The EMBO Journal vol.9 no.4 pp.1035 - 1040, 1990

Telomere interactions may condition the programming of antigen expression in *Trypanosoma brucei*

Aline Van Der Werf, Suzanne Van Assel, Diane Aerts[1], Maurice Steinert and Etienne Pays

Department of Molecular Biology, University of Brussels, 67 rue des Chevaux, B1640 Rhode St Genèse and [1]Institute for Tropical Medicine, B2000 Antwerp, Belgium

Communicated by M.Steinert

The AnTat 1.1 antigen type typically occurs late in a chronic infection by the EATRO 1125 stock of *Trypanosoma brucei*. The AnTat 1.1 gene, which is located 24 kb from a chromosome end, seems exclusively expressed by acting as a donor in gene conversion events targeted to the telomeric expression site. We report that this gene is sufficiently provided with the homology blocks required for recombination with the expression site, and is not interrupted by stop codons up to the 3′ block of homology. A possible reason for its low probability of activation is an inverse orientation with respect to the proximal chromosome end, since, if correctly positioned, it is readily expressed at an early stage of infection, following gene conversion. This suggests that interactions between chromosome ends may precede and favour the rearrangements leading to antigenic variation.

Key words: antigenic variation/gene expression/trypanosomes/telomeres

Introduction

Chronic infection by African trypanosomes depends on the sequential expression of a large repertoire of antigen variants, which precludes an efficient immune response from the mammalian host. The variant-specific antigens, or VSGs (for variable surface glycoproteins), are encoded by different genes, which are clustered in the genome. Many VSG genes, referred to as telomeric, are found next to chromosome ends. Since the trypanosome genome is carried on a total of >100 chromosomes, the collection of telomeric VSG genes could be extensive. Some of these chromosome ends are potential expression sites for the VSG gene they contain. Indeed, transcription of the VSG genes occurs exclusively in a few particular telomeric transcription units, probably between 5 and 20, generally only one of these being actively transcribed at any one time. Different DNA rearrangements, most frequently a gene conversion, allow non-telomeric VSG genes, or telomeric genes not present in potential expression sites, to have access to the active telomeric transcription unit. The mechanism allowing the alternate use of different expression sites is not understood (for recent reviews on the genetic control of antigenic variation, see Borst, 1986; Pays and Steinert, 1988).

Antigenic variation does not occur completely randomly, since antigen types can be classified as either early or late within a given repertoire (Van Meirvenne *et al.*, 1975; Capbern *et al.*, 1977; Hajduk and Vickerman, 1981). This differential probability of antigen gene expression is essential for the maintenance of long lasting infections, since it avoids a rapid exhaustion of the antigen repertoire. A late antigen may be expressed early if the corresponding gene is translocated to a telomere, indicating that the probability of antigen expression may depend on the gene environment (Laurent *et al.*, 1984a). In particular, genes not surrounded by sequences homologous to the expression site, such as the 70 bp repeats generally preceding VSG genes or the conserved 300 bp terminal region of the gene, may have a low probability of early expression, due to a degree of homology with the active expression site insufficient to allow recombination (Laurent *et al.*, 1984a). These genes can nevertheless gain access to the expression site if they share homology, and thus can recombine with the resident VSG gene itself. This means that these genes can only be expressed following the expression of a few other genes, probably a rare event that appears late in the infection. The same mechanisms may even allow pseudogenes to be expressed (Pays *et al.*, 1985a; Longacre and Eisen, 1986; Roth *et al.*, 1986). In these different cases, the recombinations within VSG genes generate chimaeric sequences.

We report here that the AnTat 1.1 VSG gene, although typically expressed late, does not appear defective and is normally flanked by sequences homologous to the expression site. Although frequently activated through partial recombinations with the VSG gene residing in the expression site, this gene can also gain access *in toto* to the expression site by means of a gene conversion initiated in the region of the 70 bp repeats (variant AnTat 1.1D). A possible reason for its low probability of expression is the inverse orientation of the gene with respect to the telomere, taking as reference the orientation of all other telomeric VSG genes described so far, and of the telomeric VSG gene expression site in particular. This suggests that the telomere plays a role in the mechanism of gene conversion underlying antigenic variation, even if the telomeric sequence itself is neither a donor nor a target in the conversion event.

Results

Characterization of the AnTat 1.1 gene and 5′ environment

The AnTat 1.1 gene belongs to a family of five members, which comprises the telomeric AnTat 1.10 gene and three non-telomeric pseudogenes (Pays *et al.*, 1983b, 1985a; S.Van Assel and E.Pays, unpublished data). All members of the family are carried by large chromosomes. Although located not far from a chromosome end (~24 kb), the AnTat 1.1 gene differs from all other known telomeric VSG genes in that it is separated from the terminal telomeric repeats by a sequence of ~11 kb, which contains a copy of the

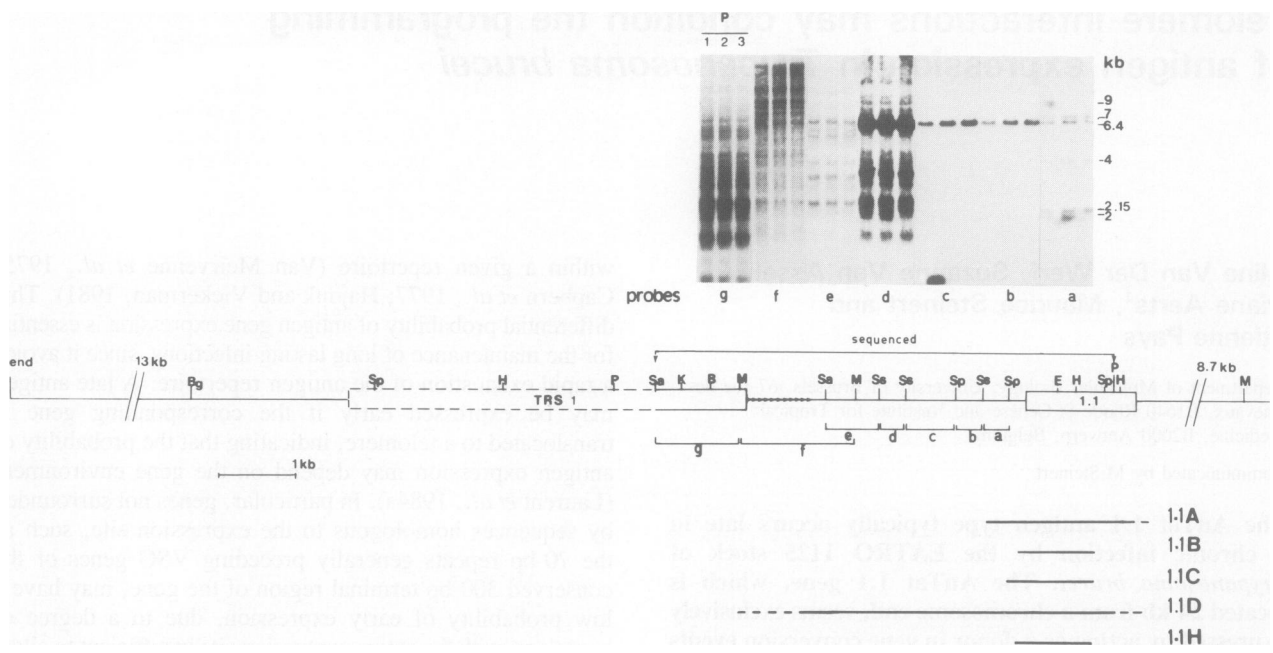

Fig. 1. Hybridization pattern and restriction map of the AnTat 1.1 gene and 5′ environment. Southern blots of *Pst*I DNA digests from the bloodstream variants AnTat 1.1A, 1.1B and 1.3A (respectively 1–3 in each panel) have been hybridized with several probes from the AnTat 1.1 gene environment (a–g), as shown in the map. The extent of the nucleotide sequence presented in Figure 2 is indicated above the map. The thick lines under the map present the relative extent of the donor sequence for gene conversions linked to VSG expression in different AnTat 1.1 clones (Pays *et al.*, 1985a). Boxes are for the TRS-1 retrotransposon (Murphy *et al.*, 1987) and AnTat 1.1 VSG sequence, as indicated. The array of small arrowheads, covered by probe f, represents the 10.5 tandemly repeated 70 bp motifs. The abbreviations for the restriction endonuclease sites, in this and subsequent figures, are: B = *Bgl*I; Bg = *Bgl*II; Dr = *Dra*I; E = *Eco*RI; H = *Hin*dIII; K = *Kpn*I; M = *Msp*I; P = *Pst*I; Sa = *Sau*3A; Sp = *Sph*I; Ss = *Sst*I.

repeated TRS-1 retrotransposon (Figure 1). Moreover, it is the only known example of a VSG gene oriented with its 5′ extremity towards the telomere (Pays *et al.*, 1983b). While the three non-telomeric family members were cloned in full, the AnTat 1.1 and 1.10 genes could only be partially cloned, due to the presence of seemingly unclonable sequences just downstream. In both cases, the cloned region extends to a *Pst*I site located 264 bp upstream from the stop codon in the corresponding cDNAs (Pays *et al.*, 1983b, 1985a; S.Van Assel and E.Pays, unpublished). Figures 1 and 2 show, respectively, the restriction map and nucleotide sequence of the AnTat 1.1 gene and its 5′ environment. The translation frame of this gene is open up to the 3′ *Pst*I site, and its nucleotide sequence perfectly matches that of the AnTat 1.1A cDNA. Downstream from *Pst*I, the gene appears identical to the cDNA over ~285 bp, since this is the size of the region protected against S1 digestion following hybridization with the cDNA (Figure 3). This means that the gene (basic copy or BC) has been completely copied to generate the expression-linked copy (ELC) in the AnTat 1.1A variant (Pays *et al.*, 1983b). The 3′ limit of this ELC is in a conserved region frequently found as gene conversion endpoint in antigenic variation (Michels *et al.*, 1982). Upstream from the gene the sequence does not exhibit significant open reading frames (the largest is 257 bp long) and appears unique in the genome over ~1600 bp; upstream from that point the DNA is repeated (Figure 1). The highly repeated pattern seen with the most 5′ probes is due to the presence of 10.5 copies of the 70 bp repeat characteristic of the 5′ environment of VSG genes (Liu *et al.*, 1983; Campbell *et al.*, 1984; Aline *et al.*, 1985; Bernards *et al.*, 1985; Florent *et al.*, 1987; Shah *et al.*, 1987) and also of a copy of the repeated TRS-1 retrotransposon (Kimmel *et al.*, 1987; Murphy *et al.*, 1987) (Figures 1 and 2). Both the 70 bp repeats and TRS-1 are in the same orientation as the VSG gene.

The AnTat 1.1 gene and environment can be used as such for conversion of the VSG expression site

From the original EATRO 1125 stabilate, which is expressing AnTat 1.1 as homotype (Pays *et al.*, 1981, 1983b) two independent AnTat 1.1-expressor clones have been analysed (AnTat 1.1A and 1.1D). As mentioned before (Pays *et al.*, 1983a), in these two clones the same VSG gene is activated, but through different recombination events. While in AnTat 1.1A the gene conversion domain (ELC) extends only 450 bp upstream from the gene, in AnTat 1.1D the ELC extends ~2800 bp upstream. The data in Figure 4 show that the AnTat 1.1D gene conversion initiates in the region of the 70 bp repeats, and terminates in the 3′ conserved region of the VSG gene. Both these blocks of homology are frequently found to be gene conversion endpoints early in infection.

Once in a correct orientation, the AnTat 1.1 gene can be expressed early, as gene conversion donor

AnTat 1.1 is not found early in infections initiated by different clones from the EATRO 1125 stabilate (Hajduk and Vickerman, 1981). However, this antigen type can be observed early if the clone used for infection contains the AnTat 1.1 gene in the correct orientation in a telomere. Such is the case in AnTat 1.6D, selected among the heterotypes arising from the AnTat 1.1D clone. The telomeric AnTat 1.6 gene is easily activable *in situ*, leaving the former

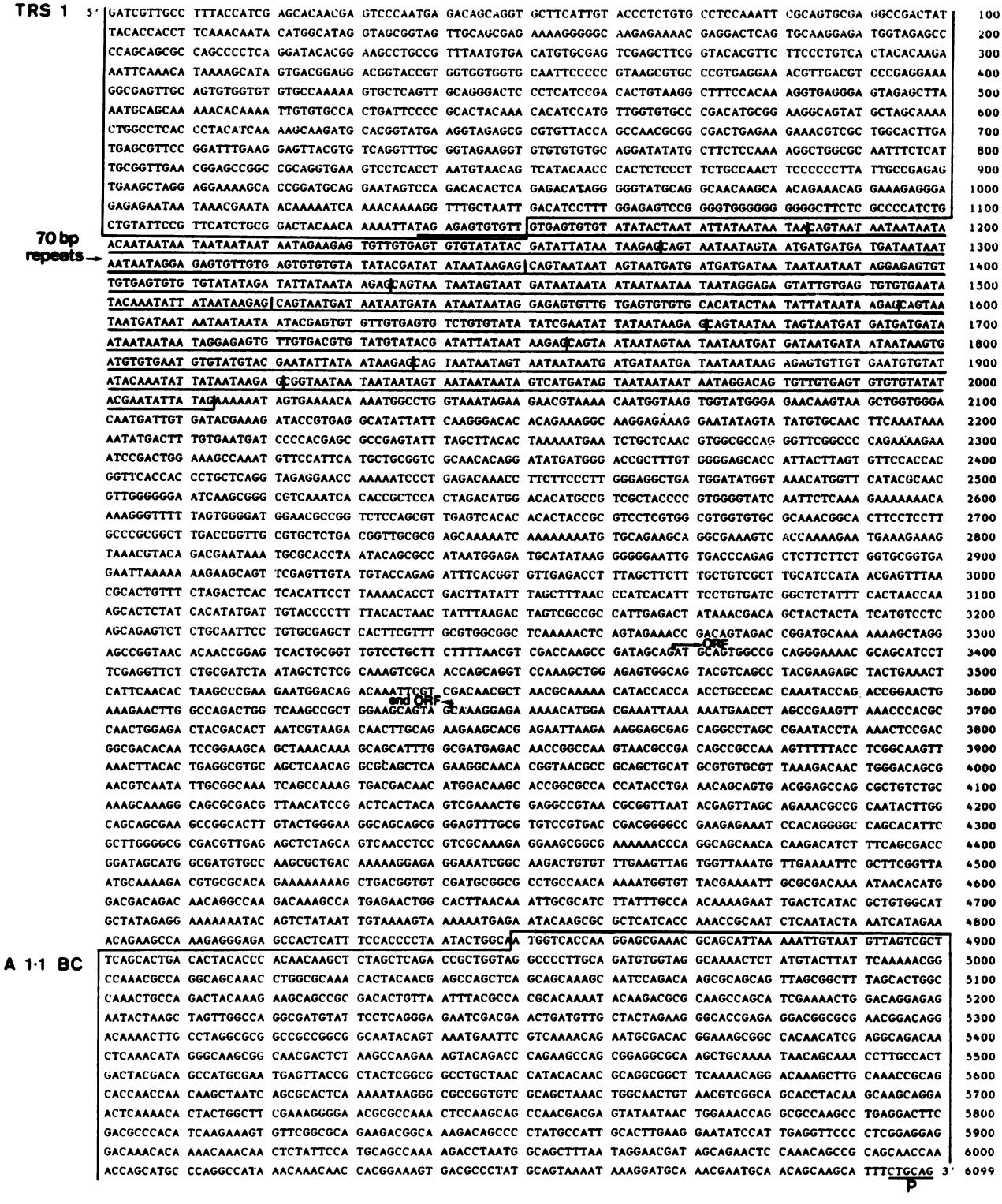

Fig. 2. Nucleotide sequence of the AnTat 1.1 basic copy and 5′ environment. The 3′ end of the TRS-1 copy and the 5′ end of the AnTat 1.1 open reading frame are boxed. The 10.5 copies of the '70 bp' repeat, characteristic of barren regions upstream from VSG genes, are underlined.

expression site with its resident ELC unchanged but silent ('ex-ELC') (Laurent *et al.*, 1984a,b; Pays *et al.*, 1985b). Therefore, in AnTat 1.6D, the AnTat 1.1D ELC is present as a silent telomeric VSG gene. If AnTat 1.6D is then used for infection, the AnTat 1.1 antigen type can be detected early, representing ~10% of the trypanosome population after 7 days. The activation mechanism is a gene conversion, with the AnTat 1.1D ex-ELC as donor (Figure 5). This shows that the AnTat 1.1 gene and upstream environment, if correctly positioned, can readily recombine with the active VSG gene expression site.

Discussion

Among a collection of 13 different variants of the EATRO 1125 *Trypanosoma brucei* isolate, AnTat 1.1 is, with AnTat

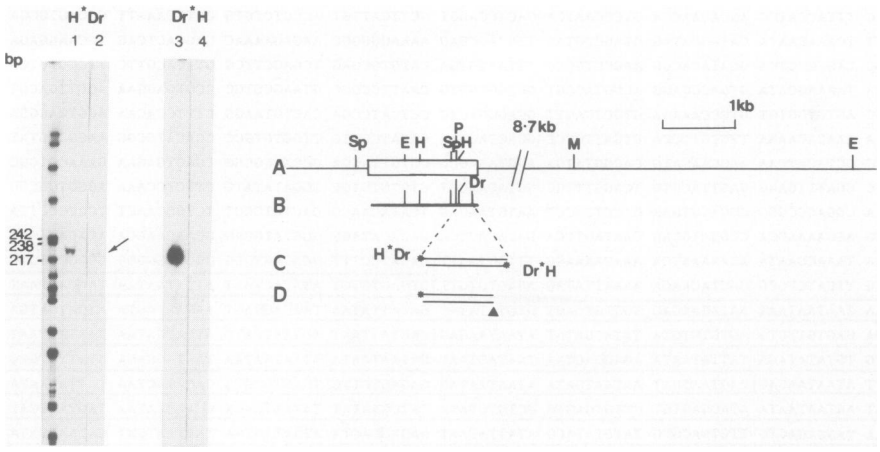

Fig. 3. The AnTat 1.1 basic copy appears identical to the AnTat 1.1A cDNA up to the gene stop codon. A 234 bp *Hin*dIII−*Dra*I fragment from the 3′ end of the AnTat 1.1A cDNA has been ^{32}P labelled by kinasing, then the two DNA strands were separated by electrophoresis through polyacrylamide. These probes have been independently hybridized with 6 μg of uncloned 13.5 kb *Eco*RI fragments from AnTat 1.3A genomic DNA, which contain the 3′ ends of the AnTat 1.1 basic copy. After hybridization, the probes have been treated by the S1 nuclease (0 or 1000 units, in 1 and 2 respectively), then electrophoresed through polyacrylamide. (**A**) Restriction map of the AnTat 1.1 basic copy and 3′ environment, showing the 13.5 kb *Eco*RI fragment. (**B**) Extent of the AnTat 1.1A cDNA. (**C**) The 234 bp *Hin*dIII−*Dra*I fragment, with asterisks for terminal labelling. The interpretation of the data is illustrated in (**D**) where fragment H*Dr has been protected down the 3′ limit of gene conversion, indicated by the arrowhead, and is visible in lane 2 (arrows); although fragment Dr*H was similarly protected, it has lost its labelling and cannot be detected in lane 4.

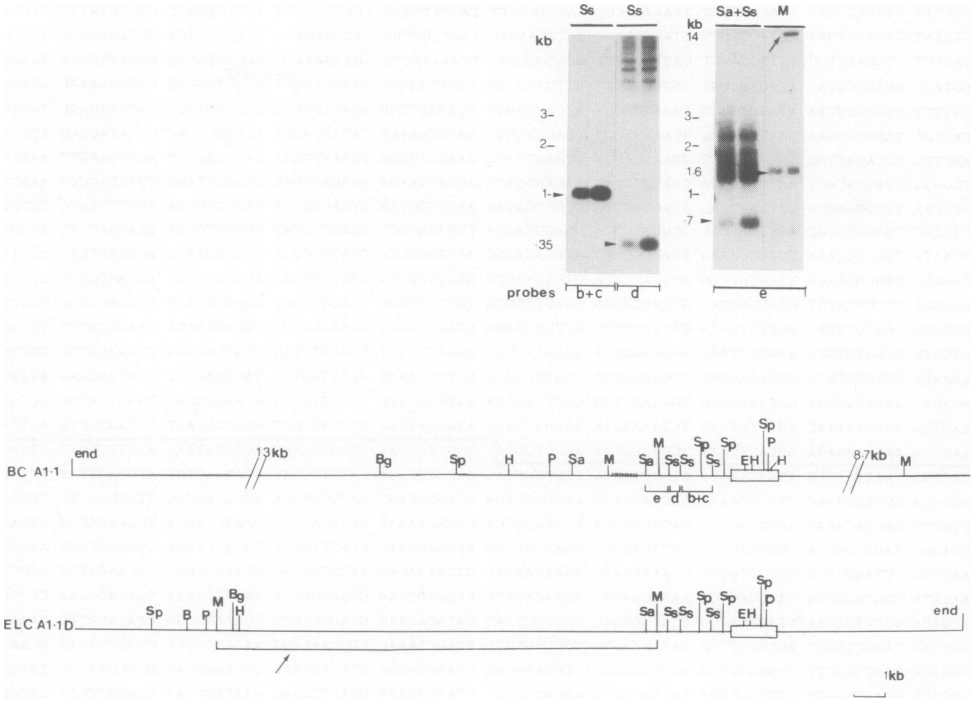

Fig. 4. Extent of the AnTat 1.1 gene conversion in the AnTat 1.1D variant, showing that the region of 70 bp repeats ahead of the basic copy gene can be used as gene conversion endpoint. Southern blots of genomic digests from AnTat 1.1A and 1.1D (first and second lane in each panel, respectively) have been hybridized with probes from the 5′ environment of the AnTat 1.1 basic copy (b+c, d and e), as shown in the map (same probes as in Figure 1). A doubling of the hybridization intensity in AnTat 1.1D indicates that the corresponding DNA fragments (arrowheads) belong to the AnTat 1.1D ELC, that is, are within the gene conversion domain. The arrowed *Msp*I fragment in 'e' spans the 5′ gene conversion endpoint; its extent is indicated under the map of the AnTat 1.1D ELC and environment. The 5′ limit of the ELC template is in the region of the 70 bp repeats, represented by the array of small arrowheads.

1.13, the only antigen type not detectable within 10 days of infection. This is true if infection is initiated either by cyclical transmission (Hajduk and Vickerman, 1981), or by mechanical injection (Van Meirvenne *et al.*, 1975). In experimental infection of a rabbit, AnTat 1.1 is still not detected at day 46 (Van Meirvenne *et al.*, 1975), and can therefore be considered as a late variant. The analysis of eight independent AnTat 1.1 clones has shown that the corresponding VSG gene can only be activated through the synthesis of an ELC (gene conversion) (Pays *et al.*, 1985a), except in clones where the ELC has been conserved in a silent form, and can be re-expressed without rearrangement

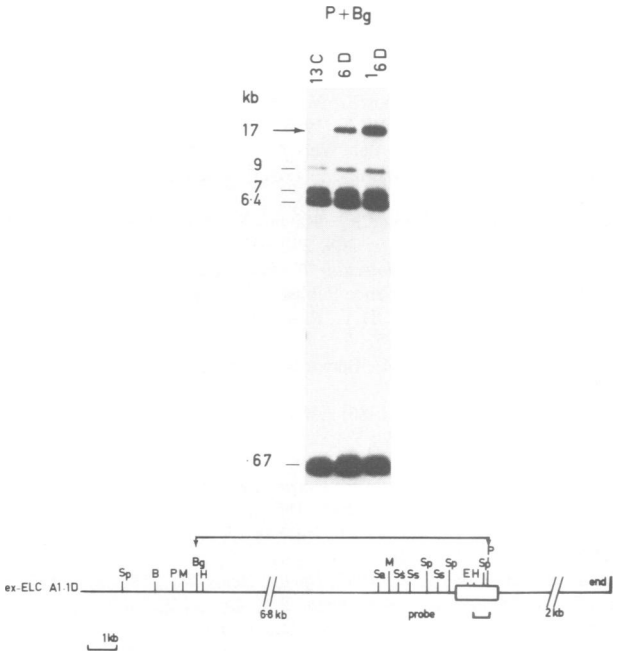

Fig. 5. The AnTat 1.1D ELC, conserved but silent in the AnTat 1.6D clone, can act as early donor for gene conversion of the VSG gene expression site. The DNA probe shown under the map has been hybridized with Southern blots of *Pst*I + *Bgl*II digests of clones AnTat 1.13C (13C) and AnTat 1.6D (6D), and of an uncloned population containing >95% AnTat 1.1, obtained from the AnTat 1.6D clone by immunolysis with an anti-AnTat 1.6 antiserum (1-6D). The arrowed fragment is characteristic of the AnTat 1.1D expression site (see Figure 4), and has been conserved, but silent, in AnTat 1.6D ('ex-ELC') (Pays *et al.*, 1985b). Its doubling in (1-6D) indicates that this sequence is used as donor in the gene conversion leading to AnTat 1.1 expression.

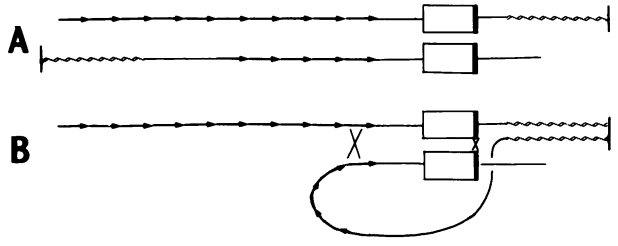

Fig. 6. Telomere interactions may impede the sequence alignment necessary for gene conversion. (**A**) The VSG gene expression site (top) is aligned with the telomere carrying the AnTat 1.1 BC (bottom), taking as guidelines the homologous sequences flanking the genes: the 5′ 70 bp repeats (arrows), and the 3′ conserved sequence of VSG genes (thick vertical bar). Based on these homologies only, one would not expect the AnTat 1.1 gene to be expressed late (see text). Notice that the chromosome ends are in opposite locations. (**B**) If the alignment of the telomeric sequences is primarily dictated by interactions between chromosome ends (wavy lines), the recombinations between the VSG genes would require a sequence inversion. This event, which is possible as evidenced by the production of AnTat 1.1D, would only occur at low frequency, accounting for the late expression of AnTat 1.1.

(Delauw *et al.*, 1985; Pays *et al.*, 1985b). This paper deals with the possible reasons for the low probability of expression of this gene.

Since the VSG gene expression site appears to be necessarily telomeric, non-telomeric VSG genes cannot be expressed *in situ* and must recombine with the active telomere. This may explain why non-telomeric VSG genes are generally not expressed at the beginning of the infection. In addition, all non-telomeric VSG genes do not seem to be expressed with an equal probability. We have proposed (Laurent *et al.*, 1984a) that a possible reason for the late expression of some VSG genes could be their limited ability to recognize and recombine with the telomeric expression site, due to a depletion or total lack of the sequences usually conserved among VSG genes and their environment, which allow recombination to occur. These sequences are the 70 bp repeats normally found ~1.5 kb upstream from the gene, and the 3′ terminal sequence of the gene. This hypothesis might suffice to explain the late expression of the AnTat 1.13 gene, the 5′ environment of which only contains a single 70 bp repeat (A.Van Der Werf, unpublished data). We show here that this model does not apply to the AnTat 1.1 gene, since this gene contains the usual 3′ region of homology, and is preceded by 10.5 copies of the 70 bp repeat. This number should normally allow the gene to be expressed early, since other non-telomeric genes expressed relatively early, such as gene AnTat 1.8 in our stock (A.Van Der Werf, unpublished), or gene 118 in another stock (Liu *et al.*, 1985), are preceded by a comparable array of repeats.

Another explanation pertains to intrinsic defects in the VSG coding sequence, such as the presence of stop codons (Longacre and Eisen, 1986; Roth *et al.*, 1986). These pseudogenes cannot be expressed, except through partial conversions of the active VSG gene, generating chimaeric but functional new sequences. Obviously this is possible only if the target gene shares homology with the donor, and this can be a rare event, occurring late. This model can be ruled out here, since we have shown that the AnTat 1.1 gene is not interrupted by stop codons up to the 3′ block of homology.

Finally, the analysis of the AnTat 1.1D variant shows that a copy of the AnTat 1.1 gene and 5′ environment, if translocated to a potential expression site, such as in the AnTat 1.6D clone, can be expressed early. This unambiguously demonstrates that the low probability of expression of the AnTat 1.1 gene does not depend on its sequence, its proximal environment or the antigen type it specifies.

An apparent anomaly which may explain the late expression of the AnTat 1.1 gene is its reverse orientation with respect to the proximal chromosome end. This anomaly might be linked to the presence, between the VSG gene and the chromosome end, of a TRS-1 retrotransposon whose insertion may have led to DNA rearrangement in this telomere. Such an effect of the VSG gene orientation would suggest that the alignment of the telomeric partners, namely the donor and the target in the gene conversion events leading to expression of early antigen types, not only depends on the extent and location of the 5′ and 3′ blocks of homology, but may be conditioned primarily by a pairing between telomeres. That telomeres interact has been observed in several instances in different organisms (Lima-de-Faria, 1983; Blackburn and Szostak, 1984; Horowitz *et al.*, 1984; Henderson *et al.*, 1987; Oka and Thomas, 1987; Sen and Gilbert, 1988; Pluta and Zakian, 1989). The telomere-to-telomere interactions may override all other possible sequence recognition events near chromosome ends, due to the extensive length and particular nucleotide sequence composition of the arrays of telomeric repeats (Sen and

Gilbert, 1988; for a review, see Blackburn and Szostak, 1984). Since the telomeric VSG genes are usually in the same orientation, the pairing of telomeres would allow a direct and efficient positioning of the sequences which are involved in recombination. In the case of non-telomeric VSG genes, the orientation would be without major influence, except for genes relatively close to a telomere, such as AnTat 1.1, where the alignment of the donor and target would be impeded by telomere pairing (Figure 6). The hypothesis that telomere pairing influences recombinations between VSG genes is also supported by the analysis of the terminal size variations in telomeres carrying VSG genes. Although not absolute, a correlation has been found between the occurrence of large telomere deletions and the involvement of the telomere in gene conversion leading to antigenic switching (Pays *et al.*, 1983c; Myler *et al.*, 1988). Thus, even if the telomere is inactive, it is frequently subject to rearrangements when carrying the donor VSG gene in conversion events. This also suggests that telomere interactions are associated with the DNA recombinations underlying antigenic variation.

Materials and methods

The trypanosome clones AnTat 1.1A-H, 1.3A and 1.6D have been characterized elsewhere (Laurent *et al.*, 1983; Delauw *et al.*, 1985; Pays *et al.*, 1985a). The procedures for DNA isolation, Southern and blot hybridization as well as DNA cloning, were as described (Pays *et al.*, 1980). The nucleotide sequence of DNA fragments, subcloned in bacteriophage M13 derivatives, as determined on both strands (Sanger *et al.*, 1980), using a modified T7 DNA polymerase ('Sequenase', USB). S1 nuclease protection experiments have been conducted as described in Gilmour (1984). The computer analysis of DNA has been performed using the DNASIS/PROSIS programs of LKB/HITACHI. The sequences have been compared with the following database releases: GenBank R52.0, EMBL R13.0 and NBRF-PIR R14.0.

Acknowledgements

We acknowledge Dr D.Jefferies for helpful comments on the manuscript. A.V.D.W. received financial support from the 'Fondation Universitaire David et Alice van Buuren' (Brussels). This work has been supported by the Belgian 'Fonds de le Recherche Scientifique Médicale', by the UNDP/World Bank/WHO special programme for research and training in tropical diseases, and also by research contracts between the University of Brussels and the Commission of the European Communities (TDS-M-023B), and between the University and Solvay & Cie (Brussels).

References

Aline,R., Jr, MacDonald,G., Brown,E., Allison,J., Myler,P.J., Rothwell,V. and Stuart,K. (1985) *Nucleic Acids Res,* **13**, 3161–3177.

Bernards,A., Kooter,J.M., and Borst,P. (1985) *Mol. Cell. Biol.*, **5**, 545–553.

Blackburn,E.H. and Szostak,J.W. (1984) *Annu. Rev. Biochem.*, **53**, 163–194.

Borst,P. (1986) *Annu. Rev. Biochem.*, **55**, 701–732.

Campbell,D.A., van Bree,M.P. and Boothroyd,J.C. (1984) *Nucleic Acids Res,* **12**, 2759–2774.

Capbern,A., Giroud,C., Baltz,T. and Mattern,P. (1977) *Exp. Parasitol.*, **42**, 6–13.

Delauw,M.F., Pays,E., Steinert,M., Aerts,D., Van Meirvenne,N. and Le Ray,D. (1985) *EMBO J.*, **4**, 989–993.

Florent,I., Baltz,T., Raibaud,A. and Eisen,H. (1987) *Gene,* **53**, 55–62.

Gilmour,R.S. (1984) In Hames,B.D. and Higgins,S.J. (eds), *Transcription and Translation—A Practical Approach.* IRL Press, Oxford, pp. 131–152.

Hajduk,S.L. and Vickerman,K. (1981) *Parasitology,* **83**, 609–621.

Henderson,E., Hardin,C.C., Walk,S.K., Tinoco, I., Jr and Blackburn,E.H. (1987) *Cell,* **51**, 899–908.

Horowitz,H., Thorburn,P. and Haber,J.E. (1984) *Mol. Cell. Biol.*, **4**, 2509–2517.

Kimmel,B.E., Ole-Moiyoi,O. and Young,J.R. (1987) *Mol. Cell. Biol.*, **7**, 1465–1475.

Laurent,M., Pays,E., Magnus,E., Van Meirvenne,N., Matthyssens,G., Williams,R.O. and Steinert,M. (1983) *Nature,* **302**, 263–266.

Laurent,M., Pays,E., Van der Werf,A., Aerts,D., Magnus,E., Van Meirvenne,N. and Steinert,M. (1984a) *Nucleic Acids Res,* **12**, 8319–8328.

Laurent,M., Pays,E., Delinte,K., Magnus,E., Van Meirvenne,N. and Steinert,M. (1984b) *Nature,* **308**, 370–373.

Lima-de-Faria,A. (1983) *Molecular Evolution and Organization of the Chromosome.* Elsevier Science Publishers, Amsterdam, pp. 701–721.

Liu,A.Y.C., Van der Ploeg,L.H.T., Rijsewijk,F.A.M. and Borst,P. (1983) *J. Mol. Biol.*, **167**, 57–75.

Liu,A.Y.C., Michels,P.A.M., Bernards,A. and Borst,P. (1985) *J. Mol. Biol.*, **175**, 383–396.

Longacre,S. and Eisen,H. (1986) *EMBO J.*, **5**, 1057–1063.

Michels,P.A.M., Bernards,A., Van der Ploeg,L.H.T. and Borst,P. (1982) *Nucleic Acids Res.*, **10**, 2353–2366.

Murphy,N.B., Pays,A., Tebabi,P., Coquelet,H., Guyaux,M., Steinert,M. and Pays,E. (1987) *J. Mol. Biol.*, **195**, 855–871.

Myler,P.J., Aline,R.F., Scholler,J.K. and Stuart,K.D. (1988) *Mol. Biochem. Parasitol.*, **29**, 243–250.

Oka,Y. and Thomas,J.C.A. (1987) *Nucleic Acids Res.*, **15**, 8877–8897.

Pays,E. and Steinert,M. (1988) *Annu. Rev. Genet.*, **22**, 107–126.

Pays,E., Delronche,M., Lheureux,M., Vervoort,T., Bloch,J., Gannon,F. and Steinert,M. (1980) *Nucleic Acids Res.*, **8**, 5965–5981.

Pays,E., Van Meirvenne,N., Le Ray,D. and Steinert,M. (1981) *Proc. Natl. Acad. Sci. USA,* **78**, 2673–2677.

Pays,E., Van Assel,S., Laurent,M., Dero,B., Michiels,F., Kronenberger,P., Matthyssens,G., Van Meirvenne,N., Le Ray,D. and Steinert,M. (1983a) *Cell,* **34**, 359–369.

Pays,E., Van Assel,S., Laurent,M., Darville,M., Vervoort,T., Van Meirvenne,N. and Steinert,M. (1983b) *Cell,* **34**, 371–381.

Pays,E., Laurent,M., Delinte,K., Van Meirvenne,N. and Steinert,M. (1983c) *Nucleic Acids Res.*, **11**, 8137–8147.

Pays,E., Houard,S., Pays,A., Van Assel,S., Dupont,F., Aerts,D., Huet-Duvillier,G., Gomes,V., Richet,C., Degand,P., Van Meirvenne,N. and Steinert,M. (1985a) *Cell,* **42**, 821–829.

Pays,E., Laurent,M., Delauw,M.F. and Steinert,M. (1985b) In Simon,M. and Herskowitz,I. (eds), *Genome Rearrangement—UCLA Symposia on Molecular and Cellular Biology, New Series, Vol. 20.* Alan R.Liss, Inc., NY, pp. 173–187.

Pluta,A.F. and Zakian,V.A. (1989) *Nature,* **337**, 429–433.

Roth,C.W., Longacre,S., Raibaud,A., Baltz,T. and Eisen,H. (1986) *EMBO J.*, **5**, 1065–1070.

Sanger,F., Coulson,A.R., Barrell,B.G., Smith,A.J.H. and Roe,B. (1980) *J. Mol. Biol.*, **143**, 161–178.

Sen,D. and Gilbert,W. (1988) *Nature,* **334**, 364–366.

Shah,J.S., Young,J.R., Kimmel,B.E., Iams,K.P. and Williams,R.O. (1987) *Mol. Biochem. Parasitol.*, **24**, 163–174.

Van Meirvenne,N., Janssens,P.G. and Magnus,E. (1975) *Ann. Soc. Belge Med. Trop.*, **55**, 1–23.

Received on July 17, 1989; revised on September 8, 1989