Send Orders for Reprints to reprints@benthamscience.ae

Current Genomics, 2018, *19*, 119-132

REVIEW ARTICLE

Transcriptional Regulation of Telomeric Expression Sites and Antigenic Variation in Trypanosomes

Igor Cestari[1,*] and Ken Stuart[1,2]

[1]*Center for Infectious Disease Research, Seattle, WA 98109, USA;* [2]*Department of Global Health, University of Washington, Seattle, WA 98195, USA*

ARTICLE HISTORY

Received: March 23, 2017
Revised: April 03, 2017
Accepted: May 04, 2017

DOI:
10.2174/1389202918666170911161831

Abstract: ***Introduction*:** *Trypanosoma brucei* uses antigenic variation to evade the host antibody clearance by periodically changing its Variant Surface Glycoprotein (VSGs) coat. *T. brucei* encode over 2,500 VSG genes and pseudogenes, however they transcribe only one VSG gene at time from one of the 20 telomeric Expression Sites (ESs). VSGs are transcribed in a monoallelic fashion by RNA polymerase I from an extranucleolar site named ES body. VSG antigenic switching occurs by transcriptional switching between telomeric ESs or by recombination of the VSG gene expressed. VSG expression is developmentally regulated and its transcription is controlled by epigenetic mechanisms and influenced by a telomere position effect.

***Conclusion*:** Here, we discuss 1) the molecular basis underlying transcription of telomeric ESs and VSG antigenic switching; 2) the current knowledge of VSG monoallelic expression; 3) the role of inositol phosphate pathway in the regulation of VSG expression and switching; and 4) the developmental regulation of Pol I transcription of procyclin and VSG genes.

Keywords: Trypanosoma, Antigenic variation, Variant surface glycoproteins, Transcriptional regulation, RNA polymerase I, Epigenetic, Telomere position effect, Allelic exclusion.

1. INTRODUCTION

Trypanosoma brucei sp. are protozoa parasites that cause Human African Trypanosomiasis, also known as sleeping sickness, and Nagana in cattle resulting in devastating health and economic burdens in sub-Saharan Africa [1]. *T. brucei* alternate between a mammalian host and the tsetse fly vector (Fig. **1**). In mammals, *T. brucei* proliferate as slender Bloodstream Forms (BFs) and employ antigenic variation by periodically switching its Variant Surface Glycoprotein (VSG) surface coat to avoid host antibody clearance. *T. brucei* have a large repertoire of ~2,500 VSG genes and pseudogenes [2, 3]; however, they only express one VSG gene at a time from one of the 20 telomeric expression sites (ES) (Fig. **2**). The VSG gene is unusually transcribed by Pol I in a monoallelic fashion from an extranucleolar Pol I focus [4-6], and VSG antigenic switching occurs by transcriptional switching between telomeric ESs or by recombination of the VSG gene expressed [7-9].

Proliferating slender BFs develop into non-dividing short stumpy forms [10]. After transmission to the fly, stumpy BFs develop in procyclic forms (PFs) and replace their VSG surface coat with procyclins [11, 12], which are also transcribed by Pol I [4, 6]. PFs develop into epimastigotes, accompanied by changes in the type of procyclins expressed on the surface, *i.e.* EPs and GPEETs [11, 13, 14]. In the insect salivary gland, epimastigotes develop into mammalian infectious metacyclic forms which replace the procyclin surface coat with VSGs, which are expressed from telomeric metacyclic ESs (MESs) [15, 16]. These changes in surface coat expression occur in parallel with other developmental changes that include alterations in cell morphology and metabolism, such as the use of glycolysis and oxidative phosphorylation for the main source of energy by the mammalian and insect forms, respectively [17]. Hence, VSG expression is developmentally regulated throughout the life cycle. The precise regulation of VSG and procyclin expression is thus critical for the successful transmission and infection of *T. brucei*.

Here, we discuss 1) the molecular basis underlying transcription of telomeric ESs and antigenic switching in *T. brucei*; 2) the current understanding of VSG monoallelic expression; 3) inositol phosphate regulation of VSG expression and switching; and 4) the developmental regulation of Pol I transcription during *T. brucei* life cycle progression.

2. GENOMIC STRUCTURE OF TELOMERIC EXPRESSION SITES AND MECHANISMS OF ANTIGENIC SWITCHING

The *T. brucei* genome contains 11 diploid megabase-sized chromosomes (1-6 Mbp), ~5 intermediate-sized chromosomes (200-900 Kbp), and ~100 minichromosomes (50-

*Address correspondence to this author at the Center for Infectious Disease Research, Seattle, WA 98109, USA; Tel: (206) 256-7351; Fax: (206) 256-7229; E-mail: igor.cestari@cidresearch.org

1389-2029/18 $58.00+.00

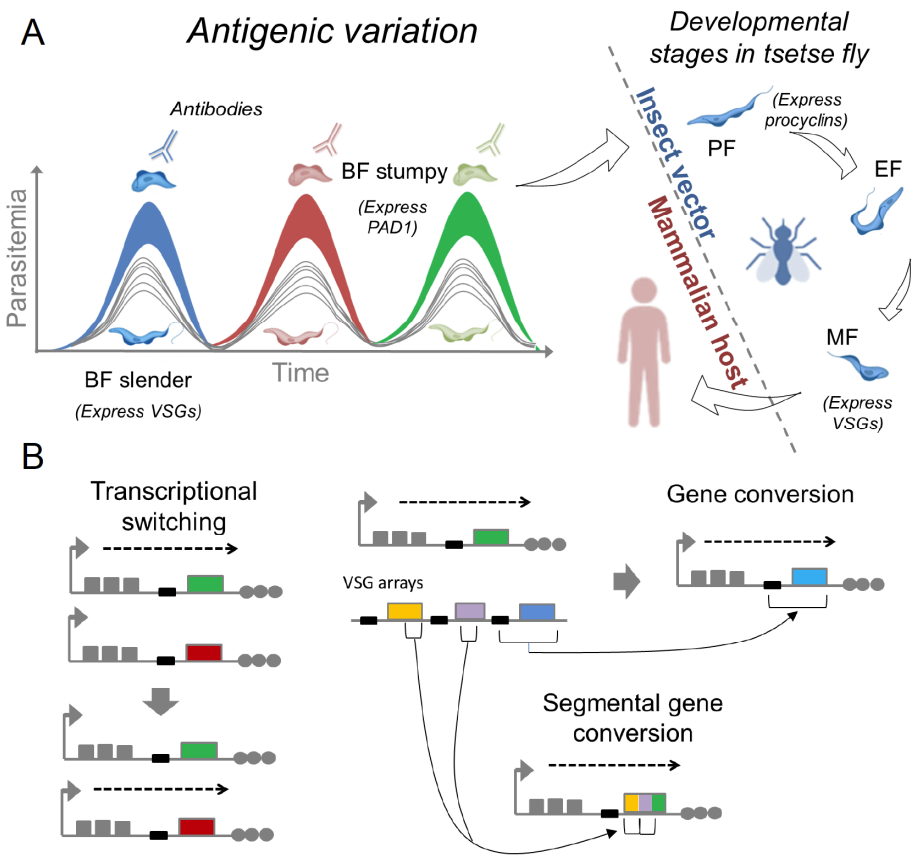

Fig. (1). *T. brucei* life cycle and mechanisms of antigenic variation. A) *T. brucei* slender BFs periodically switch its VSG coat to evade antibody clearance. Parasites expressing different individual VSGs (gray lines) may occur at the same time during infection [58, 153]. Colored thicker lines indicate combined parasitemia. Antibodies against variant types are produced periodically. At high density, slenders differentiate to non-dividing stumpies, which after uptake by tsetse flies develop into PFs in the fly midgut and into Epimastigotes Forms (EF) and then to Metacyclic Forms (MF) in the fly's salivary glands. PFs and EFs express procyclins but MFs resume VSG expression and are infective to mammals. A sexual stage may occur between EF and MF [154]. **B**) Antigenic variation occurs by transcriptional switching between telomeric ESs or by recombination of VSG genes. Recombination may happen by gene conversion or segmental gene conversion between ES VSG genes and VSG genes or pseudogenes at other loci including intrachromosomal VSG arrays and minichromosomes. (*For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.*)

150 Kbp) [3] (Fig. **2**). The megabase chromosomes contain arrays of protein-coding genes that are organized in long polycistronic units that are separated by Strand Switch Regions (SSRs) [3, 18-20]. Arrays of VSG genes or pseudogenes comprise ~20% of the genome [3]. They are located at subtelomeric sites and at telomeric ESs, and an archive of VSG genes and pseudogenes also exists in intermediate and minichromosomes [2, 7]. Almost all *T. brucei* genes lack introns and the polycistronic primary transcripts are processed into mRNAs by trans-splicing, *i.e.* the addition of a 5' 39 nucleotide splice leader sequence, and polyadenylation [21]. Pol II transcription is constitutive and Pol II promoters have not been identified [22, 23], with one exception [24]. Pol II transcription starts at SSRs that are either between two divergent polycistronic units or at the distal ends of convergent polycistronic units [18, 19, 25-27] (Fig. **2A**). Epigenetic marks, *e.g.* base J and H3 variant (H3.V) and H4 variant (H4.V), are located at the boundaries of the transcription units and have been implicated in Pol II transcription termination [28-32]. In contrast, procyclin and ribosomal RNAs (rRNA) genes are located intrachromosomally in smaller polycistronic units that are transcribed by Pol I [3, 4].

The telomeric ESs of the *T. brucei* 427 strain are in megabase and intermediate chromosomes [33-36]. There are ~15 Bloodstream ESs (BESs) and ~5 MESs in the 427 strain [2, 34, 37], and their overall organization is conserved among ESs of other strains [33, 38] (Fig. **2B**). The VSG gene is located within ~1 kb of the telomeric repeats, whereas the ES promoter is located between 40 - 60 kbp upstream of the VSG gene [34, 35]. Upstream of the VSG gene there is an array of 70 bp repeats (~1-20 kb) followed by Expression Site Associated Genes (ESAGs). In contrast, MESs are monocistronic units that only contain the VSG gene and a Pol I promoter [37]. The ESAG genes encode transferrin receptors [39] and adenylate cyclases [40] but not all ESAGs have an assigned function [41, 42], although some are implicated in parasite development and host interaction [40, 43-45].

There are also differences in the sequence of BESs, MESs, procyclins, and rRNA promoters [46-52]. The procyclin and rRNA promoters are longer (~250 bp) and contain within their 5' 70 bps, two regions that are essential for promoter activity [49, 52], whereas BES promoters are shorter (~70 bp) with two regions that are involved in the recruitment of Pol I basal transcription factors [46, 47, 49, 53].

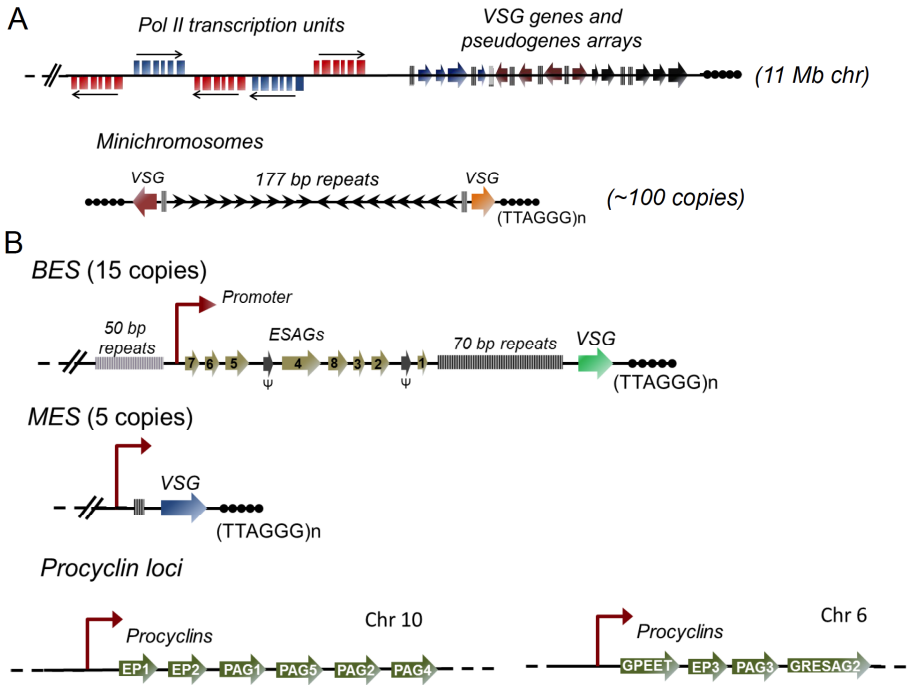

Fig. (2). Genomic organization of Pol I and Pol II transcribed genes in *T. brucei*. **A**) Pol II transcribed genes are organized in long co-transcribed gene clusters that are separated by SSRs in 11 megabase (Mb) size chromosomes (chr). Arrows indicate direction of Pol II transcription. VSG genes or pseudogenes are organized in subtelomeric regions or megabase chromosomes or at the telomeres of minichromosomes. **B**) Telomeric BESs and MESs and procyclin loci. BESs contain ESAG genes which are located between the promoter (arrow) and 70 bp repeats. Pseudogenes are indicated by ψ. VSG gene are near telomeric repeats. MES lack ESAGs and are expressed in MFs. PF express procyclins from two procyclin loci which encode *EPs* or *GPEET* procyclins.

Moreover, only very short sequences within these regions are conserved between the ES, procyclin, and rRNA promoters [49]. MES promoters (~87 bp) appear to have a less conserved sequence [50, 51, 54]. Despite the differences, BES or procyclin promoters can be functionally replaced by an rRNA promoter which is subject to regulation that is influenced by chromosome location and developmental stage [51, 52, 55].

Antigenic switching can occur by transcriptional switching between telomere ESs or homologous recombination of the VSG gene expressed [56] (Fig. **1B**). Transcriptional switching involves the transcriptional activation of a silent ES and concomitant repression of the active ES, and hence does not involve changes in DNA sequence [8, 9]. At least three types of recombination are implicated in VSG switching: VSG gene conversion, reciprocal VSG gene recombination, and segmental VSG gene conversion [8, 56-60]. The large repertoire of VSG genes in the subtelomeric arrays as well as in minichromosomes is a source of VSG sequences for recombination [57, 61]. In VSG gene conversion, a copy of a silent VSG open reading frame sequence replaces the VSG gene within the active ES [56, 57]. In reciprocal recombination, a VSG gene from a silent ES is exchanged with the VSG gene in the active ES, which unlike gene conversion does not result in a loss of VSG sequence [56, 62]. The conserved ES organization likely favors its recombination [56, 63] and the 70 bp repeats contribute to VSG switching by providing homologous sequences for recombination [63-65]. In segmental gene conversion only portions of a VSG gene or pseudogene are recombined with the VSG gene in the active ES, thereby resulting in new mosaics of VSG genes. These mechanisms have almost unlimited potential for generating new VSG sequences [58, 59, 66].

3. MOLECULAR BASIS UNDERLYING VSG ALLELIC EXCLUSION AND TELOMERE SILENCING

Several molecules have been identified that affect the transcription of VSG ESs (Table **1**). Some of these molecules regulate Pol I recruitment to the ES promoter, whereas others control assembly and remodeling of the ES chromatin or are associated with telomeres and contribute to the repression of transcription or switching of VSG genes (Fig. **3**). For clarity, we will discuss the roles of these molecules in the context of 1) Pol I recruitment and ES monoalellic transcription, 2) chromatin remodeling and Pol I elongation through the ES, and 3) repression of ES transcription by a telomere position effect.

3.1. Monoallelic ES Transcription and the Expression Site Body

The VSG gene is transcribed by Pol I in a monoallelic fashion from an extranucleolar site termed as the ES Body (ESB) [5]. Silent ESs lack detectable Pol I by immunofluorescence analysis, and the ESB is not detected in PFs [5, 67]. A single ESB is maintained during S and G2 phases and early mitosis [68]. Prior nuclear division, the ES sister chromatids are segregated with subsequent Pol I association with the new ES [68]. Knockdown of the cohesin complex subunit sister chromatid cohesion 1 (SSC1) affects Pol I co-localization with the active ES and results in transcriptional

Table 1. Genes involved in the regulation of *T. brucei* VSG gene expression or antigenic switching.

Gene Name Abbreviation[a]	Protein Function	Genetic Modification	Phenotype[b]
CIFTA subunit *1, 2* and *7* (CIFTA complex)	Basal transcription factor	RNA interference	Decrease expression of active VSG and rRNA genes [53, 73, 74, 155]
SIZ1 (SUMOylation)	Posttranslational modification	RNA interference	Decrease expression of active VSG and rRNA genes [75]
H1	Nucleosome assembly	RNA interference	Derepression of VSG and ESAG genes from silent BESs and procyclins genes [85, 91]
H3	Nucleosome assembly	RNA interference	Derepression of a reporter downstream a silent BES promoter [92]
H3V	Nucleosome assembly	Null	Derepression of VSG and ESAG genes from silent BESs and VSG genes from subtelomeric arrays or minichromosomes [29, 31]
TDP1	Nucleosome assembly	RNA interference	Decrease expression of active VSG and rRNA genes [78]
DOT1B	Chromatin modification	Null	Derepression of VSG and ESAG genes from silent BESs [86]
JBP1 & *JBP2* (Base J)	Chromatin modification	Null for both *JBP* genes (or chemical inhibition of JBPs)	Derepression of VSG genes from subtelomeric arrays or minichromosomes [30]
JBP1 & *JBP2* (Base J) & *H3V*	Chromatin modification	Null for both *JBP* genes (or chemical inhibition of JBPs) and null for *H3V*	Derepression of VSG genes from subtelomeric arrays or minichromosomes and VSG genes from BESs [29-31]
BDF2	Chromatin modification	Null or chemical inhibition	Derepression of VSG and ESAG genes from silent BESs, VSG*s* from MESs, procyclins and various Pol II transcribed genes [94]
BDF3	Chromatin modification	RNA interference or chemical inhibition	Derepression of VSG and ESAG genes from silent BESs, VSG*s* from MESs and VSG*s* from subtelomeric arrays, procyclins and various Pol II transcribed genes [94]
ISWI (ISWI complex)	Chromatin modification	RNA interference	Derepression of a reporter downstream a silent BES promoter [83]
NLP (ISWI complex)	Chromatin modification	RNA interference	Derepression of a reporter downstream a silent BES promoter [149]
SIR2RP1	Chromatin modification	Null	Derepression of a reporter gene downstream an rRNA promoter [111]
DAC3	Chromatin modification	RNA interference	Derepression of a reporter downstream a silent BES promoter [112]
SPT16 (FACT complex)	Histone chaperone	RNA interference	Derepression of a reporter downstream a silent BES promoter [121, 122]
POB3 (FACT complex)	Histone chaperone	RNA interference	Derepression of a reporter downstream a silent BES promoter [121]
CAF-1b	Histone chaperone	RNA interference	Derepression of a reporter downstream a silent BES promoter [92]
ASF1A	Histone chaperone	RNA interference	Derepression of a reporter downstream a silent BES promoter [92]
NUP-1	Nuclear lamina	RNA interference	Derepression of VSG and ESAG genes from silent BESs, and procyclins; increases VSG switching [128]
NUP-2	Nuclear lamina	RNA interference	Derepression of VSG genes from silent BESs, and procyclins [129]

(Table 1) contd….

Gene Name Abbreviation[a]	Protein Function	Genetic Modification	Phenotype[b]
RAP1	Telomere protein	RNA interference	Derepression of VSG and ESAG genes from silent BESs and VSG*s* from MESs [71, 134]
TRF	Telomere protein	RNA interference	Increases VSG switching [131]
TIF2	Telomere protein	RNA interference	Increases VSG switching [132, 133]
TRAF	Telomere protein	RNA interference	Derepression of VSG and ESAG genes from BESs [79]
PIP5K	Inositol phosphate pathway	Conditional null	Derepression of VSG and ESAG genes from silent BESs and VSG*s* from MESs, and VSG switching [70]
PIP5Pase	Inositol phosphate pathway (telomere protein)	Conditional null	Derepression of VSG and ESAG genes from silent BESs and VSG*s* from MESs [70]
PLC	Inositol phosphate pathway	Overexpression	Derepression of VSG*s* from BESs and MESs [70]
VEX1	Telomere protein	RNA interference or overexpression	Derepression of VSG and ESAG genes from silent BESs and VSG*s* from MESs [79]
ORC1/CDC6	Chromosome replication	RNA interference	Derepression of VSG genes from silent BESs and increase VSG switching [135, 136]
MCM-BP	Chromosome segregation	Conditional null	Derepression of VSG genes and genes encoding procyclins [137]
SSC1 (cohesin complex)	Chromosome segregation	RNA interference	VSG switching [68]

Notes: [a] See text for complete gene name; [b] Phenotype related to transcription of VSG genes or procyclin loci or VSG switching; other phenotypes were not included.

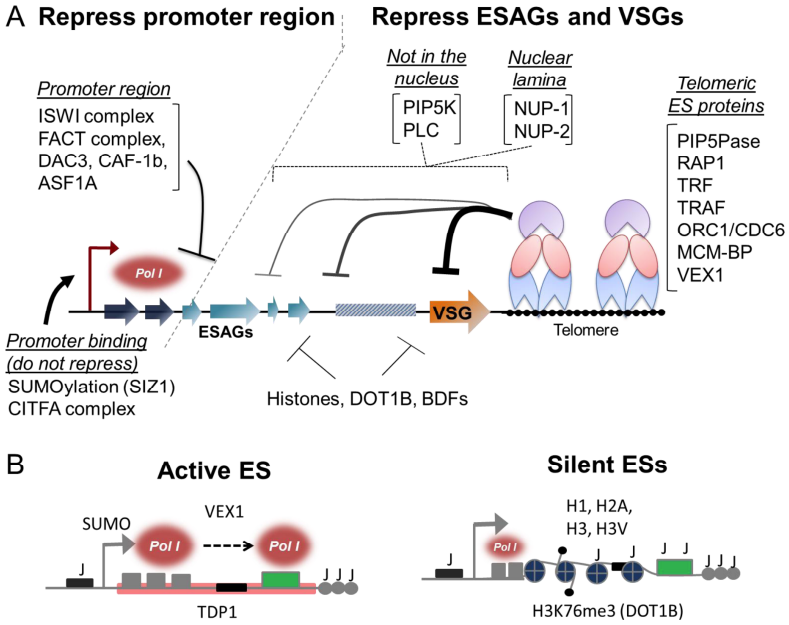

Fig. (3). Mechanisms of telomeric ES transcriptional regulation. A) Telomeric ESs are regulated by proteins that function in chromatin regulation, signaling or in nuclear lamina (Table **1**). Various chromatin regulatory proteins repress transcription near the promoter in repressed ESs. Telomeric ES proteins repress transcription of the whole ES and thus the VSG genes. A repressive gradient occurs which is strong near the telomeres (thick bars) and weak distal the telomeres (thin bars). The precise binding sites of some telomeric ES proteins are unknown. However, TRF and RAP1 bind to telomeric repeats [71], PIP5Pase binds to and colocalizes with RAP1 and telomeric repeats [70], TRAF and VEX1 co-localizes with TRF [79]. ORC1/CDC6 binds to telomeric repeats independently of RAP1 and TRF [135, 136]. Note that VEX1 is only present in the active ES but its knockdown or overexpression derepress silent ESs [79]. PIP5K and PLC are not in the nucleus but regulate ES transcription [70], as it does the NUP-1 and 2 (indicated by dotted brackets) [128, 129]. **B**) VSG regulation involves molecules that are present at the active ES and positively regulate ES transcription, *i.e.* TDP-1, VEX1 and SUMOylation (SUMO), and the active ES is depleted of nucleosomes. In contrast, silent ESs are enriched in histones, base J and chromatin regulatory proteins (depicted in A) which regulates Pol I elongation throughout the ES. Transcription initiates in silent ESs but does not elongate through the ES, and Pol I occupancy is higher in the active ES (indicated by size of Pol I, red circle) [73].

switching between ESs [68], implying that the cohesin complex is essential for epigenetic inheritance of the active ES during cell division. It has been postulated that limited access to the ESB and its singularity explain why only one telomeric ES is fully active at a time [5, 69]. However, simultaneous Pol I transcription of multiple ESs at different nuclear sites has been detected by knockdown of molecules that regulate the ES repression [70, 71], indicating that Pol I transcription of the ES is not restricted to a specific nuclear site. Single cell analysis also showed that transcription initiates simultaneously on multiple ESs but only elongates through one ES [72]. The data suggest that monoallelic ES control is unlikely determined by ES transcription initiation, but involves regulation of Pol I transcriptional elongation through the ES.

In addition to Pol I, the promoter of the active ES is enriched in the multi-subunit class I basal transcription initiation factor A (CIFTA) [53, 73, 74]. CIFTA has seven trypanosome-specific proteins, named CITFA-1 to 7, in addition to the dynein light chain DYNLL1. CIFTA was shown to bind the ES promoter sequence *in vitro* [53]. Chromatin immunoprecipitation (ChIP) assays showed that CIFTA subunit 7 was enriched in the active ES promoter compared to the silent ESs [73]. Localization studies of CIFTA subunit 7 showed its presence in the ESB and the nucleolus. RNAi knockdown of genes encoding CIFTA subunits 1, 2 or 7 reduced Pol I occupancy at the active ES and decreased the levels of VSG mRNAs and rRNAs [73, 74]. Thus, CIFTA is required for Pol I recruitment and transcription initiation of the active ES and rRNAs in BFs, and likely also plays a role in transcribing procyclins [53].

The active ES was also shown to be enriched in the Small Ubiquitin-like MOdifier (SUMO) posttranslational modification [75]. SUMOylation is a reversible posttranslational protein modification that entails the covalent ligation of 12 kDa SUMO proteins to lysine residues of protein targets in a process that is catalyzed by an E1 activating enzyme, E2 conjugating enzyme and E3 ligase [76, 77]. SUMOylation was enriched at the active ES, *i.e.* from the promoter region through the VSG gene, compared to silent ESs or the rRNA promoter [75]. The SIZ1 protein, which contains a SP-RING domain that is conserved in SUMO E3 ligases, also localizes in the ESB (although not exclusively). *SIZ1* knockdown reduced SUMOylation of the ESB, Pol I association, and transcription of the active ES. The largest Pol I subunit RPA1 is also SUMOylated which is reduced by RNAi knockdown of *SIZ1* [75]. Hence, SUMOylation of RPA1 may affect its association with other Pol I subunits or with regulatory factors that may function in Pol I recruitment to the active ES. Given the role of SUMO in regulating protein localization and interactions [77], it is possible that SUMOylation may affect targeting or recruitment of proteins to the active ES.

Trypanosome DNA binding protein 1 (TDP1), a high mobility group box protein, is enriched in the active ES compared with silent ESs and co-localizes with Pol I at the ESB [78]. TDP1 has been implicated in facilitating Pol I transcription and was also detected downstream of the rRNA promoters, and within the procyclin loci in both BFs and PFs. RNAi knockdown of *TDP1* in BFs results in a dramatic reduction (up to 90%) in transcripts from the active ES and also reduced rRNA transcripts [78]. Depletion of TDP1 is inversely correlated with enrichment in histones H1, H2A and H3 in the active ES [78]. TDP1 also binds the Pol II spliced leader and Pol III 5S rRNA transcription units, although to a lesser extent than the Pol I transcription units. TDP1 appears to maintain an open chromatin structure by substituting histones at the active ES and other Pol I transcription units, thereby facilitating Pol I transcription.

Glover *et al.* [79] identified VSG exclusion 1 (VEX1), a protein exclusively associated with the active ES in BFs. VEX1 contains a SWIM-type Zn-finger commonly found in the SWI2/SNF2 family ATPases, MuDR transposase, and MEK kinase [80, 81], which has been described to bind to DNA, RNA, or lipids [82]. VEX1 co-localized with the telomeric repeat binding factor (TRF) in both BFs and PFs. However, in BFs, VEX1 was exclusively detected at the active ES, whereas in PFs, VEX1 had a broader distribution in the nucleus. Either knockdown of *VEX1* or its overexpression resulted in derepression of silent ESs and expression of many VSG proteins in the cell population, but it did not affect VSG switching rates. A "winner takes all" model of VSG gene allelic exclusion was proposed by which VEX1 recruitment to one ES results in monoallelic Pol I transcription of the entire ES, and repression of the remaining ESs. The authors proposed that VEX1 silencing of ESs occurs by homology-dependent silencing exerted by an unidentified transcript from the active ES that initiates repression of the remaining ESs [79]. Although the molecular basis underlying the proposed mechanism remains speculative, it is clear that VEX1 plays an important role in regulating VSG allelic exclusion. In summary, the regulation of ES monoallelic transcription likely entails various molecules involved in Pol I recruitment, transcription initiation and elongation along the ES.

3.2. ES Chromatin Structure and Pol I Transcription

Epigenetic modifications of the ES chromatin also contribute to regulation of its transcription [78, 83-86]. Navarro and Cross [84] showed that replacement of the active BES promoter by a T7 polymerase promoter results in efficient ES repression during development of BFs to PFs, whereas insertion of the T7 promoter at a non-telomeric locus, *e.g.* an rDNA spacer or silent VSG array, does not affect T7 promoter-driven transcription. Similarly, replacement of the ES promoter with an rDNA promoter also results in ES repression during development [87, 88]. These results indicate that remodeling of the chromatin structure is involved in transcriptional repression of the ES. Subsequently, a series of chromatin modifying proteins was implicated in regulating Pol I transcription of the active ES (Fig. **3**, Table **1**). The active *T. brucei* ES is depleted of nucleosomes [89, 90], whereas silent ESs are enriched in histones H1, H2A, H3 and H3V [28, 78, 89, 90]. Knockdown of histone H1, H3 or knockout of H3V all result in derepression of silent ESs [29, 31, 85, 91, 92]. Some of these histones are also enriched in other genomic silent regions such as the boundaries of RNA polymerase II transcription units [28, 93]. In contrast, nucleosome depletion at active ESs is inversely correlated with TDP1, whose knockdown reduces Pol I transcription and increases deposition of histones H1, H2A and H3 in the active ES [78] (Fig. **3B**). Knockdown or inhibition of bromo-

domain factors (BDF) 2 or 3 also affected expression of VSG genes (Table **1**), but also affected expression of procyclins and other Pol II transcribed genes [94]. The enzyme DOT1B, a methyltransferase that trimethylates lysine 76 of histone H3 (H3K76me3), is also involved in repression of silent ESs [86]. *DOT1B* null cells partially derepress ESs without any effects on non-telomeric loci. Battram *et al.* [95] showed that attenuation of the active ES after ectopic overexpression of a VSG gene from a non-telomeric locus, by a process as yet unknown, was also dependent on DOT1B. A hypermodified DNA base called J, β-D-glucopyranosyloxymethyluracil, is enriched at the telomeres and silent ESs of *T. brucei* BFs but absent in the transcriptionally active ES and in PFs [96-98]. Base J is synthesized by sequential hydroxylation and glucosylation of thymidine catalyzed by SWI/SNF hydroxylases, known as J-binding protein (JBP) 1 and 2, and a glucosyl transferase (GT), respectively [99-101]. In the silent ESs, base J spanned the telomeres, the VSG gene and the 70 bp and 50 bp repeats, whereas it is absent throughout the transcribed regions in the active ES, *i.e.* between the promoter and VSG gene, but present at the telomeres and 50 bp repeats [98]. Base J is also found to be enriched in the SSRs of *Leishmania* sp. and knockout of *JBP2* results in transcription through Pol II transcription termination sites [32]. Base J elimination affects Pol II transcription termination within polycistronic units without affecting transcription at convergent SSRs in *T. brucei* [30], suggesting a somewhat conserved function of base J in controlling Pol II transcription termination. Depletion of J with dimethyloxalylglycine (DMOG), a chemical inhibitor of the thymidine hydroxylase domains of JBP1 and JBP2, affected transcription of VSG*s* but not specifically those at ESs [29, 30]. However, depletion of J by DMOG or by deletion of the *JBP1* and *JBP2* genes in combination with deletion of the *H3V* gene resulted in derepression of ES VSG genes, although most of these effects have been attributed to *H3V* elimination [29, 31].

Hughes *et al.* [83] identified an SWI2/SNF2-related chromatin-remodeling protein (ISWI), the RNAi knockdown of which resulted in increased transcription of the promoter region of silent ESs in BFs and PFs *T. brucei*. However, transcription did not extend to the VSG genes, indicating that multiple factors function in the regulation of ES transcription wherein increased transcription at the promoter may be necessary but insufficient for Pol I elongation through the ES and thus VSG gene (Fig. **3A**). Subsequent work by the same group showed that ISWI forms a protein complex that also contains the nucleoplasmin-like protein (NLP), the regulator of chromosome condensation 1-like protein (RCCP) and a phenylalanine/tyrosine-rich protein (FYRP) [102]. The *T. brucei* ISWI complex is present in both BFs and PFs and is generally associated with multiple Pol I-transcribed loci [83, 102] and with Pol II SSRs [102, 103]. ISWI complexes are also present in other organisms from yeast to humans where variations in the composition of the complex result in regulation of Pol II transcription initiation, elongation, or termination [104-106]. For example, yeast has four ISWI complexes, whereas *Drosophila* has six [107, 108]. The presence of only one ISWI complex in *T. brucei*, whose composition differs from those in fungi or animals, may be related to the absence of regulation of Pol II transcription initiation in *T. brucei*, and may also reflect the early evolutionary divergence of this eukaryote [109]. *T. brucei* silent information regulator 2-related protein 1 (SIR2RP1), which functions as a NAD^+-dependent enzyme and catalyzes ADP-ribosylation and deacetylation of histones H2A and H2B, is located in the nucleus and co-localizes with telomeres in procyclic forms [110]. SIR2RP1 has been implicated in DNA repair in PFs [110]; its knockdown in BFs affects derepression of a reporter gene immediately downstream of an rRNA promoter but not a VSG gene that is located ~3kb downstream of this promoter [111]. Similarly, histone deacetylase 3 (*DAC3*) knockdown in BFs also affects transcription of a promoter proximal reporter gene, but not that of the VSG gene [112]. SIR2 has been implicated in telomere silencing in yeast and *Plasmodium* [113-116]. In yeast, SIR2 forms a complex with the SIR3-SIR4 dimer that is spread along silent chromatin in a manner that is dependent on SIR2 deacetylation activity [117-120]. The lack of SIR2RP1 repression of VSG*s* in *T. brucei* may be due to its distribution at the ESs or the absence of other SIR complex proteins.

The facilitates chromatin transcription (FACT) complex of histone chaperones, which regulate nucleosome assembly, also contributes to ES transcriptional control [121, 122]. *T. brucei* FACT contains two subunits, SPT16 and POB3, and knockdown of either gene results in derepression of a reporter gene downstream the ES promoter [121]. SPT16 is enriched at the promoter region of silent ESs [122] and its knockdown decreases histone H2A and H3 deposition in the silent ESs, indicating an open chromatin for Pol I transcription [121]. Derepression of the ES promoter reporter is associated with cells at G2/M, suggesting cell cycle-specific chromatin remodeling within ESs [122]. Similarly, knockdown of chromatin assembly factor 1b (*CAF-1b*) or of antisilencing factor 1A (*ASF1A*) also results in derepression of the ES promoter region, but not the VSG gene [92]. Hence, *T. brucei* ES chromatin is regulated at the promoter region by various chromatin regulatory proteins (*e.g.* SIR2, ISWI, FACT) that affect nucleosome assembly and thus Pol I transcription downstream the promoter, whereas other regulatory proteins (*e.g.* H3V, DOT1B, TDP1) appear to control Pol I elongation along the entire ES and thus VSG transcription.

3.3. Telomere Position Effect and VSG Transcriptional Silencing

Telomere position effect was first shown in yeast, and consists of transcriptional repression of genes located near the chromosome ends [123]. It was subsequently shown to regulate repression of VSG genes in *T. brucei* and gene expression at telomeres in other organisms [87, 124]. Telomere clustering and tethering to the nuclear envelope have been associated with repression of the subtelomeric loci in yeast and *Plasmodium* [113, 125]. In *T. brucei* BFs, telomeres are dynamically distributed in the nucleus throughout the cell cycle, but are generally clustered and located in the nucleoplasm, whereas in PFs they are positioned near the nuclear periphery [93, 126, 127]. Moreover, the active ES is relocated from the nucleoplasm to the nuclear periphery during BF to PF development [67]. Knockdown of the nuclear peripheral protein-1 (*NUP-1*) or *NUP-2* genes affects telomere segregation and results in derepression of VSG genes and genes encoding procyclins [128, 129] (Table **1**). Knockdown

of a phosphatidylinositol 5-kinase (*PIP5K*) or a phosphatidylinositol 5-phosphatase (*PIP5Pase*) also derepresses ES VSG genes and results in repositioning of telomeres and the telomeric proteins repressor activator protein 1 (RAP1) and TRF in the nucleus [70] (See topic 4 for details). PIP5Pase localizes at telomeres and interacts with RAP1 [70]. The telomere repositioning after *PIP5Pase* knockdown correlated with the appearance of multiple extranucleolar Pol I foci at the telomeres [70]. Multiple extranucleolar Pol I foci were also detected after *RAP1* knockdown [71]. PIP5Pase or RAP1 knockdown parasites express more than one VSG protein on the surface implying that transcripts from the derepressed ESs were processed and translated into proteins [70, 71, 79]. The data indicate that nuclear organization plays a role in the control of VSG gene expression in *T. brucei*.

Some proteins identified at *T. brucei* telomeres contribute to regulate ES transcription. Others appear to control VSG switching and/or telomere end protection. The *T. brucei* TRF is functionally related to mammalian TRF2 and is involved in telomere end protection [130]. *TRF* knockdown in *T. brucei* BFs results in reduction of single-stranded G-overhangs at the telomeres. It does not affect ES transcription but increases VSG switching [130, 131]. *T. brucei* TRF-Interacting Factor 2 (TIF2), a functional homolog of mammalian TIN2, interacts with TRF and *TIF2* knockdown also increases VSG switching but not VSG expression [132, 133]. RAP1 co-localizes with TRF and helps regulate VSG gene transcription [71]. Knockdown of RAP1 in BFs and PFs results in derepression of all silent ESs, but does not affect the active ES, or procyclin or rRNA genes [71, 134]. A telomeric TTAGGG Repeat-Associated Factor (TRAF) also co-localizes with TRF in *T. brucei* BFs and its knockdown results in a moderate derepression of silent ESs [79]. In addition, the subunit ORC1/CDC6 of the Origin Recognition Complex (ORC), which binds to replication origin sites and functions in DNA replication [135], is enriched in subtelomeric regions of *T. brucei* [135, 136]. ORC1/CDC6 is associated with *T. brucei* telomeres independently of RAP1 and TRF [136], and its knockdown results in derepression of ES VSG*s* and expression of more than one VSG on the cell surface [135, 136]. Notably the replication origin sites in *T. brucei* also collocate with SSRs [135], which are also enriched for H3V and H4V [28], subunits of ISWI protein complex [102] and base J [30, 32]. In addition, knockdown of Mini-Chromosome Maintenance-binding protein (*MCM-BP*) [137], which is part of the MCM complex involved in chromosome replication [138], also results in derepression of ES VSG*s* and genes encoding procyclins [137]. Given that knockdown of *ORC1/CDC6* and *MCM-BP* both affect ES transcription and switching [135-137], and knockdown of the cohesin complex component SSC1 also affects ES switching [68], it is likely that ES regulation is functionally associated with chromosome integrity in *T. brucei*.

Hence, a series of proteins that affect ES expression in *T. brucei* localize at the telomeres and some of these interact directly with telomeric DNA (*e.g.* TRF) while others likely primarily associate with other proteins (*e.g.* PIP5Pase and RAP1). Some proteins are not exclusively at telomeres but are also distributed at other chromosomal loci (*e.g.* ORC1/CDC6). Nevertheless, perturbation of the function of these proteins results in either transcription of silent VSG genes or VSG switching, supporting their roles in antigenic variation. The precise mechanism by which these proteins silence telomeric ESs and results in only a single ES being transcribed in BFs remains unknown, but it likely entails control of telomeric chromatin structure, conformation and/or recruitment to transcriptionally repressed nuclear sites.

4. INOSITOL PHOSPHATES AND REGULATION OF VSG EXPRESSION AND SWITCHING

We recently found that the inositol phosphate pathway (IP pathway, *i.e.* the inositol phosphate and phosphatidylinositol pathways) contributes to the regulation of VSG gene expression and VSG switching. Knockdown of *PIP5K* or *PIP5Pase* in BF *T. brucei* results in derepression of silent BESs and MESs with no effect on rRNA or procyclin expression [70]. The PIP5K enzyme catalyzes phosphorylation of PI4P to PI(4,5)P2 [70, 139]. *PIP5K* knockdown results in decreased levels of PI(4,5)P2 and an accumulation of PI4P both at the plasma membrane [70, 139]. PI(4,5)P2 is predominantly at the plasma membrane in *T. brucei* and in other organisms, although it also occurs in organelles of yeast and mammalian cells [70, 139-144]. Conversely, PIP5Pase removes the 5-phosphate from PI(4,5)P2 and PI(3,4,5)P3 [70], indicating that both PIP5K and PIP5Pase control the synthesis of phosphatidylinositol molecules. In addition, overexpression of PLC, the enzyme that cleaves PI(4,5)P2 into Ins(1,4,5)P3, also results in derepression of VSGs. Thus, PI(4,5)P2 is likely a key molecule in the regulation of ES transcription. PIP5K and PLC are both located on the plasma membrane as is PI(4,5)P2, whereas PIP5Pase is in the nucleus at the telomeres, where it interacts with RAP1 [70]. *PIP5K* knockdown affects the intra-nuclear locations of RAP1 and TRF and results in telomere repositioning within the nucleus along with RAP1 and TRF. It also alters glycerol gradient sedimentation of RAP1 and TRF, implying that interactions among these telomeric proteins are affected by perturbation of the IP pathway. Hence, the localization of PIP5Pase at telomeres, its interaction with RAP1, and the effects on ES transcription and on telomeric proteins indicates that IP enzymes function in the regulation of telomere silencing and VSG monoalellic expression in *T. brucei*.

The nuclear location of PIP5Pase implies that some PI(4,5)P2 or PI(3,4,5)P3 may be present in the nucleus. Phosphatidylinositol is in the nucleus in yeast and mammalian cells [141-144], although whether these molecules are synthesized there or transported to the nucleus is unknown. In mammals, PI(4,5)P2 and Ins(1,3,4,5)P4 molecules bind to the nuclear receptor steroidogenic factor 1 and to histone deacetylases complexes, respectively, and regulate transcription [143, 145]. PI(4,5)P2 also interacts with Upstream Binding Factor (UBF) forming an anchor for the Pol I pre-initiation complex [141, 142], and is also involved in RNA splicing [140]. Neither catalytic nor non-enzymatic functions for PIP5Pase in the nucleus of *T. brucei* cannot be eliminated; however genetic studies with PIP5K and PLC imply a role for IP metabolites in the regulation of VSG expression and telomere silencing [70].

We recently found that the IP pathway also regulates *T. brucei* development. Knockdown of inositol polyphosphate

multikinase (*IPMK*), the enzyme that phosphorylates Ins(1,4,5)P3 and Ins(1,3,4,5)P4 [146], results in development of BFs to PFs. This process is accompanied by VSG gene repression and expression of procyclin proteins, in addition to metabolic and morphological changes to PF characteristics (Cestari *et al*., manuscript under review). Monoallelic VSG expression and life cycle development are likely coordinately regulated in *T. brucei,* and IP molecules may be part of the regulatory network that controls gene expression and development. While the molecular mechanism whereby the IP pathway regulates ES silencing, monoallelelic ES transcription and antigenic variation remains incompletely understood, this finding offers a new perspective on how these various processes may be coordinated.

5. DEVELOPMENTAL REGULATION OF TRANSCRIPTION OF PROTEIN CODING GENES BY RNA POLYMERASE I

VSG ES and procyclin genes are transcribed by Pol I in *T. brucei*, which is unusual, and their expression is developmentally regulated during life cycle which results in expression of VSGs in BFs and of procyclins in PFs [4, 6]. While complete transcription of the active ES occurs in slender BFs, transcription of VSG*s* is downregulated during the development of slender to stumpy BFs [147]. Nuclear run on experiments show that in stumpy BFs Pol I elongates through a portion of the ES, *e.g.* from promoter to ESAG*8* (Fig. **2B**), but fails to transcribe the VSG gene of the active ES [147]. In addition, ESs are repressed in PFs, although transcription still occurs at the promoter region [87, 88, 147]. The data suggest that repression of the active ES during development spans through the VSG gene to a region somewhat downstream of the promoter but not the promoter region itself. ES repression in PFs is also accompanied by repositioning of the active ES from the nucleoplasm to the perinuclear region and the absence of ES and Pol I colocalization and transcription, as shown by the lack of Pol I-dependent BrUTP incorporation [5, 67, 147]. The relocation of the active ES to the perinuclear region indicates that the ES chromatin environment may play a role in the developmental regulation of ES transcription, perhaps by controlling its accessibility to areas of active transcription and RNA processing. In contrast to ESs, procyclin loci which are also transcribed by Pol I localize at the nucleolar periphery [67]. Repression of telomeric ESs in PFs is also dependent on RAP1 as indicated by ES VSG derepression upon *RAP1* knockdown in this stage. The *RAP1* knockdown in PFs increases accessibility of ES chromatin to micrococcal nuclease [134], suggesting that RAP1 contributes to the maintenance of a chromatin structure in PFs that represses ES transcription. Interestingly, VEX1 is located at multiple telomeres in PFs while it is specifically located at the active ES in BFs [79]. The association of VEX1 with multiple telomeres in PFs may provide the ability of any ES to be activated in BFs [50]. It is noteworthy that *T. brucei* usually reactivates the same ES in BFs after infection [148], and thus other features of the ES may play a role in determining ES reactivation after cyclic transmission. The molecular basis underlying the coordinated regulation of VSG monoallelic transcription in BFs and procyclin transcription in PFs is unknown. However, many of the factors that regulate repression of ESs are also at the procyclin and rRNA loci, *e.g.* NLP, ISWI, MCM-BP [102, 103, 137, 149], and they may regulate procyclin gene expression. Even less is known regarding regulation of transcription by Pol I in metacyclic forms, which reactivate VSG expression from MESs in the insect salivary gland prior to mammalian infection. Overall, the regulation of Pol I transcription of ESs and procyclin loci in *T. brucei* appears to depend, at least in part, on their genomic location, the nuclear organization and distribution of telomeres in the nucleus, chromatin structure and factors that regulate chromatin accessibility to Pol I.

6. FINAL REMARKS: THE COMPLEX SYSTEM REGULATING ANTIGENIC VARIATION

The regulation of VSG gene expression during antigenic variation and cell development is a multifaceted process that involves control of Pol I transcription, chromatin structure, nuclear organization, allelic exclusion and telomere silencing. These processes are inherently integrated with other cellular processes such as the cell cycle, DNA replication and recombination. They are also coordinated with physiological changes that occur throughout the life cycle, *e.g.* gene expression, cell morphology and metabolism. This complex regulatory system may also involve cell signaling and quorum sensing, both of which may have significant roles in coordinating the processes controlling VSG expression during antigenic variation and development.

CONCLUSION

The current data suggest a model of ES transcriptional control whereby Pol I transcription initiates at all ESs but only elongates through one ES [72, 88]. The enrichment in basal transcription factors at the active ES promoter, or post-translational modifications such as SUMOylation, may also favor Pol I recruitment and transcription of the active ES compared to silent ESs [73, 110]. The elongation of Pol I through silent ESs is in part controlled by the structure of the ES chromatin, which is enriched with histones and chromatin modifying enzymes that contribute to form a compacted structure that represses Pol I transcription [83, 85, 102, 111, 121]. The distribution of chromatin modifying proteins along the ES appears to be somewhat distinct, since knockdown of some genes affects only transcription of genes proximal to the promoter [83, 111, 122], whereas knockdown of other genes affects transcription of the entire ES and thus the VSG gene [31, 70, 71, 79] (Fig. **3**). This may also be due to ES repression by telomeric proteins, *e.g.* RAP1, TRAF and PIP5Pase, implying that the location of ESs near the telomeres is critical for its regulation [87]. It is unknown whether these telomeric proteins bind only to the telomeric repeats or also bind to other ES sequences. Yeast telomeres have been proposed to fold onto the subtelomeric chromatin in a manner that is dependent on chromatin binding proteins and modifications, *e.g.* the SIR2 complex and RAP1, which blocks transcription [150, 151]. *T. brucei* has telomere loops [152] but it is unknown if this functions in telomere silencing or other ES conformations are involved. The sub-nuclear location of telomeres also appears to contribute to the control of monoallelic VSG expression [70, 128, 129] and ES transcriptional repression during life cycle development [67]. The location of telomeres in the nucleoplasm in BFs may favor transcription and potentially switching between ESs, perhaps due to ES accessibility to transcriptional and RNA

processing machineries, whereas ES re-location to the nuclear periphery in PFs may contribute to its repression. The mechanisms underlying the selective transcription of only one ES at time are still poorly understood. The identification of VEX1 [79], TDP1 [78] and SUMOylation [75] at the site of expression, *i.e.* ESB, suggests that multiple proteins, and potentially multiple levels of regulation, may be involved in the selection of a single ES for transcription. Interestingly, some of the molecules regulating ES transcription are also present at procyclin loci and SSRs, some of which also regulates Pol II transcription [31, 94, 102], suggesting that multiple factors including genomic context and chromosome locus are important for transcriptional regulation in trypanosomes. Finally, the finding that the IP pathway controls VSG expression and cell development provides a new dimension for our understanding of VSG gene regulation [70]. It suggests that IPs may be part of a complex regulatory network that controls VSG expression and switching, and perhaps the coordination of multiple molecular processes, *e.g.* chromatin structure, telomere silencing and Pol I monoallelic transcription, during antigenic variation and development. There are still many unanswered fundamental questions related to the regulation of antigenic variation, allelic exclusion and developmental regulation of Pol I transcription. Answering these questions will likely provide novel insights into mechanisms that regulate gene expression and antigenic variation in *T. brucei*.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This work was supported by NIH grant R01AI078962 to KS and supplement R01AI014102-37S1 to KS and IC, and by the Center for Infectious Disease Research.

REFERENCES

[1] Franco, J.R.; Simarro, P.P.; Diarra, A.; Jannin, J.G. Epidemiology of human African trypanosomiasis. *Clin. Epidemiol.,* **2014**, *6*, 257-275. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4130665/

[2] Cross, G.A.; Kim, H.S.; Wickstead, B. Capturing the variant surface glycoprotein repertoire (the VSGnome) of *Trypanosoma brucei* Lister 427. *Mol. Biochem. Parasitol.,* **2014**, *195*(1), 59-73.

[3] Berriman, M.; Ghedin, E.; Hertz-Fowler, C.; Blandin, G.; Renauld, H.; Bartholomeu, D.C.; Lennard, N.J.; Caler, E.; Hamlin, N.E.; Haas, B.; Bohme, U.; Hannick, L.; Aslett, M.A.; Shallom, J.; Marcello, L.; Hou, L.; Wickstead, B.; Alsmark, U.C.; Arrowsmith, C.; Atkin, R.J.; Barron, A.J.; Bringaud, F.; Brooks, K.; Carrington, M.; Cherevach, I.; Chillingworth, T.J.; Churcher, C.; Clark, L.N.; Corton, C.H.; Cronin, A.; Davies, R.M.; Doggett, J.; Djikeng, A.; Feldblyum, T.; Field, M.C.; Fraser, A.; Goodhead, I.; Hance, Z.; Harper, D.; Harris, B.R.; Hauser, H.; Hostetler, J.; Ivens, A.; Jagels, K.; Johnson, D.; Johnson, J.; Jones, K.; Kerhornou, A.X.; Koo, H.; Larke, N.; Landfear, S.; Larkin, C.; Leech, V.; Line, A.; Lord, A.; Macleod, A.; Mooney, P.J.; Moule, S.; Martin, D.M.; Morgan, G.W.; Mungall, K.; Norbertczak, H.; Ormond, D.; Pai, G.; Peacock, C.S.; Peterson, J.; Quail, M.A.; Rabbinowitsch, E.; Rajandream, M.A.; Reitter, C.; Salzberg, S.L.; Sanders, M.; Schobel, S.; Sharp, S.; Simmonds, M.; Simpson, A.J.; Tallon, L.; Turner, C.M.; Tait, A.; Tivey, A.R.; Van Aken, S.; Walker, D.; Wanless, D.; Wang, S.; White, B.; White, O.; Whitehead, S.; Woodward, J.; Wortman, J.; Adams, M.D.; Embley, T.M.; Gull, K.; Ullu, E.; Barry, J.D.; Fairlamb, A.H.; Opperdoes, F.; Barrell, B.G.; Donelson, J.E.; Hall, N.; Fraser, C.M.; Melville, S.E.; El-Sayed, N.M. The genome of the African trypanosome *Trypanosoma brucei*. *Science,* **2015**, *309*, 416-422. Available from: science.sciencemag.org/content/309/5733/416

[4] Gunzl, A.; Bruderer, T.; Laufer, G.; Schimanski, B.; Tu, L.C.; Chung, H.M.; Lee, P.T.; Lee, M.G. RNA polymerase I transcribes procyclin genes and variant surface glycoprotein gene expression sites in *Trypanosoma brucei*. *Eukaryot. Cell,* **2003**, *2*(3), 542-551.

[5] Navarro, M.; Gull, K. A pol I transcriptional body associated with VSG mono-allelic expression in *Trypanosoma brucei*. *Nature,* 2001, *414*, 759-763. Available from: https://www.nature.com/articles/414759a

[6] Rudenko, G.; Bishop, D.; Gottesdiener, K.; Van der Ploeg, L.H. Alpha-amanitin resistant transcription of protein coding genes in insect and bloodstream form *Trypanosoma brucei*. *EMBO J.,* **1989**, *8*(13), 4259-4263.

[7] McCulloch, R.; Field, M.C. Quantitative sequencing confirms VSG diversity as central to immune evasion by *Trypanosoma brucei*. *Trends Parasitol.,* **2015**, *31*(8), 346-349.

[8] Myler, P.J.; Allison, J.; Agabian, N.; Stuart, K. Antigenic variation in African trypanosomes by gene replacement or activation of alternate telomeres. *Cell,* **1984**, *39*(1), 203-211.

[9] Myler, P.; Nelson, R.G.; Agabian, N.; Stuart, K. Two mechanisms of expression of a predominant variant antigen gene of *Trypanosoma brucei*. *Nature,* **1984**, *309*(5965), 282-284. Available from: http://www.sciencedirect.com/science/article/pii/0166685185900453

[10] Mony, B.M.; MacGregor, P.; Ivens, A.; Rojas, F.; Cowton, A.; Young, J.; Horn, D.; Matthews, K. Genome-wide dissection of the quorum sensing signalling pathway in *Trypanosoma brucei*. *Nature,* **2014**, *505*(7485), 681-685. Available from: https://www.nature.com/nature/journal/v505/n7485/full/nature12864.html

[11] Acosta-Serrano, A.; Vassella, E.; Liniger, M.; Renggli, C.K.; Brun, R.; Roditi, I.; Englund, P.T. The surface coat of procyclic *Trypanosoma brucei*: Programmed expression and proteolytic cleavage of procyclin in the tsetse fly. *Proc. Natl. Acad. Sci. U.S.A.,* **2001**, *98*(4), 1513-1518.

[12] Roditi, I.; Schwarz, H.; Pearson, T.W.; Beecroft, R.P.; Liu, M.K.; Richardson, J.P.; Buhring, H.J.; Pleiss, J.; Bulow, R.; Williams, R.O. Procyclin gene expression and loss of the variant surface glycoprotein during differentiation of *Trypanosoma brucei*. *J. Cell Biol.,* **1989**, *108*(2), 737-746.

[13] Vassella, E.; Acosta-Serrano, A.; Studer, E.; Lee, S.H.; Englund, P.T.; Roditi, I. Multiple procyclin isoforms are expressed differentially during the development of insect forms of *Trypanosoma brucei*. *J. Mol. Biol.,* **2001**, *312*(4), 597-607.

[14] Vassella, E; Den Abbeele, J.V.; Butikofer, P.; Renggli, C.K.; Furger, A.; Brun, R.; Roditi, I. A major surface glycoprotein of *Trypanosoma brucei* is expressed transiently during development and can be regulated post-transcriptionally by glycerol or hypoxia. *Genes Dev.,* **2000**, *14*(5), 615-626.

[15] Ramey-Butler, K.; Ullu, E.; Kolev, N.G.; Tschudi, C. Synchronous expression of individual metacyclic variant surface glycoprotein genes in *Trypanosoma brucei*. *Mol. Biochem. Parasitol.,* **2015**, *200*(1-2), 1-4.

[16] Graham, S.V.; Barry, J.D. Transcriptional regulation of metacyclic variant surface glycoprotein gene expression during the life cycle of *Trypanosoma brucei*. *Mol. Cell. Biol.,* **1995**, *15*(11), 5945-5956.

[17] Ginger, M.L. Niche metabolism in parasitic protozoa. *Philos. Trans. R Soc. Lond. B Biol. Sci.,* **2006**, *361*(1465), 101-118. Available from: https://www.medscape.com/viewpublication/9982_265

[18] Martinez-Calvillo, S.; Yan, S.; Nguyen, D.; Fox, M.; Stuart, K.; Myler, P.J. Transcription of *Leishmania* major Friedlin chromosome 1 initiates in both directions within a single region. *Mol. Cell.,* **2003**, *11*(5), 1291-1299.

[19] Worthey, E.A.; Martinez-Calvillo, S.; Schnaufer, A.; Aggarwal, G.; Cawthra, J.; Fazelinia, G.; Fong, C.; Fu, G.; Hassebrock, M.; Hixson, G.; Ivens, A.C.; Kiser, P.; Marsolini, F.; Rickel, E.; Salavati, R.; Sisk, E.; Sunkin, S.M.; Stuart, K.D.; Myler, P.J. *Leishmania* major chromosome 3 contains two long convergent polycistronic gene clusters separated by a tRNA gene. *Nucleic Acids Res.,* **2003**, *31*(14), 4201-4210.

[20] El-Sayed, N.M.; Ghedin, E.; Song, J.; MacLeod, A.; Bringaud, F.;

Larkin, C.; Wanless, D.; Peterson, J.; Hou, L.; Taylor, S.; Tweedie, A.; Biteau, N.; Khalak, H.G.; Lin, X.; Mason, T.; Hannick, L.; Caler, E.; Blandin, G.; Bartholomeu, D.; Simpson, A.J; Kaul, S.; Zhao, H.; Pai, G.; Van Aken, S.; Utterback, T.; Haas, B.; Koo, H.L.; Umayam, L.; Suh, B.; Gerrard, C.; Leech, V.; Qi, R.; Zhou, S.; Schwartz, D.; Feldblyum, T.; Salzberg, S.; Tait, A.; Turner, C.M.; Ullu, E.; White, O.; Melville, S.; Adams, M.D.; Fraser, C.M. Donelson, J.E. The sequence and analysis of *Trypanosoma brucei* chromosome II. *Nucleic Acids Res.,* **2003**, *31*(16), 4856-4863.

[21] Gunzl, A. The pre-mRNA splicing machinery of trypanosomes: Complex or simplified? *Eukaryot. Cell,* **2010**, *9*(8), 1159-1170.

[22] Clayton, C.E. Life without transcriptional control? From fly to man and back again. *EMBO J.,* **2002**, *21*(8), 1881-1888.

[23] Palenchar, J.B.; Bellofatto, V. Gene transcription in trypanosomes. *Mol. Biochem. Parasitol.,* **2006**, *146*(2), 135-141.

[24] Gilinger, G.; Bellofatto, V. Trypanosome spliced leader RNA genes contain the first identified RNA polymerase II gene promoter in these organisms. *Nucleic Acids Res.,* **2001**, *29*(7), 1556-1564.

[25] Wright, J.R.; Siegel, T.N.; Cross, G.A. Histone H3 trimethylated at lysine 4 is enriched at probable transcription start sites in *Trypanosoma brucei*. *Mol. Biochem. Parasitol.,* **2010**, *172*(2), 141-144.

[26] Siegel, T.N.; Hekstra, D.R.; Wang, X.; Dewell, S.; Cross, G.A. Genome-wide analysis of mRNA abundance in two life-cycle stages of *Trypanosoma brucei* and identification of splicing and polyadenylation sites. *Nucleic Acids Res.,* **2010**, *38*(15), 4946-4957.

[27] Kolev, N.G.; Franklin, J.B.; Carmi, S.; Shi, H.; Michaeli, S.; Tschudi, C. The transcriptome of the human pathogen *Trypanosoma brucei* at single-nucleotide resolution. *PLoS Pathog.,* **2010**, *6*(9), e1001090. Available from: journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1001090

[28] Siegel, T.N.; Hekstra, D.R.; Kemp, L.E.; Figueiredo, L.M.; Lowell, J.E.; Fenyo, D.; Wang, X.; Dewell, S.; Cross, G.A. Four histone variants mark the boundaries of polycistronic transcription units in *Trypanosoma brucei*. *Genes Dev.,* **2009**, *23*(9), 1063-1076.

[29] Reynolds, D.; Hofmeister, B.T.; Cliffe, L.; Alabady, M.; Siegel, T.N.; Schmitz, R.J.; Sabatini, R. Histone H3 variant regulates RNA polymerase II transcription termination and dual strand transcription of siRNA loci in *Trypanosoma brucei*. *PLoS Genet.,* **2016**, *12*(1), e1005758. Available from: journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1005758

[30] Reynolds, D.; Cliffe, L.; Forstner, K.U.; Hon, C.C.; Siegel, T.N.; Sabatini, R. Regulation of transcription termination by glucosylated hydroxymethyluracil, base J, in *Leishmania* major and *Trypanosoma brucei*. *Nucleic Acids Res.,* **2014**, *42*(15), 9717-9729.

[31] Schulz, D.; Zaringhalam, M.; Papavasiliou, F.N.; Kim, H.S. Base J and H3.V regulate transcriptional termination in *Trypanosoma brucei*. *PLoS Genet.,* **2016**, *12*(1), e1005762. Available from: journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1005762

[32] van Luenen, H.G.; Farris, C.; Jan, S.; Genest, P.A.; Tripathi, P.; Velds, A.; Kerkhoven, R.M.; Nieuwland, M.; Haydock, A.; Ramasamy, G.; Vainio, S.; Heidebrecht, T.; Perrakis, A.; Pagie, L.; van Steensel, B.; Myler, P.J.; Borst, P. Glucosylated hydroxymethyluracil, DNA base J, prevents transcriptional readthrough in *Leishmania*. *Cell,* **2012**, *150*(5), 909-921.

[33] Hertz-Fowler, C.; Figueiredo, L.M.; Quail, M.A.; Becker, M.; Jackson, A.; Bason, N.; Brooks, K.; Churcher, C.; Fahkro, S.; Goodhead, I.; Heath, P.; Kartvelishvili, M.; Mungall, K.; Harris, D.; Hauser, H.; Sanders, M.; Saunders, D.; Seeger, K.; Sharp, S.; Taylor, J.E.; Walker, D.; White, B.; Young, R.; Cross, G.A.; Rudenko, G.; Barry, J.D.; Louis, E.J.; Berriman, M. Telomeric expression sites are highly conserved in *Trypanosoma brucei*. *PLoS One,* **2008**, *3*(10), e3527. Available from: journals.plos.org/plosone/article?id=10.1371/journal.pone.0003527

[34] Becker, M.; Aitcheson, N.; Byles, E.; Wickstead, B.; Louis, E.; Rudenko, G. Isolation of the repertoire of VSG expression site containing telomeres of *Trypanosoma brucei* 427 using transformation-associated recombination in yeast. *Genome Res.,* **2004**, *14*(11), 2319-2329.

[35] Berriman, M.; Hall, N.; Sheader, K.; Bringaud, F.; Tiwari, B.; Isobe, T.; Bowman, S.; Corton, C.; Clark, L.; Cross, G.A.; Hoek, M.; Zanders, T.; Berberof, M.; Borst, P.; Rudenko, G. The architecture of variant surface glycoprotein gene expression sites in *Trypanosoma brucei*. *Mol. Biochem. Parasitol.,* **2002**, *122*(2), 131-140.

[36] Kooter, J.M.; van der Spek, H.J.; Wagter, R.; d'Oliveira, C.E.; van der Hoeven, F.; Johnson, P.J.; Borst, P. The anatomy and transcription of a telomeric expression site for variant-specific surface antigens in *T. brucei*. *Cell,* **1987**, *51*(2), 261-272.

[37] Pedram, M.; Donelson, J.E. The anatomy and transcription of a monocistronic expression site for a metacyclic variant surface glycoprotein gene in *Trypanosoma brucei*. *J. Biol. Chem.,* **1999**, *274*(24), 16876-16883.

[38] Young, R.; Taylor, J.E.; Kurioka, A.; Becker, M.; Louis, E.J.; Rudenko, G. Isolation and analysis of the genetic diversity of repertoires of VSG expression site containing telomeres from *Trypanosoma brucei* gambiense, *T. b. brucei* and *T. equiperdum*. *BMC Genom.,* **2008**, *9*, 385. Available from: https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-9-385

[39] Salmon, D.; Geuskens, M.; Hanocq, F.; Hanocq-Quertier, J.; Nolan, D.; Ruben, L.; Pays, E. A novel heterodimeric transferrin receptor encoded by a pair of VSG expression site-associated genes in *T. brucei*. *Cell,* **1994**, *78*(1), 75-86.

[40] Salmon, D.; Bachmaier, S.; Krumbholz, C.; Kador, M.; Gossmann, J.A.; Uzureau, P.; Pays, E.; Boshart, M. Cytokinesis of *Trypanosoma brucei* bloodstream forms depends on expression of adenylyl cyclases of the ESAG4 or ESAG4-like subfamily. *Mol. Microbiol.,* **2012**, *84*(2), 225-242.

[41] Hoek, M.; Engstler, M.; Cross, G.A. Expression-site-associated gene 8 (ESAG8) of *Trypanosoma brucei* is apparently essential and accumulates in the nucleolus. *J. Cell. Sci.,* **2000**, *113*(Pt 22), 3959-3968.

[42] Redpath, M.B.; Windle, H.; Nolan, D.; Pays, E.; Voorheis, H.P.; Carrington, M. ESAG11, a new VSG expression site-associated gene from *Trypanosoma brucei*. *Mol. Biochem. Parasitol.,* **2000**, *111*(1), 223-228.

[43] Salmon, D.; Vanwalleghem, G.; Morias, Y.; Denoeud, J.; Krumbholz, C.; Lhomme, F.; Bachmaier, S.; Kador, M.; Gossmann, J.; Dias, F.B.; De Muylder, G.; Uzureau, P.; Magez, S.; Moser, M.; De Baetselier, P.; van den Abbeele, J.; Beschin, A.; Boshart, M.; Pays, E. Adenylate cyclases of *Trypanosoma brucei* inhibit the innate immune response of the host. *Science,* **2012**, *337*(6093) 463-466. Available from: science.sciencemag.org/content/early/2012/06/15/science.1222753.full

[44] Rolin, S.; Paindavoine, P.; Hanocq-Quertier, J.; Hanocq, F.; Claes, Y.; Le Ray, D.; Overath, P.; Pays, E. Transient adenylate cyclase activation accompanies differentiation of *Trypanosoma brucei* from bloodstream to procyclic forms. *Mol. Biochem. Parasitol.,* **1993**, *61*(1), 115-125.

[45] Xong, H.V.; Vanhamme, L.; Chamekh, M.; Chimfwembe, C.E.; van den Abbeele, J.; Pays, A.; van Meirvenne, N.; Hamers, R.; De Baetselier, P.; Pays, E. A VSG expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. *Cell,* **1998**, *95*(6), 839-846.

[46] Zomerdijk, J.C.; Ouellette, M.; ten Asbroek, A.L.; Kieft, R.; Bommer, A.M.; Clayton, C.E.; Borst, P. The promoter for a variant surface glycoprotein gene expression site in *Trypanosoma brucei*. *EMBO J.,* **1990**, *9*(9), 2791-2801.

[47] Zomerdijk, J.C.; Kieft, R.; Shiels, P.G.; Borst, P. Alpha-amanitin-resistant transcription units in trypanosomes: A comparison of promoter sequences for a VSG gene expression site and for the ribosomal RNA genes. *Nucleic Acids Res*., **1991**, *19*(19), 5153-5158.

[48] Biebinger, S.; Rettenmaier, S.; Flaspohler, J.; Hartmann, C.; Pena-Diaz, J.; Wirtz, L.E.; Hotz, H.R.; Barry, J.D.; Clayton, C. The PARP promoter of *Trypanosoma brucei* is developmentally regulated in a chromosomal context. *Nucleic Acids Res.,* **1996**, *24*(7). 1202-1211.

[49] Brown, S.D.; Huang, J.; Van der Ploeg, L.H. The promoter for the procyclic acidic repetitive protein (PARP) genes of *Trypanosoma brucei* shares features with RNA polymerase I promoters. *Mol. Cell Biol.,* **1992**, *12*(6), 2644-2652.

[50] Graham, S.V.; Wymer, B.; Barry, J.D. A trypanosome metacyclic VSG gene promoter with two functionally distinct, life cycle stage-specific activities. *Nucleic Acids Res.,* **1998**, *26*(8), 1985-1990.

[51] Graham, S.V.; Wymer, B.; Barry, J.D. Activity of a trypanosome metacyclic variant surface glycoprotein gene promoter is dependent upon life cycle stage and chromosomal context. *Mol. Cell Biol.,* **1998**, *18*, 1137-1146. Available from: mcb.asm.org/content/18/3/1137.full

[52] Janz, L.; Clayton, C. The PARP and rRNA promoters of *Trypanosoma brucei* are composed of dissimilar sequence elements that are

functionally interchangeable. *Mol. Cell Biol.,* **1994**, *14*(9) 5804-5811.

[53] Brandenburg, J.; Schimanski, B.; Nogoceke, E.; Nguyen, T.N.; Padovan, J.C.; Chait, B.T.; Cross, G.A.; Gunzl, A. Multifunctional class I transcription in *Trypanosoma brucei* depends on a novel protein complex. *EMBO J.,* **2007**, *26*(23), 4856-4866.

[54] Nagoshi, Y.L.; Alarcon, C.M.; Donelson, J.E. The putative promoter for a metacyclic VSG gene in African trypanosomes. *Mol. Biochem. Parasitol.,* **1995**, *72*(1-2), 33-45.

[55] Rudenko, G.; Blundell, P.A.; Dirks-Mulder, A.; Kieft, R.; Borst, P. 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,* **1995**, *83*(4), 547-553.

[56] McCulloch, R.; Morrison, L.J.; Hall, J.P. DNA recombination strategies during antigenic variation in the African trypanosome. *Microbiol. Spectr.,* **2015**, *3*(2), MDNA3-0016-2014. Available from: http://www.asmscience.org/content/journal/microbiolspec/10.1128/microbiolspec.MDNA3-0016-2014

[57] Scholler, J.K.; Myler, P.J.; Stuart, K.D. A novel telomeric gene conversion in *Trypanosoma brucei*. *Mol. Biochem. Parasitol.,* **1989**, *35*(1), 11-19.

[58] Hall, J.P.; Wang, H.; Barry, J.D. Mosaic VSGs and the scale of *Trypanosoma brucei* antigenic variation. *PLoS Pathog.,* **2013**, *9*(7), e1003502. Available from: journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1003502

[59] Marcello, L.; Barry, J.D. Analysis of the VSG gene silent archive in *Trypanosoma brucei* reveals that mosaic gene expression is prominent in antigenic variation and is favored by archive substructure. *Genome Res.,* **2007**, *17*(9), 1344-1352.

[60] Conway, C.; Proudfoot, C.; Burton, P.; Barry, J.D.; McCulloch, R. Two pathways of homologous recombination in *Trypanosoma brucei*. *Mol. Microbiol.,* **2002**, *45*(6), 1687-1700.

[61] Aline, R.F.Jr.; Myler, P.J.; Stuart, K.D. *Trypanosoma brucei*: Frequent loss of a telomeric variant surface glycoprotein gene. *Exp. Parasitol.,* **1989**, *68*(1), 8-16.

[62] Gottesdiener, K.M.; Goriparthi, L.; Masucci, J.P.; Van der Ploeg, L.H. A proposed mechanism for promoter-associated DNA rearrangement events at a variant surface glycoprotein gene expression site. *Mol. Cell Biol.,* **1992**, *12*(10), 4784-4795.

[63] Hovel-Miner, G.; Mugnier, M.R.; Goldwater, B.; Cross, G.A.; Papavasiliou, F.N. A conserved DNA repeat promotes selection of a diverse repertoire of *Trypanosoma brucei* surface antigens from the genomic archive. *PLoS Genet.,* **2016**, *12*(5), e1005994. Available from: journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1005994

[64] Glover, L.; Alsford, S.; Horn, D. DNA break site at fragile subtelomeres determines probability and mechanism of antigenic variation in African trypanosomes. *PLoS Pathog.,* **2013**, *9*(3) e1003260. Available from: journals.plos.org/plospathogens/article?id= 10.1371/journal.ppat.1003260

[65] Boothroyd, C.E.; Dreesen, O.; Leonova, T.; Ly, K.I.; Figueiredo, L.M.; Cross, G.A.; Papavasiliou, F.N. A yeast-endonuclease-generated DNA break induces antigenic switching in *Trypanosoma brucei*. *Nature,* **2009**, *459*(7244), 278-281. Available from: http://www.nature.com/nature/journal/v459/n7244/pubmed/nature07982.html

[66] Gjini, E.; Haydon, D.T.; Barry, J.D.; Cobbold, C.A. The impact of mutation and gene conversion on the local diversification of antigen genes in African trypanosomes. *Mol. Biol. Evol.,* **2012**, *29*(11), 3321-3331.

[67] Landeira, D.; Navarro, M. Nuclear repositioning of the VSG promoter during developmental silencing in *Trypanosoma brucei*. *J. Cell Biol.,* **2007**, *176*(2), 133-139.

[68] Landeira, D.; Bart, J.M.; Van Tyne, D.; Navarro, M. Cohesin regulates VSG monoallelic expression in trypanosomes. *J. Cell Biol.,* **2009**, *186*(2), 243-254.

[69] Navarro, M.; Penate, X.; Landeira, D. Nuclear architecture underlying gene expression in *Trypanosoma brucei*. *Trends Microbiol.,* **2007**, *15*(6), 263-270.

[70] Cestari, I.; Stuart, K. Inositol phosphate pathway controls transcription of telomeric expression sites in trypanosomes. *Proc. Natl. Acad. Sci. U.S.A.,* **2015**, *112*(21), E2803-E2812.

[71] Yang, X.; Figueiredo, L.M.; Espinal, A.; Okubo, E.; Li, B. RAP1 is essential for silencing telomeric variant surface glycoprotein genes in *Trypanosoma brucei*. *Cell,* **2009**, *137*(1), 99-109.

[72] Kassem, A.; Pays, E.; Vanhamme, L. Transcription is initiated on silent variant surface glycoprotein expression sites despite monoallelic expression in *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. U.S.A.,* **2014**, *111*(24), 8943-8948.

[73] Nguyen, T.N.; Muller, L.S.; Park, S.H.; Siegel, T.N.; Gunzl, A. Promoter occupancy of the basal class I transcription factor A differs strongly between active and silent VSG expression sites in *Trypanosoma brucei*. *Nucleic Acids Res.,* **2014**, *42*(5), 3164-3176.

[74] Nguyen, T.N.; Nguyen, B.N.; Lee, J.H.; Panigrahi, A.K.; Gunzl, A. Characterization of a novel class I transcription factor A (CITFA) subunit that is indispensable for transcription by the multifunctional RNA polymerase I of *Trypanosoma brucei*. *Eukaryot. Cell,* **2012**, *11*(12), 1573-1581.

[75] Lopez-Farfan, D.; Bart, J.M.; Rojas-Barros, D.I.; Navarro, M. SUMOylation by the E3 ligase TbSIZ1/PIAS1 positively regulates VSG expression in *Trypanosoma brucei*. *PLoS Pathog.,* **2014**, *10*(12), e1004545. Available from: journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1004545

[76] Ye, K.; Zhang, X.; Ni, J.; Liao, S.; Tu, X. Identification of enzymes involved in SUMOylation in *Trypanosoma brucei*. *Sci. Rep.,* **2015**, *5*, 10097. Available from: https://www.nature.com/articles/srep10097

[77] Flotho, A.; Melchior, F. SUMOylation: A regulatory protein modification in health and disease. *Annu. Rev. Biochem.,* **2013**, *82*, 357-385. Available from: http://www.annualreviews.org/doi/abs/10.1146/annurev-biochem-061909-093311

[78] Narayanan, M.S.; Rudenko, G. TDP1 is an HMG chromatin protein facilitating RNA polymerase I transcription in African trypanosomes. *Nucleic Acids Res.,* **2013**, *41*(5), 2981-2992.

[79] Glover, L.; Hutchinson, S.; Alsford, S.; Horn, D. VEX1 controls the allelic exclusion required for antigenic variation in trypanosomes. *Proc. Natl. Acad. Sci. U.S.A.,* **2016**, *113*(26), 7225-7230.

[80] Klug, A. Zinc finger peptides for the regulation of gene expression. *J. Mol. Biol.,* **1999**, *293*(2), 215-218.

[81] Laity, J.H.; Lee, B.M.; Wright, P.E. Zinc finger proteins: new insights into structural and functional diversity. *Curr. Opin. Struct. Biol.,* **2001**, *11*(1), 39-46.

[82] Gamsjaeger, R.; Liew, C.K.; Loughlin, F.E.; Crossley, M.; Mackay, J.P. Sticky fingers: Zinc-fingers as protein-recognition motifs. *Trends Biochem. Sci.,* **2007**, *32*(2) 63-70.

[83] Hughes, K.; Wand, M.; Foulston, L.; Young, R.; Harley, K.; Terry, S.; Ersfeld, K.; Rudenko, G. A novel ISWI is involved in VSG expression site downregulation in African trypanosomes. *EMBO J.,* **2007**, *26*(9), 2400-2410.

[84] Navarro, M.; Cross, G.A. Wirtz, E. *Trypanosoma brucei* variant surface glycoprotein regulation involves coupled activation/inactivation and chromatin remodeling of expression sites. *EMBO J.,* **1999**, *18*(8), 2265-2272.

[85] Povelones, M.L.; Gluenz, E.; Dembek, M.; Gull, K.; Rudenko, G. Histone H1 plays a role in heterochromatin formation and VSG expression site silencing in *Trypanosoma brucei*. *PLoS Pathog.,* **2012**, *8*(11), e1003010. Available from: journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1003010

[86] Figueiredo, L.M.; Janzen, C.J.; Cross, G.A. A histone methyltransferase modulates antigenic variation in African trypanosomes. *PLoS Biol.,* **2008**, *6*(7), e161. Available from: journals. plos.org/plosbiology/article?id=10.1371/journal.pbio.0060161

[87] Horn, D.; Cross, G.A. A developmentally regulated position effect at a telomeric locus in *Trypanosoma brucei*. *Cell,* **1995**, *83*(4), 555-561.

[88] Rudenko, G.; Blundell, P.A.; Taylor, M.C.; Kieft, R.; Borst, P. VSG gene expression site control in insect form *Trypanosoma brucei*. *EMBO J.,* **1994**, *13*(22), 5470-5482.

[89] Stanne, T.M.; Rudenko, G. Active VSG expression sites in *Trypanosoma brucei* are depleted of nucleosomes. *Eukaryot. Cell,* **2010**, *9*(1), 136-147.

[90] Figueiredo, L.M.; Cross, G.A. Nucleosomes are depleted at the VSG expression site transcribed by RNA polymerase I in African trypanosomes. *Eukaryot. Cell,* **2010**, *9*(1), 148-154.

[91] Pena, A.C.; Pimentel, M.R.; Manso, H.; Vaz-Drago, R.; Pinto-Neves, D.; Aresta-Branco, F.; Rijo-Ferreira, F.; Guegan, F.; Pedro Coelho, L.; Carmo-Fonseca, M.; Barbosa-Morais, N.L.; Figueiredo, L.M. *Trypanosoma brucei* histone H1 inhibits RNA polymerase I transcription and is important for parasite fitness *in vivo*. *Mol. Microbiol.,* **2014**, *93*(4), 645-663.

[92] Alsford, S.; Horn, D. Cell-cycle-regulated control of VSG expression site silencing by histones and histone chaperones ASF1A and

CAF-1b in *Trypanosoma brucei*. *Nucleic Acids Res.,* **2012**, *40*(20), 10150-10160.

[93] Lowell, J.E.; Cross, G.A. A variant histone H3 is enriched at telomeres in *Trypanosoma brucei*. *J. Cell. Sci.,* **2014**, *117*(Pt 24), 5937-5947.

[94] Schulz, D.; Mugnier, M.R.; Paulsen, E.M.; Kim, H.S.; Chung, C.W.; Tough, D.F.; Rioja, I.; Prinjha, R.K.; Papavasiliou, F.N.; Debler, E.W. Bromodomain proteins contribute to maintenance of bloodstream form stage identity in the African trypanosome. *PLoS Biol.,* **2015**, *13*(12), e1002316. Available from: journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1002316

[95] Batram, C.; Jones, N.G.; Janzen, C.J.; Markert, S.M.; Engstler, M. Expression site attenuation mechanistically links antigenic variation and development in *Trypanosoma brucei*. *Elife,* **2014**, *3*, e02324. Available from: https://elifesciences.org/articles/02324

[96] Gommers-Ampt, J.H.; Van Leeuwen, F.; de Beer, A.L.; Vliegenthart, J.F.; Dizdaroglu, M.; Kowalak, J.A.; Crain, P.F.; Borst, P. beta-D-glucosyl-hydroxymethyluracil: A novel modified base present in the DNA of the parasitic protozoan *T. brucei*. *Cell,* **1993**, *75*(6), 1129-1136.

[97] Gommers-Ampt, J.; Lutgerink, J.; Borst, P. A novel DNA nucleotide in *Trypanosoma brucei* only present in the mammalian phase of the life-cycle. *Nucleic Acids Res.,* **1991**, *19*(8), 1745-1751.

[98] van Leeuwen, F.; Wijsman, E.R.; Kieft, R.; van der Marel, G.A.; van Boom, J.H.; Borst, P. Localization of the modified base J in telomeric VSG gene expression sites of *Trypanosoma brucei*. *Genes Dev,* **1997**, *11*(23), 3232-3241.

[99] Cliffe, L.J.; Kieft, R.; Southern, T.; Birkeland, S.R.; Marshall, M.; Sweeney, K.; Sabatini, R. JBP1 and JBP2 are two distinct thymidine hydroxylases involved in J biosynthesis in genomic DNA of African trypanosomes. *Nucleic Acids Res.,* **2009**, *37*(5), 1452-1462.

[100] Bullard, W.; Lopes da Rosa-Spiegler, J.; Liu, S.; Wang, Y.; Sabatini, R. Identification of the glucosyltransferase that converts hydroxymethyluracil to base J in the trypanosomatid genome. *J. Biol. Chem.,* **2014**, *289*(29), 20273-20282.

[101] Sekar, A.; Merritt, C.; Baugh, L.; Stuart, K.; Myler, P.J. Tb927.10.6900 encodes the glucosyltransferase involved in synthesis of base J in *Trypanosoma brucei*. *Mol. Biochem. Parasitol.,* **2014**, *196*(1), 9-11.

[102] Stanne, T.M.; Narayanan, M.S.; Ridewood, S.; Ling, A.; Witmer, K.; Kushwaha, M.; Wiesler, S.; Wickstead, B.; Wood, J.; Rudenko, G. Identification of the ISWI chromatin remodeling complex of the early branching eukaryote *Trypanosoma brucei*. *J. Biol. Chem.,* **2015**, *290*(45), 26954-26967.

[103] Stanne, T.M.; Kushwaha, M.; Wand, M.; Taylor, J.E.; Rudenko, G. TbISWI regulates multiple polymerase I (Pol I)-transcribed loci and is present at Pol II transcription boundaries in *Trypanosoma brucei*. *Eukaryot. Cell,* **2011**, *10*(7), 964-976.

[104] Morillon, A.; Karabetsou, N.; O'Sullivan, J.; Kent, N.; Proudfoot, N.; Mellor, J. Isw1 chromatin remodeling ATPase coordinates transcription elongation and termination by RNA polymerase II. *Cell,* **2003**, *115*(4), 425-435.

[105] Haushalter, K.A.; Kadonaga, J.T. Chromatin assembly by DNA-translocating motors. *Nat. Rev. Mol. Cell. Biol.,* **2003**, *4*(8), 613-620.

[106] Racki, L.R.; Narlikar, G.J. ATP-dependent chromatin remodeling enzymes: Two heads are not better, just different. *Curr. Opin. Genet. Dev.,* **2008**, *18*(12), 137-144.

[107] Bouazoune, K.; Brehm, A. ATP-dependent chromatin remodeling complexes in *Drosophila*. *Chromosome Res.,* **2006**, *14*(4), 433-449.

[108] Vary, J.C.Jr.; Gangaraju, V.K.; Qin, J.; Landel, C.C.; Kooperberg, C.; Bartholomew, B.; Tsukiyama, T. Yeast Isw1p forms two separable complexes *in vivo*. *Mol. Cell. Biol.,* **2003**, *23*(1), 80-91.

[109] Hampl, V.; Hug, L.; Leigh, J.W.; Dacks, J.B.; Lang, B.F.; Simpson, A.G.; Roger, A.J. Phylogenomic analyses support the monophyly of Excavata and resolve relationships among eukaryotic "supergroups". *Proc. Natl. Acad. Sci. U.S.A.,* **2009**, *106*(10), 3859-3864.

[110] Garcia-Salcedo, J.A.; Gijon, P.; Nolan, D.P.; Tebabi, P.; Pays, E. A chromosomal SIR2 homologue with both histone NAD-dependent ADP-ribosyltransferase and deacetylase activities is involved in DNA repair in *Trypanosoma brucei*. *EMBO J.,* **2003**, *22*(21), 5851-5862.

[111] Alsford, S.; Kawahara, T.; Isamah, C.; Horn, D. A sirtuin in the African trypanosome is involved in both DNA repair and telomeric gene silencing but is not required for antigenic variation. *Mol. Microbiol.,* **2007**, *63*(3), 724-736.

[112] Wang, Q.P.; Kawahara, T.; Horn, D. Histone deacetylases play distinct roles in telomeric VSG expression site silencing in African trypanosomes. *Mol. Microbiol.,* **2010**, *77*(5), 1237-1245.

[113] Freitas-Junior, L.H.; Hernandez-Rivas, R.; Ralph, S.A.; Montiel-Condado, D.; Ruvalcaba-Salazar, O.K.; Rojas-Meza, A.P.; Mancio-Silva, L.; Leal-Silvestre, R.J.; Gontijo, A.M.; Shorte, S.; Scherf, A. Telomeric heterochromatin propagation and histone acetylation control mutually exclusive expression of antigenic variation genes in malaria parasites. *Cell,* **2005**, *121*(1), 25-36.

[114] Duraisingh, M.T.; Voss, T.S.; Marty, A.J.; Duffy, M.F.; Good, R.T.; Thompson, J.K.; Freitas-Junior, L.H.; Scherf, A.; Crabb, B.S.; Cowman, A.F. Heterochromatin silencing and locus repositioning linked to regulation of virulence genes in *Plasmodium falciparum*. *Cell,* **2005**, *121*(1), 13-24.

[115] Chien, C.T.; Buck, S.; Sternglanz, R.; Shore, D. Targeting of SIR1 protein establishes transcriptional silencing at HM loci and telomeres in yeast. *Cell,* **1993**, *75*(3), 531-541.

[116] Braunstein, M.; Rose, A.B.; Holmes, S.G.; Allis, C.D.; Broach, J.R. Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. *Genes Dev.,* **1993**, *7*(4), 592-604.

[117] Hecht, A.; Strahl-Bolsinger, S.; Grunstein, M. Spreading of transcriptional repressor SIR3 from telomeric heterochromatin. *Nature, 383*(6595), 92-96. Available from: www.nature.com/articles/383092a0

[118] Strahl-Bolsinger, S.; Hecht, A.; Luo, K.; Grunstein, M. SIR2 and SIR4 interactions differ in core and extended telomeric heterochromatin in yeast. *Genes Dev.,* **1997**, *11*(1), 83-93.

[119] Tanny, J.C.; Dowd, G.J.; Huang, J.; Hilz, H.; Moazed, D. An enzymatic activity in the yeast Sir2 protein that is essential for gene silencing. *Cell,* **1999**, *99*(7), 735-745.

[120] Imai, S.; Armstrong, C.M.; Kaeberlein, M.; Guarente, L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature,* **2000**, *403*(6771), 795-800. Available from: www.nature.com/articles/35001622

[121] Denninger, V.; Rudenko, G. FACT plays a major role in histone dynamics affecting VSG expression site control in *Trypanosoma brucei*. *Mol. Microbiol.,* **2014**, *94*(4), 945-962.

[122] Denninger, V.; Fullbrook, A.; Bessat, M.; Ersfeld, K.; Rudenko, G. The FACT subunit TbSpt16 is involved in cell cycle specific control of VSG expression sites in *Trypanosoma brucei*. *Mol. Microbiol.,* **2010**, *78*(2), 459-474.

[123] Gottschling, D.E.; Aparicio, O.M.; Billington, B.L.; Zakian, V.A. Position effect at S. cerevisiae telomeres: Reversible repression of Pol II transcription. *Cell,* **1990**, *63*(4), 751-762.

[124] Blasco, M.A. The epigenetic regulation of mammalian telomeres. *Nat. Rev. Genet.,* **2007**, *8*(4), 299-309.

[125] Figueiredo, L.M.; Freitas-Junior, L.H.; Bottius, E.; Olivo-Marin, J.C.; Scherf, A. A central role for *Plasmodium falciparum* subtelomeric regions in spatial positioning and telomere length regulation. *EMBO J.,* **2002**, *21*(4), 815-824.

[126] Chung, H.M.; Shea, C.; Fields, S.; Taub, R.N.; Van der Ploeg, L.H.; Tse, D.B. Architectural organization in the interphase nucleus of the protozoan *Trypanosoma brucei*: Location of telomeres and mini-chromosomes. *EMBO J.,* **1990**, *9*(8), 2611-2619.

[127] Perez-Morga, D.; Amiguet-Vercher, A.; Vermijlen, D.; Pays, E. Organization of telomeres during the cell and life cycles of *Trypanosoma brucei*. *J. Eukaryot. Microbiol.,* **2001**, *48*(2), 221-226.

[128] DuBois, K.N.; Alsford, S.; Holden, J.M.; Buisson, J.; Swiderski, M.; Bart, J.M.; Ratushny, A.V.; Wan, Y.; Bastin, P.; Barry, J.D.; Navarro, M.; Horn, D.; Aitchison, J.D.; Rout, M.P.; Field, M.C. NUP-1 is a large coiled-coil nucleoskeletal protein in trypanosomes with lamin-like functions. *PLoS Biol.,* **2012**, *10*(3), e1001287. Available from: journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1001287

[129] Maishman, L.; Obado, S.O.; Alsford, S.; Bart, J.M.; Chen, W.M.; Ratushny, A.V.; Navarro, M.; Horn, D.; Aitchison, J.D.; Chait, B.T.; Rout, M.P.; Field, M.C. Co-dependence between trypanosome nuclear lamina components in nuclear stability and control of gene expression. *Nucleic Acids Res.,* **2016**, *44*(22), 10554-10570.

[130] Li, B.; Espinal, A.; Cross, G.A. Trypanosome telomeres are protected by a homologue of mammalian TRF2. *Mol. Cell. Biol.,* **2005**, *25*(12), 5011-5021.

[131] Jehi, S.E.; Li, X.; Sandhu, R.; Ye, F.; Benmerzouga, I.; Zhang, M.; Zhao, Y.; Li, B. Suppression of subtelomeric VSG switching by *Trypanosoma brucei* TRF requires its TTAGGG repeat-binding ac-

tivity. *Nucleic Acids Res.,* **2014**, *42*(20), 12899-12911.

[132] Jehi, S.E.; Wu, F.; Li, B. *Trypanosoma brucei* TIF2 suppresses VSG switching by maintaining subtelomere integrity. *Cell Res.,* **2014**, *24*(7), 870-885.

[133] Jehi, S.E.; Nanavaty, V.; Li, B. *Trypanosoma brucei* TIF2 and TRF suppress VSG switching using overlapping and independent mechanisms. *PLoS One,* **2016**, *11*(6), e0156746. Available from: journals.plos.org/plosone/article?id=10.1371/journal.pone.0156746

[134] Pandya, U.M.; Sandhu, R.; Li, B. Silencing subtelomeric VSGs by *Trypanosoma brucei* RAP1 at the insect stage involves chromatin structure changes. *Nucleic Acids Res*., **2013**, *41*(16), 7673-7682.

[135] Tiengwe, C.; Marcello, L.; Farr, H.; Dickens, N.; Kelly, S.; Swiderski, M.; Vaughan, D.; Gull, K.; Barry, J.D.; Bell, S.D.; McCulloch, R. Genome-wide analysis reveals extensive functional interaction between DNA replication initiation and transcription in the genome of *Trypanosoma brucei*. *Cell Rep.,* **2012**, *2*(1), 185-197.

[136] Benmerzouga, I.; Concepcion-Acevedo, J.; Kim, H.S.; Vandoros, A.V.; Cross, G.A.; Klingbeil, M.M.; Li, B. *Trypanosoma brucei* Orc1 is essential for nuclear DNA replication and affects both VSG silencing and VSG switching. *Mol. Microbiol.,* **2013**, *87*(1), 196-210.

[137] Kim, H.S.; Park, S.H.; Gunzl, A.; Cross, G.A. MCM-BP is required for repression of life-cycle specific genes transcribed by RNA polymerase I in the mammalian infectious form of *Trypanosoma brucei*. *PLoS One,* **2013**, *8*(2), e57001. Available from: journals.plos.org/plosone/article?id=10.1371/journal.pone.0057001

[138] Bochman, M.L.; Schwacha, A. The Mcm complex: Unwinding the mechanism of a replicative helicase. *Microbiol. Mol. Biol. Rev.* **2009**, *73*(4), 652-683.

[139] Demmel, L.; Schmidt, K.; Lucast, L.; Havlicek, K.; Zankel, A.; Koestler, T.; Reithofer, V.; de Camilli, P.; Warren, G. The endocytic activity of the flagellar pocket in *Trypanosoma brucei* is regulated by an adjacent phosphatidylinositol phosphate kinase. *J. Cell Sci.,* **2014**, *127*(Pt 10), 2351-2364.

[140] Mellman, D.L.; Gonzales, M.L.; Song, C.; Barlow, C.A.; Wang, P.; Kendziorski, C.; Anderson, R.A. A PtdIns4,5P2-regulated nuclear poly(A) polymerase controls expression of select mRNAs. *Nature,* **2008**, *451*(7181), 1013-1017. Available from: https://www.nature.com/articles/nature06666

[141] Sobol, M.; Yildirim, S.; Philimonenko, V.V.; Marasek, P.; Castano, E.; Hozak, P. UBF complexes with phosphatidylinositol 4,5-bisphosphate in nucleolar organizer regions regardless of ongoing RNA polymerase I activity. *Nucleus,* **2003**, *4*(6), 478-486.

[142] Yildirim, S.; Castano, E.; Sobol, M.; Philimonenko, V.V.; Dzijak, R.; Venit, T.; Hozak, P. Involvement of phosphatidylinositol 4,5-bisphosphate in RNA polymerase I transcription. *J. Cell. Sci.,* **2013**, *126*(Pt 12), 2730-2739.

[143] Watson, P.J.; Fairall, L.; Santos, G.M.; Schwabe, J.W. Structure of HDAC3 bound to co-repressor and inositol tetraphosphate. *Nature,* **2012**, *481*(7381), 335-340. Available from: https://www.nature.com/articles/nature10728

[144] Irvine, R.F. Nuclear lipid signalling. *Nat. Rev. Mol. Cell Biol.,* **2003**, *4*(5), 349-360.

[145] Blind, R.D.; Suzawa, M.; Ingraham, H.A. Direct modification and activation of a nuclear receptor-PIP(2) complex by the inositol lipid kinase IPMK. *Sci. Signal.,* **2012**, *5*(229), ra44. Available from: http://stke.sciencemag.org/content/5/229/ra44

[146] Cestari, I.; Haas, P.; Moretti, N.S.; Schenkman, S.; Stuart, K. Chemogenetic characterization of inositol phosphate metabolic pathway reveals druggable enzymes for targeting kinetoplastid parasites. *Cell Chem. Biol.,* **2016**, *23*(5), 608-617.

[147] Amiguet-Vercher, A.; Perez-Morga, D.; Pays, A.; Poelvoorde, P.; Van Xong, H.; Tebabi, P.; Vanhamme, L.; Pays, E. Loss of the mono-allelic control of the VSG expression sites during the development of *Trypanosoma brucei* in the bloodstream. *Mol. Microbiol.,* **2004**, *51*(6), 1577-1588.

[148] Delauw, M.F.; Pays, E.; Steinert, M.; Aerts, D.; Van Meirvenne, N.; Le Ray, D. Inactivation and reactivation of a variant-specific antigen gene in cyclically transmitted *Trypanosoma brucei*. *EMBO J.,* **1985**, *4*(4), 989-993.

[149] Narayanan, M.S.; Kushwaha, M.; Ersfeld, K.; Fullbrook, A.; Stanne, T.M.; Rudenko, G. NLP is a novel transcription regulator involved in VSG expression site control in *Trypanosoma brucei*. *Nucleic Acids Res.,* **2011**, *39*(6), 2018-2031.

[150] de Bruin, D.; Kantrow, S.M.; Liberatore, R.A.; Zakian, V.A. Telomere folding is required for the stable maintenance of telomere position effects in yeast. *Mol. Cell Biol.,* **2000**, *20*(21), 7991-8000.

[151] de Bruin, D.; Zaman, Z.; Liberatore, R.A.; Ptashne, M. Telomere looping permits gene activation by a downstream UAS in yeast. *Nature,* **2001**, *409*, 109-113. Available from: https://www.nature.com/articles/35051119

[152] Munoz-Jordan, J.L.; Cross, G.A.; de Lange, T.; Griffith, J.D.) t-loops at trypanosome telomeres. *EMBO J.,* **2001**, *20*(3), 579-588.

[153] Mugnier, M.R.; Cross, G.A.; Papavasiliou, F.N. The *in vivo* dynamics of antigenic variation in *Trypanosoma brucei*. *Science,* **2015**, *347*(6229), 1470-1473. Available from: http:// science.sciencemag.org/content/347/6229/1470

[154] Peacock, L.; Bailey, M.; Carrington, M.; Gibson, W. Meiosis and haploid gametes in the pathogen *Trypanosoma brucei*. *Curr. Biol.* **2014**, *24*(4), 181-186.

[155] Park, S.H.; Nguyen, B.N.; Kirkham, J.K.; Nguyen, T.N.; Gunzl, A. A new strategy of RNA interference that targets heterologous sequences reveals CITFA1 as an essential component of class I transcription factor A in *Trypanosoma brucei*. *Eukaryot. Cell,* **2014**, *13*(6), 785-795.