Microbiology Spectrum
American Society for Microbiology Press

DNA Recombination Strategies During Antigenic Variation in the African Trypanosome

RICHARD McCULLOCH,[1] LIAM J. MORRISON,[1,2] and JAMES P.J. HALL[1,3]

[1]Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK; [2]Roslin Institute, University of Edinburgh, UK; [3]Department of Biology, University of York, York, UK

ABSTRACT Survival of the African trypanosome in its mammalian hosts has led to the evolution of antigenic variation, a process for evasion of adaptive immunity that has independently evolved in many other viral, bacterial and eukaryotic pathogens. The essential features of trypanosome antigenic variation have been understood for many years and comprise a dense, protective Variant Surface Glycoprotein (VSG) coat, which can be changed by recombination-based and transcription-based processes that focus on telomeric *VSG* gene transcription sites. However, it is only recently that the scale of this process has been truly appreciated. Genome sequencing of *Trypanosoma brucei* has revealed a massive archive of >1000 *VSG* genes, the huge majority of which are functionally impaired but are used to generate far greater numbers of VSG coats through segmental gene conversion. This chapter will discuss the implications of such VSG diversity for immune evasion by antigenic variation, and will consider how this expressed diversity can arise, drawing on a growing body of work that has begun to examine the proteins and sequences through which VSG switching is catalyzed. Most studies of trypanosome antigenic variation have focused on *T. brucei*, the causative agent of human sleeping sickness. Other work has begun to look at antigenic variation in animal-infective trypanosomes, and we will compare the findings that are emerging, as well as consider how antigenic variation relates to the dynamics of host–trypanosome interaction.

WHAT IS ANTIGENIC VARIATION?

One of the most powerful drivers of evolutionary change is the process of adaptation and counter-adaptation by interacting species (1). The so-called "arms race" between parasites and their hosts is a prime example of such reciprocal coevolution: host adaptations that reduce or attempt to remove parasites select for parasite adaptations that enable evasion of host defences. Elaborate, powerful and sometimes elegant mechanisms of host immunity and parasite infectivity are thought to have arisen from many iterations of this process. A case in point is the mammalian adaptive immune system, perhaps one of the more complex host defence mechanisms detailed to date, which uses directed DNA rearrangements, mutagenesis and selection during the development of T and B immune cells to generate vast numbers of genes encoding immunoglobulin receptors capable of recognizing the huge range of antigens in infecting pathogens (2). Parasites, on the other hand, have evolved various means of evading adaptive immunity. One such mechanism of immune evasion that is widely recorded among viruses and bacterial and eukaryotic pathogens is antigenic variation. Because parasite killing often depends on a match between circulating host immunity and parasite antigen, individual parasites that no longer

Received: 23 April 2014, **Accepted:** 29 April 2014, **Published:** 5 March 2015

Editors: Martin Gellert, National Institutes of Health, Bethesda, MD, and Nancy Craig, Johns Hopkins University, Baltimore, MD

Citation: McCulloch R, Morrison LJ, Hall JPJ. 2014. DNA recombination strategies during antigenic variation in the african trypanosome. *Microbiol Spectrum* 3(2):MDNA3-0016-2014. doi:10.1128/microbiolspec.MDNA3-0016-2014.

Correspondence: Richard McCulloch, Richard.McCulloch@glasgow.ac.uk

© 2014 American Society for Microbiology. All rights reserved.

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

express that antigen variant, but instead express an antigenically different variant in its place, survive and can proliferate. However, this advantage tends to be short-lived because immune responses will develop against the different antigen in turn. Hence, members of parasite lineages inhabiting an immunocompetent host are repeatedly being selected for antigenic novelty over the course of infection.

In contrast to depending on general processes of mutation to generate this novelty, antigenic variation *sensu stricto* is applied to cases where it is believed that pathogens have developed mechanisms to facilitate evolvability; that is, selection has acted on a "higher order" phenotype, and features and mechanisms have evolved to more effectively generate and express adaptive variation to evade adaptive immunity. Evolvability is likely to be an adaptation in only a quite restricted set of circumstances (3), but it is increasingly clear that the generally high degree of relatedness between co-infecting pathogens and the intense selective pressures imposed by host immunity can favour evolvability in antigenically variant pathogens (4). In shaping an antigenic variation phenotype, selection is likely to act on individual pathogens by favouring individuals that restrict the number of different variants they express (5), and on a lineage of clonally related pathogens by favouring those lineages that express a range of antigenically distinct variants (6). Because adaptive immunity is a selective force interacting with a range of organisms, common features of antigenic variation have convergently evolved in many phylogenetically disparate pathogens. These include (7, 8): the possession of a "family" of silent antigen genes, either explicitly encoded as variant genes or implicitly encoded by pseudogenes or gene fragments; monoallelic expression, which is that a single antigen gene is expressed by an individual at a time; and genetic or epigenetic strategies to "switch" exclusive antigen gene expression to another, previously silent member of the antigen gene family. Beyond these features, we can add that antigenic variation typically occurs at high rates, above background mutation, and that the process is stochastic and preemptive of immune recognition of the expressed antigen. These latter features are shared with other processes of phenotypic change (8–10), such as phase variation, but may not be inviolate in antigenic variation. For instance, though antigenic variation in African trypanosomes occurs at rates up to 10^{-2} events/division it can be reversibly reduced 1,000-fold by prolonged growth (11); in bacterial spirochetes of the genus *Borrelia* antigenic variation occurs when the pathogens infect mammals, but is not observed when grown *in vitro* (12). Irrespective of these variations, when interacting with the selective forces of the immune system, antigenic variation manifests as a pathogen population continually switching "forwards" through different antigen variants, as individuals expressing "old" variants (either by not switching, or by "switching back") are neutralized by immunity.

ANTIGENIC VARIATION IN TRYPANOSOMES

'African' Salivarian trypanosomes of the *Trypanosoma brucei* clade represent one of the best-studied systems of antigenic variation. *Trypanosoma brucei* are kinetoplastid (protozoan flagellate) parasites that are usually transmitted between mammal hosts by insect vectors (tsetse flies, genus *Glossina*) and cause disease in humans (human African trypanosomiasis, or sleeping sickness; caused by *T. b. gambiense* and *T. b. rhodesiense*) and animals (animal African trypanosomiasis, or Nagana; caused by all *T. brucei* trypanosomes as well as by *Trypanosoma congolense* and *Trypanosoma vivax*) in numerous foci across sub-Saharan Africa (13). Besides *T. brucei*, several other "species" of Salivarian trypanosomes have been studied in the context of antigenic variation. *T. b. evansi* and *T. b. equiperdum*, both non-human infective derivatives of *T. brucei* adapted to mechanical and sexual transmission (14), respectively, possess many if not all of the features of *T. brucei* antigenic variation. *Trypanosoma congolense* and *T. vivax* are more distantly related to *T. brucei* and are known to undergo antigenic variation but the details, particularly at the molecular and mechanistic levels, are less well understood in these species (15, 16).

The key biochemical and genetic features of trypanosome antigenic variation have been known for around 40 years. Pioneering studies by K. Vickerman demonstrated that trypanosomes survive in mammals through changes in a dense surface "coat" (17, 18), which was revealed in the mid-1970s to be composed of variant surface glycoprotein (VSG) (19). Rapidly thereafter, the genes encoding the VSG were identified and the molecular basis of *VSG* gene switching was established (20). Antigenic variation in *T. brucei* was discussed by P. Borst in the previous version of this book, *Mobile DNA II* (7), and therefore we will attempt in this chapter to update that report by reviewing the findings that have been published since then. For more detailed discussion of specific aspects of *T. brucei* antigenic variation, the reader is referred to several recent reviews (21–27).

Transmission by hematophagous biting insects tends to select for parasite mechanisms that prolong presence

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

in the blood, because it is those parasites that are most likely to be transmitted (28). Salivarian trypanosomes, unlike other chronic pathogens such as *Plasmodium* or the American trypanosome *T. cruzi*, do not invade host cells, so their residence in the blood leaves them exposed for the entirety of their existence in the mammalian host, were it not for $\sim 5 \times 10^6$ VSG dimers covering their entire surface. The VSG coat acts as a physical barrier, blocking immune effectors from other elements of the parasite surface (29). Individual VSG dimers can move across the parasite surface, and the coat is constantly turning over through endocytosis and exocytosis occurring in the flagellar pocket at the posterior of the parasite (30) and a limited degree of shedding (31). For a long time it was believed that the thickness and depth of the VSG coat was key to physically shielding all other surface molecules, as well as the parasite surface itself, from immune effectors. It now appears that this is not the whole story because some invariant surface molecules are predicted to protrude from the VSG layer (32), and the coat of *T. vivax*, which appears considerably less dense than that of *T. brucei* (33), is able to resist innate immune effectors. It is currently unclear whether these findings will change the paradigm of VSG's putative sole function as a physical barrier—at any rate, all attempts to use vaccines to direct immune responses against alternative and perhaps more productive targets have so far not been promising (34).

Each trypanosome cell expresses a single *VSG*, and it is against the encoded VSG that host immunity mounts a most vigorous attack, primarily an antibody response capable of destroying parasites by activating complement and by recruiting phagocytes (35). In fact, VSG appears to function solely as an antigen and does not perform any important biochemical activity, meaning that it is remarkably free to vary in sequence, with different functional VSGs sharing as little as 20% primary sequence identity (36). Still, there are clearly some limitations to VSG structure because all *VSG*s identified to date share strong features of secondary structure (37), in particular long α helices, which presumably stand perpendicular to the plasma membrane and contribute to the intact coat's depth and density (26, 38).

African trypanosome antigenic variation comes about when individual trypanosomes "switch" VSG, resulting in diversity emerging in the expanding lineage; a contingency that preempts the immune responses being mounted by the host. Below, we will discuss the molecular events that underpin switching, and how these contribute to antigenic variation and the survival strategies of African trypanosomes.

THE GENOMIC COMPONENTS OF ANTIGENIC VARIATION IN *TRYPANOSOMA BRUCEI*

Antigenic variation in *T. brucei* is remarkable for the huge levels of elaboration in the genome resources devoted to the process, and potentially therefore in the machinery that acts upon these resources to execute the reactions involved. In contrast with other organisms, where antigenic variation among surface antigen genes appears normally to be executed either by transcriptional or recombination mechanisms, *T. brucei* employs both strategies. Moreover, the sites of *VSG* transcription have evolved to drive the expression of not merely the VSG antigen but also to co-express many other proteins, and the number of silent *VSG* genes that act as "donors" for generating and expressing new VSG variants by recombination is of an unparalleled size. As the available evidence suggests that transcription-based and recombination-based VSG switching reactions are mechanistically distinct, these are considered in turn below.

VSG genes are expressed in the mammal from multigenic, telomeric transcription sites termed bloodstream expression sites (BES), as shown in Fig. 1. Multigenic transcription is not unusual in *T. brucei* or in related kinetoplastids, as virtually all RNA polymerase (Pol) II transcribed protein-coding genes are expressed in this way, with extensive *trans*-splicing and coupled polyadenylation acting to generate mature mRNAs from precursor RNAs (39, 40). However, the promoter that drives *VSG* transcription is unusual in that it is recognized by RNA Pol I (41), which also conventionally transcribes *rRNA* genes in the genome. Despite this, the BES promoter displays limited sequence homology with the *rRNA* promoter (42) or, indeed, with the promoter of *procyclin* genes, which are also RNA Pol I transcribed (41) and encode surface coat proteins found in the tsetse fly. In fact, though each promoter appears to bind similar, somewhat diverged, RNA Pol I transcription factors (43), the subnuclear sites of *rRNA* and BES transcription are distinct: *T. brucei* possess a conventional nucleolus for *rRNA* transcription, but some RNA Pol I is recruited to the active BES in a separate, discrete nuclear location termed the expression site body (44, 45). Whether or not BES promoter sequence specificity contributes to expression site body recruitment, or whether it reflects further adaptations for antigenic variation remains unknown.

Changes in the identity of the single transcribed BES provides one route for antigenic variation because *T. brucei* possesses not one BES, but many, with different *VSG*s occupying each BES. Whether the number

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

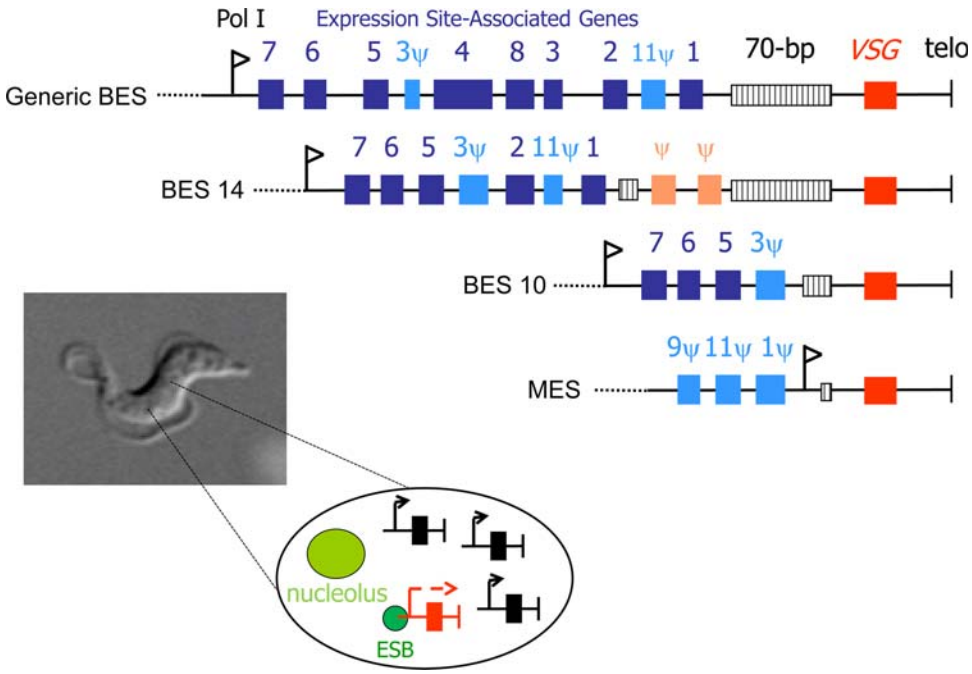

FIGURE 1 Architecture and singular transcription of variant surface glycoprotein (*VSG*) gene expression sites in *Trypanosoma brucei*. The four line diagrams show cartoon representations of telomeric *VSG* expression sites. The top diagram shows a generic bloodstream expression site (BES), while the two diagrams below display examples of variant BES (48) in which *VSG* pseudogenes (ψ, peach box) are found (BES 14) or where there has been loss of several expression site associated genes (*ESAG*s; dark blue box) or pseudogenes (light blue box) (BES 10). The final line diagram shows a *VSG* expression site (MES) used in metacyclic form *T. brucei*, which are found in the tsetse; here, the RNA polymerase I (Pol I) promoter (flag) does not drive expression of ESAGs, as it does in the BES, but only the *VSG* (red box), which in all cases is found adjacent to the telomere (telo; vertical line). Upstream of the MES promoter, several *ESAG* pseudogenes have been described, suggesting that these sites were derived from the BES. Arrays of 70-bp DNA repeats in the BES and MES are shown (hatched box), which always appear to be upstream of *VSG* genes or pseudogenes. Only one BES or MES is actively transcribed at a time in a single cell. A bloodstream form *T. brucei* cell is shown, in which the nucleus is diagrammed. The single active BES (red, extended arrow denotes transcription) is shown associated with the expression site body (ESB, small green circle), which is spatially distinct from the nucleolus (large green circle), though both subnuclear structures are sites of RNA Pol I transcription. Silent BES (three are shown in black; truncated arrow denotes limited transcription) do not associate with the ESB or nucleolus. doi:10.1128/microbiolspec.MDNA3-0016-2014.f1

of BES in the genome is fixed remains somewhat uncertain, as does the extent to which they vary in sequence composition during evolution. This is because the BES are telomeric and frequently large (many 10s of kbp), meaning that they are underrepresented in the bacterial clones used in published whole-genome *T. brucei* sequencing strategies (46, 47) and are frequently not found as assembled entities. For this reason, Hertz-Fowler et al. (48) specifically targeted the BES for cloning and sequencing by a transformation-associated recombination approach, and this remains the most complete survey of BES number and content to date. Fourteen distinct BES were found by this approach in *T. b. brucei* strain Lister 427, slightly less than estimates made by a different approach in the same strain (49) and by the same method in a different *T. b. brucei* strain (50). The BES are undoubtedly a focus for recombination (see below) and rearrangements occurring between them, which could lead to changes in number, would be consistent with subtelomeres being a rich source of gene diversification in

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

many organisms (51–55). Nonetheless, gene and sequence feature order within the BES appears to be rather conserved and to follow the general model of BES structure shown in Fig. 1 (48). In all cases, the *VSG* is found most proximal to the telomere repeats. Moreover, despite the wealth of *VSG* pseudogenes in the *T. brucei* genome (see below), the telomere-proximal *VSG*s described were all functional, with any *VSG* pseudogenes more distal to the telomere. Upstream of the *VSG* there is invariably a stretch of 70 bp repeats, which appear to be uniquely associated with the huge majority (~90%) of *VSG*s throughout the genome (37). Individual 70-bp repeats within arrays show considerable size variation and sequence complexity, including (TRR) repeats and GT- and AT-rich elements (56). The triplet repeat component has been shown to have a propensity to become non-H bonded (57) and may promote recombination, at least in bacterial plasmids and when transcribed (58); features that may tally with these elements being able to provide upstream homology during *VSG* recombination (see below).

BES are not just sites of VSG transcription, as each also contains a variable suite of Expression Site-Associated Genes (*ESAG*s). *ESAG*s are invariably found upstream of the 70-bp repeats, meaning that the size of the BES (from telomere to promoter) can be up to ~60 kbp and can include up to 13 genes (48). The order of *ESAG*s is broadly conserved between BES, but this is complicated by *ESAG* duplications, the presence of some *ESAG*s in only a minority of BES, *ESAG* pseudogenes, and the fact that some BES have suffered truncations in which *ESAG*s (but not *VSG*s) are lost. These variations suggest that recombination processes in the BES may not be limited to the *VSG*. An alternative suggestion is that variations in *ESAG* composition between BES may be an adaptation to different *T. brucei*–host species interactions, given the promiscuity of *T. brucei* in terms of infecting different mammalian species (59, 60). Testing this hypothesis is complicated by our limited understanding of ESAG function, though many *ESAG*s encode confirmed or predicted cell surface receptors (61). It seems plausible that RNA Pol I has been co-opted for BES transcription to allow for high level expression (43), so contributing to the generation of the 10^7 VSG proteins needed for the bloodstream form coat; indeed, the VSG accounts for a very large proportion of the bloodstream form mRNA population (62). Nonetheless, a need for high level expression does not readily explain the co-expression of the *ESAG*s with the *VSG*, as *ESAG* mRNAs appear not to be notably abundant (62). Bitter et al. (63) showed that when *T. brucei* is grown in culture media containing serum from different mammalian hosts, parasites are selected that have switched to transcribe a different BES. Switching correlates with expression of a different heterodimeric transferrin receptor (TfR) encoded by *ESAG6* and *ESAG7*, the genes that are most proximal to the BES promoter and in which sequence variation has been detected (64). Hence, it is proposed that multiple BES provide a range of TfRs that enable *T. brucei* to bind the variant transferrin molecules found in different host mammals that the parasite infects, thereby maximizing iron uptake in the face of anti-TfR antibodies (65). This hypothesis has been questioned (66, 67), and attempts to evaluate whether levels of *ESAG* sequence variation correlate with *Trypanosoma* species' host range have been somewhat unclear (50). Nonetheless, *in vitro* selection for BES switches is also been seen when *T. b. gambiense* is grown in differing host serum (68). Moreover, *ESAG4* genes, which encode novel, variable receptor-like adenylate cyclases, have been correlated with controlling the early immune response of the host (69), and BES-specific expression of a VSG-related serum resistance-associated factor allows *T. b. rhodesiense* to survive lysis by human serum components (70), suggesting that ESAGs may provide a variety of host-specific adaptations.

We clearly have much to learn about ESAG function, but this is central to any discussion of the role of multiple BES in antigenic variation. It is clear that *T. brucei* has evolved strategies to ensure expression of only one BES at a time and can execute a switch in transcription status from one BES to another, thereby changing the VSG coat (discussed below). Have these mechanisms evolved to serve immune evasion or do they underlie host adaptation, or both? Evolutionary pressures appear to have selected for drastic measures to secure increased numbers of BES in the *T. brucei* genome, since some are found in aneuploid "intermediate" chromosomes that are structurally distinct from the core genome, which is composed of 11 predominantly diploid "megabase" chromosomes (71) (Fig. 2). Moreover, *T. brucei* has adapted the BES to generate distinct *VSG* expression sites (metacyclic ES; MES) (Fig. 1) that are used to express a VSG coat in metacyclic stage parasites in the tsetse fly; a preadaptation for survival in the mammal for these infective stages (8, 72–74). However, recent trypanosome genome sequencing studies suggest that *ESAG*-rich BES may be a *T. brucei*-specific feature. In *T. vivax* and *T. congolense*, which also use antigenic variation for survival, homologues of most *T. brucei ESAG*s can be found, but they are not detectably linked to *VSG* in BES and may even provide distinct functions

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

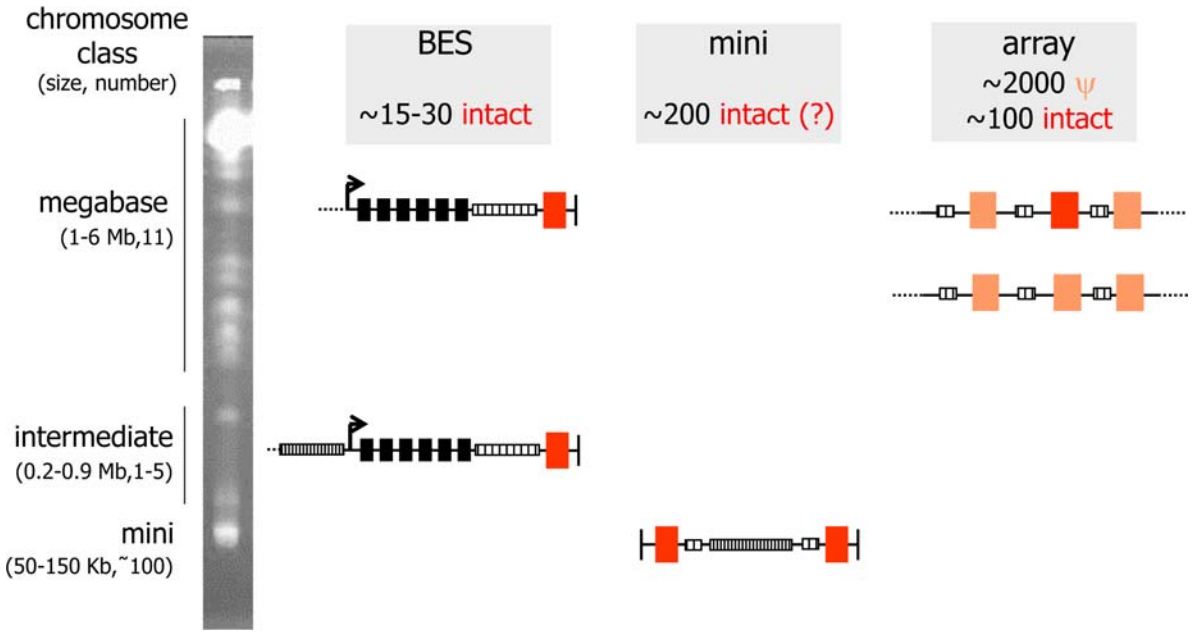

FIGURE 2 The variant surface glycoprotein (*VSG*) gene archive in *Trypanosoma brucei*. Whole *T. brucei* chromosomes are shown separated by pulsed field gel electrophoresis and stained with ethidium bromide. To the left of the gel, the positions of the megabase chromosomes, intermediate chromosomes and minichromosomes that comprise the nuclear genome are indicated, including the size and number of the different chromosome classes. To the right of the gel, the different loci in which *VSG*s are found are indicated (bloodstream expression site (BES), mini, array), including the number of *VSG*s in each locus type and whether they are functional (intact, red box) or are pseudogenic (ψ, peach box). BES denotes *VSG*s in expression sites that are used in the mammalian bloodstream and are found in the megabase and intermediate chromosomes. Mini denotes *VSG*s found in the minichromosomes, and array denotes *VSG*s found in the subtelomeres of the megabase chromosomes. In each case the presence or absence of a number of sequence features in addition to the *VSG* is shown: the telomere (vertical line), 70-bp repeats (widely hatched box), expression site-associated genes (black box), the RNA Pol I promoter (arrow) and 177-bp repeats (narrow hatched box). doi:10.1128/microbiolspec.MDNA3-0016-2014.f2

(75). Examining how *T. vivax* and *T. congolense* prosper in their mammal hosts will reveal much about the relationship between VSG and ESAG co-expression and the role of transcriptional switching in antigenic variation.

Despite their complexity in structure, and their potential occupation of all telomeres in the megabase chromosomes, the *VSG* expression sites (both BES and MES) provide only a minor component of the *T. brucei* armoury of genes for antigenic variation. The total number of *VSG*s in the *T. brucei* genome is enormous: the sequence of *T. b. brucei* strain TREU927 suggests 1,000 to 2,000 *VSG*s (37, 46), of which around half have been annotated (76). Remarkably, this "archive" represents around 20% of the coding capacity of the genome, an investment in variable antigens dwarfing that of other pathogens that employ antigenic variation. The *VSG*s outside the BES and MES—that is, the huge majority—are silent, as they are found in two locus types in which transcription has rarely been detected (Fig. 2). The first of these locus types encompasses *VSG*s found adjacent to the telomeres in ~200 minichromosomes: linear molecules ~30 to 150 kbp in size that are related to intermediate chromosomes in possessing central 177-bp repeats that may provide stability during cell division (71, 77, 78). Though it has been reported that a minichromosomal *VSG* can be expressed *in situ* (79), the lack of BES-related features other than 70-bp repeats in the minichromosomes that have been examined to date suggests that they evolved to provide a repertoire of silent, telomeric *VSG*s that are easily activated; indeed, in all cases examined to date the minichromosomes contain intact, functional *VSG*s. The sequence of the whole repertoire of minichromosomes has not yet

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

been reported for any trypanosome strain, and this approach is needed to evaluate whether the above generalizations about these molecules are widely held. It is also possible that such sequencing will determine how these minichromosomes evolved: for instance, did this part of the *VSG* archive arise from the BES or MES?

The other locus type comprises huge arrays of *VSG*s found in the subtelomeres of the megabase chromosomes (46) (Fig. 2). These *VSG*s have been referred to as "basic copy", "chromosomal-internal" or "array" VSGs, reflecting the fact that they are substrates for switching by recombination and differ from BES *VSG*s in their genomic context. Like the minichromosome *VSG*s, virtually all array *VSG*s are flanked by 70-bp repeats (37), though typically the number of repeats is lower than is found in the BES. However, genome sequencing has revealed that most array *VSG*s appear distinct from telomere-adjacent *VSG*s, because only a very small fraction (~5%) of this part of the archive encodes intact, functional VSGs (37, 46). Most of the array *VSG*s (and therefore most of the archive) are pseudogenes in which the open reading frame is interrupted by stop codons or frameshifts (~65%), or where the gene is truncated (~20%) and lacks one of the two VSG protein domains. The remainder of the array *VSG*s (~10%) appear atypical, encoding products with predicted folding or posttranslational modifications that differ from known VSGs. Another, distinct, set of ~30 *VSG*-related genes appear not to contribute to antigenic variation, because they are transcribed in both bloodstream and procyclic form *T. brucei* cells, all of them lack associated 70-bp repeats and they localize where the chromosome cores and *VSG* array-containing subtelomeres abut (37). Nonetheless, the demonstration that most of the *VSG* archive is composed of pseudogenes has huge implications for how we consider the mechanisms, dynamics and functions of *VSG* antigenic variation (see below).

Sequence features of the *VSG*s and their subtelomeric location appear to promote diversification of the archive. The sizes of individual megabase chromosomes vary considerably between *T. brucei* isolates, a variation that extends to considerable size differences between chromosome homologues within a cell (80). Though some of this variation can be attributed to changes in gene and sequence copy number throughout the genome, most is accounted for by expansions and contractions in the *VSG* arrays (81). In part, this variation is due to partitioning the core and the subtelomere, allowing ectopic recombination in the latter and limiting this in the former, where it could have deleterious effects (27, 51). Beyond subtelomeric recombination, it is possible that specific mechanisms are in place to further promote diversification: mutagenic processes favoured by higher-order selection (82). Though *VSG*s share very limited sequence homology, and the most related genes are dispersed in the subtelomeres rather than being adjacent to each other (46), around a third of *VSG*s are in families of at least two genes that share around ~75% nucleotide identity (37). Hence, duplication of *VSG*s may be a prevalent process, and appears to be associated with gene conversions extending from the 70-bp repeats to regions of C-terminal homology. Whether other dispersed sequences, such as ingi transposable elements, are also involved is less clear. Smaller-scale mutations have also been inferred from *VSG* sequence comparisons within a single archive (83), though whether these are related to *VSG* gene conversion or have a different source is unknown. It is clear that we have only just begun to examine the processes that shape and diversify the *VSG* archive, and do not fully understand the events that operate during replication, over the course of an infection and through the life cycle. Understanding this will determine the extent to which archives compare or diverge in extant trypanosome isolates (47, 84). Just as importantly, we do not yet understand if and how these largely silent processes within the archive intersect with our growing understanding of how *VSG*s are recombined into the BES.

A BRIEF DISCUSSION OF TRANSCRIPTIONAL *VSG* SWITCHING

As discussed above, while the evolutionary selection for multiple *VSG* BES is still being debated, the multiplicity of these sites provides a route for *T. brucei* antigenic variation. Since this book is primarily devoted to DNA rearrangement processes, we will provide only a brief overview of transcriptional *VSG* switching; more detailed discussion can be found in the previous version of this book (*Mobile DNA II*; chapter authored by Piet Borst: Chapter 40) (7) and in several excellent reviews (23, 25, 85–89). It is clear that *T. brucei* possesses an active mechanism for ensuring that only a single VSG is expressed: attempts to select for *T. brucei* cells in which two or more BES are actively transcribed have failed (90, 91), but this is not because a mixed VSG coat is toxic (92, 93). The nature of this counting mechanism remains uncertain, however. A range of factors have been detailed whose loss through mutation or RNAi elevates transcription from the silent BES. These factors act in diverse functions, including telomere (94), chromatin (95–98)

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

or nuclear envelope (99) activities. Despite this, perturbation of these activities shares a common outcome: though the silent BES become transcribed, the levels of this upregulation never reach transcription rates seen in the active BES. It is possible, then, that these factors influence processes that follow from, rather than dictate, singular BES expression. To date, the expression site body (Fig. 1) remains the best candidate for establishing monoallelic control (45), but its composition and association with the BES remains elusive. Indeed, the mechanistic focus of transcriptional control is still to be clarified. The observation that some transcripts can in fact be detected from silent BES, even in the absence of the above perturbations, suggests that selective RNA Pol I elongation may be the key (100). However, other studies have shown that RNA Pol I occupation levels are higher at the promoter of the active BES, suggesting that selective transcription initiation may also contribute (101).

Beyond the mechanism(s) for BES counting, the process of transcriptional switching between BES is arguably even more poorly understood. Only one factor has so far been described that influences the transfer of active transcription from one BES to another. Mutation of one of two related histone methyltransferases, DOT1B, causes ~10-fold elevated levels of transcription from silent BES, similar to the effects seen for the factors above. However, in DOT1B mutants transcription derepression is found throughout the transcription unit and, strikingly, loss of DOT1B appears to delay the time *T. brucei* takes to switch its VSG coat (102), potentially because normally unstable switch intermediates (90) are stabilized. How stabilization might occur remains uncertain (103), in part because the trigger and timing of transcriptional *VSG* switching is unknown. DNA replication and segregation may provide a window for transcriptional change, which would be consistent with observations that RNAi targeting of factors involved in nuclear replication and chromatid cohesion functions can elevate levels of silent *VSG* expression and switching (104–107). However, whether directed or random events can precipitate a transcriptional switch remains unknown. Indeed, though this switch reaction does not involve DNA rearrangements, it remains possible that it shares repair-related or replication-related functions that act in recombination-based switching (see below). Such a link would explain why mutation of core homologous recombination (HR) factors not only impairs VSG switching by recombination (see below), but also VSG switching by transcription (108–110), and why multiple treatments that cause DNA damage or replication arrest in *T. brucei* can elevate silent BES expression (111).

ACTIVATION OF INTACT *VSGS* INVOLVES HOMOLOGOUS RECOMBINATION

Activation of silent *VSG*s by their recombination into the BES is the primary route by which antigenic variation occurs, and recombination is the only route that can access all the *VSG*s in the archive for the formation of new VSG coats. Three recombination reactions have been described (Figs. 3, 4 and 5), which to some extent reflect different features of *VSG*s localized at telomeres and in subtelomeric arrays, but our understanding of how interrelated these reactions are is incomplete. What seems increasingly clear, however, is that *VSG* location and whether or not the gene is intact or pseudogenic strongly influences the timing of its activation (see below).

One recombination pathway is *VSG* gene conversion, where a copy is generated of a silent, functional *VSG* and replaces the BES-resident *VSG* (Fig. 3). This reaction is capable of activating functional *VSG*s throughout the archive. To generate a wholly new VSG, the extent of sequence copied must extend beyond the *VSG* open reading frame. Upstream, the 70-bp repeats can provide homology for both telomeric (BES, MES or or minichromosomal) and array *VSG*s (112), though gene conversions from a silent BES are frequently seen where homology is further upstream within the *ESAG*s (113, 114), even encompassing the *VSG* promoter (48). Downstream, gene conversion of array genes can end in 3′ coding or noncoding parts of the *VSG* (115), while conversion of telomeric *VSG*s can extend to the chromosome end (116, 117). A second pathway is reciprocal *VSG* recombination (118), where chromosome ends are exchanged by crossover without sequence loss (Fig. 5): a silent *VSG* moves into the BES and is activated, while the previously active *VSG* moves to the other chromosome end. This reaction, though readily detected (119), can only occur between telomere-adjacent *VSG*s, and so seems a minor pathway of *VSG* switching. The third recombination pathway, segmental gene conversion (SGC), is distinct from the two above in that it does not activate intact *VSG*s, but instead pieces together segments of multiple pseudogenes to generate novel, functional *VSG* "mosaics" (120–122) (Figs 3 and 4). This has substantial implications. First, the substrates used in SGC are different from activation of intact *VSG*s, in that at least one "end" of the reaction must be engaged through sequence homology between recombining *VSG* open reading frames, rather than through flanking homology. Since sequence homology between any two *VSG*s is normally very limited (26, 38), this raises questions about the nature, efficiency and substrate preferences of the recombination reaction; for instance, can

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

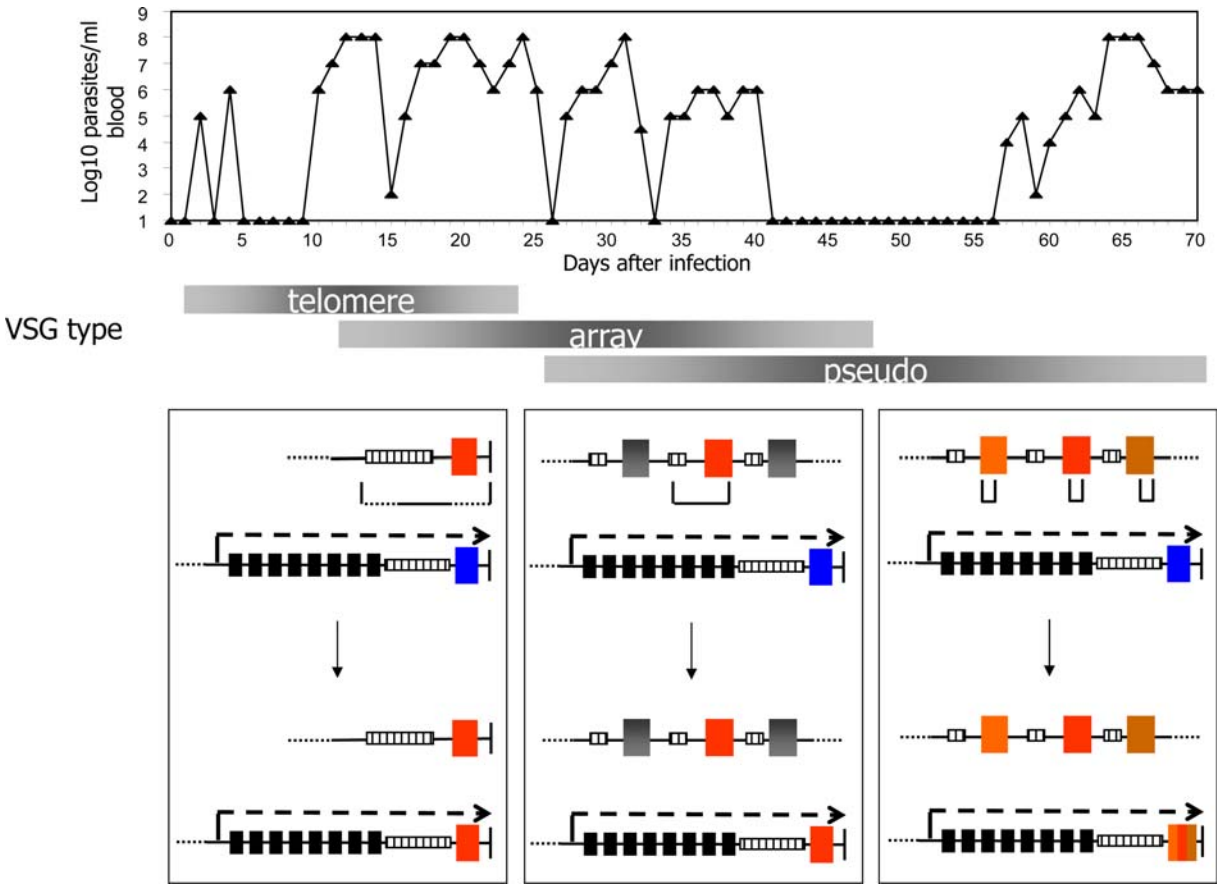

FIGURE 3 Hierarchy of variant surface glycoprotein (*VSG*) gene switching by recombination during infections by *Trypanosoma brucei*. The graph depicts the log of the number of *T. brucei* cells in a cow up to 70 days after infection (day 0). The schematic below details the timing of activation, by switching, of the different *VSGs* found in the genome (*VSG* type): silent telomeric *VSG*s (telomere) are activated more frequently than intact, subtelomeric array *VSG*s (array), which in turn are activated more frequently than *VSG* pseudogenes (pseudo). Gene conversion is the most frequent route for the above activation events, and the features associated with gene conversion of each *VSG* type are diagrammed. The *VSG* expressed before a switch (blue box) is transcribed (dotted arrow) from a bloodstream expression site (BES), in which the *VSG* is adjacent to the telomere (vertical line) and flanked upstream by 70-bp repeats (hatched box) and expression site associated genes (*ESAG*s; black boxes). The amount of sequence copied during *VSG* gene conversion is shown. For telomeric *VSG*s the sequence copied normally encompasses the *VSG* open reading frame (red box) and extends upstream to the 70-bp repeats, but also can extend further upstream into the *ESAG*s if the silent *VSG* is in an inactive BES; the downstream conversion limit may be the end of the *VSG*, but can also extend to the telomere from either a minichromosome *VSG* or inactive BES. Gene conversion of an intact subtelomeric array *VSG* is more limited in the range of sequence copied. In segmental *VSG* gene conversion parts of multiple, normally nonfunctional *VSG* pseudogenes (orange, red or brown boxes) are combined to generate a novel mosaic *VSG*; though this is shown to occur in the BES, it is not known if this is the location of gene assembly. Note also, the *VSG* pseudogene donors are shown for convenience as a contiguous array; in fact, segmental gene conversions using adjacent genes have never been observed. doi:10.1128/microbiolspec.MDNA3-0016-2014.f3

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

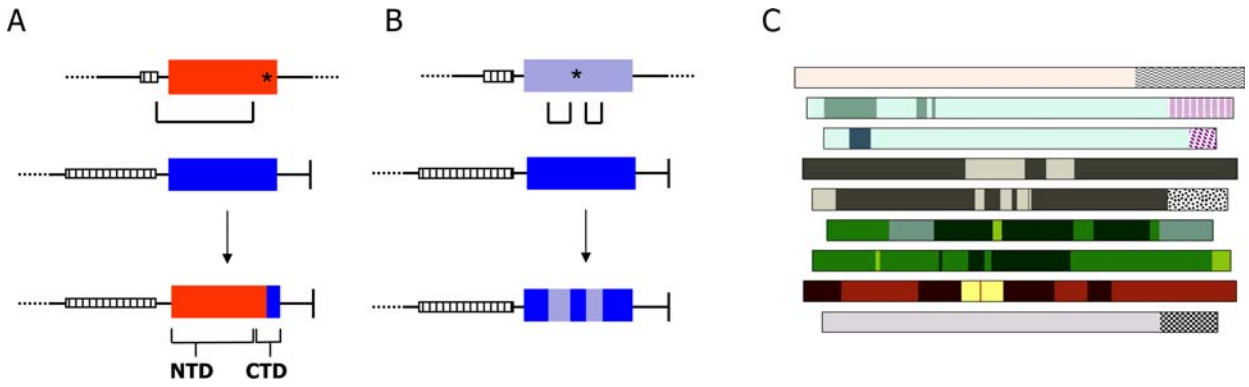

FIGURE 4 Complexity of variant surface glycoprotein (*VSG*) mosaics formed by segmental gene conversion in *Trypanosoma brucei*. (A) Where the 3′ boundary of segmental gene conversion occurs within the coding sequence of the *VSG* (3′ donation), part or all of the previously expressed C-terminal-domain-(CTD) -encoding region of *VSG* is retained, allowing the expression of a large contingent of silent *VSG*s (red box) that contain frameshifts or stop codons towards their 3′ ends (frameshift or premature stop codon indicated by an asterisk); as in Fig. 3, the recipient *VSG* (blue) is shown in the bloodstream expression site (BES) and the extent of conversion is indicated (NTD denotes N-terminal domain). Donors of *VSG*s formed in this way were found to share little sequence similarity over their whole sequence. (B) Mosaic *VSG*s can allow (partial) expression of pseudogene *VSG*s. Donors of *VSG*s (pink box) formed in this way share relatively high levels of sequence similarity (73% identity at the nucleotide level). (C) Segmental gene conversion yields diverse products: the diagram shows nine different *VSG*s detected during chronic infections (124); different donors are indicated in different colours, with 3′ donors indicated by hatching. doi:10.1128/microbiolspec.MDNA3-0016-2014.f4

RAD51-directed HR act in this process (see below)? Second, segmental *VSG* conversion has the capacity to generate many-fold larger numbers of VSG coats than merely the number of *VSG* genes in the archive (123) and, in this sense, has a comparable purpose and amplification of scale to the rearrangements used to generate mature immunoglobulin genes (120). Recent studies have built upon the genome sequence of *T. brucei* to show that segmental *VSG* conversion comes to predominate as *T. brucei* infections progress and that VSG diversity appears enormous (37, 124). These studies have confirmed far-sighted predictions by Kamper and Barbet, which predated whole genome sequencing and suggested that mosaic VSG formation is the key to infection chronicity and, most likely, even longer-term parasite–host interaction (125, 126).

To date, genetic studies to dissect the recombination machinery and sequences that act in *VSG* switching (Fig. 5) have been limited to understanding the activation of intact *VSG*s (106, 108, 110, 114, 117, 127–129). This stems from the fact that these studies have used *in vivo* or *in vitro* selection strategies that detect the most frequently activated *VSG*s; the lack of detectable mosaic *VSG* formation in these studies may suggest that SGC is less efficient than intact *VSG* conversion, though a mechanistic switch during an infection cannot be currently ruled out. A number of these studies make it clear that switching of intact *VSG*s is largely catalyzed by HR. In other words, though antigenic variation is a specialized recombination reaction targeting *VSG*s, it is executed by a nonspecific, general repair pathway rather than a specifically evolved *VSG* recombination reaction. The same conclusion has been reached regarding pilin antigenic variation in *Neisseria gonorrhoeae*, which occurs by gene conversion of silent, nonfunctional *pilS* genes to a *pilE* expression locus and where similar gene knockout approaches have been adopted (130, 131). A role for HR in VSG switching was first demonstrated by generating *T. brucei* mutants of RAD51; loss of this central enzyme of HR (132) significantly impairs *VSG* switching (110). This phenotype is similar, but not identical, to the effect that loss of RecA (the bacterial homologue of Rad51) has on *N. gonorrhoeae* antigenic variation. In *N. gonorrhoeae*, RecA mutants are effectively unable to switch their pili by recombination (133), whereas *T. brucei* VSG switching, though reduced approximately 10-fold, can still be detected in RAD51 mutants, including by gene conversion (110).

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

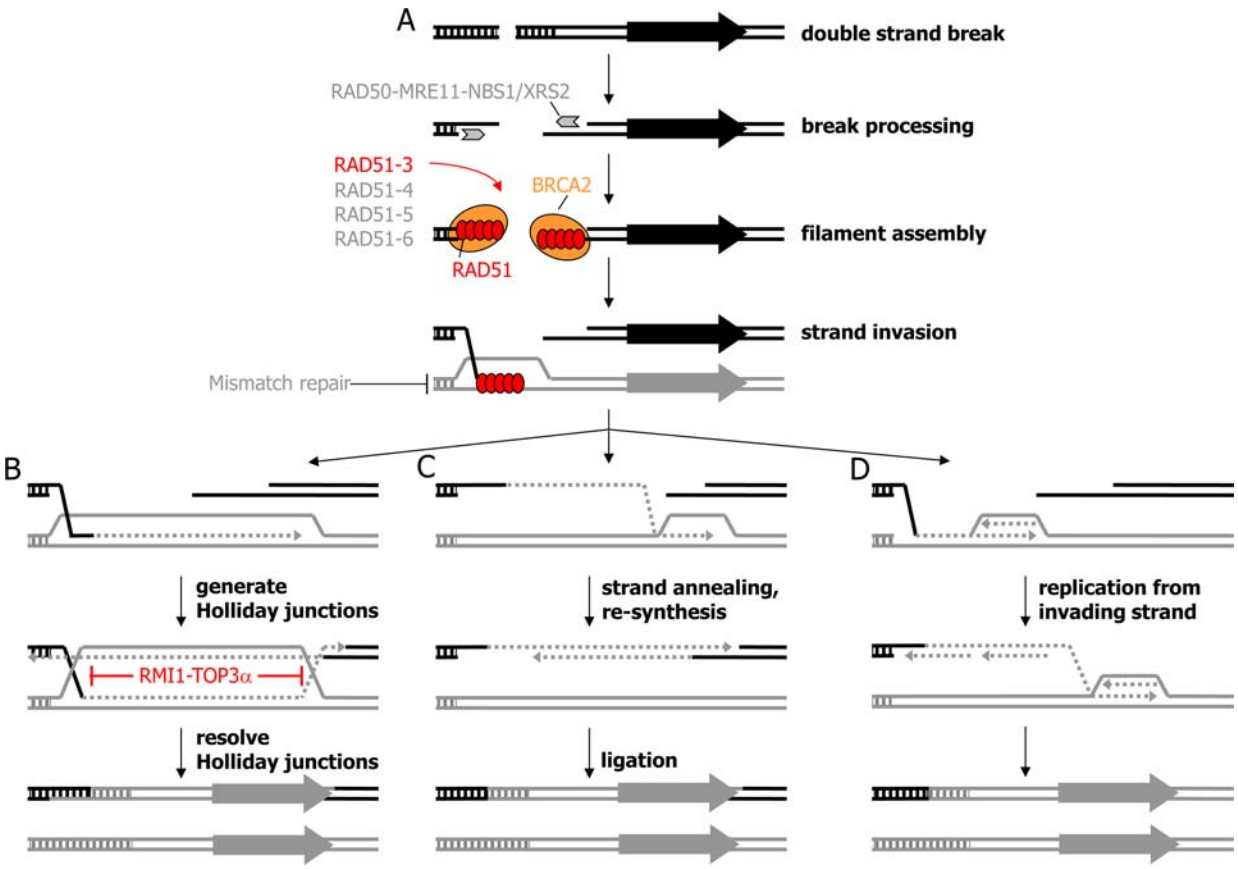

FIGURE 5 Models for variant surface glycoprotein (*VSG*) recombination during antigenic variation in *Trypanosoma brucei*. (A) Recombination is shown initiated by a DNA double-strand break (DSB) in the 70 bp repeats (hatched box) upstream of the *VSG* (black arrow) in the bloodstream expression site (BES) (*ESAG*s and promoter are not shown). Only factors that have been examined for a role in *VSG* switching are indicated; those shown in color have been found to act, while those for whom no evidence of a role in *VSG* switching has been found are shown in gray. DSB processing to reveal 3′ single-stranded ends is, in part, catalyzed by MRE11-RAD50-XRS2/NBS1 (MRX), generating a substrate on which RAD51 forms a nucleoprotein filament; note, however, that a further exonuclease (not shown) normally acts with MRX of both ends of the DSB are processed. RAD51 function is mediated by a number of factors: BRCA2 influences RAD51 filament dynamics, while the detailed roles of RAD51 paralogs (RAD51-3, RAD51-4, RAD51-5 and RAD51-6 in *T. brucei*) are unclear. RAD51 catalyzes repair by homology-dependent invasion of the single-stranded end into intact DNA (gray lines), containing a silent *VSG* (gray arrow). Mismatch repair constrains homologous recombination to act only on sufficiently homologous sequences. Three pathways for DSB repair have been described and may contribute to *VSG* switching. (B) DSB repair; here, newly synthesized DNA is copied from the intact DNA duplex and remains base-paired, generating Holliday junction structures whose enzymatic resolution can lead to gene conversion with (not shown) or without (shown) crossover of flanking sequence. In *T. brucei*, RMI1-TOP3 has been shown to suppress crossover, by perhaps acting on the Holliday junctions. (C) Synthesis-dependent strand annealing; here, newly synthesized DNA is displaced from the intact duplex and reanneals with homologous sequence at the DSB, allowing synthesis of the other strand. Break-induced replication is shown in (D); in this mechanism, an origin-independent replication fork forms on the strand invasion intermediate allowing replication to the chromosome end. doi:10.1128/microbiolspec.MDNA3-0016-2014.f5

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

This appears to suggest greater flexibility in the recombination pathways that can lead to antigenic variation in *T. brucei*. Indeed, *N. gonorrhoeae* antigenic variation appears to be a rather defined form of HR, because it only uses a sub-pathway of RecA-dependent HR initiated by a RecF-like machinery (134), and not RecBCD (135).

Further HR-related factors have been tested for their contribution to *T. brucei* antigenic variation, confirming the role of HR but suggesting some specialization. RAD51 activity *in vivo* is mediated by a number of factors (132), including BRCA2 and a collection of Rad51-related proteins termed Rad51 paralogs. BRCA2 interacts with Rad51 via conserved BRC repeats and a distinct, less-conserved motif that is found towards the C-terminus of the mammalian protein, and these interactions appear to guide the formation and disassembly of Rad51 nucleoprotein filaments on broken DNA (136) (Fig. 5). Mutation of BRCA2 in *T. brucei* impairs VSG switching to a very similar extent to RAD51 mutants (109). In *T. brucei*, but not in related African trypanosomes or more distantly related trypanosomatids, BRCA2 possesses a remarkable expansion in the number of BRC repeats relative to most eukaryotes, suggesting a need for pronounced interaction with RAD51. However, this expansion appears not to be an adaptation for gene conversion of intact *VSG*s, because cells expressing BRCA2 engineered to have only one BRC repeat still support wild-type levels of such switching (109). To date, BRC repeat number has only been demonstrated to be important in the efficiency of RAD51 relocalization after induced DNA damage (137), but in what way this might relate to antigenic variation remains elusive. Nonetheless, there are intriguing parallels between BRCA2 function in *T. brucei* and RecX in *N. gonorrhoeae*. Loss of RecX in *N. gonorrhoeae* impairs pilin switching (138) and, though RecX is unrelated in sequence to BRCA2, it acts in a related way, modulating RecA nucleoprotein filament formation (139, 140). Hence, it seems likely that in both pathogens antigen gene recombination is controlled in ways not yet understood through the size or stability of the filaments formed by the related recombinases. The functions of Rad51 paralogs remain somewhat mysterious. These factors are related to Rad51 but, unlike a meiosis-specific cousin of the HR recombinase termed Dmc1, there is no evidence that they catalyze recombination. Instead, they appear to aid Rad51-dependent HR (141) and show some overlap with BRCA2 function (142, 143), though potentially also broader functions in genome maintenance. The numbers of Rad51 paralogs vary widely in eukaryotes, with five in mammals but only one in *Caenorhabditis elegans*. Among single-celled eukaryotes, trypanosomes appear to possess a relatively large repertoire of four proteins (108, 144). In *T. brucei* each RAD51 paralog acts in DNA recombination and repair. However, mutants of only one RAD51 paralog, RAD51–3, show a pronounced impairment in intact *VSG* switching, suggesting some specificity in the HR reaction(s) used. In contrast with the above factors that promote VSG switching, one HR enzyme complex has been shown to suppress the reaction. Mutation of TOP3a or RMI1, which interact and are probably two components of the *T. brucei* RTR (RecQ/Sgs1-Top3/TOPO3a- Rmi1/BLAP75/18) complex described in yeast and mammals, results in increased levels of VSG switching, as well as recombination elsewhere in the genome (117, 145). Though the extent of elevated VSG switching is surprisingly different in the two mutants, each shares common mechanisms that are, in part, RAD51-dependent: increased levels of crossovers within the *ESAG*s and increased *VSG* gene conversion delimited by the 70-bp repeats (Fig. 5). Again, comparison of these findings with *N. gonorrhoeae* is revealing, since RecQ has been implicated in pilin antigenic variation (146, 147). However, loss of *N. gonorrhoeae* RecQ or mutation of the expanded number of helicase domains in the bacterial protein do not result in an elevation of antigenic variation rates, as in *T. brucei*, but in a reduction. *Neisseria gonorrhoeae* RecQ acts to unwind guanine quartet (or G4) structures that initiate pilin gene switching (148–150), meaning that the different phenotypes in *T. brucei* might indicate a distinct strategy for antigenic variation initiation. However, eukaryotes typically possess more than one RecQ-like factor, and indeed no *T. brucei* RECQ factors have so far been examined, so there is the potential for redundancy in this aspect of HR-directed VSG switching.

Despite the above broad association of HR with switching of intact *VSG*s, not all factors that might have been predicted to act behave in the expected ways. A key factor in the early steps of eukaryotic DNA double-strand break (DSB) repair is the MRE11 complex (Fig. 5), composed of MRE11, RAD50 and Xrs2/NBS1 in eukaryotes, with roles in processing DSBs, activating the DNA damage response through the kinase ATM, bridging DNA molecules and suppressing non-HR repair (151). MRE11 mutants in *T. brucei* display DNA damage repair and genome instability phenotypes (152, 153), including gross chromosomal rearrangements due to loss of subtelomeric *VSG*s, an instability also seen in bloodstream form *T. brucei* BRCA2 mutants (109, 137). Despite this, loss of MRE11 has no detectable effect on VSG switching, questioning whether DSBs can initiate

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

the reaction (see below) or whether loss of MRE11 alleviates suppression of alternative DSB repair reactions that can be upregulated and affect switching. Mismatch repair (MMR) recognizes and repairs base mismatches, which can occur during replication and during HR between DNA molecules that are diverged in sequence (Fig. 5). In this latter context, MMR suppresses HR (154), including in *T. brucei* (155, 156). Again, however, *T. brucei* mutations of MSH2 or MLH1 (central factors in MMR) do not detectably alter the frequency of *VSG* switching. Given that *VSG* recombination appears to frequently use highly variable flanking sequences for homology, such as the 70-bp repeats, this observation is perhaps surprising, and remains unexplained. For instance, these data might implicate an MMR-independent HR reaction (157) in VSG switching. Alternatively, MMR mutation might be neutral if the repair machinery functions not merely to survey for poorly matched *VSG*s during the downstream strand exchange steps of HR, but also acts in the initiation of the process, perhaps akin to the contribution of MMR to immunoglobulin rearrangements and transcription-associated triplet repeat expansions (158). Whatever the explanation, the lack of a detectable effect of MMR mutation may warrant further investigation, as these findings mirrored initial observations in *N. gonorrhoeae* antigenic variation, where mutants of MutS, the bacterial ortholog of MSH2, also appeared to show unaltered pilin switching (159). However, later analysis showed that MutS mutants do result in increased pilin switching, explained by altered recombination tract lengths (160). *Trypanosoma brucei* MMR may yet be revealed to contribute to *VSG* donor selection, but the far greater numbers of available silent *VSG*s mean that any effects of MMR mutation may not be seen by simply measuring switch efficiency.

A further complexity in *VSG* switching is the relatively modest effect of any single HR-associated gene mutation detailed to date. A consistent finding from many studies is that in the absence of RAD51-dependent HR, *VSG* switching by recombination continues to operate, albeit at reduced levels (110, 145). To date, the nature of this RAD51-independent HR is unknown. DSBs in eukaryotes can be repaired by at least three routes: HR, nonhomologous end-joining (NHEJ), and a still poorly understood pathway (or pathways) termed alternative end-joining or microhomology-mediated end-joining (MMEJ) (161). It seems likely that NHEJ can be discounted as a route for *VSG* gene conversion; not only does this appear mechanistically incompatible, but mutants of the Ku heterodimer that recognize DSBs in this reaction have no effect on *VSG* switching (162, 163). In fact, no study to date has successfully detected NHEJ in *T. brucei*, and it may well be absent, like in some other eukaryotes (164), due to the absence of key factors (165). In contrast, MMEJ is readily detected in *T. brucei*, and may have assumed the role of NHEJ in DSB repair (165–168). Testing whether MMEJ provides a route for *VSG* gene conversion in the absence of RAD51-dependent HR is hampered by the lack of clarity in the machinery involved, but it is increasingly clear that this reaction is highly flexible, for instance contributing to many forms of structural genome variation and acting in immunoglobulin rearrangements (161). Beyond MMEJ, some evidence suggests that bonafide HR occurs in *T. brucei* in the absence of RAD51, since homologous integration following transformation is readily detected in RAD51 mutants (166). Moreover, transformation assays have suggested that two HR reactions might operate in *T. brucei*, one that has a minimal homology length requirement of ~100 bp and another, less efficient reaction that needs around ~30 bp of homology (157). To date, however, the machinery needed for an HR reaction of such a short length is unknown. Gene conversion during antigenic variation that is independent of the HR recombinase is not unique to *T. brucei*: *Borrelia burgdorferi* is a tick-borne spirochete where recombination of silent *vls* gene segments into the single *vlsE* expression site is unaffected by mutation of RecA (169), though it does require RuvAB, a helicase that processes Holliday junction HR intermediates (170, 171). Like *T. brucei*, how homology-directed strand transfer initiates in *B. burgdorferi* without RecA/RAD51 is unknown, though the potential involvement of a novel, telomere-directed resolvase would be very interesting (172).

HOW IS *T. BRUCEI* VSG SWITCHING BY RECOMBINATION INITIATED?

The high rate of VSG switching suggests that antigenic variation must be promoted by some process(es) to elevate the frequency of coat changes above general mutation levels. In common with programmed rearrangements such a mating type switching in yeast and immunoglobulin gene maturation, DSBs in the BES have long been suspected as likely initiating lesions in *T. brucei*, promoting *VSG* recombination through HR (59, 173) (Fig. 5). Boothroyd et al. provided the first evidence that DSBs might indeed act in this way: by controllably expressing an endonuclease, ISceI, and localizing its recognition sequence within the BES, it was possible to show that an experimentally-induced DSB elevates

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

VSG switching through gene conversion (127). Moreover, elevation of switching was dependent on localizing the ISceI target sequence adjacent to the 70-bp repeats and upstream of the *VSG*, and was abolished if the 70-bp repeats were removed from the BES. Further evidence for the role of DNA breaks was found using ligation-mediated PCR, which suggested that such lesions were readily detectable in the 70-bp repeats within the actively transcribed BES, but were less detectable in a silent BES or, indeed, within the *ESAG*s of the active BES (127). These data appear to implicate the 70-bp repeats as the culprit for promoting the formation of DNA breaks, perhaps even DSBs, and that this role is associated with, or seen more readily, when the BES is traversed by RNA Pol I. This is an attractive model, but several questions and areas of uncertainty remain (174).

The foremost question promoted by the above model, is how might the 70-bp repeats promote the formation of DSBs? One possibility is that they are the target of an endonuclease (173). To date, such an enzyme has escaped detection, perhaps because it is novel or its activity or expression is tightly regulated. Endonuclease-independent break formation might also be considered, such as seen by Activation-Induced (Cytidine) Deaminase (AID) targeting of the repair machinery during immunoglobulin class switching (175). Indeed, given the association between the breaks and BES expression, transcription-associated breakage, also mediated by repair such as has been proposed for triplet repeat expansion, is possible (158). Alternatively, the 70-bp repeats, perhaps due to the structurally unstable (TRR) motif, could stall DNA replication and promote HR. Certain DNA sequences, termed fragile sites, are regions of elevated genomic instability, and at least some these are associated with stalling of replication (176, 177). Indeed, the capacity of replication stalling to induce rearrangements has been harnessed in other organisms to promote locus-specific genetic changes: in *Schizosaccharomyces pombe* a specific replication-blocking lesion promotes *mat* switching by HR (178), and replication barriers are needed for gene amplification in *Tetrahymena* macronuclear development (179). As tractable as such mechanisms are to experimental investigation, the centrality of the 70-bp repeats for switching and the suggestion that it is DSBs that form on these sequences, is not supported by all data. Though deletion of the 70-bp repeats impedes the elevation of *VSG* switching after ISceI targeting, removal of the repeats from the active BES in the absence of ISceI appears not to affect *VSG* switching rate (114, 127). Moreover, though a later study confirmed that ISceI-mediated DSB formation can activate switching in a BES location-dependent fashion, strict localization of breaks within the active BES and around the 70-bp repeats was not confirmed, and instead ligation-mediated PCR suggested that all BES are fragile adjacent to the telomere (128). Finally, as noted above, the lack of a role for MRE11 in *VSG* switching is perplexing, given its central function in DSB detection and repair (152). One explanation for these inconsistencies, as we have argued throughout, is that *VSG* recombination cannot be explained by a single mechanism, or by a sequence to direct that mechanism, and the system acts flexibly through several routes. Such flexibility may be compatible with the suggestion that telomere length, and not the 70-bp repeats, is the determinant of VSG switching (180, 181). This suggestion was promoted by studies in telomerase-deficient *T. brucei*, which gradually lose telomere repeats over time (182). In cells with critically short telomeres, *VSG* switching rate is higher than in the same cells with longer telomeres (181). How gene conversion is promoted in these cells is unclear, given that ISceI-mediated removal of the telomere tract does not elicit this response (128), though short telomeres might precipitate subtelomeric breaks or switching might relate to a process of telomere stabilization (183). How significant such telomere processes are in telomerase-proficient cells is unclear, but it may be one route among many for *VSG* switching.

A larger area of uncertainty regarding the model of DSB induction of *VSG* switching lies in selection of the *VSG* donors that provide the substrates for recombination. As we have discussed, *T. brucei* has a large, diverse archive of *VSG*s found in BES, minichromosomes and in subtelomeric arrays (Fig. 2). Despite this, the overwhelming majority of *VSG* switching events that were characterized following ISceI DSB induction used BES *VSG*s as donors (~85%); minichromosomal *VSG*s, despite their ~10-fold numerical superiority, were used infrequently (~15%) and array *VSG*s, whether intact or pseudogene, were not detected (127). This may reflect the hierarchy of switching events that are seen during long-term infections (see below), suggesting that there is a probability spectrum of donor preference and it is immune selection that allows the emergence of less frequently accessed genes in the archive. Alternatively, it is possible that activation of some *VSG*s may be initiated by a distinct route. Answers to such questions will be informed by in-depth profiling of *VSG*s expressed during infections, but require detailed assays of switch rates and mechanisms of distinct *VSG* types for a definitive answer.

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

SEGMENTAL GENE CONVERSION PRODUCES FUNCTIONAL VSG SURFACE COATS

The SGC in *VSG* switching appears to be a remarkably flexible reaction, judging by its products (Fig. 4). In some instances SGC can be simple: in cases where the 3′ boundary of gene conversion occurs within the coding sequence, SGC causes only the part of the gene encoding the exposed N-terminal domain of the antigen to be exchanged ("3′ donation") (124), a process similar to the exchange of antigen variable region "cassettes" by pathogens such as *Anaplasma marginale* (184), *Treponema pallidum* (185), and *Mycoplasma genitalum* (186–188). In addition, SGC can form a "mosaic" *VSG*, exchanging segments across the gene and introducing variation into the antigenically-important N-terminal domain. Mosaic *VSG*s identified from chronic infections can involve some quite complex rearrangements, involving up to four donors and more than ten segments (124). Both patterns of SGC—mosaicism and 3′ donation—appear to have been facilitated by homology: donors in both cases shared at least 85% nucleotide identity at the boundary of conversion, and the donors for mosaic *VSG*s were at least 73% identical across their N-terminal domain-encoding regions (124).

Segmentally converted *VSG*s identified from infections share the structural features of other expressed *VSG*s: they are not markedly different in length to intact *VSG*s, they possess a similar domain structure, and appear to form functional surface coats (124, 189). This is significant, because unlike gene conversion of intact, full-length *VSG*s, both 3′ donation and mosaicism readily use segments of pseudogene *VSG*s. Most likely, the lack of incorporation of the mutations that would impair the VSG coat or its expression reflects selection: those cells that generate *VSG* mosaics in which mutations are present are rapidly killed. Death may be immune-mediated, or may result from a cessation of protein synthesis following detection of nonfunctional VSG expression (190191). Mosaic VSGs tend to appear only relatively later in infection (after 3 weeks in mice, although the kinetics of antigenic variation are likely to vary significantly between hosts) (37, 124). The "late" appearance of mosaic VSGs suggests either that they have a lower probability of becoming expressed than intact VSGs, perhaps because the SGC events are less efficient and tend not to occur earlier in infection, and/or that trypanosomes expressing mosaic VSGs grow more slowly. The former is more likely, as previous experimental studies found poor correlation between trypanosome growth rate and expressed VSGs (192). However, these studies did not examine mosaic VSGs constructed during an infection, which may be suboptimally expressed compared with intact VSGs. This latter point may also explain why the products of SGC identified to date have been fairly conservative, with the replacement of part of one gene with the homologous region from another and with no dramatic changes to the features or structure of VSG: more radical variants may occur, but impose such a growth defect on their expressers that they are outcompeted.

The molecular mechanisms underlying *T. brucei* SGC remain mysterious. It is unknown whether *VSG* SGC products are rare consequences of "normal" recombination processes that act during intact *VSG* gene conversion, or whether the reaction involves a distinct, dedicated machinery. Some clues can be gleaned from the homology between mosaics and their donors: similarity is required, but perfect identity is not. Classical HR in *T. brucei* seems too demanding to explain this process, being relatively intolerant of base mismatches and requiring extensive regions of homology (157). The RAD51-independent MMEJ pathway, requiring only ~5 to 15 bp of homology and tolerant of mismatches (166–168), appears a more promising candidate, but the machinery involved in this process is unknown. Just as importantly, it is unclear if the form of strand transfer and break repair in MMEJ is capable of catalyzing *VSG* SGC; for instance, are mosaic *VSG*s generated in a stepwise fashion (perhaps over a number of cell cycles) involving successive gene conversions (37), or can they result from a single construction event? Understanding where this process occurs would be very revealing: are the array *VSG* pseudogenes "assembly intermediates" that arise from ongoing subtelomeric recombination, or might the reaction be rapid enough to allow assembly within the active BES without leading to protracted expression of nonfunctional VSG protein (87)? Loss of array *VSG*s during growth of *T. brucei* BRCA2 and MRE11 mutants hints at recombination within the subtelomeres, but the nature and scale of such events is unclear (109, 137, 152).

WHAT IS THE FUNCTION OF *VSG* SEGMENTAL GENE CONVERSION?

The finding that *VSG* SGC predominates in chronic infections raises many questions. Most important is: what is the function of this reaction in general—and mosaic VSGs in particular—in trypanosome antigenic variation? There are several different ways to answer this question (193). Clearly SGC contributes to antigenic

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

variation by allowing the expression of a different VSG, but as discussed above, *T. brucei* has several other mechanisms for switching between intact *VSG*s, and SGC poses the potentially lethal risk of causing expression of a dysfunctional *VSG* (191). Therefore, we want to ask: what are the advantages of expressing *VSG*s by SGC, *rather than as full-length genes*? What aspect(s) of SGC (if any) have made it a selected trait, causing trypanosomes with this phenotype to have an edge over their competitors, and what aspects are simply consequences of SGC?

Three theories have been advanced for the function of SGC and of mosaic VSGs, none of which are mutually exclusive. Each theory appeals to the ability of SGC to increase the diversity phenotype, either by increasing the number of variants possible (combinatorial variation, expression of pseudogenes) or by increasing the evenness of diversity over time (hierarchy).

Combinatorial variation

The first explanation for SGC in antigenic variation is that it increases the effective VSG repertoire by introducing combinatorial variation, allowing either prolonged infection within a host, reinfection or superinfection of a previously-infected host, or both (194); the sheer size of the *VSG*, and particularly the pseudogene *VSG*, repertoire suggests that this aspect has been a strong selective force. Specifically, combinatorial variation proposes that x donors can interact by segmental gene conversion to produce y antigenically distinct variants, where $x < y$. Many different patterns of *VSG* SGC between the same donors have been identified in individual infections, demonstrating the ability of SGC to introduce combinatorial genetic variation. Combinatorial variation as an explanation for SGC has gained substantial support in other antigenically variant pathogens, in particular *A. marginale* and *B. burgdorferi* (195), and it is well known that the mammalian immune system harnesses combinatorial variation during B-cell maturation, generating perhaps 10^{10} different antibody idiotypes within a single host (196, 197). As an explanation for trypanosome SGC, combinatorial variation has considerable appeal. However, the data are somewhat more ambiguous. Mosaic *VSG*s identified to date use donors with relatively high levels of identity, and as a consequence mosaic *VSG*s tend to be antigenically similar: four mosaic *VSG*s constructed from four different donors isolated by Barbet et al. (189) were found to be cross-reactive using polyclonal rabbit antisera ($x = 4, y = 1; x \not< y$), and of the five mosaic *VSG*s constructed from four different donors isolated by Hall et al. (124), only one was antigenically distinct from the others when tested with polyclonal mouse antiplasma ($x = 4, y = 2; x \not< y$). In the latter case, related mosaics were antigenically distinct, but not as a consequence of combinatorial variation *per se*, as antigenic variation could be explained by differences between the donors. It is possible that the acute immune responses used in these tests are not representative of those mounted during a complex natural infection—indeed, examination of chronic infection showed that antibody responses against the segmentally generated *Anaplasma* surface antigen MSP2 were highly variable, with poor maintenance of antibody against individual gene segments (198). Neither may these responses be representative of the immunological memory of previously infected host; indeed, there is evidence of trypanosomiasis causing substantial immune dysfunction in laboratory models of infection, though the effects in natural hosts are less well understood (34). Either way, the data collected to date do not show SGC during VSG expression to be a particularly efficacious means of *generating* antigenic novelty. Furthermore, the most frequently identified SGC event in chronic infections was 3′ donation (124), which introduced combinatorial genetic variation into an antigenically unimportant region of VSG (although it is possible that mosaicism and 3′ donation are acted on by different selective pressures). Whether combinatorial variability is the specific property of SGC that caused it to be selected is therefore unclear.

Hierarchy

For many years it has been recognized that there is a semi-predictable order to the different VSGs that predominate as an infection progresses, referred to as the "expression hierarchy" (8, 199–202). An advantage of this phenotype, as opposed to one in which each *VSG* has an equal probability of being activated, is that infection is prolonged because the host cannot mount responses against all the antigen variants at once. SGC has been proposed to contribute to hierarchy in two ways: (i) by generating "strings" of mosaics (that is, the progressive accumulation of segments in the expressed *VSG* over time has an implicit order to it) (203); and (ii) by causing pseudogenes to be activated at low probability, and therefore causing them to be expressed "late" in the hierarchy (204). With regards the first mechanism, there is good theoretical support (6) and some experimental evidence for a progressive increase in mosaic *VSG* complexity over time (124, 189), consistent with SGC in other pathogens (205, 206). With regards the second mechanism, there is an obvious benefit to the expression of pseudogenes (see below) and a consequence of SGC is that pseudogenes are less likely to be activated

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

and therefore tend not to appear until later in infection. However, the resulting hierarchy being the selected feature of SGC implies that trypanosome clones with delayed expression of particular genes (call them $T_{delayed}$) had an advantage over their peers ($T_{not\ delayed}$), which could not be the case if the competition were occurring within an infection. For $T_{not\ delayed}$, on expressing the antigen variant relatively earlier, would induce immunity that would also neutralize $T_{delayed}$ on expressing the antigen variant relatively later. The benefit of the $T_{delayed}$ phenotype would be seen only if selection were occurring at a between-infection level, which is not impossible, but less likely to occur than selection at the between-individual level (194, 207).

Expression of pseudogenes

An obvious beneficial effect of SGC is that it allows the expression of pseudogene *VSG*s, which would otherwise be junk, greatly increasing the potential for antigenic variation. Yet, one might suppose that if trypanosomes were being selected for increased archive size, the most straightforward adaptation would have been an increased number of intact archive *VSG*s, rather than the evolution of an archive of pseudogenes activated by risky SGC. This supposition does not take account of how selection is likely to act on the *VSG* archive, however. The strength of selection for function on an individual *VSG* gene in the archive is likely to be weak: an individual *VSG* is seldom expressed, and it usually requires to be copied for expression (208). On the other hand, genetic drift is likely to be strong: the *VSG* archive is subtelomeric, a genomic region subject to high mutation rates (that probably cause a beneficial increase in archive diversity) (51–55); and over the course of its lifecycle *T. brucei* goes through dramatic population bottlenecks (209). A consequence is that intact archive *VSG*s are likely to be under constant risk of becoming pseudogenes. In fact, like other multigene families, the *VSG* archive is probably subject to a process of birth-and-death evolution, where *VSG*s are "born" by gene duplication, and eventually "die" by the acquisition of mutations (210). Under these dynamics, the ability to use a "dead" *VSG*—which has acquired pseudogenizing mutations during the process of evolution—represents an obvious benefit of SGC that would give an individual trypanosome clone an advantage over its competitors within an individual infection. Consequently, expressing the archive in segments would further relax the strength of selection for intact genes, possibly explaining the high proportion of pseudogenes among *VSG*s when compared with other multigene families. The most severe selective bottleneck in the lifecycle of *T. brucei* is migration to the tsetse salivary glands, with perhaps only a handful of individuals surviving the journey (209)—a journey that seems to be absent from the lifecycles of *T. congolense* and *T. vivax* (which colonize the mouthparts) (211). Intriguingly, the proportion of pseudogenes in the *VSG* archives of *T. congolense* and *T. vivax* is much lower than in *T. brucei* (84), consistent with genetic drift driving pseudogenization of the *T. brucei* archive.

Comparative analyses may help to resolve these questions. It remains to be seen, for example, whether *T. congolense* and *T. vivax* use SGC to express VSG to the extent that *T. brucei* does. Finding that they do, in spite of their lower pseudogene content, would suggest that the primary function of SGC is in generating combinatorial variation and in the production of strings of mosaics, whereas finding that they do not would be more consistent with SGC performing a role primarily associated with pseudogene expression and archive evolution.

CAN VSG SWITCHING BE EXPLAINED BY A SINGLE RECOMBINATION MODEL?

Description of the locations of *VSG*s in the *T. brucei* genome, as well as the demonstration that the genes are flanked by blocks of homology (notably, the 70-bp repeats) and can be telomeric, prompted the proposal of HR mechanism models to encapsulate the process of *VSG* switching. Synthesis-dependent strand-annealing (SDSA) was initially favoured, as this process does not generate crossovers and would therefore avoid potentially lethal translocations of subtelomeric array *VSG*s into the BES (173, 212). More recently, it has been suggested that break-induced replication might be a better model for the activation of telomeric *VSG*s, where gene conversions can extend to include the telomeric repeats (8, 180). These suggestions are, at least in part, supported by the finding that loss of HR factors impairs switching. However, as we have increasing knowledge about the factors involved in *VSG* switching, it appears increasingly likely that no single mechanism is used, and instead *T. brucei* antigenic variation can exploit a number of recombination pathways (Fig. 5). Multiple lines of evidence support such reaction flexibility. First, mutation of RAD51-dependent HR impairs, but does not abolish, VSG recombination (108–110, 157). Second, though the 70-bp repeats make an important contribution to VSG switching, their removal is not an impediment (114, 127). Third, though induction of a DSB promotes VSG switching, the reaction(s) induced may not use all available *VSG* donors (127). Finally,

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

no experiments to date have examined the machinery involved in *VSG* mosaic formation, and it is unclear if this can be accounted for by BES-focused recombination models.

Given the above, it seems plausible that all the mechanisms shown in Fig. 5 can be used in VSG switching. SDSA remains the most robustly supported: it is catalyzed by RAD51 and associated factors, is able to account for recombination of any intact *VSG* in the archive, and can lead to crossover events. Break-induced replication is kinetically distinct from SDSA (213) and differs mechanistically in that it uses a processive DNA replication fork established from one end of a DSB (214). Such a reaction could only account for switching of telomeric *VSG*s, but appears highly plausible, since it could more readily explain long-range gene conversions that span much of the BES (48, 215), can be catalyzed without Rad51 (216–218) and, in yeast, has been shown to provide a route for telomere stabilization (213, 215). Nonetheless, an experiment that shows by genetic or mechanistic dissection that break-induced replication is distinct from SDSA in *VSG* switching is lacking. Elevated switching by crossover in *T. brucei* RTR mutants (117, 145) provides evidence not only that reciprocal recombination can occur but also that it is normally suppressed between BES. However, the formation and resolution of Holliday junction intermediates has not been demonstrated, such as by testing for a role of Holliday junction resolvases (219), which have been shown to act in both *N. gonorrhoeae* and *B. burgdorferi* antigenic variation (131, 170, 171). It should be noted that it remains unclear whether any of the above reactions can account for *VSG* SGC, and this will be an important next phase of investigation, given the importance of this reaction to the parasite.

THE INTERPLAY BETWEEN DIFFERENTIATION AND VSG SWITCHING

A classical characteristic of trypanosome infections, shared with many other pathogens that undergo antigenic variation, is the establishment of very chronic infections; for example, cattle experimental infections with trypanosomes can last hundreds of days to years. Bearing in mind that these organisms are permanently extracellular in the mammalian host, and therefore constantly exposed to the multi-faceted assault of the host's immune system (in particular, with respect to VSGs, the adaptive immune response) the ability to employ the armoury of the VSG expression system is clearly an essential part of establishing chronicity. However, a critical component that must be considered when analyzing how *VSG* archive use and switching mechanisms influence *in vivo* dynamics, is the ability of the trypanosome to self-regulate its growth by terminally differentiating from the long slender multiplicative life cycle stage to the short stumpy stage, which is preadapted for transmission to the tsetse fly. While the exact identity of the cAMP-sensitive molecular trigger that mediates this differentiation, termed the "stumpy identification factor" (220, 221), remains elusive, there have been significant recent advances in identifying the mechanisms underlying the process (222–224). It is becoming clear that the interplay between the differentiation and antigenic variation is even more critical than previously considered in shaping the infection dynamics of trypanosome infections (225, 226); one cannot consider the influence of *VSG* archive utilization on chronicity without taking into account the population effects of differentiation, particularly as recent data suggest that differentiation has a profound impact upon the proportion of the population in which switching is occurring.

Estimates of the contribution of differentiation have been explicitly incorporated into previous modelling analyses of *in vivo* infection dynamics (6, 227, 228), but these have been limited by relatively low-resolution measurements (e.g. microscopy-based morphological analysis) and parameter estimations based upon a linear density-dependent effect. The recent identification of stumpy-specific expression markers, in particular the surface-expressed carboxylate transporter Protein Associated with Differentiation 1 (PAD1) (222), has allowed much finer resolution analysis of the role that differentiation plays in determining the infection kinetics (229). This work quantified relative PAD1 expression during chronic (30-day) infections in mice, and generated a mathematical model to analyze the role of differentiation within infection using this much more refined input. These data revealed that stumpy forms predominate to a much greater degree than previously thought, and this is particularly marked in the chronic stages of infection; modeling predictions were validated by morphological analysis with only approximately 15% of trypanosomes being long and slender by day 15 of infection. This has a significant impact upon our understanding of the utilization of the *VSG* archive, as only long slender trypanosomes switch VSGs. The work further suggested that the early period of infection, when long slender forms predominate, is where most of the population will be able to switch VSGs, and the authors suggest that this is a period of initial adaptation to the new environment enabling the trypanosome to overcome, for example, antibodies

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

present due to previous infections or coinfections. After this period of putative rapid switching and adaptation, short stumpy forms predominate and only a minority of the population are able to switch to a new variant for the remainder of the infection timescale (230). These observations emphasize the requirement for further analysis of the chronic stages of infection, and in particular how the observation that only a small proportion of trypanosomes are switching VSGs may potentially influence the mechanistic processes during infection. Analyzing this interrelationship between *VSG* switching and differentiation may provide clues to any mechanistic switch between the utilization of intact *VSG*s early in infection and the predominance of SGC and mosaic *VSG* formation later in infection. However, it also raises the question of why the repertoire of *VSG*s in *T. brucei* is relatively so enormous: if only a fraction of cells are undergoing switching during the majority of the infection lifetime, what selective pressures have resulted in formation and retention of ~2000 *VSG* genes?

WHAT DO WE KNOW ABOUT ANTIGENIC VARIATION IN OTHER SPECIES OF TRYPANOSOME?

Antigenic variation as a phenotype is expressed and has been studied in all three species of African trypanosome (*T. brucei*, *T. congolense*, and *T. vivax*), and findings from the more laboratory-tractable and therefore intensively studied *T. brucei* have been largely assumed to translate to the other two species. Genomic analyses have challenged this assumption, revealing significant differences between the *VSG* archives in the three species (75, 84). Whole genome comparisons revealed that in *T. congolense* there is no equivalent of the *T. brucei* a-VSG subfamily; in contrast, there are two b-VSG subfamilies and a much greater range of distinct C-terminal domains (15–20 CTD types, each of which are found to be associated with particular *VSG* subtypes, in contrast to the single CTD found in *T. brucei*). This finding suggests that there is much greater structural heterogeneity in *T. congolense* VSGs, and that the *T. brucei* common CTD has evolved through transfer from one subfamily to the other, perhaps due to *VSG* switching. *Trypanosoma vivax* appears to possess the most structurally diverse *VSG* repertoire, with a-*VSG*s and b-*VSG*s, as well as two further types. Jackson and colleagues also compared the phylogenies of *VSG* families, which suggests differences in the role that recombination has played in shaping the archives in the three species. The data suggest that in *T. brucei* recombination occurs across the whole repertoire of *VSG*s, whereas in *T. congolense* recombination is focused within *VSG* clades, and in *T. vivax* there is relatively little evidence of *VSG* recombination. Finally, given our previous discussion of the increasing weight given to SGC, mosaic gene formation and use of the pseudogene repertoire in the chronic (or post-acute) stages of *T. brucei* infections, it is notable that the proportion of *VSG*s that are pseudogenes is markedly lower in either *Trypanosoma congolense* (21% and 29% of the two *VSG* subfamilies) or *T. vivax* (15% and 27%of two of the four *VSG* subfamilies) compared with *T. brucei* (69% and 72% of a- and b-VSGs, respectively). These findings raise intriguing questions about the different selective pressures that have shaped the size and organization of the different *VSG* archives, whether these relate to any mechanistic differences in *VSG* usage, and how these divergent systems express a similarly efficient antigenic variation phenotype that results in establishment of chronic infections and onwards transmission. It is clear that much insight will be gained from examining VSG expression diversity and the prevalent switch mechanisms in all three trypanosome species.

CONCLUSIONS

The 10 years that have passed since the publication of *Mobile DNA II* have seen substantial progress in our understanding of antigenic variation in African trypanosomes. In part these advances have stemmed from increasingly sophisticated tools for manipulation of, in particular, *T. brucei*, but they have also been accelerated by sequencing the genomes of the African trypanosomes and related kinetoplastid parasites. This has firmly connected *VSG* switching by recombination with general repair of genome damage through HR, aligning this strategy with antigenic variation as it operates in bacterial pathogens. A major shift in our understanding of the strategy of *VSG* switching is the realization that segmental gene conversion, frequently piecing together gene segments from multiple pseudogenes, allows for enormous coat diversity and is a major element of how antigenic variation promotes trypanosome survival; again, this aligns antigen switching in the African trypanosome with many other pathogens. The full extent of VSG diversity in trypanosomes, and how it is generated, remains unclear, but ever-improving next-generation strategies for analysis of DNA, RNA and protein content in all cells will provide routes to answer these questions. The same approaches are likely to allow us to identify putative trypanosome-specific features of antigenic variation.

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

ACKNOWLEDGMENTS

We thank the Wellcome Trust, the Royal Society, the BBSRC, the MRC and GALVmed/DfID for the predominant funding of our work over the last 10 years. We are grateful to many colleagues for their discussions over this time, but wish to particularly acknowledge Dave Barry, Piet Borst and Andy Tait for their guidance and support as each of us began our studies in trypanosomes.

REFERENCES

1. Brockhurst MA, Koskella B. 2013. Experimental coevolution of species interactions. *Trends Ecol Evol* **28:**367–375.

2. Hirano M, Das S, Guo P, Cooper MD. 2011. The evolution of adaptive immunity in vertebrates. *Adv Immunol* **109:**125–157.

3. Sniegowski PD, Murphy HA. 2006. Evolvability. *Curr Biol* **16:**R831–R834.

4. Graves CJ, Ros VI, Stevenson B, Sniegowski PD, Brisson D. 2013. Natural selection promotes antigenic evolvability. *PLoS Pathog* **9:** e1003766.

5. Nuismer SL, Otto SP. 2005. Host–parasite interactions and the evolution of gene expression. *PLoS Biol* **3:**e203.

6. Gjini E, Haydon DT, Barry JD, Cobbold CA. 2010. Critical interplay between parasite differentiation, host immunity, and antigenic variation in trypanosome infections. *Am Nat* **176:**424–439.

7. Borst P. 2002. Antigenic Variation in Eukaryotic Parasites, pp. 953–971. *In* Craig NL, Berg DE. (ed) *Mobile DNA II*. ASM Press, Washington.

8. Barry JD, McCulloch R. 2001. Antigenic variation in trypanosomes: enhanced phenotypic variation in a eukaryotic parasite. *Adv Parasitol* **49:**1–70.

9. Deitsch KW, Moxon ER, Wellems TE. 1997. Shared themes of antigenic variation and virulence in bacterial, protozoal, and fungal infections. *Microbiol Mol Biol Rev* **61:**281–293.

10. Deitsch KW, Lukehart SA, Stringer JR. 2009. Common strategies for antigenic variation by bacterial, fungal and protozoan pathogens. *Nat Rev Microbiol* **7:**493–503.

11. Turner CM. 1997. The rate of antigenic variation in fly-transmitted and syringe-passaged infections of *Trypanosoma brucei*. *FEMS Microbiol. Lett.*, **153**, 227–231.

12. Norris SJ. 2006. Antigenic variation with a twist—the *Borrelia* story. *Mol Microbiol* **60:**1319–1322.

13. Barrett MP, Burchmore RJ, Stich A, Lazzari JO, Frasch AC, Cazzulo JJ, Krishna S. 2003. The trypanosomiases. *Lancet* **362:**1469–1480.

14. Lai DH, Hashimi H, Lun ZR, Ayala FJ, Lukes J. 2008. Adaptations of *Trypanosoma brucei* to gradual loss of kinetoplast DNA: *Trypanosoma equiperdum* and *Trypanosoma evansi* are petite mutants of *T. brucei*. *Proc Natl Acad Sci USA* **105:**1999–2004.

15. Barbet AF, McGuire TC. 1978. Crossreacting determinants in variant-specific surface antigens of African trypanosomes. *Proc Natl Acad Sci USA* **75:**1989–1993.

16. Barry JD. 1986. Antigenic variation during *Trypanosoma vivax* infections of different host species. *Parasitology* **92:**51–65.

17. Vickerman K. 1978. Antigenic variation in trypanosomes. *Nature* **273:**613–617.

18. Vickerman K, Luckins AG. 1969. Localization of variable antigens in the surface coat of *Trypanosoma brucei* using ferritin conjugated antibody. *Nature* **224:**1125–1126.

19. Cross GA. 1975. Identification, purification and properties of clone-specific glycoprotein antigens constituting the surface coat of *Trypanosoma brucei*. *Parasitology* **71:**393–417.

20. Borst P, Cross GA. 1982. Molecular basis for trypanosome antigenic variation. *Cell* **29:**291–303.

21. Morrison LJ, Marcello L, McCulloch R. 2009. Antigenic variation in the African trypanosome: molecular mechanisms and phenotypic complexity. *Cell Microbiol* **11:**1724–1734.

22. Horn D. 2009. Antigenic variation: extending the reach of telomeric silencing. *Curr Biol* **19:**R496–R498.

23. Rudenko G. 2011. African trypanosomes: the genome and adaptations for immune evasion. *Essays Biochem* **51:**47–62.

24. Horn D, McCulloch R. 2010. Molecular mechanisms underlying the control of antigenic variation in African trypanosomes. *Curr Opin Microbiol* **13:**700–705.

25. Glover L, Hutchinson S, Alsford S, McCulloch R, Field MC, Horn D. 2013. Antigenic variation in African trypanosomes: the importance of chromosomal and nuclear context in VSG expression control. *Cell Microbiol* **15:**1984–1993.

26. Higgins MK, Carrington M. 2014. Sequence variation and structural conservation allows development of novel function and immune evasion in parasite surface protein families. *Protein Sci* **23:**354–365.

27. Barry JD, Hall JP, Plenderleith L. 2012. Genome hyperevolution and the success of a parasite. *Ann NY Acad Sci* **1267:**11–17.

28. Barbour AG, Restrepo BI. 2000. Antigenic variation in vector-borne pathogens. *Emerg Infect Dis* **6:**449–457.

29. Schwede A, Jones N, Engstler M, Carrington M. 2011. The VSG C-terminal domain is inaccessible to antibodies on live trypanosomes. *Mol Biochem Parasitol* **175:**201–204.

30. Engstler M, Pfohl T, Herminghaus S, Boshart M, Wiegertjes G, Heddergott N, Overath P. 2007. Hydrodynamic flow-mediated protein sorting on the cell surface of trypanosomes. *Cell* **131:**505–515.

31. Seyfang A, Mecke D, Duszenko M. 1990. Degradation, recycling, and shedding of *Trypanosoma brucei* variant surface glycoprotein. *J Protozool* **37:**546–552.

32. Higgins MK, Tkachenko O, Brown A, Reed J, Raper J. Carrington M. 2013. Structure of the trypanosome haptoglobin-hemoglobin receptor and implications for nutrient uptake and innate immunity. *Proc Natl Acad Sci USA* **110:**1905–1910.

33. Greif G, Ponce de LM, Lamolle G, Rodriguez M, Pineyro D, Tavares-Marques LM, Reyna-Bello A, Robello C, Alvarez-Valin F. 2013. Transcriptome analysis of the bloodstream stage from the parasite *Trypanosoma vivax*. *BMC Genomics* **14:**149.

34. La GF, Magez S. 2011. Vaccination against trypanosomiasis: can it be done or is the trypanosome truly the ultimate immune destroyer and escape artist? *Hum Vaccin* **7:**1225–1233.

35. Guirnalda P, Murphy NB, Nolan D, Black SJ. 2007 Anti-*Trypanosoma brucei* activity in Cape buffalo serum during the cryptic phase of parasitemia is mediated by antibodies. *Int J Parasitol* **37:**1391–1399.

36. Blum ML, Down JA, Gurnett AM, Carrington M, Turner MJ, Wiley DC. 1993. A structural motif in the variant surface glycoproteins of *Trypanosoma brucei*. *Nature* **362:**603–609.

37. Marcello L, Barry JD. 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.

38. Metcalf P, Blum M, Freymann D, Turner M, Wiley DC. 1987. Two variant surface glycoproteins of *Trypanosoma brucei* of different sequence classes have similar 6 A resolution X-ray structures. *Nature* **325:**84–86.

39. Daniels JP, Gull K, Wickstead B. 2010. Cell biology of the trypanosome genome. *Microbiol Mol Biol Rev* **74:**552–569.

40. Siegel TN, Gunasekera K, Cross GA, Ochsenreiter T. 2011. Gene expression in *Trypanosoma brucei*: lessons from high-throughput RNA sequencing. *Trends Parasitol* **27:**434–441.

41. Gunzl A, Bruderer T, Laufer G, Schimanski B, Tu LC, Chung HM, Lee PT, Lee MG. 2003. RNA polymerase I transcribes procyclin genes and variant surface glycoprotein gene expression sites in *Trypanosoma brucei*. *Eukaryot Cell* **2:**542–551.

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

42. Zomerdijk JC, Ouellette M, Ten Asbroek AL, Kieft R, Bommer AM, Clayton CE, Borst P. 1990. The promoter for a variant surface glycoprotein gene expression site in *Trypanosoma brucei*. *EMBO J* **9:**2791–2801.

43. Brandenburg J, Schimanski B, Nogoceke E, Nguyen TN, Padovan JC, Chait BT, Cross GA, Gunzl A. 2007. Multifunctional class I transcription in *Trypanosoma brucei* depends on a novel protein complex. *EMBO J* **26:**4856–4866.

44. Chaves I, Zomerdijk J, Dirks-Mulder A, Dirks RW, Raap AK, 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.

45. Navarro M, Gull K. 2001. A pol I transcriptional body associated with VSG mono-allelic expression in *Trypanosoma brucei*. *Nature* **414:**759–763.

46. Berriman M, Ghedin E, Hertz-Fowler C, Blandin G, Renauld H, Bartholomeu DC, Lennard NJ, Caler E, Hamlin NE, Haas B, Böhme U, Hannick L, Aslett MA, Shallom J, Marcello L, Hou L, Wickstead B, Alsmark UC, Arrowsmith C, Atkin RJ, Barron AJ, Bringaud F, Brooks K, Carrington M, Cherevach I, Chillingworth TJ, Churcher C, Clark LN, Corton CH, Cronin A, Davies RM, Doggett J, Djikeng A, Feldblyum T, Field MC, Fraser A, Goodhead I, Hance Z, Harper D, Harris BR, Hauser H, Hostetler J, Ivens A, Jagels K, Johnson D, Johnson J, Jones K, Kerhornou AX, Koo H, Larke N, Landfear S, Larkin C, Leech V, Line A, Lord A, Macleod A, Mooney PJ, Moule S, Martin DM, Morgan GW, Mungall K, Norbertczak H, Ormond D, Pai G, Peacock CS, Peterson J, Quail MA, Rabbinowitsch E, Rajandream MA, Reitter C, Salzberg SL, Sanders M, Schobel S, Sharp S, Simmonds M, Simpson AJ, Tallon L, Turner CM, Tait A, Tivey AR, Van Aken S, Walker D, Wanless D, Wang S, White B, White O, Whitehead S, Woodward J, Wortman J, Adams MD, Embley TM, Gull K, Ullu E, Barry JD, Fairlamb AH, Opperdoes F, Barrell BG, Donelson JE, Hall N, Fraser CM, Melville SE, El-Sayed NM. 2005. The genome of the African trypanosome *Trypanosoma brucei*. *Science* **309:**416–422.

47. Jackson AP, Sanders M, Berry A, McQuillan J, Aslett MA, Quail MA, Chukualim B, Capewell P, MacLeod A, Melville SE, Gibson W, Barry JD, Berriman M, Hertz-Fowler C. 2010. The genome sequence of *Trypanosoma brucei* gambiense, causative agent of chronic human african trypanosomiasis. *PLoS Negl Trop Dis*, **4:**e658.

48. Hertz-Fowler C, Figueiredo LM, Quail MA, Becker M, Jackson A, Bason N, Brooks K, Churcher C, Fahkro S, Goodhead I, Heath P, Kartvelishvili M, Mungall K, Harris D, Hauser H, Sanders M, Saunders D, Seeger K, Sharp S, Taylor JE, Walker D, White B, Young R, Cross GA, Rudenko G, Barry JD, Louis EJ, Berriman M. 2008. Telomeric expression sites are highly conserved in *Trypanosoma brucei*. *PLoS ONE* **3:** e3527.

49. Navarro M, Cross GA. 1996. DNA rearrangements associated with multiple consecutive directed antigenic switches in *Trypanosoma brucei*. *Mol Cell Biol* **16:**3615–3625.

50. Young R, Taylor JE, Kurioka A, Becker M, Louis EJ, 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.

51. Barry JD, Ginger ML, Burton P, McCulloch R. 2003. Why are parasite contingency genes often associated with telomeres? *Int J Parasitol* **33:**29–45.

52. Linardopoulou EV, Williams EM, Fan Y, Friedman C, Young JM, Trask BJ. 2005. Human subtelomeres are hot spots of interchromosomal recombination and segmental duplication. *Nature* **437:**94–100.

53. Fan C, Zhang Y, Yu Y, Rounsley S, Long M, Wing RA. 2008. The subtelomere of *Oryza sativa* chromosome 3 short arm as a hot bed of new gene origination in rice. *Mol Plant* **1:**839–850.

54. Brown CA, Murray AW, Verstrepen KJ. 2010. Rapid expansion and functional divergence of subtelomeric gene families in yeasts. *Curr Biol* **20:**895–903.

55. Moraes Barros RR, Marini MM, Antonio CR, Cortez DR, Miyake AM, Lima FM, Ruiz JC, Bartholomeu DC, Chiurillo MA, Ramirez JL,da Silveira JF. 2012. Anatomy and evolution of telomeric and subtelomeric regions in the human protozoan parasite *Trypanosoma cruzi*. *BMC Genomics* **13:**229.

56. Shah JS, Young JR, Kimmel BE, Iams KP, Williams RO. 1987. The 5′ flanking sequence of a *Trypanosoma brucei* variable surface glycoprotein gene. *Mol Biochem Parasitol* **24:**163–174.

57. Ohshima K, Kang S, Larson JE, Wells RD. 1996. TTA.TAA triplet repeats in plasmids form a non-H bonded structure. *J Biol Chem* **271:** 16784–16791.

58. Pan X, Liao Y, Liu Y, Chang P, Liao L, Yang L, Li H. 2010. Transcription of AAT*ATT triplet repeats in *Escherichia coli* is silenced by H-NS and IS1E transposition. *PLoS One* **5:**e14271.

59. Borst P, Rudenko G, Blundell PA, van Leeuwen F, Cross MA, McCulloch R, Gerrits H, Chaves IM. 1997. Mechanisms of antigenic variation in African trypanosomes. *Behring Inst Mitt* 1–15.

60. Pays E, Lips S, Nolan D, Vanhamme L, Perez-Morga D. 2001. The VSG expression sites of *Trypanosoma brucei*: multipurpose tools for the adaptation of the parasite to mammalian hosts. *Mol Biochem Parasitol* **114:**1–16.

61. McCulloch R, Horn D. 2009. What has DNA sequencing revealed about the VSG expression sites of African trypanosomes? *Trends Parasitol* **25:**359–63.

62. Siegel TN, Hekstra DR, Wang X, Dewell S, Cross GA. 2010. Genome-wide analysis of mRNA abundance in two life-cycle stages of *Trypanosoma brucei* and identification of splicing and polyadenylation sites. *Nucleic Acids Res* **38:**4946–4957.

63. Bitter W, Gerrits H, Kieft R, Borst P. 1998. The role of transferrin-receptor variation in the host range of *Trypanosoma brucei*. *Nature* **391:**499–502.

64. van Luenen HG, Kieft R, Mussmann R, Engstler M, ter Riet B, Borst P. 2005. Trypanosomes change their transferrin receptor expression to allow effective uptake of host transferrin. *Mol Microbiol* **58:**151–165.

65. Gerrits H, Mussmann R, Bitter W, Kieft R, Borst P. 2002. The physiological significance of transferrin receptor variations in *Trypanosoma brucei*. *Mol Biochem Parasitol* **119:**237–247.

66. Steverding D. 2006. On the significance of host antibody response to the *Trypanosoma brucei* transferrin receptor during chronic infection. *Microbes Infect* **8:**2777–2782.

67. Salmon D, Paturiaux-Hanocq F, Poelvoorde P, Vanhamme L, Pays E. 2005. *Trypanosoma brucei*: growth differences in different mammalian sera are not due to the species-specificity of transferrin. *Exp Parasitol* **109:**188–194.

68. Cordon-Obras C, Cano J, Gonzalez-Pacanowska D, Benito A, Navarro M, Bart JM. 2013. *Trypanosoma brucei gambiense* Adaptation to Different Mammalian Sera Is Associated with VSG Expression Site Plasticity. *PLoS ONE* **8:**e85072.

69. Salmon D, Vanwalleghem G, Morias Y, Denoeud J, Krumbholz C, Lhommé F, Bachmaier S, Kador M, Gossmann J, Dias FB, De Muylder G, Uzureau P, Magez S, Moser M, De Baetselier P, Van Den Abbeele J, Beschin A, Boshart M, Pays E. 2012. Adenylate cyclases of *Trypanosoma brucei* inhibit the innate immune response of the host. *Science* **337:**463–466.

70. Xong HV, Vanhamme L, Chamekh M, Chimfwembe CE, Van den AJ, Pays A, Van Meirvenne N, Hamers R, De Baetselier P, Pays E. 1998. A VSG expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. *Cell* **95:**839–846.

71. Wickstead B, Ersfeld K, Gull K. 2004. The small chromosomes of *Trypanosoma brucei* involved in antigenic variation are constructed around repetitive palindromes. *Genome Res* **14:**1014–1024.

72. Ginger ML, Blundell PA, Lewis AM, Browitt A, Gunzl A, Barry JD. 2002. Ex Vivo and In Vitro Identification of a Consensus Promoter for

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

VSG Genes Expressed by Metacyclic-Stage Trypanosomes in the Tsetse Fly. *Eukaryot Cell* **1:**1000–1009.

73. Sharma R, Gluenz E, Peacock L, Gibson W, Gull K, Carrington M. 2009. The heart of darkness: growth and form of *Trypanosoma brucei* in the tsetse fly. *Trends Parasitol* **25:**517–524.

74. Kolev NG, Ramey-Butler K, Cross GA, Ullu E, Tschudi C. 2012. Developmental progression to infectivity in *Trypanosoma brucei* triggered by an RNA-binding protein. *Science* **338:**1352–1353.

75. Jackson AP, Allison HC, Barry JD, Field MC, Hertz-Fowler C, Berriman M. 2013. A cell-surface phylome for African trypanosomes. *PLoS Negl Trop Dis* **7:**e2121.

76. Marcello L, Menon S, Ward P, Wilkes JM, Jones NG, Carrington M, Barry JD. 2007. VSGdb: a database for trypanosome variant surface glycoproteins, a large and diverse family of coiled coil proteins. *BMC Bioinformatics* **8:**143.

77. Weiden M, Osheim YN, Beyer AL, Van der Ploeg LH. 1991. Chromosome structure: DNA nucleotide sequence elements of a subset of the minichromosomes of the protozoan *Trypanosoma brucei*. *Mol Cell Biol* **11:**3823–3834.

78. Akiyoshi B, Gull K. 2014. Discovery of Unconventional Kinetochores in Kinetoplastids. *Cell* **156:**1247–1258.

79. Rothwell V, Aline R, Jr, Parsons M, Agabian N, Stuart K. 1985. Expression of a minichromosomal variant surface glycoprotein gene in *Trypanosoma brucei*. *Nature* **313:**595–597.

80. Melville SE, Gerrard CS, Blackwell JM. 1999. Multiple causes of size variation in the diploid megabase chromosomes of African trypanosomes. *Chromosome Res* **7:**191–203.

81. Callejas S, Leech V, Reitter C, Melville S. 2006. Hemizygous subtelomeres of an African trypanosome chromosome may account for over 75% of chromosome length. *Genome Res* **16:**1109–1118.

82. MacLean RC, Torres-Barcelo C, Moxon R. 2013. Evaluating evolutionary models of stress-induced mutagenesis in bacteria. *Nat Rev Genet* **14:**221–227.

83. Gjini E, Haydon DT, Barry JD, Cobbold CA. 2012. The Impact of Mutation and Gene Conversion on the Local Diversification of Antigen Genes in African Trypanosomes. *Mol Biol Evol* **29:**3321–3331.

84. Jackson AP, Berry A, Aslett M, Allison HC, Burton P, Vavrova-Anderson J, Brown R, Browne H, Corton N, Hauser H, Gamble J, Gilderthorp R, Marcello L, McQuillan J, Otto TD, Quail MA, Sanders MJ, van Tonder A, Ginger ML, Field MC, Barry JD, Hertz-Fowler C, Berriman M. 2012. Antigenic diversity is generated by distinct evolutionary mechanisms in African trypanosome species. *Proc Natl Acad Sci USA* **109:**3416–3421.

85. Borst P, Ulbert S. 2001. Control of VSG gene expression sites. *Mol Biochem Parasitol* **114:**17–27.

86. Borst P. 2002. Antigenic variation and allelic exclusion. *Cell* **109:**5–8.

87. Pays E. 2005. Regulation of antigen gene expression in *Trypanosoma brucei*. *Trends Parasitol* **21:**517–520.

88. Navarro M, Penate X, Landeira D. 2007. Nuclear architecture underlying gene expression in *Trypanosoma brucei*. *Trends Microbiol* **15:**263–270.

89. Schwede A, Carrington M. 2010. Bloodstream form Trypanosome plasma membrane proteins: antigenic variation and invariant antigens. *Parasitology* **137:**2029–2039.

90. Chaves I, Rudenko G, Dirks-Mulder A, Cross M, Borst P. 1999. Control of variant surface glycoprotein gene-expression sites in *Trypanosoma brucei*. *EMBO J* **18:**4846–4855.

91. Ulbert S, Chaves I, Borst P. 2002. Expression site activation in *Trypanosoma brucei* with three marked variant surface glycoprotein gene expression sites. *Mol Biochem Parasitol* **120:**225–235.

92. Baltz T, Giroud C, Baltz D, Roth C, Raibaud A, Eisen H. 1986. Stable expression of two variable surface glycoproteins by cloned *Trypanosoma equiperdum*. *Nature* **319:**602–604.

93. Munoz-Jordan JL, Davies KP, Cross GA. 1996. Stable expression of mosaic coats of variant surface glycoproteins in *Trypanosoma brucei*. *Science* **272:**1795–1797.

94. Yang X, Figueiredo LM, Espinal A, Okubo E, Li B. 2009. RAP1 is essential for silencing telomeric variant surface glycoprotein genes in *Trypanosoma brucei*. *Cell* **137:**99–109.

95. Denninger V, Fullbrook A, Bessat M, Ersfeld K, Rudenko G. 2010. The FACT subunit TbSpt16 is involved in cell cycle specific control of VSG expression sites in *Trypanosoma brucei*. *Mol Microbiol* **78:**459–474.

96. Povelones ML, Gluenz E, Dembek M, Gull K, Rudenko G. 2012. Histone H1 Plays a Role in Heterochromatin Formation and VSG Expression Site Silencing in *Trypanosoma brucei*. *PLoS Pathog* **8:**e1003010.

97. Alsford S, Horn D. 2012. Cell-cycle-regulated control of VSG expression site silencing by histones and histone chaperones ASF1A and CAF-1b in *Trypanosoma brucei*. *Nucleic Acids Res* **40:**10150–10160.

98. Narayanan MS, Rudenko G. 2013. TDP1 is an HMG chromatin protein facilitating RNA polymerase I transcription in African trypanosomes. *Nucleic Acids Res* **41:**2981–2992.

99. DuBois KN, Alsford S, Holden JM, Buisson J, Swiderski M, Bart JM, Ratushny AV, Wan Y, Bastin P, Barry JD, Navarro M, Horn D, Aitchison JD, Rout MP, Field MC. 2012. NUP-1 Is a large coiled-coil nucleoskeletal protein in trypanosomes with lamin-like functions. *PLoS Biol* **10:**e1001287.

100. Vanhamme L, Poelvoorde P, Pays A, Tebabi P, Van Xong H, Pays E. 2000. Differential RNA elongation controls the variant surface glycoprotein gene expression sites of *Trypanosoma brucei*. *Mol Microbiol* **36:**328–340.

101. Nguyen TN, Muller LS, Park SH, Siegel TN, Gunzl A. 2013. Promoter occupancy of the basal class I transcription factor A differs strongly between active and silent VSG expression sites in Trypanosoma brucei. *Nucleic Acids Res* **42:**3164–3176.

102. Figueiredo LM, Janzen CJ, Cross GA. 2008. A histone methyltransferase modulates antigenic variation in African trypanosomes. *PLoS Biol* **6:**e161.

103. Stockdale C, Swiderski MR, Barry JD, McCulloch R. 2008. Antigenic variation in *Trypanosoma brucei*: joining the DOTs. *PLoS Biol* **6:** e185.

104. Landeira D, Bart JM, Van Tyne D, Navarro M. 2009. Cohesin regulates VSG monoallelic expression in trypanosomes. *J Cell Biol* **186:** 243–254.

105. Tiengwe C, Marcello L, Farr H, Dickens N, Kelly S, Swiderski M, Vaughan D, Gull K, Barry JD, Bell SD, McCulloch R. 2012. Genome-wide Analysis Reveals Extensive Functional Interaction between DNA Replication Initiation and Transcription in the Genome of *Trypanosoma brucei*. *Cell Rep* **2:**185–197.

106. Benmerzouga I, Concepcion-Acevedo J, Kim HS, Vandoros AV, Cross GA, Klingbeil MM, LiB. 2013. *Trypanosoma brucei* Orc1 is essential for nuclear DNA replication and affects both VSG silencing and VSG switching. *Mol Microbiol* **87:**196–210.

107. Kim HS, Park SH, Gunzl A, Cross GA. 2013. MCM-BP is required for repression of life-cycle specific genes transcribed by RNA polymerase I in the mammalian infectious form of Trypanosoma brucei. *PLoS ONE* **8:** e57001.

108. Dobson R, Stockdale C, Lapsley C, Wilkes J, McCulloch R. 2011. Interactions among *Trypanosoma brucei* RAD51 paralogues in DNA repair and antigenic variation. *Mol Microbiol* **81:**434–456.

109. Hartley CL, McCulloch R. 2008. Trypanosoma brucei BRCA2 acts in antigenic variation and has undergone a recent expansion in BRC repeat number that is important during homologous recombination. *Mol Microbiol* **68:**1237–1251.

110. McCulloch R, Barry JD. 1999. A role for RAD51 and homologous recombination in *Trypanosoma brucei* antigenic variation. *Genes Dev* **13:**2875–2888.

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

111. Sheader K, te VD, Rudenko G. 2004. Bloodstream form-specific up-regulation of silent vsg expression sites and procyclin in *Trypanosoma brucei* after inhibition of DNA synthesis or DNA damage. *J Biol Chem* **279:**13363–13374.

112. Liu AY, Van der Ploeg LH, Rijsewijk FA, Borst P. 1983. The transposition unit of variant surface glycoprotein gene 118 of *Trypanosoma brucei*. Presence of repeated elements at its border and absence of promoter-associated sequences. *J Mol Biol* **167:**57–75.

113. Pays E, Van Assel S, Laurent M, Dero B, Michiels F, Kronenberger P, Matthyssens G, Van Meirvenne N, Le Ray D, Steinert M. 1983. At least two transposed sequences are associated in the expression site of a surface antigen gene in different trypanosome clones. *Cell* **34:**359–369.

114. McCulloch R, Rudenko G, Borst P. 1997. Gene conversions mediating antigenic variation in Trypanosoma brucei can occur in variant surface glycoprotein expression sites lacking 70- base-pair repeat sequences. *Mol Cell Biol* **17:**833–843.

115. Bernards A, Van der Ploeg LH, Frasch AC, Borst P, Boothroyd JC, Coleman S, Cross GA. 1981. Activation of trypanosome surface glycoprotein genes involves a duplication-transposition leading to an altered 3′ end. *Cell* **27:**497–505.

116. de Lange T, Kooter JM, Michels PA, Borst P. 1983. Telomere conversion in trypanosomes. *Nucleic Acids Res* **11:**8149–8165.

117. Kim H.S, Cross GA. 2010. TOPO3alpha influences antigenic variation by monitoring expression-site-associated VSG switching in *Trypanosoma brucei*. *PLoS Pathog* **6:**e1000992.

118. Pays E, Guyaux M, Aerts D, Van Meirvenne N, Steinert M. 1985. Telomeric reciprocal recombination as a possible mechanism for antigenic variation in trypanosomes. *Nature* **316:**562–564.

119. Rudenko G, McCulloch R, Dirks-Mulder A, Borst P. 1996. Telomere exchange can be an important mechanism of variant surface glycoprotein gene switching in *Trypanosoma brucei*. *Mol Biochem Parasitol* **80:**65–75.

120. Thon G, Baltz T, Giroud C, Eisen H. 1990. Trypanosome variable surface glycoproteins: composite genes and order of expression. *Genes Dev* **4:**1374–1383.

121. Roth C, Bringaud F, Layden RE, Baltz T, Eisen H. 1989. Active late-appearing variable surface antigen genes in Trypanosoma equiperdum are constructed entirely from pseudogenes. *Proc Natl Acad Sci USA* **86:**9375–9379.

122. Thon G, Baltz T, Eisen H. 1989. Antigenic diversity by the recombination of pseudogenes. *Genes Dev* **3:**1247–1254.

123. Roth C, Jacquemot C, Giroud C, Bringaud F, Eisen H, Baltz T. 1991. Antigenic variation in *Trypanosoma equiperdum*. *Res Microbiol* **142:**725–730.

124. Hall JP, Wang H, Barry JD. 2013. Mosaic VSGs and the scale of *Trypanosoma brucei* antigenic variation. *PLoS Pathog* **9:**e1003502.

125. Barbet AF, Kamper SM. 1993. The importance of mosaic genes to trypanosome survival. *Parasitol Today* **9:**63–66.

126. Kamper SM, Barbet AF. 1992. Surface epitope variation via mosaic gene formation is potential key to long-term survival of *Trypanosoma brucei*. *Mol Biochem Parasitol* **53:**33–44.

127. Boothroyd CE, Dreesen O, Leonova T, Ly KI, Figueiredo LM, Cross GA, Papavasiliou FN. 2009. A yeast-endonuclease-generated DNA break induces antigenic switching in *Trypanosoma brucei*. *Nature* **459:**278–281.

128. Glover L, Alsford S, HornD. 2013. DNA break site at fragile subtelomeres determines probability and mechanism of antigenic variation in african trypanosomes. *PLoS Pathog* **9:**e1003260.

129. Aitcheson N, Talbot S, Shapiro J, Hughes K, Adkin C, Butt T, Sheader K, Rudenko G. 2005. VSG switching in *Trypanosoma brucei*: antigenic variation analysed using RNAi in the absence of immune selection. *Mol Microbiol* **57:**1608–1622.

130. Cahoon LA, Seifert HS. 2011. Focusing homologous recombination: pilin antigenic variation in the pathogenic *Neisseria*. *Mol Microbiol* **81:**1136–1143.

131. Vink C, Rudenko G, Seifert HS. 2011. Microbial antigenic variation mediated by homologous DNA recombination. *FEMS Microbiol Rev* **36:**917–948.

132. San Filippo J, Sung P, Klein H. 2008. Mechanism of Eukaryotic Homologous Recombination. *Annu Rev Biochem* **77:**229–257.

133. Koomey M, Gotschlich EC, Robbins K, Bergstrom S, Swanson J. 1987. Effects of recA mutations on pilus antigenic variation and phase transitions in *Neisseria gonorrhoeae*. *Genetics* **117:**391–398.

134. Mehr IJ, Seifert HS. 1998. Differential roles of homologous recombination pathways in *Neisseria gonorrhoeae* pilin antigenic variation, DNA transformation and DNA repair. *Mol Microbiol* **30:**697–710.

135. Helm RA, Seifert HS. 2009. Pilin antigenic variation occurs independently of the RecBCD pathway in *Neisseria gonorrhoeae*. *J Bacteriol* **191:**5613–5621.

136. Roy R, Chun J, Powell SN. 2012. BRCA1 and BRCA2: different roles in a common pathway of genome protection. *Nat Rev Cancer* **12:**68–78.

137. Trenaman A, Hartley C, Prorocic M, Passos-Silva DG, van den Hoek M, Nechyporuk-Zloy V, Machado CR, McCulloch R. 2013. *Trypanosoma brucei* BRCA2 acts in a life cycle-specific genome stability process and dictates BRC repeat number-dependent RAD51 subnuclear dynamics. *Nucleic Acids Res* **41:**943–960.

138. Stohl EA, Seifert HS. 2001. The recX gene potentiates homologous recombination in *Neisseria gonorrhoeae*. *Mol Microbiol* **40:**1301–1310.

139. Gruenig MC, Stohl EA, Chitteni-Pattu S, Seifert HS, Cox MM. 2010. Less is more: *Neisseria gonorrhoeae* RecX protein stimulates recombination by inhibiting RecA. *J Biol Chem* **285:**37188–37197.

140. Cardenas PP, Carrasco B, Defeu SC, Cesar CE, Herr K, Kaufenstein M, Graumann PL, Alonso JC. 2012. RecX facilitates homologous recombination by modulating RecA activities. *PLoS Genet* **8:**e1003126.

141. Suwaki N, Klare K, Tarsounas M. 2011. RAD51 paralogs: roles in DNA damage signalling, recombinational repair and tumorigenesis. *Semin Cell Dev Biol* **22:**898–905.

142. Jensen RB, Ozes A, Kim T, Estep A, Kowalczykowski SC. 2013. BRCA2 is epistatic to the RAD51 paralogs in response to DNA damage. *DNA Repair (Amst)* **12:**306–311.

143. Chun J, Buechelmaier ES, Powell SN. 2013. Rad51 paralog complexes BCDX2 and CX3 act at different stages in the BRCA1-BRCA2-dependent homologous recombination pathway. *Mol. Cell Biol* **33:**387–395.

144. Proudfoot C, McCulloch R. 2005. Distinct roles for two RAD51-related genes in *Trypanosoma brucei* antigenic variation. *Nucleic Acids Res* **33:**6906–6919.

145. Kim HS, Cross GA. 2011. Identification of *Trypanosoma brucei* RMI1/BLAP75 Homologue and Its Roles in Antigenic Variation. *PLoS One* **6:**e25313.

146. Killoran MP, Kohler PL, Dillard JP, Keck JL. 2009. RecQ DNA helicase HRDC domains are critical determinants in *Neisseria gonorrhoeae* pilin antigenic variation and DNA repair. *Mol Microbiol* **71:**158–171.

147. Cahoon LA, Manthei KA, Rotman E, Keck JL, Seifert HS. 2013. *Neisseria gonorrhoeae* RecQ helicase HRDC domains are essential for efficient binding and unwinding of the pilE guanine quartet structure required for pilin antigenic variation. *J Bacteriol* **195:**2255–2261.

148. Cahoon LA, Seifert HS. 2009. An alternative DNA structure is necessary for pilin antigenic variation in *Neisseria gonorrhoeae*. *Science* **325:**764–767.

149. Cahoon LA, Seifert HS. 2013. Transcription of a cis-acting, noncoding, small RNA is required for pilin antigenic variation in *Neisseria gonorrhoeae*. *PLoS Pathog* **9:**e1003074.

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

150. Kuryavyi V, Cahoon LA, Seifert HS, Patel DJ. 2012. RecA-binding pilE G4 sequence essential for pilin antigenic variation forms monomeric and 5′ end-stacked dimeric parallel G-quadruplexes. *Structure* **20:**2090–2102.

151. Stracker TH, Petrini JH. 2011. The MRE11 complex: starting from the ends. *Nat Rev Mol Cell Biol* **12:**90–103.

152. Robinson NP, McCulloch R, Conway C, Browitt A, Barry JD. 2002. Inactivation of Mre11 Does Not Affect VSG Gene Duplication Mediated by Homologous Recombination in *Trypanosoma brucei*. *J Biol Chem* **277:**26185–26193.

153. Tan KS, Leal ST, Cross GA. 2002. *Trypanosoma brucei* MRE11 is non-essential but influences growth, homologous recombination and DNA double-strand break repair. *Mol Biochem Parasitol* **125:**11–21.

154. Jiricny J. 2013. Postreplicative mismatch repair. *Cold Spring Harb Perspect Biol* **5:**a012633.

155. Bell JS, McCulloch R. 2003. Mismatch repair regulates homologous recombination, but has little influence on antigenic variation, in *Trypanosoma brucei*. *J Biol Chem* **278:**45182–45188.

156. Bell JS, Harvey TI, Sims AM, McCulloch R. 2004. Characterization of components of the mismatch repair machinery in *Trypanosoma brucei*. *Mol Microbiol* **51:**159–173.

157. Barnes RL, McCulloch R. 2007. Trypanosoma brucei homologous recombination is dependent on substrate length and homology, though displays a differential dependence on mismatch repair as substrate length decreases. *Nucleic Acids Res* **35:**3478–3493.

158. Slean MM, Panigrahi GB, Ranum LP, Pearson CE. 2008. Mutagenic roles of DNA "repair" proteins in antibody diversity and disease-associated trinucleotide repeat instability. *DNA Repair (Amst)* **7:**1135–1154.

159. Hill SA, Davies JK. 2009. Pilin gene variation in *Neisseria gonorrhoeae*: reassessing the old paradigms. *FEMS Microbiol Rev* **33:**521–530.

160. Criss AK, Bonney KM, Chang RA, Duffin PM, LeCuyer BE, Seifert HS. 2010. Mismatch correction modulates mutation frequency and pilus phase and antigenic variation in *Neisseria gonorrhoeae*. *J Bacteriol* **192:**316–325.

161. Ottaviani D, Lecain M, Sheer D. 2014. The role of microhomology in genomic structural variation. *Trends Genet* **30:**85–94.

162. Conway C, McCulloch R, Ginger ML, Robinson NP, Browitt A, Barry JD. 2002. Ku is important for telomere maintenance, but not for differential expression of telomeric VSG genes, in African trypanosomes. *J Biol Chem* **277:**21269–21277.

163. Janzen CJ, Lander F, Dreesen O, Cross GA. 2004. Telomere length regulation and transcriptional silencing in KU80-deficient *Trypanosoma brucei*. *Nucleic Acids Res* **32:**6575–6584.

164. Gill EE, Fast NM. 2007. Stripped-down DNA repair in a highly reduced parasite. *BMC Mol Biol* **8:**24.

165. Burton P, McBride DJ, Wilkes JM, Barry JD, McCulloch R. 2007. Ku Heterodimer-Independent End Joining in *Trypanosoma brucei* Cell Extracts Relies upon Sequence Microhomology. *Eukaryot Cell* **6:**1773–1781.

166. Conway C, Proudfoot C, Burton P, Barry JD, McCulloch R. 2002. Two pathways of homologous recombination in *Trypanosoma brucei*. *Mol Microbiol* **45:**1687–1700.

167. Glover L, McCulloch R, Horn D. 2008. Sequence homology and microhomology dominate chromosomal double-strand break repair in African trypanosomes. *Nucleic Acids Res* **36:**2608–2618.

168. Glover L, Jun J, Horn D. 2011. Microhomology-mediated deletion and gene conversion in African trypanosomes. *Nucleic Acids Res* **39:**1372–1380.

169. Liveris D, Mulay V, Sandigursky S, Schwartz I. 2008. *Borrelia burgdorferi* vlsE antigenic variation is not mediated by RecA. *Infect Immun* **76:**4009–4018.

170. Dresser AR, Hardy PO, Chaconas G. 2009. Investigation of the genes involved in antigenic switching at the vlsE locus in *Borrelia burgdorferi*: an essential role for the RuvAB branch migrase. *PLoS Pathog* **5:**e1000680.

171. Lin T, Gao L, Edmondson DG, Jacobs MB, Philipp MT, Norris SJ. 2009. Central role of the Holliday junction helicase RuvAB in vlsE recombination and infectivity of *Borrelia burgdorferi*. *PLoS Pathog* **5:**e1000679.

172. Mir T, Huang SH, Kobryn K. 2013. The telomere resolvase of the Lyme disease spirochete, *Borrelia burgdorferi*, promotes DNA single-strand annealing and strand exchange. *Nucleic Acids Res* **41:**10438–10448.

173. Barry JD. 1997. The relative significance of mechanisms of antigenic variation in African trypanosomes. *Parasitol Today* **13:**212–218.

174. Barry D, McCulloch R. 2009. Molecular microbiology: a key event in survival. *Nature* **459:**172–173.

175. Keim C, Kazadi D, Rothschild G, Basu U. 2013. Regulation of AID, the B-cell genome mutator. *Genes Dev* **27:**1–17.

176. Durkin SG, Glover TW. 2007. Chromosome fragile sites. *Annu Rev Genet* **41:**169–192.

177. Ozeri-Galai E, Lebofsky R, Rahat A, Bester AC, Bensimon A, Kerem B. 2011. Failure of origin activation in response to fork stalling leads to chromosomal instability at fragile sites. *Mol Cell* **43:**122–131.

178. Klar AJ. 2007. Lessons learned from studies of fission yeast mating-type switching and silencing. *Annu Rev Genet* **41:**213–236.

179. Yakisich JS, Kapler GM. 2006. Deletion of the *Tetrahymena thermophila* rDNA replication fork barrier region disrupts macronuclear rDNA excision and creates a fragile site in the micronuclear genome. *Nucleic Acids Res* **34:**620–634.

180. Dreesen O, Li B, Cross GA. 2007. Telomere structure and function in trypanosomes: a proposal. *Nat Rev Microbiol* **5:**70–75.

181. Hovel-Miner GA, Boothroyd CE, Mugnier M, Dreesen O, Cross GA, Papavasiliou FN. 2012. Telomere Length Affects the Frequency and Mechanism of Antigenic Variation in *Trypanosoma brucei*. *PLoS Pathog* **8:**e1002900.

182. Dreesen O, Li B, Cross GA. 2005. Telomere structure and shortening in telomerase-deficient *Trypanosoma brucei*. *Nucleic Acids Res* **33:**4536–4543.

183. Dreesen O, Cross GA. 2006. Telomerase-independent stabilization of short telomeres in *Trypanosoma brucei*. *Mol Cell Biol* **26:**4911–4919.

184. Meeus PF, Brayton KA, Palmer GH, Barbet AF. 2003. Conservation of a gene conversion mechanism in two distantly related paralogues of *Anaplasma marginale*. *Mol Microbiol* **47:**633–643.

185. Giacani L, Molini BJ, Kim EY, Godornes BC, Leader BT, Tantalo LC, Centurion-Lara A, Lukehart SA. 2010. Antigenic variation in *Treponema pallidum*: TprK sequence diversity accumulates in response to immune pressure during experimental syphilis. *J Immunol* **184:**3822–3829.

186. Iverson-Cabral SL, Astete SG, Cohen CR, Totten PA. 2007. mgpB and mgpC sequence diversity in *Mycoplasma genitalium* is generated by segmental reciprocal recombination with repetitive chromosomal sequences. *Mol Microbiol* **66:**55-73.

187. Ma L, Jensen JS, Myers L, Burnett J, Welch M, Jia Q, Martin DH. 2007. *Mycoplasma genitalium*: an efficient strategy to generate genetic variation from a minimal genome. *Mol Microbiol* **66:**220–236.

188. Burgos R, Wood GE, Young L, Glass JI, Totten PA. 2012. RecA mediates MgpB and MgpC phase and antigenic variation in *Mycoplasma genitalium*, but plays a minor role in DNA repair. *Mol Microbiol* **85:**669–683.

189. Barbet AF, Myler PJ, Williams RO, McGuire TC. 1989. Shared surface epitopes among trypanosomes of the same serodeme expressing different variable surface glycoprotein genes. *Mol Biochem Parasitol* **32:**191–199.

190. Sheader K, Vaughan S, Minchin J, Hughes K, Gull K, Rudenko G. 2005. Variant surface glycoprotein RNA interference triggers a precytokinesis cell cycle arrest in African trypanosomes. *Proc Natl Acad Sci USA* **102:**8716–8721.

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01

191. Smith TK, Vasileva N, Gluenz E, Terry S, Portman N, Kramer S, Carrington M, Michaeli S, Gull K, Rudenko G. 2009. Blocking variant surface glycoprotein synthesis in Trypanosoma brucei triggers a general arrest in translation initiation. *PLoS ONE* **4:**e7532.

192. Aslam N, Turner CM. 1992. The relationship of variable antigen expression and population growth rates in *Trypanosoma brucei*. *Parasitol Res* **78:**661–664.

193. Wouters A. 2005. The function debate in philosophy. *Acta Biotheor* **53:**123–151.

194. Marcello L, Barry JD. 2007. From silent genes to noisy populations-dialogue between the genotype and phenotypes of antigenic variation. *J Eukaryot Microbiol* **54:**14–17.

195. Palmer GH, Bankhead T, Lukehart SA. 2009. 'Nothing is permanent but change'- antigenic variation in persistent bacterial pathogens. *Cell Microbiol* **11:**1697–1705.

196. Glanville J, Zhai W, Berka J, Telman D, Huerta G, Mehta GR, Ni I, Mei L, Sundar PD, Day GM, Cox D, Rajpal A, Pons J. 2009. Precise determination of the diversity of a combinatorial antibody library gives insight into the human immunoglobulin repertoire. *Proc Natl Acad Sci USA* **106:**20216–20221.

197. Ueti MW, Tan Y, Broschat SL, Castaneda Ortiz EJ, Camacho-Nuez M, Mosqueda JJ, Scoles GA, Grimes M, Brayton KA, Palmer GH. 2012. Expansion of variant diversity associated with a high prevalence of pathogen strain superinfection under conditions of natural transmission. *Infect Immun* **80:**2354–2360.

198. Zhuang Y, Futse JE, Brown WC, Brayton KA, Palmer GH. 2007. Maintenance of antibody to pathogen epitopes generated by segmental gene conversion is highly dynamic during long-term persistent infection. *Infect Immun* **75:**5185–5190.

199. Gray AR. 1965. Antigenic variation in a strain of *Trypanosoma brucei* transmitted by *Glossina morsitans* and *G. palpalis*. *J Gen Microbiol* **41:**195–214.

200. Van MN, Janssens PG, Magnus E. 1975. Antigenic variation in syringe passaged populations of *Trypanosoma (Trypanozoon). brucei*. 1. Rationalization of the experimental approach. *Ann Soc Belg Med Trop* **55:**1–23.

201. Capbern A, Giroud C, Baltz T, Mattern P. 1977. [*Trypanosoma equiperdum*: antigenic variations in experimental trypanosomiasis of rabbits]. *Exp Parasitol* **42:**6–13.

202. Morrison LJ, Majiwa P, Read AF, Barry JD. 2005. Probabilistic order in antigenic variation of *Trypanosoma brucei*. *Int J Parasitol* **35:** 961–972.

203. Barry JD, Marcello L, Morrison LJ, Read AF, Lythgoe K, Jones N, Carrington M, Blandin G, Bohme U, Caler E, Hertz-Fowler, C, Renauld H, El Sayed N, Berriman M. 2005. What the genome sequence is revealing about trypanosome antigenic variation. *Biochem Soc Trans* **33:**986–989.

204. Pays E. 1989. Pseudogenes, chimaeric genes and the timing of antigen variation in African trypanosomes. *Trends Genet* **5:**389–391.

205. Futse JE, Brayton KA, Knowles DP, Jr, Palmer GH. 2005. Structural basis for segmental gene conversion in generation of *Anaplasma marginale* outer membrane protein variants. *Mol Microbiol* **57:**212–221.

206. Coutte L, Botkin DJ, Gao L, Norris SJ. 2009. Detailed analysis of sequence changes occurring during vlsE antigenic variation in the mouse model of Borrelia burgdorferi infection. *PLoS Pathog* **5:**e1000293.

207. West SA, Griffin AS, Gardner A, Diggle SP. 2006. Social evolution theory for microorganisms. *Nat Rev Microbiol* **4:**597–607.

208. Van Dyken JD, Wade MJ. 2010. The genetic signature of conditional expression. *Genetics* **184:**557–570.

209. Oberle M, Balmer O, Brun R, Roditi I. 2010. Bottlenecks and the maintenance of minor genotypes during the life cycle of *Trypanosoma brucei*. *PLoS Pathog* **6:**e1001023.

210. Nei M, Rooney AP. 2005. Concerted and birth-and-death evolution of multigene families. *Annu Rev Genet* **39:**121–152.

211. Peacock L, Cook S, Ferris V Bailey M, Gibson W. 2012. The life cycle of *Trypanosoma (Nannomonas) congolense* in the tsetse fly. *Parasit Vectors* **5:**109.

212. Borst P, Rudenko G, Taylor MC, Blundell PA, van Leeuwen F, Bitter W, Cross M, McCulloch R. 1996. Antigenic variation in trypanosomes. *Arch Med Res* **27:**379–388.

213. Lydeard JR, Jain S, Yamaguchi M, Haber JE. 2007. Break-induced replication and telomerase-independent telomere maintenance require Pol32. *Nature* **448:**820–823.

214. Donnianni RA, Symington LS. 2013. Break-induced replication occurs by conservative DNA synthesis. *Proc Natl Acad Sci USA* **110:** 13475–13480.

215. Malkova A, Ira G. 2013. Break-induced replication: functions and molecular mechanism. *Curr Opin Genet Dev* **23:**271–279.

216. Malkova A, Signon L, Schaefer CB, Naylor ML, Theis JF, Newlon CS, Haber JE. 2001. RAD51-independent break-induced replication to repair a broken chromosome depends on a distant enhancer site. *Genes Dev* **15:**1055–1060.

217. Signon L, Malkova A, Naylor ML, Klein H, Haber JE. 2001. Genetic requirements for RAD51- and RAD54-Independent Break-Induced Replication Repair of a Chromosomal Double-Strand Break. *Mol Cell Biol* **21:**2048–2056.

218. Davis AP, Symington LS. 2004. RAD51-dependent break-induced replication in yeast. *Mol Cell Biol* **24:**2344–2351.

219. Wyatt HD, Sarbajna S, Matos J, West SC. 2013. Coordinated actions of SLX1-SLX4 and MUS81-EME1 for Holliday junction resolution in human cells. *Mol Cell* **52:**234–247.

220. Reuner B, Vassella E, Yutzy B, Boshart M. 1997. Cell density triggers slender to stumpy differentiation of *Trypanosoma brucei* bloodstream forms in culture. *Mol Biochem Parasitol* **90:** 269–280.

221. Vassella E, Reuner B, Yutzy B, Boshart M. 1997. Differentiation of African trypanosomes is controlled by a density sensing mechanism which signals cell cycle arrest via the cAMP pathway. *J Cell Sci* **110:**2661–2671.

222. Dean S, Marchetti R, Kirk K, Matthews KR. 2009. A surface transporter family conveys the trypanosome differentiation signal. *Nature* **459:**213–217.

223. Macgregor P, Matthews KR. 2012. Identification of the regulatory elements controlling the transmission stage-specific gene expression of PAD1 in Trypanosoma brucei. *Nucleic Acids Res* **40:**7705–7717.

224. Mony BM, Macgregor P, Ivens A, Rojas F, Cowton A, Young J, Horn D, Matthews K. 2014. Genome-wide dissection of the quorum sensing signalling pathway in *Trypanosoma brucei*. *Nature* **505:**681–685.

225. Matthews KR. 2011. Controlling and coordinating development in vector-transmitted parasites. *Science* **331:**1149–1153.

226. Rico E, Rojas F, Mony BM, Szoor B, Macgregor P, Matthews KR. 2013. Bloodstream form pre-adaptation to the tsetse fly in Trypanosoma brucei. *Front Cell Infect Microbiol* **3:**78.

227. Tyler KM, Higgs PG, Matthews KR, Gull K. 2001. Limitation of *Trypanosoma brucei* parasitaemia results from density-dependent parasite differentiation and parasite killing by the host immune response. *Proc Biol Sci* **268:**2235–2243.

228. Lythgoe KA, Morrison LJ, Read AF, Barry JD. 2007. Parasite-intrinsic factors can explain ordered progression of trypanosome antigenic variation. *Proc Natl Acad Sci USA* **104:**8095–8100.

229. Macgregor P, Savill NJ, Hall D, Matthews KR. 2011. Transmission stages dominate trypanosome within-host dynamics during chronic infections. *Cell Host Microbe* **9:**310–318.

230. Macgregor P, Szoor B, Savill NJ, Matthews KR. 2012. Trypanosomal immune evasion, chronicity and transmission: an elegant balancing act. *Nat Rev Microbiol* **10:**431–438.

Downloaded from www.asmscience.org by
IP: 144.32.240.1
On: Tue, 30 Aug 2016 15:16:01