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Genomic Organization of Variant Surface Glycoprotein Genes in Trypanosoma brucei Procyclic Culture Forms

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The production of the variant surface glycoprotein coat of bloodstream form African trypanosomes ceases after conversion to the procyclic form. In the bloodstream stage alternate expression of different variant surface glycoprotein genes is responsible for the antigenic variation that occurs during relapse infections in the mammalian host. We have examined procyclic stage populations, derived from different bloodstream variant antigen types, for the two types of genomic alterations associated with variant surface glycoprotein genes in the bloodstream stage. Transcriptional activation of some variant antigen genes is accompanied by the generation of a new copy of the gene, the expression-linked copy. We find that the expression-linked copy is maintained after conversion to procyclic form, indicating that the presence of an expression-linked copy is not sufficient for the expression of a surface coat. Sequences 3′ to other variant surface glycoprotein genes show expression-independent variation in bloodstream stage trypanosomes. The same genes showed variation between procyclic populations of different origin, and between procyclics and their bloodstream parent. These data are discussed in light of observations on the sequence of variant antigen expression after cyclic transmission.

Key words: Trypanosoma brucei, variant surface glycoprotein genes, procyclic forms, genomic organization in procyclic form, expression-linked copy in procyclic form

The alternate expression of single variant surface glycoprotein (VSG) genes from the large VSG gene repertory results in trypanosome surface coats with markedly different antigenic properties. This antigenic variation allows mammalian bloodstream stage trypanosome populations to evade the host antibody response. At the molecular level, the expression of some VSG genes results from the duplication of a nonexpressed basic copy of the gene [1–4] and the insertion of the new copy at a distinct genomic location [5]. The new copy, termed an expression-linked copy (ELC), is transcribed [6]. Other VSG genes are transcriptionally activated without detectable duplication or transposition [4,7]. Interestingly, regions 3′ to these genes have been shown to undergo alterations independent of homologous VSG expression [4,8]. Both the nonduplication activated genes [9] and the ELC [10] reside in regions

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of the genome preferentially sensitive to Bal 31 nuclease, implying that they are adjacent to telomeres.

Any complete model of antigenic variation must account for certain biological findings. One of these is that there exists a loose order in the sequence of bloodstream variant antigen types (VATs) which arise after cyclic transmission through the insect host, the tsetse fly [11]. The VAT ingested by the fly is preferentially reexpressed in the first bloodstream parasitemia [12]. An early VAT is expressed in the next relapse, regardless of whether the ingested type was an early, middle, or late VAT. The mechanism that determines the temporal program of VSG gene expression remains unknown. A return to a basic genomic organization during the insect stages of the life cycle could dictate the subsequent sequence of VSG gene activation in the mammalian host. Alternatively, the VSG gene organization might remain unaltered during cyclic transmission, with the order of VAT occurrence being determined by preferential gene expression and/or selection. In order to ascertain the role of the ELC and of 3′ DNA alterations (which are expression independent in bloodstream forms) in contributing to these phenomena, we have examined the genomic organization of VSG genes in another stage of the trypanosome life cycle, the procyclic form. The procyclic forms, which normally live in the midgut of the fly, lack a VSG surface coat [14]. Under appropriate in vitro conditions bloodstream stage trypanosomes can be converted to procyclic culture forms (PCFs) [13] which appear biochemically and morphologically identical to the insect midgut form [14]. Two basic findings emerge from our analysis of PCFs derived from several different bloodstream VATs: 1) Procyclic culture forms derived from different VATs differ with regard to expression independent variation, and 2) the expression-linked copy is maintained in the procyclic form.

METHODS

Cells

Bloodstream VATs 3, 5, 7, and 11 of the IsTat serodeme of Trypanosoma brucei brucei were maintained as previously described [15]. Procyclic cultures [16] were initiated at a density of 2–5 $\times$ 10^6 cells/ml, using cells of each VAT at the same passage number as that used for isolation of bloodstream form DNA. Five percent to 40% of the inoculum survived the initial transition to procyclic form. After 2 months in culture at 28°C, cells were harvested and DNA was prepared.

Southern Analysis

Genomic DNA was isolated from bloodstream and culture form cells as previously described [15]. DNA samples were digested to completion with restriction enzymes [4] and the resultant fragments separated by electrophoresis in agarose gels. After transfer to nitrocellulose, the fractionated samples were hybridized to nick-translated VSG cDNA probes corresponding to the early IsTat VSGs as previously detailed by Parsons et al [4].

RESULTS

VSG 1 of the IsTat serodeme is expressed without apparent gene duplication [4]. In Southern hybridization analysis of Sal 1–digested DNA derived from IsTat bloodstream VATs 3, 5, 7, and 11, a 1 VSG cDNA probe hybridizes strongly to a single restriction fragment. In each VAT the 1 gene resides on a uniquely sized

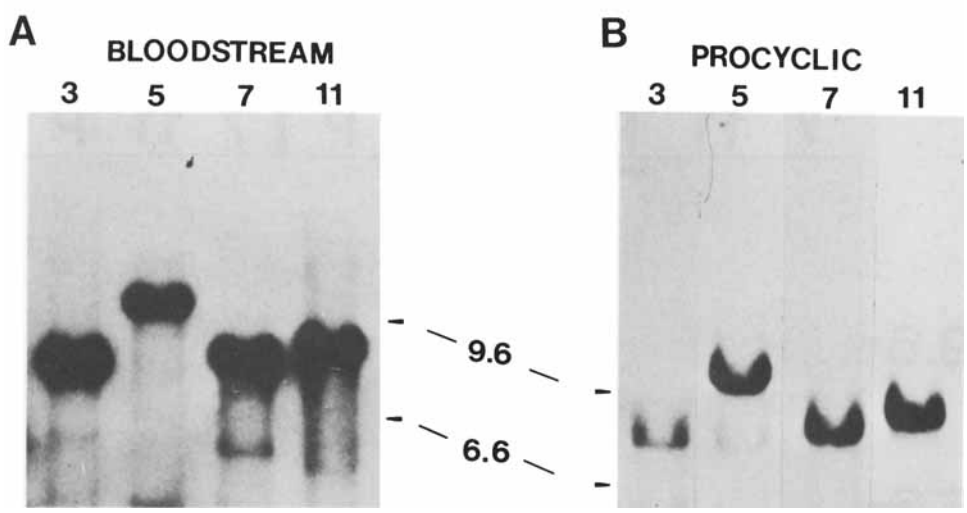

Fig. 1. Genomic Southern analysis of the VSG 1 gene. Sal 1-restricted DNA was hybridized with the 1060 bp probe pTb1.1-c1 which contains no Sal 1 sites. A) The VSG 1 gene in different bloodstream VATs. The VAT origin of each DNA sample is indicated above the lane. B) The VSG 1 gene in procyclic forms derived from different bloodstream VATs. The bloodstream origin of each procyclic population is indicated above the lane.

restriction fragment (Fig. 1A). Thus, during growth in the mammalian host, sequences flanking the VSG 1 gene undergo extensive alterations. Figure 1B shows the same analysis performed using PCF DNA. The VSG 1 gene is also on uniquely sized fragments in PCFs derived from VATs 3, 5, 7, and 11, the pattern closely resembling that observed in the parent bloodstream forms. Additional restriction fragments which weakly hybridize to the probe were occasionally observed. A clonal analysis [15] suggests that these fragments reflect heterogeneity arising within the cloned populations in sequences 3′ to the VSG 1 coding region (see below), rather than the presence of genes slightly homologous to the VSG 1 cDNA.

The nature of the DNA alterations demonstrated in Figure 1 was examined in genomic Southerns using restriction enzymes which cleaved the VSG 1 cDNA. Three such restriction digests of DNA derived from bloodstream VATs 7 and 11, as well as the procyclic counterpart to VAT 11, are shown in Figure 2. The VSG 1 gene resides on Pst 1 fragments of 6.7 kb in VAT 7, 7.25 kb in VAT 11, and 7.7 kb in PCF 11. The sizes of the Kpn 1 and Bgl 11 fragments containing the VSG 1 gene in VAT 7 and VAT 11 also differ by approximately 450 bp, as do those in VAT 11 and PCF 11. Data such as these were used to construct the genomic map of the 1 gene shown in Figure 3. All of the enzymes used appear to cleave at the same point 3′ to the VSG 1 gene in a given DNA sample. However, the distance between the VSG 1 gene and this apparent cluster of restriction sites varied between each DNA, whether of bloodstream or procyclic origin.

Variation in the distance between a VSG gene and an apparent cluster of restriction sites is not limited to the VSG 1 gene. In bloodstream forms a member of the IsTat VSG A gene family also shows variation in 3′ flanking sequences [4]. Experiments similar to those described above have established that these alterations

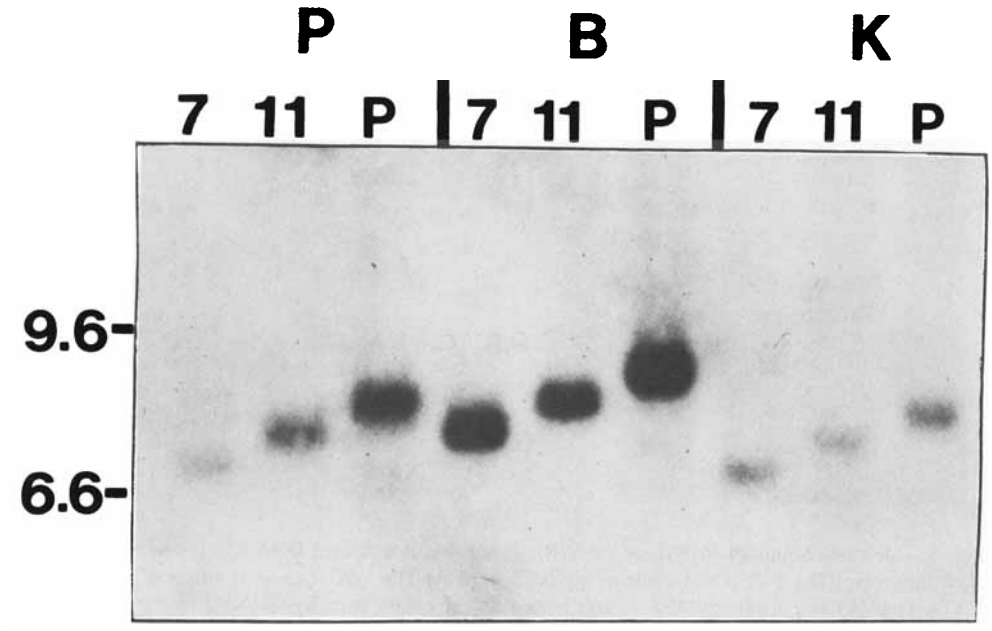

Fig. 2. Genomic rearrangements 3′ to the VSG 1 gene. DNA from VAT 7 (indicated above the corresponding lane as 7), VAT 11 (11), or procyclic forms derived from VAT 11 (P) were restricted with enzymes known to cleave the VSG 1 cDNA. The enzyme Bgl 11 recognizes a site approximately 200 bp from the 5′ end of the complete VSG 1 cDNA, Pst 1 recognizes a site at 400 bp, and Kpn 1 recognizes a site at 740 bp. After separation on agarose gels, the samples were hybridized with nick-translated pTb1.1-c1. Only the portion of the gel containing VSG 1 sequences 3′ to the restriction sites is shown (the 5′ fragments generated by each of these enzymes are very small and have migrated off the bottom of the gel). Restriction analyses such as these were used to construct the genomic map shown in Figure 3.

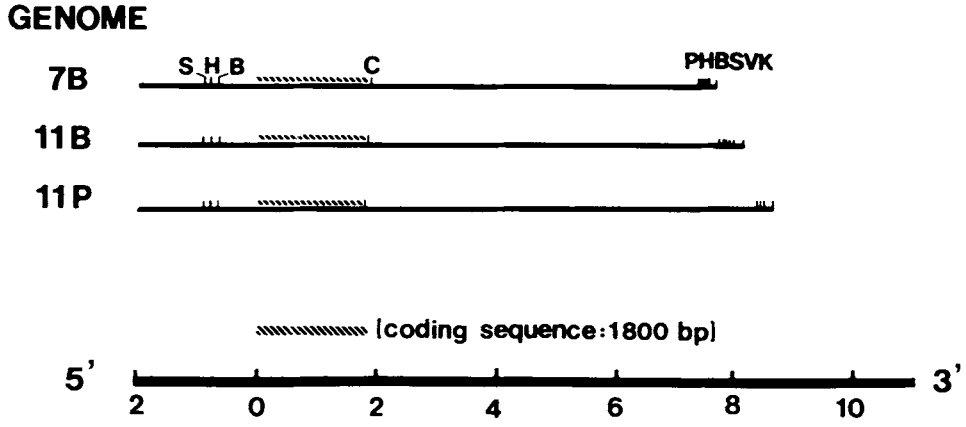

Fig. 3. Genomic map of the VSG 1 gene. The context of the VSG 1 gene in VAT 7 (7B), VAT 11 (11B), and procyclic forms derived from VAT 11 (11P) was determined by genomic Southern analyses as shown in Figure 2. B, Bgl 11; C, Hinc 11; H, Hind III; K, Kpn 1; P, Pst 1; S, Sal 1; V, Pvu 11.

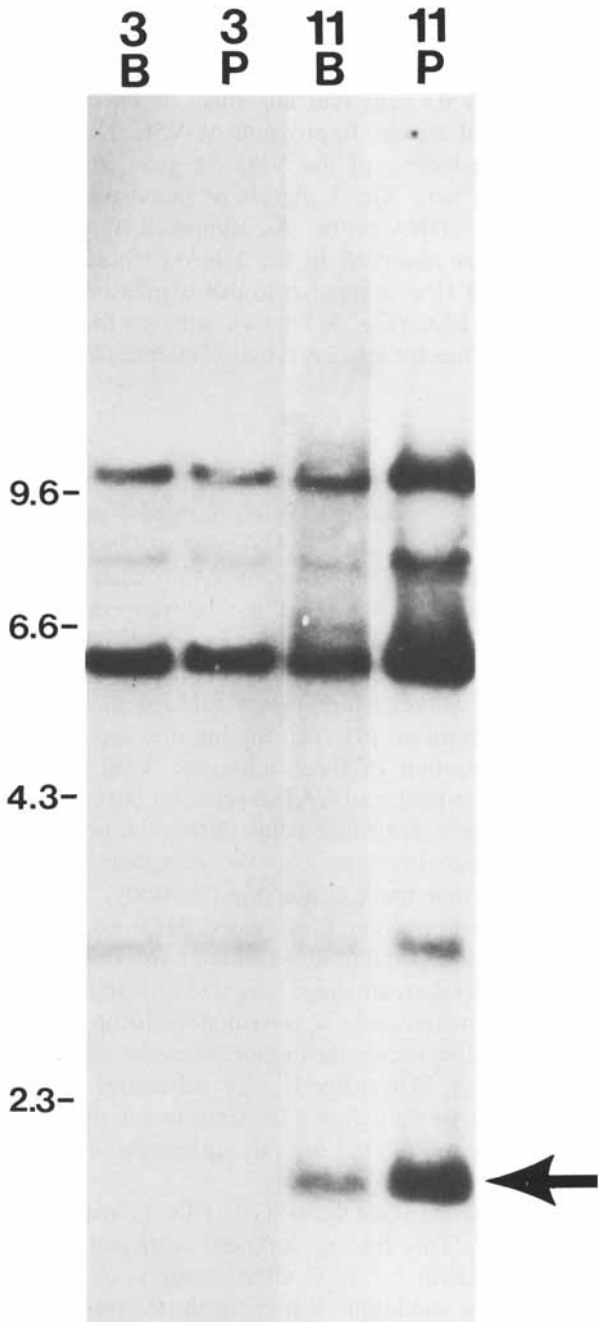

Fig. 4. Retention of the VSG 11 ELC in procyclic culture form. Genomic DNA from VATs 3 (3B) and 11 (11B) and their procyclic counterparts (3P and 11P) were digested with the enzyme Xba 1. After separation on agarose gels, the samples were hybridized to nick-translated pTb1.11-c1. There is an Xba 1 site approximately 200 bp from the 5′ end of a 5′ terminal VSG 1.11 cDNA. The 630 bp pTb1.11-c1 does not contain this Xba 1 site, and detects only sequences 3′ to it. An arrow marks the 11 ELC.

also occur in PCFs, which differ both from one another and from their bloodstream parent (data not shown). Similarly, variations 3′ to a member of the IsTat 5 gene family (previously observed in bloodstream VATs [4]) are also seen in PCFs.

The second type of VSG gene rearrangement observed in bloodstream forms results in the generation of ELCs. Expression of VSG 11 is accompanied by the appearance of an additional copy of the VSG 11 gene, the 11 ELC [4]. This is demonstrated in Figure 4; here Xba 1 digests of bloodstream VATs 3 and 11 are hybridized with a VSG 11 cDNA probe. An additional hybridizing sequence, on a 1.6 kb Xba 1 fragment, is observed in the DNA derived from the bloodstream population expressing VSG 11 as compared to that expressing VSG 3. PCFs derived from VAT 11 retain the 11 ELC (Fig. 3, lane 4), whereas those derived from VAT 3 do not show the 11 ELC. Thus the ELC is retained after conversion from mammalian to insect form.

DISCUSSION

In this report we have shown that PCFs derived from different bloodstream VATs show differences in sequences 3′ to VSG genes. Only those genes previously observed to show 3′ alterations in bloodstream VATs, such as the IsTat 1 gene [4], showed variation in PCFs. The nature of the rearrangements appears similar in procyclic and bloodstream forms; restriction fragments differ by addition or deletion of DNA in regions proximal to an apparent cluster of restriction sites. These clusters have been shown by other workers to represent double-stranded breaks in the DNA, and are presumed to be telomeres [9]. The finding that each procyclic DNA differs with regard to the organization of these telomeric VSG genes implies that any mechanism that resets the sequence of VAT expression during cyclic transmission is not effected through telomeric rearrangements during the procyclic stage of the life cycle. If a reset mechanism involves genomic reorganization of VSG genes, it functions on a scale larger than that examined in this study.

Our analyses show that procyclic forms can differ from their bloodstream parent with regard to expression-independent DNA alterations. Borst and co-workers have demonstrated that in the bloodstream stage, the size of restriction fragments containing telomeric VSG genes increases by approximately 10 bp per generation [17]. As yet we do not know if this also occurs during the procyclic stage, when the generation time is substantially longer. Alternatively, the difference between procyclic and bloodstream DNAs could arise only during the transition to the culture form. Analysis of DNA from procyclic forms cultured for various lengths of time should answer this question.

We have also demonstrated that the VSG 11 ELC is maintained after conversion from bloodstream to PCF. This finding contrasts with that of Pays et al [2], who observed the loss of the AnTat 1.1 ELC after transition to the procyclic stage was induced by in vitro culture conditions similar to those employed here. Preliminary data obtained in our laboratory indicate that other IsTat ELCs are maintained in homologous PCFs (Parsons, unpublished results). This suggests that the loss of the AnTat 1.1 ELC might have resulted from an antigenic variation event rather than a step in the life cycle differentiation process. Alternatively, retention of the ELC may not be obligatory during procyclic differentiation.

Although the previous work on the AnTat 1.1 gene might have been taken to suggest that the lack of a surface coat in procyclics results from the loss of the ELC,

we have shown this not to be the case. Our work further shows that the presence of a duplicate VSG gene (ELC) is not sufficient for the expression of the VSG surface coat. However, the previous studies suggesting that the VSG genes are not transcribed in PCFs must now be viewed with caution, particularly since they were performed with uncharacterized populations [18,19]. An analysis of the transcriptional status of the procyclic ELC is now in progress. Data presented here do not bear upon the sequences flanking the ELC, which are presumed to contain the transcriptional control sequences. Preliminary genomic maps of the regions 5′ to the bloodstream and corresponding procyclic ELCs appear identical for over 15 kb (Parsons, unpublished results). However, the 35 nucleotide mini-exon sequence found thus far on all mature VSG mRNAs [20] (hypothesized to be furnished by the VSG expression site) has not been detected within 22 kb of the ELC [21]. Thus, it is still possible that inactivation of the procyclic ELC is accomplished by removal from the expression site by transposition or some other process. Alternatively, the procyclic ELC may still be in a VSG expression site, but remain unexpressed for reasons yet to be discovered. If true, this could explain why the VAT ingested by the fly is preferentially reexpressed in the first bloodstream parasitemia.

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