Characteristics of trypanosome variant antigen genes active in the tsetse fly

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ABSTRACT

Trypanosoma brucei contains a repertoire of more than 100 different genes for Variant Surface Glycoproteins (VSGs). A small and strain-specific fraction of these genes is expressed in the salivary glands of the tsetse fly (M-genes), giving rise to metacyclic Variable Antigen Types (M-VATs). Antibodies produced in a chronic trypanosome infection initiated by syringe inoculation of bloodstream forms into mammals (*i.e.* against B-VATs), will react with most of the M-VATs suggesting that these B-VATs express VSG genes that are similar or identical to M-genes. We have cloned DNA complementary to the VSG mRNA of four of such B-VATs and used this to characterize the corresponding VSG genes. In three of the four VATs we find a single VSG gene hybridizing with the cDNA probe and we provide supporting evidence that this gene is expressed as an M-gene. In the bloodstream repertoire these genes appear to be activated by duplicative translocation to another telomere. In all four variants the putative M-genes are telomeric and in the three cases where the location of the genes on chromosome-sized DNA molecules could be determined, the genes were located in large DNA, whereas the majority of the telomeric VSG genes are in chromosomes < 1000 kb. Our results are best explained by models for M-gene activation involving telomeric expression sites for these genes which are separate from those used by bloodstream forms. The implications of these results for vaccination are discussed.

INTRODUCTION

Antigenic variation provides the main defence of *Trypanosoma brucei* against the antibodies of its mammalian host (1, 2). This unicellular eukaryotic parasite is completely covered by a surface coat, which consists of a single protein species, the Variant Surface Glycoprotein or VSG (3, 4). By repeatedly changing the VSG made, a small fraction of the trypanosomes escape destruction by host antibodies (5). Analysis of chronic infections in rabbits infected with *Trypanosoma equiperdum*, a trypanosome closely related to *T.brucei*, has shown that the progeny of one trypanosome can produce more than 100 different coats that have no exposed antigenic determinant in common (6). Molecular hybridization indicates that the genome of *T.brucei* may contain as many as 1000 different genes for VSGs (7).

Although several aspects of the control of VSG gene expression have been clarified in the past five years, one major problem has hardly been touched: how does the trypanosome genome determine which VSG gene is to be activated next? Some form of timing is essential in chronic infections to avoid premature expression of the entire VSG repertoire.

The VSG genes which are expressed early in infection, that is following injection of bloodstream trypanosomes into a mammal, are telomeric in all cases examined so far. A telomeric position does not itself dictate early expression, however, as only a minority of VATs encoded by telomeric genes arises early in a chronic infection. Other factors, including antibody selection, growth rate competition, distribution throughout the host and the genetic programme resulting in the preferential activation of certain VSG genes, affect the trypanosome population during a chronic infection (see refs. 6, 8-19 for discussion). The relative contributions of each of these factors are unknown and difficult to investigate, because of the complexity of the biological system.

To understand in more detail the selection of VSG genes for expression we have investigated the sub-set of VATs (M-VATs) produced in the tsetse fly salivary gland. This sub-set consists of 12 VATs in one stock of T.congolense (20) and approximately 10-13 major VATs in T.brucei (21). This sub-set of M-VATs is predictable in composition and is produced independently of the VAT originally used to infect the tsetse fly. This suggests genetic programming, the elucidation of which may also throw light on the interacting genetic factors that determine the time at which B-VATs appear in a chronic infection.

Homogeneous populations of M-VATs are difficult to obtain in large numbers. A single fly produces only about 10^4 trypanosomes and these are antigenetically heterogeneous. Although M-VATs continue to be expressed a few days in the trypanosome population developing in mammals after the tsetse fly bite, the switching of individual M-VATs to other M-VATs and early B-VATs (22) makes their cloning difficult.

To circumvent this problem we have made use of the observation that antibodies raised against some B-VATs will mediate lysis of some M-VATs and vice versa, suggesting that these two VATs make similar or identical VSGs (23). If the trypanosome contained a single gene for such a VSG, only that copy of the gene would have to be expressed both in the bloodstream and the metacyclic populations. In that case, the bloodstream version of the gene could be cloned and used to study properties of a M-gene. To test this

strategy we have analysed four B-VATs that correspond to four VATs of the M-repertoire. We show here that three of these B-VATs each contain a single gene for its VSG, implying that the same gene can be expressed in the bloodstream and the M-repertoire. The putative M-genes studied here lie close to chromosome telomeres and are expressed by duplicative transposition in the four B-VATs studied. We discuss possible features that make a gene part of the M-repertoire.

MATERIALS AND METHODS

Trypanosomes. GUTat (Glasgow University Trypanozoon antigen types) 7.1 and 7.2 were isolated as bloodstream trypanosome clones from a mouse adapted line of the T.b.rhodesiense stock EATRO (East African Trypanosomiasis Research Organization) 2340 (21). These two VATs interswitch frequently (22). GUTat 7.13 is a metacyclic form clone. To overcome the problems of antigenic instability associated with fly-transmissible trypanosomes, this clone was adapted to a stable form by subjection to 29 rapid syringe passages in mice, being recloned a further 7 times in the early passages when other VATs threatened to overgrow as determined by immunofluorescence. ILTat (ILRAD Trypanozoon antigen type) 1.61 was isolated in a similar manner, except that it was cloned originally as a bloodstream trypanosome 2 days after tsetse fly bite. VSG 1.61 is indistinguishable of gene 7.15 in the GUTat serodeme. The isolation of variant ILTat 1.22 from EATRO stock 795 and ETat (Edinburgh Trypanozoon antigen type) 1.2 from EATRO stock 3, have been described by Miller and Turner (11) and Lumsden and Herbert (24), respectively. All these trypanosome stocks belong to the same serodeme (i.e. have the same VSG repertoire) as described by Barry et al. (21). All VATs were checked for homogeneity by immunofluorescence and immune lysis tests using specific antisera and monoclonal antibodies. The uncloned population 221ar (r for relapse) was derived of VAT 221a from stock 427 of T.brucei (25). Trypanosomes were grown in rats or mice, their purification from blood elements was by published procedures (13).

Isolation and blotting of nucleic acids. Trypanosomal DNA and RNA were purified as described by Auffray and Rougeon (26), Bernards et al. (27) and Van der Ploeg et al. (7). Poly(A)$^+$ RNA was purified by oligo(dT) cellulose chromatography as described by Hoeijmakers et al. (28). Restriction endonuclease digestion, electrophoresis, transfer of DNA to nitrocellulose filters and prehybridization of DNA blots were as described by Southern (29) and Bernards et al. (27). Pulsed-field gradient gel electrophoresis (30) of intact chromosomes was carried out as described by Van der Ploeg et al. (31-33) in 1% agarose gels run at 20°C, alternating the electric fields (17.5 V/cm N-S; 6.25 V/cm W-E) every 35 seconds. Currently we use oligomers of bacteriophage lambda DNA as size markers for chromosome-sized DNA molecules. In PFG gels run with a 35 second interval oligomers ranging from 50 to 650 kb are separated. Longer oligomers - > 700 kb - are present, but compressed at the "2 Megabasepairs" position (A. Bernards and P. Borst, unpublished observations). At a switching interval of 45 seconds, however, the compression area of "2 Mb" contains oligomers > 900 kb. This indicates that calibration of large chromosomes with the PFG technique is still somewhat uncertain as pointed out previously (31-33). DNA fragments to be used as hybridization probes were obtained by isolation of the appropriate restriction fragments from low-melting temperature agarose gels (34) and labelled by nick-translation (35). Post-hybridizational washes were at 65°C in 0.1 x SSC.

cDNA cloning. A library of cDNA from trypanosome clones 7.1, 7.2, 7.13 and 1.61 was constructed essentially as described by Maniatis et al. (36) using oligo(dT) as primer and a 1:1:1:1 mixture of poly(A)$^+$ RNAs from these four variants as template. The cDNAs were annealed in the PstI site of the vector pBR 322 by GC tailing and introduced in Escherichia coli C 600. Recombinant DNA clones containing DNA complementary to VSG mRNAs were selected from this library by using ^{32}P-labelled cDNA prepared on poly(A)$^+$ RNA with a 14-nucleotide as primer that is complementary to a 3' conserved sequence found at the 3' terminus of all VSG mRNAs (13, 14).

Recombinant DNA clones. A complete HindIII digest of DNA from variant GUTat 7.1, was used to construct a recombinant DNA library in pAT 153, essentially as described by Maniatis et al. (36). The vector DNA and nuclear DNA were mixed at a ratio of 5 to 1 and ligated for 15 h at 15°C. The recombinant plasmids were then used to transform E.coli DH1. The library was screened with nick-translated cDNA probes TcV 7.2-7 and TcV 7.1-14, using hybridization conditions as described above with the addition of pAT 153 and E.coli DNA as competitor. Recombinant plasmids were isolated from clonal cultures using the procedure described by Birnboim and Doly (37).

Bal 31 digestion of nuclear DNA. Large trypanosomal DNA was incubated with Bal 31 (Boehringer) as described by Yao and Yao (38) using the modified procedure described by De Lange and Borst (39). The purified DNA was digested with EcoRI (GUTat 7.2) or HindIII (GUTat 7.1), size-fractionated and transferred to nitrocellulose as described above.

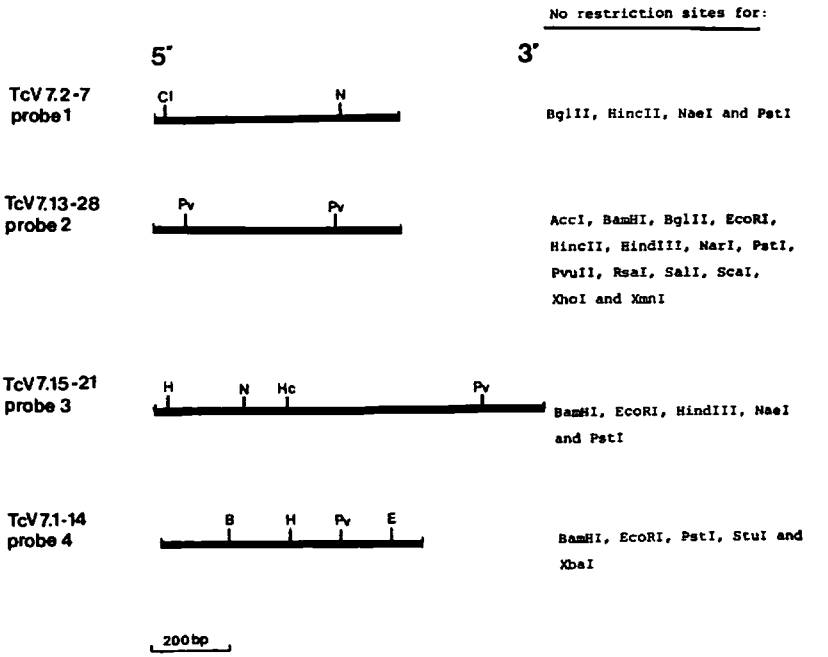

Figure 1. Physical maps of cDNA plasmids TcV 7.2-7, TcV 7.13-28, TcV 7.15-21 and TcV 7.1-14; only the insert DNA is shown. The numbering of the probes is as used in the text. Abbreviations of restriction sites: see Figure 3.

RESULTS

We have previously obtained several cloned B-VATs which elicit antibodies that react with the surface coat of the M-VATs of our T.brucei stock. Four of these, 7.1, 7.2, 7.13 and 1.61, were used to isolate recombinant DNAs complementary to the corresponding VSG mRNAs. These recombinant DNAs were hybridized to restriction digests of the nuclear DNAs of a series of VATs to characterize the corresponding VSG genes. Figure 1 shows the physical maps of the cDNA probes used and Figs 2-4 summarize some of the results.

A simple hybridization pattern was obtained with the probe for the 7.2 VSG gene. Panel A of Figure 2 shows that this probe hybridizes to a single band in restriction digests of the VATs that do not express the VSG gene (lanes 1 and 3), if a restriction endonuclease is used that does not cut in the probe DNA. The 7.2 expressor (lane 2) yields a second hybridizing band, suggesting that this gene is activated by duplicative transposition. To analyse the nature of this transposition a genomic clone (pMG 7.2-1) of the

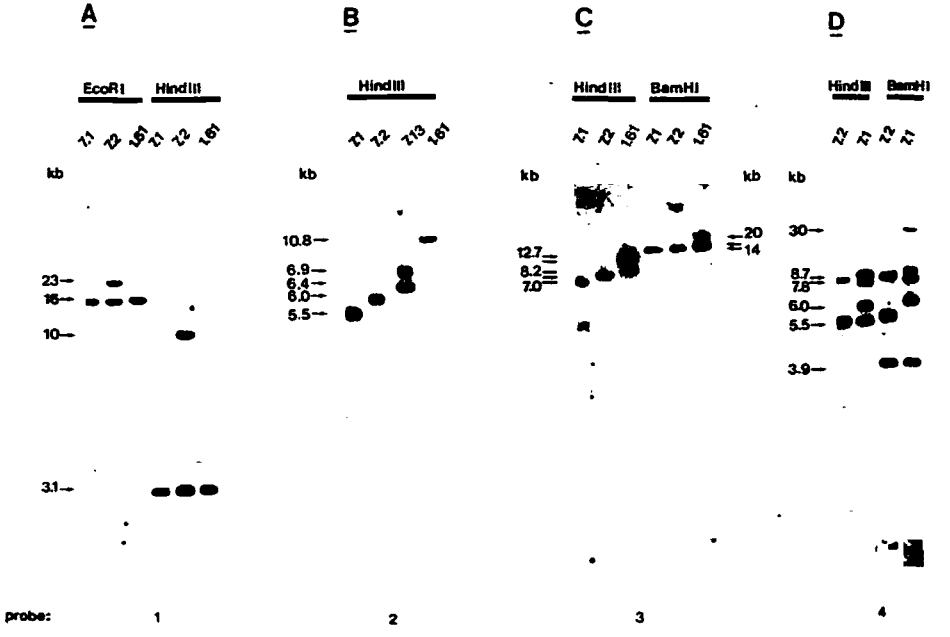

Figure 2. Blot analysis of the BC and ELC genes in variants 7.2 (panel A), 7.13 (panel B), 1.61 (panel C) and 7.1 (panel D). Nuclear DNA of each variant was digested with the restriction enzyme indicated, size-fractionated on agarose gels, blotted onto nitrocellulose filters and hybridized to ^{32}P-labelled cDNA probes complementary to each VSG mRNA (see Figure 1).

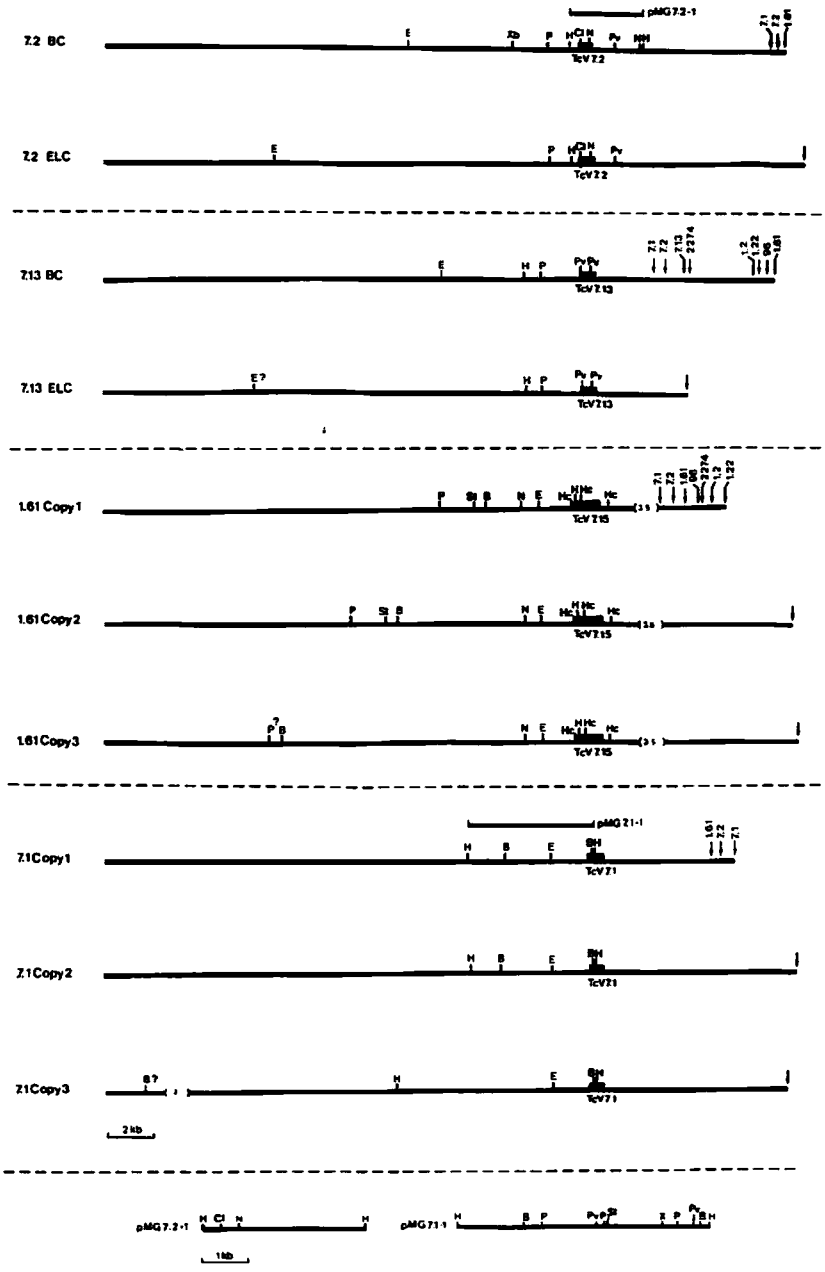

Figure 3. Physical maps of the gene copies for VSG 7.2, 7.13, 1.61 and 7.1. The restriction sites were mapped with cDNAs as hybridization probes. Additional sites for VSG 7.2 and 7.1 (BC) genes were mapped using the

genomic clones pMG 7.2-1 and pMG 7.1-1 (enlarged at the bottom part of the figure). The arrows indicate the chromosome end for each variant or stock analysed;? indicates that site position or order is not certain. Abbreviations of restriction enzymes: B, BamHI; Cl, ClaI; E, EcoRI; H, HindIII; Hc, HincII; N, NaeI; P, PstI; Pv, PvuII; St, StuI; X, XhoI; Xb, XbaI.

basic copy 7.2 gene was isolated and physical maps of both basic copy and expression-linked extra copy were constructed. Fig. 3 shows that both gene copies look like telomeric genes with the characteristic downstream region devoid of restriction enzyme cleavage sites ending in a site where all restriction enzymes seem to cut. The telomeric location of the genes was confirmed by digestion of the DNA with exonuclease Bal 31 prior to restriction enzyme cleavage, as shown in Figure 4A.

The maps in Figure 3 show that the size of the duplicated DNA segment of the 7.2 gene that gave rise to an ELC lies between 2.7 kb (size of PstI-PvuII fragment) and 5.2 kb (size of the XbaI-NaeI fragment). In previous duplicative transpositions of telomeric genes we have thusfar always found that all restriction sites downstream of the gene are co-transposed. This is obviously not the case in Figure 3A, where the NaeI and HindIII sites 3' of the gene are not found in the expression-linked copy. It is possible, however, that these were originally present, but removed by a later abortive telomeric conversion (cf. 40).

The results obtained with the probe for VSG gene 7.13 are similar to those for gene 7.2. Panel B of Figure 2 shows that the three VATs that do not express VSG gene 7.13 contain a single fragment that hybridizes strongly with the probe against a background of weakly cross-hybridizing fragments. This fragment varies in size in the four VATs, a property characteristic of telomeric restriction fragments in trypanosomes (41-43). Also in this case the VAT that expresses the 7.13 gene contains an extra copy of the gene. The physical maps (Figure 3, panel B) and digestion with Bal 31 (not shown) establish that both copies are telomeric.

Analogous results were obtained with VSG gene 7.15. In the non-expressors 7.1 and 7.2 (both GUTat) the probe recognizes a single band (panel C, Figure 2). In the non-expressor ILTat 1.22, however, the same probe recognizes two bands (not shown), whereas the expressor ILTat 1.61 yields three bands (panel C, Figure 2). All three 7.15 genes mapped in ILTat 1.61 are telomeric, as judged from the physical maps (Figure 3) and Bal 31 digestion (not shown).

Panel D of Figure 2 shows that the 7.1 gene probe gives more complex

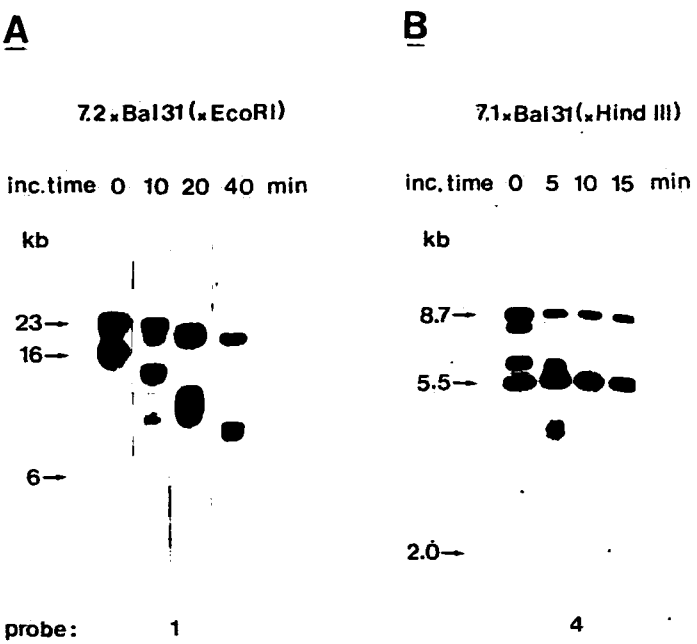

Figure 4. Autoradiograms of Southern blots showing preferential digestion of VSG genes 7.2 and 7.1 by Bal 31 nuclease. The DNA was incubated with Bal 31 for the times indicated, collected by ethanol precipitation and digested with EcoRI (variant 7.2; panel A) or HindIII (variant 7.1; panel B). The restricted DNA was size-fractionated, blotted onto a nitrocellulose filter and hybridized with the probes indicated (see Figure 1).

results than the other probes. In this case restriction enzymes were used that cut in the probe and each gene yields two hybridizing fragments. The non-expressor VAT 7.2 contains two genes, the expressor VAT 7.1 three. The physical maps in panel D of Figure 3, constructed with the aid of the genomic recombinant-DNA clone pMG 7.1-1, indicate that each of the three genes is telomeric and this was confirmed by Bal 31 digestion in panel B of Figure 4. Note that in this experiment the 3' fragment of one of the three genes comigrates with one of the 5' fragment in the 8.7 kb double band, which decreases two fold in intensity after 5 min incubation.

These results show that in 3 of the 4 VATs studied only a single gene is usually present in non-expressors. Does this imply that the same gene is expressed in the M- and B-repertoire? Two alternative interpretations can be considered. First, it is theoretically possible that two copies of each gene were present in the M-repertoire, but that one of these - the metacyclic

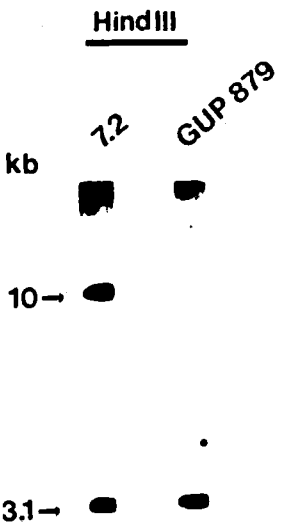

Figure 5. Blot analysis of gene copy number of gene 7.2 in a trypanosome population derived from a cloned metacyclic trypanosome (GUP 879) after 3 mice passages (total 15 days) since the last tsetse transmission. Nuclear DNAs (variant 7.2 was used as control) were digested with HindIII, size-fractionated on an agarose gel, blotted onto a nitrocellulose filter and hybridized with a 7.2 gene-specific probe.

probe: 1

copy - was lost when these trypanosomes were grown for prolonged periods in mammals. This was tested by transmitting the progeny of a cloned metacyclic trypanosome through tsetse flies. The tsetse salivary probe of this clone reacted for approximately 12% with antibody against VSG 7.2. Bloodstream trypanosomes derived from a line of this clone were isolated. Figure 5 shows a blot of DNA isolated from this limited passaged population, called GUP 879. There is still a single gene hybridizing with the 7.2-specific probe. These bloodstream trypanosomes were again used for transmission through tsetse flies and as expected the presence of GUTat 7.2 was demonstrated using immunofluorescence, indicating that loss of metacyclic gene copies through prolonged passage - as suggested above - is highly improbable. This implies that the single 7.2 gene present in the fly-transmitted population can be expressed both in the M- and the B-VAT repertoire. A second alternative interpretation for our results is that the M- and B-VSGs that react with the same antibodies, are encoded by two different genes that do not cross-hybridize under our conditions. There are, however, no reported precedents for common epitopes on very different VSG genes, despite the application of

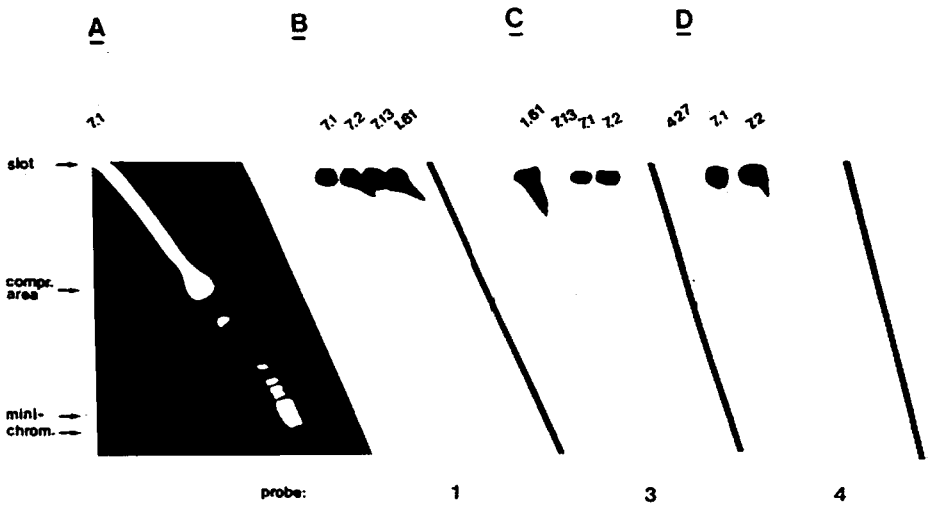

Figure 6. The chromosomal location of VSG genes 7.2, 1.61 and 7.1. Intact chromosomes of each variant (indicated at the top of the figure) were fractionated in a PFG-gel (see ethidium bromide stained lane in panel A), blotted onto a nitrocellulose filter and hybridized with the probes indicated (see Figure 1). At the pulse frequency of 35 seconds used, the mini-chromosomes migrate at an apparent size of 50-150 kb, the compression area contains DNA of 700 kb and larger. See Methods for experimental procedures.

monoclonal antibodies to the study of epitopes (44). Common epitopes have been demonstrated only on VSGs with very similar genes (45, 46). We therefore conclude that a single VSG gene can be active in both the M- and the B-repertoire.

It follows from this conclusion that our data provide information on the properties on M-genes. The most striking property is that all M-genes are telomeric. To test whether these telomeres belong to a specific class of chromosomes, we have located the genes on chromosome-sized T.brucei DNA molecules size-fractionated by pulsed-field gradient gel electrophoresis (30). Figure 6 shows that the 7.1, 7.2 and 1.61 probes all hybridize predominantly with material near the slot, with some hybridization in the 2-Mb area. This is typical for genes that are located in large DNA that is trapped near the slot. The hybridization near the slot is not due to hybridization to related genes, because these three probes give relatively clean Southern blots under the hybridization conditions used. This is also supported by the result for the 1.61 probe on VAT 7.13 DNA which does not contain the 1.61

gene. In this case only weak hybridization is observed in the PFG gels. This confirms the immunological analysis of Barry et al. (21), who showed that the 7.15 (= 1.61) VAT was lost from the metacyclic population from which VAT 7.13 was cloned (9th tsetse transmission). A similar control is available for the 7.1 probe, because this gene is absent in the 427 stock. The 7.13 probe was not useful because it hybridizes to too many related genes (cf. Figure 2B).

DISCUSSION

We have set out to characterize VSG genes expressed in the M-repertoire. To achieve this we have used two approaches: cloning of bloodstream trypanosomes expressing VSG genes homologous to M-VATs (7.1 and 7.2) and cloning of metacyclic trypanosomes (7.13) or their immediate bloodstream succesors (1.61) which were subsequently rendered stable for VAT by passaging in mice. The four B-VATs obtained in this way reacted with polyclonal- and monoclonal antibodies directed against their respective homologous M-VAT (21). Southern analysis showed that probes for the VSG gene expressed hybridized to a single basic copy VSG gene for three of the four B-VATs. We conclude that this gene must be expressed both in the B- and in the M-repertoire. Hence, there are no separate B- and M-genes.

What makes a VSG gene a M-gene? Table 1 summarizes the main features of the VSG genes studies here. All genes identified are telomeric and in the

TABLE I: Chromosomal position, mode of activation and copy number of the four metacyclic VSG genes studies in T.brucei (GUTAR repertoire of stock 2340 (21)).

VAT	VSG gene expressed	Number of BC genes in non-expressors*	Chromosomal position of activated BC gene	Mode of activation	Position in PFG gels BC/ELC
7.2	7.2	1	telomeric	duplicative transposition	slot/slot
7.13	7.13	1	telomeric	duplicative transposition	?
7.1	7.1	2*	telomeric	duplicative transposition	slot/slot
1.61	7.15	1	telomeric	duplicative transposition	slot/slot

? interpretation of results is uncertain since the 7.13 probe hybridizes to many related genes (see Figure 2B)
* both copies are telomeric
BC = basic copy
ELC = expression-linked extra copy

three cases where the chromosomal location of the genes was identified, they were located in the large DNA fraction close to the slot. Of the total repertoire of telomeric VSG genes, the majority is located in small chromosomes (31). This raises the possibility that the M-genes may represent a specific sub-set of telomeric VSG genes located in the large DNA fraction. It is not known what makes the large DNA fraction remain near the slot. If it is merely a matter of size, the chromosomes involved would probably have to be at least 3 Mb. A very crude estimate of the amount of DNA near the slot, led Van der Ploeg et al., (31) to conclude that twothirds of the DNA remains near the slot, i.e. nearly 30 Mb. This is probably an underestimate because they disregarded in their calculation that T.brucei is probably diploid for the slot-chromosomes, which contain housekeeping genes, while it is functionally haploid for the smaller chromosomes (47). There could therefore be a sufficient number of telomeres near the slot to code for the limited number of VATs thought to be expressed at a high percentage in the M-repertoire (20, 21).

Why is this specific sub-set of telomeric VSG genes activated in the fly? Telomeric genes active in the B-repertoire can be activated with or without duplication (e.g. 15, 25, 48, 49). Duplicative activation involves the duplicative transposition of the gene and a varying stretch of surrounding sequence to a telomeric expression site. This probably inserts the gene downstream of an active promoter and displaces the gene previously present in the expression site. Non-duplicative activation activates transcription of a previously silent expression site by an unknown mechanism. More than one telomere can act as an expression site; the number of telomeres which can express a VSG gene is not known (31, 32).

On the basis of these data, two extreme models can be considered for the activation of M-genes. In model one there is a single expression site activated in the fly and genes move to the expression site by telomere conversion (14, 32, 50). Specificity would be conferred in two ways: by the single M-promoter and by the ability of genes to enter the M-expression site. Model two postulates that there are a limited number of M-telomeres and that only the VSG genes that happen to reside in the M-telomere can be activated. Obviously the actual situation could be between these extremes.

A problem with model one is the requirement of two separate events that are not easily combined, at least conceptually: activation of a separate M-expression site and the preferential insertion of a limited fraction of the large number of telomeric VSG genes into this site. The telomeric gene

conversion involved is probably mediated by sequence homology (although this has not been directly proven) and it is not obvious why this should involve a different sub-set of genes in the M- and B-repertoires. This is underlined by the recent finding that telomere conversion may be a more general process in eukaryotes and not restricted to trypanosomes (51). Model two requires a mechanism for inter-telomeric exclusion; if there is a limited set of M-telomeres, there must be a way to prevent their simultaneous activity in one trypanosome.

If M-genes are present in expression sites that are preferentially activated in situ in the metacyclic phase of development, such genes might require duplicative transposition to another expression site for activation in the B-repertoire. This is essentially what we find for the genes analysed (Table I) although we have not verified that the extra copy in VATs expressing these genes in the B-repertoire are indeed the genes expressed. In contrast, Laurent et al. (58) have observed no duplication of the putative AnTat 1.6 M-gene in the B-repertoire of another trypanosome strain. Possibly, a telomere may contain separate signals allowing activation in the M- and B-repertoire.

VSG genes can be lost abruptly from the M-repertoire (21). This can readily be explained by their telomeric position. Both Pays and co-workers and Bernards et al. have shown previously that single copy telomeric genes can be readily lost by telomeric conversion in which these genes are displaced by another telomeric gene and destroyed (25, 50). When the telomere in itself is the determining factor, one would expect that the new gene that has displaced the old M-gene will now become part of the M-repertoire.

There are several aspects of this model which need to be verified. Are M-genes indeed activated without duplication? Do telomeres active in the M-repertoire differ systematically in sequence from telomeres active in the B-repertoire? Despite intensive efforts in several laboratories a VSG gene promoter has yet to be found. It may therefore take some time until we know in detail what characterizes a metacyclic expression site.

After this work was completed, Lenardo et al. (52) reported properties of two M-VATs of another T.brucei stock. Both are telomeric genes and the authors tentatively conclude that both genes are activated in the M-repertoire without duplication. They also suggest that telomeres containing M-genes may not undergo much size variation. This is what we observe for the 7.2 gene but not for the other ones (see Fig. 3). The authors note that their M-genes are not preceded by a region without restriction enzyme cleavage

sites (a so-called "barren" region), as is usual for telomeric genes of the B-repertoire. Such regions are now known to consist of long tandem arrays of the imperfect 70-bp repeats (7, 25, 53, 54) first observed in few copies before chromosome-internal VSG genes (55). Although we have not systematically looked at this aspect, it is clear from Fig. 3 that none of the basic copy VSG gene that we have studied contains a barren region comparable to that present in front of ELC genes of the B-repertoire. It should be realized, however, that the imperfect nature of the 70-bp repeats leads to the presence of occasional restriction enzyme cleavage sites. Moreover, we have verified by hybridization that at least some 70-bp are present in front of the 7.1 gene in the PvuII fragment shown in the map in Figure 3 (unpublished observations). Further analysis of M-genes and a larger selection of telomeric B-genes will be required to see if lack of long arrays of 70-bp repeats is indeed a characteristic of M-genes.

The data presented here and the models invoked to account for them also have some bearing on the pressing question whether vaccination against trypanosomiasis is feasible. If the M-repertoire were stable enough, one could in principle prepare a polyvalent vaccine based on M-VATs and prevent the spread of trypanosomes by tsetse flies. If our data are representative for M-genes in general and if our interpretation of the data is correct, then vaccination prospects are not good. The extensive analysis of B-repertoires of different trypanosome stocks in several laboratories in recent years has shown that there is considerable movement of VSG genes through telomeres (e.g. 40, 56, 57). Chromosome internal genes can move by duplicative transposition into a telomere and telomeric genes can move to other telomeres by telomeric conversion. Hence, one would expect that in principle any VSG gene of the vast repertoire of 10^3 genes, could end up in a telomere which acts as an expression site for metacyclic VSG genes. Indeed, antibody analysis has already shown instability of the M-VAT repertoire. This makes development of a generally useful vaccine improbable.

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REFERENCES

1. Vickerman, K. (1978) Nature 273, 613-617.
2. Rifkin, M.R. (1978) Exp. Parasitol. 46, 189-206.
3. Cross, G.A.M. (1975) Parasitology 71, 393-417.
4. Cross, G.A.M. (1978) Proc. R. Soc. Lond. B. 202, 55-72.
5. Doyle, J.J. (1977) In: Immunity of Blood Parasites of animals and man. (Miller, L.H., Pino, J.A. and McKelvey, J. Jr., Eds.) Plenum Press, New York, 27-63.
6. Capbern, A., Giroud, C., Baltz, T. and Mattern, P. (1977) Exp. Parasitol. 42, 6-13.
7. Van der Ploeg, L.H.T., Valerio, D., De Lange, T., Bernards, A., Borst, P. and Grosveld, F.G. (1982) Nucl. Acids Res. 10, 5905-5923.
8. Van Meirvenne, N., Janssens, P.G. and Magnus, E. (1975) Ann. Soc. Belge Med. Trop. 55, 1-23.
9. Van Meirvenne, N., Janssens, P.G., Magnus, E., Lumsden, W.H.R. and Herbert, W.J. (1975) Ann. Soc. Belge Med. Trop. 55, 25-30.
10. Kosinski, R.J. (1980) Parasitology 80, 343-357.
11. Miller, E.N. and Turner, M.J. (1981) Parasitology 82, 63-80.
12. Laurent, M., Pays, E., Magnus, E., Van Meirvenne, N., Matthyssens, G., Williams, R.O. and Steinert, M. (1983) Nature 302, 263-266.
13. Michels, P.A.M., Liu, A.Y.C., Bernards, A., Sloof, P., Van der Bijl, M.M.W., Schinkel, A.H., Menke, H.H., Borst, P., Veeneman, G.H., Tromp, M.C. and Van Boom, J.H. (1983) J. Mol. Biol. 166, 537-556.
14. Michels, P.A.M., Van der Ploeg, L.H.T., Liu, A.Y.C. and Borst, P. (1984) EMBO J. 3, 1345-1351.
15. Myler, P., Nelson, R.G., Agabian, N. and Weber, H. (1984) Nature 309, 282-284.
16. Longacre, S., Hibner, U., Raibaud, A., Eisen, H., Baltz, T., Girard, C. and Baltz, D. (1983) Mol. Cell. Biol. 3, 399-409.
17. Raibaud, A., Gaillard, C., Longacre, S., Hibner, U., Buck, G., Bernardi, G. and Eisen, H. (1983) Proc. Natl. Acad. Sci. USA 80, 4306-4310.
18. Seed, J.R., Edwards, R. and Sechelski, J. (1984) J. Protozool. 31, 48-53.
19. Liu, A.Y.C., Michels, P.A.M., Bernards, A. and Borst, P. (1985) J. Mol. Biol., in press.
20. Crowe, J.S., Barry, J.D., Luckins, A.G., Ross, C.A. and Vickerman, K. (1983) Nature 306, 389-391.
21. Barry, J.D., Crowe, J.S. and Vickerman, K. (1984) Nature 306, 699-701.
22. Barry, J.D., Crowe, J.S. and Vickerman, K. (1985) Parasitology 90, 79-88.
23. Barry, J.D., Hajduk, S.L., Vickerman, K. and Le Ray, D. (1979) Trans. R. Soc. Trop. Med. Hyg. 73, 205-208.
24. Lumsden, W.H.R. and Herbert, W.J. (1975) Trans. R. Soc. Trop. Med. Hyg. 69, 205-208.
25. Bernards, A., De Lange, T., Michels, P.A.M., Huisman, M.J. and Borst, P. (1984) Cell 36, 163-170.
26. Auffray, C. and Rougeon, F. (1980) Eur. J. Biochem. 107, 303-314.
27. Bernards, A., Van der Ploeg, L.H.T., Frasch, A.C.C., Borst, P., Boothroyd, J.C., Coleman, S. and Cross, G.A.M. (1981) Cell 27, 497-505.
28. Hoeijmakers, J.H.J., Borst, P., Van den Burg, J., Weissmann, C. and Cross, G.A.M. (1980) Gene 8, 391-417.
29. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
30. Schwartz, D.C. and Cantor, C.R. (1984) Cell 37, 67-75.
31. Van der Ploeg, L.H.T., Schwartz, D.C., Cantor, C.R. and Borst, P. (1984) Cell 37, 77-84.
32. Van der Ploeg, L.H.T., Cornelissen, A.W.C.A., Michels, P.A.M. and Borst, P. (1984) Cell 39, 213-221.
33. Van der Ploeg, L.H.T., Cornelissen, A.W.C.A., Barry, J.D. and Borst, P. (1984) EMBO J. 3, 3109-3115.

34. Wieslander, L. (1979) Anal. Biochem. 98, 305-309.
35. Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237-251.
36. Maniatis, T., Frisch, E.F. and Sambrook, J. (Eds.) (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
37. Birnboim, H.C. and Doly, J. (1979) Nucl. Acids Res. 7, 1513-1523.
38. Yao, M.C. and Yao, C.H. (1981) Proc. Natl. Acad. Sci. USA 78, 7436-7439.
39. De Lange, T. and Borst, P. (1982) Nature 299, 451-453.
40. De Lange, T., Kooter, J.M., Michels, P.A.M. and Borst, P. (1983) Nucl. Acids Res. 11, 8149-8165.
41. Bernards, A., Michels, P.A.M., Lincke, C.R. and Borst, P. (1983) Nature 303, 592-597.
42. Pays, E., Laurent, M., Delinte, K., Van Meirvenne, N. and Steinert, M. (1983) Nucl. Acids Res. 11, 8137-8147.
43. Van der Ploeg, L.H.T., Liu, A.Y.C. and Borst, P. (1984) Cell 36, 459-468.
44. Hall, T. and Esser, K. (1984) J. Immunol. 132, 2059-2063.
45. Pays, E., Lheureux, M., Vervoort, T. and Steinert, M. (1981) Mol. Biochem. Parasitol. 4, 349-357.
46. Pays, E., Lheureux, M. and Steinert, M. (1983) Nucl. Acids Res. 9, 4225-4238.
47. Gibson, W.C., Osinga, K.A., Michels, P.A.M. and Borst, P. (1985) Mol. Biochem. Parasitol., in press.
48. Williams, R.P., Young, J.R., Majiwa, P.A.O., Doyle, J.J. and Shapiro, S.Z. (1980) Am. J. Trop. Med. Hyg. 29 (Supplement) 1037-1042.
49. Penncavage, N.A., Julius, M.A. and Marcu, K.B. (1983) Nucl. Acids. Res. 12, 5235-5247.
50. Pays, E., DeLauw, M.F., Van Assel, S., Laurent, M., Vervoort, T., Van Meirvenne, N. and Steinert, M. (1983) Cell 35, 721-731.
51. Horowitz, H., Thorburn, P. and Haber, J.E. (1984) Mol. Cell. Biol. 4, 2509-2517.
52. Lenardo, M.J., Rice-Ficht, A.C., Kelly, G., Esser, K.H. and Donelson, J.E. (1984) Proc. Natl. Acad. Sci. USA 81, 6642-6646.
53. Campbell, D.A., Van Bree, M. and Boothroyd, J.C. (1984) Nucl. Acids. Res. 12, 2759-2774.
54. Bernards, A., Kooter, J.M. and Borst, P. (1985) Mol. Cell. Biol. 5, 545-553.
55. Liu, A.Y.C., Van der Ploeg, L.H.T., Rijsewijk, F.M. and Borst, P. (1983) J. Mol. Biol. 167, 57-75.
56. Frasch, A.C.C., Borst, P. and Van den Burg, J. (1982) Gene 17, 197-211.
57. Borst, P., Bernards, A., Van der Ploeg, L.H.T., Michels, P.A.M., Liu, A.Y.C., De Lange, T. and Sloof, P. (1984) In: Molecular Biology and Host-Parasite Interactions (Agabian, N. and Eisen, H., Eds.) Liss, New York, 205-217.
58. Laurent, M., Pays, E., Delinte, K., Magnus, E., Van Meirvenne, N. and Steinert, M. (1984) Nature 308, 370-373.