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The contribution of chromosomal translocations to antigenic variation in *Trypanosoma brucei*

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Genomic rearrangements influencing gene expression occur throughout nature. Several of these rearrangements disrupt normal gene expression, as exemplified by the genetic alterations caused by the mobile genetic elements of maize or *Drosophila* (see Shapiro 1983). Other rearrangements are part of the normal developmental programme of an organism. An understanding of the control of genomic rearrangements and their effects on gene expression should contribute to our insight into the mechanism of genetic programming and cellular development.

The protozoan parasite *Trypanosoma brucei* exhibits a variety of genomic rearrangements that influence the expression of genes that code for versions of the variant surface glycoprotein (v.s.g.), which makes up the cell surface coat. V.s.g. genes are expressed in a mutually exclusive manner. Several v.s.g. genes are activated by duplicative transposition of the gene to a telomeric expression site where they are transcribed, while others can be activated without detectable genomic rearrangements. Recently we have been able to fractionate the chromosomes of *T. brucei* in agarose gels (Van der Ploeg *et al.* 1984*a*). This led to the observations that duplicative transpositions occur inter-chromosomally and that the chromosomes of *T. brucei* are subject to frequent recombinations that displace hundreds of kilobase pairs. At least two and possibly more telomeric expression sites can be used for v.s.g. gene transcription. How these sites are activated and inactivated is still unsolved, but this does not depend on recombinations in the vicinity of the gene. Gross genomic rearrangements occur sometimes in correlation with antigenic switching and this suggests that such rearrangements have a function in regulating the mutually exclusive transcription of the different expression sites.

V.s.g. genes consist of two exons. No physical linkage of the 35 nucleotide (n.t.) mini-exon to the v.s.g. gene main exon occurred within 15 kilobase pairs in variant 118a and possibly 150 kilobase pairs in variant 1.8b. These mapping data give additional support for the hypothesis that both exons might represent separate transcription units. Transcription initiation of v.s.g. genes would thus be from a promotor other than the mini-exon repeat unit. We propose that transcription of the v.s.g. gene in the expression site can be regulated by a position effect on the gene.

Taxonomy

Species belonging to the order of the Kinetoplastida are unicellular flagellates with a characteristic organelle, the kinetoplast. Most members of this order are parasitic, although some free-living species are known in the suborder Bodonina. Members of the other suborder, Trypanosomatina, are all parasitic and comprise a single family, the Trypanosomatidae. One genus, *Trypanosoma*, contains parasites of the blood and tissues of vertebrates, among which are several species causing important diseases of man and his domestic animals. On the basis of their life cycle, these parasites are divided into two groups: the Stercoraria, containing species

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which spend part of their life cycle in the hindgut of the transmitting insect vector; and the Salivaria, which develop in the mouthparts or salivary glands of the transmitting insect vector, the tsetse fly (Hoare 1972; Lumsden & Evans 1976). *T. evansi* and *T. equiperdum* have lost the ability to undergo cyclical development in the tsetse fly and are transmitted by other means.

Trypanosomes have a wide distribution in the Old and the New World and are a major problem in these continents both medically and economically. The African species *Trypanosoma brucei* is infective to cattle. Like all trypanosomes of the *brucei* group it survives life in the bloodstream by defying the host's immune response (Vickerman 1969, 1978). For that purpose the parasite is covered with a dense layer of a single glycoprotein, the variant surface glycoprotein or v.s.g. (Cross 1978). Through the periodic expression of immunologically distinct cell surface coats, the trypanosome alters its antigenic identity and thus evades the immune response. This process, known as antigenic variation, allows the trypanosome to populate the host's bloodstream. Immunologically, over one hundred different v.s.g.s have been identified in *T. equiperdum* (Capbern *et al.* 1977). In *T. brucei* up to 1000 potential v.s.g. genes are present as estimated by molecular hybridization (Van der Ploeg *et al.* 1982*a*). These extensive repertoires of v.s.g. genes meet the demands for variability of the v.s.g. coat in prolonged infections. The different coats appear in a more or less predictable order, with certain v.s.g.s that always appear early and others always late (Cross 1978; Van Meirvenne *et al.* 1975*a*, *b*, 1977; Turner, this symposium). The rate of switching is independent of the immune response, which only applies selection on the growing population. Parasitaemias can reach up to several million organisms per millilitre of blood. If untreated, the disease generally has a fatal outcome.

The phenomenon of antigenic variation in Trypanosomatidae is restricted to the species of the section Salivaria. It is found in all trypanosomes of the *brucei* group, and in *T. equiperdum*, *T. evansi*, *T. congolense* and *T. vivax*. Other protozoa, *Paramecium*, *Plasmodium* and *Babesia* also exhibit antigenic variation but in a less copious manner (Bloom 1979).

The variant surface glycoprotein

The v.s.g. coat makes up 7–10% of the total cellular protein and consists of one single type of v.s.g. only (Cross 1975, 1977). All v.s.g.s have a molecular mass of approximately 50 KDa. The protein is directed to the membrane with its C terminus, which is conserved between different v.s.g.s (Borst *et al.* 1981; Rice Ficht *et al.* 1981; Ferguson & Cross 1984; Cross, this symposium). The membrane form of the v.s.g. contains a phosphoglycolipid moiety, attached to the C-terminal carboxyl group. The lipid moiety is easily removed during isolation, resulting in the soluble v.s.g. usually obtained (Cardoso de Almeida & Turner 1983). Ferguson & Cross (1984) have recently shown that this lipid moiety contains myristic acid and they have argued that this is responsible for the binding of the v.s.g. to the membrane. The N terminus varies widely in amino acid composition between different v.s.g.s. This part is exposed on the outside of the membrane and is involved in immunorecognition.

Antigenic variation; the mutually exclusive expression of v.s.g. genes

The first cDNA clones complementary to a v.s.g. mRNA were independently isolated by Williams *et al.* (1979) and Hoeijmakers *et al.* (1980*a*). Hoeijmakers obtained cDNA clones specific for the mRNAs of the v.s.g. genes 117, 118, 121 and 221 of *T. brucei* stock 427. The

analysis of the cDNAs showed that the regulation of v.s.g. expression is at the transcriptional level and that each v.s.g. gene is encoded as such in the genome (Hoeijmakers *et al.* 1980*b*). For transcriptional regulation, several routes are open to the trypanosome. From the repertoire of roughly a thousand, often tandemly linked (Van der Ploeg *et al.* 1982*a*), non-transcribed v.s.g. genes (or basic copy (b.c.) genes), one can be duplicated and transposed to an expression site, creating an expression linked copy (e.l.c.) which is transcribed (Hoeijmakers *et al.* 1980*b*; Pays *et al.* 1981*a*, *b*; Bernards *et al.* 1981; Mayiwa *et al.* 1982; Van der Ploeg *et al.* 1982*a*; Michels *et al.* 1982, 1983; Bernards *et al.* 1984*a*). This e.l.c. is located at a chromosome end or telomere (Borst & Cross 1982; Williams *et al.* 1982; De Lange & Borst 1982; Bernards *et al.* 1983; Van der Ploeg *et al.* 1984*c*). The v.s.g. genes 117, 118 and 121 can all be activated in this way (Van der Ploeg *et al.* 1982*b*, 1984*d*; Michels *et al.* 1983; Bernards *et al.* 1984*a*).

Telomeric v.s.g. genes, like gene 221, can be activated by a variant of the duplicative activation mode called telomere conversion (Borst *et al.* 1983*a*, *b*; De Lange *et al.* 1983*b*; Bernards *et al.* 1984*a*).

However, these are not the only ways to activate v.s.g. genes. B.c. genes, like v.s.g. gene 221, which are located at telomeres, can be activated without duplication and without DNA rearrangements in the vicinity of the gene (Williams *et al.* 1979, 1981, 1982; Borst *et al.* 1981; Young *et al.* 1982; Bernards *et al.* 1984*a*). These apparently different routes for v.s.g. gene expression have to be related somehow to prevent expression of multiple v.s.g. genes in one organism, which has not yet been observed. One model invoked to explain the mutually exclusive regulation of transcription, involves a reciprocal exchange of telomeric v.s.g. genes with the expression site (Borst *et al.* 1983*a*, *b*).

Because trypanosomal chromosomes do not condense, it is not possible to locate genes on chromosomes by cytological hybridization. However, size fractionation of the chromosomes of *T. brucei* by a new type of agarose gel electrophoresis, illustrated in figure 1, allowed a critical testing of the v.s.g. gene activation routes (Schwartz *et al.* 1983; Schwartz & Cantor 1984; Borst *et al.* 1983*b*; Van der Ploeg *et al.* 1984*d*). This analysis showed *T. brucei* to have over 100 chromosomes. The majority of these (roughly a hundred) are small mini-chromosomes of 50–150 kilobase pairs. Probably most of the telomeres of these mini-chromosomes have v.s.g. genes, making the repertoire of telomeric b.c. genes extensive. The e.l.c. of the duplicatively activated v.s.g. genes 117, 118, 121 and 221 have similar physical maps and are located in one chromosomal fraction of 2000 kilobase pairs, indicating that there is indeed only one expression site for these genes (Van der Ploeg *et al.* 1984*d*). The telomeric v.s.g. gene 221 is located in the large DNA fraction that migrates close to the slot. When activated without duplication in VAT 221a, this gene does not move to the 2000 kilobase pairs chromosomes.

The presence of expressed genes in different chromosomal fractions proves that trypanosomes have more than one expression site, rendering the *one* expression site models for regulation of v.s.g. gene transcription incorrect (Van der Ploeg *et al.* 1984*d*). This had already been proposed on the basis of restriction enzyme analysis of expressed telomeric v.s.g. genes (Young *et al.* 1983; Longacre *et al.* 1983). However, since the physical maps do not give information on the chromosomal position of the telomere, these data could not be interpreted in a straightforward manner, as will be shown below. Other experiments indicating the presence of multiple expression sites concern the fate of the e.l.c. in antigenic switching. Most often the preceding gene in the e.l.c. is displaced by the incoming gene and destroyed. Sometimes the old e.l.c. is retained (Michels *et al.* 1984; Young *et al.* 1983; Buck *et al.* 1984) when a telomeric gene is

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activated. It is therefore possible to switch e.l.c.s off without losing them. The expression of one v.s.g. gene out of a large repertoire of v.s.g. genes thus requires the selective activation and inactivation of one of several telomeric expression sites.

The transposed segment

The segment transposed to the expression site contains the v.s.g. gene coding sequence plus 1–2 kilobase pairs of DNA in front of the gene (Van der Ploeg *et al.* 1982*a*; Pays *et al.* 1982). The ends of the transposed segment have been mapped. At the 5′ side the edge falls in a 70 base pair tandem repeat, thought to be located in front of all v.s.g. genes (Van der Ploeg *et al.* 1982*a*; Liu *et al.* 1983; Campbell *et al.* 1984). The 5′ end of the transposed segment is located in this repeat (Campbell *et al.* 1984; T. De Lange, personal communication).

The 3′ end of the transposed segment is known to have a variable position. It can be located anywhere from within the 3′ end of the gene to just beyond it (Bernards *et al.* 1981; Michels *et al.* 1982, 1983).

The first observation indicating that the duplication activated v.s.g. genes might be put downstream of a promoter in the expression site came from the discovery of transcripts that covered the complete transposed segment (Majiwa *et al.* 1982; Van der Ploeg *et al.* 1982*b*; Pays *et al.* 1982). These transcripts could be precursors to the mature v.s.g. mRNA and be initiated in the expression site. B.c. genes would then be silent because they lack the v.s.g. gene promoter (Van der Ploeg *et al.* 1982*b*; Liu *et al.* 1983; De Lange *et al.* 1983*a*).

Sequence analysis of the v.s.g. mRNA showed it to consist of two exons. At the 5′ end of the RNA a small 35 n.t. mini-exon is found, which is not encoded in the transposed segment (Van der Ploeg *et al.* 1982*b*; Boothroyd *et al.* 1982; Liu *et al.* 1983; Bernards *et al.* 1984). The intron–exon border contains a splice acceptor consensus sequence (Liu *et al.* 1983). To localize the putative v.s.g. gene mini-exon promoter, the genomic location of the mini-exon was determined.

Mini-exons are repetitive in the genome

A small 22mer synthetic oligonucleotide, complementary to part of the mini-exon sequence, was used to probe nuclear DNA to determine the location of the putative v.s.g. gene promoter. The 22mer recognizes a 1.35 kilobase pair tandem repeat in the genome of *T. brucei* (De Lange *et al.* 1983*a*; Nelson *et al.* 1983). Cosmid clones containing this repeat were shown, by sequence analysis, to contain the 35 n.t. mini-exon sequence as part of the 1.35 kilobase pairs repeat (De Lange *et al.* 1983*a*). The mini-exon is bordered by a splice donor consensus sequence, thus flanking the putative intron by the correct splice signals.

The genome of *T. brucei* contains approximately 200 copies of the repeat linked in tandem arrays, as shown by analysis of nuclear DNA and cosmid clones containing the repeats (De Lange *et al.* 1983*a*). Because of their close linkage, only 10–20 arrays of the repeats can be present in the genome and such an array has been proposed to function as a multiple promoter for v.s.g. gene transcription (Van der Ploeg *et al.* 1982*c*; Pays *et al.* 1982; Liu *et al.* 1983; De Lange *et al.* 1983*a*).

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Are mini-exons mobile?

The mutually exclusive transcription of multiple expression sites demands a tight regulation of the different transcriptional units. A movable promoter (for example, mini-exon repeat) or enhancer could explain the ordered expression.

Alterations in the physical maps that might indicate the presence of a movable promoter or enhancer have not been observed in a region of at least 15 kilobase pairs flanking the 5′ side of the genes. Minor variations in the restriction enzyme pattern of the mini-exon repeats have been detected by us and others (T. De Lange, unpublished results; Nelson *et al.* 1983), but these do not seem to correlate with antigenic switching. Moreover, the lack of physical linkage of the v.s.g. gene main exon and the mini-exon, as shown below, make it unlikely that the mini-exon could function as a movable promoter. Also, rearrangements take place at the 3′ end of telomeric v.s.g. genes that cannot be correlated with transcription regulation (Michels *et al.* 1982, 1983; Williams *et al.* 1982; Donelson *et al.* 1982; Laurent *et al.* 1983; Van der Ploeg *et al.* 1984*b*). We have therefore no evidence that hints to a movable promoter or enhancer that regulates the mutually exclusive expression of telomeric v.s.g. genes.

The transcription units of telomeric v.s.g. genes

Several recent experiments have failed to substantiate the proposal that transcribed v.s.g. genes are located downstream of a mini-exon array, and have raised the possibility that transcription of v.s.g. genes is discontinuous. No mini-exon has been detected within 15 kilobase pairs of the 118 e.l.c. in variant 118a (De Lange *et al.* 1983*a*), or within 8.5 kilobase pairs of the telomeric 221 gene (Bernards *et al.* 1984*a*).

Further evidence for discontinuous transcription was obtained recently by analysis of the separated chromosomes of variant 1.8b, which expresses the 1.8 gene (Michels *et al.* 1984; L. H. T. Van der Ploeg, unpublished results). In switching from v.s.g. 118 to v.s.g. 1.8, the trypanosome acquired an extra copy of the 1.8 telomeric b.c. gene. This 1.8 e.l.c. is located on a 550 kilobase pairs chromosome that contains no mini-exons (less than 0.5 copies). Because both telomeric 1.8 alleles are exact duplicates we have been unable to prove that this extra copy is the one transcribed. However, this seems a reasonable assumption. Since the v.s.g. 1.8 mRNA in this variant starts with the mini-exon sequence, mini-exon and main exon in this case appear not to be present on the same chromosome.

Additional experiments have shown that the mini-exon is not only found at the 5′ end of v.s.g. mRNAs. In Northern blots of trypanosomal RNA an abundance of transcripts that hybridize with the mini-exon can be detected (De Lange *et al.* 1983*a*, 1984). They range in length from 700 to 5000 n.t. These RNAs do not represent read-through products of the 1.35 kilobase pairs repeat because they are not detected by the 1.35 kilobase pairs repeat in Northern blots. Moreover, several non v.s.g. mRNAs have been isolated from cDNA clone banks which all have the 35 n.t. mini-exon, but they do not contain the remainder of the 1.35 kilobase pairs repeat coding sequence (De Lange *et al.* 1984). Only one of these genes has been localized in the vicinity of the 1.35 kilobase pairs repeat sequences. Moreover, a comparison of the hybridization intensities of v.s.g. mRNA and the other transcripts that are detected with the synthetic 22mer, indicate the mini-exon to be present on most if not all mRNA molecules.

How do all these mRNAs get a mini-exon sequence, if at most 10–15 arrays of mini-exon

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repeats are present per nucleus? Either most genes are tandemly linked and transcribed in large precursor RNA molecules from the mini-exon promoters. By differential termination and splicing these would yield the different mature mRNAs. Alternatively, mini-exon and main exon RNAs are initially transcribed from separate genes (De Lange *et al.* 1984; Campbell *et al.* 1984), and joined in one mRNA molecule at a later stage. Although there is no conclusive evidence against either possibility we favour the second at present. If transcription of v.s.g. genes is discontinuous, selection of the v.s.g. gene transcribed is not due to its postulated position downstream of a mini-exon cluster, but to another activating event. We return to this point in the next section.

The mini-exon repeat is a characteristic of most genera of the Trypanosomatidae (De Lange *et al.* 1983*b*, 1984; Nelson *et al.* 1983) and it is also found in the poly(A)+RNA from these genera. mRNAs starting with a mini-exon sequence may therefore be a general feature of the order Kinetoplastida.

The molecular karyotype of *T. brucei* is frequently altered by recombinations that displace hundreds of kilobase pairs

Figure 1 shows an agarose gel comparing the size distribution of chromosomes from *T. brucei* stock 427 and *T. brucei rhodesiense* GUTat 7, which can be considered a second *T. brucei* stock (Frasch *et al.* 1982), and *T. equiperdum*. The chromosome separation of variants from *T. brucei* stock 427 typically results in a fraction of chromosomes that migrates close to the slot, several chromosomes of 2000 kilobase pairs, about six intermediate-sized chromosomes of 200–700 kilobase pairs, and roughly a hundred 50–150 kilobase pairs mini-chromosomes (Van der Ploeg *et al.* 1984*d*). All these are present in both *brucei* stocks but the intermediate-sized chromosomes are different in length in *T. brucei rhodesiense*, where they range from 200 to 2000 kilobase pairs.

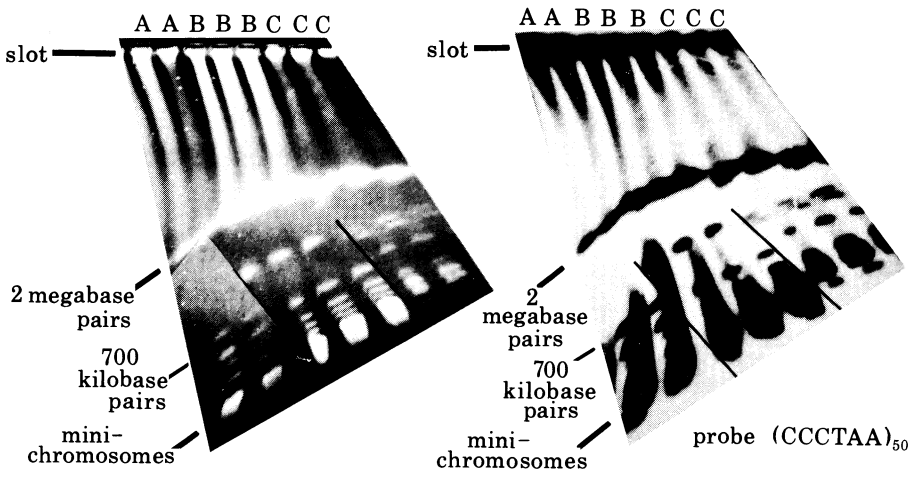

Figure 1. Pulsed field gradient gelelectrophoresis of chromosomes of *T. brucei* variant 118a (lanes A), *T. brucei rhodesiense* (GUTat 7, B) and *T. equiperdum* (C). Trypanosomes were lysed in agarose blocks as described by Schwartz & Cantor (1984) and Van der Ploeg *et al.* (1984*d*) and placed in the slots of a 1% agarose gel. Electrophoresis was performed at 20 °C for 18 h with 17.5 V cm^{-1} in the north–south and 6 V cm^{-1} in the west–east direction and a pulse frequency of 35 s. After electrophoresis the gel was stained with ethidium bromide (left hand panel) and the DNA blotted to nitrocellulose. The recovery of chromosomes in the left hand lanes (*T. brucei* stock 427, variant 118a) is lower than those in the right half of the gel owing to differences in the strength of the electrical fields. Hybridization was performed as described by Van der Ploeg *et al.* (1982*a*) with the insert of clone pT6, containing a telomeric $(CCCTAA)_{50}$ insert only (Van der Ploeg *et al.* 1984*b*). Post-hybridizational washes were performed at 65 °C and 3 × SSC stringency (right-hand panel).

T. equiperdum also has the intermediate sized chromosomes but mini-chromosomes are much less abundant. Since *T. equiperdum* exhibits antigenic variation the abundance of mini-chromosomes is apparently not essential for this process.

The large differences in the intermediate-sized chromosomes in the two *T. brucei* stocks shown in figure 1 (and an additional one not shown) are most likely the results of recombinations that displace large stretches of DNA. These recombinations can also be detected in variants of one stock. They often correlate with antigenic switching as summarized in Table 1. All recombinations that correlate with an antigenic switch are given in the top part of the table. The activation of v.s.g. gene 1.8 in the switch of variant 118b to variant 1.8b correlates with a 150 kilobase pairs duplicative translocation of the 1.8 telomeric gene to a small (550 kilobase pairs) chromosome. In the subsequent inactivation of gene 1.8 in the switch of variant 1.8b to variant 118b′, alterations cannot be detected in the Southern blots probed for gene 1.8. The physical maps of both telomeric v.s.g. genes remain unchanged in an area of at least 25 kilobase pairs upstream of the genes. The extra 1.8 gene copy on the 550 kilobase pairs chromosome is, however, displaced in the karyotype owing to a recombination that moves part of the 550 kilobase pairs chromosome to one of the chromosomes of 2000 kilobase pairs or larger. Since these gross alterations take place outside the detection limit of the physical maps, made by Southern blotting of restriction enzyme digested DNA, they can only be visualized at the molecular karyotype level. Also two other translocations involving another telomeric v.s.g. gene, gene 221, may correlate with its antigenic switch. In one case the 221 gene is translocated when the gene is switched off; in the other case the gene is lost from the v.s.g. gene repertoire.

TABLE 1

antigenic switch	description of recombination
recombinations that correlate with antigenic switching	
118b → 1.8b	activation of v.s.g. gene 1.8 associated with a 150 kilobase pairs duplicative translocation of the 1.8 telomere
1.8b → 118b′	inactivation of gene 1.8 correlating with a reciprocal translocation that leads to the disappearance of the 550 kilobase pairs chromosome, containing the 1.8 gene extra copy
221a → 1.8d	inactivation of v.s.g. 221 concomitant with a 15 kilobase pair insertion in front of the gene. It is translocated to a 400 kilobase pair chromosome in one case and retained in the slot in another (1.8 d′)
221a → 1.8f	loss of gene 221 and at least 8.5 kilobase pairs upstream
recombinations not linked to antigenic switching	
118b → 1.207	duplication of telomeric 1.8 gene copy without activation of the gene
0 52 → 1.8a	200 kilobase pairs deletion in a 300 kilobase pairs chromosome unlinked to gene 1.8
antigenic switches without detectable chromosomal rearrangements	
118b → 1.205 118b → 1.206 118a → 1.8c → 118a′	no detectable alterations

Variants of *T. brucei* stock 427 were analysed for their karyotype alterations by visual inspection of the ethidium stained chromosome separation gels and by hybridization of the chromosomes that were transferred to nitrocellulose with cDNA probes for the v.s.g. genes 118, 1.8 and 221. (see figure 1 for additional details). Based on unpublished results (Van der Ploeg *et al.* 1984*b*).

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However, not all recombinations can be correlated with antigenic switching. In the switch of variant 118b′ to variant 1.207, gene 1.8 is duplicated, but the duplicate is located in the large chromosomes (larger than 2000 kilobase pairs) and is not switched on. In another recombination a 200 kilobase pairs deletion has taken place in a 300 kilobase pairs chromosome (in the relapse of variant 052 to variant 1.8a). Since this chromosome does not carry a 1.8 gene, the importance of the recombination for the antigenic switch is unclear. In the bottom half of the table, antigenic switches are presented that do not show any detectable karyotype alterations and we cannot tell whether gene translocations have occurred during these switches. Perhaps the chromosomes involved are not separated in the gel system, or the recombinations may be intrachromosomal. Since about 15% of the antigenic switches analysed so far show a detectable chromosomal recombination, it is possible that most antigenic switches correlate with chromosomal recombinations, displacing the telomeric gene of interest. The vast differences in the molecular karyotypes of different stocks of *T. brucei* are indicative that these recombinations are frequent. The two recombinations that are apparently not correlated with antigenic switching may have important evolutionary implications since they alter the repertoire of telomeric v.s.g. genes. A detailed description of the translocated DNA may give insight in the importance of chromosomal recombinations for v.s.g. gene activation. As background, we shall first discuss the general structure of the trypanosome telomeres.

The structure of telomeres

Figure 2 shows the physical maps of the e.l.c. of the v.s.g. genes 117a, 118a, 118c, 1.8b and 221b and the 221a b.c. gene which can be activated *in situ*. These telomeres are compared with the generalized telomere structure depicted below the physical maps.

Cloned telomeres carry repeats that have been shown, by hybridization, to be localized on most telomeres of *T. brucei* (Van der Ploeg *et al.* 1984*b*; Blackburn & Challoner 1984). Figure 3 shows a hybridization of trypanosomal nuclear DNA that has been treated with exonuclease Bal 31 before digestion with the restriction endonuclease BspRI. This experiment allows the specific visualization of telomeric sequences because of their preferential sensitivity to exonuclease Bal 31. The telomere repeats CCCTAA (panel A), the 29mer sub-telomeric repeats and the more complex AT rich segment (panel B) can by this means be shown to be common elements of trypanosomal telomeres because they predominantly recognize Bal 31-sensitive bands which gradually decrease in length in the Bal 31 incubation series. The increasing number of non-Bal 31-sensitive bands, detected with probes located more internally result from sequences that are cut loose from their telomeres by the restriction enzyme and the presence of internal repeats. Hybridization of the chromosome separation gel in figure 1 with the CCCTAA repeat shows the hybridization signal of the chromosomes to be roughly stoichiometric with chromosome number, indicating that most likely all telomeres have CCCTAA repeats.

The repeats that are located more internally are not as common between different telomeres, as can be seen from the decrease in the number of bands that are detected in the hybridization of the Bal 31-treated nuclear DNA (panel C). Because most telomeres of *T. brucei* have v.s.g. genes, and the AT-rich segment has been found flanking a telomeric v.s.g. gene (De Lange *et al.* 1983*b*) these repeats (CCCTAA, the 29mer and AT-rich subtelomeric repeats) describe the DNA segments adjacent to telomeric v.s.g. genes.

Upstream of all v.s.g. genes analysed, we have found a 70 b.p. repeat, recently shown by Campbell *et al.* (1984) to contain the 5′ end of the transposed segment of the duplication

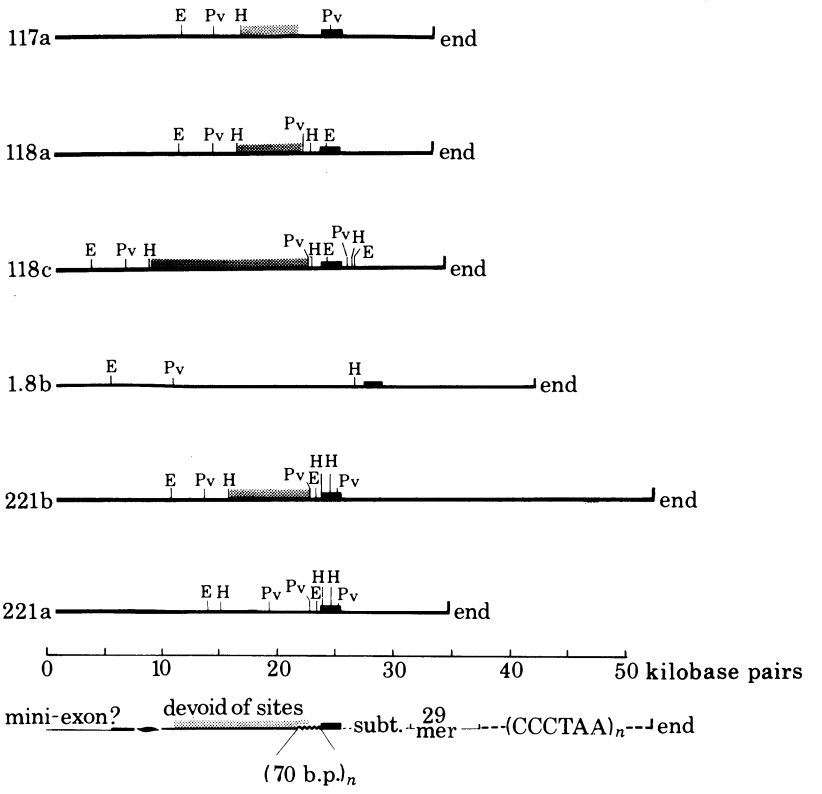

FIGURE 2. Physical maps of the e.l.c.s of v.s.g. genes 117a, 118a (Van der Ploeg *et al.* 1982*b*), 118c and 1.8b (Michels *et al.* 1983, 1984) and 221b and the b.c. of v.s.g. gene 221a (Bernards *et al.* 1984*a*). A schematic outline of the repetitive elements in a trypanosome telomere based on Van der Ploeg *et al.* (1984*c*) is indicated below the physical maps. Abbreviations are: E, *EcoRI*; H, *Hind*III; P, *Pvu*II; end indicates the chromosome end; the black box indicates the v.s.g. gene coding sequence; the shaded region up stream of the gene the uncuttable barren region; 70 b.p., the 70 b.p. repeats found at the 5′ side of v.s.g. genes; subt., the subtelomeric repeats found on some telomeres, as found downstream of v.s.g. 118 in variant 118c (the region with restriction enzyme sites); 29mer, the 29 nucleotide CCCTAA derived repeat found on trypanosome telomeres and CCCTAA the tandem repeat of the chromosome ends.

activated v.s.g. gene 117, an observation confirmed in this laboratory for another e.l.c. of v.s.g. gene 117 (T. De Lange, unpublished results). This repeat is a common element of the mini-chromosomes that carry most telomeric v.s.g. genes. Hybridization of a chromosome separation gel with this 70 b.p. repeat shows a major signal located in the mini-chromosomal fraction (figure 4). Most telomeric v.s.g. genes are therefore likely to contain the 70 b.p. repeat flanking the 5′ side of the gene.

Upstream of the 70 b.p. repeats we find DNA of variable nature. The v.s.g. genes that are activated by duplicative transposition are flanked by large stretches of uncuttable DNA that differs in length in different variants (Michels *et al.* 1982, 1983; Williams *et al.* 1982; Donelson *et al.* 1982; Laurent *et al.* 1983). These large uncuttable stretches of DNA and at least 5 kilobase pairs of the flanking sequences are devoid of mini-exon repeat units (De Lange *et al.* 1983*a*). Moreover, when gene 1.8 is duplicated and transposed to the 550 kilobase pairs chromosome in variant 1.8, it lacks mini-exon repeats on the 550 kilobase pairs chromosome. The physical maps of the telomeric 1.8 alleles on the 2000 and 550 kilobase pairs chromosomes are identical in an area of at least 25 kilobase pairs. Since the length of the duplicated segment is about 150 kilobase pairs, an area of at least 25 kilobase pairs and probably as much as 150 kilobase pairs upstream of both alleles may lack the coding sequence for the 35 n.t. mini-exon.

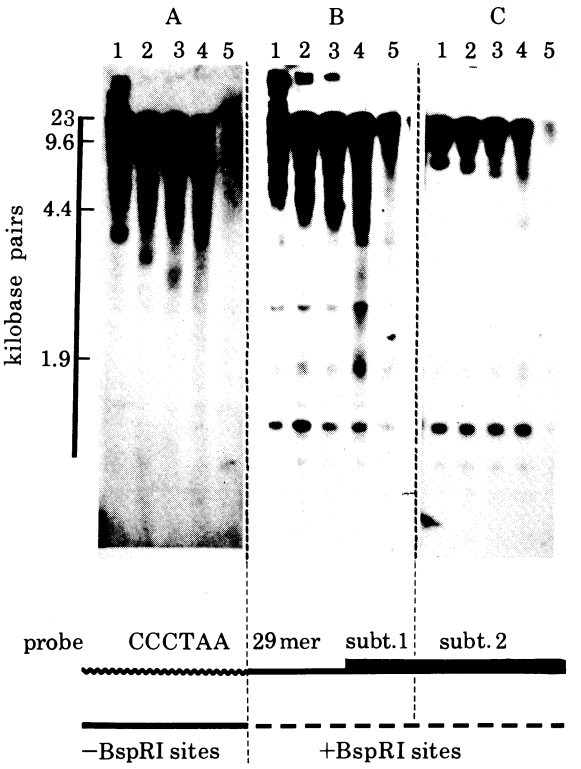

FIGURE 3. Hydridization of exonuclease Bal 31-treated nuclear DNA with telomere-specific probes. Nuclear DNA was incubated with the exonuclease Bal 31 as described (Van der Ploeg *et al.* 1984*c*) and shortened for 0 (lanes 1), 400 (2), 700 (3), 2200 (4) and 5500 b.p. (5) respectively. The DNA was subsequently digested with the restriction endonuclease BspRI, size fractionated in a 0.7% agarose gel and transferred to nitrocellulose filters. Hybridizations were with the CCCTAA-specific probe derived from plasmic pT6 (panel A), the subtelomeric repeats on the 900 b.p. *Alu*I fragment of clone pT1 (B) and the subtelomeric repeats on the 340 b.p. *Alu*I fragment of clone pT1 (C, Van der Ploeg *et al.* 1984*c*). Post-hybridizational washes were performed at 65 °C and 3 × SSC stringency of hybridization. Control hybridizations with chromosome internal probes did not detect Bal 31-sensitive bands (not shown).

A SPECULATIVE MODEL FOR REGULATION OF TRANSCRIPTION AT TELOMERES

Translocations and deletions are known to influence the regulation of transcription of a number of eucaryotic genes, ranging from the yeast-mating type genes to several oncogenes (Hicks *et al.* 1979; Nasmyth *et al.* 1981; Robertson 1983; Rowley 1983; Heisterkamp *et al.* 1983; Yunis 1983; Nusse *et al.* 1983). Some of these chromosomal aberrations exhibit their effects over large areas (tens of kilobase pairs).

Similarly, translocations of trypanosome telomeres may be involved in control of v.s.g. gene expression, these genes being regulated by position effects on the gene rather than by promoter addition.

How could translocations influence v.s.g. gene expression? Since only some of the antigenic switches are duplicative, a duplication is not essential for gene activation. Some translocations result in activation of the v.s.g. gene involved, others are silent or inactivate a telomeric v.s.g. gene. What then determines an 'active telomere' and how does the trypanosome manage to have only *one* active telomere at a time? Two considerations seem important in relation to these questions.

(i) The active telomere has a different chromatin structure as illustrated by its preferential

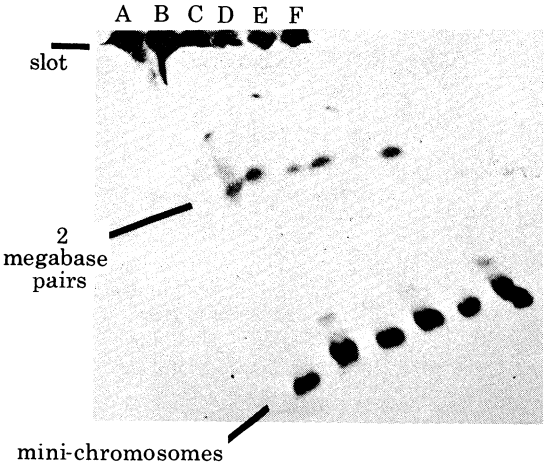

FIGURE 4. Location of the 70 b.p. repeats on the chromosomes of *T. brucei*. Chromosomes were separated as described in the legend to figure 1. *T. brucei* (stock 427) variants 1.86 (lane A; Michels *et al.* 1984), 221a and 221b (B and C; Bernards *et al.* 1984*a*), 118a and 118b (D and E; Michels *et al.* 1983) and variant 121a (F; Cross 1975; Michels *et al.* 1983) were used. The DNA was blotted to nitrocellulose and hybridized with the 70 b.p. specific repeat on a 1 kilobase pairs *Pvu*II fragment derived from the 5′ end of the transposed segment of v.s.g. gene 118 (Van der Ploeg *et al.* 1982*a*). Post-hybridizational washes were performed at 65 °C and 3 × SSC stringency of hybridization.

DNase I sensitivity (Pays *et al.* 1981*b*); its likelihood to undergo length alterations at heat shock and its different telomere growth rate (Bernards *et al.* 1983; Pays *et al.* 1983; Van der Ploeg *et al.* 1984*b*). It is likely that a translocation that elongates a chromosome by almost 40% has a large influence on chromosome and chromatin structure and thus transcription. The apparent variability of the effect of translocations on v.s.g. gene expression could be due to the many parameters affecting the status of the chromosome after the translocation. Length and sequence of the translocated segment and acceptor chromosome might all influence the outcome for telomere structure. This may explain some apparently contradictory effects, for example the putative activation of v.s.g. gene 1.8 owing to its translocation in a 150 kilobase pair segment to a 400 kilobase pair chromosome and its inactivation in a recombination that displaces the complete chromosome and takes place at least 25 kilobase pairs upstream of the gene (table 1).

(ii) Since the switch rate is approximately 10^{-5} per division, the synchronous activation and inactivation of the new and old telomere is unlikely to occur. Trypanosomes that express multiple or no v.s.g. genes at all should therefore exist. As these are selected against in a parasitaemia, only growth of trypanosomes in immunosuppressed animals can finally lead to a population containing trypanosomes with a mixed coat. Because at least three telomeres on different chromosomes (v.s.g. 221 in the large DNA, v.s.g. 1.8 on the 550 kilobase pairs or 2000 kilobase pairs chromosome and the expression site of the genes 117, 118 and 221b on the 2 megabase pair chromosome) are transcribed, it is possible that several telomeres contain all sequences needed for v.s.g. gene transcription. Because *T. equiperdum* has probably only one mini-chromosome and *T. vivax* none (compare table 2 and figure 1), we propose that mini-chromosomes might function to extend the pool of telomeric v.s.g. genes only. The percentage of multiple expressors might remain low when only a fraction of the telomeres, excluding the mini-chromosomes, can function as 'expression site' telomeres.

Table 2. Size range and number of mini-chromosomes in species of the order of the Kinetoplastida

genus and species	ref.	no. of mini-chromosomes	chromosome size range (kilobase pairs)
Trypanosoma			
T. rangeli	1	—	*ca.* 700–4000
T. cyclops	2	—	*ca.* 700–4000
T. vivax	3	—	*ca.* 2000
T. equiperdum	4	*ca.* 1, 50 kilobase pairs	*ca.* 50–2000
T. brucei (427)	5	*ca.* 100, 50–150 kilobase pairs	*ca.* 50–2000
T. brucei (rhod.)	6	*ca.* 100, 50–150 kilobase pairs	*ca.* 50–2000
Leptomonas			
L. ctenocephali	7	—	*ca.* 700–4000
Herpetomonas			
H.m. muscarum	8	—	*ca.* 700–4000
Crithidia			
C. fasciculata	9	—	*ca.* 700–2000
Leishmania			
L. tropica minor	10	—	*ca.* 700–4000

All organisms tested contain several chromosomes of undetermined molecular mass. These are retained in the slot of the chromosome separation gel, together with circular molecules, like the maxi- and minicircles of the kinetoplast. All molecular masses were determined using yeast or *T. brucei* (stock 427) DNAs as a marker. The molecules of 4000 kilobase pairs are measured inaccurately because no markers are available larger than 2000 kilobase pairs. Their molecular mass is extrapolated from the size markers that go up to 2000 kilobase pairs. References: 1, Ellis *et al.* 1980; 2, Weinman 1972; 3, Leeflang *et al.* 1976; 4, Hajduk & Cosgrove 1979; 5, Opperdoes *et al.* 1976; 6, Barry *et al.* 1983; 7, strain LV 117, Liverpool School of Tropical Medicine, Liverpool, U.K.; 8, Rogers & Wallace 1971; 9, obtained from Dr M. Steinert, Free University Brussels, Belgium; 10, obtained from Dr D. T. Hart, International Institute of Cellular and Molecular Pathology, Brussels, Belgium (Van der Ploeg *et al.* 1984*a*).

However, v.s.g. genes can also be activated by other routes. For instance, the duplicative transposition of v.s.g. genes to the expression site on the 2000 kilobase pair chromosome does not involve a telomere translocation. These genes could however be transposed into a telomere that is already open for transcription owing to a previous translocation. A separate factor, for example, chromatin structure, might determine the preferential duplicative translocations to the expression site on the 2000 kilobase pair chromosome.

However, translocations do not explain all antigenic switches. Trypanosomes put into culture lose their v.s.g. mRNA within 2 h (Overath *et al.* 1983). This complete halt of transcription would mean a translocation of the transcribed telomere in every member of the population. This is very unlikely when translocations occur infrequently. Moreover the trypanosome often switches back to reexpression of a lingering or 'old e.l.c.' (Michels *et al.* 1984). This memory function cannot be explained by the translocations that we have observed. A second type of regulation of v.s.g. gene transcription is therefore needed to account for the memory function and the rapid switch-off of transcription in culture.

An explanation for switching without recombinations might be found in the recent observations of Bernards *et al.* (1984*b*) who showed that telomeres of *T. brucei* contain a modified base unless they harbour an expressed v.s.g. gene. Most likely these modifications are indicative of the altered chromatin structure at the active telomere. Raibaud *et al.* (1983) had previously shown that most telomeres of *T. equiperdum* contain modified bases. As telomeres have been shown to be variably associated with the nuclear membrane (for example, Hughes-Schrader

1943; Hinton 1945), the different modification levels could indicate a nuclear compartmentalization on which activation and inactivation of telomeric v.s.g. genes may depend. When translocations alter the status of the chromosome in respect to this nuclear compartmentalization, as should environmental stimuli like growth in culture, *in situ* switching could be explained.

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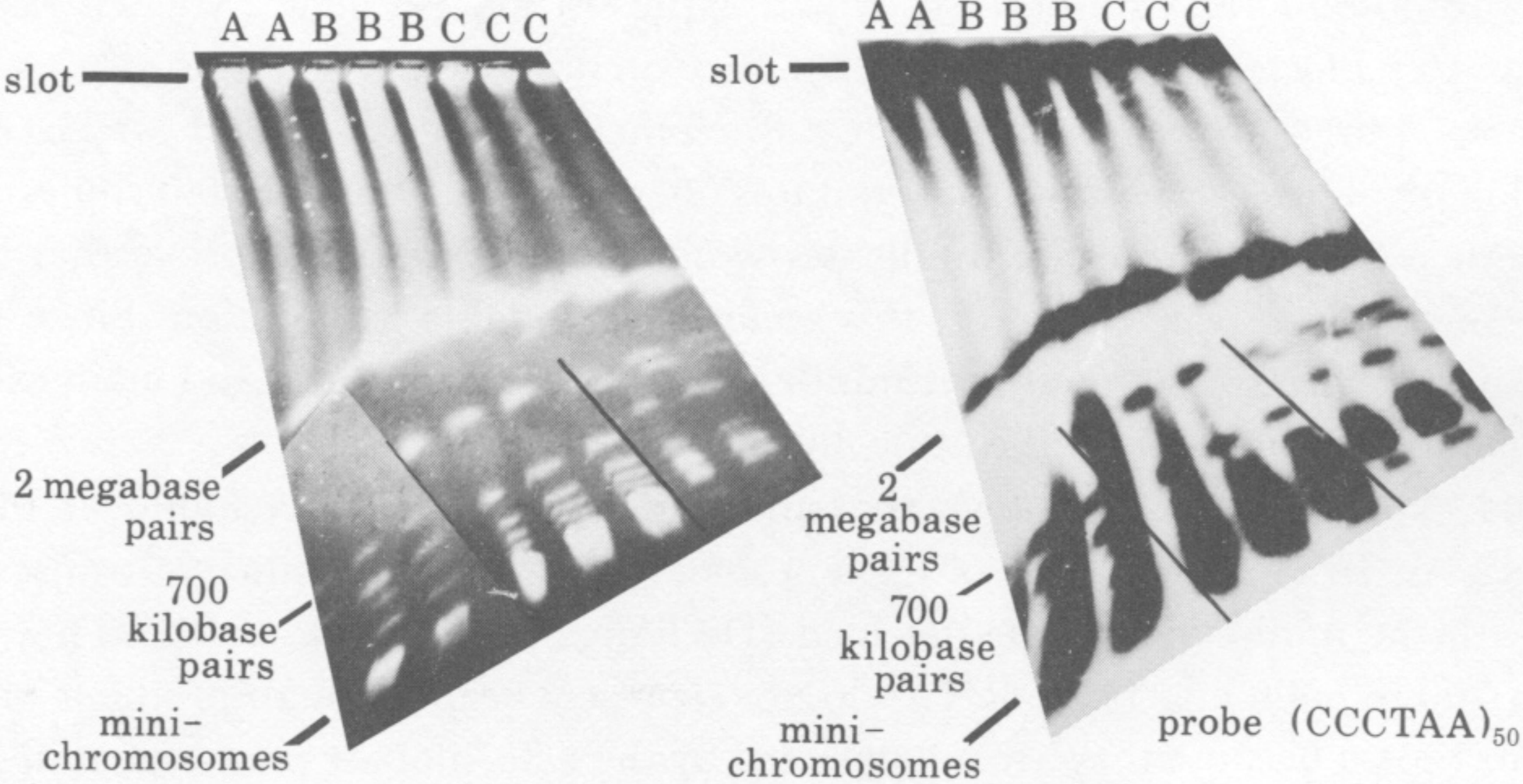


FIGURE 1. Pulsed field gradient gelelectrophoresis of chromosomes of *T. brucei* variant 118a (lanes A), *T. brucei rhodesiense* (GUTat 7, B) and *T. equiperdum* (C). Trypanosomes were lysed in agarose blocks as described by Schwartz & Cantor (1984) and Van der Ploeg *et al.* (1984*d*) and placed in the slots of a 1% agarose gel. Electrophoresis was performed at 20 °C for 18 h with 17.5 V cm^{-1} in the north–south and 6 V cm^{-1} in the west–east direction and a pulse frequency of 35 s. After electrophoresis the gel was stained with ethidium bromide (left hand panel) and the DNA blotted to nitrocellulose. The recovery of chromosomes in the left hand lanes (*T. brucei* stock 427, variant 118a) is lower than those in the right half of the gel owing to differences in the strength of the electrical fields. Hybridization was performed as described by Van der Ploeg *et al.* (1982*a*) with the insert of clone pT6, containing a telomeric $(CCCTAA)_{50}$ insert only (Van der Ploeg *et al.* 1984*b*). Post-hybridizational washes were performed at 65 °C and 3 × SSC stringency (right-hand panel).

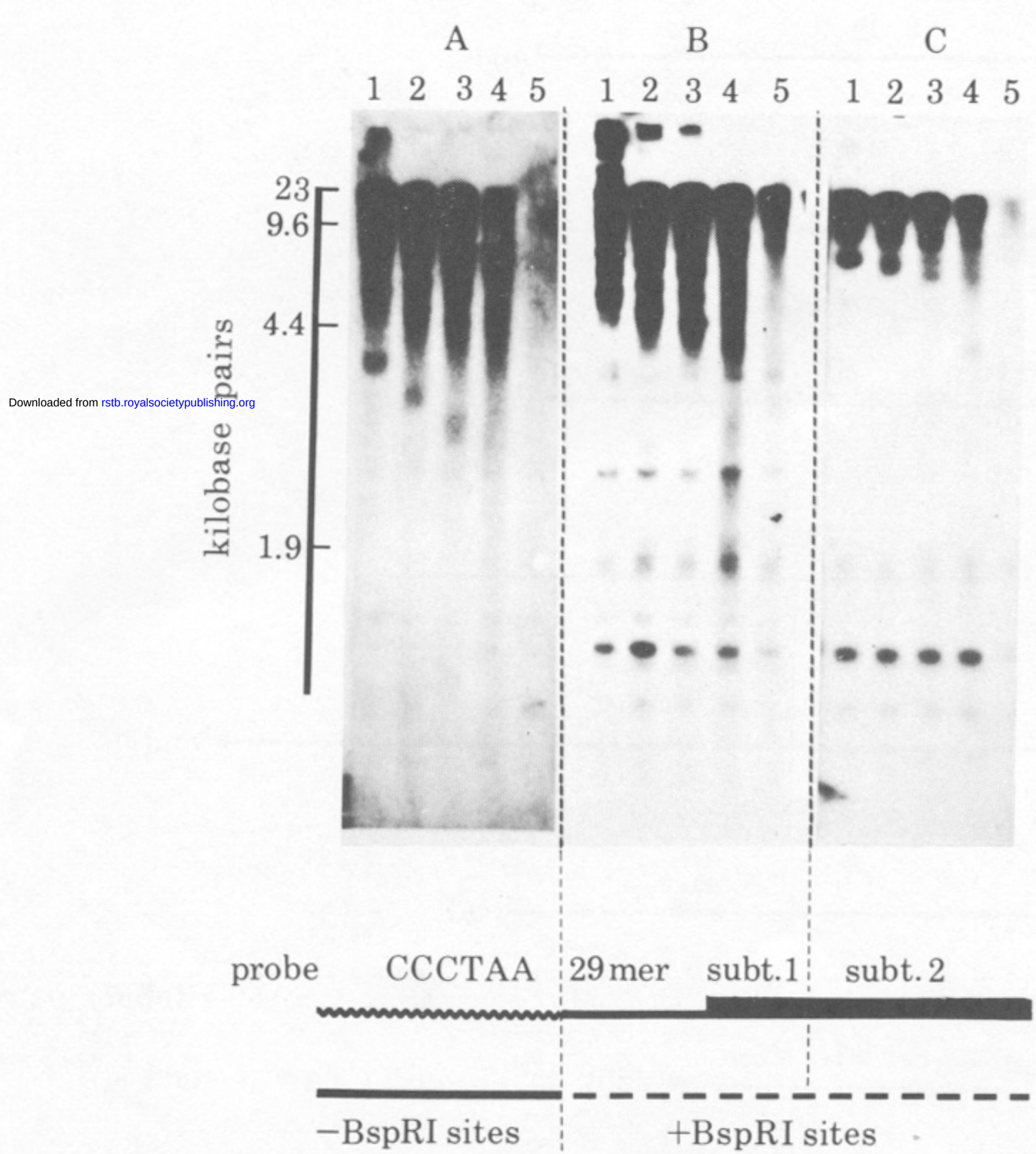
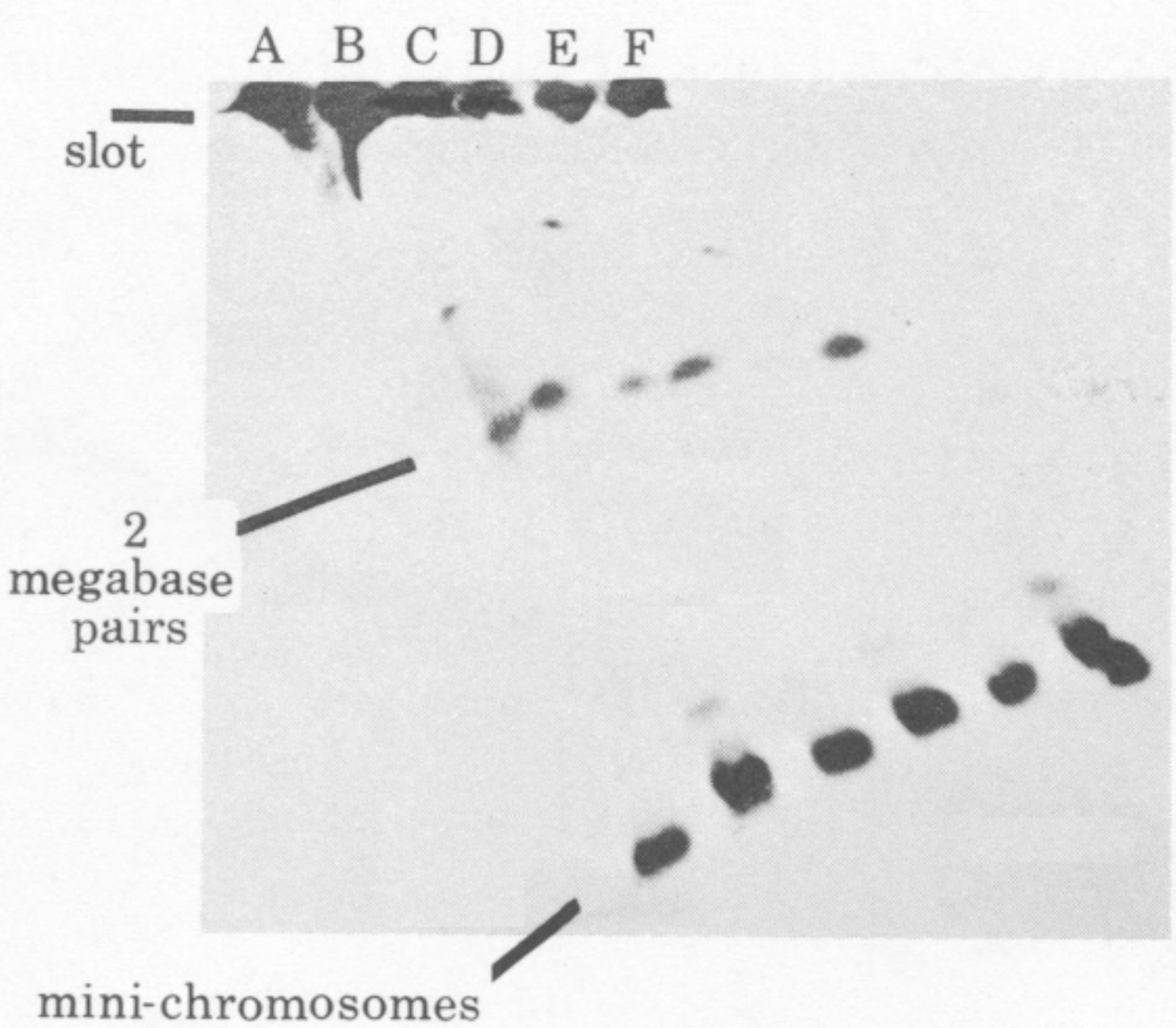


FIGURE 3. Hydridization of exonuclease Bal 31-treated nuclear DNA with telomere-specific probes. Nuclear DNA was incubated with the exonuclease Bal 31 as described (Van der Ploeg *et al.* 1984*c*) and shortened for 0 (lanes 1), 400 (2), 700 (3), 2200 (4) and 5500 b.p. (5) respectively. The DNA was subsequently digested with the restriction endonuclease BspRI, size fractionated in a 0.7% agarose gel and transferred to nitrocellulose filters. Hybridizations were with the CCCTAA-specific probe derived from plasmic pT6 (panel A), the subtelomeric repeats on the 900 b.p. *Alu*I fragment of clone pT1 (B) and the subtelomeric repeats on the 340 b.p. *Alu*I fragment of clone pT1 (C, Van der Ploeg *et al.* 1984*c*). Post-hybridizational washes were performed at 65 °C and 3 × SSC stringency of hybridization. Control hybridizations with chromosome internal probes did not detect Bal 31-sensitive bands (not shown).



GURE 4. Location of the 70 b.p. repeats on the chromosomes of *T. brucei*. Chromosomes were separated as described in the legend to figure 1. *T. brucei* (stock 427) variants 1.86 (lane A; Michels *et al.* 1984), 221a and 221b (B and C; Bernards *et al.* 1984*a*), 118a and 118b (D and E; Michels *et al.* 1983) and variant 121a (F; Cross 1975; Michels *et al.* 1983) were used. The DNA was blotted to nitrocellulose and hybridized with the 70 b.p. specific repeat on a 1 kilobase pairs *Pvu*II fragment derived from the 5′ end of the transposed segment of v.s.g. gene 118 (Van der Ploeg *et al.* 1982*a*). Post-hybridizational washes were performed at 65 °C and 3 × SSC stringency of hybridization.

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