We demonstrate, by gene deletion analysis, that Mre11 has a critical role in maintaining genomic integrity in Trypanosoma brucei. mre11Δ null mutant strains exhibited retarded growth but no delay or disruption of cell cycle progression. They showed also a weak hyporecombination phenotype and the accumulation of gross chromosomal rearrangements, which did not involve sequence translocation, telomere loss, or formation of new telomeres. The trypanosome mre11Δ null strains were hypersensitive to phleomycin, a mutagen causing DNA double strand breaks (DSBs) but, in contrast to mre11Δ null mutants in other organisms and T. brucei rad51Δ/Δ mutants, displayed no hypersensitivity to methyl methanesulfonate, which causes point mutations and DSBs. Mre11 therefore is important for the repair of chromosomal damage and DSBs in trypanosomes, although in this organism the intersection of repair pathways appears to differ from that in other organisms. Mre11 inactivation appears not to affect VSG gene switching during antigenic variation of a laboratory strain, which is perhaps surprising given the importance of homologous recombination during this process.

Multiple functions have been ascribed to the tripartite Mre11 complex (1), including roles in DNA repair, cell cycle control, meiosis, and telomere maintenance. The core of the complex consists of Mre11 and Rad50, both of which remain highly conserved across all kingdoms (2–5), whereas the third component, Xrs2 (Nbs1), exists only in eukaryotes and is quite divergent between species (6). It appears that Mre11 is the central protein in this complex, since it can interact with either of the other two proteins, whereas Rad50 requires Mre11 for Xrs2 binding (7).

DNA double strand breaks (DSBs) arise frequently during replication (8–10) and can be induced by ionizing radiation, mutagenic chemicals, or free radicals generated during cell metabolism (11). Additionally, DSBs occur as natural intermediates during specific processes such as mating type switching in Saccharomyces cerevisiae (12) and mammalian V(D)J recombination (13). DSB repair is mediated by two independent pathways, non-homologous end-joining and homologous recombination (HR), both of which mediate repair via the Mre11 complex in S. cerevisiae (14–16). Inactivation of the Mre11 complex results in the accumulation of DSBs in a number of organisms (2, 10, 17, 18), and these DNA lesions cause lethality in vertebrate mre11Δ/Δ mutants (10, 17–19). DSB repair defects also occur in mre11Δ−/− or rad50Δ−/− mutants, as manifest by their extreme sensitivity to ionizing radiation and chemical mutagens causing DSBs, and they are sensitive also to the mutagen methyl methanesulfonate (MMS), which causes point mutations and DSBs (2, 3, 7, 10, 18, 20–22).

The involvement of the Mre11 complex (to which we refer as the M/R complex) in DSB repair operates at several levels, including nucleolytic processing of the break, acting as a DNA damage signal sensor/transducer, and determining cell cycle checkpoints. Mre11 is involved in nucleolytic processing of DNA ends at DSBs during HR and possibly non-homologous end-joining (1–5, 23), whereas a DNA binding activity of the complex may facilitate strand exchange and end-joining reactions (1, 11, 23, 24). The M/R complex forms foci at sites of DNA damage (7, 25–28). These DNA damage detection and repair functions are essential for maintaining genomic integrity (11, 28–30), and M/R complex mutants display an increase in spontaneous gross chromosomal rearrangements (GCRs) (18, 30, 31), notably in the human cancer predisposition syndromes ataxia telangiectasia-like disorder and Nijmegen breakage syndrome. These conditions, which are initiated by mutation in the MRE11 gene (32) and the NBS1 (XRS2) gene (33), respectively, also result in hypersensitivity to ionizing radiation. The complex also plays a role in chromosome integrity at telomeres, such that deletion of any one of its three proteins results in telomere shortening in yeast (15, 34, 35). In this role, the MRE11 complex interacts epistatically with telomerase but synergistically with the Ku proteins (35, 36).

Homologous recombination is important in the survival of African trypanosomes, unicellular parasites that diverged early during eukaryotic evolution and display peculiarities in their gene expression and metabolism. Bloodstream form trypanosomes are protected by a variant surface glycoprotein (VSG) coat, which masks the underlying invariant surface antigens and inhibits the nonspecific immune mechanisms of the mammalian host. Although only one VSG species is present on the cell surface at any particular time, the parasite evades destruction by periodically switching to another VSG, using a process known as antigenic variation (for recent reviews see Refs. 37–41). Each trypanosome potentially can produce prob-
ably hundreds of distinct surface coats (42, 43). The majority of antigenic variation is driven by HR, where silent VSG genes are duplicated and transposed into a transcriptionally active telomeric locus (the bloodstream expression site (BES)). The importance of HR in this switching is shown by the central involvement of Rad51, a major HR enzyme (44).

Given the involvement of Mre11 in DNA repair and recombination, as well as in telomere biology, it is important to understand how the protein acts in trypanosome antigenic variation, a process involving recombination and transcriptional regulation conducted largely at telomeres. Here we show that mre11^+/− null mutant strains display significant growth retardation, resulting from decreased cell viability. The strains showed no change in VSG gene switching or in telomere length but did display hypersensitivity to DNA damage and developed GCRs. Our work demonstrates the significance of Mre11 in maintaining integrity of the Trypanosoma brucei genome, and the absence of a detectable role in VSG switching implies that DSBs either are not involved or are processed independently of Mre11 in this process.

**EXPERIMENTAL PROCEDURES**

**Trypanosome Strains, Transformation, and VSG Switching—Mono-morphic bloodstream form T. brucei 221a trypanosomes of the strain 3174.2 (44, 45), a derivative of strain Lister 427 (46), were grown in HMI-9 medium (47). Pleomorphic bloodstream form transformants were selected by plating 10^6 transformed cells in 36 ml of medium over a 24-well dish. VSG switching frequencies and loci were measured in the marked strain 3174.2 as described (44).

**Generation and Analysis of mre11^+/− Null Mutant Lines—The entire MRE11 ORF was substituted with cassettes for puromycin N-acetyltransferase (puromycin resistance) or blasticidin S deaminase (blasticidin resistance) encompassed by flanks specific to the MRE11 locus. These cassettes, derived from pTBT and pTPT (gifts of M. Cross and P. Borst, the Netherlands Cancer Institute), were used over two successive rounds of transformation to replace both alleles of the gene. Targeting flanks were amplified from genomic DNA by PCR using Pfu DNA polymerase (Stratagene). 5′ flank primers: 5′-ggaggttcctcgagttg-agaggtgctg-3′ (EcoRI), and 5′-ccgagacgctttaaccgtaaagg-agcggcggtagtga (HindIII/HpaI). 3′ flank primers: 5′-ccgagagctcgtttgctggcttgtgtctttctttctttctttctcttgtaaagcggcggtagtga (HindIII/NsiI, and 5′-ccgagagctcgtctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttctttcc
null mutant cells accumulate gross chromosomal rearrangements—To determine possible causes of the growth phenotype in mre11−/− cells, we studied their molecular karyotype after prolonged growth. Wild type cells and the two independent T. brucei 3174.2 MRE11+/+ and mre11−/− lines were cloned, grown in vitro for ~550 generations, and re-cloned, and genomic DNA was prepared (19 subclones in total: 3 wild type, 3 from each independent MRE11+/+ and 5 from each independent mre11−/−). Pulsed field gel (PFG) electrophoresis, utilizing conditions that resulted in a broad chromosomal separation, demonstrated the occurrence of gross chromosomal rearrangements (Fig. 4). It is apparent, even from the ethidium bromide-stained gels, that the karyotypes of the wild type (lanes 1–3) and MRE11+/+ mutant clones (lanes 4–6 and 12–14) are indistinguishable from each other. This chromosome pattern was consistent with PFG separations of DNA from 427 wild type cells (the progenitor of 3174.2) (50), indicating that no visible gross chromosomal changes had occurred during the extensive passaging period. In contrast, the subclones from the two independent mre11−/− mutant lines
displayed a high degree of chromosome rearrangement (lanes 6–11 and 15–19). Only one mre11−/− mutant subclone (lane 15) demonstrated any resemblance to the wild type karotype, whereas all others appeared to have accumulated chromosomes in the 1-Mb to 1.9-Mb range, perhaps coincident with the apparent loss of many of their larger chromosomes.

To understand how these GCRs were occurring, we determined the genomic distribution of two housekeeping genes, located 0.55 Mb apart on chromosome I. The three phosphoglycerate kinase (PGK) genes are arranged in tandem between 190,189 and 192,583 bp on the chromosome, whereas the second marker gene, glucose-6-phosphate isomerase (GPI), is unique and resides at 742,127 to 743,950 bp. Southern analysis with a PGK probe (Fig. 4) revealed two bands of constant size across the wild type and MRE11+/− lanes, representing the two alleles of the locus. This demonstrates that the two homologues of chromosome I are very different in size, at 1.8 and 3.5 Mb, as shown previously for 427 (50). In contrast, for the mre11−/− trypanosomes, at least 7 and possibly all 10 of the mutant clones exhibited significant reduction in the size of the smaller chromosome I homologue, whereas 8 of the mutant clones displayed reduction in the larger homologue; between 5 and 8 of the clones had reduction of both chromosomes. Additionally, it appeared that 3 of the mutant clones had lost one allele of the PGK locus (Fig. 4, lanes 8, 15, and 16). The one mutant clone that resembled the wild type karyotype upon ethidium bromide...
staining (lane 15) clearly displayed GCRs when probed for
PGK. With the exception of one mutant subclone, an identical
hybridization pattern was generated when the blots were
stripped and re-probed with GPI (Fig. 4), indicating that link-
age of the two markers was retained in 9 of the 10 mutant
clones. The one discrepant clone (lane 9) had lost one GPI
allele, but had retained both PGK alleles. Finally, the blots
were stripped again and reprobed with DNA specific to
VSG 221 (Fig. 4), which is the telomeric VSG in the active blood-
stream expression site located on chromosome VI (50). Hybirdiza-
tion of this haploid gene revealed a significant reduction in
length of chromosome VI in 8 of the 10 mutant clones, but in
none of the wild type or heterozygous clones.

We next investigated the effect of GCR on the VSG 121
family, which in 3174.2 cells consists of 5 genes in separate loci.
Five bands were observed in all the wild type and MRE11+/−
lanes (Fig. 5, lanes 1–9); four of these are chromosome-internal genes, of constant
size, whereas the largest is the telomeric copy, lying in an
inactive BES, and for which differences in telomere tract length
result in size variation of the fragment. Five of the nine
mre11−/− clones examined had lost one internal VSG 121 gene
copy (Fig. 5, II in lanes 16 and 17, and III in lanes 10, 12, and
13), indicating that the GCRs can result in the loss of non-
essential genetic material. Notably, the telomeric gene copy
was never lost.

In summary, it appears that the GCRs arising in mre11−/−
trypanosomes are associated with deletions within, and short-
ening of, chromosomes, and it is evident from the PFG analysis
that all of the megabase chromosomes can be affected. Interest-
estingly, the smaller intermediate and minichromosomal rep-
ertoire of the parasites appeared largely unaffected in the null
mutant lines (data not shown). In contrast to studies in S.
cerevisiae (31), there were no obvious cases of simple translo-
cations in mre11−/− cells, because there were no increases in
chromosome size.

Unlike with T. brucei KU70 or KU80 (69), mutation of
MRE11 appears not to result in shorter telomeres (Fig. 5).
Telomere length was also investigated at the VSG 221 active BES, but again no significant shortening was observed in clones derived after 115 or 550 cell generations (data not shown). Genomic DNA was also prepared from trypanosome clones that had grown in culture for 550 generations but had not been re-cloned. This displayed a telomere smear, which was longer (extending to shorter fragments) in mre11−/− cells than in MRE11+/− or wild type cells (data not shown). This observation implies that Mre11 plays a minor role in telomere regulation in T. brucei.

Effects of Mre11 Inactivation on Mutagen Sensitivity and Recombination—Previously, we have demonstrated (44) that T. brucei rad51−/− mutant clones display MMS sensitivity. Unexpectedly, we could not detect any MMS sensitivity in the mre11−/− cells. A typical dose-dependent inhibition of growth was observed in wild type, MRE11+/−, and mre11−/− cells grown in 0.0002, 0.0003, and 0.0004% MMS, but this was not more severe in mre11−/− cells (data not shown). Confirmation of this negative phenotype was achieved using a clone viability assay. One trypanosome was plated into each well of a 96-well plate at the mutagen concentrations shown. A, MMS sensitivity. The number of wells in the culture plates presenting growth after 20 generations; figures are shown as means ± S.D. of wells growing/96, from at least three independent experiments. Only rad51−/− mutants were investigated at 0.0001% MMS. B, phleomycin sensitivity. The data are means ± S.D. from two experiments each with two independent heterozygous clones and with homozygous clones derived from them.

fig. 6. Effect of mutagens on growth of MRE11 mutants. MRE11 wild type (+/+), MRE11−/−, mre11−/−, and rad51−/− trypanosomes were plated at 1 cell/well−1 in a 96-well plate at the mutagen concentrations shown. A, MMS sensitivity. The number of wells in the culture plates presenting growth after 20 generations; figures are shown as means ± S.D. of wells growing/96, from at least three independent experiments. Only rad51−/− mutants were investigated at 0.0001% MMS. B, phleomycin sensitivity. The data are means ± S.D. from two experiments each with two independent heterozygous clones and with homozygous clones derived from them.

expected increased MMS sensitivity relative to wild type (Fig. 6A). In contrast to the lack of sensitivity to MMS, the homozygous mre11−/− mutant clones were hypersensitive to phleomycin (Fig. 6B). These findings indicate a different from usual involvement of Mre11 in HR, because Mre11 absence in a number of species causes MMS hypersensitivity. To investigate this further, we characterized the effect of Mre11 absence on general HR by assaying the efficiency of transformation by plasmid DNA. For this, we used the construct tubBLEtub, which normally should integrate a bleomycin phosphotransferase cassette into the tubulin gene array using terminal sequence homology to the tubulin intergenic sequences. In the wild type and MRE11−/− lines, the transformation efficiency was ~0.95 × 10−6 (Table II), which is comparable with the efficiencies observed in similar investigations using other drug selection markers.2 The mre11−/− cells displayed a reduced tubBLEtub integration efficiency of ~0.25 × 10−6 although, considering that the viability of these cells is around 50% that of wild type trypanosomes, the actual transformation efficiency could be ~0.5 × 10−6 (52.6% wild type integration efficiency). rad51−/− mutant cells have an even poorer transformation efficiency, on average 0.12–0.15 × 10−6 but in some instances as low as 0.03 × 10−6 (12–50% of mre11−/− targeting efficiency) (44)2 (Table II). These data indicate that Mre11 plays a role in trypanosome HR but not as centrally as does Rad51.

We next determined the tubBLEtub integration sites in the

2 C. Conway, C. Proudfoot, P. Burton, J. D. Barry, and R. McCulloch, submitted for publication.
wild type, MRE11<sup>−/−</sup> and mre11<sup>−/−</sup> cells. In addition to the TUBULIN array, the tubBLEtub construct could integrate into the TUBULIN processing signals present in the transgenic BES (particular to 3174.2 cells (45)) and into the TUBULIN flanks at the deleted MRE11 locus alleles. Table III indicates that in wild type and heterozygous cells, about 75% of the integrations occurred into the tubulin gene array, and this high proportion may reflect the greater number of TUBULIN copies compared with the other targets. In contrast, the tubBLEtub construct appeared to integrate equally into all of the three available homologous loci in the null mutant cells. Similarly, rad51<sup>−/−</sup> trypanosomes demonstrate decreased targeting to the TUBULIN array,<sup>2</sup> perhaps indicating that Mre11 and Rad51 have some common influence on trypanosome HR.

Finally, we investigated whether Mre11 absence from monomorphic 427 trypanosomes affects VSG gene switching. VSG switching can be achieved by several different mechanisms, including transcriptional switching between BESs and gene duplication into the active BES, and in the 427 strain used here the switch rate occurs at background level (1 × 10<sup>−6</sup> to 1 × 10<sup>−7</sup> switch/cell/generation). The protocol used enables determination of both switching rate and activation mechanisms, and Table IV illustrates that we found no differences in VSG switching between wild type, MRE11<sup>−/−</sup> and mre11<sup>−/−</sup> lines.

**DISCUSSION**

Our findings add to the emerging picture that T. brucei generally has the recombination and repair pathways observed in other eukaryotes but with some large differences. The Rad51 pathway dominates, and the role of other pathways appears to be modified, perhaps in relation to special requirements of the trypanosome genome. The phenotype of the trypanosome mre11<sup>−/−</sup> null mutant requires interpretation in the context of the M/R complex acting in a number of interacting pathways. Prominent among the mutant phenotype traits are the formation of GCRs and the reduced growth rate. We believe these are related. The GCRs are not duplicative, because we see only decreases in chromosome size, and do not result from loss of terminal fragments, as is evident from the retention of the VSG 221 and VSG 121 telomeric genes in all clones. This is in striking contrast to GCRs in S. cerevisiae mre11<sup>−/−</sup> mutant cells, where 60% are duplicative translocations and 30% are additions of new telomeres (31). It is chromosome-internal sequence that is lost in trypanosome rearrangements, and this happens perhaps randomly, as seen by the disappearance of internal VSG 121 genes being only occasional and GPI absence arising only once. This genome is diploid, which can allow a high degree of loss of genetic material, as long as essential genes are maintained in at least one copy. Although the loss of even one complete chromosome copy would not be lethal, we believe this is not happening at a high frequency in the mre11<sup>−/−</sup> null mutant clones. A casual glance at the ethidium bromide-stained pulsed field gels might suggest a spectacular attrition, but more careful examination reveals otherwise, as is evident also from Fig. 3 where the null mutant clones do not have a noticeably reduced DNA content. The reason for GCR formation in the mutants most likely is the same as in S. cerevisiae; replication errors cause DSBs or gaps that are incorrectly repaired in the absence of a functional M/R complex. This would be consistent with the fact that GCRs do not arise as frequently in either T. brucei or S. cerevisiae rad51<sup>−/−</sup> mutants (29).<sup>2</sup> Why the types of rearrangement that arise through mre11<sup>−/−</sup> mutation are so different in the two organisms is interesting and we believe due to specific requirements for DNA repair and recombination in T. brucei (see below). We can at least exclude trivial explanations for the lack of a short telomere phenotype and the absence of translocations during GCRs in the parasite. Telomerase activity and new telomere seeding have both been detailed in T. brucei (51, 52), so there is no problem with creating new chromosome ends following their deletion. Moreover, antigenic variation involves the duplication of VSG genes between chromosomes, so translocation is not only possible in T. brucei but is central to survival. The mre11<sup>−/−</sup> growth phenotype we observed is fairly major, yielding approximately a 36% increase in population doubling time. Our cell cycle analysis has revealed no obvious alteration in progression through the cell cycle, and we believe that trypanosomes are dying as a consequence of the lethal event of deletion of any essential gene for which the other allele has already been lost. Alternatively, loss of one allele could prevent it being used as a template for the repair of a DSB at the remaining allele.

The difference in nature of GCR when compared with budding yeast may be related to our unexpected finding of lack of sensitivity of the null mutant to the DNA damage-inducing agent MMS. Usually, inactivation of Mre11 leads to increased sensitivity to MMS (3, 7, 20, 22, 53), although this is not always the case when the NBS1 component of the human M/R complex is mutated (54). Nonetheless, the sensitivity of the trypanosome null mutant to phleomycin and its altered integration patterns do show that Mre11 is involved in repair and recombination. The lack of MMS phenotype agrees with our finding that Mre11 has no detectable involvement in VSG switching, which is mediated by Rad51, the null mutant of which is MMS-sensitive (44). In other organisms, the exact role of the M/R complex in the various processes where it is featured is unclear. Although its nuclease activities are often thought to be essential for generating 3′-overhangs that initiate Rad51-mediated HR (1–5, 23), there is evidence that it plays another role, bridging the two ends in the DSB (1, 11, 23, 24, 55, 56). Our data from the mre11<sup>−/−</sup> null mutant point against the trypanosome enzyme performing resection or bridging as a prelude to Rad51-mediated HR repair of MMS damage or in VSG switching, and other activities must account for these roles. This is different from the active role trypanosome Mre11 plays in repair of DSBs during replication and in promoting the integration of linear DNA by HR. Here again, however, there are differences, because GCR formation largely is Rad51-independent, whereas most HR integration is Rad51-dependent. The similarities in relaxation in stringency of insertion target site and reduction in transformation efficiency, as observed in T. brucei mre11<sup>−/−</sup> and rad51<sup>−/−</sup> null mutants (44), suggest these proteins act in a common pathway. It may be that when the M/R complex is presented with two individual free ends on a linear molecule (57), it performs exonucleolytically, in conjunction with Rad51, rather than acting as a bridge. When Mre11 is absent, shorter 3′-overhangs are created, driving the
repair mechanism toward a Rad51-insensitive pathway that operates on substrates with shorter end homology, resulting in a wider range of targets being available, as we observed for TUBULIN sequence insertions. In the different situation of DSBs arising during replication, the wild type repair pathway would involve Mre11 but not Rad51. Here the main role of Mre11 may be as a sensor or signal of damage, perhaps using its bridging activity, and coordinating repair of the damage without RAD51, which relies upon long homology stretches (30). Such a separation of function in MRE11 between recognition/signaling and repair of damage has been suggested before (28, 58). The distinct phenotypes of mre11-/- (and rad51-/-) mutants to the three situations (MMS-induced damage, replication defects, and recombination of free ends) is suggestive of differences between the trypanosome and other organisms.

Even though it is in direct contrast with what happens in S. cerevisiae (15, 34, 35, 59), the absence of a telomere phenotype in trypanosomes mre11-/- null mutant cells is not completely unexpected, in the sense that telomere maintenance and recombinational events catalyzed by Mre11 differ in some ways. Trypanosomes differ from the fungus in the structure of the chromosome end, having a t-loop (60), so it is not necessarily the case that the parasite uses the same proteins, such as Mre11, for telomere maintenance. We have shown that Ku is important for telomere maintenance in trypanosomes (69), and there may not be a need for Mre11. It is not yet known whether, in any organism, Mre11 is important for t-loop formation.

The lack of involvement of Mre11 in VSG switching might be indicative of a greater than usual demarcation between DNA repair and recombination pathways in T. brucei. VSG gene switching, most of which is driven by recombination at a high rate, involves a flow of VSG duplications toward telomeres. In fact, it appears that most, if not all, the telomeres of the megabase trypanosome chromosomes have been set aside to harbor VSG transcription units (61). For proteins such as the M/R complex and Ku, clear functional distinctions between their multifarious roles in telomere function and in recombination might be necessary. It is interesting, therefore, that we have found that neither the M/R complex nor Ku (69) has a detectable role in VSG switching, at least in the background-switching strain examined. This is in contrast to the important role played by Rad51 (44). Perhaps a novel pathway of recombination, or a modification of a general damage repair pathway, has evolved to enable high frequency switching. Indeed, modification of general pathways of recombination-based repair may explain the limitation of individual GCRs to the same chromosome in mre11-/- mutants and the lack of a role for Mre11 in responding to MMS damage. A second genomic feature in the trypanosome that may relate to this proposed demarcation between repair and recombination is the abundance of repetitive sequence, estimated at 32% (62). Much of this is the satellite sequence that constitutes most of the structure of the ~100 nuclear minichromosomes (63, 64), whereas other highly repetitive regions flank VSG genes (65, 66) and bloodstream VSG transcription units (61). The latter two repeat sequences are thought to have important roles in gene rearrangements and as insulators, respectively. The potential importance of the minichromosome repeats is seen in the fact that they are remarkably stable in size, despite the repeats being identical in sequence over most of their length (64). Even though Mre11 is thought to help in the removal of secondary structures arising in some types of repeat, thus preventing replication errors (67, 68), we find no such problems arise in trypanosome minichromosomes when Mre11 is absent (data not shown). It would not be surprising to find that maintenance of such important repeated regions during trypanosome replication depends on, besides a robust mismatch repair system, a strict demarcation between the different repair and recombination processes.

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