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## Telomere length in *Trypanosoma brucei*

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### Abstract

**Trypanosoma brucei**—thwarts the host immune response by replacing its variant surface glycoprotein (VSG). The actively transcribed VSG is located in one of ~20 telomeric expression sites (ES). Antigenic variation can occur by transcriptional switching, reciprocal translocations, or duplicative gene conversion events among ES or with the large repertoire of telomeric and non-telomeric VSG. In recently isolated strains, duplicative gene conversion occurs at a high frequency and predominates, but the switching frequency decreases dramatically upon laboratory-adaptation. Uniquely, *T. brucei* telomeres grow—apparently indefinitely—at a steady rate of 6–12 base pairs (bp) per population doubling (PD), but the telomere adjacent to an active ES undergoes frequent truncations. Using two-dimensional gel electrophoresis, we demonstrate that all of the chromosome classes of fast-switching and minimally propagated *T. brucei* have shorter telomeres than extensively propagated Lister 427 clones, suggesting a link between laboratory adaptation, telomere growth, and VSG switching rates.

### Index Descriptors

*Trypanosoma brucei*; Antigenic Variation; Isolation History; Propagation History; Telomere Length; Strain Phylogeny; Variant Surface Glycoprotein

## 1. Introduction

Antigenic variation allows several pathogens to evade immune responses and prolong an infection. *Borrelia burgdorferi*, *Pneumocystis carinii* and *Trypanosoma brucei*, all evade the host immune response by sequential expression of different surface antigens that, in *T. brucei*, form a homogeneous, dense, coat, overlying the cytoplasmic membrane (Cross, 1975; Kutty, et al., 2001; Singh and Girschick, 2004). The *T. brucei* genome encodes hundreds of VSG (Berriman, et al., 2002), and the single transcribed VSG is located in a telomere-proximal expression site (ES) (de Lange and Borst, 1982). *T. brucei* has ~20 telomeric ES, whose transcription is strictly monoallelic (Vanhamme, et al., 2000). Antigenic switching can occur by a coupled activation and inactivation of transcription between two ES (*in-situ* switch), by reciprocal DNA translocation between two ES, or by a duplicative gene conversion event that places a new VSG into the transcribed ES (Horn and Cross, 1997; McCulloch, et al., 1997; Myler, et al., 1984; Rudenko, et al., 1996; van der Ploeg, et al., 1984a). Gene-conversion-mediated switching predominates in pleomorphic strains (Robinson, et al., 1999). Although we know the general molecular mechanisms underlying antigenic switching, little is known about its regulation.

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The rate of VSG switching varies substantially among strains of *T. brucei*. Recent isolates have a high switching frequency of  $\sim 10^{-2}$ – $10^{-4}$ /population doubling (PD) (Barry, 1997; Robinson, et al., 1999; Turner, 1997; Turner and Barry, 1989), and grow to lower densities *in vitro*, which complicates laboratory studies. Thus, isolates were often passaged sequentially in mice in the absence of immune selection (Turner, 1997). After several months, such parasites become monomorphic and more virulent, with as much as a 10,000-fold reduction of switching frequency (Horn and Cross, 1997; Lamont, et al., 1986; McCulloch, et al., 1997; Turner, 1997; Turner and Barry, 1989; van Deursen, et al., 2001). Unfortunately, nothing is known about the molecular changes that occur during laboratory adaptation — especially what mediates the reduction in switching frequency. Identifying the mediators of these changes would improve our understanding of antigenic variation.

The intriguing location of VSG ES led us to investigate, from several directions, whether telomere structure might play a role in regulating antigenic variation. *T. brucei* telomeres consist of hexameric TTAGGG-repeats that, together with their interacting proteins, protect chromosome ends from being recognized as double stranded breaks (Blackburn and Challoner, 1984; de Lange, 2002; van Steensel, et al., 1998). Uniquely, *T. brucei* telomeres grow at a steady rate of 6–12 bp/PD, but the ES telomere adjacent to the single transcribed VSG undergoes frequent truncations (Bernards, et al., 1983; Dreesen and Cross, 2006a; Horn and Cross, 1997; Myler, et al., 1988; Pays, et al., 1983; van der Ploeg, et al., 1984b). Although the mechanism and frequency of these truncations remains unknown, if they fall within the telomeric TTAGGG repeats they are elongated by telomerase at a rate that is inversely proportional to telomere length (Dreesen and Cross, 2006b; Glover and Horn, 2006; Horn, et al., 2000).

When the reverse-transcriptase component of telomerase is deleted, telomeres shorten at a rate of 3–6 bp/PD (Dreesen, et al., 2005). Upon reaching a discreet length, short silent ES telomeres stabilize within a distinct size range, maintained by a telomerase-independent mechanism (Dreesen and Cross, 2006b). Although a short telomere at an active ES is also stabilized, at least temporarily, subsequent breakage appears to catalyze its replacement by a different VSG and its associated telomere, through duplicative gene conversion (Dreesen and Cross, 2006a). To explain the apparent difference between active and silent ES, we recently proposed (Dreesen and Cross, 2006a; Dreesen, et al., 2007) that a double-stranded break at the transcribed ES could fall into the subtelomeric region and be repaired by break-induced replication, using another ES as template, with consequent duplication and activation of the resident VSG. We have speculated that telomere breakage and repair could increase the rate of antigenic switching at a short active ES telomere (Dreesen and Cross, 2006a; Dreesen, et al., 2007).

In this study, we measured telomere lengths in clones of some recent isolates whose histories were known, in the recently laboratory-adapted strain used for the genome project, and in several clones of the extensively propagated and widely used Lister 427 strain. We found that minimally propagated and recently laboratory-adapted strains have shorter telomeres than the extensively propagated Lister 427.

## 2. Materials and methods

### 2.1. History of *T. brucei* strains

The detailed lineages of *T. brucei* cell lines used in this study and by other laboratories are available at [http://tryps.rockefeller.edu/trypanosome\\_pedigrees.html](http://tryps.rockefeller.edu/trypanosome_pedigrees.html). The strain abbreviations used in this paper are indicated in parentheses and are summarized in Table 1. *T. brucei* S42 (RUMP102), STIB348 (RUMP106) and *T. b. rhodesiense* s427 (RUMP151) are isolates that have not been extensively propagated in the laboratory. S42 was isolated from a female warthog near Kirawira, Tanzania, in March 1966 (Baker, et al., 1967). It was minimally passaged before

being tsetse-transmitted in November 1974. STIB366D was derived from a single metacyclic cell and was minimally propagated before freezing (by GAMC) as RUMP102. STIB246 was isolated from a Hartebeest in Serengeti in 1971 (Geigy and Kauffmann, 1973). After growth in mice and rats for a total of ~6 weeks, and cloning as STIB348, it was transmitted through tsetse in July 1975 (Leo Jenni, Swiss Tropical Institute, Basel). STIB348U was derived from a single metacyclic cell and frozen (by GAMC) as RUMP106 after passage through 2 irradiated mice. *T. b. rhodesiense* RUMP151 is derived from the true s427, which was isolated in 1960 from a sheep in south-east Uganda (Cunningham and Vickerman, 1962) and frozen as EATRO217. After its initial isolation, s427/EATRO217 was passaged through ~20 mice before being frozen (by GAMC) as RUMP151, which is highly resistant to human serum (GAMC, unpublished data), consistent with its isolation from an area of epidemic human sleeping sickness ((Cunningham and Vickerman, 1962) and M.P. Cunningham, personal communication). TREU927, was isolated in Kiboko, Kenya, in 1969 or 1970, and preserved after 3–11 passages (Goedbloed, et al., 1973). A clone of TREU927 (GUTat 10.1), after 27 passages in mice to render it more virulent (van Deursen, et al., 2001), was the source of DNA used for the first *T. brucei* complete genome sequence (Berriman, et al., 2005). The procyclic 927 cells from which we isolated DNA were derived from GUTat 10.1 that had been differentiated to procyclic forms in the laboratory of Scott Landfear and further propagated for 2–4 weeks. The origin of Lister 427 is unknown. It is not related to s427, as was generally assumed (Cross, 1975), and was maintained by frequent syringe passage in mice during indeterminate intervals from 1961–1967. Lister 427 ‘single-marker’ (SM) bloodstream and 29-13 procyclic forms (Wirtz, et al., 1999) are closely related to other extensively propagated clones of Lister 427 that have a low switching frequency of  $\sim 10^{-6}$ – $10^{-7}$ /PD (Horn and Cross, 1997; Lamont, et al., 1986). The relative positions of clones RUMP150 and RUMP501 (these were tsetse-transmitted in 1983 and 2004, respectively) in the Lister 427 lineage are clear ([http://trys.rockefeller.edu/trypanosome\\_pedigrees.html](http://trys.rockefeller.edu/trypanosome_pedigrees.html)), and their genotypic identity has been verified on several occasions (C.M.R. Turner and G.A.M. Cross, unpublished data). RUMP150 is genetically indistinguishable from the SM and 29-13 clones, but the degree of propagation of Lister 427 prior to its earlier incarnation as RUMP501 cannot be verified. RUMP100 and RUMP502 are descendants of EATRO795, isolated from a Zebu cow in Kenya (September, 1964), which has a high switching frequency (Onyango, et al., 1966; Robinson, et al., 1999; Turner, 1997; Turner and Barry, 1989). The ancestors of RUMP100 were re-cloned several times, with minimal passaging, prior to infecting a mouse with a single metacyclic cell, yielding STIB367H, from which the RUMP100 population was frozen (by GAMC) after one additional mouse passage. RUMP502 is one passage removed from GUP2900 (GUG359). Although we lack documentation of the lineage prior to GUP2900, RUMP502 was recently verified to be genetically indistinguishable from a verified sample of EATRO795 (C. M. R. Turner, personal communication).

## 2.2. Cultivation of *T. brucei*

Lister 427 clones were cultured in HMI-9 at 37°C (Doyle, et al., 1980; Hirumi and Hirumi, 1989) or in SDM-79 at 27°C (Brun and Schonenberger, 1979). TREU927 procyclic forms were grown in Cunningham’s medium (Cunningham, 1977). All other strains were grown for three days in female CD-1 AKA ICR (Charles River) mice. Where larger quantities were required, trypanosomes were grown for a further 3 days in male Sprague-Dawley rats (Charles River), then purified by centrifugation and passage through a DEAE cellulose column (Taylor, et al., 1974).

## 2.3. DNA isolation and terminal restriction fragment analysis

DNA was isolated from freshly harvested cells, as previously described (Munoz-Jordan, et al., 2001), except for clones 100 and 502, where it was isolated from frozen cell pellets that had been stored at  $-70^{\circ}\text{C}$ , which did not affect DNA quality. Briefly,  $\sim 2 \times 10^8$  cells were washed

twice in TDB (5 mM KCl, 80 mM NaCl, 1 mM MgSO<sub>4</sub>, 20 mM Na<sub>2</sub>HPO<sub>4</sub>, 2 mM NaH<sub>2</sub>PO<sub>4</sub>, 20 mM glucose, pH 7.7), resuspended in 1 ml TNE (10 mM Tris pH 7.4, 100 mM NaCl, 10 mM EDTA), lysed by adding 1 ml TNES (TNE with 1% SDS) supplemented with 100 µg/ml proteinase K (Roche), and incubated overnight at 55°C. DNA was extracted with phenol/chloroform/isoamyl-alcohol (PCI), precipitated and resuspended in TNE with 100 µg/ml RNase A (Sigma). After incubation for 3 h at 37°C, TNES with 100 µg/ml proteinase K was added, and samples were incubated overnight at 55°C. Lastly, DNA was PCI-extracted, precipitated and resuspended in T<sub>10</sub>E<sub>1</sub> (10 mM Tris pH 7.4, 1 mM EDTA). Restriction enzyme digestion took place overnight according to the manufacture's conditions (New England Biolabs). DNA restriction fragments were separated on a 0.8% agarose gel. Equal loading was ensured by measuring DNA concentration after digestion and by staining of agarose gels with Ethidium Bromide. Bulk telomere length was analyzed by denaturing in-gel hybridization: gels were dried at room temperature, denatured, neutralized, and hybridized at 50°C using a radiolabeled (TTAGGG)<sub>4</sub>-probe in Church Mix [0.5 M NaPO<sub>4</sub> (pH 7.2), 1 mM EDTA (pH 8.0), 7% SDS and 1% BSA]. Gels were subsequently washed three times for 30 min with 4x SSC (1x SSC is 15 mM tri-sodium citrate and 150 mM NaCl) and once for 30 min with 4x SSC + 0.1% SDS at 20°C, then exposed to a phosphorimager screen for various times (Dreesen, et al., 2005; Hemann and Greider, 1999). Signals were quantified using ImageQuant software (Molecular Dynamics).

#### 2.4. 2-dimensional DNA electrophoresis

DNA agarose plugs were prepared and chromosome-sized DNA was separated by Rotating Agarose Gel Electrophoresis (Stratagene, Inc), as described previously (Navarro and Cross, 1996; Navarro and Cross, 1998). Gels were stained with 2 µg/ml Ethidium bromide in 0.5 × TAE. Entire lanes, containing MBC, IC and MC were cut from the gel, placed in 15 ml Falcon tubes, pre-incubated in digestion buffer, and digested overnight with RsaI, MboI and AluI, using gentle rotation. Fresh aliquots of enzymes were added the next morning and incubation extended for 20 h. Digested plugs were equilibrated in 0.5 × TAE for 30 min and embedded in 0.8% Agarose in 0.5 × TAE. Digested fragments were separated in the second dimension for 1 h at 90 v and 15 h at 40 v. In-gel hybridization was performed as described above (Dreesen, et al., 2005; Hemann and Greider, 1999).

#### 2.5 Telomere size distribution

Using Image Quant software, each gel lane was graphically partitioned into 30 equally sized rectangle. The hybridization signal in each compartment was quantified and subsequently represented as the percentage of the total signal in the entire lane.

### 3. Results

#### 3.1 Telomere length in laboratory-adapted less-propagated strains

Based on our experimental results with telomerase-deficient cells, we had the idea that telomere length at the active ES might affect the frequency of antigenic variation. This notion led us to measure telomere length in clones derived from some well-characterized pleomorphic and monomorphic strains, whose history was fairly clear.

The telomeres of the four Lister 427-derived clones (only 150 and 501 are shown in Fig. 1A) are strikingly similar in length, ranging from 3–20 kB, with an average of ~15 kB, as previously shown (Dreesen, et al., 2005; Munoz-Jordan, et al., 2001). Telomeres of less propagated isolates ranged in size from 3–12 kB, with an average of ~8–10 kB (Fig. 1A), and quantification of these data confirmed that the average telomere length was shorter in less propagated strains than in two clones of Lister 427 (Fig. 1B). TREU927 is of particular interest, since it was recently laboratory-adapted and used for the *T. brucei* genome project (Berriman, et al.,

2005). As described elsewhere (van Deursen, et al., 2001), TREU927 clone 4 was passaged 27 times in mice to increase its virulence, and predominately expresses VSG GUTat 10.1. Even after 4 more months *in vitro* culture and ~ 75 additional doublings as procyclic forms, our sample of TREU927 has much shorter telomeres than the extensively *in vitro* cultured Lister 427 SM (Fig. 1C).

Laboratory-adapted TREU927 and Lister 427 SM stably express one particular VSG. Constitutive elongation of all non-transcribed telomeres could account for the telomere length difference between the extensively propagated Lister 427 and the recently adapted strain TREU927. We predict that continuous *in vitro* growth of TREU927, with stable expression of GUTat 10.1, would eventually result in similarly elongated telomeres.

### 3.2 Telomere length analysis by 2-dimensional gel electrophoresis

The *T. brucei* genome consists of 11 diploid megabase chromosomes (MBC), which contain most VSG ES and all essential housekeeping genes, a few intermediate chromosomes (IC), whose function remains unclear but which often contain an ES, and ~ 100 minichromosomes (MC) that contain only 177-bp repeats, VSGs and telomeric repeats. To address whether telomere length varied among the different chromosome types, we used 2-dimensional gel electrophoresis to separate chromosomes and then their telomeres. In the first dimension, MBC, IC and MC were separated by Rotating Agarose Gel Electrophoresis. Under the conditions used, MBC were well separated and, as previously described, we observed extensive size polymorphisms among different strains (Fig. 2A) (Melville, et al., 1998; Melville, et al., 2000). MC are not well separated under these conditions and migrate at the bottom of the gel (Fig. 2A). To analyze telomere lengths on individual chromosome types of Lister 427 clones, entire lanes (shown in Fig. 2A) were cut out of the gel, digested with restriction enzymes, and perpendicularly embedded in a conventional 0.8% agarose gel. The Ethidium-bromide-stained chromosomal separations are graphically inserted on top of each gel and serve as a loading indicator for the telomere blots (Fig. 2B and C). Telomere restriction fragments were separated in the second dimension and visualized by in-gel hybridization (Dreesen and Cross, 2006b; Dreesen, et al., 2005). Within the well-separated MBC telomeres, individual spots representing clusters of telomeres of particular lengths, can be distinguished (Fig. 2B, arrows). Consistent with previous observations using individual telomeric VSG as hybridization probes, all MBC telomeres migrate well above the 12 kB molecular weight marker (Dreesen and Cross, 2006a; Dreesen and Cross, 2006b; Dreesen, et al., 2005; Horn and Cross, 1997; Munoz-Jordan, et al., 2001). The abundant MC telomeres (~200) migrate within the same size range (12–23 kB) (Fig. 2B, circle). As a control, we visualized telomere length in telomerase-deficient *T. brucei* that had been continuously cultured for over 2.5 years (Fig. 2C). We previously reported that long-term cultured telomerase-deficient cells lose MC (Dreesen and Cross, 2006b), so the telomere signal was very low (Fig. 2C). Furthermore, this control confirmed that the telomere signal observed in Lister 427 SM was derived from terminal TTAGGG repeats.

Next, we compared telomere lengths between the Lister 427 SM clone, the recently laboratory-adapted genome strain TREU927, and minimally propagated strains represented by RUMP clones 100, 102 and 151 (Fig. 3). Two-dimensional gel electrophoresis confirmed that the telomeres of TREU927, especially those in the MC range, are very short, ranging from 3–10 kB (Fig. 1C and 3A). Similarly, in RUMP100, a clone of the well-characterized rapid-switching EATRO795 strain, the telomeres run from 5–10 kB (Fig. 3B, left panel). Most 102 and 151 MBC telomeres also run at significantly lower sizes (Fig. 3B, middle and right panels). No systematic size differences were observed between MBC, IC and MC telomeres.

## 4. Discussion

In this study, we analyzed telomere length in clones representing five ‘recent’ *T. brucei* isolates, the recently laboratory-adapted TREU927, and the extensively propagated Lister 427. We found that the recent isolates have much shorter telomeres than extensively propagated clones, and this is true for MBC, IC and MC. This is the most extensive analysis of telomere length in different *T. brucei* isolates. Telomeres in different *T. cruzi* isolates vary in size from 1–10 kB (Freitas-Junior, et al., 1999). More recently, telomere length was monitored during growth of *Leishmania major*, *L. tarentolae* and *Crithidia fasciculata* (Genest and Borst, 2007). Telomeres of *L. major* and *L. tarentolae*, but not *C. fasciculata*, grew by ~1 bp/PD. In contrast, *T. brucei* telomeres grow by 6–12 bp/PD. Furthermore, a short transcribed ES telomere is exceedingly rapidly elongated by telomerase, but elongation is counteracted by frequent telomere breakage (Bernards, et al., 1983; Dreesen and Cross, 2006b; Glover and Horn, 2006; Horn and Cross, 1997; Horn, et al., 2000; Pays, et al., 1983).

Regular and apparently unlimited growth of transcriptionally silent telomeres, which is so far unique to *T. brucei*, can explain why clones that have been extensively propagated *in vitro* have long telomeres. We would predict that longer propagation of more recently adapted strains, in the absence of immune selection, would also lead to an increase in telomere length, although we cannot exclude the possibility that the observed differences are due to inherent differences in telomere biology among various strains. We cannot yet explain why the non-transcribed telomeres of more recent isolates would be inherently shorter. However, if trypanosomes in the wild are switching *VSG* at far higher rates than after laboratory adaptation, which is a highly favored idea, supported by experimental data (Horn and Cross, 1997; Lamont, et al., 1986; Robinson, et al., 1999; Turner, 1997; Turner and Barry, 1989), the frequent breakage of transcriptionally active ES telomeres, coupled with frequent inter-telomeric *VSG* recombination events among all chromosome classes, would counter the intrinsic lengthening of silent telomeres that occurs in the absence of selection for such recombination events, under the usual *in-vitro* propagation conditions. Furthermore, the generally elevated rate of recombination at chromosome ends, as observed in other organisms, could lead to a general involvement non-expression site telomeres, resulting in their overall shortening.

We therefore speculate that telomere growth and *VSG* switching are inversely related.

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### Abbreviations

<b>EATRO</b>	East African Trypanosomiasis Research Organization
<b>ES</b>	expression site
<b>IC</b>	intermediate-size chromosomes
<b>GU</b>	Glasgow University

<b>MBC</b>	megabase chromosomes
<b>MC</b>	minichromosomes
<b>PD</b>	population doubling
<b>RUMP</b>	Rockefeller University Molecular Parasitology
<b>STIB</b>	Swiss Tropical Institute Basel
<b>TREU</b>	Trypanosomiasis Research Edinburgh University
<b>VSG</b>	variant surface glycoprotein

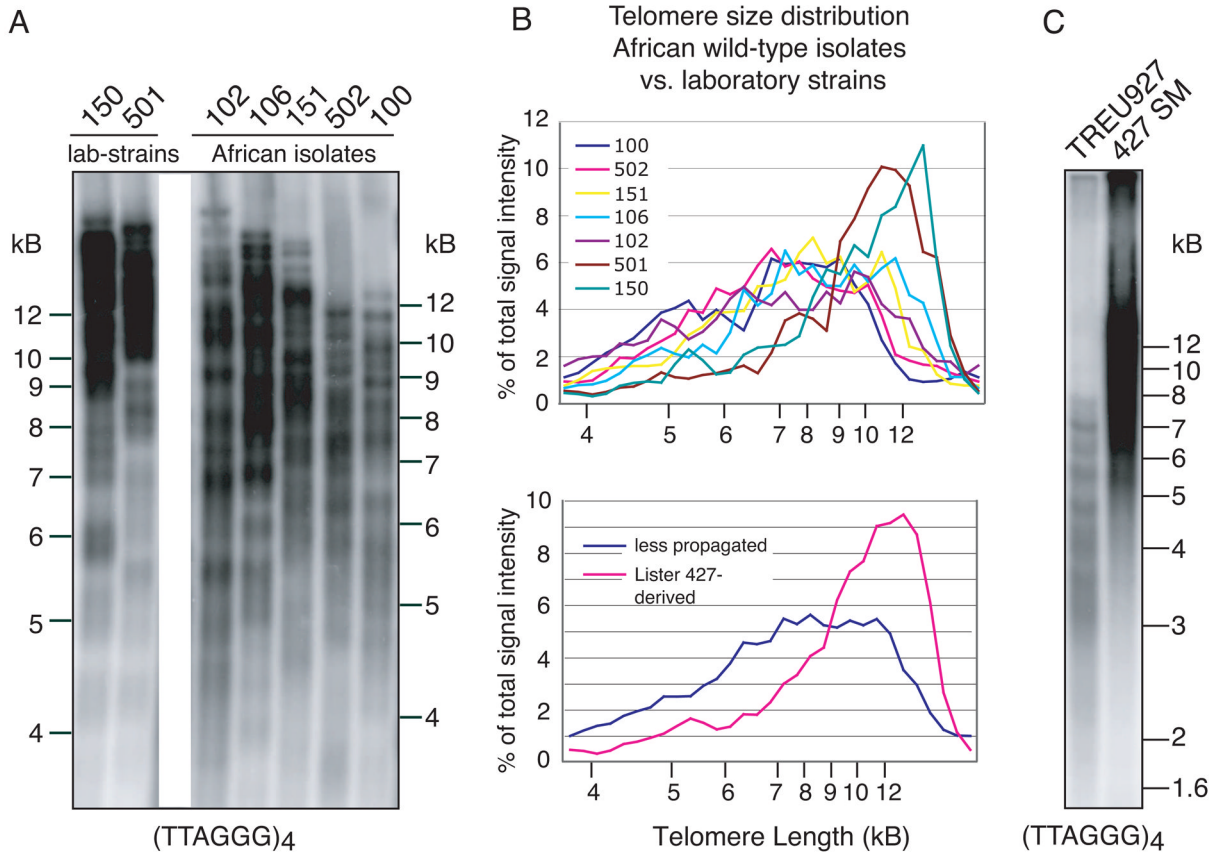
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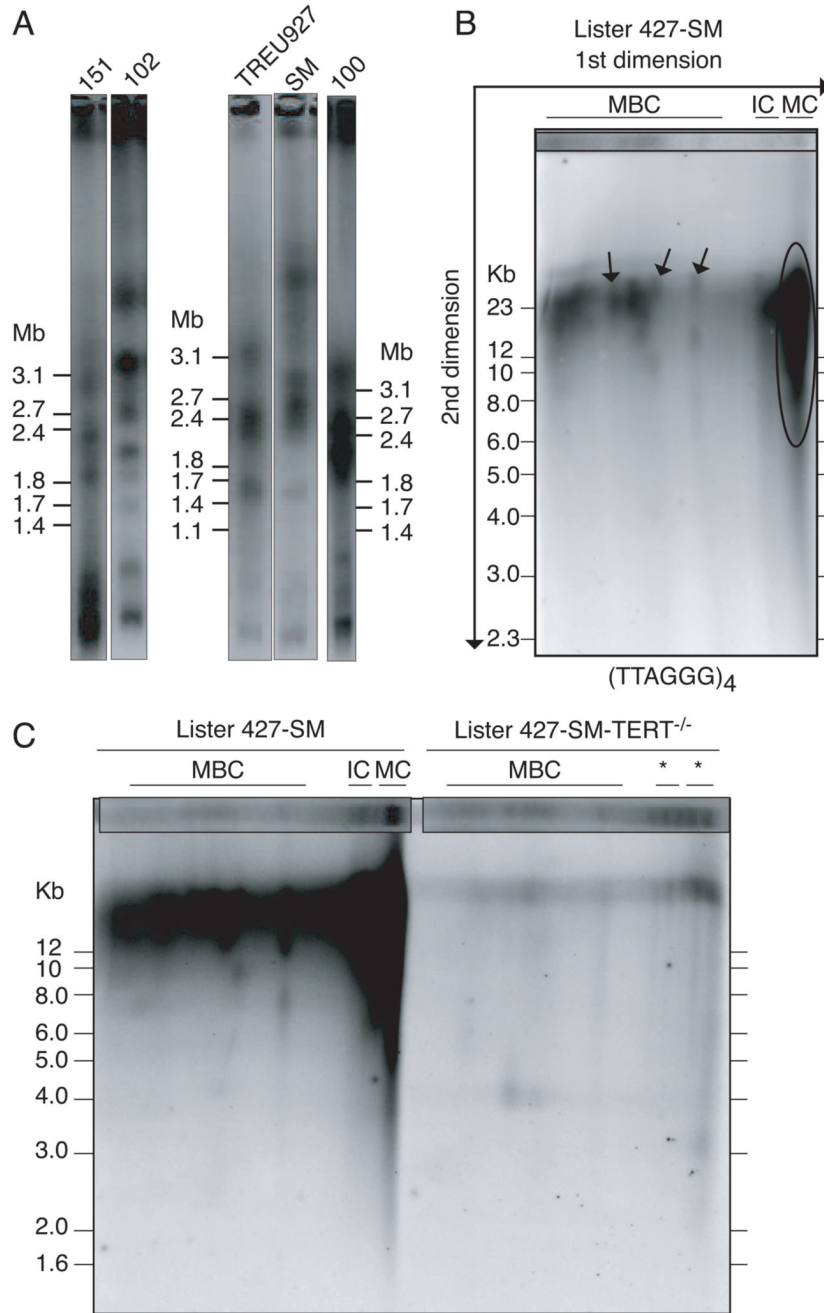
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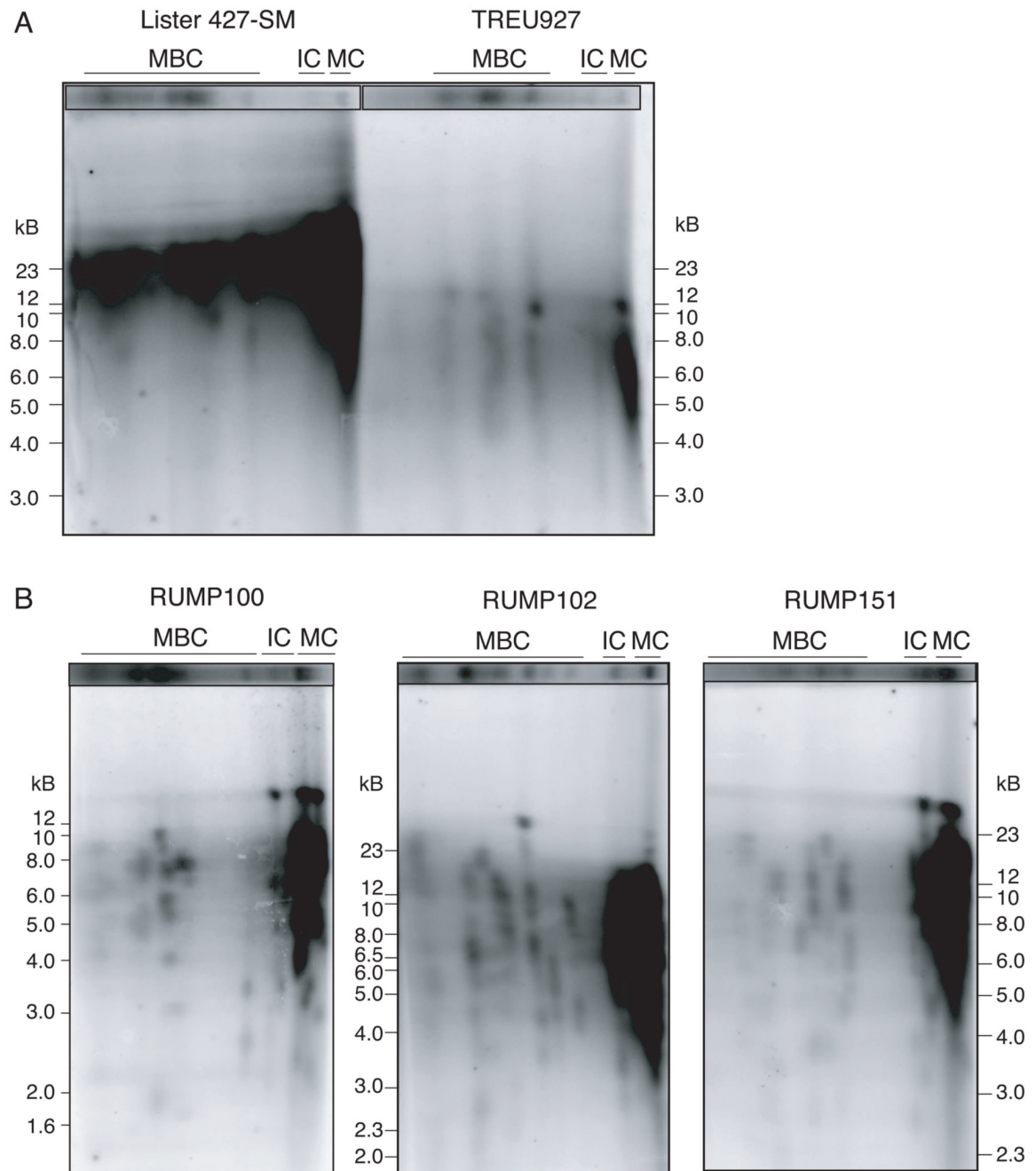
**Fig. 1.**

Analysis of telomere length in recently isolated and laboratory-adapted *T. brucei*. Genomic DNA from laboratory-adapted strains and wild-type isolates was digested with AluI plus MboI and separated on an 0.8% agarose gel. Telomere restriction fragments were visualized by in-gel hybridization using a radiolabeled (TTAGGG)<sub>4</sub> probe. (A) Lister 427 clones RUMP150 and 501 have long telomeres (average ~15 kB). Less propagated isolates (clones RUMP102, 106, 151, 502 and 100) have shorter telomeres (average ~8–10 kB). (B) Upper panel: telomere size distribution as a percentage of total telomere signal. Lower panel: average telomere size distribution of two laboratory-adapted clones (RUMP150 and 501) and five recent isolates (RUMP102, 106, 151, 502 and 100). (C) Comparison of telomere length between recently laboratory-adapted TREU927 and extensively propagated Lister 427 SM clone. Despite equal loading, signal intensity for TREU927 is lower, due to its shorter telomere tracts. Please note that the resolving power of agarose gels at sizes larger than ~15 kB is limited, which is the average telomere length in Lister 427 SM (Munoz-Jordan, et al., 2001). Size markers are as indicated.



**Fig. 2.** Telomere size distribution among chromosome classes analyzed by 2-dimensional gel electrophoresis. (A) Chromosome size polymorphism among *T. brucei* clones RUMP151, 102, 100, TREU927 and Lister 427 SM. Chromosomes were separated by Rotating Agarose Gel Electrophoresis and stained with Ethidium bromide. (B) Entire lanes from (A) were digested with a mixture of MboI, AluI and RsaI, and perpendicularly embedded in 0.8% agarose to separate telomere terminal restriction fragments (Ethidium-bromide-stained chromosomal DNA profiles were graphically inserted on top of the gel). Telomere restriction fragments were visualized using a (TTAGGG)<sub>4</sub>-probe. MBC are well separated (arrows) whereas IC and MC (circled) are not. Exposure time was 6 h. (C) Direct comparison of Lister 427-SM parental and

extensively propagated telomerase-deficient clone. Exposure time was 15 hours. Size markers are as indicated. It is unclear whether the genomic rearrangements (\*) in the TERT null clone are derived from MC or IC.



**Fig. 3.** Telomere size distribution among chromosome classes of Lister 427-SM and several less propagated clones, analysed by two-dimensional gel electrophoresis. (A) SM and TREU927. Ethidium-bromide-stained chromosomal separations are shown on the top and serve as a loading indicator. Exposure time was ~48 hours. Size markers are indicated on the right. (B) RUMP100 (left), 102 (middle) and 151 (right). Exposure times were optimized to most effectively visualize individual MBC telomeres and telomere clusters.

**Table 1**  
**T. brucei**  
 strains and clone designations

Strain and clone	Origin	Year	References
<i>Minimally propagated clones</i>			
EATRO795-RUMP100/502	Kenya	1964	Onyango et al, 1966; Turner, 1997; Robinson et al., 1999.
S42-RUMP102	Tanzania	1966	Baker et al., 1967.
STIB246/348-RUMP106	Tanzania	1971	Geigy and Kauffmann, 1973.
s427/EATRO217-RUMP151	Uganda	1960	Cunningham and Vickerman, 1962.
<i>Intermediate laboratory-adapted clone</i>			
TREU927	Kenya	1969/70	Goedbloed et al., 1973; van Deursen et al., 2001; Berriman et al., 2005.
<i>Extensively propagated (origin unknown) Lister 427 clones</i>			
SM (bloodstream forms)			Wirtz et al., 1999.
29-13 (procyclic forms)			
RUMP150			
RUMP501			