Differential size variations between transcriptionally active and inactive telomeres of *Trypanosoma brucei*

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ABSTRACT

We have studied the genes coding for the variant-specific surface antigen (VSA) in a series of seven trypanosome clones derived from AnTat 1.1: 1.1 → 1.3 → 1.6 → 1.16 → 1.1C → 1.3B → 1.18

These genes are all telomeric (1-5), and their surrounding, although sometimes similar, differs in each case. The length between these antigen genes and the corresponding DNA end appears to increase at each antigenic switch, with however occasional sharp size reductions, often linked to the involvement of the telomere in gene expression. This increase is due to a constant "growth" of the telomeres, at a rate of about 28 bp per day in at least four cases and probably linked to chromosome duplication. The telomere harbouring the transcribed VSA gene is growing slightly faster (about 36 bp per day), and it is the only one whose size reduction is progressive, leading to a terminal length heterogeneity within a clone. As a result, the active VSA gene is found in a population of telomeres which, as the trypanosomes divide, becomes increasingly heterogeneous, with however a preferred discrete size class about 1.4 kb smaller. The fact that the "active" telomere is the only one in a chromatin conformation highly sensitive to DNaseI (1-4, 6), suggests that chromatin structure influences the rate and extent of both size increase and shortening of telomeres.

INTRODUCTION.

Antigenic variation is one of the mechanisms developed by African trypanosomes to evade the immunological defense of their hosts. Chronic infection by these parasites is indeed maintained by their ability to repeatedly and completely change their uniform surface coat (see review in 7). At least one hundred different antigens can be synthesized sequentially during the development of trypanosome populations derived from one single cell (8). It has been reported that this adaptability is due to the possibility to express, in alternation, a large collection of genes, and that this expression is associated with genome rearrangements (reviewed in 7). We provided evidence that gene conversion is one of the recombinational mechanisms involved in these rearrangements and that interactions between telomeric sequences...
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seem to be very important in these processes (2).

Using variant-specific antigen (VSA) gene probes, trypanosome telomeric DNA sequences have been studied in some detail (9-11). It has been observed that trypanosome telomeres are continuously "growing", a property which could be common to all telomeres (11). We report here that, although the growth of different trypanosome telomeres seems similar, the terminal size variations of the telomere carrying the active VSA gene exhibit several distinct features. This particular telomere is subject to an active process of terminal shortening, which compensates for its continuous growth while leading to an ever increasing size heterogeneity within the clone. This particular behaviour of the active telomere is speculatively linked to its different chromatin conformation.

MATERIAL AND METHODS.

Trypanosomes. The different antigenic variants were cloned at the Institute of Tropical Medicine (Antwerp) from the same stock (EATRO 1125) of Trypanosoma b. brucei. All trypanosome populations were grown in mice. Clones of successive VATs were derived by immunological selection (12) from heterotypes arising in the diversifying clone of the previous VAT. The time of cultivation elapsed during the cloning procedure, between the first variant detection and its DNA isolation, is undetermined for AnTat 1.1, more than 46 days for AnTat 1.3, between 48 and 98 days for AnTat 1.6, between 38 and 48 days for both AnTat 1.16 and 1.18, 41 days for AnTat 1.1C and 29 days for AnTat 1.3B. Reference clones used for DNA analysis were serologically homogeneous for more than 99%. An abridged pedigree of the different clones is given in Figure 1. Their nomenclature follows the rules recommended by Lumsden (13).

DNA isolation and analysis have been performed as described (4, 14).

Molecular cloning. The AnTat 1.1, 1.3, 1.6, 1.16, 1.1C, 1.3B and 1.18 cDNAs have been synthesized, cloned and characterized as described previously (2-5, 14).

Probes. Specific parts of the cloned cDNAs have been isolated by preparative electrophoresis on low melting point agarose, then ($^{32}$P)-labelled by nick-translation (15).

Hybridization of the probes with Southern blots (16) of digested genomic DNAs was performed as in previous work (14). As size markers we used $^{32}$P-labelled HindIII and SalI digestion products of lambda and SV40 DNAs.
RESULTS.

We have undertaken a comparative study of terminal size variations affecting different telomeres, either activated or not, which are involved in the succession of seven antigenic variants of T. b. brucei:

Stock EATRO 1125 ↔ AnTat 1.1 ↔ 1.3 ↔ 1.6 ↔ 1.16 ↔ 1.1C ↔ 1.3B

Description of the telomeres

Thorough descriptions of the genes expressed in the first two variants of this series, and of their activation mechanism, have already been provided (1-6,14,17). The maps in Figure 1 summarize most of the informations collected about these seven genes.

All the sequences involved are telomeric, and, with one exception, all are oriented with their 3' end towards the DNA terminus. The activation of five of them (AnTat 1.1, 1.3, 1.16, 1.18 and 1.3B) is linked to the production of an additional, "expression-linked" copy (ELC) from a "basic copy" (BC) of the gene. The length of the ELC (i.e. the extent of the duplicated or converted region) is about 2 kb for AnTat 1.1, and at least 3, 1.5 and 40 kb, for AnTat 1.3, 1.16 and 1.3B respectively.

No AnTat 1.6 and 1.1C ELCs could be detected, but the corresponding genes seem instead to be activated by a reciprocal recombination with the preceding ELC (4,5); indeed, in each of these cases the preceding ELC (AnTat 1.3 and 1.16, respectively) is conserved, though in an inactive form ("ex-ELCs"), whereas the AnTat 1.6 or 1.1C genes are lost from the ensuing variants, being chased by the incoming ELC (4,5).

The restriction maps of most of these telomeres show similarities: except for the telomeres containing the AnTat 1.1 BC and AnTat 1.1C gene, and taking into account that all sites could not be mapped in each case, due to the unavailability of adequate probes, the restriction maps can be aligned on a common SphI/BglI/PvuII/PstI/BglII/HindIII block (dots in Figure 1) located in front of the variable "barren" region which flanks the antigen-specific sequence.

Moreover, in some cases the restriction maps of the sequence preceding this block are so similar that we suppose that the corresponding telomeres are identical: this is true for the AnTat 1.1 and 1.3 ELC surroundings, as previously stressed (1); these telomeres furthermore appear to be identical to the AnTat 1.3B BC, which is an AnTat 1.3 ex-ELC (4,5). Similarly, the AnTat 1.6 environment seems identical to that of the AnTat 1.16 ELC. When observed in different variants, apart from terminal size variations, the restriction maps of these telomeres reveal no significant alteration at
least up to about 50 kb upstream from the DNA end.

**Terminal size variations**

The results, presented in Figure 2, show that with some exceptions, the stretch between the VSA sequence and the DNA end seems to increase in length, by about 0.8 kb, at each antigenic switch. The best case is the AnTat 1.3 BC-containing telomere, whose terminal length is increased by 3.2 kb from AnTat 1.3 to AnTat 1.3B clones; similarly, the terminal length of the telomere containing the AnTat 1.3 ex-ELC increases by 2.4 from AnTat 1.6 to AnTat 1.3B (double arrow-head in Figure 2). The telomeres harbouring the AnTat 1.16 BC and ex-ELC, as well as the AnTat 1.1C and 1.18 BCs also show such regular extensions in at least three successes variants in each case. The telomere of the AnTat 1.1 BC makes no exception, despite the fact that in this case the VSA gene is oriented with its 5' end towards the DNA terminus.

Breaks in these progressions are observed for the telomeres carrying the AnTat 1.3, 1.16, 1.1C and 1.18 BCs, in connection with their involvement in gene expression; the telomeres are then shortened by about 6, 4, 0.2 and 3 kb, respectively. This is not a general rule, since the telomere harbouring the AnTat 1.3B BC is "normally" extended by 0.8 kb in the AnTat 1.3B clone, whereas the telomere harbouring the AnTat 1.18 BC is shortened in two successive steps in clones which do not express the AnTat 1.18 gene (AnTat 1.16 and 1.1C) and, similarly, the AnTat 1.1 BC-containing telomere is shortened in AnTat 1.16 and 1.3B clones.

In order to know whether telomeric growth is linked to the antigenic switch, or reflects a more continuous increase occurring within the growing...
Figure 2. Comparison of different telomeres in a series of successive trypanosome clones. The genomic DNA has been extracted from trypanosome clones AnTat 1.1, 1.3, 1.6, 1.16, 1.1C and 1.3B (respectively from left to right in each block; AnTat 1.18 DNA is added at the right of the last block), then digested by different restriction endonucleases, as indicated above each block (EcoRI, E; PvuII, Pv; SphI, Sp; StuI, Ss), electrophoresed in agarose gels, transferred to nitrocellulose (16) and hybridized with (32)P-labelled AnTat 1.1C (5' or 3'), 1.3, 1.6, 1.16 and 1.18 cDNA probes, as indicated under each block; the origin of each probe is shown in Figure 1. The asterisks label the DNA from the variants corresponding to the probe. Arrowheads and double arrowheads point to fragments carrying an ELC or an ex-ELC, respectively. In all cases, the fragments harbouring the VSA gene BCs are of a different size in each variant (arrows). In AnTat 1.1 DNA (first lane of the fifth panel), the AnTat 1.10 BC ("9 kb" AnTat 1.1 gene family member: see ref. 2) is found in two versions of the same telomere, differing by 5 kb in its 3' terminal part (see discussion and ref. 2). The AnTatt 1.1C probe restricted to the 5' part of the gene weakly recognizes the AnTat 1.10 BC (lower bands in AnTat 1.1, 1.3, 1.6 and 1.16 DNAs in the first panel), in addition to the AnTat 1.1 BC (strongly hybridizing bands).
Figure 3. Terminal growth of the telomeres carrying the AnTat 1.6, 1.16 and 1.18 VSA genes in AnTat 1.3 DNA. The AnTat 1.3 clone has been maintained in rats during 18 passages of three days each. The DNAs extracted from rats at passages 1, 5, 9, 13 and 18 (five first lanes in each block, respectively) have been digested by SphI, EcoRI or ClaI, and hybridized with a AnTat 1.6, 1.16 or 1.18 cDNA probe, as indicated. The last lane in each block contains the DNA from an independent first passage of the same clone. Arrows point to the fragments carrying the respective VSA genes.

trypanosome clone, we analyzed the terminal size of different telomeres (carrying the AnTat 1.3, 1.6, 1.16 and 1.18 BCs), at different time intervals during the propagation of the AnTat 1.3 clone. Figures 3 and 4 show that within this clone, the four different telomeres are continuously growing, each at a rate of approximatively 7 bp per generation period (assumed to be 6 h). The gain of about 0.8 kb that we observed between the different clones considered above, could be accounted for by this regular growth, since it is compatible with the time (from 29 to about 50 days), and thus the number of cell divisions elapsed between antigen type switching and DNA isolation.

Another striking observation pertains to the length of the "active" telomere, which harbours the expressed sequence. As revealed clearly in at least six cases (AnTat 1.1, 1.3, 1.6, 1.16, 1.3B, Figure 2, arrowheads; also AnTat 1.18, not shown), the terminal fragment containing the expressed gene is the only one to be accompanied by a "smear" of hybridizing sequences. This observation indicates that, within the same clone, the size of the
Terminal size variations of the telomeres harbouring the AnTat 1.3 BC (arrow) and ELC (arrowheads). DNA samples have been prepared at each of the 18 passages (three days each) performed on the AnTat 1.3 clone (see legend of fig. 3); after PstI digestion, it was hybridized with an AnTat 1.3 cDNA probe. The last lane contains the DNA from an independent first passage of the same clone. The bands and smear characteristic of the AnTat 1.3 ELC (arrowheads; see also ref. 3) are discussed in the text.

barren sequence between the active gene and the DNA terminus is highly heterogeneous.

The experiment in Figure 4, carried out on the telomere carrying the AnTat 1.3 ELC, shows how this heterogeneity arises during clone propagation. In the original population (first track), a majority of the terminal fragments of the telomere are 6.6 kb long and form the distinct upper band. A second band, containing fragments about 1.4 kb shorter, has a blurred aspect indicative of size heterogeneity. These two bands are finally accompanied by a continuous smear of still smaller terminal fragments. The kinetics illustrated in Figure 4 shows an upward displacement of the bands, indicating a terminal size increase of about 9 bp per 6 h., thus slightly faster than the other telomeres. However, simultaneously, the upper band fades progressively, as more DNA appears in the second band and in the smear. Thus, as the size of the telomere is regularly growing in the whole population in steps of about 9 bp per cell division, deletions (with high frequency of a 1.4 kb deletion) appear to randomly affect an ever increasing fraction of this population, with the result that the size of the telomere is kept within...
acceptable limits while it becomes more and more heterogeneous.

It should be stressed that the trypanosomes of the last passage in this experiment are still fully expressing the AnTat 1.3 antigen type, as measured by the immune lysis and immunofluorescence tests (data not shown). Moreover, the expressed AnTat 1.3 gene as well as its upstream "barren" region, are conserved unaltered through the 18 passages, as judged by restriction mapping (not shown). The terminal size variations of the telomere thus do not alter the gene structure nor its expression.

**DISCUSSION.**

It is highly probable that the terminal portion of these telomeres is made up of very simple sequences, since almost no restriction sites can be mapped within these regions (see Figure 1). Furthermore, any trial to clone these sequences has failed (2), presumably due to some peculiarity in the DNA structure (18). Some possible analogies with telomeric sequences from other protozoa could be pointed out: in the macronucleus of Hypotrichida (ciliates), the genome is fragmented into minichromosomes with a size of single genes (19, 20). The mechanism of this chromosome fragmentation and amplification involves DNA end extension, the oligonucleotides CCCCCAA (21, 22) or CCCCCAAAA (23, 24) being indeed repeated at both ends of each minichromosome to generate telomeric structures. It is conceivable that these structures as well as the barren DNA region in trypanosome telomeres belong to a class of essential elements of all chromosome ends (11).

The interpretation of the telomere length variations reported herein is still conjectural; it could depend on the succession of sequence unpairing and foldback pairing within telomeric repeated units after sub-terminal cutting during replication (11, 25). The terminal extension rate seems identical (about 7 bp per assumed generation time) for all the studied telomeres which are not involved in gene expression, namely the telomeres carrying the AnTat 1.3, 1.6, 1.16 and 1.18 VSA genes (this study) and the one carrying the 221 gene (11). This growth rate is slightly lower than that observed for the active VSA gene: 10 bp per division in case of the 118 gene (11), and 9 bp per division for the AnTat 1.3 ELC (this study). Moreover, in our AnTAR 1 repertoire, the active VSA genes have always been found in size heterogeneous telomeric populations, in contrast with all the transcriptionally inactive telomeres. This particular feature has also been observed for the 118 ELC-containing telomere (11). In this case, the terminal segment flanking the ELC was found increasingly heterogeneous as the trypanosomes multiply,
an instability which was supposed to be related to gene expression (11). We show here that this instability is typical of the active telomeres; indeed, in the telomeres carrying the silent genes, progressive length shortening and increasing size heterogeneity have not been observed. In these inactive telomeres, breaks in the regular increase occur suddenly and, often but not always, in connection with the recombinational event which leads to its activation and the expression of the gene it carries. In this respect, the case of the telomere carrying the "9 kb" AnTat 1.1 gene family member in the AnTat 1.1 clone (2) is worth mentioning: this clone exhibits two distinct variants of the same telomere, one of which has been shortened by about 5 kb (2, and see fig. 2). We hypothesize that a deletion has taken place in the "9 kb"-containing telomere early in the history of the AnTat 1.1 clone, yielding two cell populations differing only in this respect.

There are thus clear differences between "active" and "inactive" telomeres, with regard to both their growth rate and the way size reduction is operated. The "active" telomere exhibits several other particular features: being the only one to express a VSA gene, its chromatin is the only one to be in an active conformation, highly sensitive to DNAaseI (1-4, 6). Moreover, it also appears the only one to act as a target for gene conversion (2, 4): for instance, we have described two variants where the very same sequence, the "9 kb" AnTat 1.1 gene family member, is either a receptor or a donor in the gene conversion process, depending on whether it is transcribed or not (AnTat 1.10 or AnTat 1.1C clones, respectively: refs. 2, 4). This is reminiscent of an observation made for the mating type interconversion mechanism in yeast: when the silent HML and HMR loci are allowed to express, they become effectively converted to the opposite mating type, as it occurs normally only at the "active" MAT locus (26). It is clear therefore that a sequence can be a target for gene conversion only when it is in active chromatin. Similarly, one could conceive that the DNA rearrangements involved in the terminal size variations, as the deletions leading to telomere shrinking, could be influenced by the accessibility of DNA in chromatin.

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