

The molecular karyotype of the megabase chromosomes of *Trypanosoma brucei* stock 427

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Accepted 1 August 2000

Abstract

We present the molecular karyotype of the megabase chromosomes of *Trypanosoma brucei* stock 427, clone 221a. This cloned stock is most commonly used in research laboratories in genetic manipulation experiments and in studies of antigenic variation. Using 116 previously characterised chromosome-specific markers, we identify 11 diploid pairs of megabase chromosomes and detect no loss of synteny in EST and gene marker distribution between this stock and the genome project reference stock TREU 927/4. Nevertheless, the chromosomes of 427 are all larger than their homologues in 927, except chromosomes IIa and IXa. The greatest size variation is seen in chromosome I, the smallest of which is 1.1 Mb (927-Ia) and the largest 3.6 Mb (427-Ib). The total nuclear DNA content of both stocks has been estimated by comparison of the mobility of *T. brucei* and yeast chromosomes. Trypanosomes of stock 427 contain approximately 16.5 Mb more megabase chromosomal DNA than those of stock 927. We have detected the presence of bloodstream-form expression-site-associated sequences on eight or more megabase chromosomes. These sequences are not found on the same chromosomes in each stock. We have determined the chromosomal band location of nine characterised variant surface glycoprotein genes, including the currently expressed *VSG* 221. Our results demonstrate both the stability of the *T. brucei* genome, as illustrated by the conservation of syntenic groups of genes in the two stocks, and the polymorphic nature of the genomic regions involved in antigenic variation. We propose that the chromosomes of stock 427 be numbered to correspond to their homologues in the genome project reference stock TREU 927/4. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: *Trypanosoma brucei*; Karyotype; Megabase chromosomes; Variant surface glycoprotein genes; Expression sites

Abbreviations: VSG, variant surface glycoprotein; *VSG*, variant surface glycoprotein gene; PFGE, pulsed field gel electrophoresis; kb, kilobase pairs; Mb, million base pairs; EST, expressed sequence tag; EtBr, ethidium bromide; B-ES, bloodstream-form *VSG* expression site; TREU, Trypanosome Research Edinburgh University; STIB, Swiss Tropical Institute Basel.

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1. Introduction

Trypanosoma brucei contains three size classes of chromosomes, which can be resolved by pulsed field gel electrophoresis (PFGE). Minichromosomes (25–100 kb) contain silent ‘basic copies’ of variant surface glycoprotein genes (*VSGs*) and simple repeat sequences [1]. Intermediate chromosomes (100–800 kb) may carry *VSG* expression sites [2,3], but it is not known if there are genes outside the expression sites of these chromosomes. The megabase chromosomes contain all of the ‘housekeeping’ genes that have currently been mapped [[4], and unpublished data] and both bloodstream-form and metacyclic telomeric *VSG* expression sites (reviewed in [5–7]). Some also carry large arrays of *VSG* basic copies, which are not transcribed until they are transposed into an expression site [8,9]. These arrays may contain haploid genes, indicating that these sections of otherwise homologous chromosomes are probably aneuploid and polymorphic [9].

The karyotypes of the megabase chromosomes of several cloned stocks of *T. brucei* have been characterised previously [4,10]. Homologous chromosomes may vary in size both within and between stocks and comigrating chromosomes are not necessarily homologues. A nomenclature has been proposed whereby chromosomes are numbered to correspond to their homologues in the genome project reference stock, TREU927/4, which are numbered in increasing size [4,11]. However, chromosomes are not necessarily in the same size order in other stocks. The size variation greatly exceeds that reported in other protozoan parasites, in which size differences of the scale described here may be observed between, but only rarely within, species (e.g. *Leishmania* [12], *Plasmodium* [13], *Trypanosoma cruzi* [14]). Comparative mapping of size-polymorphic *T. brucei* chromosomes has shown that size variation affects all regions of the chromosome [15], in contrast to similar studies in *Leishmania* [16] and *Plasmodium* [17]. In *T. brucei*, all gene probes remain in identical syntenic groups across a range of stocks, but regions associated with antigenic variation are highly polymorphic and contribute considerably to chromosome plasticity [2,4,15].

The origin of ‘strain 427’ remains unclear and may never be resolved (Cross, G.A.M. et al., unpublished information). However, it almost certainly is not derived from S427 [18], as previously implied [19]. The confusion and misnaming of ‘strain 427’ has been noted previously [20], but without resolving its true origins. This virulent rodent-adapted and generally monomorphic stock does not differentiate into procyclic forms as efficiently as some pleomorphic stocks. However, reports of transmission through tsetse flies of several individual laboratory stocks of 427 have been communicated to GAMC (F.R. Opperdoes, 1975; J.J. Doyle 1983; W. Gibson, 1994, personal communications). Midgut infection rates of 13–56% and salivary-gland infection rates of 2.3–6.4% of dissected tsetse were recorded by S.K. Moloo (personal communication, 1994).

Stock 427 is particularly important because it has been widely used for laboratory studies of antigenic variation and trypanosome biochemistry and cell biology. It was the first strain of *T. brucei* to be grown as procyclic forms in semi-defined culture medium [19], it was the first strain to be grown as bloodstream forms in culture, initially with feeder cells [21] and subsequently under axenic conditions [22]. The property of ‘strain 427’ that is perhaps most relevant in the present context is that it has so far proved to be the most facile strain in which to perform genetic manipulations [23–27].

We present the karyotype of the megabase chromosomes of *T. brucei* stock 427 clone 221a, including the chromosomal locations of bloodstream-form expression site-associated sequences and some characterised *VSGs*, and compare it with that of the genome project reference stock TREU927/4. Stocks of 427 exist in laboratories throughout the world, and the data presented here will help researchers to compare their stocks with the carefully characterised 427–221a.

2. Materials and methods

2.1. Parasites

Based on contemporaneous information, stock

427 was previously described [19] as originating from isolate S427 [18]. Recent investigations and enquiries (G.A.M. Cross, unpublished information) suggest that it corresponds to the cloned stock designated Lister 427, although the origin of Lister 427 is also obscure. The derivation of clone 221a has been described previously [28]. It was isolated as the predominant, fastest growing variant in the first cultures of bloodstream-form trypanosomes [21]. Bloodstream-form trypanosomes of stock 427 clone 221a were cultured in HMI-9 [22], prior to preparing chromosomal DNA agarose blocks.

Cloned bloodstream-form stocks of TREU (Trypanosomiasis Research Edinburgh University) 927/4 (GPAL/KE/70/EATRO 1534) were transformed to procyclic stocks by growth in SDM-79 [29] supplemented with 10% foetal calf serum (FCS) and 3 mM cis-aconitate, 1 mM pyruvate at 28°C. Log phase procyclic parasites were frozen in liquid nitrogen with 20% glycerol. The DNA of this stock and its single VAT derivative TREU927/4GUTat 10.1, is the sequencing substrate for the African trypanosome genome project [30].

2.2. Pulsed field gel electrophoresis

Chromosomal-DNA agarose blocks of the 427 strain, antigenic variant 221a, were prepared as described previously for mammalian cells [31] at a final cell concentration of $2 \times 10^8 \text{ ml}^{-1}$. In brief, bloodstream-form parasites were resuspended in LB (0.1M EDTA pH8, 0.01 M Tris-HCl, 0.02 M NaCl) and mixed with 1 vol. of 1.6% low melting point agarose (Sigma) in LB. Lysis was carried out twice in LB plus 1% lauroyl sarcosine and 1 mg ml^{-1} proteinase K for 48h at 50°C. 927/4 chromosome blocks were prepared by embedding $2 \times 10^8 \text{ ml}^{-1}$ washed procyclic parasites in 1.4% low melt agarose (SeaPlaque GTG, FMC Bio-products) and lysing them in situ in NDS buffer pH 9 (0.5M EDTA, 0.01M Tris, 1% lauroyl sarcosine), followed by incubation with 2 mg ml^{-1} proteinase K at 55°C for 48 h. Blocks were stored in NDS pH 8 at 4°C until required and dialysed in 10 mM Tris-HCl, 1 mM EDTA before use.

Pulsed field gel electrophoresis (PFGE) was performed in a contour clamped homogeneous electric field apparatus (CHEF DRII, BioRad). Agarose gels were prepared in 0.089 M Tris-borate pH 8, 0.2 mM EDTA ($1 \times \text{TB}(0.1)\text{E}$) or 0.045 M Tris-borate pH 8, 1 mM EDTA ($0.5 \times \text{TBE}$), and were electrophoresed with buffer recirculation at constant temperature. *S. cerevisiae* (New England Biolabs), *S. pombe* (BioRad) and/or *H. wingei* (BioRad) chromosomes were electrophoresed concurrently. Precise conditions are given in the legends of figures illustrating each separation. Chromosome bands were visualised by staining with 0.5 mg ml^{-1} ethidium bromide (EtBr) and photographed on a UV transilluminator; photographs of actual size were produced to allow correct determination of chromosome position within a band. Gels were blotted onto Hybond-N membranes (Amersham) by standard procedures.

2.3. DNA probes

Expressed sequence tags (ESTs) derived from a cloned population of the bloodstream-form *T.b.rhodesiense* stock WRATat 1.24 (LVH/75/USAMRU-L/18) were provided as cDNA fragments amplified by the polymerase chain reaction by N. El-Sayed and J. Donelson [32]. All partially sequenced cDNAs (ESTs) are identified by a T or OT number originating in the sequencing laboratory and this number can be used to search for the EST in dbEST. A few cloned gene probes were also used: phospholipase C (PLC) [33]; actin [34]; glycosomal glyceraldehyde phosphate dehydrogenase (gGAPDH) [35]; GDH, glycerol phosphate dehydrogenase [36]; 5 s ribosomal RNA gene (5srRNA) [37]. The DNA probes which identify bloodstream-form VSG expression sites (B-ES) are: B-ES promoter region 315-bp *HpaI-SalI* fragment, position 1359–1673 in [38], isolated from plasmid pRK8(+); 50-bp repeats *XbaI-ScaI* fragment subcloned from ES2X in [39] by G. Rudenko.

Four DNA probes derived from variant surface antigen genes were prepared by the polymerase chain reaction using synthetic oligonucleotides:

VSG60: 5'GTAACACTTCCAGCGACGACA3' and 5'GCTCTCGAGCCCACCGTTTGT3';
VSGT3: 5'CGGATTCTCTACTAACTCAGT3' and 5'TTTATCGTTTCAATCGCTTTG3';
VSGBR2: 5'CAACATAGGAACTGGCGACA-A3' and 5'GGCCCCGCGAGCTTGTATTCTT3';
VSG222: 5'CGCAAATCGAAAATCTTATGT3' and 5'CCGCCGGTGTAGTCCCTGAAC3'. To generate other *VSG*-specific probes, the 5' regions of cloned *VSGs* were released by restriction enzyme digestion and gel-purified.

2.4. Hybridisation of cDNA and *VSG* gene probes to southern blots of PFGs

Hybridization of cDNA and gene probes to Southern blots was carried out by one of the following methods:

1. the ECL™ direct nucleic acid labelling and detection system manufactured by Amersham International plc, used according to manufacturer's instructions except that the primary wash buffer was prepared with $0.1 \times$ SSC and the incubation period for hybridisation was 2 h. Signal was detected by exposure of the processed blot to KODAK XAR-5 film for between 30 s and 1 h. Blots were re-probed after allowing the signal to decay overnight.
2. probes prepared using $\alpha^{32}\text{P}$ -dCTP and the PRIME-IT II labelling kit from Stratagene, hybridised to chromosomal DNA overnight in 0.5M (Na)PO₄ pH 7.5, 7% SDS, and washed to a stringency of $0.1 \times$ SSC, 0.1% SDS at 65°C prior to exposure to XAR-5 film.
3. *VSG* probes were prepared and hybridised as (2) but the membranes were washed to a stringency of $1 \times$ SSC, 0.1% SDS at 65°C.

3. Results and discussion

We have previously identified 11 pairs of diploid megabase chromosomes in cloned stocks of *T. brucei* and assigned cDNA markers to all [4]. We now present the characterisation of the homologous chromosomes in *T. brucei* strain 427 clone 221a using 116 of those markers, and compare the two genomes in terms of size, synteny

and the location of sequences involved in antigenic variation.

3.1. The molecular karyotype of *T. brucei* strain 427–221a

Each of the 11 diploid megabase chromosome pairs identified in *T. brucei* is defined by at least 10 chromosome-specific markers [4,11]. Hybridisation of 116 markers to the PFGE-separated chromosomal bands of 427 has identified 11 consistent hybridisation patterns. Therefore, 11 diploid pairs of megabase chromosomes have been identified in *T. brucei* strain 427–221a, ranging in size from 1 Mb to ca. 6 Mb. Fig. 1 shows separation of the chromosomal DNA by PFGE using four different sets of separation parameters. Fig. 1A shows the separation of the eight smallest chromosomes in eight separate PFG bands (IVa and Vb are clearly distinguished by overlaying autoradiographs). In Fig. 1B, four of these chromosomes are compressed in two PFG bands, but three larger chromosomes are completely separated. Fig. 1C shows the resolution of the largest chromosomes. In 427–221a, the three largest remain compressed in one band. The PFGE parameters used to produce the gels in Fig. 1A, B and C were used previously for the separation of the chromosomes of stocks TREU927/4, STIB247, STIB386 and four cloned hybrids [4], and this facilitates a direct comparison to our previous work. We performed another separation not shown here (4500–3600 s, 1.2 V cm^{-1} , 0.8% agarose, in $0.5 \times$ TBE, at 16°C for 192 h) that further separated the largest chromosomes of strain 427–221a. This revealed that they were somewhat larger than the largest *S. pombe* chromosome of 5.7 Mb, but failed to resolve chromosomes Xb, XIa and XIb from each other. Fig. 1D shows a PFG of chromosomal bands separated using a novel set of PFGE parameters, aimed at resolving the two compressions containing VIb, VIIa, VIIb and VIIIa (band 10 in Fig. 1B and band 2 in Fig. 1C) and Ib and VIIIb (band 3 in Fig. 1C). Although the separation is not clear on the EtBr-stained gel, it is possible to distinguish a band containing VIb and VIIIa from another

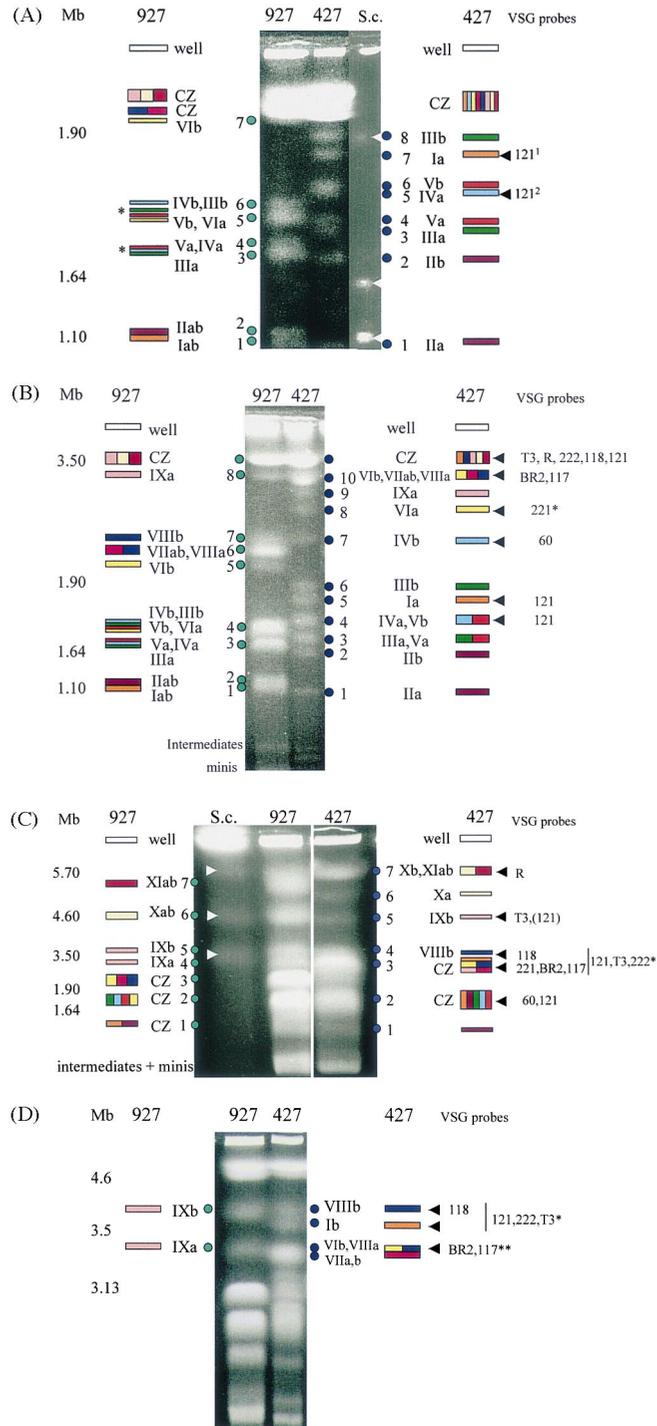


Fig. 1.

containing VII (presumably two homologues a and b) by overlaying autoradiographs following hybridisation with chromosome-specific markers. Therefore it is likely that further adjustment could separate these chromosomes more completely. The upper compression containing Ib and VIIIb has resolved into two bands, each containing one chromosome. In total, we have succeeded in completely resolving 15 individual homologues with only two compressions remaining (VIb/VIIab/VI-IIa and Xb/XIab) and have accounted for the chromosome content of all PFG bands shown (Table 1). In some gels, the strength of the EtBr fluorescence of the top band of 427 (Xb, XIab) is greater than expected, but EtBr staining is an unreliable method of determining DNA content in PFG gels and this result is not consistent between gels (data not shown). Nevertheless we must consider the possibility that a twelfth chromosome exists. We have no other data that suggest there are undetected megabase chromosomes in strain 427–221a.

The karyotypes of *T. brucei* strains 427–221a and 427–60 have been partially resolved previously [10,40]. Gottesdiener et al. identified seven chromosome pairs. They labelled each PFG band

and this nomenclature system has been used by other researchers working with stock 427, e.g. [39,41]. However, this system is not applicable to separations of chromosomal DNA using different PFGE parameters nor for cross-comparison between stocks. Therefore it is important that the chromosomes of stock 427 are also defined by the assignment of chromosome markers and comparison with the genome project reference stock 927.

Table 1 gives an estimate of the size of each megabase chromosome. The sizes shown are consistent with those used to calculate the DNA content of 927, STIB247 and STIB386 megabase chromosomes [4]. However, the estimates vary from those shown in several other papers where 427 chromosomes were separated by PFGE. The size estimates of chromosomes 427-I–V (excluding Ib) are lower in reference [10] than shown in Table 1. However, the estimated sizes of chromosomes 427-IVa (expression site containing the 121 *VSG*) and VIa (expression site containing the 221 *VSG*) in reference [42] are 2.2 and 3.2 Mb respectively. In both cases these estimates are higher than shown in Table 1 (1.78 and 2.8 Mb respectively). However, size estimates obtained by comparing the relative mobility of DNA standards

Fig. 1. The 11 diploid megabase chromosomes of *T. brucei* stocks 927 and 427. (A) The central panel shows the EtBr-stained chromosomal bands of 927 (left) and 427 (centre) separated by PFGE. Electrophoresis parameters were 1% agarose, 850–450 s linear ramped pulse times, 144 h, 2.5 V cm⁻¹, 1 X TB(0.1)E, 16°C. Individual PFG bands are given Arabic numerals and marked by blue-green (927) and purple (427) circles. The content of PFG bands varies under different PFGE separation parameters (compare Fig. 1B,C,D). Roman numerals show the position of diploid chromosome pairs, of which the smaller is labelled a and the larger b. The positions of the chromosomes are shown in diagrammatic form and colour-coded to enable comparison between 427 and 927 (*, the size order of these chromosomes has been determined previously: for full details on the 927 karyotype, see [4]). The chromosomal location (427) of only the 121 *VSG* is shown (¹basic copy; ²expression site copy [42]). The size markers on the left (Mb) show the positions of *S. cerevisiae* strain YPH80 chromosomes XV, IV and XII arrowed in lane 3 (S.c.). CZ = compression zone containing unresolved chromosomes. (B) As Fig. 1A, except electrophoresis parameters were 1.2% agarose, 1400–700 s linear ramped pulse times, 144 h, 2.5 V cm⁻¹, 1 X TB(0.1)E, 15°C. The chromosomal locations (427) of all *VSG*s used in this study are shown. *The 221 gene is located in the active expression site. Only three of the *VSG* probes hybridised consistently to the chromosomal bands of 927: *VSG*60 to band 4 (IIIb/IVb/Vb/VIa); *VSG*BR2 to band 4; and *VSG*T3 to the CZ. *VSG* 121 hybridised weakly to bands 4 and 6. The size markers are as shown in A (data not shown, but see Fig. 2A in [4]), except the largest size marker given (3.5 Mb) is *S. pombe* strain 972 h-chromosome III. (C) As Fig. 1A, except electrophoresis parameters were 0.8% agarose, 3600 s pulse times, 192 h, 1.2 V cm⁻¹, 0.5 X TBE, 15°C. The size markers given on the left (Mb) are *S. cerevisiae* chromosomes IV and XII (data not shown) and *S. pombe* chromosomes III, II and I (arrowed in lane 1, S.c.). The chromosomal locations (427) of all *VSG*s are shown. **VSG*s 121, T3 and 222 may hybridise to more than one band. Inclusion within brackets implies weak hybridisation to that chromosome relative to others. *VSG*T3 hybridises to band 5 (chromosome IXb) in 927. (D) As Fig. 1A, except electrophoresis parameters were 0.8% agarose, 2000 s pulse times, 144 h, 1.5 V cm⁻¹, 1 X TB(0.1)E, 15°C. The size markers given on the left (Mb) are *S. pombe* chromosomes and the largest *Hansenula wingei* chromosome (data not shown). Only the positions of those chromosomes and *VSG*s indicated have been determined. *It is not clear if *VSG*s 121, 222 and T3 are on one or both chromosomes. **The division of the lower compression containing VIIab, VIb and VIIIa into two halves as shown can be confirmed by overlaying autoradiographs after probing with chromosome-specific probes.

Table 1
The size and probe content of *T. brucei* stock 427 megabase chromosomes^a

Chr #	Band #			Complete separation	Approx. size (Mb)	% size difference of 927	ES	VSGs	Gene and EST markers
	A	B	C						
Ia	7	5	2	Y	1.85	+68	N	121	OT07, OT08, OT14, OT29, OT43, T238, T392, T558, T665, T764
Ib	cz	cz	3	Y	3.6	+200	N	121? 222? T3?	
IIa	1	1	1	Y	1	-23	Y	-	PLC, OT45, T007, T009, T114, T292, T338, T419, T496, T623
IIb	2	2	2	Y	1.65	+23	Y	-	
IIIa	3	3	2	Y	1.7	+04	Y	-	T027, T336, T366, T374, T762, T061, T081, T236, T427, T688
IIIb	8	6	2	Y	1.88	+07	Y	-	
IVa	5	4	2	Y	1.78	+08	Y	121	T200, T317, T381, T434, T741, T031, T050, T067, T086, T221
IVb	cz	7	2	Y	2.3	+29	N	60	
Va	4	3	2	Y	1.72	+01	Y?*	-	T144, T245, T275, T404, T425, T446, T476, T564, T165, T763
Vb	6	4	2	Y	1.79	+04	N	-	
VIa	cz	8	3	Y	2.8	+60	Y	221	gGAPDH, T281, T335, T384, T770, T799, T130, T253, T426,
VIb	cz	10	3	N	3.4	+70	?	BR2? 117?	
VIIa	cz	10	3	N	3.4	+57	?	-	OT15, OT33, T252, T321, T341, T346, T641, T683, T773, T804
VIIb	cz	10	3	N	3.4	+62	?	BR2? 117?	5srRNA, GDH, T263, T280, T410, T416, T457, T630, T654
VIIIa	cz	10	3	N	3.4	+66	N	118, 121? 222? T 3?	ACTIN, OT02, OT12, T025, T265, T291, T296, T312, T318, T332, T411, T417, T431, T443, T601, T614, T634, T684, T742, T808
VIIIb	cz	cz	4	Y	3.6	+66	N	-	
IXa	cz	9	3	Y	3	-10	N	-	T324, T394, T396, T436, T449, T526, T547, T651, T657
IXb	cz	cz	5	Y	4.5	+29	N	T3 (121)	
Xa	cz	cz	6	Y	5	+14	N	-	
Xb	cz	cz	7	N	6	+36	N	R?	
XIa	cz	cz	7	N	6	+15	N	R?	T069, T187, T257, T278, T339, T356, T368, T379, T380, T580
XIb	cz	cz	7	N	6	+15	N	R?	

^a Table shows the PFG band location of all chromosomes in the different PFGE separations presented here. The size of each chromosome has been estimated by comparison with *S. cerevisiae* and *S. pombe* chromosomes, in each case from PFGs in which the chromosome was located in the main window of resolution (not all shown). The size variation relative to the chromosomes of 927 is calculated by comparing a homologues and b homologues in both stocks, and therefore does not indicate the greatest size variations observed in Fig. 1 (for example, chromosome 427-Ib (3.6 Mb) compared to 927-Ia (1.1 Mb) shows a variation of +227%). The chromosomal locations of B-ES and VSGs are shown; ? indicates that it was not possible to resolve two or more chromosomes (see Fig. 1 and Fig. 2). ?*, see text. The EST and gene markers used to determine syntenic relationships between the genomes of 427 and 927 are listed.

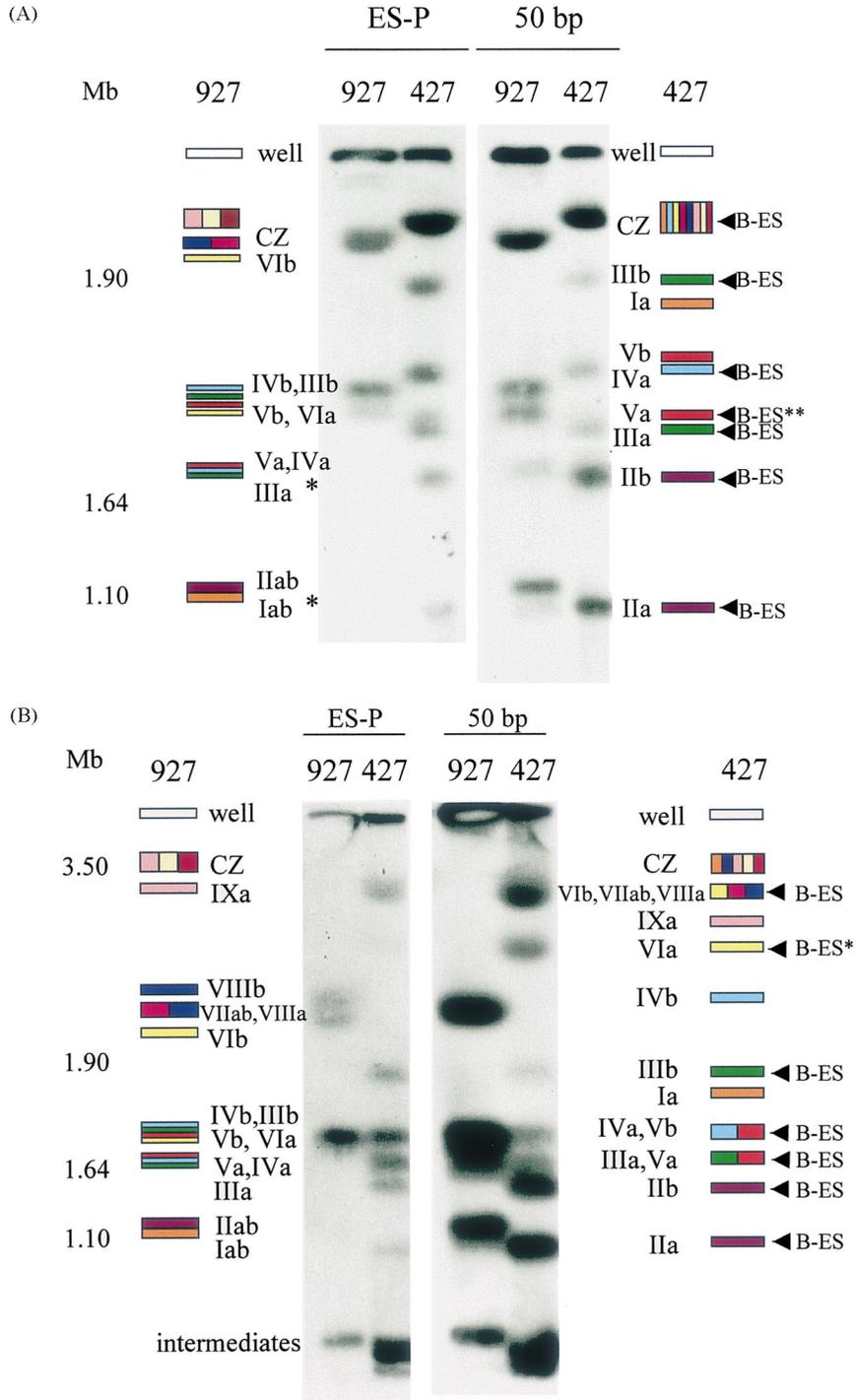


Fig. 2.

(such as yeast chromosomes) vary considerably depending on whether the chromosome falls within the main window of resolution or is in a region of compression. For example, in [42], Fig. 2, chromosome 427-IVa is compressed with several other chromosomes while in Fig. 1A it is in the region of greatest resolution. Where there are insufficient markers in the size range separated, it is also not simple to determine the extent of the area of gel in which fragment mobility is linear with respect to fragment size. The most accurate estimates derive from separations produced using switch times specific to the fragments under consideration. Migration of markers and samples is also affected by the relative amounts of DNA loaded on the gels.

The estimated DNA content of the 11 pairs is 70 Mb. If there are 50–100 minichromosomes of 50–100 kb (2.5–10 Mb) and an estimated 1.5 Mb of intermediate-sized chromosomes (data not shown), the total DNA content of a 427 nucleus is approximately 7.8×10^7 base pairs. Previous estimates from DNA–DNA renaturation, cytophotometry and restriction analysis were $3.5\text{--}4.0 \times 10^7$ bp (haploid) [43,44].

Except for chromosomes that are unambiguously resolved, the chromosomal location of a DNA sequence in stock 427 is currently determined by the hybridisation of the probe to the PFGE-separated chromosomes of two size-polymorphic cloned *T. brucei* stocks, e.g. 427 and 927. Reference probes (Table 1) should be used for confirmation when trying novel separation parameters. Using the PFGE parameters described here this will necessitate two separate gels, electrophoresed using different PFGE conditions (as Fig. 1B and Fig. 1C). However, we have now determined a single set of parameters that separates all chromosomes sufficiently to allow us to

use inter-stock chromosomal size polymorphism to determine the chromosomal location of any probe in any stock with one hybridisation (manuscript in preparation).

3.2. Comparison of the karyotypes of *T. brucei* strain 427–221a and the genome project reference stock TREU927

All 427 chromosomes differ in size from their homologues excepting chromosomes VII and XI. The size differences vary from an additional 4% (70 kb, chromosome V) to almost twice the size (1.75 Mb, chromosome I). 427 chromosomes are larger than their homologues in 927 with the exception of IIa and IXa. Table 1 shows the percentage size variation between 427 and 927 chromosomes, comparing a homologues and b homologues in each stock. Therefore, maximum size variation is greater than shown (in most cases, between 927-a and 427-b homologues). The greatest difference in size is that seen in chromosome I, which varies from 1.1 Mb (Ia in 927) to 3.6 Mb (Ib in 427). The smallest chromosome I identified so far is 0.9 Mb, found in stock H9 [4]. This is one quarter the size of chromosome Ib in strain 427–221a. Nevertheless, all cDNA and gene markers hybridised to *T. brucei* chromosomes resolved by PFGE have remained in identical groups in all stocks examined, including 427 ([4], Table 1 and unpublished data). We have noted no large translocations between chromosomes using gene probes, suggesting that the additional DNA consist of amplified sequence, insertion of new sequence elements, and/or recombination in gene-poor regions. We have shown in a study of chromosome I of field isolates [15] that almost ALL regions of the chromosomes are affected. Part of the size variation is due to

Fig. 2. The location of bloodstream-form *VSG* expression site-associated sequences on the chromosomal bands of 427. (A) The left autoradiograph shows the chromosomal bands of 927 (left) and 427 separated as described in Fig. 1A, Southern blotted and probed with B-ES promoter DNA (ES-P). The right-hand autoradiograph shows a similar blot probed with a tract of 50 bp repeats. The chromosomes of 927 (left) and 427 are shown in diagrammatic form and the chromosomal locations of the B-ES-associated sequences in 427 are indicated. *Hybridisation of the promoter probe to 927-Ia, Iib and Va is visible only after longer exposure of the blot to X-ray film [4]. **Hybridisation of the 50 bp repeat probe to 427-Va is weak or absent at this exposure. (B) As Fig. 2A, except the chromosomal bands were separated as in Fig. 1B. *Chromosome VIa carries the active expression site. Hybridisation of the B-ES-associated sequences to the intermediate chromosomes is also evident.

amplification of a region of DNA found on all megabase chromosomes, which contains many putative transposons, but the content of the extra DNA in the gene-rich regions is not yet known.

The estimated DNA content of the megabase chromosomes of stock 927 is 53.4 Mb (diploid). Therefore 427 trypanosomes contain almost 30% more megabase chromosomal DNA. No differences have been observed in PFGE karyotype after replication in laboratory rodents (427, 927), after passage through tsetse flies (927), in procyclic culture (927), nor between many cloned variant antigen types ([4,42,45] and this paper). The probes used here are available to any researcher wishing to compare the karyotype of their own 'strain 427' laboratory stock.

3.3. The location of bloodstream-form expression sites on the chromosomes of *T. brucei* strain 427–221a

The number of bloodstream-form *VSG* expression sites (B-ES) in *T. brucei* 427 has been estimated previously. Hybridisation of ES-derived probes indicated 14–25 copies in the genome [46], and hybridisation of a B-ES promoter sequence to restriction digested DNA followed by phosphorimaging indicated 29 ± 3 copies [42]. However, the promoter and other expression-site sequences may be duplicated within a B-ES [47,48]. Upstream of all characterized expression sites there are long tracts of 50-bp repeats [38,42]. We attempted to determine which chromosomes may carry B-ES by hybridising a 315-bp promoter sequence and a cloned region of 50-bp repeats to PFGE-separated chromosomes (Fig. 2A and Fig. 2B). The strength of hybridisation of both probes varies, and this may be due to copy number variation and/or sequence divergence between chromosomes, and/or variable transfer of UV-nicked chromosomal DNA of different sizes to the membrane.

B-ES-associated sequences are found on 427 chromosomes IIa, IIb, IIIa, IIIb, IVa, Va, VIa, and one or more of VIIb, VIIa, VIIb and VIIIa (although this compression can be partly resolved (Fig. 1D), we could not be certain whether the B-ES-associated sequences hybridised to one or

both bands). Therefore at least two of these eight or more chromosomes are segmental aneuploids. The promoter probe hybridises clearly to chromosome Va, but it is not clear that the 50 bp repeats have hybridised. This has been observed previously in chromosome 927-VIIIb [4] and we speculated that this may be due to sequence divergence, but it remains possible that there are no 50 bp repeats associated with a B-ES on chromosome Va. It is not known if this putative ES is complete or functional. The promoter probe hybridises only weakly to chromosome VIa, which is the active expression site in 427–221a. Very weak hybridisation of the promoter probe to 927-I/II (visible on an autoradiograph only after very long exposure) has been observed previously [4]. *T. brucei* stock 927 chromosomes Ia, IIb, IVb, Va, VIa, VIIa and/or b and VIIIb carry B-ES-associated sequences ([4] and Fig. 2A, Fig. 2B). B-ES-associated sequences are found on neither 427 nor 927 chromosomes IX, X or XI, but we have noted the hybridisation of these probes to chromosomes X/XI in other stocks ([4] and unpublished data).

Loss of telomeric and subtelomeric sequences from chromosomes has been reported in other organisms, both in culture and in varying environmental conditions [49–51]. The telomeres of trypanosomes are uniquely devoted to *VSG* expression sites, and we currently have no evidence that there is any loss of B-ES or other telomeric DNA due to long periods in culture, although there have been two reports of loss of B-ES after antigenic switching in strain 427 in laboratory experiments [52,53]. The variable locations of the B-ES in different isolates are as likely to be due to illegitimate recombination between multicopy sequences on non-homologous chromosomes [15], as the number of megabase chromosomes carrying B-ES in 427 is within the range of 7–13 identified in a selection of isolates ([4] and unpublished data). The presence of an aneuploid B-ES on a chromosome obviously affects the size of the chromosome, but such chromosomes are not always larger than their homologues (compare 427-IV, 427-V, 927-I, 927-V, 927-VI diploid homologues). We do not know if B-ES are found at one or both ends of these chromosomes. B-ES are also found on intermediate chromosomes in 427 [2] and in the AnTat 1.3 strain [3].

3.4. The location of characterised variant surface glycoprotein genes on the chromosomes of *T. brucei* strain 427–221a

We hybridised 10 distinct *VSGs* to the chromosomal DNA of 427, resolved using the same PFGE parameters as described in Fig. 1A and Fig. 1B and Southern-blotted. *T. brucei* strain 427–221a is expressing the 221 *VSG* from an expression site on chromosome VIa (Fig. 1B; compare [42], Fig. 2). This is equivalent to band 14 in [10]. However, we note that several published papers state that this expression site is found in 427 PFG band 15, for example [39,41]. This corresponds to the compression of VIb/VI-Iab/VIIIa (Fig. 1B, Fig. 1D). The 221 probe may have been wrongly assigned to band 15, due to variation between PFGs and the lack of chromosome-specific markers, or it may be due to chromosomal variation in the 427–221a strains that has arisen during clonal growth in one or the other laboratory. The chromosome markers are available to all researchers wishing to investigate chromosome sizes and assignments, and this is a good example of the urgent need to adopt the recommended chromosome nomenclature [11] and the use of markers [4] to facilitate comparison of data from different laboratories.

One copy of the 121 *VSG* is found in the inactive expression site on chromosome IVa and a basic copy on chromosome Ia (compare [42], Fig. 7). Other characterised *VSGs* are located on chromosomes IVb, VIb or VIIIa, VIIIb, IXa and IXb. It has been shown previously that some *VSGs* are present in long tandem arrays in megabase chromosomes and that they may be haploid [8,9]. It is not known if arrays containing different *VSGs* are present at the same positions on homologous chromosomes, nor if such arrays vary in length between homologues. Our results confirm that the *VSGs* are found on only one of a homologous pair, although *VSGs* 121, 222 and T3 are duplicated on non-homologous chromosomes. Weak hybridisation of *VSG* 121 to other chromosomes probably indicates related members of a gene family. It is not known if the *VSGs* are located near telomeres or elsewhere

within the chromosome, with the exception of 221 and 121/IVa, which are in telomeric expression sites. The others are all located on chromosomes to which the expression site-associated sequences have not hybridised (although we could not determine the chromosomal location of the B-ES(s) in the VIb/VIIab/VIIIa compression). This may be important in the process of homologous recombination, which effects the transposition of the basic copy to a B-ES; or it may have no significance, as the locations of B-ES vary between stocks [4].

Only three of the *VSG* probes hybridised strongly to the chromosomal DNA of 927 (see legend to Fig. 1B) and the results indicate the possibility that these *VSGs* are on homologous chromosomes in the two stocks (927 compressions prevent us identifying exact locations). The genomic locations of *VSG* basic copies in stock 927 are not known, except for uncharacterised arrays on chromosomes IXa and b (unpublished results). However, hybridisation data detected only telomeric *VSGs* on chromosomes 927-Ia and 927-Ib and no internal arrays [15], while recent sequence data has detected only the telomeric *VSGs* and some genes and gene fragments in the subtelomeric regions (EMBL Acc. No. AL359782).

T. brucei stocks show a remarkable plasticity in their genomes, which may vary by 30% or more in DNA content. We have found that all gene and EST probes remain in identical syntenic groups between stocks. However, sequences associated with antigenic variation are found in highly polymorphic regions of the genome: the *VSG* repertoire of individual trypanosomes varies; the basic copy *VSGs* are mostly haploid and therefore these regions of the chromosomes are aneuploid; expression sites are sometimes found on only one of a diploid pair of chromosomes and expression sites are found on different chromosomes in different stocks. Antigenic variation has been most carefully characterised in *T. brucei* stock 427 and this comparison of its genome with that of the genome project reference stock will be useful to further studies aimed at clarifying the genomic recombination processes involved.

Acknowledgements

This investigation received financial support from the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (SEM and VL) and the National Institutes of Health (grant number AI 21729 to GAMC). We thank M. Carrington, J. Donelson, N. El-Sayed, K. Ersfeld, P. Michels and G. Rudenko for gifts of probes, and G. Rudenko for helpful discussions. Chromosome-specific markers may be requested from S. Melville.

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