EUKARYOTIC CELL, Jan. 2010, p. 148–154 Vol. 9, No. 1
1535-9778/10/$12.00 doi:10.1128/EC.00282-09

Nucleosomes Are Depleted at the *VSG* Expression Site Transcribed by RNA Polymerase I in African Trypanosomes▽†

Luisa M. Figueiredo and George A. M. Cross*

Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Avenue, New York, New York 10065

Received 29 September 2009/Accepted 5 November 2009

In most eukaryotes, RNA polymerase I (Pol I) exclusively transcribes long arrays of identical rRNA genes (ribosomal DNA [rDNA]). African trypanosomes have the unique property of using Pol I to also transcribe the variant surface glycoprotein *VSG* genes. VSGs are important virulence factors because their switching allows trypanosomes to escape the host immune system, a mechanism known as antigenic variation. Only one *VSG* is transcribed at a time from one of 15 bloodstream-form expression sites (BESs). Although it is clear that switching among BESs does not involve DNA rearrangements and that regulation is probably epigenetic, it remains unknown why BESs are transcribed by Pol I and what roles are played by chromatin structure and histone modifications. Using chromatin immunoprecipitation, micrococcal nuclease digestion, and chromatin fractionation, we observed that there are fewer nucleosomes at the active BES and that these are irregularly spaced compared to silent BESs. rDNA coding regions are also depleted of nucleosomes, relative to the rDNA spacer. In contrast, genes transcribed by Pol II are organized in a more compact, regularly spaced, nucleosomal structure. These observations provide new insight on antigenic variation by showing that chromatin remodeling is an intrinsic feature of BES regulation.

Chromatin structure is dynamic, adopting a more condensed conformation at transcriptionally silent regions (closed chromatin) than at transcriptionally active regions (open chromatin) (6). Studies of genes transcribed by RNA polymerase II (Pol II) have shown that open and closed chromatin structures are dynamically regulated through multiple mechanisms, including histone modifications, histone variant incorporation, and DNA methylation (reviewed in reference 17). In addition, the density and positioning of nucleosomes have also been linked to transcriptional regulation. Genome-wide studies in *Saccharomyces cerevisiae*, for example, have revealed that promoters of most actively transcribed genes are depleted of nucleosomes and that histone density is inversely proportional to the transcription rate within coding regions, suggesting that nucleosomes are dynamically disassembled and reassembled at each passage of the polymerase (16). Regulation of nucleosome density is therefore an important mechanism to enable the transcription machinery to access the DNA.

Chromatin also regulates Pol I transcription, although its mechanistic details are less understood. In most organisms, Pol I exclusively transcribes rRNA genes, of which there are usually 100 to 1,000 copies arranged in tandem. Only ~50% of ribosomal DNA (rDNA) is transcribed in proliferating cells. Active and silent rDNAs have distinct chromatin states at the promoter and within the transcribed region. In mammalian cells, active and silent rDNA promoters are characterized by specific histone modifications, DNA methylation, and positioning of the promoter-bound nucleosome (reviewed in reference 22). Coding regions of active rDNA were initially thought to be devoid of nucleosomes, as suggested by electron microscopy and psoralen cross-linking experiments (4). Recent studies have both corroborated and contradicted these observations. Chromatin endogenous cleavage (ChEC) and psoralen experiments indicated that active rDNA is devoid of histones (23), whereas chromatin immunoprecipitation (ChIP) and genetic studies led to the conclusion that active rDNA has at least a few histones and is associated with chromatin-remodeling enzymes (15, 31, 34). Thus, although there appear to be differences between transcribed and nontranscribed rDNA coding regions, their exact nature remains to be elucidated.

Trypanosoma brucei is a unicellular eukaryote that, in the human host, lives in the bloodstream and extracellular tissue spaces, causing the fatal disease called human African trypanosomiasis, or sleeping sickness. As far as we know, *T. brucei* is the only eukaryote that uses Pol I to transcribe genes other than rDNA. This is possible because transcription and mRNA capping are uncoupled in this parasite and other *Kinetoplastidae*, which enables Pol I to transcribe rDNA and genes that encode proteins, including the variant surface glycoproteins (VSGs) and procyclins (reviewed in reference 30).

Tight regulation of *VSG* transcription by Pol I would seem to be essential for parasite survival. *T. brucei* evades the immune system by periodically switching among antigenically distinct VSGs, a mechanism known as antigenic variation. Although there are hundreds of *VSG* genes in the genome, only one VSG is expressed at a time (reviewed in reference 7). *VSG* genes are transcribed only if they are positioned in a specialized subtelomeric locus called a bloodstream-form expression site (BES), which is transcribed polycistronically from a Pol I promoter that is located ~50 kb upstream from the *VSG* (10). Identification of the first complex of Pol I transcription factors suggests that BES and rDNA share at least the basic Pol I transcription machinery (3).

* Corresponding author. Mailing address: Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Avenue, New York, NY 10065. Phone: (212) 327-7571. Fax: (212) 327-7572. E-mail: george.cross@rockefeller.edu.

† Supplemental material for this article may be found at http://ec.asm.org/.

▽ Published ahead of print on 13 November 2009.

BES transcriptional status is inherited from one generation to the next, and switching between BESs does not involve DNA rearrangements, suggesting that it is regulated epigenetically. The first players involved in the epigenetic control of VSG regulation have been recently identified: a chromatin remodeller (ISWI), a histone methyltransferase (DOT1B), and a telomeric protein (RAP1) (8, 13, 33). Depletion of these genes is associated with an increase in transcripts from silent BESs, indicating a role in BES silencing. However, it is still unclear exactly how these factors affect the BES chromatin structure. Further evidence that active and silent BESs probably have different chromatin conformations comes from two studies that showed the active BES to be more sensitive to single-strand-specific endonucleases and pancreatic DNase than silent BESs (9, 27).

It remains a mystery why *T. brucei* uses Pol I to transcribe VSGs, although it is commonly accepted that this RNA polymerase may allow for a faster rate of VSG mRNA synthesis, which is probably necessary to produce the 7 to 10% of cellular protein that VSG represents. In this study we investigated the chromatin structure of coding regions in active and silent BESs and we compared it to that of the rDNA and Pol II-transcribed genes. We used pairs of isogenic cell lines in which a specific BES was either transcriptionally active or silent. Using three independent methods, we demonstrated that the chromatin of the active BES is dramatically different from that of silent BESs. The active BES was depleted of nucleosomes, and those that remained were irregularly spaced. Coding sections of rDNA were also nucleosome depleted, whereas actively transcribed Pol II genes showed a regularly organized nucleosomal structure. Our study provides new insight on monoallelic expression by showing that chromatin remodeling is an intrinsic feature of BES regulation.

MATERIALS AND METHODS

Strains and growth medium. *T. brucei* bloodstream form cells (strain Lister 427, antigenic type MiTat 1.2, clone 221a) (14) were cultured at 37°C in HMI-9 medium (11). BES double-tagged cell lines were generated as described previously (10). Each cell line contains a puromycin resistance gene (*PUR*) 242 bp downstream of the active BES1 promoter and a neomycin resistance gene (*NEO*) 242 bp downstream of a silent BES promoter. Drug selection was used to obtain isogenic cell lines in which the active BES is the one tagged with *NEO*. In this report, the names of the cell lines reflect which drug resistance and *VSG* genes are actively transcribed (details are given in Table 1). The histone H3 variant knockout (H3V KO) cell line was generated by Lowell and Cross (18).

Chromatin immunoprecipitation. ChIP was carried out essentially as described elsewhere (18) but with several modifications. A total of 2×10^8 cells were fixed for 10 min in 1% formaldehyde in HMI-9 medium. DNA was sonicated with a BioRuptor for 15 min total (30-s on and off cycles), such that the resulting chromatin fragments averaged 500 bp in length. Lysate was incubated with Dynabeads sheep anti-rabbit IgG M-280 beads (Dynal) combined with 5 μg of rabbit H3 antibody (ab1791; Abcam) overnight at 4°C. This commercial antibody was raised against a peptide at the C terminus of human H3, and it recognizes *T. brucei* histone H3 but not H3V (data not shown). No posttranslational modifications have been identified at the C terminus of *T. brucei* H3 (20), suggesting that this antibody can be used as a general H3 antibody. Immunoprecipitated material was quantified by real-time PCR with SYBR green (Applied Biosystems) in an Applied Biosystems 7900HT instrument. Amplification reactions were performed in duplicates of three consecutive dilutions. Primer sequences are listed in Table S1 in the supplemental material. Primer pair efficiencies were determined according to Applied Biosystems instructions. Relative levels of gene expression were measured using the $\Delta\Delta C_T$ (where C_T is threshold cycle) method.

MNase analysis. Micrococcal nuclease (MNase) digestion was carried out essentially as described elsewhere (19), with a few changes. MNase (0.2 units; Sigma) was added to the cell suspension and incubated for up to 60 min at 25°C. DNA was isolated using a Qiagen Mini Elute PCR purification Kit, run on a 1.5% agarose gel, transferred to a nylon membrane, and detected with random-primer-labeled probes. Sequences are available upon request.

TABLE 1. *T. brucei* cell lines used in this study

Cell line	Drug resistance gene (BES location)	Studied genes and transcription status		Original name[a]
		Active	Silent	
PUR-VSG2	*PUR* (BES1) *NEO* (BES7)	*PUR*, *VSG2*	*NEO*, *VSG3*	BF-LF17.9
NEO-VSG3	*PUR* (BES1) *NEO* (BES7)	*NEO*, *VSG3*	*PUR*, *VSG2*	BF-LF17.9s1.3
PUR-VSG2(b)	*PUR* (BES1) *NEO* (BES4)	*PUR*, *VSG2*	*NEO*, *VSG21*	BF-LF17.21
NEO-VSG21	*PUR* (BES1) *NEO* (BES4)	*NEO*, *VSG21*	*PUR*, *VSG2*	BF-LF17.21s1
PUR-VSG2(c)	*PUR* (BES1) *NEO* (BES17)	*PUR*, *VSG2*	*NEO*, *VSG13*	BF-LF17.13
NEO-VSG13	*PUR* (BES1) *NEO* (BES17)	*NEO*, *VSG13*	*PUR*, *VSG2*	BF-LF17.13s2

[a] According to reference 10.

FAIRE. Formaldehyde-assisted isolation of regulatory elements (FAIRE) was adapted to *T. brucei* from the original protocol (24). Chromatin from 2×10^7 to 5×10^7 cells was prepared as described above for ChIP analysis. For the final results to be independent of gene copy number, a "total DNA" control was included in which the same protocol was followed except that cross-linking was omitted. Sonicated chromatin was submitted to two consecutive phenol-chloroform extractions, and DNA was ethanol precipitated overnight in the presence of 20 μg/ml of glycogen, resuspended in elution buffer (Qiagen), and treated with 100 μg/ml of RNase at 37°C for 1 h. Quantification of the FAIRE and total DNA samples was performed by real-time PCR as described above.

RESULTS

The active BES is depleted of histone H3. Chromatin immunoprecipitation experiments were performed using an anti-H3 antibody in the cell line PUR-VSG2, where the actively transcribed expression site (BES1) contains a puromycin-resistance gene (*PUR*) downstream of the promoter and *VSG427-2* (abbreviated to *VSG2*) proximal to the telomere (Fig. 1A). Quantification of immunoprecipitated DNA by real-time PCR showed that both *PUR* and *VSG2* were immunoprecipitated very inefficiently (0.5% of input, which is very close to background levels) (Fig. 1B). In contrast, *VSG* genes located at silent BESs (*VSG3* and *VSG18*) (10) were immunoprecipitated much more efficiently (8% of input) (Fig. 1B). These results suggested that the actively transcribed BES contains at least 10-fold less H3 than the silent BESs.

To test if H3 depletion is a general phenomenon of Pol I transcription, we measured the H3 content at rDNA loci using PCR primers for the rDNA spacer, 18S, and 28Sα rDNA subunits (Fig. 1A). The actively transcribed 18S and 28Sα rDNA regions were also depleted of H3 relative to the rDNA spacer although the difference was less dramatic (~5-fold) than the difference between active and inactive BESs (Fig. 1B). The immunoprecipitation efficiency was 4- to 10-fold higher for 18S and 28Sα than for *PUR* and *VSG2*, implying that rDNA is less depleted of nucleosomes than the active BES. This value is independent of gene copy

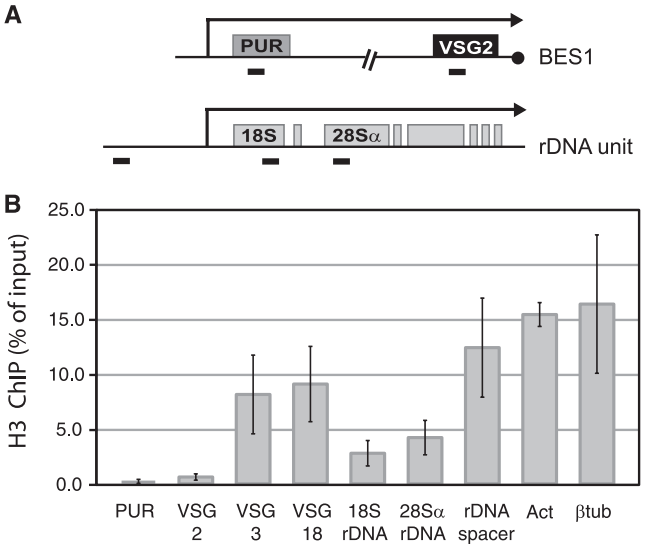

FIG. 1. Histone H3 is depleted from the active BES and rDNA coding regions. (A) Diagram of the actively transcribed BES1 and an rDNA unit of the PUR-VSG2 cell line. Horizontal bars denote the regions amplified by real-time PCR. Exact locations and sizes of PCR products are detailed in Table S1 in the supplemental material. The diagram is not to scale. (B) H3 immunoprecipitation in PUR-VSG2 cells. *PUR* and *VSG2* are located in BES1, which is the actively transcribed expression site. DNA was quantified by real-time PCR and compared to the total input material. PUR, puromycin resistance gene; Act, actin; βTub, β-tubulin.

number since the ChIP efficiency is expressed as a percentage of the input material.

Experiments in which reporter constructs were integrated at different Pol II and Pol I loci have shown that transcription of a typical Pol II locus is about 10-fold less efficient than that of a Pol I-transcribed locus (21). To test if H3 depletion extended to Pol II-transcribed regions, we used ChIP to detect H3 at β-tubulin and actin genes. We found that both genes were very efficiently immunoprecipitated (~15%) (Fig. 1B), suggesting that not all transcribed regions of the *T. brucei* genome are depleted of H3 but that H3 depletion is a feature of highly transcribed regions.

As reported previously (19; also T. N. Siegel, personal communication) and by Stanne and Rudenko in this issue (29), H2A and H4 are also depleted at transcribed *VSG* genes, further suggesting that histone H3 content is a good indicator of nucleosome density.

The active BES is mostly organized in irregularly spaced nucleosomes. To confirm that the active BES is an unusually nucleosome-depleted structure, we compared the MNase digestion patterns of BESs in isogenic cell lines in which BES1 was either active (PUR-VSG2) or silent (NEO-VSG3) (Fig. 2A). PUR-VSG2 and NEO-VSG3 were grown in the presence of puromycin and G418, respectively, to ensure that the entire population of cells uses the intended BES. Permeabilized cells were digested with MNase for increasing periods of time, and DNA was isolated and analyzed. In both cell lines, ethidium bromide staining or a Southern blot hybridized with β-tubulin revealed a ladder of digestion products corresponding to regularly spaced nucleosomes (Fig. 2B). In contrast, dramatic differences between the two cell lines were observed using probes for the *VSG2* and *PUR* genes, which are located close to the beginning and end of the BES1 transcription unit, respectively. When these genes were silent (NEO-VSG3 cell line), their chromatin showed a regular MNase digestion pattern. When they were actively transcribed (PUR-VSG2 cell line), we mainly observed a smear overlapped with a faint ladder. The sensitivity of this assay does not allow us to determine whether there are significant differences in nucleosomal laddering between the two probes. For both probes, the size of the smear became progressively smaller with increasing MNase digestion, but after 1 h it did not get smaller than ~150 bp, which corresponds to the length of DNA required to wrap around the core histones to form a nucleosome. These results indicate that the transcribed BES contains fewer nucleosomes, although a small number of units must exist because a faint ladder could still be detected, and a mononucleosome MNase-resistant band remained after prolonged digestion. The same differences between the nucleosomal laddering of active and silent BESs were also observed in two other isogenic cell lines expressing VSG2 or VSG13 (data not shown).

Chromatin at the active BES is more open than at silent BESs. FAIRE identifies nucleosome-depleted or naked regions such as promoters, the transcribed regions of rDNA, or mitochondrial DNA (24). Formaldehyde cross-linking preserves histone-DNA interactions. When cross-linked and sheared chromatin is extracted with phenol-chloroform, fragments of DNA that were naked or loosely associated with histones or other proteins are enriched in the aqueous phase. We used FAIRE to confirm that active BES and rDNA in *T. brucei* are nucleosome depleted, and we quantified the fractionated DNA by real-time PCR for two isogenic cell lines with different active BESs (Fig. 3). For both pairs of cell lines, *VSG* genes were 20- to 40-fold more enriched in the aqueous phase when the BES was active than when it was silent (Fig. 3A and B). The drug resistance genes located downstream of the BES promoter showed a slightly lower difference in FAIRE enrichment (3- to 15-fold), suggesting that chromatin undergoes more pronounced changes at the *VSG* genes than close to the promoter.

Two nuclease-hypersensitive sites have been described at both active and silent BES promoters (25), suggesting that the BES promoter region may be constitutively depleted of nucleosomes. To investigate the promoter region using FAIRE, we designed a pair of primers for real-time PCR that amplifies the core promoter, which is highly conserved among all BESs. The FAIRE enrichment for the core promoter region was about 4-fold (Fig. 3A), which is intermediate between the FAIRE enrichments of active and silent *VSG* genes, consistent with the idea that the chromatin of the promoter region of a silent BES is partially depleted of nucleosomes. This is consistent with the results of Stanne and Rudenko (29) and with data suggesting that most or all BES promoters are occupied by a transcription complex (32).

The FAIRE enrichment for the active 18S and 28Sα rDNA was 10- to 50-fold higher than for the silent rDNA spacer (Fig. 3A and B). Quantification with a primer pair that amplifies immediately downstream of the transcription

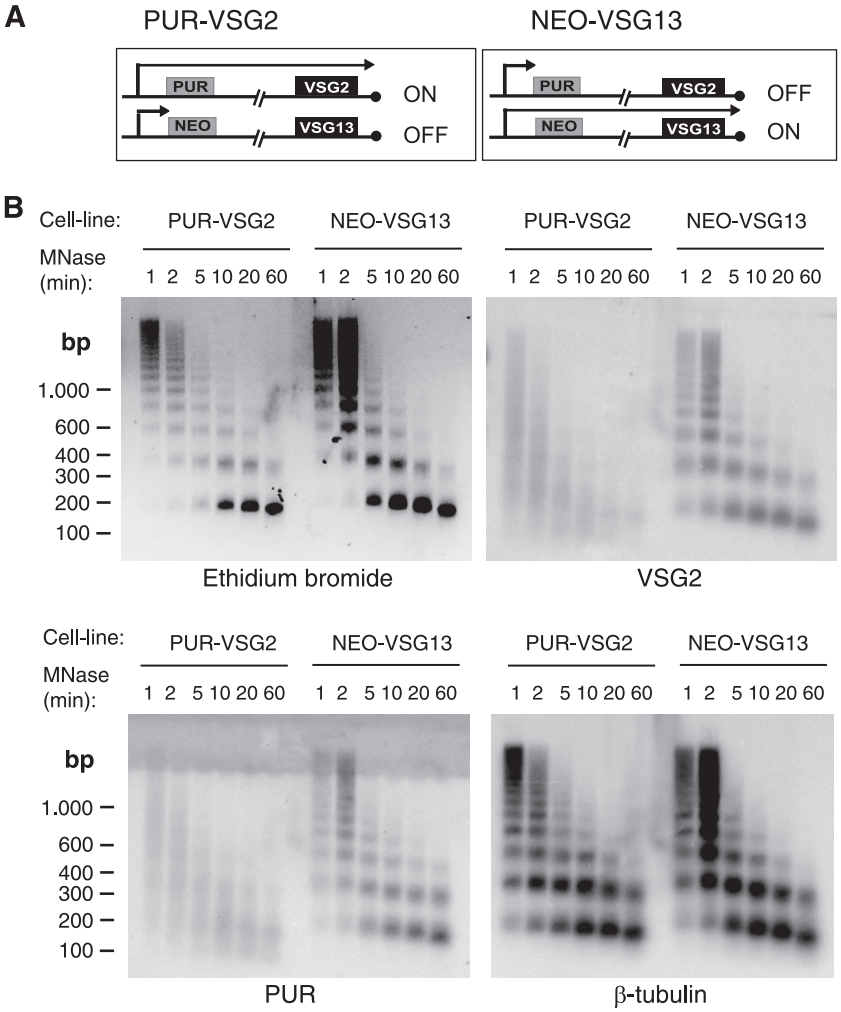

FIG. 2. Regularly spaced nucleosomes are mostly missing from the active BES. (A) Diagram of two isogenic cell lines with different active BESs. In PUR-VSG2, *PUR* and *VSG2* are actively transcribed from BES1. In NEO-VSG3, *NEO* and *VSG3* are actively transcribed from BES7, whereas *PUR* and *VSG2* are silent (Table 1). BES1 and BES7 are about 60 and 45 kb long, respectively. The diagram is not to scale. (B) After progressive treatment of permeabilized cells with 0.2 units of MNase, Southern blotting was performed with *VSG2*, *PUR*, and β-tubulin probes.

start site revealed a FAIRE enrichment around 40-fold higher than for the rDNA spacer and a level similar to that for the 18S and 28Sα coding regions (data not shown), confirming that actively transcribed rDNA is less tightly bound to nucleosomes.

Histone H3 variant is not responsible for the open chromatin structure at the active BES. *T. brucei* histone H3 variant (H3V) has been mapped to telomeres (18) and to the ends of Pol II polycistronic units by genome-wide studies (28). Although H3V was undetectable at the actively transcribed BES with previously used techniques (18), we used ChIP and FAIRE to confirm its absence from the active BES. Because H3V is not essential for viability, we were concerned that it might be replaced by the canonical H3 in the *H3V* knockout (H3V KO) cell line. If this were correct, a ChIP experiment with an anti-canonical H3 antibody in an H3V KO background should reveal an increase in H3 ChIP at the active BES. However, no changes were detected in the levels of canonical H3 at the actively transcribed *VSG* between the wild-type and H3V KO cell lines (18) (Fig. 4A), suggesting that there was no replacement of putative H3V by H3 at the active BES. As expected, the H3 ChIP profile of other coding regions known to lack H3V (two silent *VSGs*, actin, and β-tubulin) was identical in the two cell lines.

If H3V was simply lost from the active BES in the KO cell line, without being replaced, we would expect the chromatin of this locus to become even more open. FAIRE analysis was performed to compare the chromatin conformation at the active BES between an H3V KO and a wild-type background (Fig. 4B). The FAIRE enrichment of the actively transcribed *VSG* was identical regardless of the presence or absence of H3V in the cell line, further suggesting that the active BES chromatin is not composed of H3V. Chromatin of coding regions from a silent *VSG*, actin, and β-tubulin also remained unchanged, which was expected since no H3V was detected at these loci (28).

Our results show that the low level of H3 at the active BES is not due to its replacement by H3V. Together with the

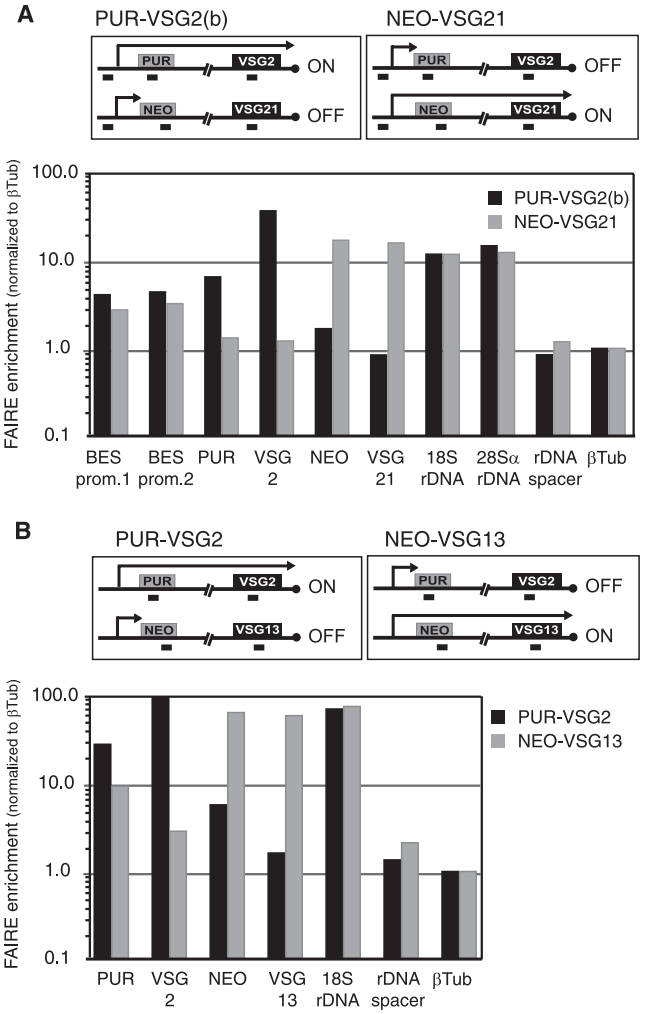

FIG. 3. FAIRE analysis shows that active BES and rDNA coding regions are organized in an open chromatin structure. (A) FAIRE analysis of two isogenic cell lines with different active BESs. In PUR-VSG2(b) (Table 1), *PUR* and *VSG2* are actively transcribed from BES1, while *NEO* and *VSG21* are silent. In NEO-VSG21, *NEO* and *VSG21* are actively transcribed from BES4, whereas *PUR* and *VSG2* are silent (Table 1). DNA isolated by FAIRE was quantified by real-time PCR. The approximate positions of amplicons for the BES promoter (prom), *PUR*, *V221*, *NEO*, and *VSG21* are indicated by horizontal bars. BES prom simultaneously amplifies the core promoter of all 15 BESs. Amplicons for rDNA are as indicated in the legend of Fig. 1A. FAIRE enrichment is shown as the *n*-fold difference between the amount of DNA quantified in the aqueous phase in the cross-linked sample relative to the non-cross-linked (total) sample. (B) FAIRE analysis of another pair of isogenic cell lines described in Table 1. Act, actin; βTub, β-tubulin.

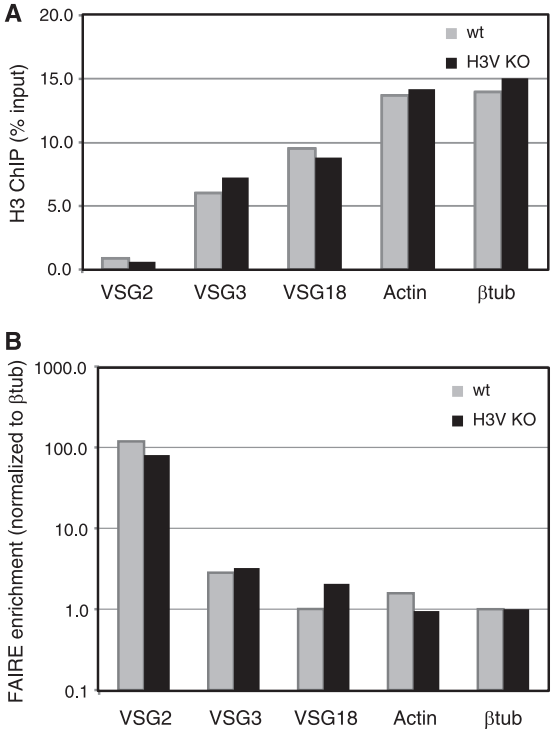

FIG. 4. Histone H3V is absent from the active BES. (A) H3 immunoprecipitation in VSG2-expressing cells in either a wild-type or H3V KO genetic background. *VSG2* is located in the actively transcribed BES1, while *VSG3* and *VSG18* are located in silent BESs (BES7 and BES5). DNA was quantified by real-time PCR and compared to the total input material. (B) FAIRE analysis of the same two cell lines. FAIRE enrichment is shown as the *n*-fold difference between the amount of DNA quantified in the aqueous phase in the cross-linked sample relative to the non-cross-linked (total) sample. βtub, β-tubulin.

FAIRE and MNase experiments described above, we can conclude that the active BES has fewer nucleosomes than silent BESs.

DISCUSSION

Chromatin remodeling and antigenic variation. Our study, together with the concurrent work of Stanne and Rudenko (29), provides new insight on monoallelic expression by showing that chromatin remodeling is an intrinsic feature of BES regulation. We observed that chromatin of active and silent BESs was dramatically different, with the active BES having almost undetectable levels of histones and showing a disorganized and open conformation. These results explain why the active BES is more sensitive to single-strand-specific endonucleases and pancreatic DNase (9, 27). Previous micrococcal nuclease digestions had failed to show a difference in nucleosomal patterns between active and silent BESs (9). It is not clear why such differences were not detected, but if, for example, the active *VSG* was a not a single-copy gene, two overlapping signals would have been detected: a smear from the active allele and a regular nucleosome ladder from the silent allele(s). The combination of the two signals would probably look like a nucleosome ladder from a silent single-copy *VSG*. Besides, because the authors did not have isogenic cell lines, the active and silent *VSG* genes were detected with two different probes, which may have hybridized differently.

The active BES is transcribed in a subnuclear site known as the expression site body (26), and this unique environment may provide an optimum environment to sequester the Pol I transcriptional machinery and the chromatin-related factors necessary to keep the active BES in an open conformation, such as remodeling enzymes, histone chaperones, and histone-modifying enzymes. In contrast to the chromatin of Pol I-transcribed

genes, Pol II-transcribed genes are organized in a regularly spaced nucleosomal structure. This further suggests that a specialized machinery may exist to exclusively open the chromatin of Pol I-transcribed genes.

In a closely related species, *Leishmania tarentolae*, spliced leader RNA genes, which are transcribed by Pol II, were also depleted of nucleosomes at both promoter and coding regions, with only one nucleosome present at the nontranscribed region (12). However, because these genes are transcribed monocistronically and each unit is very small (300 to 360 bp), there is space for a maximum of only two nucleosomes. It is therefore difficult to predict if the process that displaces one of the two nucleosomes is identical to the one that we observed in this study, in which nucleosomes are most likely depleted from throughout BES (~40 kb).

For antigenic variation to occur, a few cells of a population need to express an antigenically distinct VSG. This is most commonly achieved by homologous recombination (30). It was recently shown that an artificial double-strand break (DSB) induces *VSG* switching and that natural DSBs occur frequently at the active BES but not at silent BESs (2). It has been previously proposed that the frequency of such breaks is related to telomere length (5). Here, we propose an alternative model in which the chromatin structure of the actively transcribed BES makes it more susceptible to DSBs and to switching.

***T. brucei* as a model organism to study chromatin status during Pol I transcription.** Studying the chromatin structure of regions transcribed by Pol I in higher eukaryotes has been difficult due to the lack of rapid and sensitive assays that can distinguish individual active and silent rDNA units. Several features make the BES family (10) an ideal system to study the regulation of Pol I transcription: 14 of the 15 BESs have unique sequences, which allows them to be distinguished by molecular means; only one member is transcribed at a time instead of a variable number; and there are molecular tools to select the active BES. This system allowed us to unequivocally determine the chromatin structure of the single active BES and to compare it to the structure of silent BESs and other genomic loci.

In yeast and mammalian cells, several studies have provided contradictory evidence regarding the role of chromatin in the regulation of Pol I transcription (reviewed in reference 22). Because these studies were unable to examine individual rDNA units, the data represent the average of hundreds of rDNA units, and, therefore, it remains unclear if transcribed and nontranscribed rDNAs have comparable nucleosome densities. In this report, using ChIP, MNase digestion, and FAIRE, we provide unequivocal evidence that the chromatin of the active BES is nucleosome depleted. We could not determine the chromatin structure of the expression site-associated genes (ESAGs), which are located between the promoter and the *VSG* gene because they are highly conserved among BESs. An open chromatin structure was also found within actively transcribed *T. brucei* rDNA. Transcriptionally silent BES and the rDNA nontranscribed spacer, in contrast, are nucleosome rich, as are Pol II-transcribed genes. Our results demonstrate, at a single-locus level, that chromatin transcribed by Pol I is dramatically depleted of nucleosomes.

It is noteworthy that the levels of H3 at the rDNA coding regions are significantly higher than at the genes in the active BES (Fig. 1B), as was also observed by Stanne and Rudenko (29). This could be because not all nine rDNA units in *T. brucei* are transcribed. This hypothesis has been proposed before (1) but has never been formally tested because rDNA units are almost identical in sequence and therefore impossible to study individually.

In summary, we have demonstrated that *T. brucei* opens new avenues to study chromatin at Pol I-transcribed loci while we obtained critical information relevant to antigenic variation. Future studies should aim to reveal the players involved in chromatin remodeling at BESs, which are also likely to be important for rDNA regulation in *T. brucei* and other eukaryotes.

ACKNOWLEDGMENTS

We thank members of the Cross lab and Nina Papavasiliou for helpful discussions; Nicolai Siegel, Joanna Lowell, and Christian Janzen for critical reading of the manuscript; Tara Stanne and Gloria Rudenko for communicating unpublished data; and the Robert Darnell and Titia de Lange labs for sharing instrumentation.

This work was supported by grant number R01AI021729 from the National Institute of Allergy and Infectious Diseases (NIAID) of the U.S. National Institutes of Health (NIH). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIAID or the NIH.

REFERENCES

1. **Alsford, S., T. Kawahara, L. Glover, and D. Horn.** 2005. Tagging a *T. brucei* RRNA locus improves stable transfection efficiency and circumvents inducible expression position effects. Mol. Biochem. Parasitol. **144:**142–148.
2. **Boothroyd, C. E., et al.** 2009. A yeast-endonuclease-generated DNA break induces antigenic switching in *Trypanosoma brucei*. Nature **459:**278–281.
3. **Brandenburg, J., et al.** 2007. Multifunctional class I transcription in *Trypanosoma brucei* depends on a novel protein complex. EMBO J. **26:**4856–4866.
4. **Dammann, R., R. Lucchini, T. Koller, and J. M. Sogo.** 1993. Chromatin structures and transcription of rDNA in yeast *Saccharomyces cerevisiae*. Nucleic Acids Res. **21:**2331–2338.
5. **Dreesen, O., B. Li, and G. A. M. Cross.** 2007. Telomere structure and function in trypanosomes: a proposal. Nat. Rev. Microbiol. **5:**70–75.
6. **Elgin, S. C., and S. I. Grewal.** 2003. Heterochromatin: silence is golden. Curr. Biol. **13:**R895–R898.
7. **Figueiredo, L. M., G. A. M. Cross, and C. J. Janzen.** 2009. Epigenetic regulation in African trypanosomes: a new kid on the block. Nat. Rev. Microbiol. **7:**504–513.
8. **Figueiredo, L. M., C. J. Janzen, and G. A. M. Cross.** 2008. A histone methyltransferase modulates antigenic variation in African trypanosomes. PLoS Biol. **6:**e161.
9. **Greaves, D. R., and P. Borst.** 1987. *Trypanosoma brucei* variant-specific glycoprotein gene chromatin is sensitive to single-strand-specific endonuclease digestion. J. Mol. Biol. **197:**471–483.
10. **Hertz-Fowler, C., et al.** 2008. Telomeric expression sites are highly conserved in *Trypanosoma brucei*. PLoS One **3:**e3527.
11. **Hirumi, H., and K. Hirumi.** 1989. Continuous cultivation of *Trypanosoma brucei* blood stream forms in a medium containing a low concentration of serum protein without feeder cell layers. J. Parasitol. **75:**985–989.
12. **Hitchcock, R. A., S. Thomas, D. A. Campbell, and N. R. Sturm.** 2007. The promoter and transcribed regions of the *Leishmania tarentolae* spliced leader RNA gene array are devoid of nucleosomes. BMC Microbiol. **7:**44.
13. **Hughes, K., et al.** 2007. A novel ISWI is involved in VSG expression site downregulation in African trypanosomes. EMBO J. **26:**2400–2410.
14. **Johnson, J. G., and G. A. M. Cross.** 1979. Selective cleavage of variant surface glycoproteins from *Trypanosoma brucei*. Biochem. J. **178:**689–697.
15. **Jones, H. S., et al.** 2007. RNA polymerase I in yeast transcribes dynamic nucleosomal rDNA. Nat. Struct. Mol. Biol. **14:**123–130.
16. **Lee, C. K., Y. Shibata, B. Rao, B. D. Strahl, and J. D. Lieb.** 2004. Evidence for nucleosome depletion at active regulatory regions genome-wide. Nat. Genet. **36:**900–905.
17. **Li, B., M. Carey, and J. L. Workman.** 2007. The role of chromatin during transcription. Cell **128:**707–719.
18. **Lowell, J. E., and G. A. M. Cross.** 2004. A variant histone H3 is enriched at telomeres in *Trypanosoma brucei*. J. Cell Sci. **117:**5937–5947.
19. **Lowell, J. E., F. Kaiser, C. J. Janzen, and G. A. M. Cross.** 2005. Histone H2AZ dimerizes with a novel variant H2B and is enriched at repetitive DNA in *Trypanosoma brucei*. J. Cell Sci. **118:**5721–5730.

20. **Mandava, V., et al.** 2007. Histone modifications in *Trypanosoma brucei*. Mol. Biochem. Parasitol. **156:**41–50.
21. **McAndrew, M., S. Graham, C. Hartmann, and C. Clayton.** 1998. Testing promoter activity in the trypanosome genome: isolation of a metacyclic-type VSG promoter, and unexpected insights into RNA polymerase II transcription. Exp. Parasitol. **90:**65–76.
22. **McStay, B., and I. Grummt.** 2008. The epigenetics of rRNA genes: from molecular to chromosome biology. Annu. Rev. Cell Dev. Biol. **24:**131–157.
23. **Merz, K., et al.** 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.
24. **Nagy, P. L., M. L. Cleary, P. O. Brown, and J. D. Lieb.** 2003. Genomewide demarcation of RNA polymerase II transcription units revealed by physical fractionation of chromatin. Proc. Natl. Acad. Sci. U. S. A. **100:**6364–6369.
25. **Navarro, M., and G. A. M. Cross.** 1998. In situ analysis of a variant surface glycoprotein expression-site promoter region in *Trypanosoma brucei*. Mol. Biochem. Parasitol. **94:**53–66.
26. **Navarro, M., and K. Gull.** 2001. A Pol I transcriptional body associated with VSG mono-allelic expression in *Trypanosoma brucei*. Nature **414:**759–763.
27. **Pays, E., M. Lheureux, and M. Steinert.** 1981. The expression-linked copy of a surface antigen gene in *Trypanosoma* is probably the one transcribed. Nature **292:**265–267.
28. **Siegel, T. N., et al.** 2009. Four histone variants mark the boundaries of polycistronic transcription units in *Trypanosoma brucei*. Genes Dev. **23:**1063–1076.
29. **Stanne, T. M., and G. Rudenko.** 2010. Active *VSG* expression sites in *Trypanosoma brucei* are depleted of nucleosomes. Eukaryot. Cell **9:**136–147.
30. **Stockdale, C., M. R. Swiderski, J. D. Barry, and R. McCulloch.** 2008. Antigenic variation in *Trypanosoma brucei*: joining the DOTs. PLoS Biol. **6:**e185.
31. **Tongaonkar, P., et al.** 2005. Histones are required for transcription of yeast rRNA genes by RNA polymerase I. Proc. Natl. Acad. Sci. U. S. A. **102:**10129–10134.
32. **Vanhamme, L., et al.** 2000. Differential RNA elongation controls the variant surface glycoprotein gene expression sites of *Trypanosoma brucei*. Mol. Microbiol. **36:**328–340.
33. **Yang, X., L. M. Figueiredo, A. Espinal, E. Okubo, and B. Li.** 2009. RAP1 is essential for silencing telomeric Variant Surface Glycoprotein genes in *Trypanosoma brucei*. Cell **137:**99–109.
34. **Yuan, X., W. Feng, A. Imhof, I. Grummt, and Y. Zhou.** 2007. Activation of RNA polymerase I transcription by cockayne syndrome group B protein and histone methyltransferase G9a. Mol. Cell **27:**585–595.