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Two Modes of Activation of a Single Surface Antigen Gene of Trypanosoma brucei

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Summary

Several genes for variant antigens in trypanosomes are activated by duplicative translocation to a telomeric expression site. A second—nonduplicative—mode of activation is restricted to telomeric antigen genes. We show here that the single telomeric gene for antigen 221 can be activated in both ways. We also show that gene 221 is split and that the 5′ 35 nucleotide sequence, common to all surface antigen mRNAs, is not encoded within 8.5 kb upstream of the 221 coding region. No major rearrangements are observed within 55 kb upstream of the 221 coding region upon nonduplicative activation. Gene inactivation is usually accompanied by deletion of the gene and at least 8.5 kb upstream and may involve conversion by another telomere. These results are not readily explained by a single expression site model. The duplicative gene 221 activation differs from conventional duplicative activation in the extent of the transposed segment, which is larger and may include the entire segment between gene and telomere.

Introduction

The surface coat that envelopes salivarian trypanosomes during their stay in the vertebrate host (Vickerman, 1969) consists mainly of a single protein, the variant surface glycoprotein (VSG) (Cross, 1975). Each trypanosome can express at least 100 (Capbern et al., 1977), but contains possibly as many as 1000 VSG genes (Van der Ploeg et al., 1982a). The repeated switching of the VSG gene expressed gives rise to antigenic variation that allows Trypanosoma brucei and other salivarian trypanosomes to maintain a chronic relapsing infection in the face of an active host immune response (reviewed by Vickerman, 1978; Englund et al., 1982; Borst and Cross, 1982).

To control the expression of this multigene family, trypanosomes employ two modes of antigen gene activation. The best understood is a mechanism involving gene duplication and insertion of the duplicated gene into an expression site (Hoeijmakers et al., 1980a; Borst et al., 1981; Pays et al., 1981a; Van der Ploeg et al., 1982b; Majiwa et al., 1982) that is located adjacent to a chromosome end (De Lange and Borst, 1982). The incoming gene appears to displace the preceding one from the expression site by a gene conversion process involving limited sequence homology at the edges of the duplicated segments (Bernards et al., 1981; Van der Ploeg et al., 1982b; Michels et al., 1982, 1983; Liu et al., 1983). The duplicated genes in the expression site are bracketed by segments of DNA that are not cut by most restriction endonucleases and that differ in length in different trypanosome clones (Michels et al., 1982, 1983). The length variation of the downstream segment is explained by a continuous growth of chromosome ends in trypanosomes during cell multiplication, a process balanced by occasional deletions (Bernards et al., 1983). The variability of the upstream segment may be a consequence of the switching process itself.

All VSG messenger RNAs (mRNAs) in T. brucei strain 427 carry the same 35 nucleotide sequence at their 5′ end (Van der Ploeg et al., 1982c; Boothroyd and Cross, 1982). This common sequence is not encoded in the duplicated segment of the VSG gene and, therefore, probably stems from a mini-exon in the expression site (Van der Ploeg et al., 1982c; Liu et al., 1983). Hybridization of trypanosome DNA with a synthetic mini-exon probe reveals the presence of about 200 copies of the mini-exon sequence, mostly in a tandemly linked configuration (Borst et al., 1983a; De Lange et al., 1983). Although a direct linkage between mini-exons and the expression site has not been established, we have proposed that this class of VSG genes is activated by inserting a duplicated gene downstream of a promoter and mini-exon repeat (De Lange et al., 1983). The duplication-activated VSG genes include all the presently studied antigen genes that are not adjacent to chromosome ends and at least one of the telomerically located genes (Laurent et al., 1983).

A second mode of antigen gene activation was first described by Williams and coworkers and does not lead to a change in the antigen gene copy number (Williams et al., 1979, 1981; Young et al., 1982). A gene with similar properties was found in our trypanosome strain to be adjacent to a chromosome end (Bernards, 1982) and the same conclusion has been reached by Williams et al. (1982) for their set of genes. This mode of activation therefore seems restricted to telomerically located antigen genes. We have proposed that nonduplicative activation results from a telomere-exchange process, involving the expression site telomere and telomeric VSG genes (Borst et al., 1983a).

Here, we show that both the duplicative and the nonduplicative mode of activation may be used by the single telomeric gene for antigen 221. The two modes of activation, therefore, operate on overlapping gene families.

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Results

The Nonduplicative Mode of Gene 221 Activation

To analyze possible DNA rearrangements accompanying the expression of VSG gene 221, detailed maps of the restriction enzyme cleavage sites in and around the gene in nuclear DNA were constructed, using the complementary DNA (cDNA) plasmids described previously (Hoeijmakers et al., 1980b). Figure 1A shows that plasmids TcV221.1 and TcV221.5 together cover the entire coding and 3′ noncoding part of VSG 221 mRNA (Boothroyd et al., 1980). In addition to the cDNA restriction fragments indicated in Figure 1A, fragments of the genomic clones TgB221.1 (Van der Ploeg et al., 1982a), TgB221.2, and TgB221.3 were used as hybridization probes (Figure 1B). No clones were obtained containing the 3′ flanking sequences of the 221 gene, but telomeric VSG genes are known to be selected against in recombinant DNA clone banks (Van der Ploeg et al., 1982a; Young et al., 1982).

The blot hybridization analysis of the 221 gene in two VSG 118 expressors (trypanosome clones 118a and 118b) and one VSG 221 expressor (clone 221a) yielded the maps presented in Figure 2. At the 3′ side the 221 gene is flanked by a DNA segment that differs in length in different trypanosome clones. This segment is not cut by 26 restriction endonucleases (see legend to Figure 2); it ends at a position where all these enzymes appear to cut, and it is preferentially shortened after incubation of intact trypanosome DNA with Bal 31 nuclease (results not shown). The 221 gene is therefore located adjacent to an interruption in the DNA molecule, presumably the end of a chromosome (Bernards, 1982; Bernards et al., 1983). The "barren" region devoid of restriction sites does not start immediately downstream of the 221 gene, however, as does a similar region downstream of most genes occupying the expression site in this trypanosome strain (see Michels et al., 1983). Instead, the 221 gene is flanked by a 0.8 kb DNA segment cut by Ava II, Msp I, Nar I, Sph I, Taq I, and Xba I (Figure 2).

The main conclusion to emerge from the analysis of the 221 gene is, however, that no extra expression-linked copy (ELC) of the gene is formed when it is activated in clone 221a. To exclude that the 221 ELC evades detection in blots, either because the duplicated segment of the gene is so large that all tested restriction sites are contained within it or because the flanking regions of the 221 ELC are unstable and have a different length in individual trypanosomes (see Borst and Cross, 1982), we determined the copy number of the 221 gene in expressor and nonexpressor trypanosome clones (Figure 3). In this experiment we calculated the number of 221 genes per nucleus to be 1.33 in 118b and 1.29 in 221a (see legend to Figure 3). This result confirms that the 221 gene is activated without gene duplication.

To test whether any upstream rearrangements accompany the expression of the 221 gene in trypanosome clone 221a, we extended the maps of the area upstream of the 221 coding region using fragments of clone TgB221.1 as

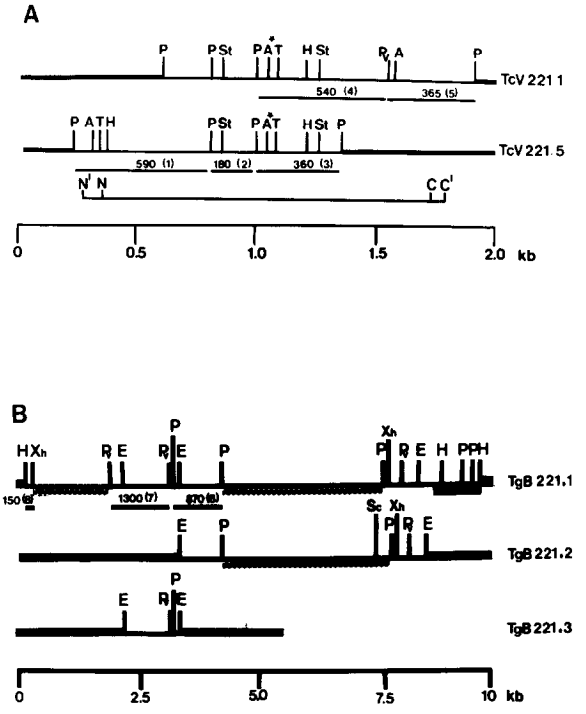

Figure 1. Physical Maps of cDNA Plasmids TcV221.1 and TcV221.5 Indicating Probe Fragments and Restriction Maps of Genomic Recombinant DNA Plasmids Containing Fragments Mapping Upstream of the 221 Gene

(A) Vector DNA is drawn with thick lines, insert DNA with thin ones. The restriction fragments used as hybridization probes, the length of these fragments in bp and the numbering of the probes used in the text (in brackets) is indicated beneath the maps. VSG 221 is synthesized as a precursor with both an NH_2- and a COOH-terminal extension (Boothroyd et al., 1980). The position of the 221 coding region is indicated. Abbreviations: N and C, NH_2- and COOH-termini of mature VSG; N′ and C′, NH_2- and COOH-termini of primary translation product. Abbreviations of restriction enzymes are given in the legend to Figure 2. A* denotes an Ava II site that is only very partially cut (1%–5%) in DNA replicated in Escherichia coli HB101 and was only detected by blot hybridization analysis. The partial cutting may be due to overlap with a methylated Eco RII site, since this prevents cutting by Ava II (Snyder et al., 1982). (B) Restriction maps of genomic recombinant DNA plasmids containing fragments mapping upstream of the 221 gene. Vector DNA, insert DNA, and fragments used as hybridization probes are drawn as in Figure 1A. The black rectangle beneath the map in TgB221.1 denotes the position of the VSG 221 main exon. The wavy lines underneath the maps of TgB221.1 indicate restriction fragments that hybridize to repeated DNA in genomic blots. Clone TgB221.1 was isolated from a recombinant DNA clone bank made of trypanosome clone 118a DNA; clones TgB221.1 and TgB221.3 are derived from a 221a DNA clone bank. The Eco RI fragment cloned in TgB221.2 is 0.15 kb larger than the corresponding Eco RI fragment in TgB221.1 because of an insertion in this fragment in trypanosome clone 221a DNA from which TgB221.2 is derived. This insertion may carry the extra Sca I (Sc) site in TgB221.2 (see text).

hybridization probes (see Figure 1B). In essence we obtained the same overall map (Figure 2) whether the 221 gene was active or not, but the maps are complicated somewhat by the presence of polymorphisms. First, there is a 0.15 kb insertion in 221a DNA at the position indicated in Figure 2 that is absent in 15 trypanosome variants that do not express this gene. This insertion probably carries the extra Sca I site in 221a DNA (indicated by an asterisk) since both the insertion and the Sca I site map in a 0.6 kb

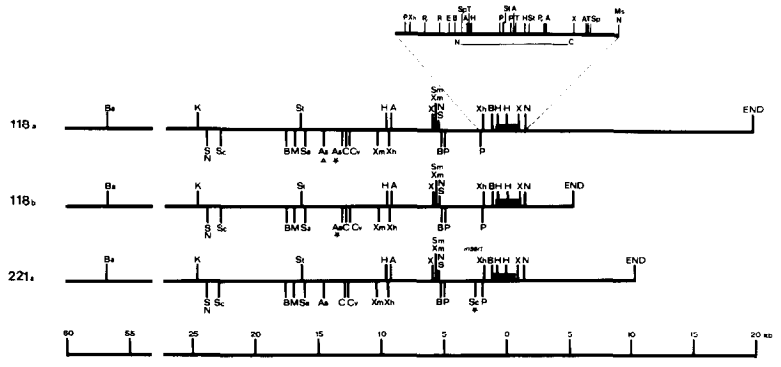

Figure 2. Physical Maps of the 221 Gene in Trypanosome Clones 118a, 118b, and 221a

The restriction sites indicated above the lines were mapped with cDNA fragments as hybridization probes. To map the additional sites drawn below the lines, probe fragments from the genomic clone TgB221.1 (see figure 1B) were used. To show more detail, the immediate surroundings of the 221 coding region (indicated with a box in the maps and with a line beneath the enlarged section) is drawn enlarged above the 118a map. The kb scale is centered on the 3′ gene-internal Hind III site. *: polymorphic Asu II and Sca I sites; Δ: a second Asu II site, detected in variant 118a by analysis of partial Asu II digests. The presence of this site in variant 118b has not been verified. The approximate position of the 0.15 kb insert just in front of the 221 main exon in 221a DNA is indicated. "END" denotes the Bal 31-sensitive end of the chromosome. The DNA segment between the 3′ end of the 221 gene and the end of the chromosome is not cut by the restriction enzymes Asu II, Bam HI, Bcl I, Bgl II, Cla I, Cvn I, Eca I, Eco RI, Eco RV, Hind III, Kpn I, Mlu I, Mbo II, Nco I, Pst I, Pvu II, Rsa I, Sac I, Sac II, Sal I, Sca I, Sma I, Stu I, Xho I, Xma III, and Xmn I. Abbreviations: A, Ava II; As, Asu II; B, Bgl II; Ba, Bam HI; C, Cla I; Cv, Cvn I; E, Eco RI; H, Hind III; K, Kpn I; M, Mlu I; Ms, Msp I; N, Nar I; P, Pst I; Pv, Pvu II; R, Rsa I; S, Sal I; Sa, Sac I; Sc, Sca I; Sm, Sma I; Sp, Sph I; St, Stu I; T, Taq I; X, Xba I; Xh, Xho I; Xm, Xmn I. Not all Msp I sites in the 221 coding region are indicated.

Mbo II fragment (not shown). A second complication in the maps results from the absence of an Asu II site in 221a DNA (marked with an asterisk). This is not due to an inversion in variant 221a, because the upstream Asu II site (marked with a triangle) is also present in variant 118a, as determined by the analysis of DNA partially digested with Asu II (not shown). All the above-mentioned polymorphisms are also found in the DNA of a trypanosome population that has switched off the expression of gene 221 (population $221ar_2$, see below). There is, therefore, no strict correlation between the expression of the 221 gene and the presence of the polymorphisms.

The most upstream site that was accurately mapped is the Bam HI site at approximately 55 kb in front of the 221 coding region. This site was mapped in a Bam HI × Sma I double digest that yields a 50 kb fragment detectable with probe 7 (Figure 1B) that comigrates in 0.3% agarose gels at low-voltage gradients in all DNAs tested. The Sma I fragment detected with this probe has the shear length of the DNA preparations (more than 100 kb) and is therefore too large for mapping purposes.

The 221 Gene Contains an Intervening Sequence

The mRNAs of the transposition-activated VSG genes 117 and 118 have an identical 35 nucleotide sequence at their 5′ end (Van der Ploeg et al., 1982c; Boothroyd and Cross, 1982). This sequence is not present in the transposed segment of the 118 gene and we have therefore concluded that it derives from a mini-exon in the expression site (Van der Ploeg et al., 1982c; Liu et al., 1983). An analysis of the 221 mRNA, isolated from trypanosome clone 221a, has established the presence of the same 5′ 35 nucleotide sequence (Boothroyd and Cross, 1982). To test whether this sequence is encoded contiguously with the remainder of the 221 gene we determined the DNA sequence of the region corresponding to the 5′ border of the 221 coding region present in recombinant clone TgB221.1 (see Figure 1B). Figure 4 presents this sequence in parallel with the 5′ end of the mRNA sequence determined by Boothroyd and coworkers (Boothroyd and Cross, 1982; Boothroyd et al., 1980). The sequences are identical but for the first 34 nucleotides of the mRNA which are not present contiguous with the remainder of the gene. At the position where the gene diverges from the mRNA, the DNA sequence reads CTCTTGAAG/A, the slash between nucleotides 35 and 36 indicating the probable divergence point as determined from the sequence of cloned mini-exons (De Lange et al., 1983). This sequence is similar to the intron–exon boundary of transposition-activated VSG genes (De Lange et al., 1983) and resembles the consensus sequence for splice acceptor sites in eucaryotes (see Mount, 1982).

The recombinant clone used to determine the DNA sequence of the 221 gene was isolated from a clone bank made of variant 118a DNA. We can exclude, however, that the 35 bp segment is encoded contiguously with the remainder of the gene in other variants, because the 35 bp mini-exon contains an Xmn I site and the first Xmn I site lies 5 kb upstream of the border of the main 221 exon in all variants analyzed here (Figure 2). To test whether the 35 bp mini-exon is present as a continuous sequence elsewhere in the TgB221.1 clone or in clone TgB221.2, containing the polymorphic 0.15-kb insertion, we hybridized these clones with a synthetic 22-mer deoxynucleotide probe complementary to the mini-exon. No hybridization was found (not shown) under conditions that allow ready detection of the mini-exon in other recombinant DNA clones or blots of total trypanosome DNA.

Switching Off Gene 221 Often Leads to Its Deletion

To test whether the inactivation of a VSG gene that is

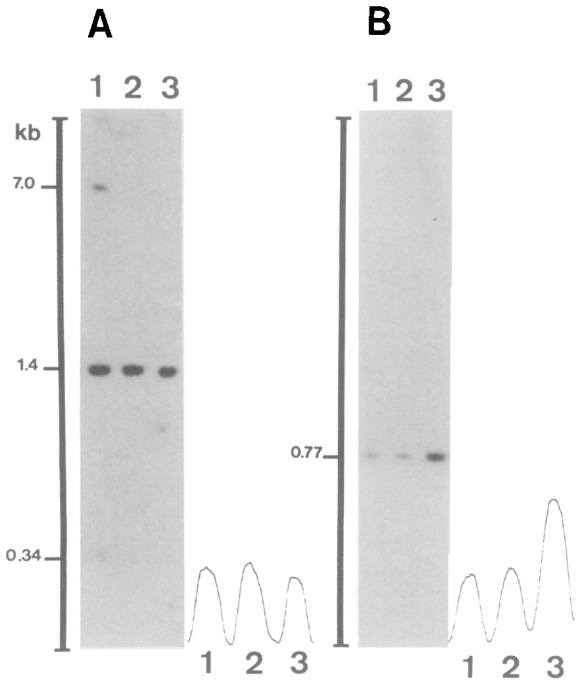

Figure 3. Determination of the 221 Gene Copy Number in Three Trypanosome Clones

The blot contains 1.0 μg Taq I-digested 118b (lane 1), 221a (lane 2), and 221b (lane 3) DNA and was probed with a VSG 118 cDNA probe (A). After autoradiography the probe was removed and the blot rehybridized with VSG 221 cDNA probe 1 (Figure 1A). The hybridization to a 1.4 kb VSG 118 BC fragment and a 0.77 kb VSG 221 gene-internal fragment was quantitated by scanning the autoradiographs. The scans are shown. The VSG 118 BC fragment was used to compare the amounts of DNA in the three lanes. In addition, the 118 cDNA probe detects a 0.34 kb Taq I fragment common to the 118 BC and ELC and a 7.0 kb fragment derived from the 118 ELC. The hybridization to the 0.77 kb 221 gene fragment was compared to the hybridization in adjacent lanes containing known amounts of Taq I-digested TcV221.5 plasmid. The amount of DNA per nucleus in T. brucei was assumed to be 86 × 10^6 bp (Borst et al., 1982). The calculated copy number of the 221 gene is 1.33 genes per nucleus in clone 118b, 1.29 in clone 221a, and 2.13 in clone 221b.

activated without duplication leads to detectable DNA rearrangements, we studied the 221 gene in five independent trypanosome populations that have switched off the expression of gene 221. These five "first-relapse" trypanosome populations were obtained by in vitro immune lysis of approximately 10^6 clone 221a trypanosomes with anti-VSG 221 antiserum. The small fraction of trypanosomes that does not express gene 221 survives the immune lysis and is grown up in rats. The inactivation of gene 221 was confirmed by an indirect immunofluorescence assay and by RNA blotting. Only population $221ar_4$ ("r" for relapse) still contained a small fraction (approximately 5%) of VSG 221-producing trypanosomes. Figure 5 shows a blot hybridization analysis of DNA isolated from the five switched populations in parallel with DNA from the parent clone 221a. The unexpected result is that the 1.9 kb Pvu II fragment representing the 221 gene is not detected in four out of the five populations. The faint 1.9 kb fragment in $221ar_4$ DNA probably stems from the small fraction of unrelapsed 221 expressors in this trypanosome

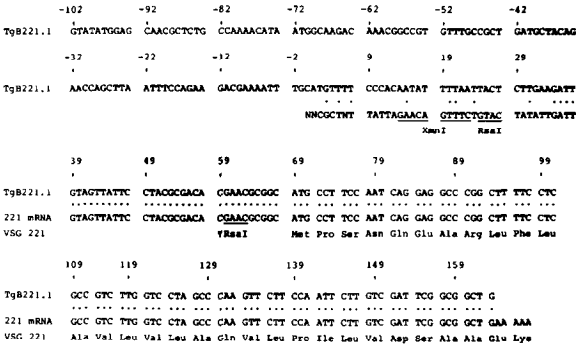

Figure 4. DNA Sequence of the 5′ End of the 221 Main Exon Plus Adjacent DNA Segment from Recombinant Clone TgB221.1, Lined Up with the 221 mRNA Sequence and the Amino Acid Sequence Determined by Boothroyd et al. (1980) and Boothroyd and Cross (1982)

The Xmn I and Rsa I sites in the 5′ most 35 bp of the 221 mRNA are indicated. At the position marked ψ Rsa I, an Rsa I site is present in TcV221.5 (Boothroyd et al., 1980), but not in TgB221.1 or in the 221 mRNA sequence (J. C. Boothroyd, personal communication). Its presence in TcV221.5 may be due to a cloning artifact. Abbreviations as given in the legend to Figure 2.

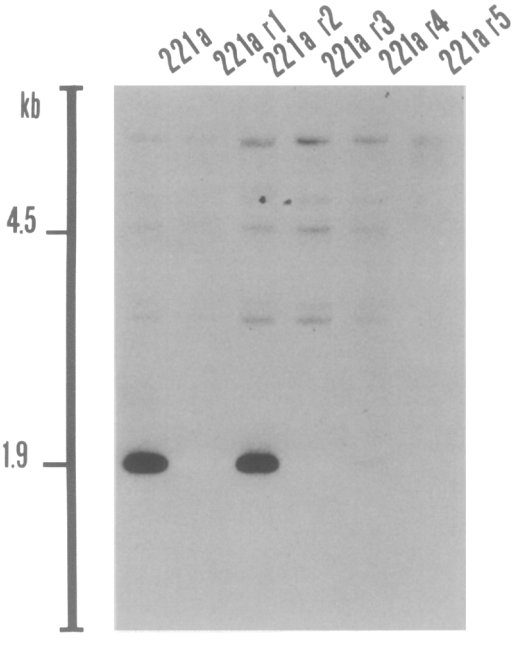

Figure 5. Blot Showing the Deletion of the 221 Gene in Four Out of Five Trypanosome Populations that Have Switched Off the Production of VSG 221

The isolation of these trypanosome populations is described in the text. The blot contains a size-fractionated Pvu II digest and was probed with cDNA probe 4. The fragments that cross-hybridize to this probe (see text) serve as a control for the presence of DNA. The 1.9 kb Pvu II fragment detected with this probe is indicated in the enlarged section of Figure 2.

population. The only fragments detected in these DNAs are fragments that weakly cross-hybridize to the probe.

To determine the extent of the deletion of the 221 gene in the four populations without the gene and to test whether upstream rearrangements accompany the inactivation of the 221 gene in $221ar_2$, we hybridized analogous blots as in Figure 5 with probe fragments derived from TgB221.1.

Fragments derived from the 221 gene area complementary to probe 6, 7, or 8 (Figure 1B) were not detected in the four DNAs from which the gene is missing (not shown). At least 8.5 kb of the segment upstream of the 221 main exon has therefore been codeleted. In $221ar_2$ DNA—containing the 221 gene—no rearrangements are observed with these probes. The most upstream restriction site that has been checked and continues to be present is the Sal I site at 23 kb upstream of the 221 coding region. A small fraction (approximately 5%) of the trypanosomes in relapse population $221ar_2$ may, however, have a rearranged 221 telomere, since a faint extra band (not detected in 221a DNA) is seen with upstream probes in most restriction digests (not shown). The analysis of this rearrangement awaits the cloning of a representative of this fraction of the population.

The Duplicative Mode of Gene 221 Activation

To further analyze DNA rearrangements associated with gene 221 activation we isolated and analyzed an additional trypanosome clone expressing gene 221. Trypanosome clone 221b was isolated as described by Michels et al. (1983) from a chronically infected rabbit 9 days after infection with 10^6 trypanosomes of variant 121a. VSG 221 cDNA probe 1 (Figure 1A) detects a similar RNA species in clone 221a and 221b RNA and this 1800 nucleotide RNA also hybridized with the synthetic 22 nucleotide miniexon probe (results not shown). The antigenic identity of clone 221b was further checked by S1 nuclease digestion of 221b RNA–221a cDNA hybrids. No differences between 221a and 221b mRNA were detected.

To determine whether DNA rearrangements are associated with the activation of gene 221 in trypanosome clone 221b, the DNA was subjected to an analogous blot hybridization analysis as described for clone 221a. The results are summarized in the physical maps presented in Figure 6. The most conspicuous point to emerge is the presence of a duplicated 221 ELC in 221b DNA. This extra gene manifests itself in the gene quantitation experiment shown in Figure 3 (2.13 copies per nucleus in 221b; 1.29 copies in 221a). It is also detected in blots hybridized with 3′ probes, since the gene-to-chromosome-end distance is different for the two gene copies, and in blots hybridized with 5′ probes since the duplicated gene has moved to a different telomere (see Figure 6).

The extent of the duplicated segment of the 221 gene is indicated with a line beneath the map in Figure 6. The upstream border of the duplicated segment falls in a 3.6 kb Pst I fragment that shows homology at the hybridization level with the fragment containing the upstream border of the duplicated segment of VSG gene 118 (Van der Ploeg et al., 1982a). Downstream of the 221 gene all mapped sites are coduplicated.

The duplicated ELC of gene 221 uses the same or a very similar expression site as the other duplication-activated genes (see Figure 6). To test which of the two 221 genes is transcribed we determined the susceptibility of the two genes to digestion by DNAase I (see Pays et al., 1981b). In this experiment, shown in Figure 7, a 8.2 kb Hind III fragment derived from the 221 ELC (see Figure 6)

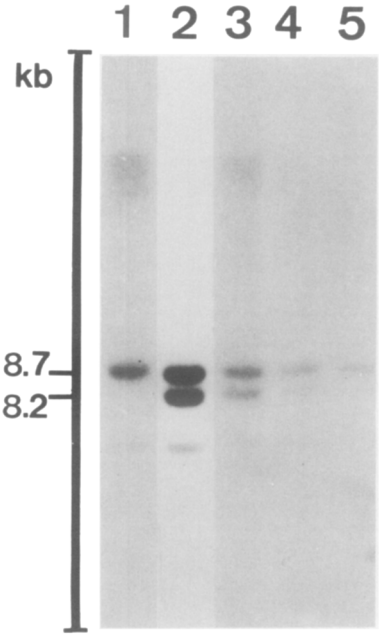

Figure 7. DNAase I Digestion of Trypanosome Clone 221b Nuclei Showing Preferential Degradation of the 221 ELC

Trypanosome clone 221b nuclei were prepared as described (Pays et al., 1981b) and incubated with DNAase I for 0 sex (lane 3), 30 sec (lane 4), and 60 sec (lane 5). The DNA was then purified, cut by Hind III and size-fractionated by agarose gel electrophoresis. The blot was hybridized with cDNA probe 1 (see Figure 1A). Lanes 1 and 2 contain Hind III digests of trypanosome clones 221a and 221b DNA respectively.

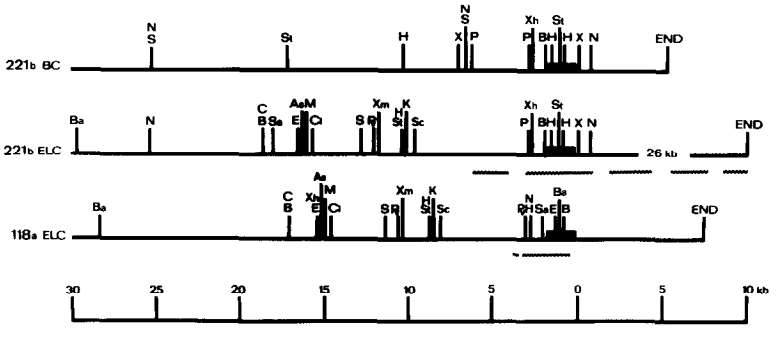

Figure 6. Physical Maps of the 221 BC and ELC in Trypanosome Clone 221b

The map of the ELC of the VSG 118 gene in trypanosome clone 118a is given for comparison. The coding regions of the 118 and 221 genes are indicated as in Figure 2. The extent of the duplicated segments of the 118 and 221 BC is indicated with a wavy line beneath the maps, the interruptions in the line denote the uncertainty in the size of the duplicated segment. The DNA segment without restriction sites downstream of the 221b ELC is 26 kb long. For abbreviations of restriction enzymes see legend to Figure 2.

is preferentially digested over a 8.7 kb Hind III fragment representing the 221 basic copy (BC). This result indicates that the 221 ELC is the active gene and shows that gene 221 can be activated in two basically different ways. The fragment detected in 221a DNA (Figure 7, lane 1) does not comigrate exactly with the BC-derived fragment in 221b DNA because of the presence of the 0.15 kb insertion in front of the 221 gene in 221a DNA (see above).

Discussion

We show here that both the duplicative and the nonduplicative mode of variant antigen gene activation may be used by the single telomeric gene for the VSG 221. The two activation mechanisms, therefore, operate on overlapping gene classes. For several reasons we consider it unlikely that the two modes of activation are exhibited by two very similar but distinct 221 gene copies. First, the one to one stoichiometry of 221 BC and ELC gene fragments is difficult to reconcile with the presence of more than one BC. Second, the polymorphic Asu II and Sca I sites and the polymorphic insertion in front of the 221 coding region indicate that in all trypanosome clones—except clone 221b—only one type of 221 gene is present per nucleus. The deletion of the 221 gene in four out of five switched trypanosome populations derived from clone 221a is also most easily explained by the presence of just one 221 gene in 221a nuclei. The values for the 221 gene copy number determined in the gene quantitation experiment (1.3 copies per nucleus in nonexpressors and 221a; 2.1 copies per nucleus in 221b) are consistent with this conclusion.

The analysis of trypanosome clone 221b presented in this paper shows that the duplicated 221 ELC occupies the same or a very similar expression site, as do two independent 117 and four independent 118 ELCs (Michels et al., 1983). The expression site presumably donates a mini-exon encoding the first 35 nucleotides of the mature VSG 117 and 118 mRNAs, since this sequence is not found in the duplicated segments of these genes (Van der Ploeg et al., 1982c; Liu et al., 1983). We show here that the duplicated segment of the 221 gene does not contain the mini-exon as a continuous sequence either and that an mRNA of the same length as mature VSG 221 mRNA hybridizes with a mini-exon probe. We therefore conclude that the duplicative activation of the telomeric 221 gene is basically similar to the duplicative activation of chromosome-internal genes, but differs in the extent of the duplicated segment. In the case of the chromosome-internal 117 and 118 genes the 3′ end of the duplication lies at a variable position somewhere in the last 150 bp of the gene (Bernards et al., 1981; Michels et al., 1983). The duplicated segment of the 221 gene, on the other hand, includes at least 0.8 kb beyond the end of the gene and may or may not include the entire downstream DNA segment up to the telomere. The upstream edge of the duplicated segment of the 221 gene falls in a DNA fragment that has homology with sequences upstream of all VSG genes tested (Van der Ploeg et al. 1982a).

To accomodate nonduplicative activation of VSG genes as shown by gene 221 in trypanosome clone 221a in a single expression site model, we previously proposed the chromosome and exchange hypothesis (Borst et al., 1983a). This hypothesis states that nonduplicative activation of VSG genes is the result of a reciprocal chromosome end translocation involving the expression site chromosome and a chromosome carrying a telomeric VSG gene. However, using a novel method to separate chromosome-sized DNA molecules (see Borst et al., 1983b, 1983c) we have recently shown that this model does not hold for gene 221. Nonduplicative activation of this gene is not accompanied by transfer of the gene to the dominant expression-site chromosome utilized by other VSG genes (Van der Ploeg, L. H. T., personal communication). Although transfer to another chromosome is not excluded, these results cannot readily be explained by a single expression site. Arguments for multiple expression sites have also been brought forward by Longacre et al. (1983) on the basis of experiments with Trypanosoma equiperdum, a trypanosome species closely related to T. brucei.

If multiple expression sites exist, there must be ways to activate and inactivate telomeric VSG genes in situ. This could be accomplished by a mobile promoter hopping from telomere to telomere. Boothroyd and Cross (1982) have found that the mRNA for VSG 221 begins with the same 35 nucleotide mini-exon sequence as the VSG mRNAs transcribed from the ELC in the dominant expression site of our strain. Our present results show that this mini-exon sequence is not present within 8.5 kb of the 221 coding region. Hence, telomeric VSG genes are split and the promoter-mini-exon unit required to complete them might come and go by transposition. If expression of the 221 gene is controlled in this way, the controlling element must be inserted at least 55 kb upstream of the gene, since we have not seen major rearrangements closer to the gene linked to expression. Moreover, if activation of the 221 gene would be controlled by a mobile element, inactivation is usually not as we find deletion of the 221 gene (and its total loss from the genome) in four of the five trypanosomes in which the expression of the 221 gene has been switched off. This loss is not simply due to displacement of the 221 gene by another VSG gene, because the conventional displacement of a VSG gene at the dominant expression site in our strain involves only the gene itself and the flanking 1.5 kb, whereas the switch-off of the 221 gene entails the loss of the gene plus at least 8 kb upstream. We think that this switch-off is due to conversion of the 221 telomere by another telomere and, indeed, in two of the four relapse populations we have demonstrated that the loss of the 221 gene is accompanied by the activation of a known telomeric gene, MITat 1.8 (unpublished experiments). Attempts to demonstrate that the 1.8 gene has moved into the 221 telomere have failed thus far because the segment converted is too long

to see the crossover point with the probes available. Although these results do not exclude a mobile promoter mode for telomeric VSG gene activation, they make one think of alternatives. These are still speculative and will be discussed elsewhere.

Experimental Procedures

Trypanosomes

Clones 118a and 221a of Trypanosoma brucei, strain 427, were obtained from Dr. G. A. M. Cross (see Michels et al., 1983). Clone 118b is an independently isolated trypanosome clone expressing VSG 118 (Michels et al., 1983). Trypanosome clone 221b was isolated following the procedure of Van Meirvenne et al. (1975) as described by Michels et al. (1983) 9 days after infecting a rabbit with 10^6 trypanosomes of clone 121a (rabbit 5 in Michels et al., 1983).

The in vitro immune lysis of trypanosomes involved: (a) Infecting a rat with about five trypanosomes of clone 221a. (b) Isolating trypanosomes 6–7 days later (more than 99% homogeneous population) by DEAE adsorption of blood cells (Lanham, 1968). (c) Incubating 10^6 parasites in 50 mM sodium phosphate, 45 mM NaCl and 55 mM glucose (pH 8.0) containing 50% guinea pig serum and anti-VSG 221 antiserum in a volume of 0.2 ml for 1–2 hr at ambient temperature. The lysis of the parasites was checked by light microscopy. (d) Infecting the suspension intraperitoneally into a rat. (e) Isolating the relapsed trypanosomes after 6–7 days.

The efficiency of the immune lysis of trypanosomes making VSG 221 was determined by immunofluorescence microscopy. This procedure will be described in more detail elsewhere (P. A. M. Michels, unpublished results).

Recombinant DNA Plasmids

The recombinant plasmids TcV221.1 and TcV221.5, containing DNA complementary to the mRNA for VSG 221 inserted into the Pst I site of plasmid pBR322 by GC tailing, have been described by Hoeijmakers et al. (1980b). Recombinant plasmid TgB221.1 was obtained from a clone bank containing a partial Hind III digest of clone 113a DNA, inserted into plasmid pBR322 (Van der Ploeg et al., 1982a). TgB221.2 and TgB221.3 contain Eco RI fragments of 221 DNA cloned in plasmid pAT153. Plasmid DNA was prepared by the alkaline lysis procedure of Birnboim and Doly (1979).

DNA and RNA Isolation

Trypanosomes were grown in rats to a density of 10^9 parasites per ml of blood and purified from blood cells as described above. Trypanosome preparations were checked for antigenic homogeneity by immunofluorescence microscopy (Michels et al., 1983).

RNA was isolated from purified trypanosomes by LiCl-urea extraction (Auffray and Rougeon, 1980). Contaminating DNA was removed by DNAase I treatment (Van der Ploeg et al. 1982c). Poly(A)$^+$ RNA was prepared by two cycles of oligo(dT)-cellulose chromatography (Hoeijmakers et al., 1980b).

DNA and RNA Blotting and Hybridization

DNA blotting and hybridization of nitrocellulose filters were performed as described (Bernards et al., 1981). DNA fragments to be used as hybridization probes were isolated by preparative agarose gel electrophoresis (Girvitz et al., 1980) and labeled by nick translation (Rigby et al., 1977). Labeling and hybridization of the 22 nucleotide synthetic mini-exon probe to nitrocellulose filters has been described by De Lange et al. (1983). RNA was size-fractionated on agarose gels and transferred to nitrocellulose filters as described by Thomas (1980). RNA blots were hybridized as described above.

DNAase I Digestion of 221b Nuclei

The isolation of nuclei from trypanosome clone 221b and the DNAase I digestion of this preparation were performed essentially as described by Pays et al. (1981b). The purified DNA was digested by Hind III, size-fractionated, and transferred to nitrocellulose filters as described above.

DNA Sequence Analysis

The DNA sequence of the 5′ part of the 221 main exon was determined by the Maxam and Gilbert (1980) procedure, using a Xho I–Hind III fragment of TgB221.1 5′ end-labeled at the Hind III site. To eliminate ambiguities, five base-specific reactions were used (G, A+G, C+T, C, and A+C).

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