis laboratory.

Plasmid pHD30 has a 5' splice acceptor region and a 3'-untranslated region from the actin genes, replacing the corresponding PARP sequences (17). The PARP promoter was synthesized by PCR (31) by using total T. brucei (strain 427) DNA as a template and primer oligonucleotides C119 (CCGAGGGTACCGCCGCAGGCGGTGCA) and C212 (TCAGGCCGGGTCTGAGAGCGGTCAGTGCT); the product was digested with KpnI and Smal and was cloned into pHD30, replacing the PARP promoter, to give pHD50. An analogous plasmid (pHDI) bearing the originally published sequence was amplified in the same way, by using plasmid pRK20+ as a template (48). Site-directed mutagenesis was performed by PCR; sequences of PCR mutants are shown in Fig. 1.Spacing mutants (Table 1; see Fig. 2) were made by cleaving promoter mutant plasmids with restriction enzymes, creating blunt ends with Klenow fragment or T4 DNA polymerase, and allowing self-reliation (pHDI 40, pHDI 161-6, and pHDI 173-6). All the plasmids listed above have actin 5'- and 3'-untranslated regions and processing signals. pHDI 265 has the RNA promoter replacing the PARP promoter in pHDI 1; in it and the domain swap plasmids, the luciferase gene has PARP 5'- and 3'-untranslated regions and processing signals. The promoters of the domain swap plasmids (see Fig. 4) were hybrids of pHDI 18 (19) and pHDI 151 (pHDI 262 and pHDI 263) or pHG 44.37 (32) and pHDI 154 (pHDI 264 and pHDI 329); spacing corrections were effected by PCR (pHDI 347 and pHDI 348).

The hygromycin resistance plasmid pHDI 132 (which contains the PARP promoter with the actin splicing signal and 3'-untranslated region surrounding the hygromycin resistance cassette [42]) was modified for insertion into the nontranscribed spacer of the RNA locus (40) by insertion of the 1.3-kb HincII fragment into the unique PvuII site, upstream of the promoter in the sense (pHDI 331) or antisense (pHDI 332) direction (see Fig. 5). The HincII fragment contains a unique Clal site that can be used for targeted recombination into the genome. The promoters of pHDI 263 and pHDI 264 were inserted into pHDI 332 by using the unique KpnI and SmaI sites to yield pHDI 340 and pHDI 341, respectively. The Kpn-SmaI promoter fragment from pHDI 262 was cloned into pHDI 331 to yield pHDI 335.

**RNA isolation and RNase protection.** RNA was isolated from UV-irradiated trypanosomes as described previously (9). RNase protection was performed as described by Zinn et al. (45). Briefly, 40 μg of total RNA was hybridized with uniformly 32P-labeled RNA probes (5 ng) in 80% formamide–40 mM PIPES [piperazine-N,N'-bis(2-ethanesulfonic acid); pH

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**TABLE 1. Deletions and insertions in the RNA promoter**

<table>
<thead>
<tr>
<th>Type of mutation and plasmid</th>
<th>Method of construction</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upstream deletion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pHDI 173</td>
<td>pHDI 151, Kpn-Xho</td>
<td>Delete-mutate to −72</td>
</tr>
<tr>
<td>pHDI 174</td>
<td>pHDI 152, Kpn-Xho</td>
<td>Delete-mutate to −62</td>
</tr>
<tr>
<td>pHDI 175</td>
<td>pHDI 153, Kpn-Stu</td>
<td>Delete-mutate to −52</td>
</tr>
<tr>
<td>pHDI 176</td>
<td>pHDI 154, Kpn-Stu</td>
<td>Delete-mutate to −42</td>
</tr>
<tr>
<td><strong>Internal spacing change</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pHDI 170</td>
<td>pHDI 153 + pHDI 154</td>
<td>Delete 10 bp, −56 to −47</td>
</tr>
<tr>
<td>pHDI 171</td>
<td>pHDI 153 + pHDI 154</td>
<td>Insert 9 bp, −43 to −44</td>
</tr>
<tr>
<td>pHDI 169</td>
<td>pHDI 153 + pHDI 154</td>
<td>Insert 10 bp, −52 to −53</td>
</tr>
<tr>
<td>pHDI 172</td>
<td>pHDI 154 + pHDI 155</td>
<td>Delete 11 bp, −46 to −36</td>
</tr>
<tr>
<td>pHDI 162</td>
<td>Fill in pHDI 151</td>
<td>Insert 1 bp at −79 to −80</td>
</tr>
<tr>
<td>pHDI 161</td>
<td>Fill in pHDI 151</td>
<td>Insert 2 bp at −79 to −80</td>
</tr>
<tr>
<td>pHDI 167</td>
<td>pHDI 152 + pHDI 151</td>
<td>Insert 9 bp at −78 to −79</td>
</tr>
<tr>
<td>pHDI 163</td>
<td>Fill in pHDI 152</td>
<td>Insert 1 bp at −68 to −69</td>
</tr>
<tr>
<td>pHDI 164</td>
<td>Fill in pHDI 152</td>
<td>Insert 3 bp at −70 to −71</td>
</tr>
<tr>
<td>pHDI 168</td>
<td>pHDI 152 + pHDI 151</td>
<td>Delete −80 to −71</td>
</tr>
<tr>
<td>pHDI 304</td>
<td>Fill in pHDI 168</td>
<td>Delete −75 to −70</td>
</tr>
</tbody>
</table>

* All numbers are relative to the position of the start site shown in Fig. 1.
FIG. 2. Effect of insertions and deletions on rRNA promoter activity. The regions shown to be most affected by 10-bp block replacements are shaded; activities are shown as percentages of that of pHD 50.

6.4]–400 mM NaCl–1 mM EDTA at 56°C for 16 h. Nonhybridized RNA was then digested either with RNases A (12 µg) and T1 (0.6 µg) for 45 min at 29°C or with RNase T2 (18 U in 50 mM sodium acetate [pH 5.0]–100 mM NaCl–2 mM EDTA) for 2 h at 30°C. The digestion mixtures were deproteinized with phenol-chloroform, ethanol precipitated, and separated on a denaturing 8% polyacrylamide gel before autoradiography. The labeled RNA probes were transcribed by T7 RNA polymerase from linearized plasmids containing the PARP or rRNA promoter with synthetic SmaI and actin 5′ splice sites, cloned into pGEM 4 (13a).

Genomic DNA analysis. Genomic DNA was isolated from hygromycin-resistant cell populations by Nonidet P-40 lysis followed by phenol-chloroform extraction (1). Southern blotting and hybridization with 32P-labeled probes were performed according to standard methods.

RESULTS

Mutation of the T. brucei rRNA promoter. As trypanosomes branched very early in eucaryotic evolution (33), to compare the PARP promoter with rRNA promoters from other species is highly unsatisfactory. A mutational analysis of a trypanosome rRNA promoter was therefore undertaken. We used genomic PCR to clone a T. brucei rRNA promoter fragment mapping from positions −257 to +32 relative to the transcription initiation site mapped initially by White et al. (40). The promoter was placed upstream of a luciferase gene flanked by actin gene RNA processing signals (to give pHD 50). The promoter sequence (Fig. 1) matched that originally published (40), with minor changes. The 3-bp deletion at position −84, a G at position −7, and a C at position −27 had been seen before, in an rRNA promoter isolated from a 2-Mb chromosome (47), and some changes further upstream (data not shown) were noted to occur in a minichromosomal promoter (47). Only the T residue at position −10 has not been seen previously.

The newly cloned rRNA promoter (pHD 50) gave between 50 and 100% of the level of luciferase expression driven by the PARP promoter and had activity similar to that of the rRNA promoter used originally by Zomerdijk et al. in their plasmid pRK20+ (subcloned by us to give pHD 181) (48). Mutation of the sequence around the start site to conform to the pRK20+ sequence (pHD 159) resulted in a slight (up to twofold) stimulation of activity. In the following discussion, all nucleotide positions are relative to the start site mapped by White et al. (40).

Previous results (48) showed that deletion to position −181 left about 14% residual reporter gene expression relative to that in plasmids containing promoters extending to position −260 or beyond; a promoter containing the region from position −75 to beyond the start site had a very low but detectable level of activity (48). We confirmed this result (pHD 173) and found that further deletion abolished expression (pHD 174) (Fig. 2). We therefore define the core promoter activity as lying downstream of position −75, with stimulatory activities further upstream. All RNA polymerase I promoters analyzed so far, as well as the PARP promoter, have a stimulatory region situated at about positions −100 to −150 that is detected by deletional analyses.

It had already been shown that mutation of the start site residue (+1) to T or C severely impaired T. brucei rRNA promoter activity (29, 48), but no other mutational analysis had been undertaken. For the PARP promoter, mutation of the upstream stimulatory region had only minor effects (3, 32), suggesting binding of factors over a broad area in a fashion that is not very sequence specific. We therefore chose to concentrate on the region from positions −3 to −82. Sequential 10-bp mutations were performed (Fig. 1). The results of these mutations, as for the deletions, resemble those previously reported for the PARP promoter (Fig. 3). The rRNA promoter core has two important regions, extending from positions −42 to −13 and −53 to −62. The discrepancies in spacing observed could in part reflect the way in which the 10-bp changes were phased; for example, more detailed mu-
FIG. 3. Effect of the 10-bp replacement mutations in the rRNA promoter on luciferase gene expression. The approximate position of each replacement relative to the putative start site is shown beneath each bar; for example, the mutant labeled −10 is pHD 159 and is actually mutated from positions −3 to −12. Luciferase activity is expressed as a percentage of the level from the wild-type promoter. All mutants were assayed at least three times in duplicate; results are means ± standard deviations (error bars). A graph showing the activity of equivalent PARP promoter mutants (32) is shown above for comparison.

taggenesis of the PARP promoter indicates that the sequence at positions −64 to −68 (according to the numbering in Fig. 1) is more important for activity than the other 5 bp affected in the "−70" mutant (19).

Spacing effects. The spacing between the upstream element and the core is crucial to PARP promoter activity (32). The fact that genomic rRNA promoters already have a variation of ±3 bp in this region (Fig. 1, pHD 50 and pRK20+) alerted us to the possibility that this might not be true for the rRNA promoter. Restoring the deleted 3 bp to pHD 50 gave at most a twofold stimulation (Fig. 1, pHD 160). All changes in the region from positions −40 to −55 abolished expression (Fig. 2, pHD 169 to pHD 172). Further upstream, insertions seemed better tolerated than deletions (compare, for instance, pHD 167 with pHD 168 in Fig. 2).

Activity of hybrid promoters. If RNA polymerase I effects transcription of both PARP and rRNA genes, one might expect the promoters to be able to bind to some common transcription factors, or at least to some of the component subunits. In particular, factors binding to the upstream element of the PARP promoter might be able to interact productively with those binding to the proximal element of the rRNA promoter, and vice versa. We tested this indirectly by exchanging different portions of the PARP and rRNA promoters. The substitution of the PARP upstream element for the rRNA upstream element, or vice versa (pHD 262 and pHD 263), had very little effect on the level of luciferase expression (Fig. 4). This was true for either actin or PARP RNA processing sequences and with either the luciferase gene or the CAT gene as the reporter gene (data not shown). Promoters for which the exchange had been effected in the mutually neutral island within the core (by using existing restriction sites near position −50 in mutant promoters) had reduced but significant activity (pHD 329 and pHD 264). In these mutants, the exchange had

FIG. 4. Activity of hybrid PARP-rRNA promoters. The pHD 1 construct is shown linearized, without the vector sequence, at the top; the start site is indicated by a flag, and the positions of the two regions most susceptible to mutation are indicated by dotted boxes. In the remainder of the figure, contributions from the PARP promoter are in grey and those from the rRNA promoter are in black. The junctions for pHD 262 and pHD 263 are at the introduced XhoI site starting at positions −81; for pHD 329 and pHD 264, the junctions are at the Smal site starting at positions −49 (PARP) and −46 (rRNA) (spacing changes indicated). Luciferase activity is expressed as a percentage of that of pHD 1.
resulted in a 3-bp insertion or deletion (assuming alignment relative to the start sites as shown in Fig. 1). Restoration of the assumed correct spacing (Fig. 3, pH D 347 and pH D 348) raised the activity of the hybrids markedly. The activity of pH D 347 was greater than that of the wild type, and, although the activity of pH D 348 was less, this promoter was more than fourfold more active than PARP or rRNA promoters bearing 10-bp mutations at the −60 or −70 region (Fig. 3). We therefore concluded that the different domains of the rRNA and PARP promoters were functionally interchangeable.

**Determination of the transcriptional start sites.** All attempts to map transcriptional start sites of our constructs after transient transfection failed. We therefore wished to test the promoters in a situation in which they were permanently integrated into a silent region of the trypanosome genome, the nontranscribed spacer of the rRNA locus (40). The promoters were placed upstream of a hygromycin resistance cassette (bearing processing signals and untranslated regions from the actin locus). Upstream of each promoter was placed a 1.3-kb fragment of the rRNA locus, in either the forward or reverse orientation with respect to rRNA transcription. In African trypanosomes, linearized plasmid DNA almost invariably integrates into the genome by homologous recombination at the site of cleavage in the construct (13, 36). We therefore targeted the constructs into the nontranscribed spacer by cleaving the input DNA at a unique (ClaI) site at the center of the rRNA spacer region. To confirm that integration occurred as expected with such constructs, genomic DNA from some of the lines was digested with StuI. This enzyme cuts the input plasmid DNA once at the 3′ extremity of the actin 3′-intergenic region, 2.65 kb downstream of the unique ClaI site. In the nontranscribed spacer, a StuI site is present 1.56 kb upstream of the ClaI site used for vector insertion (Fig. 5A). If a construct designed for integration in the forward direction integrates correctly, the hygromycin resistance gene is expected to be found on a 4.2-kb StuI fragment. This is indeed observed (Fig. 5B, lane 2). Lines containing inserts in the opposite orientation yield a band of about 10 kb; this indicates cleavage at the 3′ end of the hygromycin cassette as before, and with the 2S8 rRNA gene (approximately 7 kb downstream of the ClaI site), as predicted from the published sequences (4, 40). Digestion with ClaI yielded unit plasmid-sized bands, as expected (data not shown).

To confirm that the plasmids remained intact after integration, and in particular that the hybrid promoters had not been repaired, a PCR was performed with genomic DNA as the template and primers specifically designed to amplify the PARP or rRNA promoters joined to an actin 5′-untranslated region. All the promoters appeared to be intact. For instance, amplification with a primer that hybridized to the upstream extremity of the PARP promoter yielded products of the predicted size, not only from intact PARP promoters (Fig. 5C, lanes 4 and 6) but also from a PARP-rRNA hybrid (lane 8). (Considering the location of this hybrid in the genome, it was the strongest candidate for repair by intrachromosomal gene conversion.) Control reactions using an rRNA promoter primer yielded only background bands (Fig. 5C, lanes 3, 5, 7, and 9).

Control experiments have shown that low-level UV irradiation of trypanosomes does not affect the site of transcriptional initiation but does increase the abundance of the precursor transcript (13b). RNA was therefore prepared from the hygromycin-resistant trypanosomes after appropriate UV treatment, and sites of initiation were mapped by RNase protection. (Primer extension yielded no detectable products.) Results are shown in Fig. 6. The probe used was specific for PARP promoter–actin 5′-end hybrids, and so it yields bands of the expected size only from cells carrying integrated plasmid DNA (Fig. 6, lane 5 in both panels). Detection was extremely difficult because of the low abundance of the precursors. With RNase T1, it was clear that the orientation of the promoter within the rRNA locus did not affect the site of initiation, which was within the area previously mapped by a variety of other means.

![FIG. 5. Insertion of promoter constructs in the nontranscribed spacer.](http://mcb.asm.org/)

**Panel A** shows the rRNA locus and plasmids pH D 331 and pH D 332 (linearized), showing relevant restriction sites. The two HincII sites shown are those used for cloning the intergenic fragment. The positions of the genomic and plasmid transcription start sites are indicated by arrows. Open boxes, rRNA genes; thick lines, intergenic sequences needed for targeting; black box, promoter; hatched box, actin-hygromycin resistance-actin cassette; broken line, pGem vector. **Panel B** shows restriction maps of DNA from cells transfected with various plasmids. Lane 1, wild-type parasites; lane 2, pHD 331 (forward orientation); lane 3, pHD 332 (reverse orientation); lane 4, pH D 340 (pHD 332 with the pHD 263 rRNA-PARP hybrid promoter). **Panel C** shows the structure of the plasmid used for integration. A 0.5-ug amount of genomic DNA from cells transfected with various plasmids was used as the template in a PCR using either primer CC 25 (forward primer from the 5′ extremity of the PARP promoter) and CC 119 (forward primer from the 5′ extremity of the rRNA promoter) and Lane 1, pHD 332 (pHD 332 with the pHD 263 rRNA-PARP hybrid promoter). C) Promoter structure is retained during insertion. A 0.5-ug amount of genomic DNA from cells transfected with various plasmids was used as the template in a PCR using either primer CC 25 (forward primer from the 5′ extremity of the PARP promoter) and CC 119 (forward primer from the 5′ extremity of the rRNA promoter) and Lane 1, pHD 332 (pHD 332 with the pHD 263 rRNA-PARP hybrid promoter).
(3, 26, 32). By using either RNase A and T_1 digestion (Fig. 6A) or RNase T_2 digestion (Fig. 6B), the pattern obtained for the hybrid rRNA-PARP promoter (pHD 340; promoter structure from pHD 263) was very similar to that for the wild-type PARP promoter. Even when the rRNA-PARP junction was in the core region and had incorrect spacing (pHD 341, promoter from pHDI 264), a similar pattern could be seen after T_2 digestion, although detection was more difficult (Fig. 6B, lane 9). These results confirm that, for the hybrid rRNA-PARP promoters, the initiation sites were similar or identical to those used by the species providing the start site. Preliminary results indicate that the same is true for hybrid PARP-rRNA promoters (18). These results are therefore consistent with transcription of PARP, rRNA, and hybrid promoters by the same polymerase.

**DISCUSSION**

The experiments described above have highlighted functional similarities between the PARP and rRNA promoters. The relative positions of the core and upstream elements are similar, and hybrid promoters are active. Both the rRNA and PARP promoters were unable to function after deletions of greater than 4 bp between the proximal element and the upstream element, but the rRNA promoter appeared more tolerant of insertions. RNA polymerase I promoters from other species show various degrees of tolerance to spacing changes. Spacing is absolutely crucial in the** *Xenopus* **rRNA promoter (41), the rat rRNA promoter tolerates some changes but not others (43), and the human promoter appeared fairly tolerant of spacing changes (21).

In all species so far examined, the core element interacts with a species-specific factor known (for example) as TF-1D or SL-1 (reviewed in reference 34). The upstream element (UCE) interacts with another factor, UBF, and sometimes also with TF-1D. Various other interactions between UBF, TF-1D, the upstream element, and additional factors, as well as interactions with RNA polymerase I, have been documented (reviewed in reference 34), and any of these could be disrupted by changes in the relative positions of the upstream element and core. In all other species, there is also an enhancer element some distance upstream of the UCE. No equivalent of this has yet been found in trypanosomes.

The PARP and rRNA promoters share only very limited sequence similarity. Nevertheless, the sequences that are important for promoter activity map to the same positions relative to the transcriptional start. The start site nucleotide has been reported to be critical for rRNA promoter activity (29, 48), whereas the sequence at the PARP start site does not appear to influence the level of mature rRNAs but may affect the accuracy of transcriptional initiation (3, 13a, 32). The question is complicated by the fact that the start site may not be the same for all rRNA promoter sequences (47), and the precise position of the PARP start site is controversial (3, 26, 32). We think, however, that the promoter alignment we used (Fig. 1) is a reasonable one because hybrid promoters constructed on that basis had good activity.

Most transcriptional promoters contain sequences upstream of the initiation site that are important for activity. The resemblance between the PARP and rRNA promoters in mutational analyses is therefore not in itself evidence that they use the same polymerase. The fact that hybrid promoters are active is more persuasive. Even promoters that contain PARP-rRNA junctions within the core element were active. These hybrids contained a junction near position −50. From this evidence, one might expect the PARP and rRNA promoters to compete for transcription factors. We have been unable to confirm this by gel mobility shift analysis (19), and it is unlikely to be possible to test by transient transfection because the system is not easily saturated (48). Further analysis will require development of an in vitro transcription system.

There are 4 PARP promoters, maybe 20 or so VSG promoters, and rather more rRNA promoters in the trypanosome genome. Each class is strongly internally conserved but almost completely unrelated to the others; we do not know if any of the sequence differences have biological importance, how the unusual transcription of the PARP and VSG genes arose, or why it has been conserved. It is, however, tempting to see a
connection between this (possible) use of RNA polymerase I and the unusual properties of RNA polymerase II transcription in these organisms.

To our knowledge, there is no evidence whatsoever for regulation of RNA polymerase II transcription in trypanosomatids. Cotranscribed genes showing contrasting regulation have been found less than 200 bp apart (14, 39). RNA polymerase II transcription also appears inefficient in comparison with transcription by RNA polymerase I: a single neomycin phosphotransferase gene integrated at the rRNA locus gave 5- to 10-fold more RNA than an analogous construct integrated in the tubulin array (35), and integrated luciferase genes show a similar difference in expression (41b). For abundant RNA polymerase II transcripts, a combination of gene amplification (2, 37, 38) and posttranscriptional regulation must compensate for the lack of transcriptional activation.

For VSG, however, these methods apparently do not suffice. African trypanosomatids must synthesize sufficient VSG to cover the plasma membrane, to shield essential invariant membrane components from the immune system, and to guard against activation of complement (10). Other members of the order Kinetoplastida have surface glycolipids, so that the synthesis of adequate quantities of VSG is dependent only upon a reasonable rate of enzymatic catalysis; they lack antigenic variation, and—despite the presence of trans splicing—show no evidence of mRNA production by polymerases other than polymerase II. Ten percent of bloodstream trypanosome mRNA encodes VSG, yet the constraints of antigenic variation require that all this must be synthesized by a single gene (10, 12, 25). The rate of synthesis of mRNA from a single VSG gene (equivalent to that from about 30 tubulin genes [30, 37]) may be unattainable by using conventional trypanosome RNA polymerase II. The use of RNA polymerase I for VSG transcription, along with the ability to cap the transcript through trans splicing, could therefore have been a prerequisite for the evolution of antigenic variation.

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