Repression of polymerase I-mediated gene expression at *Trypanosoma brucei* telomeres

Lucy Glover & David Horn⁺

London School of Hygiene & Tropical Medicine, London, UK

The African trypanosome, *Trypanosoma brucei*, is a flagellated pathogenic protozoan that branched early from the eukaryotic lineage. Unusually, it uses RNA polymerase I (Pol I) for mono-telomeric expression of variant surface glycoprotein (VSG) genes in bloodstream-form cells. Many other subtelomeric VSG genes are reversibly repressed, but no repressive DNA sequence has been identified in any trypanosomatid. Here, we show that artificially seeded *de novo* telomeres repress Pol I-dependent gene expression in mammalian bloodstream and insect life-cycle stages of *T. brucei*. In a telomeric VSG expression site, repression spreads further along the chromosome and this effect is specific to the bloodstream stage. We also show that *de novo* telomere extension is telomerase dependent and that the rate of extension correlates with the expression level of the adjacent gene. Our results show constitutive telomeric repression in *T. brucei* and indicate that an enhanced, developmental stage-specific repression mechanism controls antigenic variation.

Keywords: antigenic variation; silencing; trypanosome; variant surface glycoprotein

EMBO reports (2006) 7, 93–99. doi:10.1038/sj.embor.7400575

INTRODUCTION

Silent genes encoding variant surface molecules have a striking subtelomeric arrangement in several pathogens that undergo antigenic variation (reviewed by Barry *et al*, 2003), whereas telomeric DNA is known to induce local gene repression in yeast (Gottschling *et al*, 1990; Nimmo *et al*, 1994), *Drosophila* (Levis *et al*, 1985) and humans (Baur *et al*, 2001). Generally, these effects spread only a short distance from the telomere, but in some cases, repression has an impact on native genes, such as the mating-type loci in *Saccharomyces cerevisiae* (Maillet *et al*, 1996) and genes encoding variant surface antigens involved in adhesion in *S. cerevisiae* (Halme *et al*, 2004) and in the pathogenic yeast, *Candida glabrata* (De Las Penas *et al*, 2003).

Trypanosomatids branched early from the eukaryotic lineage and gave rise to several important pathogens including *Trypanosoma*

brucei. These flagellated protozoa are spread between mammals by tsetse flies and cause human African trypanosomiasis, also known as sleeping sickness, and a cattle disease known as Nagana. They escape elimination by the host immune response through a continual process of antigenic variation that relies on a sophisticated mechanism of variant surface glycoprotein (VSG) gene expression. Of up to 2000 VSGs and VSG fragments in the genome, essentially all are subtelomeric such that most, if not all, telomeres seem to be occupied by VSGs. There are two principal subsets of VSG promoters. The 'metacyclic' set, typically within ~5 kbp of telomeres, operates during the earliest phase of an infection, whereas the 'bloodstream' set, ~50 kbp from telomeres, operates later during infection (Barry *et al*, 2003). Although there are ~40 VSG promoters per cell, expression is monoallelic in bloodstream-form cells (reviewed by Barry *et al*, 2003).

Gene expression has unusual features in trypanosomatids. Polycistronic transcription is widespread and every messenger RNA has an identical *trans*-spliced leader. This polymerase II (Pol II) transcript (Gilinger & Bellofatto, 2001) provides a 5' cap such that other polymerases may produce the remainder of the mRNA. Indeed, VSG genes are transcribed by Pol I, a polymerase confined to ribosomal RNA (RRNA) in other eukaryotes (Gunzl *et al*, 2003). The single active telomeric VSG localizes to an 'expression site body', an extranucleolar structure enriched in Pol I (Navarro & Gull, 2001), and limited occupancy within this structure has been proposed to restrict VSG expression (reviewed by Borst, 2002). The system is reminiscent of odorant receptor choice in olfactory sensory neurons (Li *et al*, 2004), but in *T. brucei*, rather than a 'one-protein rule' a 'one-telomere rule' seems to operate. Thus, the active VSG is always adjacent to a telomere. Although a second VSG inserted at the active expression site can be coexpressed (Muñoz-Jordán *et al*, 1996), simultaneous activation of two telomeres does not seem to be possible (Chaves *et al*, 1999). We have explored the relationship between gene repression and telomeric location in *T. brucei*. We show that telomeres repress Pol I transcription locally. Repression is enhanced within a VSG expression site, specifically in the life-cycle stage that undergoes antigenic variation.

RESULTS

De novo telomeres at VSG expression sites

In *T. brucei*, DNA rearrangement occurs almost exclusively by homologous recombination, and DNA fragments with terminal G-rich sequences can seed *de novo* telomeres (Horn *et al*, 2000),

London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK
⁺Corresponding author. Tel: +44 20 7927 2352; Fax: +44 20 7636 8739;
E-mail: david.horn@lshtm.ac.uk

Received 10 August 2005; revised 15 September 2005; accepted 10 October 2005;
published online 25 November 2005

Fig 1 | *De novo* telomeres at VSG loci. (A) The polycistronic VSG expression site extends from the VSG promoter (P_{VSG}) to several kilobase pairs of terminal T_2AG_3 repeats. pTMF, cut with *Sma*I (*Sm*) or *Sma*I plus *Bsp*120I (*Bs*), can be used to replace the native telomere with telomere seeds 5 and 2 kbp from the *RRNA* promoter (P_{RRNA}), respectively. 'GC' indicates the location of a conserved GC-rich element (see below). PCR assays using a T_2AG_3 repeat primer and an internal primer (see Methods) indicate that *de novo* telomeres are generated at the expected locations (inset). (B) *De novo* telomere extension. DNA extracted from cells 20 generations after transfection and a month later was digested with *Xho*I (*Xh*) or *Xho*I plus *Sca*I (*Sc*; middle panel) before Southern blotting. The probes (see the pTMF map in (A)) used for hybridization are indicated on the blots. Similar hybridization patterns with *NPT* or *VSG* probes confirm integration at the VSG locus.

facilitating targeted chromosome fragmentation. The telomere-mediated fragmentation construct used here (pTMF; Fig 1A) can be cleaved to generate an array of T_2AG_3 repeats or a short G-rich sequence immediately adjacent to a double-strand break, and both of these 'telomere seeds' allow reproducible generation of *de novo* telomeres in *T. brucei* (Horn *et al*, 2000). Thus, we can use pTMF to generate *de novo* telomeres at two different positions. Also, an *RRNA* promoter (Schimanski *et al*, 2004) driving *NPT* expression permits selection of cells that express *NPT* at only a low level (Horn & Cross, 1997b). The *RRNA* promoter recruits Pol I, as does the *VSG* promoter (Gunzl *et al*, 2003). Indeed, it can replace the *VSG* promoter and can still be switched on and off during antigenic variation in bloodstream-form cells (Rudenko *et al*, 1995). Thus, the *RRNA* promoter will reflect the presence or absence of Pol I cofactor-binding platforms.

We first generated *de novo* telomeres that replaced the native telomere at an active VSG locus (Fig 1A). A PCR assay was used to show that telomeres can be engineered 5 or 2 kbp from the promoter (Fig 1A, inset; the expected PCR products are illustrated; the cartoons shown are used throughout to illustrate promoter/telomere spacing). The extra 3 kbp of sequence in the '5 kbp' cells

is a plasmid vector sequence and there is no evidence that this DNA can block repression in *T. brucei* (see below) or in other cell types. Using another cell line expressing a VSG on another chromosome, we generated telomeres adjacent to a silent copy of the same VSG gene, as above.

In *T. brucei*, the number of telomeric DNA repeats at the chromosome end is not strictly regulated, but the rate of extension is normally limited to 8 bp per generation (Pays *et al*, 1983). Southern blotting indicates that the *NPT* gene is linked to the VSG gene as expected, and that adjacent *de novo* telomeres are rapidly extended when the active locus is targeted (Horn *et al*, 2000), but are slowly extended when the repressed locus is targeted (Fig 1B; see below).

Telomerase-dependent *de novo* telomere extension

Next, we determined whether the pTMF construct could be used to generate terminal truncations and *de novo* telomeres elsewhere in the genome. The *RRNA* promoter in the fragmentation construct may also serve as a targeting fragment. Thus, we targeted the *NPT* reporter cassette to *RRNA* loci (Fig 2A). PCR assays again indicated that *de novo* telomeres were generated at defined distances from the promoter (Fig 2A, inset). Four independent

Fig 2 | *De novo* telomeres at RRNA loci and telomere shrinkage after *TERT* disruption. (A) pTMF cut with *SalI* (Sa) is targeted to RRNA. As in Fig 1, another cut at *SmaI* (Sm) or *Bsp120I* (Bs) generates telomere seeds 5 and 2 kbp from the promoter, respectively. A PCR assay indicates that telomeres are generated as expected (inset). *Hp*, *HpaI*. (B,C) Southern blotting was carried out as in Fig 1B. (B) *De novo* telomere extension. Different hybridization patterns with NPT or VSG probes (left panels) confirm that the NPT gene is not linked to the VSG locus in these cells. (C) *TERT* disruption leads to telomere shrinkage. Genomic DNA was extracted at intervals of ~20 cell doublings before and after *TERT* disruption and was then digested with *HpaI* before Southern blotting.

clones were generated for further analysis, two with telomeres about 5 kbp from the promoter (R5^a and R5^b) and two with telomeres about 2 kbp from the promoter (R2^a and R2^b). As expected, Southern blotting indicated that telomeres formed at RRNA loci are not linked to VSG (Fig 2B, left panels).

To determine whether *de novo* telomeres were extended by a telomerase-dependent mechanism, we identified the putative telomerase reverse-transcriptase (*TERT*) gene in the *T. brucei* genome sequence. Both alleles of the gene were disrupted by deletion of the portion encoding residues 222–961 in R5 and R2 *de novo* telomere cells and in otherwise unmodified cells. Correct disruption was confirmed in all three cell types using a PCR assay (data not shown). We were unable to generate *de novo* telomeres in cells lacking *TERT* (data not shown), which suggested that initial telomere seeding is *TERT* dependent. Examination of both R5 and R2 cells indicated that telomeres are extended before *TERT* disruption, whereas length diminishes after *TERT* disruption (Fig 2C). This can be seen more clearly at the shorter R2 telomere (Fig 2C, right panel) in which length is uniformly reduced in the population. Telomere loss in these cells is consistent with the recently reported rate of loss of ~4 bp per cell division at native

telomeres in the absence of *TERT* (Dreesen *et al*, 2005). We conclude that both *de novo* telomere seeding and subsequent extension are *TERT* dependent.

De novo telomeres support VSG regulation

We first analysed VSG expression in clones with *de novo* telomeres at VSG221 loci. First, immunofluorescence analysis, using VSG antisera, indicated that active and silent VSG221 genes maintain their expression status when fused to *de novo* telomeres (Fig 3, top left). In a previous work, selection for cells with increased NPT expression indicated that antigenic variation and coactivation of a selectable marker proximal to a native telomere occurred in $<1 \times 10^6$ cells per division (Horn & Cross, 1997a). Cells that activate the current *de novo* telomere-associated VSG could be similarly selected (Fig 3, right-hand side). In insect-stage cells, VSG is not normally expressed. After differentiation to this stage, VSG genes did not produce any detectable VSG surface labelling irrespective of their prior expression status (Fig 3, bottom left). Thus, *de novo* telomeres do not interrupt VSG expression or repression, VSG activation during antigenic variation or VSG regulation during differentiation.

Fig 3 | *De novo* telomeres support VSG regulation. Schematic maps indicate VSG transcription (dashed line) in bloodstream-form cells with *de novo* telomeres at active and repressed VSG expression sites. Immunofluorescence signals representing VSG221 (red), VSG118 (green) and DNA (blue) were merged in all images. Scale bar, 5 μ m. The box at the bottom right-hand corner shows a schematic 'growth curve', illustrating how antigenically variant cells were selected, and a control for detection of cells expressing VSG118 (green), VSG221 (red) or both (yellow).

Telomeric and VSG-associated repression

We next examined the influence of telomere proximity on *NPT* reporter expression using three different assays: immunofluorescence analysis, western blotting and G418 growth-inhibitory concentration (IC_{50}) analysis. Immunofluorescence analysis indicated that *NPT* expression mirrored VSG expression at active and repressed VSG loci (Fig 4A, left panels). An examination of reporter expression at *RRNA* loci indicated that the R2 population (Fig 4A, top right panel) consistently expressed *NPT* at a low level but that expression was increased when the reporter was further from the telomere, as in R5 cells (bottom right panel). Although heterogeneous, *NPT* expression was consistently higher in most R5 cells relative to R2 cells. Similar results were obtained following G418 selection or further telomere extension (data not shown). An *NPT* western blot assay and IC_{50} assays (Fig 4B) confirmed that *NPT* expression is inversely proportional to telomere proximity (compare R5 and R2 lanes). Taken together, the data suggest that telomeric repression is rapidly diminished only a short distance from the telomere at *RRNA* loci. In contrast to repression at *RRNA* loci, repression at a VSG locus is strong when the promoter is 5 kbp from the telomere (Fig 4B, compare repressed VSG and R5 lanes). Indeed, repression within a VSG expression site was previously shown to extend over a greater distance (Horn & Cross, 1997b). We conclude that telomeres mediate repression independent of expression site context, and that additional repression operates within VSG expression sites.

Repression adjacent to VSG genes at native telomeres is developmentally regulated and seems to be bloodstream-form specific (Horn & Cross, 1995). Our analysis indicated that this was also the case in an expression site with a *de novo* telomere (Fig 4C). Thus, VSG expression site-associated repression, which is strong at 5 kbp from the telomere in the bloodstream form, was abolished in insect-stage cells and the expression level was the same whether the *NPT* gene was linked to the previously active or silent VSG gene (compare 'from active' and 'from repressed' in Fig 4C with 'active VSG' and 'repressed VSG' in Fig 4B, respectively). In striking contrast, telomeric repression, within 2 kbp of the telomere, continued to operate when cells were differentiated to the insect stage (Fig 4C, compare R5 and R2 lanes). Thus, VSG expression site-associated repression is bloodstream-form specific whereas telomeric repression is constitutive.

Telomere extension rate correlates with gene expression

Telomere extension at native telomeres, whether associated with silent or active VSG genes, is limited to about 8 bp per cell division (Pays *et al*, 1983). To examine the relationship between TERT-dependent *de novo* telomere extension and gene expression, we estimated approximate extension rates from our Southern blot data (Figs 1B,2B) and plotted these against IC_{50} values (Fig 3B) for the corresponding cells. The analysis shows that the rate of telomere extension correlates with adjacent gene expression level (Fig 5).

Fig 4 | Telomeric and VSG expression site-associated repression. (A) Immunofluorescence analysis of bloodstream-form cells. Images representing NPT (red) and DNA (green) were merged. Scale bar, 5 μ m. (B,C) Western blotting using anti-NPT II as a primary antibody. VSG on the blot, stained with amido black (B), or an equivalent gel stained with Coomassie blue (C), provides a loading control. (B) Analysis of bloodstream-form cell extracts. Corresponding IC₅₀ values (μ g/ml) are shown. (C) Analysis of insect-stage cell extracts.

DISCUSSION

No *cis*- or *trans*-acting repressor has been previously identified in a trypanosomatid. Here, we report *cis*-acting repression of Pol I transcription emanating from telomeric DNA in *T. brucei*. Our results greatly extend the phylogenetic range of organisms that

Fig 5 | *De novo* telomere extension rate correlates with gene expression.

show telomeric gene repression, suggesting either that this phenomenon had an ancient origin or that it is a general feature of chromosome ends. Similar to the situation in *S. cerevisiae*, basal telomeric repression seems to be confined to a relatively limited telomeric domain. In bloodstream-form cells, however, in which VSG repression is crucial to the survival of the parasite, repression extends further. This developmentally regulated spread of repression may represent an elaboration of 'simple' telomeric repression, but at this stage we cannot rule out the possibility that telomeric silencing and VSG expression site silencing are mechanistically distinct.

In *S. cerevisiae*, a specialized chromatin structure represses Pol II genes inserted at telomeres, mating-type loci or *RRNA*. At telomeres, transcription is blocked by repressive machinery recruited to the telomere and propagated further along the DNA. Nucleosomes modified by repressors allow transcription factors to access the DNA, but they block promoter clearance or transcription elongation (reviewed by Rusche *et al*, 2003). A similar mechanism is involved in silencing variant surface antigen genes up to 55 kbp from the telomere in *Plasmodium falciparum* (reviewed by Ralph & Scherf, 2005). Such a mechanism may also act on Pol I in *T. brucei*.

The current results also show that the rate of telomerase-dependent *de novo* telomere extension is proportional to the level of local gene expression (when a promoter is directed towards the telomere). Irrespective of length, telomere extension is limited to ~ 8 bp per cell division at transcriptionally silent *T. brucei* telomeres. In contrast, at the active expression site, extension is slightly faster at native telomeres (Pays *et al*, 1983) and initially much faster at *de novo* telomeres but inversely proportional to telomere length (Horn *et al*, 2000). We suggest a negative feedback mechanism for telomere length control at the active locus, whereby transcription increases exposure to the action of telomerase. The effect would be diminished as the terminus or terminal t-loop (Muñoz-Jordán *et al*, 2001) moves further from the promoter, possibly owing to transcription attenuation brought about by DNA base modification found in *T. brucei* telomeric DNA (van Leeuwen *et al*, 1998). This model is consistent with transcription of T₂AG₃ repeats in *T. brucei* (Rudenko & van der Ploeg, 1989) and may explain relatively large increases in telomere length seen at activated expression sites (Myler *et al*, 1988). A similar model could explain longer than average

telomeres at truncated chromosomes in the malaria parasite, *P. falciparum* (Figueiredo *et al*, 2002), but transcription does not correlate with the increased preference of telomerase for shorter telomeres seen in *S. cerevisiae* (Sandell *et al*, 1994; Teixeira *et al*, 2004).

We have shown telomeric repression of RNA Pol I transcription in *T. brucei*. Our data also indicate pronounced repression associated with VSG expression sites, a developmentally regulated effect likely to have a specific role in antigenic variation. In yeasts, native subtelomeric sequences generally limit the spread of repression (Pryde & Louis, 1999), but silencing can be propagated by subtelomeric relay elements to control the expression of a subset of genes (Maillet *et al*, 1996; De Las Penas *et al*, 2003; Halme *et al*, 2004). A similar propagation mechanism may operate in *T. brucei*. A potential *T. brucei* telomere-associated 'relay' element retained at the VSG-associated *de novo* telomeres described here is the GC-rich element (location indicated in Fig 1). This element contains a highly conserved T₂AG₃ repeat-related core and is found between VSG genes and terminal T₂AG₃ repeats within all subtelomeres examined so far (Horn & Barry, 2005). Thus, the potential for gene repression at *T. brucei* telomeres may have been usurped for VSG gene silencing to facilitate mono-allelic VSG expression and antigenic variation. Further genome manipulation should allow us to determine whether telomeres and/or GC-rich elements are involved in VSG expression site repression, and genome sequence data (Berriman *et al*, 2005) should facilitate identification of *trans*-acting repressors.

METHODS

Cells. Bloodstream-form *T. brucei* cells of the Lister 427 strain, clone 221a (MITat 1.2) and clone 118a (MITat 1.5), were maintained, cloned, genetically modified and differentiated, as described previously (Horn & Cross, 1997a).

Plasmid construction. See the supplementary information online.

DNA analysis. PCR assays were carried out using the Tel2 primer (5'-acccta-3')₅ paired with either NEO3 (5'-cgcttcttgacgagtct-3') or Vheal (5'-cccactacgtgaaccatca-3') primers, as described (Horn *et al*, 2000). A month of continuous culture represents approximately 50 generations for 118a cells and 100 generations for 221a cells.

Protein analysis. Primary anti-NPT-II was from Europa Bioproducts Ltd (Cambridge, UK). Antisera for VSG immunofluorescence were rat anti-VSG221 and mouse anti-VSG118.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

We thank R. Sabatini, S. Alsford, J. Kelly, M. Taylor and N. Dorrell for critical reading of the manuscript. We also thank G. Cross for the valuable gift of antibodies against well-characterized VSGs. This work was supported by a Research Career Development Fellowship to D.H. (052323) and a project grant (069909) from The Wellcome Trust.

REFERENCES

- Barry JD, Ginger ML, Burton P, McCulloch R (2003) Why are parasite contingency genes often associated with telomeres? *Int J Parasitol* **33**: 29–45
- Baur J, Zou Y, Shay J, Wright W (2001) Telomere position effect in human cells. *Science* **292**: 2075–2077
- Berriman M *et al* (2005) The genome of the African trypanosome *Trypanosoma brucei*. *Science* **309**: 416–422

- Borst P (2002) Antigenic variation and allelic exclusion. *Cell* **109**: 5–8
- Chaves I, Rudenko G, Dirks Mulder A, Cross M, Borst P (1999) Control of variant surface glycoprotein gene-expression sites in *Trypanosoma brucei*. *EMBO J* **18**: 4846–4855
- De Las Penas A, Pan SJ, Castano I, Alder J, Cregg R, Cormack BP (2003) Virulence-related surface glycoproteins in the yeast pathogen *Candida glabrata* are encoded in subtelomeric clusters and subject to RAP1- and SIR-dependent transcriptional silencing. *Genes Dev* **17**: 2245–2258
- Dreesen O, Li B, Cross GAM (2005) Telomere structure and shortening in telomerase-deficient *Trypanosoma brucei*. *Nucleic Acids Res* **33**: 4536–4543
- Figueiredo LM, Freitas-Junior LH, Bottius E, Olivo-Marin JC, Scherf A (2002) A central role for *Plasmodium falciparum* subtelomeric regions in spatial positioning and telomere length regulation. *EMBO J* **21**: 815–824
- Gilinger G, Bellofatto V (2001) Trypanosome spliced leader RNA genes contain the first identified RNA polymerase II gene promoter in these organisms. *Nucleic Acids Res* **29**: 1556–1564
- Gottschling DE, Aparicio OM, Billington BL, Zakian VA (1990) Position effect at *S. cerevisiae* telomeres: reversible repression of pol II transcription. *Cell* **63**: 751–762
- Gunzl A, Bruderer T, Laufer G, Schimanski B, Tu LC, Chung HM, Lee PT, Lee MG (2003) RNA polymerase I transcribes procyclin genes and variant surface glycoprotein gene expression sites in *Trypanosoma brucei*. *Eukaryot Cell* **2**: 542–551
- Halme A, Bumgarner S, Styles C, Fink GR (2004) Genetic and epigenetic regulation of the FLO gene family generates cell-surface variation in yeast. *Cell* **116**: 405–415
- Horn D, Barry JD (2005) The central roles of telomeres and subtelomeres in antigenic variation in African trypanosomes. *Chromosome Res* **13**: 525–533
- Horn D, Cross GAM (1995) A developmentally regulated position effect at a telomeric locus in *Trypanosoma brucei*. *Cell* **83**: 555–561
- Horn D, Cross GAM (1997a) Analysis of *Trypanosoma brucei* VSG expression site switching *in vitro*. *Mol Biochem Parasitol* **84**: 189–201
- Horn D, Cross GAM (1997b) Position-dependent and promoter-specific regulation of gene expression in *Trypanosoma brucei*. *EMBO J* **16**: 7422–7431
- Horn D, Spence C, Ingram AK (2000) Telomere maintenance and length regulation in *Trypanosoma brucei*. *EMBO J* **19**: 2332–2339
- Levis R, Hazelrigg T, Rubin GM (1985) Effects of genomic position on the expression of transduced copies of the white gene of *Drosophila*. *Science* **229**: 558–561
- Li J, Ishii T, Feinstein P, Mombaerts P (2004) Odorant receptor gene choice is reset by nuclear transfer from mouse olfactory sensory neurons. *Nature* **428**: 393–399
- Maillet L, Boscheron C, Gotta M, Marcand S, Gilson E, Gasser SM (1996) Evidence for silencing compartments within the yeast nucleus: a role for telomere proximity and Sir protein concentration in silencer-mediated repression. *Genes Dev* **10**: 1796–1811
- Muñoz-Jordán JL, Davies KP, Cross GAM (1996) Stable expression of mosaic coats of variant surface glycoproteins in *Trypanosoma brucei*. *Science* **272**: 1795–1797
- Muñoz-Jordán JL, Cross GAM, de Lange T, Griffith JD (2001) t-loops at trypanosome telomeres. *EMBO J* **20**: 579–588
- Myler PJ, Aline RF, Scholler JK, Stuart KD (1988) Changes in telomere length associated with antigenic variation in *Trypanosoma brucei*. *Mol Biochem Parasitol* **29**: 243–250
- Navarro M, Gull K (2001) A poll transcriptional body associated with VSG mono-allelic expression in *Trypanosoma brucei*. *Nature* **414**: 759–763
- Nimmo ER, Cranston G, Allshire RC (1994) Telomere-associated chromosome breakage in fission yeast results in variegated expression of adjacent genes. *EMBO J* **13**: 3801–3811
- Pays E, Laurent M, Delinte K, vanMeirvenne N, Steinert M (1983) Differential size variations between transcriptionally active and inactive telomeres of *Trypanosoma brucei*. *Nucleic Acids Res* **11**: 8137–8147
- Pryde FE, Louis EJ (1999) Limitations of silencing at native yeast telomeres. *EMBO J* **18**: 2538–2550
- Ralph SA, Scherf A (2005) The epigenetic control of antigenic variation in *Plasmodium falciparum*. *Curr Opin Microbiol* **8**: 434–440
- Rudenko G, van der Ploeg LH (1989) Transcription of telomere repeats in protozoa. *EMBO J* **8**: 2633–2638

- Rudenko G, Blundell PA, Dirks-Mulder A, Kieft R, Borst P (1995) A ribosomal DNA promoter replacing the promoter of a telomeric VSG gene expression site can be efficiently switched on and off in *T. brucei*. *Cell* **83**: 547–553
- Rusche LN, Kirchmaier AL, Rine J (2003) The establishment, inheritance, and function of silenced chromatin in *Saccharomyces cerevisiae*. *Annu Rev Biochem* **72**: 481–516
- Sandell LL, Gottschling DE, Zakian VA (1994) Transcription of a yeast telomere alleviates telomere position effect without affecting chromosome stability. *Proc Natl Acad Sci USA* **91**: 12061–12065
- Schimanski B, Laufer G, Gontcharova L, Gunzl A (2004) The *Trypanosoma brucei* spliced leader RNA and rRNA gene promoters have interchangeable TbSNAP50-binding elements. *Nucleic Acids Res* **32**: 700–709
- Teixeira MT, Arneric M, Sperisen P, Lingner J (2004) Telomere length homeostasis is achieved via a switch between telomerase-extendible and -nonextendible states. *Cell* **117**: 323–335
- van Leeuwen F, Kieft R, Cross M, Borst P (1998) Biosynthesis and function of the modified DNA base β -D-glucosyl-hydroxymethyluracil in *Trypanosoma brucei*. *Mol Cell Biol* **18**: 5643–5651