MOLECULAR AND CELLULAR BIOLOGY, Mar. 1983, p. 410–414
0270-7306/83/030410-05$02.00/0

Vol. 3, No. 3

DNA Rearrangements and Antigenic Variation in *Trypanosoma equiperdum*: Expression-Independent DNA Rearrangements in the Basic Copy of a Variant Surface Glycoprotein Gene

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Received 21 June 1982/Accepted 12 November 1982

Antigenic variation in *Trypanosoma equiperdum* is associated with the sequential expression of variant surface glycoprotein (VSG) genes in a process which involves gene duplication and transposition events. In this paper we present evidence that the genomic environment of the VSG-1 basic copy gene, the template for duplicated, expression-linked VSG-1 genes, differs in every trypanosome clone examined. This variation is thus independent of the expression of the VSG-1 gene, and it also appears to be restricted to the 3′ genomic environment. It is also demonstrated that the DNA located 3′ to the VSG-1 basic copy gene is moderately sensitive to digestion when the nuclei of either expressor or nonexpressor trypanosomes are treated with DNase I.

In an accompanying paper (2), it has been demonstrated that the expression of two variant surface glycoprotein (VSG) genes in *Trypanosoma equiperdum* is associated with the presence of a supplementary expression-linked gene copy which is probably transcribed. The results suggest that gene duplication and transposition events accompany the VSG switching phenomenon in these organisms. In the course of this work, two observations evoked interest regarding the nature of the VSG basic copy gene which presumably serves as a template for the synthesis of the duplicated expression-linked copy: (i) cDNA probes for two different VSGs (VSG-1 and VSG-28) both hybridized to multiple nonoverlapping bands in filter hybridization experiments done under stringent conditions, indicating the presence of several relatively homologous copies of each gene; and (ii) the expression-linked VSG-1 gene copies from each of four independently isolated Bordeaux trypanozoon antigenic type 1 (BoTat-1) trypanosome clones had different genomic environments.

In spite of the fact that there are multiple VSG-1-related sequences in the *T. equiperdum* genome, only one appears to be used as a template for the synthesis of the duplicated expression-linked copy. This was shown by analysis of *Eco*RI digests. Although digestion of DNAs from VSG-1 expressors and nonexpressors with *Eco*RI did not reveal any supplementary expression-linked bands in filter hybridizations, there was a 1.9-kilobase (kb) band which was at least twice as intense in all the expressor DNAs. These results suggested that *Eco*RI cuts twice within the DNA sequence which is duplicated in VSG-1 expressors. This interpretation was supported by the finding that DNase I treatment of chromatin from VSG-1-expressing trypanosomes reduced the relative intensity of the 1.9-kb *Eco*RI fragment to that found in DNA from nonexpressors. It is thus possible to identify fragments containing the silent basic copy of the VSG-1 gene in DNA digested with other restriction endonucleases since they should contain this characteristic 1.9-kb *Eco*RI fragment.

In the present paper it is demonstrated that the 12-kb expression-linked *Bam*HI fragment of BoTat-1[78] contains the 1.9-kb *Eco*RI fragment as predicted and that the basic copy of the VSG-1 gene is found in a *Bam*HI fragment which varies in size in all of the trypanosome DNAs examined. This variation, which is independent of the expression of VSG-1, appears to be restricted to the 3′ genomic environment. We also demonstrate that the DNA located 3′ to the basic copy VSG-1 gene is selectively degraded when nuclei of expressor or nonexpressor trypanosomes are treated with DNase I.

MATERIALS AND METHODS

The genealogy of the BoTat variants used here has been shown (2, Fig. 1). The VSG-1 cDNA clones used here were plasmids pTe1.2, pTe1.6, and pTe1.6a,

which is a subclone of the 5' *Pst*I fragment of the insert in pTe1.6. Genomic and plasmid DNAs were prepared as described (2). Electrophoresis, filter hybridization, and DNase I digestion of chromatin were performed as described (2).

DNA was isolated from agarose gels by a modification of the procedure described by Vogelstein and Gillespie (4). Trypanosome DNA (20 μg) was cleaved with the *Bam*HI restriction enzyme and was electrophoresed on a 0.5% agarose gel. Bands of the appropriate size were cut out and dissolved in 2 ml of NaI solution (90.8% NaI, 1.5% $NaSO_3$ [wt/vol]) per g at 37°C for 3 h in the dark. Glass powder, prepared as described (4), was added (25 μl per 20 μg of DNA), and the DNA was allowed to absorb in the dark at 4°C overnight with mixing. After centrifugation, the glass powder pellet was washed once with 10 volumes of NaI solution and twice with cold 50% ethanol in 0.1 M NaCl–10 mN Tris-hydrochloride (pH 7.5)–1 mM EDTA. DNA was eluted twice by suspending the pellet in two to three volumes of 5 mM Tris-hydrochloride (pH 8.0)–0.5 mM EDTA at 37°C for 30 min.

RESULTS

Identification of the VSG-1 basic copy gene. The VSG-1-specific probes hybridize with multiple fragments in *T. equiperdum* expressor or nonexpressor genomic DNA digested with restriction endonucleases which do not cleave in the cDNA sequences (2). However, experiments with *Eco*RI-digested DNAs show that a gene located within a 1.9-kb fragment is the only one which is duplicated in four independently isolated VSG-1 expressors (2). Therefore, it is this 1.9-kb *Eco*RI band which corresponds to both the basic and expression-linked copies of the VSG-1 gene. Thus, it is possible to identify the bands containing the basic copy gene in DNA digested with other enzymes since they should contain the 1.9-kb *Eco*RI fragment.

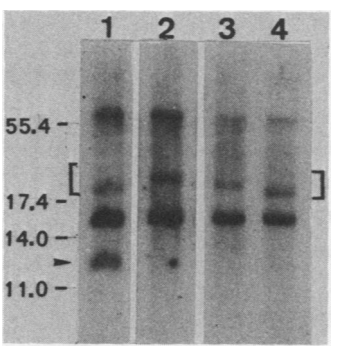

FIG. 1. Filter hybridization of *Bam*HI-digested DNA from VSG-1 expressing and nonexpressing trypanosomes. DNA from various trypanosome clones was digested with *Bam*HI, electrophoresed in a 0.5% agarose gel, and filter hybridized with ^{32}P-labeled plasmid pTe1.6 DNA (2). Lanes represent DNA from BoTat-1^{78}, (lane 1), BoTat-20 (lane 2), BoTat-28 (lane 3), and BoTat-78 (lane 4). Molecular sizes are marked in kilobases. Brackets show 19- to 21-kb band which varies in both expressors and nonexpressors. Arrow shows extra expression-linked band (see text).

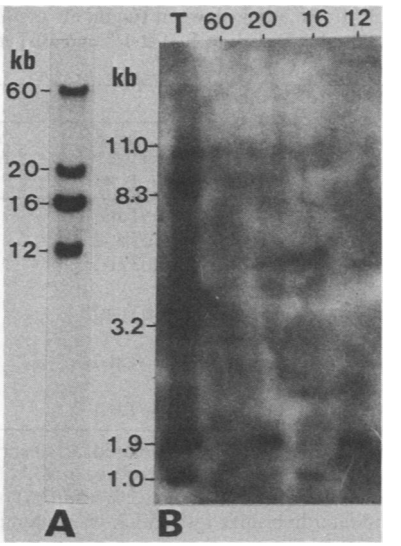

FIG. 2. *Eco*RI bands contained in fragments of BoTat-1^{78} DNA generated with *Bam*HI. (A) Filter hybridization of *Bam*HI-digested BoTat-1^{78} DNA hybridized with ^{32}P-labeled plasmid pTe1.6 DNA. (B) Filter hybridization of *Eco*RI-digested total BoTat-1^{78} DNA and of isolated *Bam*HI fragments of BoTat-1^{78} DNA. Lanes represent total DNA (T) and *Bam*HI fragments of 60, 20, 16, and 12 kb (indicated by corresponding numbers). Molecular sizes are shown in kilobases.

Trypanosome DNA was prepared from VSG-1-expressing and nonexpressing clones, all of which were derived from BoTat-1. The DNA was digested with *Bam*HI, which does not cut in the probe sequence, and was filter hybridized with nick-translated plasmid pTe1.6 DNA (2). The VSG-1 expressor clone was found to have an extra expression-linked band (Fig. 1, arrow). In addition, there is a band which varies between 19 and 21 kb in expressors and nonexpressors (Fig. 1, brackets). The four VSG-1 *Bam*HI bands from BoTat-1^{78} DNA and three bands from BoTat-78 DNA were isolated from agarose gels, cleaved with *Eco*RI, and analyzed by filter hybridization with nick-translated plasmid pTe1.6 DNA (Fig. 2, Table 1). One *Bam*HI fragment from BoTat-78 DNA and two fragments from BoTat-1^{78} DNA contained the 1.9-kb *Eco*RI fragment. In the BoTat-1^{78} DNA, the *Eco*RI 1.9-kb band was present in the 12-kb expression-linked *Bam*HI fragment, thus confirming that it is duplicated in VSG-1 expressors. It was also present in the 20-kb *Bam*HI fragment. This fragment is of a different size in all the trypanosome DNAs examined. In the BoTat-78 DNA the 1.9-kb *Eco*RI band was found

TABLE 1. Relation between fragments of *Bam*HI, *Eco*RI, and *Sau*3A in BoTat-1[78] and BoTat-78 DNAs[a]

Fragment	DNase sensitivity[b]	Origin	
		Variant antigenic type	*Bam*HI fragment (kb)
*Eco*RI 1.9	R	BoTat-78	19
	R	BoTat-1[78]	20
	S	BoTat-1[78]	12
*Sau*3A 2.7	R	BoTat-78	19
*Sau*3A 3.5	R	BoTat-1[78]	20
*Sau*3A 7.8	S	BoTat-1[78]	12

[a] *Bam*HI fragments were isolated as described in the text, digested with either *Eco*RI or *Sau*3A, electrophoresed on 0.7 (*Eco*RI) or 1.2% (*Sau*3A) agarose gels, and filter hybridized with nick-translated plasmid pTe1.2 DNA.

[b] R, Resistant; S, sensitive.

only in the variable 19-kb *Bam*HI fragment. These findings indicate that the 19- and 20-kb *Bam*HI fragments in BoTat-78 and BoTat-1[78], respectively, contain the basic copy of the VSG-1 gene.

Limitation of basic copy variation to the 3′ end. These results suggest strongly that the basic copy of the VSG-1 gene undergoes frequent rearrangements which are independent of expression. Since *Bam*HI does not cut within the cDNA sequence, it could not be determined whether the rearrangements around the VSG-1 basic copy gene were located on the 3′ or 5′ side. Therefore, filter hybridizations were carried out with probes which detect either the 5′ or 3′ end of the cDNA after digestion of the genomic DNAs with restriction enzymes which cut in the gene sequence. For analysis of the 5′ ends of the genes and their flanking sequences, genomic DNAs were digested with *Pst*I and were hybridized with plasmid pTe1.6a, which contains the 350-base pair 5′ *Pst*I fragment from plasmid pTe1.6. For analysis of the 3′ end of the genes and their flanking regions, the genomic DNAs were digested with *Sau*3A and hybridized with plasmid pTe1.2. The analysis for the VSG-1 basic copy gene with three nonexpressor DNAs is shown in Fig. 3. Whereas no variation in bands corresponding to the 5′ end of the gene and its flanking sequences were seen (Fig. 3B), one variable band corresponding to the 3′ end was detected (Fig. 3A). This band was different in each of the DNAs examined. Similar results were obtained with several other restriction endonucleases which cut within the VSG-1 cDNA sequence (data not shown). These results suggest that the variability of the VSG-1 basic copy gene is restricted to the 3′ genomic environment.

To verify that the VSG-1 basic copy gene is associated with variable 3′-specific fragments, the *Bam*HI fragments of BoTat-78 and BoTat-1[78] DNAs were eluted from agarose gels (see above), digested with *Sau*3A, and analyzed by filter hybridization. The results are summarized in Table 1. The 2.7-kb variable *Sau*3A band from BoTat-78 nonexpressor DNA was contained in the 19-kb *Bam*HI fragment. *Sau*3A digests of DNA from VSG-1 expressors show two variable fragments (2). In the case of BoTat-1[78], these fragments are 3.5 and 7.8 kb. Table 1 shows that the 7.8-kb *Sau*3A band was contained in the expression-linked 12-kb *Bam*HI fragment and that the 3.5-kb *Sau*3A band was contained in the variable 20-kb *Bam*HI fragment. Since both of these *Bam*HI fragments also contain the 1.9-kb *Eco*RI band, we conclude that the 7.8- and 3.5-kb *Sau*3A fragments are generated from the expression-linked and the basic copy *Bam*HI fragments, respectively.

So far, attempts to determine the restriction maps of the 3′ environment of the VSG-1 basic copy gene have failed. Except for the *Eco*RI site, which occurs at or very near the 3′ end of the gene, all restriction sites 3′ to the gene appear to be at the same place in a given

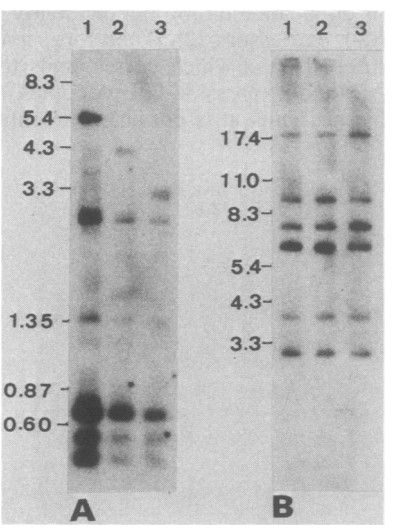

FIG. 3. DNA rearrangements on the 3′ and 5′ ends of the VSG-1 sequence in nonexpressor trypanosomes. (A) DNA at the 3′ end. Trypanosome DNA was digested with *Sau*3A, electrophoresed on a 1.2% agarose gel, and filter hybridized with ^{32}P-labeled plasmid pTe1.2. (B) DNA at the 5′ end. Trypanosome DNA was digested with *Pst*I, electrophoresed on a 0.8% agarose gel, and filter hybridized with ^{32}P-labeled plasmid pTe1.6a. Lanes in (A) and (B) represent DNA from BoTat-20 (lane 1), BoTat-28 (lane 2), and BoTat-78 (lane 3).

genomic DNA, as was found with the 3′ environment of the expression-linked copy (2). *Bam*HI also appears to cut at the same universal 3′ site since after digestion with enzymes which cut within the VSG-1 sequence, subsequent digestion with *Bam*HI does not alter the variable 3′-specific fragment. Thus, the variation in the size of the *Bam*HI-generated VSG-1 basic copy fragments can be accounted for entirely by variation on the 3′ side. This would place the *Bam*HI site 5′ to the VSG-1 basic copy gene at 14 kb from the coding sequences in all of the BoTat DNAs.

DNase sensitivity of the VSG-1 basic copy gene. As shown above, the VSG-1 basic copy gene differs from the other VSG-1-related bands in that its 3′ environment varies in all the trypanosome clones examined. Another distinguishing feature of the basic copy genes is the increased sensitivity of the 3′ sequences when the chromatin is digested with DNase I. Since *Sal*I cuts in the probe sequence, it was possible to examine both 3′ and 5′ ends. Nuclei were prepared from expressor (BoTat-1) and nonexpressor (BoTat-28) trypanosomes and were treated with DNase I as described (2). The DNA was then purified, treated with *Sal*I, and analyzed by filter hybridization with nick-translated plasmid pTe1.6 as the probe (Fig. 4). It can be seen that although the VSG-1 basic copy gene (Fig. 4, arrow) was less sensitive to DNase digestion than was the expression-linked copy (Fig. 4, double arrow), it was more sensitive than the other VSG-1-related sequences.

The moderate DNase sensitivity of the VSG-1 basic copy gene was seen in both the expressor (BoTat-1) and the nonexpressor (BoTat-28) DNAs, suggesting that it is not due to expression of the gene. The partial sensitivity was associated particularly with the 3′ ends represented by the 6.0- and 4.5-kb bands in the BoTat-1 and BoTat-28 DNAs, respectively. No partial DNase sensitivity was seen in the 1.9-kb *Eco*RI basic copy band from nonexpressor DNA (2, Fig. 8C), whereas very marked DNase sensitivity was seen in the same band for expressor DNA, presumably because of the active transcriptional state of the expression-linked gene. Since *Eco*RI appears to cut twice within the VSG-1 duplicated region, deleting surrounding genomic sequences (2), the 3′ flanking region must be responsible for the partial DNase sensitivity observed in the basic copy.

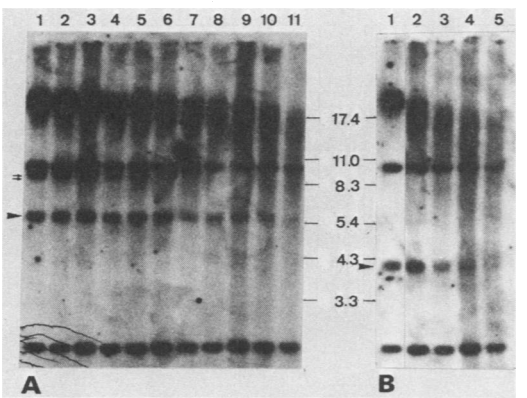

FIG. 4. DNase I sensitivity of *Sal*I bands from expressor (VSG-1) and nonexpressor (VSG-28) DNA. Nuclei from BoTat-1 and BoTat-28 trypanosomes were treated for various lengths of time with DNase I, and the DNAs were purified as described in the text. After digestion with *Sal*I, the DNAs were electrophoresed on a 0.8% agarose gel and filter hybridized with plasmid pTe1.6 DNA. (A) BoTat-1 nuclei. Lanes represent treatment times of (left to right) 0, 0.5, 1, 2, 3, 5, 7.5, 10, 15, 20, and 30 min. (B) BoTat-28 nuclei. Lanes represent treatment times of (left to right) 0, 4, 9.5, 15, and 22 min. Single arrow, Basic copy gene; double arrow, expression linked copy (see text).

DISCUSSION

The results presented here show that the basic copy gene coding for the VSG-1 antigen of *T. equiperdum* has different properties from the other VSG-1-related sequences in the genome. The 3′ environment of the basic copy gene is different in all of the trypanosome clones examined. This variation is not linked to expression of the VSG-1 antigen since it also occurs in nonexpressors. The variation must occur within 2.7 kb of the coding sequence since this is the size of the smallest variable fragment seen thus far. Variability similar to that described here also occurs in at least one other basic copy gene in *T. equiperdum*, VSG-2 (unpublished data). This is the first report of variability specifically associated with the VSG basic copy. Williams et al. (5) have observed expression-independent variability on the 3′ ends of *Trypanosoma brucei* VSG genes. However, the observed variability has not been associated with basic copy genes.

It is not yet known whether variation in the 3′ genomic environment of the VSG basic copy genes in *T. equiperdum* is associated with the mechanism of antigenic variation or whether it occurs independently of variation. However, the variable band must be relatively stable within a given clone of trypanosomes since the expansion of the clones involves 30 to 40 generations, and only a single variable 3′ VSG-1 basic copy fragment is seen in each trypanosome clone. This suggests that the variation in the 3′ flanking region of the VSG basic copy genes may be associated with the phenomenon of antigenic variation.

There is no apparent relationship between the size of the 3′-specific restriction fragments from basic and expression-linked copies in VSG-1

expressors. However, the 3′ fragments from the expression-linked genes are invariably longer than those from the basic copy, although they differ among the various expressors.

Several features of both the VSG-1 expression-linked and the basic copy 3′ genomic environments suggest that they may have unusual structures. There is a clustering of 3′ restriction enzyme sites, which suggests that the genes encoding the expression-linked and basic copies may be located near DNA termini. This hypothesis is supported by the sensitivity of expression-linked and basic copy 3′ ends to digestion by the exonuclease *Bal* 31 (manuscript in preparation). In addition, the 3′ genomic environments of both copies show a remarkable absence of restriction enzyme cleavage sites. Similar observations regarding the clustering of restriction sites and *Bal* 31 sensitivity have been made by others (1, 3, 5, 6). The significance of these unusual features on both the expression-linked and basic copy 3′ ends is unclear. It is also interesting that the chromatin located 3′ to the basic copy VSG-1 gene is moderately sensitive to DNase, whereas the coding sequences appear to be relatively insensitive. This could reflect a particular chromatin configuration of the 3′ regions.

ACKNOWLEDGMENTS

This work was supported by grants from the Centre National de la Recherche Scientifique and the Institut National de la Santé et de la Recherche Médicale, France.

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