Essays Biochem. (2010) **48**, 201–219; doi:10.1042/BSE0480201

13

Epigenetics and transcriptional control in African trypanosomes

Gloria Rudenko[1]

Division of Cell and Molecular Biology, Sir Alexander Fleming Building, Imperial College, South Kensington, London SW7 2AZ, U.K.

Abstract

The African trypanosome *Trypanosoma brucei* is a unicellular parasite which causes African sleeping sickness. Transcription in African trypanosomes displays some unusual features, as most of the trypanosome genome is transcribed as extensive polycistronic RNA Pol II (polymerase II) transcription units that are not transcriptionally regulated. In addition, RNA Pol I is used for transcription of a small subset of protein coding genes in addition to the rDNA (ribosomal DNA). These Pol I-transcribed protein coding genes include the *VSG* (variant surface glycoprotein) genes. Although a single trypanosome has many hundreds of *VSG* genes, the active *VSG* is transcribed in a strictly monoalleleic fashion from one of approx. 15 telomeric VSG ESs (expression sites). Originally, it was thought that chromatin was not involved in the transcriptional control of ESs; however, this view is now being re-evaluated. It has since been shown that the active ES is depleted of nucleosomes compared with silent ESs. In addition, a number of proteins involved in chromatin remodelling or histone modification and which play a role in ES silencing {including TbISWI [*T. brucei* ISWI (imitation-switch protein)] and DOT1B} have recently been identified. Lastly, the telomere-binding protein TbRAP1 (*T. brucei* RAP1) has been shown to establish a repressive gradient extending

[1]*email gloria.rudenko@imperial.ac.uk*

from the ES telomere end up to the ES promoter. We still need to determine which epigenetic factors are involved in 'marking' the active ES as part of the counting mechanism of monoallelic exclusion. The challenge will come in determining how these multiple regulatory layers contribute to ES control.

Introduction

African trypanosomes are unicellular flagellated eukaryotes which have evolved from an ancestor that branched off from other eukaryotes relatively early [1]. This ancient lineage plus aspects of their biology mean that transcription in trypanosomes displays some very unusual features. There is very little transcriptional control in African trypanosomes, and control of mRNA abundance occurs post-transcriptionally, primarily at the level of mRNA processing and stability [2]. In addition, unusually for a eukaryote, some trypanosome protein-coding genes are transcribed by RNA Pol I (polymerase I) [3], which in other eukaryotes exclusively transcribes rDNA (ribosomal DNA). There is transcriptional regulation of these Pol I transcription units, of which some are expressed in a strictly monoallelic fashion.

The role that epigenetics plays in the regulation of gene expression in the trypanosome genome [4], as well as in the monoallelic control of Pol I expression, is currently being investigated. Since trypanosomes are so divergent from the standard eukaryotic model organisms, studying their molecular biology not only gives insight into the diversity of biochemical pathways that have evolved, but could eventually prove useful in treating disease. Members of the trypanosome family cause maladies including Chagas disease in South America caused by *Trypanosoma cruzi*, and African sleeping sickness caused by the African trypanosome *Trypanosoma brucei*. Sleeping sickness is endemic to sub-Saharan Africa, and is transmitted by tsetse flies to humans and their livestock, as well as to a large range of reservoir hosts. While multiplying in the bloodstream of the mammalian host, trypanosomes rely on an extraordinarily sophisticated strategy of antigenic variation of a protective VSG (variant surface glycoprotein) coat to evade host antibodies [5]. Hopefully, understanding how trypanosome biochemical pathways differ from those of the host, as well as how transcription of these different *VSG*s is controlled, will eventually give us the tools to tackle these evasive disease-causing parasites.

Trypanosome genome structure and transcription

The *T. brucei* genome is approx. 30–40 MB depending on the strain, and contains 11 diploid megabase chromosomes [6–8], as well as approx. 100 nontranscribed minichromosomes of 50–100 kb each [9]. A striking feature of the *T. brucei* genome is that the bulk of it is arranged as very large constitutively transcribed polycistronic Pol II transcription units superficially similar to operons in bacteria (Figure 1). However, unlike bacterial operons that contain coregulated genes often in the same biochemical pathway,

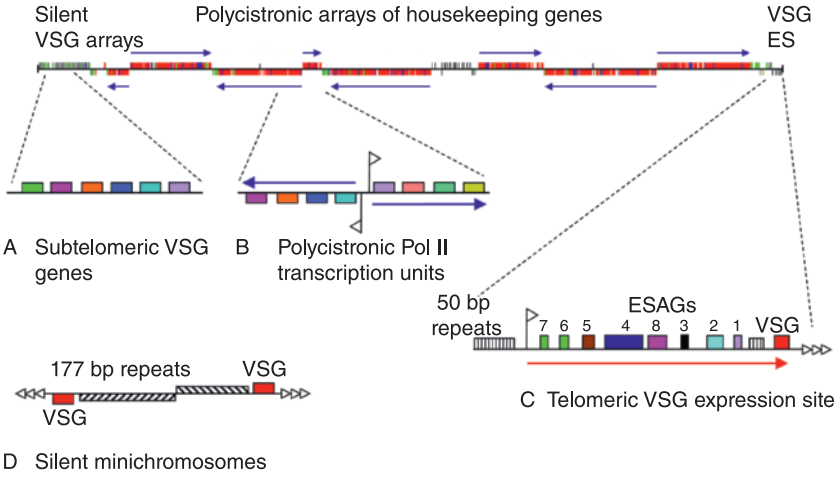

Figure 1. Organization of the genome of African trypanosomes

At the top of the Figure, a schematic of a typical *T. brucei* chromosome is indicated, with open reading frames indicated with red bars. Most of the genes within the core of the *T. brucei* chromosome are arranged as convergent or divergent polycistronic transcription units with transcription indicated with blue arrows. Various chromosomal regions are expanded in the different panels below. (**A**) The bulk of the silent *VSG* genes and pseudogenes are arranged in tandem arrays at the subtelomeres of the chromosome. Coloured boxes indicate genes. (**B**) The majority of the trypanosome house-keeping genes are present in polycistronic transcription units transcribed by RNA Pol II. The promoters are indicated with flags. (**C**) The active *VSG* gene is transcribed from a telomeric *VSG* ES transcription unit containing various ESAGs, indicated with numbered boxes, in addition to the telomeric *VSG* gene. The telomere repeats are indicated with horizontal arrowheads. Characteristic ES simple sequence repeats are indicated with striped boxes. (**D**) *T. brucei* contains approx. 100 transcriptionally silent minichromosomes of approx. 50–100 kb, which are composed primarily of large palindromes of 177 bp repeat sequences (diagonally striped boxes), with *VSG* genes at the telomeres (indicated with arrowheads).

individual polycistronic trypanosome transcription units appear to contain relatively random assortments of genes [8]. Maturation of functional transcripts from these polycistronic precursor RNAs proceeds through *trans*-splicing of a capped spliced leader RNA onto the 5′ end of each mRNA as well as polyadenylation [10]. There is no evidence for transcriptional regulation of any of these polycistronic Pol II transcription units, either within a given life-cycle stage or after drastic perturbations including knock-down of clathrin or VSG [2,11,12]. Compatible with this, bioinformatic analyses of the *T. brucei* genome have shown relatively few homologues for eukaryotic transcription factors [13,14].

Very little is known about Pol II promoters in trypanosomes, and the only one that is well characterised is the promoter for the spliced leader RNA transcription units [15,16]. Part of the difficulty of analysing trypanosome Pol II promoters has been their very weak activity in driving reporter genes in transient transfections, making some of the published data controversial. It is clear that promoters must be present at the junctions where two polycistronic transcription units diverge from each other (Figure 1B). However, mapping

these weak trypanosomatid Pol II promoters at these divergent 'strand switch' regions using strand-specific nuclear run-ons [17] has proven to be technically challenging.

Therefore, our best approximation of the location of the Pol II promoters of the *T. brucei* genome has come from a comprehensive genomic analysis of the location of several different *T. brucei* histone variants [18]. It has recently been shown that the histone modification H4K10ac, as well as different *T. brucei* histone variants (H2AZ and H2BV), and the bromodomain factor BDF3, are significantly enriched at putative Pol II transcription start sites (Figure 2) [18]. In addition, histone variants H3V and H4V are enriched at probable Pol II transcription termination sites. Mapping the location of these different epigenetic modifications and histone variants over the *T. brucei* genome allowed the validation of a previously identified actin Pol II promoter [19], as well as the localization of numerous new putative Pol II promoters. Conserved Pol II promoter sequences were not identified at these strand switch regions, although the regions upstream of putative transcription start sites consistently contain polyguanine stretches [18]. These results have led to the speculation that trypanosomes, as early branching eukaryotes lacking sophisticated

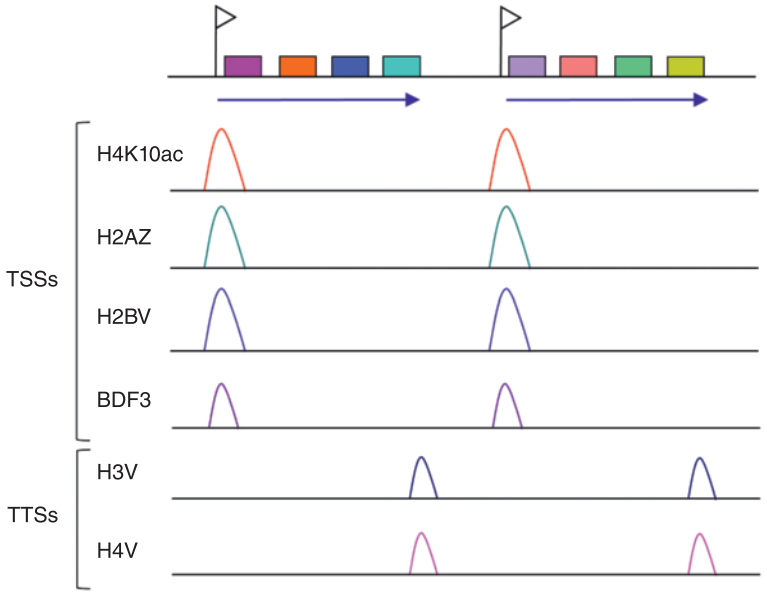

Figure 2. Distribution of various epigenetic markers over the transcription start and termination sites of *T. brucei* polycistronic Pol II transcription units

This Figure is a highly simplified schematic diagram of data presented in [18]. Two Pol II transcription units are indicated above, with the promoters indicated with flags, various genes with coloured boxes, and transcription with blue arrows. The distribution of various histone modifications (H4K10ac), histone variants (H2AZ, H2BV, H3V, H4V) or the bromodomain factor BDF3 over these transcription units is indicated below. Epigenetic modifications occurring over the transcription start sites (TSS) or transcription termination sites (TTS) are grouped separately.

transcription regulatory elements, use the deployment of histone variants to regulate transcription [4].

Monoallelic expression of VSG

Not all of the *T. brucei* genome is constitutively transcribed. Large segments of the genome containing vast repertoires of genes necessary for antigenic variation are kept transcriptionally silent. The bloodstream form trypanosome multiplying in the mammalian host is protected by a dense layer of VSG [20]. During an infection, the host eventually mounts an effective antibody response against the predominant VSG coat type, allowing the destruction of recognised trypanosomes. However, within a given trypanosome population VSG switch variants arise, which have switched to a new VSG not recognised by host antibodies. These new variants have a temporary growth advantage, until they too are recognised by host antibodies. As an individual trypanosome has more than 1000 *VSG* genes and pseudogenes [21], there is a vast wardrobe of *VSG* genes available that the trypanosome can draw upon as it changes its coat. This continuous switching of the VSG surface coat allows African trypanosomes to form chronic infections which can last for many years (reviewed in [5,22]).

Although most silent *VSG*s are located in large subtelomeric arrays [23], the active *VSG* is located in one of approx. 15 *VSG* ESs (expression sites) (Figure 1) [24]. ESs are polycistronic transcription units which are invariably located at telomeres, and contain an assortment of ESAGs (ES-associated genes) in addition to the telomeric *VSG* [24–26]. Only one of the many ESs is active at a time, and is transcribed in a strictly monoallelic fashion [27,28]. Switching expression of the active *VSG* can involve replacing the *VSG* in the active ES with a new *VSG* through gene conversion (Figure 3) [29]. This switching mechanism can allow the trypanosome to activate almost any silent *VSG* in the genome. Alternatively, the active *VSG* can be changed through exchange of two *VSG* containing telomere ends or switching to activation of a different ES.

A severe restriction operates, ensuring that only one ES is maximally active at a time, and it has not proven possible to select for trypanosomes which simultaneously and stably maintain transcription of two active ESs [27]. Drug selection for trypanosomes with two active ESs leads to the generation of trypanosomes which appear to be continuously switching between transcription of the two [27,30]. Removal of selection pressure leads to these rapidly switching trypanosomes reverting back to stable transcription of one of the two ESs. This strict monoallelic exclusion operating on the VSG ESs is critical for effective antigenic variation, as it prevents the bloodstream form trypanosome from expressing multiple VSG variants on its surface, and therefore running through its VSG coat repertoire unnecessarily quickly.

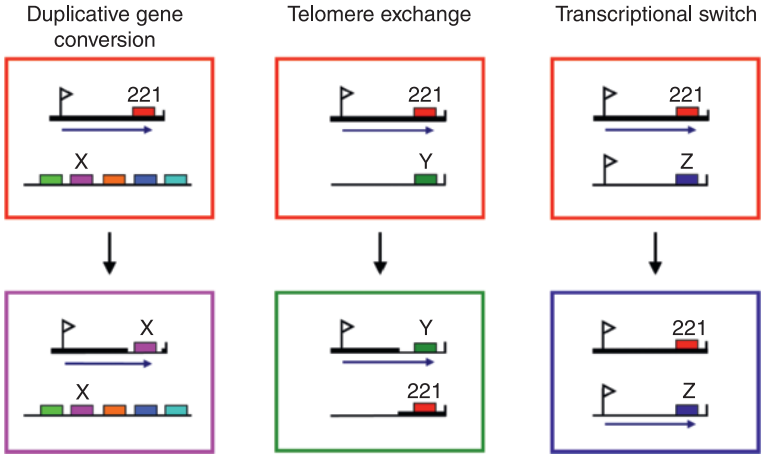

Figure 3. Major mechanisms of VSG switching in African trypanosomes
The large open boxes indicate trypanosomes which express the active *VSG221* from a telomeric *VSG221* ES. The small filled coloured boxes indicate *VSG*s. The ES promoter is indicated with a white flag and transcription with an arrow. After a duplicative gene conversion event, a copy of a silent *VSG* (*VSGX*) is inserted into the active ES, replacing the active *VSG*. After a telomere exchange, a cross-over occurs on two *VSG* containing telomere ends. This results in a previously silent *VSG* being moved into the active ES. A transcriptional switch entails the trypanosome activating a new ES, and silencing the old one.

Why are VSG ESs transcribed by RNA Pol I?

Another highly unusual feature of *T. brucei* is that not only the ribosomal DNA, but some of its protein coding genes, are transcribed by Pol I. Both the VSG ESs containing *VSG* (the major surface protein expressed in bloodstream form trypanosomes) and the procyclin loci (which encode the major surface protein in insect form trypanosomes) are transcribed by Pol I rather than the usual Pol II [3]. In general, eukaryotic Pol I transcription units can be expected to generate uncapped, and therefore potentially untranslateable, mRNA. It has been shown experimentally in yeast and mammalian cells that rDNA promoters inserted in front of protein coding genes generate high levels of unstable transcript that is very inefficiently translated [31,32]. As trypanosomes add a capped spliced leader RNA transcript to the 5′ ends of their transcripts through *trans*-splicing [10], this could potentially have allowed recruitment of an alternative RNA polymerase to transcribe a subset of their protein coding genes.

Why would *T. brucei* select Pol I rather than Pol II for the transcription of some of its protein coding genes? First of all, this could be a means for ensuring high levels of transcript, as Pol I transcription units in *T. brucei* typically appear to be transcribed at an approx. 10-fold higher rate than the polycistronic Pol II transcription units [33]. This could have resulted in trypanosomes recruiting a nonorthodox RNA polymerase for the high level expression of

specific proteins, where in the case of VSG, a single copy gene encodes the most abundant protein of the cell (making up to 10% total protein) [34]. In contrast, transcript dosage of Pol II-transcribed 'house-keeping' genes in *T. brucei* appears to be primarily regulated through amplification of gene copy number, as well as transcript RNA stability elements, rather than promoter strength [35].

An additional possibility is that Pol I in trypanosomes has been recruited for the transcription of genes where transcriptional control is required. Although there is no evident transcriptional control of trypanosome Pol II transcription units, the trypanosomal Pol I transcription units *VSG* and procyclin are regulated in a life-cycle specific fashion. The *VSG* ESs, which are essential in bloodstream form trypanosomes, are downregulated in insect form *T. brucei* where VSG is not necessary [36], as well as being controlled in a monoallelic fashion in bloodstream form *T. brucei* [28]. In addition, the procyclin loci are transcriptionally active in the insect form trypanosome present in the mid-gut of the tsetse fly insect vector, but are transcriptionally downregulated in bloodstream form trypanosomes [37]. *VSG* ESs are regulated not only at the level of transcription initiation, but are also controlled at the level of transcription elongation. Significant levels of transcription initiate from 'silent' ES promoters in both bloodstream and insect form *T. brucei,* but fully processive RNA polymerases only extend down to the telomere in the active bloodstream form ES [36,38].

Telomeric location and *VSG* ES control

How are these ESs controlled? *VSG* ES transcription units are invariably located at chromosome ends. In *Saccharomyces cerevisiae*, 'TPE' (telomere position effect) results in the unstable downregulation of subtelomeric promoters, with a gradient of repression increasing towards the telomere end [39,40]. In trypanosomes, a localized gradient of silencing appears to operate on Pol I promoters inserted immediately adjacent to telomeres in bloodstream form *T. brucei* [41]. However, there is no evidence that this phenomenon plays a role in the monoallelic exclusion of *VSG* ESs. Deletion of the telomere of the inactive ES using conditional I-Sce I meganuclease cleavage results in the loss of this localized telomeric silencing, whereas silencing of the *VSG* ES promoter is maintained [42].

ES silencing differs mechanistically from telomere position effect in yeast, as several proteins shown to be essential for telomere position effect in *S. cerevisiae* are not involved in *VSG* ES control. For example SIR2 plays a key role in telomere position effect in *S. cerevisiae* [40,43], as well as in phenotypic switching in *Candida albicans* [44], and antigenic variation of the *VAR* genes in the malaria parasite *Plasmodium falciparum* [45,46]. In trypanosomes, SIR2 does not appear to be involved in *VSG* ES control, where the ES promoter is many tens of kilobases upstream of the telomere end [47]. Instead, it does play some role in the repression of Pol I promoters inserted immediately adjacent

to telomere ends, as well as being involved in DNA repair [47,48]. Similarly, the KU70/80 heterodimer in *S. cerevisiae* is involved in the maintenance of silent telomeric chromatin and also has other functions at telomeres [49,50]. However, the *T. brucei* KU homologue, although important for telomere maintenance, including telomere length regulation, does not play a role in *VSG* ES control [51,52].

How important is a telomeric location for VSG ES control? Recent data would argue that it is probably critical, as *T. brucei* RAP1 has been shown to be essential for ES silencing [53]. RAP1 is a telomere binding protein which can either repress or activate transcription, and has been shown to play a major role in silencing at the mating type loci and telomeres in *S. cerevisiae* [54,55]. In *T. brucei*, knockdown of TbRAP1 (*T. brucei* RAP1) results in derepression of all known VSG ESs, with a stronger derepression observed in genes located closer to telomeres [53]. However, there is no indication that TbRAP1 is a general transcription regulator in trypanosomes, as none of the other Pol I or Pol II transcribed control genes or other non-ES-located *VSG*s tested in trypanosomes were derepressed when RAP1 synthesis was blocked [53].

These data lead to a model whereby a gradient of TbRAP1-dependent silencing extends from the ES chromosome ends, creating a repressive force preventing transcription elongation from partially active ES promoters [53]. TbRAP1 knockdown results in only partial ES derepression, however, indicating that additional layers of control must be involved in preventing full ES activation. Therefore, at the moment there appears to be evidence for two types of repressive gradients operating at bloodstream form trypanosome telomeres. One is relatively localized, requires SIR2 for spreading, and might involve TbRAP1 for telomere binding [47]. The other gradient extends up to the ES promoter and requires TbRAP1 for telomere binding, although it is still unclear which additional factors are necessary for spreading (Figure 4) [53,56].

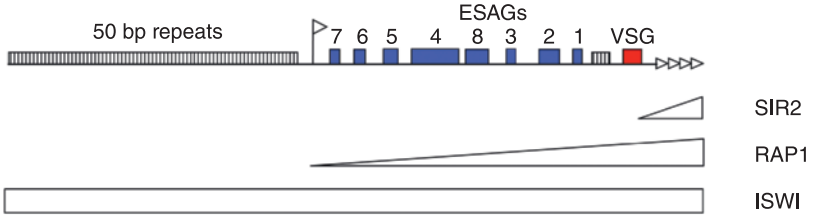

Figure 4. Silencing forces operating on *VSG* ESs

SIR2 is thought to be involved in a silencing gradient operating in the immediate vicinity of the telomeres of *VSG* ESs in bloodstream form *T. brucei*. Depletion of SIR2 does not result in derepression of the silent ES promoters. In addition, the telomere-binding protein RAP1 is involved in a second gradient of silencing extending up from the telomere and influencing transcription from the ES promoters. Knock-down of RAP1 does result in derepression of silent ES promoters. Lastly, ISWI appears to be required for full silencing of silent ESs, and could be one of the factors limiting full ES derepression after the knock-down of RAP1.

Epigenetic modifications of the *VSG* ES

Monoallelic expression of *VSG* ESs requires that one of the many highly similar ES transcription units is treated differently from the others, and that this activated transcriptional state is then heritably transmitted over many generations. What are the role of epigenetic markers in 'labelling' the silent or active *VSG* ES? Presence of the modified DNA base 5-methylcytosine at CpG dinucleotides in mammalian cells is associated with a repressed chromatin state and impaired gene expression [57]. This inhibition of gene expression can be mediated through methylation, preventing DNA-binding factors from binding to regulatory regions. Alternatively, methyl-CpG-binding proteins and associated transcriptional co-repressor molecules can be recruited to the methylated DNA, resulting in modification of the surrounding chromatin to an inactive state [57]. The modified base 5-methylcytosine has recently been found in *T. brucei*, however the abundance of this modified base is very low (estimated at approx. 1 modified base per 10000 cytosines in the *T. brucei* genome) [58]. Initial studies indicate that localization of this modified base does not appear to be restricted to silenced regions of the *T. brucei* genome, making its role in transcriptional silencing unclear. However, further analysis of the functional role of this DNA modification in *T. brucei* still needs to be performed.

Much more is known about the novel base 'J' (or β-D-glucosyl-hydroxymethyldeoxyuridine), which is unique to kinetoplastid flagellates, as well as some unicellular flagellates which are evolutionarily close to trypanosomes [59]. Base J was initially found in silent *VSG* ESs in bloodstream form *T. brucei*, where it is particularly enriched in a gradient increasing in abundance down to the ES telomere (Figure 5) [60]. This lead to the appealing hypothesis that base J could play a role in downregulation of ES transcription. In addition to being present in silent ESs, J is also abundant in various trypanosome repeat sequences, including telomeres (where it is particularly enriched) as well as the 50 bp repeats directly flanking both silent and active *VSG* ESs [60,61]. In the active ES telomere, base J is present in the 50 bp repeats directly upstream of the ES transcription unit as well as in the telomere repeats downstream, but is not present within the ES transcription unit itself [60]. DNA methylation can negatively impact on transcription elongation in mammalian cells [62]. As J was particularly enriched in the inactive ESs, it seemed plausible that the presence of the J modification could play a role in antagonising transcription elongation within silent ESs.

Base J is made in two steps. First, a thymidine hydroxylase oxidises thymidine residues, resulting in the formation of HOMedU (hydroxymethyluridine) in the DNA. HOMedU is subsequently glucosylated by a glucosyl transferase, resulting in base J [59]. Two proteins have been identified in J biosynthesis (JBP1 and JBP2), both of which have been shown to have thymidine hydroxylase activity [63]. Trypanosomes which are genetic knockouts for JBP1 and JBP2 completely lack base J, however no effect on VSG ES derepression or

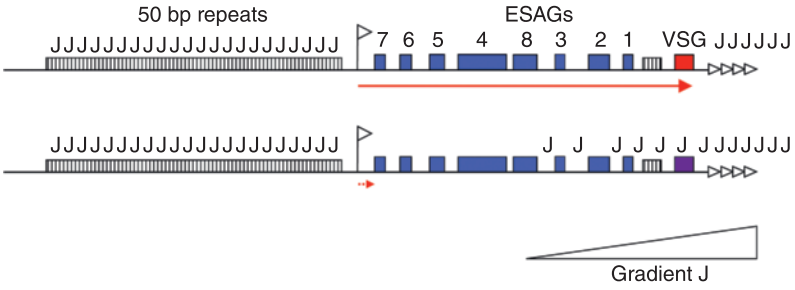

Figure 5. Distribution of base J in bloodstream form African trypanosomes
The unusual nucleotide base J (or β-D-glucosyl-hydroxymethyldeoxyuridine) is found in bloodstream form *T. brucei,* particularly in and around *VSG* ESs. A schematic diagram of an active *VSG* ES is shown above, with transcription indicated with a red arrow. Below is a silent *VSG* ES, with only minimal transcription in the immediate region of the promoter (short red arrow). Within the ESs, ESAGs are indicated with numbered blue boxes, the telomeric *VSG*s with coloured boxes, and the telomere repeats with arrowheads. Extending upstream of the ES promoter (white flag) are characteristic 50 bp simple sequence repeats which extend for many tens of kilobases upstream of all known *VSG* ESs (vertically striped box). J is particularly enriched in the 50 bp simple sequence repeats and in the telomeres of both active and silent ESs. In addition, J is also present in a gradient extending up from the telomeres of silent ESs.

switching has been observed [63]. Reduction in the amount of J within the genome also does not lead to obvious changes in DNA stability, including no changes in the size of various repeat regions [59]. The functional role of this unusual modified nucleotide within trypanosomes therefore still needs to be elucidated.

Chromatin structure and ES regulation

The role that chromatin plays in ES regulation has been a controversial topic. Initially it appeared that repressed chromatin was not important for ES silencing. Early analyses comparing the nucleosomal structure of active versus silent *VSG* ESs reported that active ESs were preferentially digested by single-strand-specific endonucleases including S_1 and Bal31 [64]. However, in that study it was argued that there is no detectably different nucleosomal organization in the active compared with the silent ESs, as investigated using micrococcal nuclease digestion experiments. Later analyses mapped a prominent DNase-I-hypersensitive site in ES core promotors, with this nuclease-sensitive site being equally prominent in both active and silent ESs [65]. In a parallel approach, the chromatin structure of active compared with silent *VSG* ESs was probed using an exogenous T7 RNA polymerase [66]. This heterologous T7 bacteriophage RNA polymerase has been used as an alternative to nuclease sensitivity experiments as a tool for determining eukaryotic chromatin accessibility. In general, levels of transcription by exogenous T7 RNA polymerase from T7 promoter sequences integrated into the genome are higher in open transcriptionally active chromatin compared

with silent chromatin [67]. That study in trypanosomes reported that T7 RNA polymerase-mediated transcription from chromosomally integrated T7 promoters was repressed along the entire length of ESs in insect form *T. brucei*, however this effect was not observed if these T7 promoters were integrated in silent ESs in bloodstream form *T. brucei* [66]. These results, together with the earlier nuclease sensitivity studies, made it appear likely that chromatin could play a role in downregulation of silent ESs in insect form *T. brucei,* but not in ES repression in bloodstream form *T. brucei*.

This viewpoint has now been re-evaluated, and it now appears likely that chromatin plays a critical role in ES downregulation. First, the nucleosomal structure of active compared with silent ESs has recently been reinvestigated and has been shown to be quite different between the two ES activation states. ChIP (chromatin immunoprecipitation) experiments to investigate histone distribution, as well as qPCR (quantatative PCR) or Southern blot analysis of micrococcal nuclease digestion experiments show that active ESs are strikingly depleted of nucleosomes [68,69]. This relative lack of nucleosomes on the active ES might be one of the factors that is involved in marking the active ES, ensuring that the active or silent epigenetic state is propagated to the next generation.

Secondly, chromatin-remodelling proteins have recently been shown to be involved in ES silencing. The chromatin remodeling protein TbISWI [*T. brucei* ISWI (imitation-switch protein)] has been shown to be involved in ES silencing in both insect and bloodstream form *T. brucei* [70]. TbISWI is a member of the ISWI family of SWI2/SNF2 (switch/sucrose non-fermentable)-related chromatin remodelling complexes, which are characterized by the presence of a highly conserved SNF2 domain [71]. The SNF2 domain is a motif found on proteins with chromatin remodelling activity either with regards to transcriptional control or DNA repair, and has DNA-dependent ATPase activity [72]. Knockdown of TbISWI using tetracycline-inducible RNAi (RNA interference) results in 30–60-fold derepression of ESs in bloodstream form *T. brucei*, and 10–17-fold ES derepression in insect form *T. brucei* [70]. Although this level of ES derepression is very striking, it represents only approx. 10% the activity of a maximally active ES in bloodstream *T. brucei*. It is therefore clear that there are other restrictions operating on full ES activation.

One of these restrictions may be TbRAP1, a telomere-binding protein which appears to be a component of a gradient of repression which increases in strength towards the ES telomere [53]. Knockdown of TbRAP1 results in an increase in VSG switching as well as in ES promoter derepression. In *S. cerevisiae*, RAP1 is essential for the telomeric heterochromatin that represses subtelomeric genes [39]. Although trypanosomes do not have a TPE analogous to *S. cerevisiae*, it is possible that some of the TPE components are being utilized in a different fashion. Possibly, TbISWI-mediated repression operates over the repressed ES promoters, while a gradient of RAP1-mediated repression also extends up from the telomeres of the silent ESs. TbISWI knockdown therefore

does not necessarily give rise to fully processive ES transcription because TbRAP1 is still present (Figure 4). This would indicate that multiple levels of control ensure that silent *VSG* ESs are kept silent.

DOT1B is another protein implicated in chromatin remodelling that has been shown to play a role in the monoallelic exclusion of ESs. DOT1B is a H3K76 (histone H3 Lys^{76}, which corresponds to H3K79 in yeast and mammals) methyltransferase. Knock-down of DOT1B results in silent telomeric ESs becoming partially derepressed when DOT1B is depleted, indicating that ES silencing has been at least partially compromised [73]. A striking feature of this DOT1B knockdown phenotype is that, although activation of a new ES can proceed at similar ES activation rates, the full changeover from one ES to another occurs significantly more slowly than normal. This led the authors to conclude that DOT1B is necessary for monoallelic expression of VSG.

Trypanosome histones are divergent from other eukaryotic histones, particularly at their N-termini [74,75]. This has meant that antibodies against histone modifications from other species have not been useful for mapping histone modifications in *T. brucei*. Compared with other eukaryotes, there appear to be relatively few histone modifications in *T. brucei*, which is consistent with the limited number of histone-modifying enzymes identified through bioinformatic studies [75,76]. Histone modifications specific for *T. brucei* are currently being determined by Edman degradation and MS [74,75]. Although some of these *T. brucei* histone modifications have proven to be cell-cycle regulated [77], or specific for the initiation sites of Pol II polycistronic transcription units [18], none has yet been shown to be specific for either active or silent ESs.

ES regulation and Pol I control

It is increasingly clear that chromatin remodelling must play a key role in *VSG* ES control, particularly in the monoallelic exclusion operating in bloodstream form *T. brucei*. One of the striking features of ES regulation is that this stringent monoallelic control is operating on Pol I transcription units. In other eukaryotes, only limited transcriptional regulation of the Pol I-transcribed rDNA transcription units has been observed, where approximately half of the rDNA units are active at any time [78]. In yeast, a stochastic process operates whereby active and silent rDNA repeats appear to be randomly distributed in the genome, with the transcriptional state continuously reset in dividing cells [78]. Inactivation of the rDNA transcription units requires the ISWI-containing NoRC (nucleolar remodelling complex), which silences the inactive rDNA repeats through nucleosome positioning [79,80]. The active rDNA repeats appear to be greatly depleted of nucleosomes [81], similar to as is observed in *T. brucei VSG* ESs [68,69]. However, the sequence of events is unknown, and it is unclear to what extent the chromatin conformation directly impacts on the transcriptional activity of the rDNA, versus a scenario whereby the transcriptional activity of the rDNA affects the chromatin state [82,83]. In trypanosomes, although it is clear that the active *VSG* ES is stripped

of nucleosomes [68,69], it is not known whether this open chromatin state precedes or is a consequence of high levels of ES transcription.

Nuclear architecture and ES monoallelic exclusion

As soon as it was realized that *VSG* ESs appeared to be transcribed by Pol I, it was proposed that subnuclear localization could play a role in their monoallelic exclusion. As Pol I transcribes rDNA within the subnuclear compartment of the nucleolus, the initial hypotheses postulated that *VSG* ES activation was a consequence of ES relocalization to the nucleolus. Here, high concentrations of Pol I could putatively allow transcription of the ES as well as the rDNA. Although no evidence was found that the active ES colocalizes with rDNA in the nucleolus [84], it appears that the active ES occupies a discrete compartment in the cell referred to as an 'ESB' (ES body) [85]. The ESB is specific to bloodstream form *T. brucei*, where Pol I is located in an additional extranucleolar body containing the active ES, as well as in the nucleolus. This ESB remains intact after DNase I digestion of the nuclei [85], arguing that the ESB is a subnuclear structure, rather than merely a consequence of Pol I associating with ES DNA.

Trypanosomes selected for the simultaneous activation of two ESs give rise to unstable double-expressors, which rapidly switch back and forth between the two ESs [27]. Possibly a restriction operates on trypanosomes, whereby the ESB can only accommodate a single ES [27]. Experiments selecting for simultaneous activation of three ESs were not able to produce unstable triple-resistant trypanosomes [30]. Instead, an unstable rapidly switching state involving two ESs was found, which the authors argued was a natural intermediate state during VSG switching. These authors also argued that silent ESs can exist in a stable, partially activated state, as has also been shown by other investigators [86].

Although the active ES appears to be located in an ESB outside of the nucleolus, this does not seem to be the case for the Pol I-transcribed procyclin genes, which are transcribed at the nucleolar periphery in insect form *T. brucei* [87]. There does not appear to be monoallelic exclusion operating on the procyclin loci, and multiple procyclin transcription units are simultaneously transcriptionally active in the same cell in insect form *T. brucei* [37]. As the bloodstream form trypanosome differentiates to the insect form stage, where ESs are to a large extent transcriptionally inactive, the ESB disappears and the ES promoter relocates to the nuclear envelope [87]. These results all argue that the ESB could be necessary for monoallelic exclusion of ESs in bloodstream form *T. brucei*.

ES monoallelic exclusion

It is still unclear how the counting mechanism behind this monoallelic exclusion operates. An attractive model could be one similar to that operating

in mammalian olfactory receptor choice. Individual olfactory sensory neurons in mice express only one of approx. 1300 olfactory receptor genes, where monoallelic exclusion ensures that a given olfactory neuron has a distinct identity [88]. The counting mechanism behind this appears to involve a single 'H enhancer' element. This regulatory region can associate with any one of multiple olfactory receptor gene promoters on different chromosomes, but is associated with only one olfactory receptor gene promoter in any given olfactory neuron [89,90]. DNA methylation is thought to mask one of the diploid copies of the H enhancer, leaving behind one non-methylated and functional enhancer in sensory neurons which can activate a single olfactory receptor promoter [90]. At the moment there is no obvious candidate for a trypanosome H enhancer element. Models that explain ES switching in *T. brucei* also need to accommodate the observation that the monoallelic exclusion operating on ES promoters does not appear to be promoter sequence specific. *T. brucei* rDNA promoters lack sequence similarity with ES promoters. Nevertheless, an rDNA promoter can direct transcription of the active ES, and can be efficiently switched on and off, indicating that it is recognised by the ES counting machinery [91].

Recently, DNA segregation has been implicated in *VSG* monoallelic expression, as partial knock-down of the cohesin subunit TbSCC1 resulted in activation of other silent telomeric *VSG* ESs [92]. That study showed that after DNA replication, sister alleles of the active *VSG* ES remain associated with each other longer than other loci, with both alleles of the active ES remaining associated with a single ESB until chromosome segregation. Knockdown of the cohesin ring resulted in one of the active *VSG* ES sister alleles not being associated with the ESB in many of the cells. The fact that this disassociation from the ESB was correlated with increased ES switching implies that continued association with the ESB is necessary for inheritance of the ES activated state. Premature ES disassociation from the ESB therefore appears to have a 'resetting' function, allowing an ESB to form *de novo* on another ES, resulting in an ES switch.

Conclusions

In summary, it is increasingly clear that chromatin must play a key role in *VSG* ES control, both in the monoallelic exclusion operating in bloodstream form *T. brucei*, as well as in the life-cycle specific silencing of all *VSG* ESs, as the bloodstream form trypanosome differentiates to the insect form. A striking feature of this ES control is that it operates on Pol I transcription units, which are normally not 'counted' in a stringent fashion in other eukaryotes [82,83]. Regulation of the rDNA transcription units is also likely to occur in *T. brucei*, although this has not yet been shown. It will be interesting to determine the extent to which the molecular mechanism of transcriptional regulation is shared between the different *T. brucei* Pol I transcription units. The ultimate challenge remains in determining how the monoallelic exclusion of *VSG* expression is so effectively achieved.

Summary

- *The bulk of the genome of African trypanosomes is transcribed as extensive polycistronic transcription units which are not transcriptionally regulated. Exceptions to this include the Pol I-transcribed telomeric ES transcription units, which are expressed in a strictly monoallelic fashion.*
- *Using new chromatin analysis methods, it has been shown that active ESs are highly depleted of nucleosomes. This makes it likely that chromatin plays a role in the epigenetic inheritance of active or silent ES activation states.*
- *Chromatin modification proteins that play a role in the maintenance of the silent ES state have been identified, including TbISWI and DOT1B.*
- *It has recently been shown that effective ES silencing requires a repressive gradient of TbRAP1 extending from the ES telomere towards the ES promoter.*
- *Multiple levels of control appear to be behind the monoallelic exclusion operating on the active ES. The challenge will come in determining how the 'counting mechanism' marking the active ES operates.*

I thank Manish Kushwaha, Megan Lindsay Povelones, Mani Narayanan, Viola Denninger, Nadina Vasileva, Alexander Fullbrook and Tara Stanne for comments on the manuscript. My research is funded by the Wellcome Trust. I am a Wellcome Senior Fellow in the Basic Biomedical Sciences.

References

1. Embley, T.M. and Martin, W. (2006) Eukaryotic evolution, changes and challenges. Nature **440**, 623–630
2. Clayton, C.E. (2002) Life without transcriptional control? From fly to man and back again. EMBO J. **21**, 1881–1888
3. Gunzl, A., Bruderer, T., Laufer, G., Schimanski, B., Tu, L.C., Chung, H.M., Lee, P.T. and Lee, M.G. (2003) RNA polymerase I transcribes procyclin genes and variant surface glycoprotein gene expression sites in *Trypanosoma brucei*. Eukaryot. Cell **2**, 542–551
4. Talbert, P.B. and Henikoff, S. (2009) Chromatin-based transcriptional punctuation. Genes Dev. **23**, 1037–1041
5. Taylor, J.E. and Rudenko, G. (2006) Switching trypanosome coats: what's in the wardrobe? Trends Genet. **22**, 614–620
6. Melville, S.E., Leech, V., Gerrard, C.S., Tait, A. and Blackwell, J.M. (1998) The molecular karyotype of the megabase chromosomes of *Trypanosoma brucei* and the assignment of chromosome markers. Mol. Biochem. Parasitol. **94**, 155–173
7. Melville, S.E., Leech, V., Navarro, M. and Cross, G.A. (2000) The molecular karyotype of the megabase chromosomes of *Trypanosoma brucei* stock 427. Mol. Biochem. Parasitol. **111**, 261–273
8. Berriman, M., Ghedin, E., Hertz-Fowler, C., Blandin, G., Renauld, H., Bartholomeu, D.C., Lennard, N.J., Caler, E., Hamlin, N.E., Haas, B. et al. (2005) The genome of the African trypanosome *Trypanosoma brucei*. Science **309**, 416–422

9. Wickstead, B., Ersfeld, K. and Gull, K. (2004) The small chromosomes of *Trypanosoma brucei* involved in antigenic variation are constructed around repetitive palindromes. Genome Res. **14**, 1014–1024
10. Liang, X.H., Haritan, A., Uliel, S. and Michaeli, S. (2003) *trans* and *cis* splicing in trypanosomatids: mechanism, factors, and regulation. Eukaryot. Cell **2**, 830–840
11. Koumandou, V.L., Natesan, S.K., Sergeenko, T. and Field, M.C. (2008) The trypanosome transcriptome is remodelled during differentiation but displays limited responsiveness within life stages. BMC Genomics **9**, 298
12. Smith, T.K., Vasileva, N., Gluenz, E., Terry, S., Portman, N., Kramer, S., Carrington, M., Michaeli, S., Gull, K. and Rudenko, G. (2009) Blocking variant surface glycoprotein synthesis in *Trypanosoma brucei* triggers a general arrest in translation initiation. PLoS One **4**, e7532
13. Ivens, A.C., Peacock, C.S., Worthey, E.A., Murphy, L., Aggarwal, G., Berriman, M., Sisk, E., Rajandream, M.A., Adlem, E., Aert, R. et al. (2005) The genome of the kinetoplastid parasite, *Leishmania major*. Science **309**, 436–442
14. Iyer, L.M., Anantharaman, V., Wolf, M.Y. and Aravind, L. (2008) Comparative genomics of transcription factors and chromatin proteins in parasitic protists and other eukaryotes. Int. J. Parasitol. **38**, 1–31
15. Das, A., Zhang, Q., Palenchar, J.B., Chatterjee, B., Cross, G.A. and Bellofatto, V. (2005) Trypanosomal TBP functions with the multisubunit transcription factor tSNAP to direct spliced-leader RNA gene expression. Mol. Cell. Biol. **25**, 7314–7322
16. Schimanski, B., Nguyen, T.N. and Gunzl, A. (2005) Characterization of a multisubunit transcription factor complex essential for spliced-leader RNA gene transcription in *Trypanosoma brucei*. Mol. Cell. Biol. **25**, 7303–7313
17. Martinez-Calvillo, S., Yan, S., Nguyen, D., Fox, M., Stuart, K. and Myler, P.J. (2003) Transcription of *Leishmania major* Friedlin chromosome 1 initiates in both directions within a single region. Mol. Cell **11**, 1291–1299
18. Siegel, T.N., Hekstra, D.R., Kemp, L.E., Figueiredo, L.M., Lowell, J.E., Fenyo, D., Wang, X., Dewell, S. and Cross, G.A. (2009) Four histone variants mark the boundaries of polycistronic transcription units in *Trypanosoma brucei*. Genes Dev. **23**, 1063–1076
19. Ben Amar, M.F., Jefferies, D., Pays, A., Bakalara, N., Kendall, G. and Pays, E. (1991) The actin gene promoter of *Trypanosoma brucei*. Nucleic Acids Res. **19**, 5857–5862
20. Cross, G.A. (1975) Identification, purification and properties of clone-specific glycoprotein antigens constituting the surface coat of *Trypanosoma brucei*. Parasitology **71**, 393–417
21. Barry, J.D., Marcello, L., Morrison, L.J., Read, A.F., Lythgoe, K., Jones, N., Carrington, M., Blandin, G., Bohme, U., Caler, E. et al. (2005) What the genome sequence is revealing about trypanosome antigenic variation. Biochem. Soc. Trans. **33**, 986–989
22. Barry, J.D. and McCulloch, R. (2001) Antigenic variation in trypanosomes: enhanced phenotypic variation in a eukaryotic parasite. Adv. Parasitol. **49**, 1–70
23. Marcello, L. and Barry, J.D. (2007) 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. **17**, 1344–1352
24. Hertz-Fowler, C., Figueiredo, L.M., Quail, M.A., Becker, M., Jackson, A., Bason, N., Brooks, K., Churcher, C., Fahkro, S., Goodhead, I. et al. (2008) Telomeric expression sites are highly conserved in *Trypanosoma brucei*. PLoS One **3**, e3527
25. Berriman, M., Hall, N., Sheader, K., Bringaud, F., Tiwari, B., Isobe, T., Bowman, S., Corton, C., Clark, L., Cross, G.A. et al. (2002) The architecture of variant surface glycoprotein gene expression sites in *Trypanosoma brucei*. Mol. Biochem. Parasitol. **122**, 131–140
26. Young, R., Taylor, J.E., Kurioka, A., Becker, M., Louis, E.J. and Rudenko, G. (2008) 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 Genomics **9**, 385
27. Chaves, I., Rudenko, G., Dirks-Mulder, A., Cross, M. and Borst, P. (1999) Control of variant surface glycoprotein gene-expression sites in *Trypanosoma brucei*. EMBO J. **18**, 4846–4855
28. Borst, P. (2002) Antigenic variation and allelic exclusion. Cell **109**, 5–8

29. Robinson, N.P., Burman, N., Melville, S.E. and Barry, J.D. (1999) Predominance of duplicative VSG gene conversion in antigenic variation in african trypanosomes. Mol. Cell. Biol. **19**, 5839–5846
30. Ulbert, S., Chaves, I. and Borst, P. (2002) Expression site activation in *Trypanosoma brucei* with three marked variant surface glycoprotein gene expression sites. Mol. Biochem. Parasitol. **120**, 225–235
31. Grummt, I. and Skinner, J.A. (1985) Efficient transcription of a protein-coding gene from the RNA polymerase I promoter in transfected cells. Proc. Natl. Acad. Sci. U.S.A. **82**, 722–726
32. Lo, H.J., Huang, H.K. and Donahue, T.F. (1998) RNA polymerase I-promoted HIS4 expression yields uncapped, polyadenylated mRNA that is unstable and inefficiently translated in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **18**, 665–675
33. Biebinger, S., Rettenmaier, S., Flaspohler, J., Hartmann, C., Pena-Diaz, J., Wirtz, L.E., Hotz, H.R., Barry, J.D. and Clayton, C. (1996) The PARP promoter of *Trypanosoma brucei* is developmentally regulated in a chromosomal context. Nucleic Acids Res. **24**, 1202–1211
34. Bohme, U. and Cross, G.A. (2002) Mutational analysis of the variant surface glycoprotein GPI-anchor signal sequence in *Trypanosoma brucei*. J. Cell Sci. **115**, 805–816
35. Jackson, A.P. (2007) Tandem gene arrays in *Trypanosoma brucei*: comparative phylogenomic analysis of duplicate sequence variation. BMC Evol. Biol. **7**, 54
36. Rudenko, G., Blundell, P.A., Taylor, M.C., Kieft, R. and Borst, P. (1994) VSG gene expression site control in insect form *Trypanosoma brucei*. EMBO J. **13**, 5470–5482
37. Roditi, I., Furger, A., Ruepp, S., Schurch, N. and Butikofer, P. (1998) Unravelling the procyclin coat of *Trypanosoma brucei*. Mol. Biochem. Parasitol. **91**, 117–130
38. Vanhamme, L., Poelvoorde, P., Pays, A., Tebabi, P., Van Xong, H. and Pays, E. (2000) Differential RNA elongation controls the variant surface glycoprotein gene expression sites of *Trypanosoma brucei*. Mol. Microbiol. **36**, 328–340
39. Gottschling, D.E., Aparicio, O.M., Billington, B.L. and Zakian, V.A. (1990) Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. Cell **63**, 751–762
40. Aparicio, O.M., Billington, B.L. and Gottschling, D.E. (1991) Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. Cell **66**, 1279–1287
41. Glover, L. and Horn, D. (2006) Repression of polymerase I-mediated gene expression at *Trypanosoma brucei* telomeres. EMBO Rep. **7**, 93–99
42. Glover, L., Alsford, S., Beattie, C. and Horn, D. (2007) Deletion of a trypanosome telomere leads to loss of silencing and progressive loss of terminal DNA in the absence of cell cycle arrest Nucleic Acids Res. **35**, 872–880
43. Brachmann, C.B., Sherman, J.M., Devine, S.E., Cameron, E.E., Pillus, L. and Boeke, J.D. (1995) The SIR2 gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. Genes Dev. **9**, 2888–2902
44. Perez-Martin, J., Uria, J.A. and Johnson, A.D. (1999) Phenotypic switching in *Candida albicans* is controlled by a SIR2 gene. EMBO J. **18**, 2580–2592
45. 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. and Scherf, A. (2005) Telomeric heterochromatin propagation and histone acetylation control mutually exclusive expression of antigenic variation genes in malaria parasites. Cell **121**, 25–36
46. Tonkin, C.J., Carret, C.K., Duraisingh, M.T., Voss, T.S., Ralph, S.A., Hommel, M., Duffy, M.F., Silva, L.M., Scherf, A., Ivens, A. et al. (2009) Sir2 paralogues cooperate to regulate virulence genes and antigenic variation in *Plasmodium falciparum*. PLoS Biol. **7**, e84
47. Alsford, S., Kawahara, T., Isamah, C. and Horn, D. (2007) 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. **63**, 724–736
48. Garcia-Salcedo, J., Gijon, P., Nolan, D.P., Tebabi, P. and Pays, E. (2003) A chromosomal SIR2 homologue with both histone NAD-dependent ADP ribosyltransferase and deacetylase activities is involved in DNA repair in *Trypanosoma brucei*. EMBO J. **22**, 5851–5862
49. Boulton, S.J. and Jackson, S.P. (1998) Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. EMBO J. **17**, 1819–1828

50. Mishra, K. and Shore, D. (1999) Yeast Ku protein plays a direct role in telomeric silencing and counteracts inhibition by rif proteins. Curr. Biol. **9**, 1123–1126
51. Conway, C., McCulloch, R., Ginger, M.L., Robinson, N.P., Browitt, A. and Barry, J.D. (2002) Ku is important for telomere maintenance, but not for differential expression of telomeric VSG genes, in African trypanosomes. J. Biol. Chem. **27**, 21269–21277
52. Janzen, C.J., Lander, F., Dreesen, O. and Cross, G.A. (2004) Telomere length regulation and transcriptional silencing in KU80-deficient *Trypanosoma brucei*. Nucleic Acids Res. **32**, 6575–6584
53. Yang, X., Figueiredo, L.M., Espinal, A., Okubo, E. and Li, B. (2009) RAP1 is essential for silencing telomeric variant surface glycoprotein genes in *Trypanosoma brucei*. Cell **137**, 99–109
54. Buck, S.W. and Shore, D. (1995) Action of a RAP1 carboxy-terminal silencing domain reveals an underlying competition between HMR and telomeres in yeast. Genes Dev. **9**, 370–384
55. Hardy, C.F., Sussel, L. and Shore, D. (1992) A RAP1-interacting protein involved in transcriptional silencing and telomere length regulation. Genes Dev. **6**, 801–814
56. Horn, D. (2009) Antigenic variation: extending the reach of telomeric silencing. Curr. Biol. **19**, R496–R498
57. Klose, R.J. and Bird, A.P. (2006) Genomic DNA methylation: the mark and its mediators. Trends Biochem. Sci. **31**, 89–97
58. Militello, K.T., Wang, P., Jayakar, S.K., Pietrasik, R.L., Dupont, C.D., Dodd, K., King, A.M. and Valenti, P.R. (2008) African trypanosomes contain 5-methylcytosine in nuclear DNA. Eukaryot. Cell **7**, 2012–2016
59. Borst, P. and Sabatini, R. (2008) Base J: discovery, biosynthesis, and possible functions. Ann. Rev. Microbiol. **62**, 235–251
60. van Leeuwen, F., Wijsman, E.R., Kieft, R., van der Marel, G.A., van Boom, J.H. and Borst, P. (1997) Localization of the modified base J in telomeric VSG gene expression sites of *Trypanosoma brucei*. Genes Dev. **11**, 3232–3241
61. van Leeuwen, F., Wijsman, E.R., Kuyl-Yeheskiely, E., van der Marel, G.A., van Boom, J.H. and Borst, P. (1996) The telomeric GGGTTA repeats of *Trypanosoma brucei* contain the hypermodified base J in both strands. Nucleic Acids Res. **24**, 2476–2482
62. Lorincz, M.C., Dickerson, D.R., Schmitt, M. and Groudine, M. (2004) Intragenic DNA methylation alters chromatin structure and elongation efficiency in mammalian cells. Nat. Struct. Mol. Biol. **11**, 1068–1075
63. Cliffe, L.J., Kieft, R., Southern, T., Birkeland, S.R., Marshall, M., Sweeney, K. and Sabatini, R. (2009) JBP1 and JBP2 are two distinct thymidine hydroxylases involved in J biosynthesis in genomic DNA of African trypanosomes. Nucleic Acids Res. **37**, 1452–1462
64. Greaves, D.R. and Borst, P. (1987) *Trypanosoma brucei* variant-specific glycoprotein gene chromatin is sensitive to single-strand-specific endonuclease digestion. J. Mol. Biol. **197**, 471–483
65. Navarro, M. and Cross, G.A. (1998) *In situ* analysis of a variant surface glycoprotein expression-site promoter region in *Trypanosoma brucei*. Mol. Biochem. Parasitol. **94**, 53–66
66. Navarro, M., Cross, G.A. and Wirtz, E. (1999) *Trypanosoma brucei* variant surface glycoprotein regulation involves coupled activation/inactivation and chromatin remodeling of expression sites. EMBO J. **18**, 2265–2272
67. Jenuwein, T., Forrester, W.C., Qiu, R.G. and Grosschedl, R. (1993) The immunoglobulin mu enhancer core establishes local factor access in nuclear chromatin independent of transcriptional stimulation. Genes Dev. **7**, 2016–2032
68. Stanne, T.M. and Rudenko, G. (2009) Active VSG expression sites in *Trypanosoma brucei* are depleted of nucleosomes. Eukaryot. Cell. **9**, 136–147
69. Figueiredo, L.M. and Cross, G.A. (2009) Nucleosomes are depleted at the VSG expression site transcribed by RNA polymerase I in African trypanosomes. Eukaryot. Cell. **9**, 148–154
70. Hughes, K., Wand, M., Foulston, L., Young, R., Harley, K., Terry, S., Ersfeld, K. and Rudenko, G. (2007) A novel ISWI is involved in VSG expression site downregulation in African trypanosomes. EMBO J. **26**, 2400–2410
71. Richmond, E. and Peterson, C.L. (1996) Functional analysis of the DNA-stimulated ATPase domain of yeast SWI2/SNF2. Nucleic Acids Res. **24**, 3685–3692

72. Lusser, A. and Kadonaga, J.T. (2003) Chromatin remodeling by ATP-dependent molecular machines. Bioessays **25**, 1192–1200
73. Figueiredo, L.M., Janzen, C.J. and Cross, G.A. (2008) A histone methyltransferase modulates antigenic variation in African trypanosomes. PLoS Biol. **6**, e161
74. Mandava, V., Fernandez, J.P., Deng, H., Janzen, C.J., Hake, S.B. and Cross, G.A. (2007) Histone modifications in *Trypanosoma brucei*. Mol. Biochem. Parasitol. **156**, 41–50
75. Janzen, C.J., Fernandez, J.P., Deng, H., Diaz, R., Hake, S.B. and Cross, G.A. (2006) Unusual histone modifications in *Trypanosoma brucei*. FEBS Lett. **580**, 2306–2310
76. Figueiredo, L.M., Cross, G.A. and Janzen, C.J. (2009) Epigenetic regulation in African trypanosomes: a new kid on the block. Nat. Rev. Microbiol. **7**, 504–513
77. Siegel, T.N., Kawahara, T., Degrasse, J.A., Janzen, C.J., Horn, D. and Cross, G.A. (2008) Acetylation of histone H4K4 is cell cycle regulated and mediated by HAT3 in *Trypanosoma brucei*. Mol. Microbiol. **67**, 762–771
78. Dammann, R., Lucchini, R., Koller, T. and Sogo, J.M. (1995) Transcription in the yeast rRNA gene locus: distribution of the active gene copies and chromatin structure of their flanking regulatory sequences. Mol. Cell. Biol. **15**, 5294–5303
79. Santoro, R., Li, J. and Grummt, I. (2002) The nucleolar remodeling complex NoRC mediates heterochromatin formation and silencing of ribosomal gene transcription. Nat. Genet. **32**, 393–396
80. Li, J., Langst, G. and Grummt, I. (2006) NoRC-dependent nucleosome positioning silences rRNA genes. EMBO J. **25**, 5735–5741
81. Merz, K., Hondele, M., Goetze, H., Gmelch, K., Stoeckl, U. and Griesenbeck, J. (2008) Actively transcribed rRNA genes in *S. cerevisiae* are organized in a specialized chromatin associated with the high-mobility group protein Hmo1 and are largely devoid of histone molecules. Genes Dev. **22**, 1190–1204
82. Birch, J.L. and Zomerdijk, J.C. (2008) Structure and function of ribosomal RNA gene chromatin. Biochem. Soc. Trans. **36**, 619–624
83. McStay, B. and Grummt, I. (2008) The epigenetics of rRNA genes: from molecular to chromosome biology. Annu. Rev. Cell. Dev. Biol. **24**, 131–157
84. Chaves, I., Zomerdijk, J., Dirks-Mulder, A., Dirks, R.W., Raap, A.K. and Borst, P. (1998) Subnuclear localization of the active variant surface glycoprotein gene expression site in *Trypanosoma brucei*. Proc. Natl. Acad. Sci. U.S.A. **95**, 12328–12333
85. Navarro, M. and Gull, K. (2001) A pol I transcriptional body associated with VSG mono-allelic expression in *Trypanosoma brucei*. Nature **414**, 759–763
86. Navarro, M. and Cross, G.A. (1996) DNA rearrangements associated with multiple consecutive directed antigenic switches in *Trypanosoma brucei*. *Mol. Cell Biol.* **16**, 3615–3625
87. Landeira, D. and Navarro, M. (2007) Nuclear repositioning of the VSG promoter during developmental silencing in *Trypanosoma brucei*. J. Cell Biol. **176**, 133–139
88. Chess, A., Simon, I., Cedar, H. and Axel, R. (1994) Allelic inactivation regulates olfactory receptor gene expression. Cell **78**, 823–834
89. Savarese, F. and Grosschedl, R. (2006) Blurring *cis* and *trans* in gene regulation. Cell **126**, 248–250
90. Lomvardas, S., Barnea, G., Pisapia, D.J., Mendelsohn, M., Kirkland, J. and Axel, R. (2006) Interchromosomal interactions and olfactory receptor choice. Cell **126**, 403–413
91. Rudenko, G., Blundell, P.A., Dirks-Mulder, A., Kieft, R. and 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
92. Landeira, D., Bart, J.M., Van Tyne, D. and Navarro, M. (2009) Cohesin regulates VSG monoallelic expression in trypanosomes. J. Cell. Biol. **186**, 243–254