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Translocation alters the activation rate of a trypanosome surface antigen gene

M.Laurent+, E.Pays, A.Van der Werf, D.Aerts*, E.Magnus*, N.Van Meirvenne* and M.Steinert

Département de Biologie Moléculaire, Université libre de Bruxelles, B-1640 Rhode St Genèse, and *Laboratorium voor Serologie, Instituut voor tropische Geneeskunde, Antwerp, Belgium

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SUMMARY

We report here the characterization of the gene coding for AnTat 1.13, a very late variable antigen type (VAT) from Trypanosoma b. brucei. This gene is chromosome-internal and it is activated by the duplicative mechanism. Like in another case of late VAT expression (1), its expression-linked copy (ELC) is flanked by "companion" sequences. It was possible to convert the late expression of this VAT into an early one, by changing the location of the gene in the genome. This has been achieved by selecting an AnTat 1.6 clone among heterotypes arising in the AnTat 1.13 cloned population. Indeed, this particular derivation leads to the conservation of the AnTat 1.13 ELC as a new telomeric member of the gene family, and this conserved ELC (or ex-ELC) appears to be preferentially activable. The telomeric position and other factors possibly involved in early or late antigen gene expression are discussed; in this respect, we propose that some antigen genes are rarely activated because their duplicative transposition requires the presence, in the expression site, of "companion" sequences only shared by a limited number of other genes.

INTRODUCTION

Antigenic variation allows african trypanosomes to escape the immune defence of their host. The different VATs of a repertoire are genetically determined and the corresponding genes are expressed, one at a time, in a semi-ordered sequence. Indeed, in a given trypanosome stock, "predominant" VATs are always observed early in chronic infections and others only late (2-8).

In an attempt to correlate the relative frequency of switching to given VATs with features of their corresponding genes, we decided to analyse and compare the genes coding for predominant and late VATs from the same Trypanosoma b. brucei repertoire. The results obtained on the gene of the early VAT AnTat 1.3 have already been presented (9).

So far the AnTat 1.13 antigenic type was consistently found to be expressed very late (5,8). We report here the characterization of the AnTat 1.13

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gene, showing that it is in a chromosome-internal position and that it is activated by the duplicative mechanism (10,11) involving gene conversion (12). From previous work (13,14) we predicted that the ELC of the AnTat 1.13 gene basic copy (BC) should be conserved in an inactive form (ex-ELC) upon switching from AnTat 1.13 to AnTat 1.6: this ex-ELC was indeed observed in the new clone AnTat 1.6B derived from AnTat 1.13. Interestingly, AnTat 1.13 has become predominant in this clone and its expression is now achieved by duplicative transposition of the AnTat 1.13 ex-ELC, which is telomeric. It is proposed that a telomeric environment is essential for a surface antigen gene to be expressed predominantly; other possible requirements are discussed.

A first account of these results has already been presented (15). The serological and parasitological features of these variants will be presented in detail elsewhere (N. Van Meirvenne et al., in preparation).

MATERIAL AND METHODS

Trypanosomes.

All the antigenic variants are from the Trypanosoma b. brucei stock EATRO 1125. They have been cloned at the Institute of Tropical Medicine, Antwerp, after immunological selection (5) from heterotypes arising in the previous clone. They were grown in mice and rats.

cDNA cloning.

Cloning of the AnTat 1.13 cDNA has been conducted as previously described (14).

Isolation and blotting of DNA.

The DNA of cloned populations was isolated (11) and cleaved with restriction endonucleases according to the manufacturers instructions. The restriction fragments were then fractionated by gel electrophoresis through 0.6 or 0.85% agarose gels, blotted onto cellulose nitrate filters and hybridized with the cDNA probes.

Sensitivity to DNAaseI.

Trypanosome nuclei, prepared as described previously (16) were digested by DNAaseI for 1, 2, 4, 6, 8, 10, 15 and 20 min. The DNA was then extracted, digested with appropriate endonucleases, transferred onto cellulose nitrate filters and hybridized with cDNA probes. The relative labelling intensity of the restriction fragments was estimated from recordings with a Joyce-Loebl microdensitometer.

Probes.

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

RESULTS

The AnTat 1.13 gene is activated duplicatively.

The analysis of the gene encoding the AnTat 1.13 antigen was conducted by hybridization of the AnTat 1.13 genomic DNA with a specific probe prepared from an AnTat 1.13 cDNA clone. The restriction map of this cloned cDNA is shown in Fig. 1. The genomic DNAs hybridized with this probe were isolated from AnTat 1.6, 1.13, 1.6B and 1.13B cloned populations of T. b. brucei.

The results show that the AnTat 1.13 surface antigen gene was activated by duplicative transposition. Indeed, the 0.22 kb MspI-MspI and 0.48 kb HindIII-HindIII fragments (Fig. 2, digests M, H), which are internal fragments of the AnTat 1.13 gene (see map Fig. 1), hybridize in the AnTat 1.13 trypanosome clone (second channel) twice as intensely as the corresponding fragment in the non-expressor AnTat 1.6 clone (first channel). Moreover, in AnTat 1.13 genomic DNA, the probe reveals additionnal restriction fragments specific to the AnTat 1.13 ELC and its environment (1.7 kb ClaI-SphI, 19 kb HindIII-HindIII, 0.7 kb MspI-MspI, 23 kb ClaI-ClaI, see Fig. 2, digests C + Sp, H, M, C).

The AnTat 1.13 BC is chromosome-internal.

Fig. 1 shows the restriction maps of the AnTat 1.13 BC and ELC in their respective environments. The AnTat 1.13 BC is not enclosed by conspicuous barren regions. In addition, as shown in Fig. 2, in neither expressor nor non-expressor variants were the restriction fragments containing the AnTat 1.13 BC of the variable size characteristic of telomeric fragments (17,18). It is concluded that the AnTat 1.13 BC is most probably located within a chromosome.

The AnTat 1.13 ELC is flanked by "companion" sequences.

The results also indicate that the AnTat 1.13 ELC is flanked by sequences copied from regions not contiguous to the BC: indeed, the existence of the 1.7 kb ClaI-SphI and 0.7 kb MspI-MspI fragments in AnTat 1.13 DNA only (Fig. 2, digests C+Sp,M, second channel) allows to map a SphI and a MspI sites respectively in the 5' and 3' vicinity of the ELC, but not of the BC (Fig. 1). These sites belong to "companion" sequences (1, see also ref. 19 for a similar case) probably transposed independently of the AnTat 1.13 ELC.

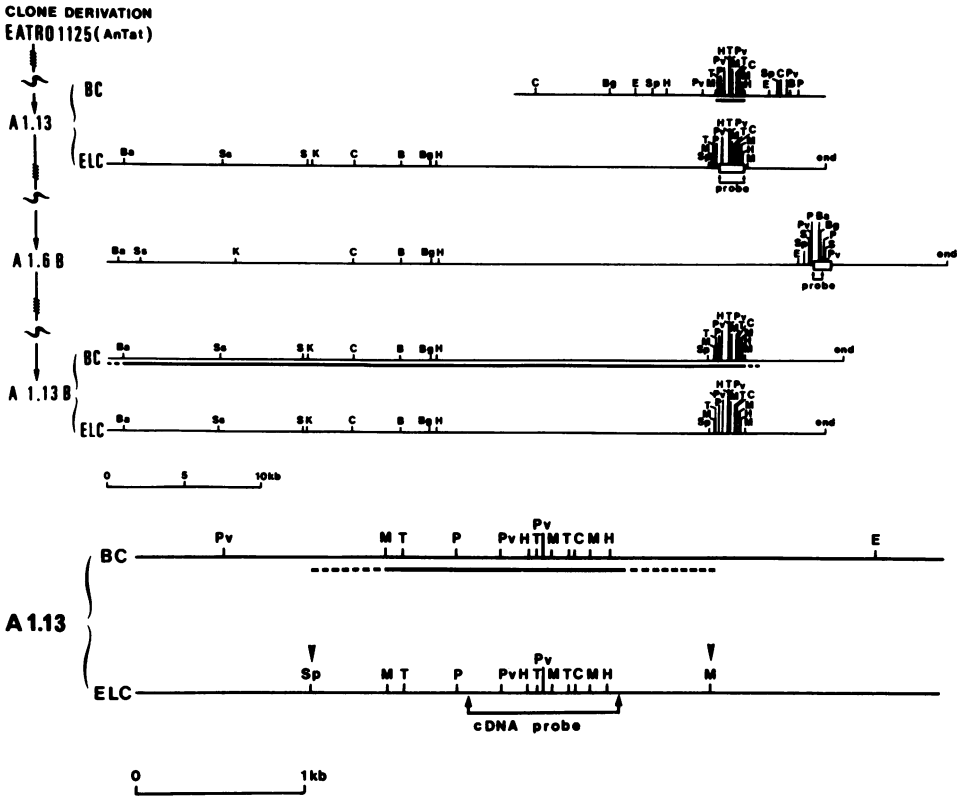

Figure 1. Restriction maps of the AnTat 1.13, AnTat 1.13B BCs and ELCs, and of the AnTat 1.6 gene. The bars under the BC maps show the known extent of the transposed sequences, dashed parts representing uncertainties at both ends. The sequences corresponding to cDNAs are indicated by boxes. Arrows point to the fragments used as probes. The pedigree of the trypanosome clones is shown schematically on the left (A is for AnTat, ~~~ and ʃ symbolise antigenic variation and cell cloning, respectively). Detailed maps of AnTat 1.13 BC and ELC are given below. Sites designated by arrowheads belong to "companion" sequences (see text).
Abbreviations: B, BglI; Ba, BamHI; Bg, BglII; C, ClaI; E, EcoRI; H, HindIII; K, KpnI; M, MspI; P, PstI; Pv, PvuII; S, SalI; Ss, SstI; T, TaqI.

Generation of a silent telomeric copy (ex-ELC) of the AnTat 1.13 gene.

Comparing the genes coding for the early VATs AnTat 1.3 (9) and AnTat 1.6 (14) with those for the late VATs AnTat 1.1 (12,1) and AnTat 1.13 (this study), it appears that the only molecular character that could be correlated to predominance is the localisation of the gene on an expression site-like telomere. The best way to test this correlation is to transpose a gene encoding a late VAT to such an environment and to examine if it then becomes pre-

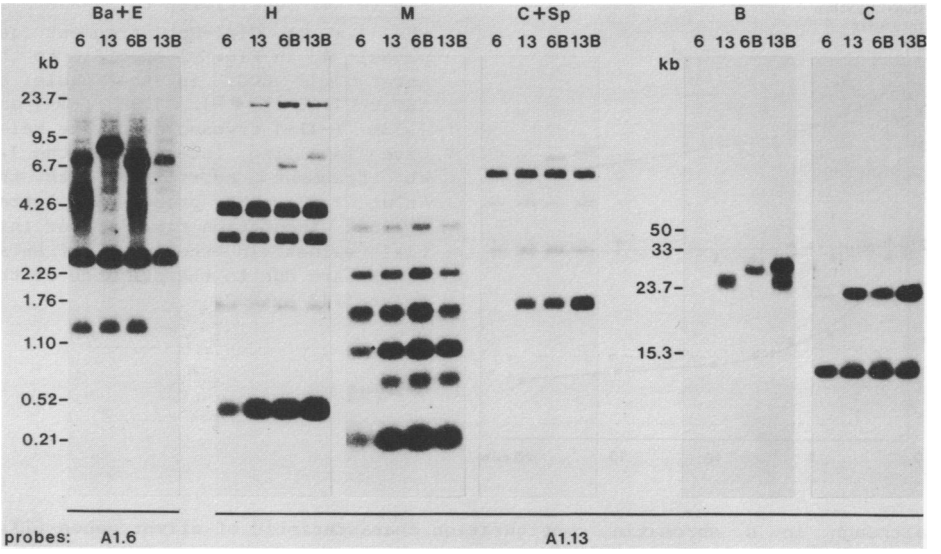

Figure 2. Analysis of the AnTat 1.13 gene. The DNAs of cloned populations expressing AnTat 1.6 (see pedigree in ref. 13), 1.13, 1.6B and 1.13B were cleaved with the restriction endonucleases indicated above each pannel (see Fig. 1 for abbreviations) and hybridized with (^{32}P)-nick translated AnTat 1.6 cDNA (first pannel) or AnTat 1.13 cDNA (next five pannels).

dominant. This could be done by an appropriate clone derivation (Fig. 3). It is known that the activation of a surface antigen gene by the non-duplicative mechanism leads to the conservation of the previously expressed ELC,

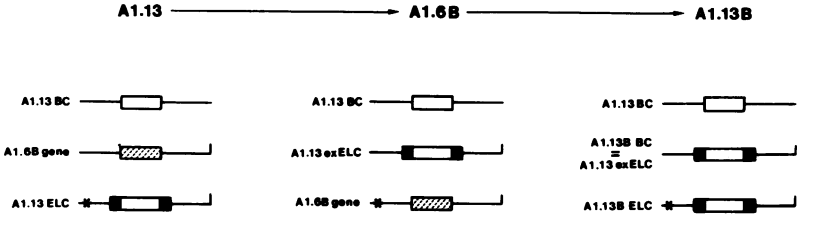

Figure 3. Scheme of the successive antigen gene rearrangements involved in the switching from AnTat 1.13 to 1.13B. The "active" telomere is in each case marked with an asterisk (*). In the first variant, the AnTat 1.13 gene is activated by duplicative transposition: the non-telomeric gene is copied into an ELC which is apparently transposed in the expression site (*) between two "companion" sequences (black boxes). In the ensuing variant, the telomeric AnTat 1.6 gene has been activated (*) without duplication; this leads to the conservation of the AnTat 1.13 ELC in an inactive form (ex-ELC). In the third clone, this ex-ELC is used as BC for the generation of a new, AnTat 1.13B ELC (*), which is at least 50 kb long. This new gene is expressed predominantly, contrary to the original non-telomeric AnTat 1.13 BC.

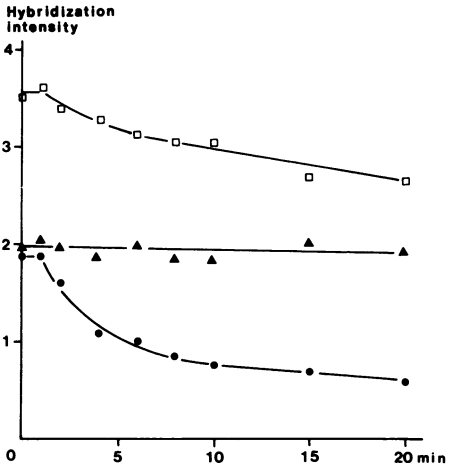

Figure 4. Sensitivity to DNAase I of the 1.6 kb ClaI-SphI fragment (see pannel 4 in Fig. 2) specific to the AnTat 1.13 ELC, in the nuclei of AnTat 1.13 (-●-), 1.6B (-▲-) and 1.13B (-□-) trypanosomes. The relative labelling intensity of the 1.6 kb fragment hybridized with the AnTat 1.13 cDNA probe was plotted against incubation time. Higher initial values in the case of AnTat 1.13B are due to the presence of the AnTat 1.13 ex-ELC.

although in a chromatin configuration characteristic of silent genes (13, 14). Since the AnTat 1.6 gene seems always activated non duplicatively (14, 20), we derived an AnTat 1.6 expressor clone, namely AnTat 1.6B, from the AnTat 1.13 clone. As expected, this led to the conservation of the AnTat 1.13 ELC in an "inactive" chromatin configuration, as shown by its relative resistance to DNAase I (Fig. 4). There are thus at least two potential AnTat 1.13 genes in AnTat 1.6B genomic DNA: the novel telomeric AnTat 1.13 "ex-ELC" and the non telomeric AnTat 1.13 BC.

The AnTat 1.13 ex-ELC can be predominantly re-expressed, by duplicative transposition.

Before this study, AnTat 1.13 had never been observed in the first relapses of an infection by trypanosomes belonging to the same EATRO 1125 stock (5,8). Originally it has been detected in a rabit after 46 days of infection (5). This VAT thus clearly belongs to the "late" class in the AnTAR 1 repertoire. Remarkably however, after switching to AnTat 1.6B, it shows up frequently among the predominant heterotypes arising in the AnTat 1.6B cloned population (Van Meirvenne et al., in preparation).

We cloned one of these predominant AnTat 1.6B-derived AnTat 1.13, designated AnTat 1.13B, and we analysed its DNA. We found that in this clone the AnTat 1.13 ex-ELC had been used as template for the generation of a new ELC: indeed, characteristic restriction fragments of the ex-ELC are specifically duplicated in AnTat 1.13B DNA (see Fig. 2, 1.7 kb ClaI-SphI and 0.7 kb MspI-MspI fragments, digests C + Sp, M, fourth channel). This new AnTat 1.13B ELC seems to have replaced the previously expressed AnTat 1.6B gene, since the

latter is no longer present in AnTat 1.13B DNA (Fig. 2, digest Ba + E, fourth channel). In addition, it appears that the AnTat 1.13B ELC is very long, since only digestions generating fragments extending to the 3' end of the telomere allow to distinguish the AnTat 1.13B ELC from its template, the AnTat 1.13 ex-ELC (Fig. 2, digest B, fourth channel): as observed previously in another case of ex-ELC reactivation (AnTat 1.3B, ref.13), the extent of gene conversion probably exceeds 50 kb (Fig. 1).

Since another AnTat 1.6 expressor clone, derived from AnTat 1.3, is known to switch preferentially and at a very high rate back to AnTat 1.3, we contemplated the possibility that the predominance of AnTat 1.13B could be due to some unexplained tendency of AnTat 1.6 expressors to revert to the parental VAT. We therefore cloned variant AnTat 1.5B, one of the heterotypes derived from AnTat 1.6B, and asked if AnTat 1.13 would still appear predominant in an infection by this new variant. The answer was clearly positive (N. Van Meirvenne et al., in preparation), strongly suggesting that the newly appeared "predominance" of AnTat 1.13 is not directly linked to the expression of the AnTat 1.6 gene, but is a genuine property of the novel telomeric AnTat 1.13 gene copy.

DISCUSSION

We have shown firstly that the gene which specifies AnTat 1.13, a very late VAT, is chromosome-internal and that it is activable by the duplicative transposition mechanism. We have also demonstrated that the ELC thus formed is retained, in a silent form, if an AnTat 1.13 trypanosome switches to AnTat 1.6, a serotype expressed non-duplicatively, and that this ex-ELC, acting as a novel and telomeric member of the gene family, can be preferentially re-expressed.

That an ex-ELC is preferentially re-expressed is not new, it has indeed been found to occur in the same (13,14) as well as in another (23) *T. b. brucei* stock. Both the AnTat 1.13 ex-ELC (this study) and the previously described AnTat 1.3 ex-ELC (13) are re-expressed by duplicative transposition, another telomere (the last expression site) being converted by the ex-ELC. However, non duplicative, apparently direct reactivation of the telomere which bears the lingering ELC has been described by others (23) and also found to occur in our own stock (AnTat 1.1G, unpublished observations).

The most striking observation reported here is that in trypanosomes which harbour the novel telomeric gene, AnTat 1.13 has become a predominant VAT, suggesting that a telomeric position is essential for predominance, in agree-

ment with other predominant VATs being also encoded by telomeric genes (13, 14,24,25). The telomeric location of a surface antigen gene could enhance its ability to recombine with an expression site. However in this respect all telomeres are not equally efficient. In the AnTat 1.3B clone for instance two identical AnTat 1.3 antigen-specific sequences, one of which is an ex-ELC, are carried by similar telomeres, yet the activation of the ex-ELC seems to be preferred to the activation of the other telomeric gene (13). A telomeric position in itself is thus not the sole factor that determines precedence in the activation of surface antigen genes, and ex-ELCs seem to be particularly marked for high predominance.

Predominance in VAT expression is not linked to the way the corresponding gene is activated, since both "late" and "early" expression of AnTat 1.13 were effected by duplicative transposition. Moreover, although both are predominant, AnTat 1.3 and 1.6 are activated by duplicative transposition (9) and by the alternative mechanism respectively (14).

The preferential expression of an ex-ELC over the BC is not apparently related to any difference in DNA modification, since the extent of modification, at least at some sites, appears identical in both copies (26). Differences in chromatin structure have also been tested for by DNAase I sensitivity and again the BC and ex-ELC do not appear to differ significantly (13).

A comprehensive explanation for the preferential activation of ex-ELCs should take into account that it occurs in both ways, duplicatively and non duplicatively. One may speculate that the ex-ELC retains a memory which characterizes the active telomere such as a preferentially reactivable transcription promoter. However the nature and location of transcription promoters in trypanosomes is still a matter of conjecture. In addition, it is not clear how a transcription promoter could be responsible for the preferential duplicative transposition of some sequences.

The ease with which a surface antigen gene is translocated and thus activated may also depend on the extent of sequence homologies shared by the environment of the gene and the expression site. Such homologies are usually provided by the 70 bp repeats found upstream of both the BC (27) and the ELC (28) and by a common sequence present at the 3' border of each variable antigen gene (29). The existence of these particular sequences in the AnTat 1.13 genes is presently being investigated, but one may speculate that the low probability for the chromosomal-internal AnTat 1.13 BC to be activated could be due to their partial or total absence.

It seems difficult to explain in similar terms the preference of an ex-ELC over another telomeric gene (as in the AnTat 1.3B case mentioned above), since both are amply provided with 5' repeats. Interestingly, the large gene conversions characteristic of ex-ELC reactivation by the duplicative process (13, this study) indicate that the recombination was most probably not initiated in the 5' barren region adjacent to the gene, but far upstream from this region. This would further seem to suggest that a sequence far upstream from the gene could be preferred for promoting conversion.

We also demonstrated that the AnTat 1.13B gene (the ex-ELC) is framed into "companion" sequences which are absent from the original BC. The fact that the gene conversion involved in the AnTat 1.6B → AnTat 1.13B switch starts at such a considerable distance upstream of the "companion" sequences makes it unlikely that the latter could in some way confer predominance to the gene. On the contrary, companion sequences could in some way be linked to the expression of late VATs. As reported in the case of the AnTat 1.1 ELC (1), a "companion" sequence is most probably the remains of a previous ELC that failed to be entirely erased by the incoming gene, following partial or segmental conversion (12). Interestingly, both AnTat 1.1 and 1.13 appear late in chronic infections, suggesting that the genes for such late VATs might perhaps require segmental conversion to be inserted in the expression site. One can for instance imagine that these genes, which would lack the 5' homology block usually involved in the transposition of ELCs, could be transposed into the expression site only if the latter contains a particular gene or sequence which provides an alternative recombination site. Such a precise prerequisite, which is presently being investigated, would obviously restrict the probability for such a gene to be activated.

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+To whom reprint requests should be sent

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