African trypanosomes evade the immune response of their mammalian hosts by switching the expression of their variant surface glycoprotein genes (vsg). The bloodstream trypanosome clone MVAT4 of *Trypanosoma brucei rhodesiense* expresses a monocistronic vsg as a monocistronic RNA from a promoter located 2 kilobases (kb) upstream of its start codon. Determination of 23 kb of sequence at the metacyclic variant antigen type 4 (MVAT) vsg expression site (ES) revealed an ES-associated gene (esag) 1 preceded by an ingi retroponon and an inverted region containing an unrelated vsg, short stretches of 70-bp repeats and a pseudo esag 3. Nuclear run-on experiments indicate that the 18-kb region upstream of the MVAT4 vsg promoter is transcriptionally silent. However, multiple members of different esag families are expressed from elsewhere in the genome. The MVAT4 vsg promoter is highly repressed in the procyclic stage, in contrast to the known polycistronic vsg ESs which undergo abortive transcription. Activation of the MVAT4 vsg ES occurs in situ without nucleotide sequence changes, although this monocistronic ES undergoes a pattern of base J modifications similar to that reported for the polycistronic ESs. The relative simplicity of the MVAT4 vsg ES and the uncoupled expression of the vsg and esags provide a unique opportunity for investigating the molecular mechanisms responsible for antigenic variation in African trypanosomes.

*Trypanosoma brucei rhodesiense*, which causes sleeping sickness in humans, is a subspecies of the flagellated parasitic protozoa commonly called African trypanosomes. These unicellular organisms survive in the hostile bloodstream environment of their host by periodically changing their protein coats, a process called antigenic variation. Each protein coat covers the entire outer surface of the parasite and is composed of about \(10^7\) copies of a unique variant surface glycoprotein (VSG). Although the vsgs are immunogenic during an infection, the time antibodies against a particular VSG are raised by the host immune system, a subpopulation of the parasites expressing a new VSG has appeared. Thus, the infection is prolonged and the parasite population as a whole manages to keep “one step ahead” of the host’s immune response (for recent reviews, see Refs. 1–8).

The life cycle of *T. brucei* alternates between a mammalian host and its transmission vector, the tsetse fly vector (9, 10). In the bloodstream of the mammalian host, each parasite expresses one of about 1000 VGS genes (vsg) (11). After a tsetse fly takes a blood meal from an infected animal, the ingested parasites differentiate into non-infective procyclic forms in the midgut of the fly and their VSG coats are replaced with an invariant glycoprotein coat composed of procyclins or procyclic acidic repetitive proteins (reviewed in Ref. 12). After about 3 weeks, the parasites migrate to the fly’s salivary glands, differentiate into highly infective, non-dividing metacyclic forms, and re-acquire a VSG coat. At this metacyclic stage, only one of about 15–20 different vsgs can be expressed (13, 14). Late in a bloodstream infection, these metacyclic variant antigen type (MVAT) vsgs can occasionally be re-expressed in the bloodstream parasites (2, 15–17).

Although the vsgs are scattered throughout the *T. brucei* genome, all expressed vsgs studied to date are located near the telomeres. These telomere-linked vsg expression sites (ES) are defined here to include the sequences that extend from the regions immediately upstream of the vsg promoters to the subtelomeric/telomeric repeats located downstream of the vsgs, *i.e.* the sequences encoding the primary transcription unit and its immediate flanking regions that might influence transcription initiation and termination. Only a few bloodstream vsg ESs have been examined in detail, and the conventional model of an ES is based primarily on a detailed characterization of one such locus, the ES for the AnTat 1.3A vsg (18). This bloodstream ES, and the few others that have been examined, contain a polycistronic transcription unit spanning 45–60 kb. Upstream of the transcription unit is a promoter preceded by several kilobases of 50-bp repeats. Downstream of the promoter is a minimum of eight ES-associated genes (esag), several kilobases of a 70-bp repeat and the vsg, followed by the subtelomeric and telomeric repeats (1). Hybridization data suggest that about 20 such bloodstream ESs occur in the *T. brucei* genome (19–21) and, presumably, a similar number of ESs for the metacyclic vsgs (22). Based on the limited characterization of several metacyclic vsg ESs (2, 23), these metacyclic ESs differ in several respects from the bloodstream vsg ESs. They are organized as short (3–5 kb) monocistronic transcription units and generally lack all or most of the 50-bp repeats, 70-bp repeats, and esags of bloodstream ESs. Although the extent of overlap between the bloodstream and metacyclic ESs remains unclear, under normal circumstances only one ES is fully activated at a given time (3, 24).

The vsgs are maneuvered into and out of the active ES by...
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**RESULTS**

**Physical Map of the MVAT4 vsg ES**

**Structure of the Active ES**—Previous screening of a λ FIX II library of genomic DNA derived from bloodstream MVAT4 trypanosomes resulted in identification of two overlapping phage clones, designated M4-g1 and -g2, that span the MVAT4 vsg ES (16). To construct a detailed physical map of this ES in its active state, we subcloned and sequenced the DNA inserts in these clones. The resulting 23 kb of contiguous DNA sequence extends from 21 kb upstream of the MVAT4 vsg ES to 290 bp downstream of its stop codon (Fig. 1).

It was previously shown that the promoter for the MVAT4...
vsg is located 2 kb upstream of the gene’s coding region, and that the start codon of an esag 1 occurs 3.9 kb further upstream of this promoter (16). GenBank searches of the newly sequenced portion of the 23 kb revealed several additional sequence elements upstream of the esag 1: a 5.2-kb ingi retroposon (37, 38), a 1-kb open reading frame (ORF) with no significant homology to known sequences in the data bases, a 1.4-kb ORF (designated ILTat 1.23), whose deduced amino acid sequence is highly similar, but not identical, to the ILTat 1.23 VSG (39), and a pseudo ESAG 3 gene (ψ esag 3) (40). In addition, two small regions of 70-bp repeats, which are thought to be involved in recombination events of at least some vsgs (7, 41, 42), were found. One region containing 2½ repeat units of 70 bp is located 0.6 kb upstream of the 1-kb ORF, and a single 70-bp unit is located 0.4 kb upstream of ILTat 1.23.

Some of the previously studied ingi elements are flanked by short (<10 bp) direct repeats which apparently result from a duplication of the integration site (37, 38, 43). The ingi shown in Fig. 1 is not flanked by any repeats. However, the 8.5-kb region upstream of the ingi appears to have been inverted. All of the sequences from this region that match sequences in the data bases are in a reverse orientation compared with that of the ingi and its downstream region including the MVAT4 vsg. A somewhat similar observation has been reported by Vassella et al. (44) where an apparent integration of an ingi into the basic copy of an expressed vsg resulted in a hybrid transcript composed of the ingi sense strand preceded by the vsg antisense strand.

A polymerase chain reaction fragment corresponding to the 1-kb unidentified ORF upstream of the ingi (probe C, Fig. 1) was used as a probe in Northern blots of total RNAs from the bloodstream MVAT4, MVAT7, and WRATat 1.1 trypanosomes but did not detect the presence of a corresponding RNA. When the same blots were stripped and reprobed for the MVAT4 vsg, histone H4 RNAs as controls, the expected signals were readily detected (not shown). In addition, no positive clones were identified when the same probe was used to screen about 100,000 clones in a bloodstream MVAT4 cDNA library. Thus, if an mRNA of this region occurs, it is too rare to be detected by these techniques.

The discovery of a second VSG gene, ILTat 1.23, in this region was unexpected. When 1500 randomly picked expressed sequence tag clones from an MVAT4 cDNA library were sequenced, 3 different expressed sequence tags were found to contain ILTat 1.23 vsg-like sequences (45). However, an alignment of the nucleotide sequences showed that none of these expressed sequence tags were derived from the ILTat 1.23 vsg near the MVAT4 vsg ES. Consistent with this observation, Southern blot experiments indicated that ILTat 1.23-like sequences are present in multiple copies in the MVAT4 genome (not shown), and nuclear run-on assays showed that this ILTat 1.23 is transcriptionally silent (see below).

Comparison of the Active and Silent MVAT4 vsg ES—To determine if there are any sequence differences between the active and silent versions of the MVAT4 vsg ES, we first conducted an extensive Southern blot analysis of this region using genomic DNAs from the expressor trypanosomes (MVAT4) and two bloodstream trypanosomes, MVAT7, and WRATat 1.1, in which the MVAT4 vsg ES is silent. The WRATat 1.1 trypanosome clone is the progenitor of the other two clones. Southern blot analysis of the region downstream of the esag 1 showed no differences between the active and silent ESs (data not shown), and confirmed earlier findings that this region is present in a single copy in all three genomes (16). Southern blot analyses of the region upstream of the ingi also did not show any differences among the three genomes. However, these blots did indicate that, in contrast to the region downstream of the esag 1, this 8.5-kb upstream region is present in multiple copies in all three genomes. One example of these Southern blots is shown in Fig. 2. Genomic DNAs from the bloodstream trypanosome clones MVAT4 (lane 4), MVAT7 (lane 7), and WRATat 1.1 (lane 1) were digested with the indicated restriction enzymes and probed with a 0.5-kb fragment containing the sequence upstream of the ψ esag 3 (probe A, Fig. 1). Lane M contains the DNA DNA markers.

![Fig. 2. At least three copies of the region upstream of ingi occurs in the genome. Genomic DNAs from the bloodstream trypanosome trypanosomes MVAT4 (lane 4), MVAT7 (lane 7), and WRATat 1.1 (lane 1) were digested with the indicated restriction enzymes and probed with a 0.5-kb fragment corresponding to the extreme 5' sequence in the physical map (probe A, Fig. 1). Because none of the four examined restriction sites occur within the probe fragment, these data indicate there are at least three similar copies of this upstream region in the MVAT4, MVAT7, and WRATat 1.1 genomes. Furthermore, the hybridization patterns for the BglII digestions suggest that this sequence similarity continues about an additional 10–15 kb upstream of the extreme 5' region shown in Fig. 1. Similar blots probed with other fragments from this inverted 8.5-kb region indicated that these sequences or their homologues are also repeated at least three times in the genome, and in one case, ψ esag 3, more than three times (not shown).

These genomic Southern blots indicated there are no gross changes in the organization of the active and silent MVAT4 vsg ES. However, this analysis does not detect point changes or other small differences outside the restriction sites. Thus, we determined if there were any changes in the DNA sequence of this ES not detectable by Southern blot analysis. To examine the sequence of the MVAT4 vsg ES in its silent state, a λ FIX II library of genomic DNA derived from WRATat 1.1 bloodstream trypanosomes was constructed. This genomic library was probed with a 1.6-kb AccI-SacI fragment containing the region upstream of the MVAT4 vsg and 130 bp of its coding region (probe E, Fig. 1). This screening resulted in identification of two overlapping phage clones, designated W1.1-g1 and W1.1-g2. End sequencing and NotI digestions of DNAs from these clones indicated that clone W1.1-g2 contains only one 13-kb NotI insert, whereas clone W1.1-g1 contains a 14.5-kb insert composed of 12- and 2.5-kb NotI fragments (Fig. 1). These NotI fragments were directly subcloned into pBluescript vectors and sequenced. A comparison of these sequences with the sequence derived previously from parasites expressing the MVAT4 vsg showed that, not only is the overall genomic organization of the region encompassing the MVAT4 vsg ES the same in its active and silent states, but also that it is highly conserved at the level
of nucleotide sequence. Only two single nucleotide changes from the previous sequence were detected in the 18-kb overlapping regions, one at nucleotide position 12,825 in clone W1.1-g2 and the other at nucleotide position 18,200 in clone W1.1-g1. These two differences are unlikely to bear any significance, especially since they are not conserved between the W1.1-g1 and -g2 clones, which are both derived from the same genome.

**Transcriptional Map of the MVAT4 vsg ES**

Previous work from our laboratory showed that there is a gap in transcription between the vsg and the esag 1 shown in Fig. 1. UV irradiation experiments suggested that the esag 1 is at the end of a large polycistronic precursor transcript, whereas the MVAT4 vsg is on a short transcript (16). Using subcloned fragments spanning the MVAT4 vsg ES, we further investigated these early findings and constructed a detailed transcription map for this ES.

pBluescript subclones containing fragments spanning the MVAT4 vsg ES were digested with appropriate restriction enzymes and used in nuclear run-on assays to map the transcribed regions (Figs. 1 and 3A, left panel). A comparison of the middle panel in Fig. 3A with the three panels in Fig. 3B shows that, except for the MVAT4 vsg transcript, the transcription pattern of this region is the same in MVAT4 parasites and those expressing other VSGs or procyclin. Furthermore, these data indicate that both the esag 1 (fragment b) and the region upstream of the ingi (fragment g) are transcriptionally silent (Fig. 3A, middle panel). Upon re-examination of the earlier data (16), we discovered that the DNA fragment which appeared to give rise to a transcription signal for the esag 1 contained 1.6 kb from the 3′ end of the ingi element in addition to the esag 1 coding region. Therefore, the hybridization signal that originally appeared to represent transcription of esag 1 was in fact the result of the associated upstream ingi sequence. Further experiments confirmed this conclusion and prompted a more extensive study of the differential expression of the ESAG 1 gene family in this serodeme, which has been reported earlier (46). This study showed that at least 20 different ESAG 1 genes are expressed in the bloodstream MVAT4 trypanosome clone, none of which is the esag 1 at the MVAT4 vsg ES.

The T. brucei genome contains as many as 400 copies of ingi (38). It has been shown previously that ingi is highly transcribed from both strands (38). Since these transcripts are heterogeneous in size with the majority being greater than the 5.2-kb size of the element itself, they are thought to initiate from external upstream promoters (37, 38, 47). Because the regions flanking the ingi upstream of the MVAT4 vsg promoter appear to be transcriptionally silent, we suspect that the signal for ingi present in our run-on assays comes from elsewhere in the genome. This view is further supported by the following lines of evidence.

Murphy et al. (38) have shown that in the T. brucei AnTat 1.3A clone ingi transcription is only moderately sensitive to α-amanitin at 1 μg/ml. In contrast, in the MVAT4 genome, ingi transcription is highly sensitive to α-amanitin (Fig. 3A, right panel). This apparent discrepancy actually supports the idea that ingi elements rely upon their fortuitous locations downstream of external promoters for their transcription.

Additionally, in contrast to the MVAT4 vsg, transcription of ingi is very sensitive to UV irradiation (Fig. 3C), which results in formation of pyrimidine dimers in the DNA. Because of the inability of RNA polymerases to traverse these dimers, transcription is increasingly impeded as it extends further away from the promoter (31). The middle panel in Fig. 3C shows a dramatic drop in ingi transcription when MVAT4 trypanosomes are exposed to even a low dose (10 μJ/cm²) of UV irradiation. This result indicates that the vast majority of the ingi transcripts are derived from distant upstream promoters in the MVAT4 genome.

Finally, when a 0.6-kb fragment from ingi (probe D, Fig. 1) was used to screen 60,000 phage clones in the MVAT4 cDNA library, three ingi-related cDNAs were identified. Partial sequence determination of these cDNAs revealed that they have differing 3′ sequences, none of which are derived from the ingi upstream of the MVAT4 vsg promoter (not shown). Thus, although we cannot completely exclude the possibility that this ingi may be transcribed at a very low level from an internal promoter, it is clear that expression of this specific ingi element is not coupled to that of the MVAT4vsg. Transcription at the MVAT4 vsg ES is exclusive to the vsg unit itself.

**Expression of ESAG Gene Family Members in MVAT4**

**ESAG 3 Gene Family—**Since at least 20 different esag 1 family members are expressed in the bloodstream MVAT4 trypanosome clone (46), we investigated whether multiple members of the esag 3 family are also expressed in this same clone. Consistent with our nuclear run-on data, Northern blot experiments using a 0.5-kb BglII-XhoI fragment (probe B, Fig. 1) from the ψ esag 3 upstream of the MVAT4 vsg promoter did not detect any signal for an RNA species of this particular esag 3, whereas the signals for the MVAT4 VSG and histone H4 mRNAs were readily detected on the same blots (not shown). However, when probe B was used to screen an MVAT4 cDNA library, four positive clones were identified in 60,000 phage clones. Sequence analysis of these cDNAs (submitted to the GenBankTM/EMBL Data Bank under accession numbers AF068694–97) confirmed that each represented a different member of the ESAG 3 gene family, and that none were derived from the ψ esag 3 upstream of the MVAT4 vsg promoter (not shown). These esag 3 sequences do not possess substantive similarity with other sequences in the data base, and the function(s) and cellular location(s) of their protein products are not known.

**ESAG 6 and 7 Gene Families—**The closely related products of the ESAG 6 and 7 genes form a heterodimeric transferrin receptor that is essential for the survival of bloodstream trypanosomes (48, 49). These genes are found in pairs near the promoters of the bloodstream polycistronic vsg ESs and form the basis for one prediction on why multiple bloodstream ESs are necessary (3, 50). Although esags 6 and 7 do not occur in the MVAT4 vsg ES, screening of the MVAT4 cDNA library showed that, similar to the esag 1 and 3 families, many different members of the esag 6 and 7 families are expressed in the MVAT4 clone (not shown). Table I shows the relative abundance of the different ESAG cDNAs identified in the MVAT4 cDNA library. For the ease of comparison, these values are normalized in the table to those present relative to 1000 MVAT4 vsg cDNAs after being rounded to the nearest integer.

**Experimental Examinations of Two Models on VSG Gene Regulation**

We used the nucleotide sequence of the MVAT4 vsg ES and its upstream region to examine two of the current models in the literature for the regulation of ESs, one concerning the inactivation of all but one ES in the bloodstream parasites (35, 36) and the other addressing ES inactivation in procyclic forms (51–53).

**The DNA Base Modification Model—**The presence of a modified DNA base, β-D-glucosylhydroxymethyluracil (also called J), and around the telomeric vsgs has been found to correlate with inactivation of the corresponding ESs in the bloodstream trypanosomes (35, 36, 54–58). The base J modification causes
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A

![EtBr](image)

- α-amanitin

(0.2 mg/mL)

B

![WRATat 1.1](image)

![MVAT 7 Rx](image)

Procylic

C

![EtBr](image)

10 mJ/cm²

50 mJ/cm²
partial resistance to cleavage by some restriction endonucleases including HindIII, PstI, and PvuII (35, 36). Fig. 4 shows Southern blots of genomic DNAs from bloodstream MVAT4, MVAT7, and WRATat 1.1 trypanosomes that were digested with the three enzymes mentioned above. The probe used in the Southern blots shown in panel A is a 1.6-kb AccI-SacI fragment (probe E, Fig. 1). Panel B shows an autoradiogram of the same filter that has been stripped and reprobed with a 0.9-kb fragment spanning the HindIII and PvuII sites (probe F, Fig. 1). In panel A, since the restriction sites are outside the region of the probe, one would expect in a complete digest to detect only a 5-kb fragment and a 5-kb fragment in the HindIII and PvuII digests, respectively. In the HindIII and PvuII panels of Fig. 4A only those lanes containing nonexpressor DNAs (lanes 7 and 1.1) show signs of partial resistance to cleavage. The high molecular weight bands in lanes 7 and 1.1 superimpose on the higher telomeric fragment bands of the corresponding lanes from panel B. The presence of these bands is consistent with the possibility that the MVAT4 vsg ES is partially modified in its inactive state. In the case of the PstI digest, since neither of the two probe fragments contains a PstI site, a complete digest should result in detection of a single fragment of 5 kb. However, once again, the presence of the high molecular weight fragments in lanes 7 and 1.1 indicates that the PstI site which occurs in the vsg coding region (PstI number 1, Fig. 1) is partially resistant to digestion when this ES is in a silent state. The 10-kb bands in the PstI digests in panels A and B are likely the result of modifications at the PstI site located 1.4 kb upstream of the MVAT4 vsg promoter (PstI number 2, Fig. 1). It appears that this site is even more heavily modified in the MVAT4 genome (lane 4) than in the nonexpressor genomes (lanes 7 and 1.1). It is important to note, however, that this particular PstI site is located upstream of the promoter sequence, in a region that is transcriptionally silent (Fig. 3). These observations are consistent with observations from another laboratory on distribution of J in the polycistronic bloodstream vsg ESs (57). Thus, these data indicate that the monocistronic metacyclic vsg ESs undergo similar DNA modifications as those observed for the large polycistronic ESs.

The Abortive Transcription Model—It has been proposed that in the procyclic stage of African trypanosomes all bloodstream vsg promoters are functionally active, but transcription is attenuated, or aborted, about 700 nucleotide downstream of the promoters (55). We examined whether transcription of the MVAT4 vsg ES was under similar control in procyclic parasites. Fig. 5 shows nuclear run-on experiments using nuclei from bloodstream and procyclic trypanosomes, respectively. Fragment k contains the promoter sequence (see Fig. 1). These run-on data indicate that the MVAT4 vsg promoter is highly repressed in the procyclic parasites. No hybridization to fragment k could be detected, even under much longer exposure times than that shown in Fig. 5. Similar results were obtained for two other metacyclic vsg promoters, i.e. the MVAT5 and MVAT7 vsg promoters (not shown). Thus, in the procyclic stage, the MVAT vsg ESs seem to be exclusively regulated at the level of transcription initiation, in contrast to bloodstream vsg ESs. Since restriction fragments containing these three MVAT vsg promoters possess extensive promoter activity when placed upstream of a reporter gene on a plasmid and transiently transfected into the procyclic trypanosomes (16, 17, 59), this regulation must be a function of their chromosomal context rather than their specific sequences.

DISCUSSION

Despite the fact that antigenic variation in African trypanosomes has been the subject of intensive investigation for the past two decades, the underlying molecular mechanisms that directly turn the vsg ESs on and off remain a mystery. Many documented examples of vsg switches are accompanied by DNA recombination events at or near the ESs. Systematic investigations of vsg switching both in vitro and in vivo, however, have shown that while such DNA rearrangements can lead to the (in)activation of an ES, they are not a requirement (21, 26). Some and perhaps all ESs in the T. brucei genome can be (in)activated in situ without attendant recombination.

To date, the MVAT vsg remains the only metacyclic vsg reported whose re-expression in the bloodstream stage is not associated with a gene conversion. However, we suspect that this rare occurrence is not because of an inherent inability of the metacyclic vsg ESs or their promoters to be activated in bloodstream form trypanosomes, as has been suggested by some investigators (23, 60). Rather, we suggest that under usual circumstances, the re-activation of a metacyclic vsg ES is likely to go unnoticed because: (i) the corresponding parasites are likely to be cleared by the immune response of a host that has been exposed to the metacyclic organisms during the initial phase of the infection; (ii) trypanosomes expressing a monocistronic ES and a diverse esag repertoire, will be at a growth disadvantage compared with those organisms expressing a polycistronic ES containing a specific set of the esags suitable for that host (3, 50); and (iii) parasites expressing low affinity transferrin receptors (ESAGs 6 and 7) are likely to be selected against by the host immune response (61). It is worth recalling that the bloodstream trypanosomes re-expressing any of the MVAT vsgs were initially detected as a very small fraction (i.e. much less than 1%) of the population and cloned only after several rounds of sequential enrichment (15, 16).

All of our data indicate that the MVAT4 vsg ES is turned on in situ, i.e., the MVAT5 and MVAT7 promoters, are not a requirement (21, 26).
and off in situ without a DNA rearrangement or a change in the nucleotide sequence of either the ES or its upstream region. Similar to other examples of in situ ES switches reported in the literature, however, we cannot completely exclude that DNA rearrangements or nucleotide differences outside the examined region may be responsible for the switch. Although it is conceivable that such changes occur far upstream of the promoter or downstream of the vsg in the telomeric repeats, our observations suggest that (in)activation of the MVAT4 vsg ES involves an epigenetic mechanism (i.e., it is dependent upon changes in the chromosomal context that are not sequence specific).

The discovery of the modified DNA base J and its enhanced presence in and around the vsgs in silent ESs of the bloodstream form T. brucei led to suggestions that J could be responsible for silencing of the vsg ESs (35, 36). Although upon repression, the MVAT4 vsg ES appears to undergo a similar DNA modification pattern as that ascribed to J (i.e., the MVAT4 vsg shows signs of modification only in its silent state), it seems unlikely that J is the cause of silencing. First, J is not exclusive to trypanosomes, but is also present in the genomes of other kinetoplastida, such as Leishmania and Crithidia, which do not undergo antigenic variation (62). Second, the bulk of J is found in telomeric repeats downstream of the vsg and in the 50-bp repeats upstream of the ES promoters regardless of the activity of the ES (58, 63). Third, J is not found in the DNA of procyclic trypanosomes where all of the vsg ESs are silent (54). Recently, Van Leeuwen et al. (58) reported that experimental clearance of J from the silent ESs resulted in only a partial activation of the ES promoters, whereas forced incorporation of J into the active ES did not result in its repression. However, the increase in the J content did lead to a significant reduction in ES switching events accompanied by DNA recombination. These investigators concluded that it is more likely that J acts as a stabilizing factor after the repression of ESs rather than serving as the cause of silencing.

The procyclic nuclear run-on data shown in Fig. 5 differ from the results of Rudenko et al. (53) who showed that in procyclic trypanosomes the polycistronic bloodstream ES promoters are moderately active but that this transcription is aborted about 700 nucleotides downstream. In contrast, we did not detect any transcription from the MVAT4, 5, and 7 vsg promoters in procyclic organisms. For example, in Fig. 5 no hybridization of procyclic run-on RNA occurs to fragment k which contains the MVAT4 vsg promoter sequence. Likewise, no procyclic RNA hybridization was detected to fragments located between the MVAT5 or MVAT7 vsgs and their respective promoters (not shown). The most notable difference between the three vsg promoters we investigated and those examined by Rudenko et al. (53) is that our promoters, at only 2 kb upstream of their vsgs, are 45 kb closer to the telomere than are their promoters. Thus, if a form of telomere silencing similar to that described in yeast (64–66) contributes to the control of the vsg ESs, promoters close to a telomere might experience a tighter silencing in procyclic organisms than promoters located 45 kb further upstream. Further support for this conclusion comes...
from work by Graham and Barry (60) who likewise did not detect abortive transcription in procyclic organisms from another metacyclic vsg with a nearby promoter.

Why do African trypanosomes possess many ESs and why are there two distinct sets of ESs with respect to mono- and polycistronic transcription? Our observations support the proposal by Borst et al. (3) that the answer to both of these questions may rely in part upon expression of the ESAGs rather than the VSGs. Bloodstream MVAT4 trypanosomes may be reminiscent of the normal situation in metacyclic organisms. Rather than the VSGs, Bloodstream MVAT4 trypanosomes may rely in part upon expression of the ESAGs from work by Graham and Barry (60) who likewise did not find complete ESAG products in MVAT4 bloodstream trypanosomes.

The relative simplicity of the MVAT4 vsg gene expression site may be the same as that which fully activates one and only one vsg from the polycistronic transcription? Our observations support the proposal by Borst et al. (3) that the answer to both of these questions may rely in part upon expression of the ESAGs rather than the VSGs. Bloodstream MVAT4 trypanosomes may be reminiscent of the normal situation in metacyclic organisms. Rather than the VSGs, Bloodstream MVAT4 trypanosomes may rely in part upon expression of the ESAGs from work by Graham and Barry (60) who likewise did not find complete ESAG products in MVAT4 bloodstream trypanosomes.

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