MOLECULAR AND CELLULAR BIOLOGY, June 1986, p. 1991–1997
0270-7306/86/061991-07$02.00/0

Vol. 6, No. 6

Metacyclic Variant Surface Glycoprotein Genes of *Trypanosoma brucei* subsp. *rhodesiense* Are Activated In Situ, and Their Expression Is Transcriptionally Regulated

MICHAEL J. LENARDO,[1†] KLAUS M. ESSER,[2] ANNE M. MOON,[1] LEX H. T. VAN DER PLOEG,[3] AND JOHN E. DONELSON[1*]

Department of Biochemistry, College of Medicine, University of Iowa, Iowa City, Iowa 52242[1]; Department of Immunology, Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20307[2]; and Department of Genetics and Development, College of Physicians and Surgeons, Columbia University, New York, New York 10032[3]

Received 3 January 1986/Accepted 17 March 1986

During the metacyclic stage in the life cycle of *Trypanosoma brucei* subsp. *rhodesiense*, the expression of variant surface glycoproteins (VSGs) is restricted to a small subset of antigenic types. Previously we identified cDNAs for the VSGs expressed in metacyclic variant antigen types (MVATs) 4 and 7 and found that these VSG genes do not rearrange when expressed at the metacyclic stage (M. J. Lenardo, A. C. Rice-Ficht, G. Kelly, K. Esser, and J. E. Donelson, Proc. Natl. Acad Sci. USA 81:6642–6646, 1984). We now provide further evidence that these genes do not rearrange and demonstrate that their 5′ upstream regions lack the 72 to 76-base-pair repeats which are considered the substrate for duplication and transposition events. Pulsed field gradient electrophoresis showed that the MVAT VSG genes were located on the largest chromosome-sized DNA molecules, and the lack of the MVAT 4 gene in one of two different serodemes suggested that one mechanism for the evolution of MVAT repertoires is gene deletion. When MVATs were inoculated into the bloodstream of a mammalian host by a bite from the insect vector, they rapidly switched into nonmetacyclic VSG types. We found that this switch was accomplished by a loss of MVAT RNA concomitant with the loss of metacyclic VSGs. Transcription studies with isolated metacyclic nuclei showed that the MVAT genes were expressed in situ from a single locus and were regulated at the level of transcription.

Antigenic variation in African trypanosomes is achieved by sequential activation of different genes from a large repertoire which encode the major surface component, the variant surface glycoprotein (VSG). The order of expression of the VSG genes appears to be more or less random in the mammalian bloodstream (4, 10). When the parasite is ingested by its insect vector, the tsetse fly, it loses its surface coat in the midgut stages and regains it after migrating to the salivary gland. During this final developmental stage in the inset salivary gland, called the metacyclic stage, expression of VSG genes is distinctly nonrandom (7, 17). Of several hundred VSG genes in the genome of the parasite, a restricted subset of 10 to 15 metacyclic variant antigen types (MVATs) is repeatedly expressed regardless of the antigenic type ingested by the fly.

The MVATs which arise in the salivary gland of the fly are the first organisms to enter the mammalian bloodstream (14). The expression of MVATs is different from that of bloodstream VATs because they are very unstable when growing in the bloodstream. Approximately 5 days following the bite from the fly, MVATs proliferating in a warm-blooded host rapidly change to nonmetacyclic VATs by an unknown mechanism (14). Previously, we isolated cDNAs for two MVAT VSG genes of *Trypanosoma brucei* subsp. *rhodesiense* (17). We found that the genes encoding these MVATs, unlike bloodstream VATs, did not rearrange when expressed at the metacyclic stage. We now address several questions about these genes. (i) Are these VSGs expressed in situ from single loci? (ii) Why do these genes not undergo typical duplication and transposition events when expressed at the metacyclic stage? (iii) How is the switch from metacyclic to bloodstream VSGs at day 5 accomplished at the molecular level?

MATERIALS AND METHODS

Trypanosomes. The geneaology of *T. brucei* subsp. *rhodesiense* clones WRATat1.1, WRATat1.14, and day 5 metacyclics (organisms bearing metacyclic VSGs taken from the bloodstream of a mouse 5 days after infection with salivary gland metacyclics), and RNA and DNA isolation have been described previously (17). Procyclic culture forms were made by inoculating trypanosomes into BSM medium (8) supplemented with 3 mM *cis*-aconitate and 1 mM pyruvate (G. Hill, personal communication) and incubating at 25°C for either 10 or 30 days. Conversion to procyclic forms was monitored by microscopic morphology, and loss of surface coat was determined by immunofluorescent assay (14). Indirect immunofluorescent assays and selective neutralization of metacyclic populations with MVAT-specific monoclonal antibodies have been described previously (17).

Recombinant DNA. Procedures for Southern blots have been described previously (17). Briefly, each lane of the genomic Southern blots contained 2.5 μg of total trypanosome DNA restricted with various endonucleases, electrophoresed on 0.9% agarose gels, and transferred to nitrocellulose by standard procedures (19). Filters were hybridized in 4× SET buffer (1× SET is 0.15 M NaCl, 30 mM Tris hydrochloride, pH 8.0, and 2.0 mM EDTA) containing 0.1% sodium dodecyl sulfate, 0.1% sodium pyrophosphate, 1 mg of salmon sperm DNA per ml, 1× Denhardt solution (19),

* Corresponding author.
† Present address: Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142.

and 4×10^6 cpm of nick-translated probe (specific activity, 0.5×10^8 cpm/μg or greater) at 65°C for 12 to 20 h. Filters were washed in 4× SET for 1 h and either 0.1× SET for 2 h with two changes at 65°C for high stringency or 1× SET at 50°C for 2 h with two changes for low stringency. Cloning of genomic regions was done by established methods (11, 19). Bacteriophage lambda clones of the upstream regions were isolated from a λ Charon 4A library of *T. brucei* subsp. *rhodesiense* (kindly provided by William J. Murphy) by screening 50,000 plaques by the method of Benton and Davis (2). Pulsed-field gradient (PFG) gel electrophoresis was performed generally as described previously (12, 21) and blotted as described above. Autoradiography was done on Kodak XAR film with a Lightning-Plus intensifying screen (Du Pont) at −70°C.

RNA analysis. RNA dot-blots were done by the method of Thomas (22). Runoff transcription assays used nuclei isolated from trypanosomes freshly harvested from infected blood by DE-52 chromatography (16). The method for nuclei transcription has been described previously (12). Hybridization of labeled RNA to DNA immobilized on nitrocellulose filters was done by a previously established procedure (12). Control experiments with this system showed that hybridization of transcripts to genes immobilized on nitrocellulose is strand specific for the coding strand (data not shown).

RESULTS

Comparison of MVAT genomic loci in expressers and nonexpressers. A unique feature of the MVAT 4 and MVAT 7 VSG genes is that they do not rearrange when expressed at the metacyclic stage. Figure 1A compares DNA from WRATat1.1 bloodstream organisms which gave rise to the MVAT population, a metacyclic population enriched for MVAT 7 (17), and WRATat1.14, a bloodstream organism which arises early after metacyclic infection digested with *Cla*I or *Eco*RI. No rearrangement was observed in more than 22 kilobases (kb) of sequence flanking the MVAT 7 gene from the *Cla*I site ca. 8 kb upstream to the telomere ca. 14 kb downstream (Fig. 1D). These results suggest that neither duplication and transposition nor nonduplicative rearrangement is associated with expression of the MVAT 7 gene at the metacyclic stage. Similar results have previously been reported for MVAT 4 (17).

This pattern suggests that MVAT genes are activated in situ without rearrangement. Since the MVAT populations were amplified for 5 days in immunosuppressed mice, it was possible that a duplicated copy of the MVAT gene had been lost. However, it was not possible to obtain enough organisms enriched for a particular MVAT directly from tsetse fly salivary glands to analyze genomic DNA. Therefore, to examine the structure of the MVAT genes in organisms at an earlier stage in the life cycle, WRATat1.1 organisms were transformed into procyclic culture forms in vitro. Procyclic forms are the stage prior to metacyclic expression, and previous studies have shown that culture forms do not differ in VSG gene shutoff from procyclic forms isolated from the insect midgut (9). Southern blots were done to compare genomic DNA from WRATat1.1 bloodstream organisms with their derivative procyclic forms (Fig. 1B and C). No rearrangements were detected at either the MVAT 7 locus (Fig. 1B) or the MVAT 4 locus (Fig. 1C). Each gene structure was the same as that in day 5 metacyclic organisms. Therefore, no rearrangement occurred at the procyclic stage that set up the MVAT genes for expression. This supported but did not prove the hypothesis that the metacyclic genes are expressed in situ. A slight deletion of DNA occurred at the MVAT 4 gene telomere in passage to the procyclic stage (Fig. 1C). In further experiments, we found no evidence for the previously reported modification of nucleotides affecting *Pvu*II or *Pst*I restriction sites (3) when comparing expressers and nonexpressers (data not shown).

Upstream regions of MVATs 4 and 7 do not possess repeats. Detailed restriction mapping of genomic subclones of the flanking regions of the MVAT genes (Fig. 1D) indicated that unlike bloodstream VSG genes, the MVAT 4 and MVAT 7 loci did not contain an obvious upstream barren region devoid of restriction sites (17). Previously it has been shown that the barren region which precedes many, if not all, rearranging VSG genes is composed of imperfect 72 to 76-base-pair (72/76-bp) repeats which provide homologous regions between expression sites and basic copy genes for gene conversion events (4–6, 18). This suggested that the MVAT genes do not undergo typical duplication and transposition events associated with expression at the metacyclic stage because they lack the appropriate molecular substrate for these rearrangements. Figure 2 shows a Southern blot of cloned DNAs from the upstream regions of MVAT 4 (lane A) and MVAT 7 (lane B) hybridized under low-stringency conditions which would detect one copy of the 72/76-bp repeat. No hybridization to a radiolabeled DNA clone containing a tandem array of 17 copies of the 72/76-bp repeat was detected. To further investigate the nature of the upstream region of the MVAT 7 gene, two phage clones, λAMM-1 and λAMM-4, were isolated from a *T. brucei* subsp. *rhodesiense* library which extended 16 kb upstream from an *Eco*RI site ca. 7 kb in front of the coding sequence. These two 16-kb inserts differed only in a single restriction site (unpublished results). Neither phage clone hybridized to the 72/76-bp repeat probe (Fig. 2, lanes C and D), establishing that there were no copies of the 72/76-bp repeat within 23 kb of the MVAT 7 coding region. Controls (lanes E through I) showed that as little as one copy of the 72/76-bp repeat would have easily been detected under these conditions. Therefore, the reason for the normal random distribution of restriction sites in the regions upstream of MVATs 4 and 7 genes is that they lack the 72/76-bp repeats typically found within 2 to 4 kb upstream of VSG genes expressed at the bloodstream stage. This lack of 72/76-bp repeats was also striking because WRATat1.1 has an abundance of these repeats in its genome (Fig. 1B) and genomic clones of other VSG genes with 72/76-bp regions located within 2 kb were easily isolated from WRATat1.1 (data not shown).

Chromosomal location of MVAT genes. To determine the location of the MVAT genes in the trypanosome karyotype, we used electrophoresis to separate chromosome-sized DNA molecules and blotted the DNA to nitrocellulose (12, 21, 23). Figure 3 shows hybridization of the filters to probes containing the coding sequences of MVAT 7 and MVAT 4. Populations of trypanosomes enriched for MVAT 7 or MVAT 4 and unselected metacyclics hybridized only to a single size class of DNA, and presumably a single gene for either MVAT 4 or MVAT 7 which was apparently unrearranged between expressers and nonexpressers. No minor bands were detected in the unselected metacyclic lanes, suggesting that rearrangements do not occur in organisms expressing other MVATs. This further supports the conclusion that metacyclic genes are expressed in situ without chromosomal rearrangements discernible by this technique.

Also tested was a mixed metacyclic population, LVH32, which was isolated 1 year later from the same geographic region as the isolate LVH18 from which the WRATat

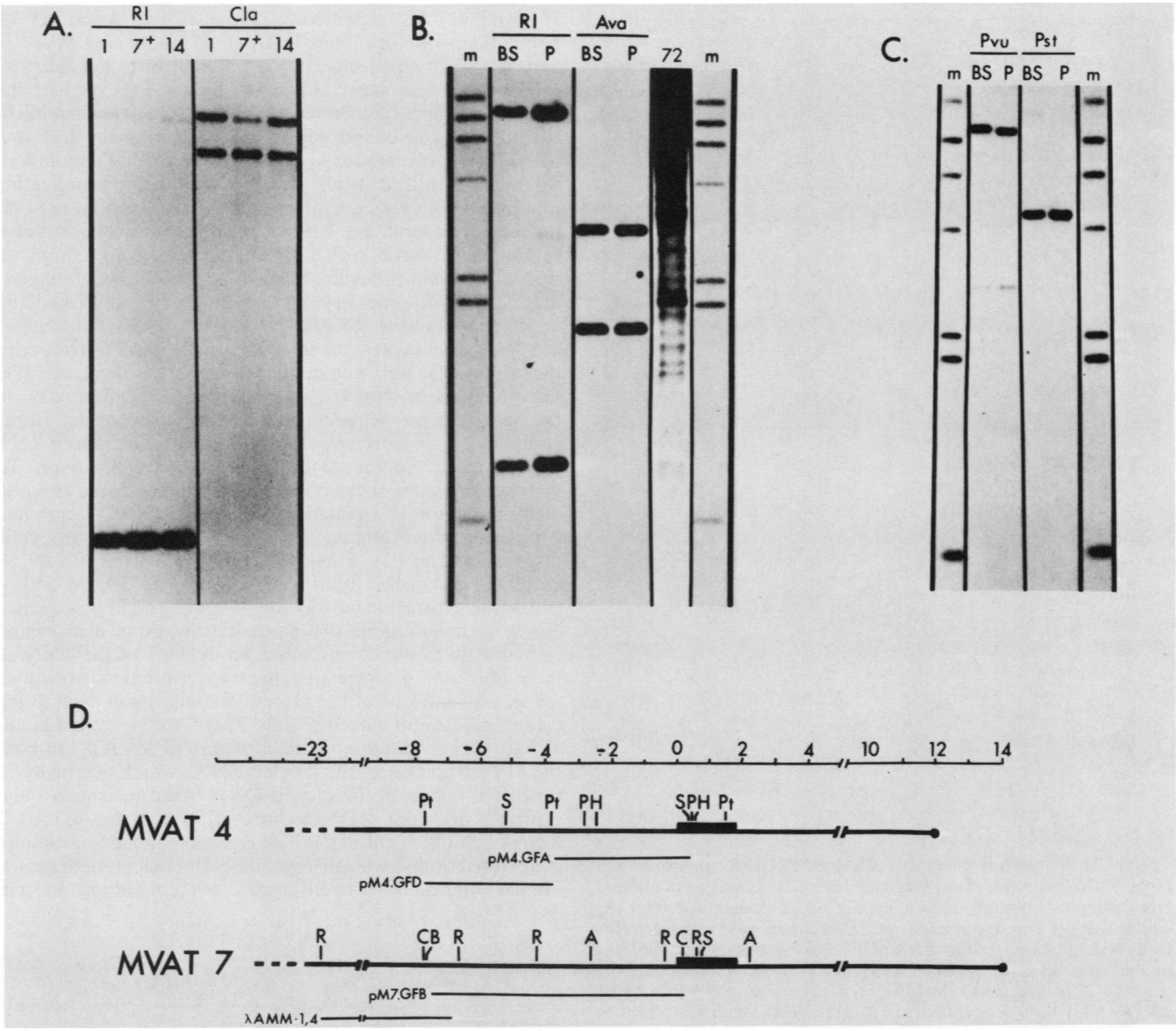

FIG. 1. Southern blot analysis of genomic DNA from *T. brucei* subsp. *rhodesiense*. Radioactive probes for the filters were MVAT 7 cDNA (plasmid insert 3L41 [17]) (A and C) and MVAT 4 cDNA (plasmid insert 2L11 [17]) (B). Lane 72 in panel B was probed with the genomic clone pGE117.6, containing the 72/76-bp repeats (5). Lanes: (A) total genomic DNA from *T. brucei* subsp. *rhodesiense* clones WRATat1.1, MVAT 7 enriched, and WRATat1.14 (lanes 1, 7+, and 14, respectively); (B and C) total genomic DNA from *T. brucei* subsp. *rhodesiense* clones WRATat1.1 and 10-day procyclic culture forms derived from these organisms (lanes BS and P, respectively); 72 is WRATat1.1 DNA cleaved with *Ava*I; m, molecular size standards (23.1, 9.4, 6.6, 4.3, 2.3, 2.0, and 0.56 kb from top to bottom). Autoradiographic exposures were between 2 and 5 days. (D) Restriction enzyme map of the metacyclic genomic loci. Genomic subclones which are discussed in this paper are indicated below each map. Cleavage sites for *Ava*I (A), *Bam*HI (B), *Cla*I (C), *Eco*RI (R), *Hin*dIII (H), *Pst*I (Pt), *Pvu*II (P), and *Sal*I (S) are shown. The scale at the top is in kilobases.

serodeme was cloned (14). This metacyclic population was found to contain ca. 50% MVAT 7 organisms but no longer expressed MVAT 4 in its repertoire (unpublished results). The MVAT 4 gene was no longer present in these organisms (Fig. 3), suggesting that one possible mechanism for the previously reported evolution of metacyclic repertoires in *T. brucei* subsp. *rhodesiense* (1) is deletion of MVAT genes from the genome.

Metacyclic switching in the bloodstream due to downregulation of the VSG mRNA. The switching of metacyclic VATs to bloodstream VATs at 5 days after a fly bite is due to individual trypanosomes changing the VSG composition of their surface coats (13). This suggests that the crucial event in the regulation of this switch in the stages of the life cycle occurs within individual trypanosomes and is not a modulation of preexisting trypanosome types within the population. To understand the molecular details of this switch, we compared organisms which at day 5 were still expressing predominantly metacyclic VATs to the same population which was allowed to propagate until day 7, when bloodstream VATs were nearly exclusively expressed. Table 1 shows the antigenic composition of three trypanosome populations, enriched for MVAT 4 and MVAT 7 and mixed (unselected), at days 5 and 7, as determined by immunofluorescent assay. While the total number of organisms increased in each population from day 5 to day 7, the

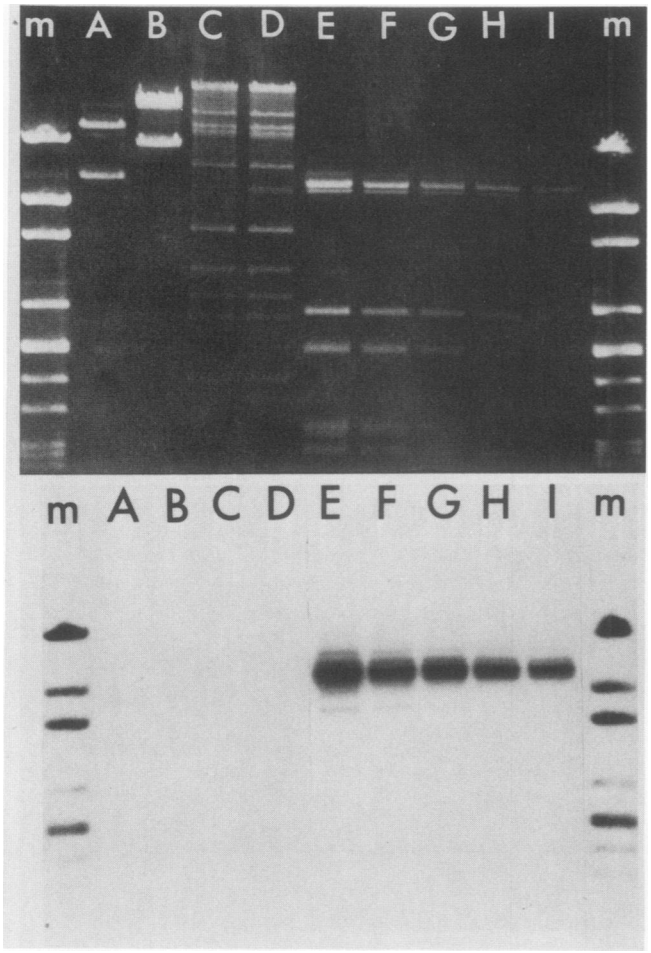

FIG. 2. Southern blot analysis of cloned DNAs for the presence of the 72/76-bp repeat. (Top) Ethidium bromide-stained gel of cloned DNA samples; (bottom) autoradiogram of a nitrocellulose filter to which the gel was transferred and hybridized with radioactively labeled purified insert from clone pGE117.6 (5) containing 17 copies of the 72/76-bp repeat. Lanes: (A) 2 μg of pM4.GFB, a clone of genomic DNA containing the 5.5-kb region between the *Sal*I sites in the MVAT 4 VSG gene shown in Fig. 1D digested with *Sal*I; (B) 4.5 μg of pM7.GFB digested with *Bam*HI and *Cla*I; (C and D) 4.5 μg of either λAMM-1 (C) or λAMM-4 (D) digested with *Ava*I; and (E through I) pGE117.6 digested with *Sau*3A and *Sal*I in the amounts 3.0 (E), 1.5 (F), 0.75 (G), 0.38 (H), and 0.16 (I) μg. To determine copy number, we assumed that if a band was observed with 2 μg of pM7.GFB that was as intense as that for 0.16 μg of pGE117.6, then approximately $(1.3 \times 0.17)/(2 \times 17) = 2$ copies would be present because the ratio of plasmid sizes is 8 to 6 kb, or 1.3, and pGE177.6 contains 17 copies of the 72/76-bp repeat. Since we observed no band even half as intense as that seen in lane I, there was less than one copy of the repeat on this plasmid. Similiarly, there was less than one copy on pM7.GFB and the λ clones since 4.5 μg of DNA was used and this autoradiogram and others exposed longer did not show bands even 1/10 as intense as that in lane E. Lanes m are molecular size standards (4,360, 2,250, 1,560, 910, 650, 510, 400, 250, 240, and 230 bp from top to bottom).

trypanosomes no longer expressed MVAT VSGs by day 7. An RNA dot-blot analysis of RNA in these populations is shown in Fig. 4. In a population enriched for MVAT 4, RNA which hybridized to MVAT 4 and not MVAT 7 coding sequences was found at day 5 but gone at day 7. Similar results were found for MVAT 7. Densitometric scanning showed that the MVAT 4 and MVAT 7 RNAs were reduced 200- and 30-fold, respectively, at day 7. The mixed MVAT population, which contained only small numbers of MVAT 4 and MVAT 7 organisms, did not significantly hybridize to the coding sequences. However, longer exposure of the hybridized filters (not shown) revealed some hybridization to MVAT 7 in the mixed population, reflecting the fact that MVAT 7 represented a low percentage of this population. Tubulin hybridized to all the RNA populations, indicating that approximately equivalent amounts of RNA were spotted for all preparations. Loss of specific transcripts was also found when RNAs from day 5 metacyclics were compared with those from procyclic forms (data not shown), suggesting that MVAT genes are not constitutively transcribed and are regulated at the RNA level.

MVAT gene expression controlled at the level of transcription. The dramatic fall in the steady-state level of VSG mRNA accompanying the loss of MVAT VSG after 5 days in the bloodstream or when converting to procyclic forms could be due to decreased transcription rate, increased RNA processing, or increased cytoplasmic RNA turnover. To distinguish among these possibilities, we compared the rate of transcription in isolated nuclei of an MVAT 4-enriched population of day 5 metacyclic organisms to that in procyclic organisms. The day 5 nascent transcripts from MVAT 4-enriched organisms hybridized strongly to the MVAT 4 coding sequence but not to the MVAT 7 coding sequence (Fig. 5). In addition, the nascent transcripts also bound significantly to the cloned upstream regions of the genomic locus of MVAT 4, suggesting that transcription may occur as far as 8 kb in front of the protein-coding region despite the lack of a 72/76-bp barren region. There was a small amount of hybridization to the upstream region of MVAT 7 in both the MVAT and procyclic labeled RNAs which mapped to a repetitive region in the cloned DNA (data not shown) and probably does not represent transcription at the MVAT 7 locus. An equivalent amount of ribosomal gene and tubulin gene transcription was observed in both nuclei preparations. Similar studies have been carried out comparing nascent

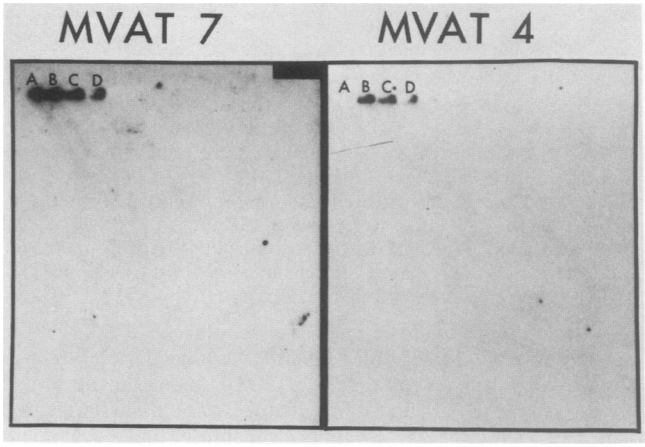

FIG. 3. PFG gel electrophoresis of chromosome-sized DNA molecules prepared from day 5 metacyclic organisms. Each panel is an autoradiogram of the same filter prepared from blotting a PFG gel onto nitrocellulose; the left was probed with MVAT7.GFA, a 900-bp MVAT 7 genomic subclone spanning the intragenic region between the *Bam*HI and *Cla*I sites (17; the right was probed with the MVAT 4 cDNA (17). Trypanosome samples were (A) LVH32 uncloned day 5 metacyclics, (B) MVAT 4-enriched day 5 metacyclics, (C) MVAT 7-enriched day 5 metacyclics, and (D) MVAT 5- and MVAT 7-depleted day 5 metacyclics.

TABLE 1. Antigenic composition of three trypanosome populations

Population	Monoclonal antibody	Fluorescence assay (% positive)[a]		No. of trypanosomes/ml[b]	
		Day 5	Day 7	Day 5	Day 7
MVAT 4 enriched	MVAT 4	83	0	5×10^6	3×10^7
	MVAT 7	0	0		
	MVAT 13	3	0		
MVAT 7 enriched	MVAT 4	0	0	1×10^7	2×10^7
	MVAT 7	90	0		
	MVAT 13	3	0		
Mixed	MVATs	>90	<10	1×10^7	2×10^7

[a] For MVAT 4- and MVAT 7-enriched populations, percentage of 1,000 organisms which give positive fluorescence with the monoclonal antibody specific for the indicated MVAT. Unless indicated, all other MVATs were <1% of each population. For the mixed population, percentage of organisms which reacted with any of a set of 14 monoclonal antibodies which define the metacyclic repertoire of WRATat1.1. (17).

[b] Total number of trypanosomes as determined by hemacytometer counts of dilutions of infected blood.

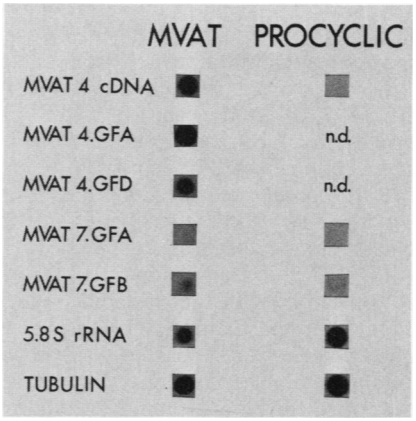

FIG. 5. Nuclear runoff assays with day 5 metacyclic trypanosomes and procyclic culture forms. Shown are filters spotted with various cloned DNAs and hybridized to [α-^{32}P]UTP-labeled total trypanosome RNA from isolated nuclei from either MVAT 4-enriched day 5 metacyclics (MVAT) or procyclic culture forms. DNAs are the MVAT 4 cDNA (plasmid 2L11 [17]), clones pM4.GFA, pM4.GFD, and pM7.GFB (Fig. 1D), and clone pM7.GFA, which is described in the legend to Fig. 3. The 5.8S rRNA was described previously (12). The tubulin clone is described in the legend to Fig. 4. n.d., not done. Hybridization was carried out with 3×10^6 cpm of labeled RNA for each filter.

transcripts from MVAT and bloodstream organisms; these also showed no transcription of the MVAT 4 or MVAT 7 genes at the bloodstream stage (data not shown). The observation that transcription of the MVAT 4 gene occurred in a population of organisms which only contained one gene for MVAT 4 strongly supports the conclusion that this gene is transcribed in situ at its single locus, which is not rearranged between expressers and nonexpressers.

DISCUSSION

The metacyclic stage of the African trypanosome life cycle is the only time there is a clear order to the expression of VSG genes. Each metacyclic parasite expresses one of about 15 VSGs, and that VSG gene is turned off approximately 5 days after entry into the bloodstream. To understand how the trypanosome repeatedly selects a very small subset of VSG genes for expression at the metacyclic stage, we investigated the molecular structure of two metacyclic VSG genes. We found that the two MVAT VSG genes did not rearrange when expressed at the metacyclic stage (17), in contrast to bloodstream VSG gene rearrangement during expression. This suggested that a possible mechanism for the selective expression of certain VSG genes at the metacyclic stage is to fix the metacyclic genes at expression loci which are active only at the metacyclic stage. The simplest form of this model is for each MVAT gene to reside at its own MVAT expression site which is specifically activated at the metacyclic stage. This model makes several predictions which we have investigated.

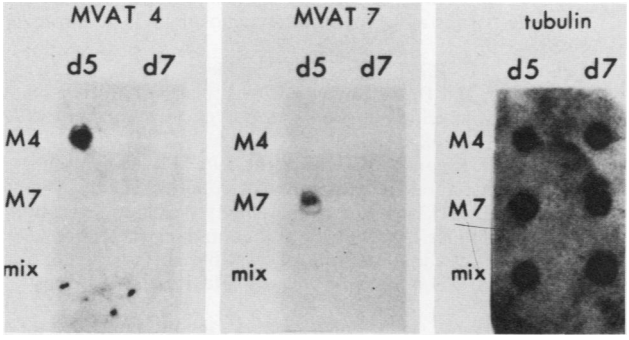

FIG. 4. RNA dot-blot analysis of total metacyclic trypanosome RNA. Lanes: d5, trypanosomes harvested at day 5; d7, trypanosomes harvested at day 7. The various trypanosome populations used were MVAT 4 enriched (M4), MVAT 7 enriched (M7), and a mixed population (mix). Identical filters were hybridized with the MVAT 4 cDNA (17); a 900-bp genomic DNA subclone described in the legend to Fig. 3 (MVAT 7 panel); the α- and β-tubulin genomic clone (15).

An important prediction of the model is that these genes should not rearrange during expression, since this could potentially move the MVAT genes to expression sites which would not be selectively activated at the metacyclic stage. We have shown here and in previous work that neither MVAT 4 or MVAT 7 genes are rearranged when expressed in day 5 metacyclic organisms (17). To address the issue of a potential rearrangement which precedes metacyclic expression and is then lost by day 5, we passaged WRATat1.1 organisms, which are the bloodstream forms from which the metacyclic organisms were generated, into the procyclic stage. Again we found no rearrangements. In addition, no large-scale chromosomal rearrangement between expressers and nonexpressers, as observed in trypanosomes at the bloodstream stage (4, 10), was detected by PFG gel electrophoresis. These findings, together with the transcriptional analysis discussed below, suggest that indeed no rearrangement is associated with expression of MVAT genes. Moreover, the results of restriction mapping and DNA sequencing (H. J. Son, personal communication) clearly establish that the MVAT 4 and MVAT 7 loci are different from each other and from the loci of other putative MVATs (unpublished results).

Another prediction of the model is that MVAT genes should not frequently undergo rearrangements which would move the MVAT genes to alternative expression sites, particularly those used at the bloodstream stage. We found that the lack of a 5′ barren region noted previously (17) is

significant in that these two MVAT genes do not contain typical 72/76-bp repeated motifs which are found within 3 kb upstream of the bloodstream VSG genomic loci which have been described (4–6, 18). In the case of MVAT 7 none can be found within a distance of over 20 kb in front of the VSG coding sequence. While it will be important to establish this as a general feature of all metacyclic VSG genes, the lack of these motifs in MVATs 4 and 7 is striking. Since these motifs have been clearly implicated in the gene conversion events associated with VSG gene expression (4, 5), the lack of these sequences is consistent with the possibility that rearrangement is not favored at metacyclic VSG gene loci. In addition, since both of these genes are apparently single copy, partial gene conversions within the protein-coding sequence which have been described for bloodstream VATs (20) would also not be favored.

A significant feature of the expression of metacyclic VSG genes is their rapid turn-off 5 days after metacyclic organisms enter the mammalian bloodstream. Esser et al. (13) have demonstrated that the switch occurs in individual organisms, which appear to replace their metacyclic VSG coats with a coat of bloodstream antigenic type. We can now attribute this switching event to a transcriptional control mechanism. This conclusion is based on three lines of evidence: (i) 5 days postinfection, nuclei from organisms expressing MVAT VSGs were still transcribing the corresponding MVAT VSG gene as well as a large portion of the upstream region; (ii) between 5 and 7 days postinfection, the amount of MVAT-specific mRNA decreased 30- to 200-fold; and (iii) the MVAT loci were unrearranged in organisms expressing early bloodstream VSGs, and the MVAT upstream regions were not constitutively transcribed, eliminating the possibility that the MVAT expression site is used for the expression of early bloodstream VATs. The finding that metacyclic genes are each transcribed in situ from independent loci suggests that transcription of bloodstream and metacyclic VSG genes is independently controlled (17; unpublished observations). This suggests that *cis*-acting control sequences, such as enhancer elements, may differ between bloodstream and metacyclic VSG genes. It is interesting that the disappearance of MVATs from the repertoire, as seems to have occurred for MVAT 4 in LVH 32, did not appear to be related to transcriptional regulation but rather to deletion of the coding sequences from the genome.

While this manuscript was in preparation, Cornelissen et al. (6) described putative MVAT cDNAs isolated from bloodstream trypanosome clones which cross-react immunologically with metacyclic organisms. Their results are consistent with ours in that a telomeric copy of the gene is found in nonexpresser organisms and this gene is present on the largest size class, chromosome-sized DNA molecules, by PFG electrophoresis. They found that these genes are duplicated and transposed when expressed in the bloodstream and undergo considerable variation in the distance to the telomere, which differs from our results. A significant difference between their experimental system and ours is that they were not studying metacyclic stage trypanosomes but bloodstream organisms expressing MVAT VSGs or MVAT-like VSGs during antigenic variation. Our studies used organisms indistinguishable antigenically from salivary gland organisms (13, 14) and have continually expressed the metacyclic VATs from the fly stage prior to the onset of antigenic variation. We have found a case in which the MVAT 7 gene has been reexpressed at the bloodstream stage, has undergone rearrangement, and has other characteristics which differ markedly from day 5 metacyclics which express this gene (Lenardo et al., manuscript submitted). This distinction is important, since regulatory factors which lead to the expression of MVAT genes at the metacyclic stage will probably not be present when these genes are expressed at the bloodstream stage. Therefore the examination of bloodstream organisms which bear immunologically cross-reactive VSGs may not accurately reflect the expression of MVAT genes at the metacyclic stage.

ACKNOWLEDGMENTS

We thank Hyeung-Jin Son, David Dorfman, and V. J. Reddy for helpful discussions, Tony Weil for help with runoff transcription assays, and Allison Rice-Ficht for assistance in the early stages of this project. We are grateful to William Murphy for preparing the *T. rhodesiense* λ library, John Boothroyd for the use of the clone pGE117.6, and Lyn Yarbrough for the use of the cloned tubulin gene.

This work was supported by grant DAMD 1782C2228 from the U.S. Army, by Public Health Service grants AI-18954, AM-25295, and AM-07018 from the National Institutes of Health, and by an award from the Burroughs-Wellcome Foundation and is dedicated to the memory of Gregory P. Lenardo.

LITERATURE CITED

1. **Barry, J. D., J. S. Crowe, and K. Vickerman.** 1983. Instability of the *Trypanosoma brucei rhodesiense* metacyclic variable antigen repertoire. Nature (London) **308**:699–701.
2. **Benton, W. D., and R. W. Davis.** 1977. Screening λgt recombinant clones by hybridization to single plaques in situ. Science **196**:180–182.
3. **Bernards, A., N. Van Harten-Loosbroek, and P. Borst.** 1984. Modification of telomeric DNA in *Trypanosoma brucei*: a role in antigenic variation? Nucleic Acids Res. **12**:4153–4169.
4. **Borst, P., A. Bernards, L. H. T. Van der Ploeg, P. A. M. Michels, A. Y. C. Liu, T. De Lange, and J. Kooter.** 1983. The control of variant surface antigen synthesis in trypanosomes. Eur. J. Biochem. **137**:383–389.
5. **Campbell, D. A., M. P. van Bree, and J. C. Boothroyd.** 1984. The 5′ limit of transposition and upstream barren region of a trypanosome VSG gene: tandem 75 base-pair repeats flanking $(TAA)_{90}$. Nucleic Acids Res. **12**:2759–2774.
6. **Cornelissen, A. W. C. A., G. A. M. Bakkeren, J. D. Barry, P. A. M. Michels, and P. Borst.** 1985. Characteristics of trypanosome variant antigen genes active in the tsetse fly. Nucleic Acids Res. **13**:4661–4676.
7. **Crowe, J. S., J. D. Barry, A. G. Luckins, C. A. Ross, and K. Vickerman.** 1983. All metacyclic variable antigen types of *Trypanosoma congolense* identified using monoclonal antibodies. Nature (London) **306**:389–391.
8. **Cunningham, I.** 1977. New culture medium for the maintenance of tsetse tissues and growth of trypanosomatids. J. Protozool. **24**:325–329.
9. **DeLauw, M. F., E. Pays, M. Steinert, D. Aerts, N. Van Meirvenne, and D. Le Ray.** 1985. Inactivation and reactivation of a variant-specific antigen gene in cyclically transmitted *Trypanosome brucei*. EMBO J. **4**:989–993.
10. **Donelson, J. E., and A. C. Rice-Ficht.** 1985. Molecular biology of trypanosome antigenic variation. Microbiol. Rev. **49**:105–125.
11. **Dorfman, D. M., and J. E. Donelson.** 1984. Characterization of the 1.35 kilobase DNA repeat unit containing the conserved 35 nucleotides at the 5′ termini of variable surface glycoprotein mRNAs in *Trypanosoma brucei*. Nucleic Acids Res. **13**: 3533–3549.
12. **Dorfman, D. M., M. J. Lenardo, L. V. Reddy, L. H. T. Van der Ploeg, and J. E. Donelson.** 1984. The 5.8S ribosomal gene of *Trypanosoma brucei*: structural and transcriptional studies. Nucleic Acids Res. **13**:3533–3549.
13. **Esser, K. M., and M. Schoenbechler.** 1985. Expression of two variant surface glycoproteins on individual African trypanosomes during antigen switching. Science **229**:190–193.
14. **Esser, K., M. J. Schoenbechler, and J. B. Gingerich.** 1982. *Trypanosoma rhodesiense* blood forms express all antigen spec-

ificities relevant to protection against metacyclic (insect form) challenge. J. Immunol. **129**:1715–1717.

15. **Kimmel, B. E., S. Samson, R. Hirschberg, and L. Yarbrough.** 1985. Tubulin genes of the African trypanosome *Trypanosoma brucei rhodesiense*: nucleotide sequence of a 3.7 kilobase fragment containing the genes for alpha and beta tubulins. Gene **35**:237–248.
16. **Lanham, S.** 1968. Separation of trypanosomes from the blood of infected rats and mice by anion exchangers. Nature (London) **218**:1273–1274.
17. **Lenardo, M. J., A. C. Rice-Ficht, G. Kelly, K. Esser, and J. E. Donelson.** 1984. Characterization of the genes specifying two metacyclic variable antigen types in *Trypanosoma brucei rhodesiense*. Proc. Natl. Acad. Sci. USA **81**:6642–6646.
18. **Liu, A. Y. C., L. H. T. Van der Ploeg, F. A. M. Rijsewijk, and P. Borst.** 1983. The transposition unit of variant surface glycoprotein gene 118 of *Trypanosoma brucei*, presence of repeated elements at its borders and absence of promoter-associated sequences. J. Mol. Biol. **167**:57–75.
19. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Pays, E., S. Houard, A. Pays, S. Van Assel, F. DuPont, D. Aerts, G. Huet-Duvillier, V. Gomes, C. Richet, P. Degand, N. Van Meirvenne, and M. Steinert.** 1985. *Trypanosoma brucei*: the extent of conversion in antigen genes may be related to the DNA coding specificity. Cell **42**:821–829.
21. **Schwartz, D. C., and C. R. Cantor.** 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell **36**:67–75.
22. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
23. **Van der Ploeg, L. H. T., A. W. C. A. Cornelissen, P. A. M. Michels, and P. Borst.** 1984. Chromosome rearrangements in *Trypanosoma brucei*. Cell **39**:213–221.