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DNA Repair Pathways in Trypanosomatids: from DNA Repair to Drug Resistance

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SUMMARY

All living organisms are continuously faced with endogenous or exogenous stress conditions affecting genome stability. DNA repair pathways act as a defense mechanism, which is essential to maintain DNA integrity. There is much to learn about the regulation and functions of these mechanisms, not only in human cells but also equally in divergent organisms. In trypanosomatids, DNA repair pathways protect the genome against mutations but also act as an adaptive mechanism to promote drug resistance. In this review, we scrutinize the molecular mechanisms and DNA repair pathways which are conserved in trypanosomatids. The recent advances made by the genome consortiums reveal the complete genomic sequences of several pathogens. Therefore, using bioinformatics and genomic sequences, we analyze the conservation of DNA repair proteins and their key protein motifs in trypanosomatids. We thus present a comprehensive view of DNA repair processes in trypanosomatids at the crossroads of DNA repair and drug resistance.

INTRODUCTION

Preserving genome integrity is crucial for adequate eukaryotic cellular homeostasis and development. During the cell cycle, it is essential to repair DNA damage properly to ensure accurate transfer of DNA integrity to daughter cells and prevent chromosomal rearrangements. This is an important challenge considering that each day, a eukaryotic cell can struggle with thousands of DNA lesions imposed by endogenous and exogenous agents (1). DNA break detection, checkpoint arrest, and DNA damage repair rely on a variety of proteins implicated in a complex DNA caretaking network. The set of proteins involved in DNA repair is well studied in humans and model organisms, with several excellent recent reviews (2–4). However, our understanding of DNA repair in human parasites is lagging behind, although important progress has been made recently and warrants this review. We present here a comprehensive view of the function of nuclear DNA repair proteins conserved through evolution, with an emphasis on the proteins found in human-pathogenic parasites belonging to the

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kinetoplastid family and with special interest on the parasite *Leishmania*.

The kinetoplastid parasites diverged early in the eukaryotic branch of life, and several of their members are responsible for some of the great scourges of humanity, including sleeping sickness (caused by *Trypanosoma brucei*), Chagas disease (caused by *Trypanosoma cruzi*), and leishmaniasis (caused by *Leishmania* spp.). There is no effective vaccine for the prevention of these parasitic diseases, and their control relies on chemotherapy. A few drugs are in clinical use against human cases of leishmaniasis (pentavalent antimonials, amphotericin B, miltefosine, pentamidine, and paromomycin), sleeping sickness (suramin, eflornithine, pentamidine, melarsoprol, and nifurtimox), and Chagas disease (nifurtimox and benznidazole). The arsenal of available drugs is thus limited, with most compounds being compromised by toxicity, cost, or resistance. Even worse, the mode of action and targets of these drugs are not known despite their use for several decades, with the exception of amphotericin B and eflornithine, which target ergosterol-containing membranes and ornithine decarboxylase, respectively (5).

Because of their medical and veterinary importance, this class of parasites has been intensively studied, leading to a novel basic concept. These organisms contain a unique mitochondrion with a complex network of interlocked DNA maxi- and minicircles constituting the kinetoplast DNA (kDNA). Studies on replication mechanisms of this complex kDNA network have been recently reviewed (6). RNA editing was first described within the mitochondria of kinetoplastid parasites (7, 8), where minicircle-encoded guide RNAs edit maxicircle-encoded transcripts by the insertion/deletion of uridine nucleotides catalyzed by a cellular machinery called the editosome (9). In addition to kDNA and RNA editing, studies of these parasites have led to many other groundbreaking discoveries, such as glycosylphosphatidylinositol (GPI)-anchored proteins (10–12), *trans*-splicing (13–15), polycistronic transcription (16, 17), and the Th1/Th2 polarization in immunology (18, 19), to name a few. Moreover, the regulation of gene expression in these early-diverging eukaryotes displays some unique features, including a lack of transcriptional control at the level of initiation.

The complete genomic sequences of *Leishmania major* (20), *Trypanosoma brucei* (21), and *Trypanosoma cruzi* (22), known as the tritryps genomes, became available in 2005. In these landmark studies, DNA repair, DNA recombination, and DNA replication machineries were analyzed (23). Many homologs of the components of the different DNA repair pathways and recombination enzymes were present, with some noticeable absent proteins, such as RAD52 and some components of the nonhomologous end-joining machinery (23). Recombination, repair, and replication enzymes of *T. brucei* were revisited (24), and more recently DNA repair enzymes in the tritryps were reviewed, adding experimental evidence pertaining to the repair enzymes and focusing on *T. cruzi* (25). Since repair and recombination in *Leishmania* were less emphasized, we discuss this here in greater detail while making connections with recent findings for both *Leishmania* and other kinetoplastids. The advent of next-generation sequencing has allowed the sequencing of several additional *Leishmania* species, including *L. infantum* and *L. braziliensis* (26). These sequences were useful when looking at the presence of DNA repair and recombination enzymes.

Intriguingly, some antitrypanosome drugs (e.g., pentamidine) may act in part by binding to kDNA (27), and several drugs directed against *Leishmania* produce reactive oxygen species (ROS) (28) that may lead to DNA damage. Both *Leishmania* and *T. cruzi* have intracellular life stages and are also likely to encounter reactive oxygen species, produced by the macrophage, which can induce DNA damages. DNA repair is a key to several biological features pertaining to kinetoplastid parasites. *T. brucei* evades the immune system by changing its protective variant surface glycoprotein (VSG) coat by antigenic variation. This process occurs close to telomeres and can be promoted by the presence of double-strand breaks (DSBs) in DNA (29, 30). *Leishmania* is distinguished from the *Trypanosoma* spp. by its extreme genome plasticity. The copy number of its chromosome may vary either in wild-type (WT) cells or in drug-resistant mutants (31–34), and the ploidy of specific chromosomes of individual cells may differ within a population, a concept known as mosaic aneuploidy (35). *Leishmania* also amplifies specific portions of its genome by gene rearrangements at the level of direct or inverted repeated sequences, leading to small extrachromosomal circular or linear amplicons (32, 34, 36, 37). Recently, we found that repeated sequences are widespread in the *Leishmania* genome and that there is constitutive amplification at the level of these repeated sequences (J. M. Ubeda et al., submitted for publication). This amplification is adaptive and can be selected with a number of chemotherapeutic drugs, and it involves DNA repair/recombination enzymes (Ubeda et al., submitted; M.-C. N. Laffitte et al., unpublished observations). The constitutive stochastic gene rearrangements in *Leishmania* and the programmed gene rearrangements in *T. brucei* are likely to require active DNA repair machineries. Similarly, drugs and an intracellular life style in oxidative environments for *Leishmania* or *T. cruzi* are likely to induce DNA damage that needs to be repaired efficiently. A better understanding of DNA repair mechanisms in parasites could have considerable impact on the development of future therapeutic strategies.

The variety of DNA damage that continually challenges the integrity of the genetic material has led to the emergence of diverse DNA repair pathways to mediate efficient repair (Fig. 1 shows an outline of the DNA damage types and the associated DNA repair pathways). In this review, we focus on machineries present in tritryps that are involved in repair of spontaneous DNA lesions arising during physiological processes, such as incorrect deoxynucleoside triphosphates (dNTPs) introduced during DNA replication (resolved by mismatch repair [MMR]), base modifications caused by deamination, depurination, or alkylation (fixed by base excision repair [BER]), oxidized DNA bases resulting from exposure to reactive oxygen species (ROS), and DNA double-strand breaks. In addition, environmental threats such as sunlight and ionizing radiation (IR) disrupt also the integrity of the DNA backbone. UV light creates helix-distorting lesions via pyrimidine dimers and 6-4 photoproducts (counteracted by nucleotide excision repair [NER]) whereas IR induces oxidation of DNA bases, single-strand breaks (SSBs), and double-strand breaks (repaired by homologous recombination [HR] and nonhomologous end joining [NHEJ]). Using both the human and the yeast DNA damage proteomes with, respectively, 129 and 84 proteins, we performed a top-five reciprocal best BLAST hit bioinformatics approach to systematically retrieve the orthologous DNA damage proteomes of kinetoplastid parasites (see Fig. S1 and S2 and text in the supplemental material). Our results (also available at http://www.crc.ulaval.ca/trypdnarepair/) complement previous analy-

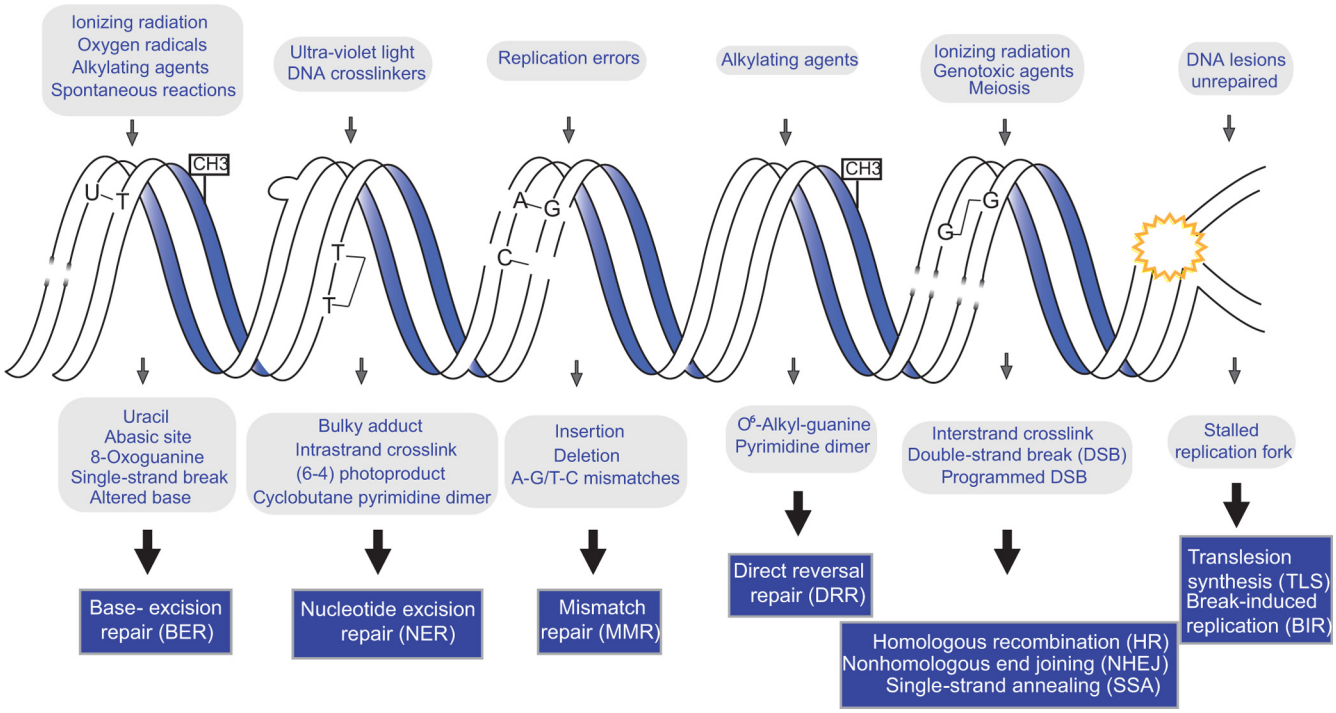

FIG 1 DNA damage and the associated DNA repair pathways. The DNA backbone can be attacked by several endogenous or exogenous agents (for instance, ionizing radiation, alkylating agents, or oxygen radicals), leading to the activation of DNA repair enzymes. (Modified from reference 292 by permission from Macmillan Publishers Ltd., copyright 2001.)

ses (24, 25) and will serve as the backbone for the current review. The relevance of DNA repair to the development of drug resistance and its potential as a drug target are also discussed.

BASE EXCISION REPAIR

Base excision repair processes damaged bases produced either spontaneously or from attack of bases by reactive oxygen species. Damaged bases are first recognized and excised by DNA glycosylases, leading to abasic sites, also known as apurinic/apyrimidinic (AP) sites (Fig. 2; see Fig. S3 in the supplemental material). Two classes of DNA glycosylases exist: monofunctional and bifunctional (Fig. 2A). Monofunctional glycosylases have only a glycosylase activity, while bifunctional glycosylases possess an additional AP lyase activity, which can convert a base lesion into a single-strand break without the need for an AP endonuclease. Next, DNA polymerases are recruited at the nick for DNA synthesis, and the DNA strand is finally sealed by DNA ligase (Fig. 2; see Fig. S3 in the supplemental material).

DNA Glycosylases and AP Endonucleases

AP endonucleases catalyze the hydrolytic cleavage of the phosphodiester bond 5′ to the AP site. If left unrepaired, AP sites block DNA replication and have both mutagenic and cytotoxic effects. There are two structurally unrelated families of AP endonucleases based on their homology to the ancestral bacterial AP endonucleases, endonuclease IV and exonuclease III. AP endonuclease 1 (APN1) is the primary type in budding yeast and is homologous to *Escherichia coli* endonuclease IV, while APN2 is related to exonuclease III. In 1999, Perez et al. provided the first report on BER enzymes by identifying AP endonuclease in *L. major* and *T. cruzi* (38). The *L. major* apurinic/apyrimidinic endonuclease (*Lm*AP) belongs to the APE1/ExoIII family (Table 1). The catalytic properties and crystal structure of *Lm*AP were reported and compared with those of human APE1 and bacterial exonuclease III (39). The analysis of *Lm*Ape1 kinetic parameters for the removal of the EndoIII AP lyase reaction product revealed that the protein possesses a 3′-phosphodiesterase activity equally robust as an AP endonuclease. These results suggest an important role for *Lm*AP in the processing of oxidative damage, providing a 3′-OH primer for repair DNA synthesis. In support of this, overexpression of *Lm*AP exerts a protective effect in the parasite against hydrogen peroxide and the antifolate drug methotrexate (MTX) (40). Methotrexate inhibits dihydrofolate reductase to produce an increase in the intracellular levels of dUTP, allowing the incorporation of uracil into DNA (40). In order to identify the residues specifically involved in the repair of oxidative DNA damage, Vidal et al. (39) generated random mutations in *Lm*AP and selected variants that conferred resistance to hydrogen peroxide. Unlike that of the wild-type protein, expression of mutant *Lm*APE^{A138D}, which has reduced 3′-phosphodiesterase activity, sensitizes the cells treated with hydrogen peroxide (41). The A138 residue corresponds to the D70 residue within the nuclease domain of human APE1. In contrast to the case for *Lm*AP, mutation of human APE1 (D70A) leads to an increased capacity to remove 3′-blocking ends *in vitro*, which reflect a divergent molecular evolution in response to oxidative damage.

Uracil metabolism is of special interest in parasite studies, since it has been reported that the modified base β-D-glucosyl-hydroxymethyluracil is a normal constituent of DNA in kinetoplastids (42). More precisely, thymine is hydroxylated and glucosylated to yield base J (β-D-glucosyl-hydroxymethyluracil).

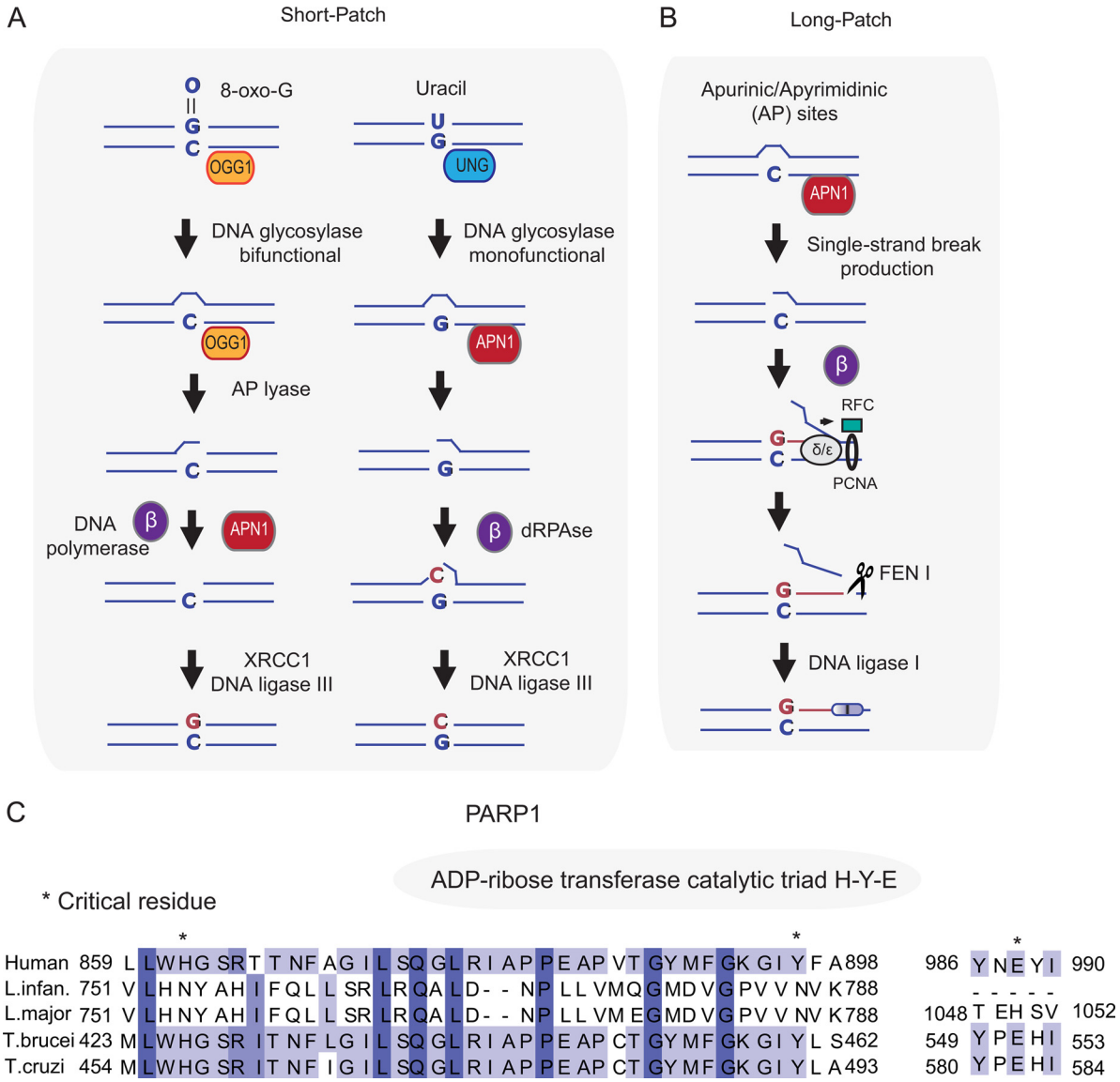

FIG 2 (A) Short-patch base excision repair pathway. This pathway involves the removal of damaged bases and leads to a repair track of a single nucleotide. (B) The long-patch base excision repair pathway produces a repair track of at least two nucleotides. (C) Alignment of PARP1 catalytic domains in human, *Leishmania infantum*, *Leishmania major*, *Trypanosoma brucei*, and *Trypanosoma cruzi*. The ADP-ribose transferase catalytic triad H-Y-E is highlighted.

Approximately 1% of thymine is replaced by base J, which is present mostly in repetitive DNA, such as telomeric repeats. Base J regulates gene expression, as its loss leads to a readthrough of normal RNA polymerase II transcription termination sites in *Leishmania* (43). Using a specific ethidium bromide fluorescence assay, recombinant *T. cruzi* uracil DNA glycosylase (*Tc*UNG) was shown to specifically excise uracil from DNA. In addition, the activity was stimulated in the presence of AP endonuclease (44), similar to what was observed with the human enzymes (45). Moreover, a functional role for the enzyme was confirmed, since the expression of *Tc*UNG in an *E. coli ung* mutant restored the WT phenotype (46). In *T. brucei*, *ung*-null mutant cell extracts did not perform excision of uracil in DNA, revealing the absence of a backup excision pathway when the specific glycosylase is not active (47). This enzyme escaped our bioinformatic analysis.

Different Classes of BER Enzymes

We found that around 65% of the human/yeast base excision repair pathway is conserved in *Leishmania* species (see Fig. S1B and C in the supplemental material). Two BER subpathways have been classified according to the length of the repair patch as either short-patch BER (SP-BER) (one nucleotide) or long-patch BER (LP-BER) (more than one nucleotide) (Fig. 2A and B). It has been shown by using a covalently closed circular DNA (cccDNA) substrate containing a uracil that only SP-BER occurs in *T. cruzi* cell extracts (46). In the LP-BER pathway, the flap endonuclease 1 (FEN-1) cleaves within the apurinic/apyrimidinic (AP) site-terminated flap (48). In 1997, Shen and colleagues found that mutations in seven conserved aspartic and glutamic acid residues in human FEN-1 (D34, D86, E158, E160, D179, D181, and D233)

TABLE 1 Trypanosomatid genes involved in base excision repair

Gene product designation (alternate designation)	Protein; function	Gene ID[a]						
		Human	*S. cerevisiae*	*S. pombe*	*L. infantum*	*L. major*	*T. brucei*	*T. cruzi*
UNG	Uracil DNA *N*-glycosylase; removes uracil	NM_003362	YML021C	SPCC1183.06 (B)	LinJ.18.0480 (B)	LmjF.18.0480 (B)	Tb927.10.13970 (B)	X
UDG2	Uracil DNA glycosylase 2; removes uracil	NM_080911	YML021C	SPCC1183.06 (H)	LinJ.18.0480 (H)	LmjF.18.0480 (H)	Tb927.10.13970 (H)	X
SMUG1	Single-strand-selective monofunctional uracil-DNA glycosylase 1	NM_014311	X	X	X	X	X	X
OGG1	8-Oxo-guanine glycosylase 1; 8-oxoG paired with C, T, G	NM_016821	YML060W	SPAPB24D3.04c (Y)	LinJ.34.1930 (B)	LmjF.34.2170 (B)	Tb927.4.2480 (B)	Tc00.1047053510229.20 (B)
TDG	Thymine DNA glycosylase; U, T, or ethenoC opposite G	NM_003211	X	SPCC965.05c (H)	X	X	X	X
MBD4	Methyl-CpG-binding domain 4 DNA glycosylase; U or T opposite G	NM_003925	X	X	X	X	X	X
MYH	Mut Y homolog DNA glycosylase; A opposite 8-oxoG	NM_012222	X	SPAC26A3.02 (H)	LinJ.28.2290 (H)	LmjF.28.2140 (H)	Tb11.01.3270 (H)	Tc00.1047053511803.20 (H)
NTH1	Endonuclease three homolog 1 DNA glycosylase; ring-saturated, oxidized, and fragmented pyrimidines	NM_002528	YAL015C	SPAC30D11.07 (B)	LinJ.09.0070 (B)	LmjF.09.0050 (B)	Tb11.01.3910 (B)	Tc00.1047053504005.10 (B)
MPG	Methyl purine DNA glycosylase; 3-MeA, 7-MeG, 3-MeG, ethenoA, hypoxanthine	NM_002434	X	X	X	X	X	X
NEIL1	Nei-like DNA glycosylase1; removes thymine glycol	NM_024608	X	X	X	X	X	X
NEIL2	Nei-like DNA glycosylase 2; removes oxidative products of pyrimidines	NM_145043	X	X	X	X	X	X
NEIL3	Nei-like DNA glycosylase 3; removes oxidative products of pyrimidines	NM_018248	YBL019W	X	X	X	X	Tc00.1047053510347.50 (H)
APE1 (APEX1, Ref-1, HAP1)	Apurinic/apyrimidinic endonuclease 1; cleavage of phosphodiester bond at 5′ side of AP site	NM_001641	YKL114C	SPBC3D6.10 (H)	LinJ.16.0680 (H)	LmjF.16.0680 (H)	Tb927.8.5510 (H)	Tc00.1047053507083.30 (H)
APE2 (APEX2)	Apurinic/apyrimidinic endonuclease 2	NM_014481	YBL019W	SPBC3D6.10 (B)	LinJ.16.0680 (B)	LmjF.16.0680 (B)	Tb927.8.5510 (B)	Tc00.1047053507083.30 (B)
LIG I	DNA ligase involved mainly in long-patch BER	NM_000234	YDL164C	SPAC20G8.01(B)	LinJ.30.3490 (B)	LmjF.30.3440 (B)	Tb927.6.4780 (B)	Tc00.1047053506945.80 (B)
LIG III	DNA ligase involved in only short-patch BER	NM_013975	X	SPAC20G8.01 (H)	LinJ.30.3490 (H)	LmjF.30.344 0(H)	Tb927.6.4780 (H)	Tc00.1047053506945.80 (H)
Polβ	DNA polymerase involved in long and short-patch BER	NM_002690	YCR014C	SPAC2F7.06c (B)	LinJ.08.0830 (B)	LmjF.08.0890 (B)	Tb927.5.2780 (H), Tb927.5.2790 (Y)	Tc00.1047053503955.20 (B)
Polε	DNA polymerase involved in long-patch BER	NM_006231	YNL262W	SPBC25H2.13c (B)	LinJ.35.4430 (B)	LmjF.35.4360 (B)	Tb09.211.1820 (B)	Tc00.1047053506147.180 (B)
Polδ	DNA polymerase involved in long-patch BER	NM_002691	YDL102W	SPBC336.04 (B)	LinJ.33.1790 (B)	LmjF.33.1690 (B)	Tb927.2.1800 (B)	Tc00.1047053510259.6 (B)
XRCC1	X-ray repair cross-complementing protein 1; DNA ligase 3 factor	NM_006297	X	SPAC23C4.18c (H)	X	X	X	X
PCNA	Proliferating cell nuclear antigen; trimeric circular DNA polymerase processivity factor that acts as sliding clamp for Polδ and Polε	NM_182649	YBR088C	SPBC16D10.09 (B)	LinJ.15.1500 (B)	LmjF.15.1450 (B)	Tb09.160.3710 (B)	Tc00.1047053508277.150 (B)
RF-C	Strand displacement and DNA synthesis (clamp loader)	NM_002913	YOR217W	SPBC23E6.07c (B)	LinJ.24.1010 (B)	LmjF.24.0990 (B)	Tb11.02.3360 (B)	Tc00.1047053508647.40 (B)
FEN1 (DNase IV)	Flap endonuclease 1; removes 5′ overhanging flap structure and 5′-3′ exonuclease	NM_004111	YKL113C	SPAC3G6.06c (B)	LinJ.27.0260 (B)	LmjF.27.0250 (B)	Tb927.3.830 (B)	Tc00.1047053511867.110 (B)

[a] H, found from human homolog only; Y, found from yeast homolog only; B, found from both human and yeast homologs; X, no homolog.

resulted in the complete loss of flap endonuclease activity (49). Importantly, all these residues are conserved in *Leishmania braziliensis* and *Leishmania infantum*, suggesting that *Leishmania* may possess the LP-BER pathway (Table 1). Using a cleverly designed LP-BER assay *in vivo*, Sattler et al. in 2003 showed that following the excision of 8-oxo-7,8-dihydroguanine (8-oxoG) or incision at an AP site, a significant proportion of the damage is processed by LP-BER (50).

It is estimated that the steady-state level of 8-oxoG in human cells is about 10^3 per day (51). Complete sequencing of the *T. cruzi* genome revealed the presence of a putative 8-oxoguanine DNA glycosylase gene (22). *Tc*OGG1 bears a helix-hairpin-helix (HhH) domain followed by a Gly/Pro-rich loop and a conserved aspartic acid (HhH-G/PD motif). These protein domains/motifs are the hallmark of the BER HhH-G/PD protein superfamily, containing essential amino acids for catalysis and substrate recognition. The expression of *TcOGG1* complemented an Ogg1-defective *Saccharomyces cerevisiae* strain when assayed for spontaneous mutation frequency. Moreover, *Tc*OGG1 reduced the levels of 8-oxoG in the nucleus and in the mitochondrion of *T. cruzi* (52).

DNA polymerase beta (Pol β), a member of family X of DNA polymerases, participates in several DNA transactions *in vivo*, e.g., DNA replication, recombination, and BER. In the last stage of BER, DNA synthesis is required. To carry out this process, Pol β requires, in addition to the polymerization domain, an 8-kDa N-terminal domain able to excise the 5′-terminal deoxyribose phosphate (dRP) residue from an incised abasic site by a β-elimination mechanism. Pol β contains subdomains involved in DNA synthesis (namely, fingers, palm, and thumb) and dRP lyase activity (8-kDa subdomain). *Leishmania infantum* Pol β (*Li*Pol β) consequently has intrinsic DNA polymerase activity (53). This was confirmed using *Li*Pol β purified and refolded from *E. coli* inclusion bodies (54). The enzyme is a DNA-dependent DNA polymerase, with an intrinsic dRP lyase activity, most likely proceeding through a β-elimination mechanism. In addition, *Li*Pol β showed a nuclear localization like that of human Pol β. The activity of *Li*Pol β varies with the parasite life cycle, being maximal in the intracellular amastigote stage. The intracellular amastigote resides inside the phagolysosome, suffering the onslaught of a cell that generates huge amounts of endogenous oxidative damage (superoxide anion, H_2O_2, and NO). Consequently, repair of DNA damage is essential for the continued survival of the organism. Therefore, the maximal activity of *Li*Pol β detected inside the macrophage is consistent with a role in BER. While it is normally assumed that Pol β is a nuclear enzyme, *T. brucei* and *T. cruzi* have distinct mitochondrial DNA polymerases β. The mitochondrial DNA of trypanosomes is a catenated network of minicircles and maxicircles named kinetoplast DNA. *Trypanosoma* Pol β proteins may have distinct and nonredundant roles in kDNA replication or maintenance (55, 56).

Kinetoplastid protozoa of the genera *Leishmania* and *Trypanosoma* are sensitive to oxidative stress, while enduring high levels of reactive oxygen species coming mainly from the host defenses. Paradoxically, during their life cycle, *L. major* cells invade the host macrophages and survive in a highly oxidative intracellular environment. Found exclusively in Trypanosomatidae, the presence of a molecule consisting of two glutathiones joined by a spermidine, named trypanothione, helps the parasite to survive against oxidative stress (57). The increase of antioxidant mechanisms in macrophages has been often explained by the presence of the oxidative burst produced by the host, but recent results demonstrated that ROS production is also linked with differentiation of the promastigote to an infective amastigote in *Leishmania amazonensis*. When exposed to H_2O_2, the parasite triggers this key transition in a process regulated by iron availability, independently of temperature and pH changes (58). Further experimental analyses need to be performed to evaluate properly the functional role of the BER pathway for parasite survival to target potential BER enzymes as antiparasitic drugs.

Poly(ADP-Ribose) Polymerase

Poly(ADP-ribose) polymerases (PARPs) constitute a large family of at least 17 protein members in humans. PARP enzymes are involved in several distinct cellular processes such as signaling mechanisms in chromatin modification, transcription, DNA damage signaling and repair, cell death, and metabolism. Poly(ADP-ribose) polymerase catalyzes the transfer of an ADP-ribose moiety from NAD^+ to a glutamate, an aspartate, or a carboxy-terminal lysine residue of target proteins. In DNA damage signaling, the automodification of PARP leads to poly(ADP-ribose) polymers (PAR), which recruit several DNA repair proteins (59, 60). Poly(ADP)-ribosylation can also regulate the activity of proteins. *T. cruzi* possesses only one ortholog of human poly(ADP-ribose) polymerases. It is 47% homologous to PARP-1, 45% to PARP-2, 32%, to PARP-3, and 33% to vPARP (61). In addition, it is 65% homologous with *T. brucei* PARP. The WGR domain, defined by the conserved Trp, Gly, and Arg residues, is conserved (62). The catalytic domain structure at the C terminus of the protein is also conserved in *L. infantum*, *L. major*, *T. brucei*, and *T. cruzi* (Fig. 2C; see Fig. S3 and Table S3 in the supplemental material). Notably, the H-Y-E triad, which constitutes the three essential amino acids required to produce an active PARP capable of poly(ADP-ribose) synthesis, is conserved in *T. brucei* and *T. cruzi* (63). The *T. cruzi* PARP (*Tc*PARP) poly(ADP-ribose) synthesis is activated by DNA strand breaks *in vitro* but also *in vivo*, as observed by the accumulation of PAR in the nuclei of *T. cruzi* epimastigotes. The activity is also inhibited by 3-aminobenzamide (61) but also by next-generation inhibitors such as olaparib (64). PARP activity is normally counterbalanced by a glycohydrolase activity, which hydrolyzes the polymers present on PARP, thereby allowing a new cycle of automodification. Biochemical analyses revealed that *T. cruzi* extracts possess an activity that degrades poly(ADP-ribose) similarly to poly(ADP-ribose) glycohydrolase (PARG) (65; Jean-Phillipe Gagné, Guy Poirier, and Sylvia Villamil, personal communication).

MISMATCH REPAIR

To permit accurate transmission of the genetic information, cells use replicative DNA polymerases, which harbor proofreading activity to replicate faithfully their genetic information and prevent mutation. Remarkably, the basal level of mutagenesis is 1 in 10^9 to 10^{10} base pairs per cell division (66). Likewise, to increase fidelity of DNA replication (up 50- to 1,000-fold), a postreplicative pathway termed DNA mismatch repair (MMR), is responsible to correct errors introduced during DNA synthesis (67). This process targets replication mistakes, such as base-base mismatches and insertion-deletion loops (IDLs) from heteroduplex molecules with microsatellite instability (MSI). In addition, it participates in homologous recombination to prevent strand exchange between nonhomologous sequences and in repairing DNA damage from

endogenous, physical, and chemical insults (for reviews, see references 66, 68, 69, and 70). Understandably, the importance of MMR is clearly illustrated in cells lacking the MMR machinery, which exhibit a mutator phenotype due to MSI.

Widely distributed throughout the genome, microsatellites are repeated sequences of $(A)_n$ or $(CA)_n$ motifs which can lead to strand slippage and produce one or more unpaired bases (71). This "replication error signature" is made by many slipped mismatches while it increases the mutation rate. Microsatellites are also present in parasites, where multilocus microsatellite typing has been used for an extensive population survey of New World *L. infantum* strains originating mainly from different regions of endemicity within Brazil but also from other countries (Paraguay, Colombia, Venezuela, Panama, Costa Rica, and Honduras) (72).

As a highly conserved pathway, as highlighted by the bioinformatics identification of all of the human/yeast mismatch repair proteome (Table 2; see Fig. S1B and C and S5 in the supplemental material), MMR operates in three steps: recognition, excision, and DNA synthesis. Because of evolutionary conservation, most of our insights on human MMR arise from yeast and *E. coli* studies (69, 73). Notably, in 1996, Blundell et al. demonstrated perfect DNA homology integration by transformation experiments and speculated on the existence of a MMR system in *T. brucei* that prevents recombination between divergent DNA sequences (74). Ten years later, Papadopoulou and Dumas presented the same evidence in *Leishmania* (75). In 2001 and 2003, the first characterizations of the key mismatch repair player MSH2 in *T. cruzi* and *T. brucei*, respectively, were reported (76, 77). Then, by interfering with the MMR mechanism, the generation of *Leishmania* hybrid species was published in 2012, showing integration of DNA as large as 45 kb (78).

DNA Mismatch Recognition

Mismatch recognition from the nascent strand is required to enable targeted incision (Fig. 3A). In *E. coli*, MutS and MutL homodimers are in charge of the initiation step, although human homologs are heterodimers. In fact, eukaryotes encode MutS homologs, MSH2, MSH3, and MSH6, depending on the nature of the substrate to repair (reviewed in reference 79). The heterodimer hMutSα (MSH2-MSH6) binds single base-base and 1- or 2-base IDL mismatches, while the redundant complex hMutSβ (MSH2-MSH3) preferentially recognizes larger IDL mismatches containing up to 16 extra nucleotides (Fig. 3B) (80). Both complexes carry the Walker ATP-binding motif, which controls their activity after the initial contact with DNA (81, 82). MutS then recruits another ATPase, MutL, to form a ternary complex. Four MutL homologs were identified in mammalian cells: MLH1, MLH3, PMS1 (postmeiotic segregation protein 1), and PMS2. These are regrouped in three distinct heterodimers (MutLα-β-γ). The most important heterodimer is MutLα (formed by MLH1 and PMS2), which provides endonuclease activity, while hMutLγ (formed by MLH1 and MLH3) is involved in meiotic recombination and MutLβ (MLH1 and PMS1) has a hitherto-unknown function related to MMR (71).

Interestingly, we noticed that almost all the MMR machinery components are conserved in trypanosomatids (Table 2; see Fig. S1B and C and S5 in the supplemental material). Based on three specific domains involved in base mismatch correction, three MutS-like proteins have been identified in *T. brucei*: MSH2, MSH3, and MSH8 (eukaryotic MSH6) (83). These domains are located in the N terminus (a mismatch-interacting domain), in the middle (a DNA-binding domain), and in the C terminus (an ATPase domain belonging to the ABC ATPase superfamily-included helix-turn-helix motif involved in dimerization) (82, 84, 85). As identified by Obmolova et al. in the crystallographic structure of *Thermus aquaticus* MutS, the binding to mispaired base required four residues, both Phe39 and Glu41 for direct interaction and Gln97 and Arg110 to anchor protein on DNA (82). Consistent with the fact that the eukaryotic MSH2 protein does not interact directly with DNA mismatches, all four residues are absent from the *T. brucei* MSH2 (*Tb*MSH2) sequence. However, *Tb*MSH8 contains all four residues, and *Tb*MSH3 retains only Gln97 and Arg110, which evolved to recognize small and longer IDLs, respectively (82). The presence of the MSH2 gene in *T. cruzi* and *Leishmania* was also reported (76, 78). Furthermore, two members of the MutL-related proteins have been annotated in the *T. brucei* genome according to two reported conserved domains (MLH1 and PMS1) (83). In fact, an ATPase domain in the N terminus and a C-terminal domain required for dimerization are conserved in both proteins. A C-terminal motif termed the "carboxy-terminal homology motif," with an unknown function, is only found in the MLH1 homolog.

Functions of MMR Genes in Tritryps

MMR also participates in the response to genotoxic agents and oxidative lesions. To better understand the role of MMR in kinetoplastids, studies were focused on MSH2 and MLH1, two crucial proteins of the recognition process. Foremost, *T. cruzi* MSH2 was the first MMR gene product investigated in tritryps by negative-dominance phenotype analyses in *E. coli*, where it interfered with the prokaryotic mismatch system (76). The existence of three isoforms of *Tc*MSH2 (A, B, and C) in different strains has been reported, with the A isoform having more efficient MMR ability under genotoxic stress (86). This may have epidemiological importance, since *T. cruzi* II strains (related to isoform C) have more nuclear genetic variability than the *T. cruzi* I lineage (with isoform A) (87). Unexpectedly, the generation of *Msh2* null mutants in *T. cruzi* was not possible. The unavailability of *TcMsh2* mutants led to complementation experiments adding a *T. cruzi Msh2* copy in the *T. brucei Msh2* null mutant, which is viable and sensitive to hydrogen peroxide. Reversion of DNA damage sensitivity by expressing MSH2 from either *T. brucei* or *T. cruzi* was obtained. However, the heterologous expression of *T. cruzi* did not revert other MMR-related phenotypes (*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine [MNNG] tolerance and microsatellite instability), in accordance with results published earlier by another group (83). Strikingly, a *T. brucei Mlh1* knockout mutant displays no H_2O_2 sensitivity (79). Only MSH2 might be involved in the response to oxidative damage in *T. brucei*, suggesting no requirement for the MMR machinery. Similarly, the increase of 8-oxoG in kDNA in a single *TcMsh2* allele mutant provides a potential mitochondrial function for MSH2 in response to oxidative stress (88).

MMR and Recombination

MMR is also a mechanism that protects the genome against DNA recombination. During mitotic homologous recombination, the resected end of the DSB invades the homologous sister chromatid in a strand invasion reaction (see Fig. 5C). This event is described in more detail in "Homologous Recombination" below. The role

TABLE 2 Trypanosomatid genes involved in mismatch repair

Gene product designation (alternate designation)	Protein; function	Gene ID[a]						
		Human	*S. cerevisiae*	*S. pombe*	*L. infantum*	*L. major*	*T. brucei*	*T. cruzi*
MSH2	MutS homolog 2; mismatch recognition (MSH2-MSH6) and loop recognition (MSH2-MSH3)	NM_000251	YOL090W	SPBC19G7.01c (B)	LinJ.33.0420 (B)	LmjF.33.0410 (B)	Tb927.10.11020 (B)	Tc00.1047053507711.320 (B)
MSH3	MutS homolog 3; loop recognition (MSH2-MSH3)	NM_002439	YCR092C	SPAC8F11.03 (B)	LinJ.15.1470 (B)	LmjF.15.1420 (B)	Tb09.160.3760 (B)	Tc00.1047053508277.180 (B)
MSH4	MutS homolog 4; specialized for meiosis	NM_002440	YFL003C	SPAC8F11.03 (H) SPBC19G7.01c (Y)	LinJ.33.0420 (B)	LmjF.33.0410 (B)	Tb927.10.11020 (H)	Tc00.1047053507711.320 (B)
MSH5	MutS homolog 5; specialized for meiosis	NM_002441	YDL154W	SPAC8F11.03 (H)	LinJ.33.0420 (H), LinJ.36.2050 (Y)	LmjF.33.0410 (H), LmjF.36.1950 (Y)	Tb927.3.4280 (B)	Tc00.1047053506237.10 (B)
MSH6 (GTBP, MSH8)	MutS homolog 6; mismatch recognition (MSH2-MSH6)	NM_000179	YDR097C	SPCC285.16c (B)	LinJ.36.2050 (B)	LmjF.36.1950 (B)	Tb927.10.6410 (H), Tb927.10.11020 (Y)	Tc00.1047053507711.320 (B)
MLH1	MutL homolog 1; forms heterodimer with PMS1, PMS2, and MLH1	NM_000249	YMR167W	SPBC1703.04 (B)	LinJ.24.1460 (H), LinJ.35.1660 (Y)	LmjF.24.1420 (B)	Tb927.8.6840 (B)	Tc00.1047053504035.140 (B)
MLH2	MutL homolog 2; forms heterodimer	X	YLR035C	SPAC19G12.02c (Y)	X	X	X	X
MLH3	MutL homolog 3; forms heterodimer with PLH1	NM_014381	YPL164C	SPAC19G12.02c (B)	X	X	X	X
PMS1	Forms heterodimer with MLH1	NM_000534	YNL082W	SPAC19G12.02c (B)	LinJ.35.1660 (B)	LmjF.35.1660 (B)	Tb927.8.6840 (H), Tb09.211.4840 (Y)	Tc00.1047053510761.10 (B)
LIG I (CDC9)	DNA ligase I; ligation	NM_000234	YDL164C	SPAC20G8.01 (B)	LinJ.30.3490 (B)	LmjF.30.3440 (B)	Tb927.6.4780 (B)	Tc00.1047053506945.80 (B)
Polε	DNA polymerase filling gap	NM_006231	YNL262W	SPBC25H2.13c (B)	LinJ.35.4430 (B)	LmjF.35.4360 (B)	Tb09.211.1820 (B)	Tc00.1047053506147.180 (B)
Polδ	DNA polymerase filling gap	NM_002691	YDL102W	SPBC336.04 (B)	LinJ.33.1790 (B)	LmjF.33.1690 (B)	Tb927.2.1800 (B)	Tc00.1047053510259.6 (B)
Polβ	DNA polymerase specialized for G-U mispairs	NM_002690	YCR014C	SPAC2F7.06c (B)	LinJ.08.0830 (B)	LmjF.08.0890 (B)	Tb927.5.2780 (H), Tb927.5.2790 (Y)	Tc00.1047053503955.20 (B)
EXO 1 (HEX1)	Exonuclease 1; excision of DNA strand containing mispaired base	NM_003686	YOR033C	SPBC29A10.05 (B)	LinJ.23.1530 (B)	LmjF.23.1270 (B)	Tb927.8.3220 (B)	Tc00.1047053510517.90 (B)

[a] H, found from human homolog only; Y, found from yeast homolog only; B, found from both human and yeast homologs; X, no homolog.

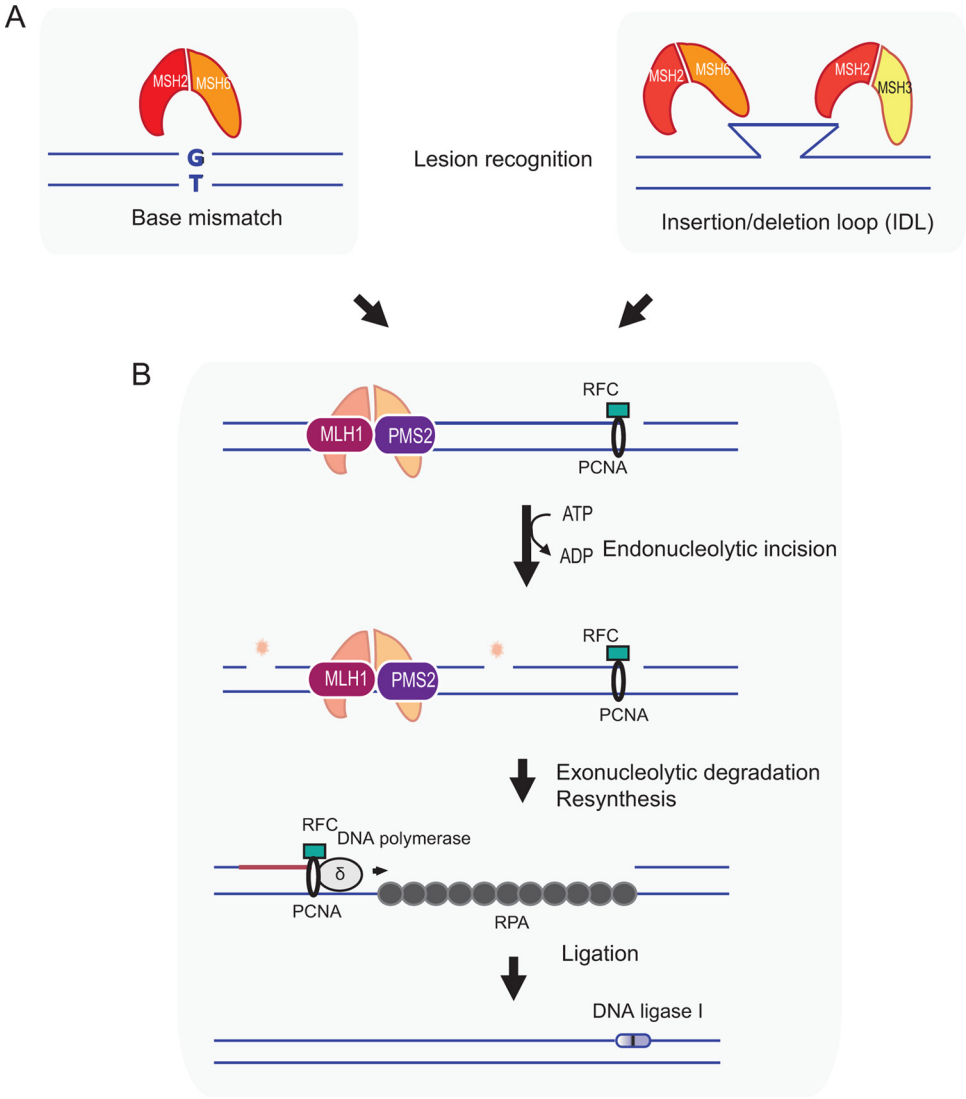

FIG 3 Mismatch repair. (A) Lesion recognition for a base mismatch or an insertion/deletion (IDL). (B) Endonucleolytic incision and exonucleolytic degradation followed by DNA synthesis.

of MMR proteins is to abort homologous recombination between nonhomologous sequences in order to prevent both point mutations and gross chromosomal rearrangements (70). The impact of MMR proteins in limiting misincorporation and promiscuous recombination between divergent sequences is important for genome stability. This is exemplified by the phenotype of *Msh2*-deficient mice, which are viable and fertile but harbor a high mutation rate that is conducive to tumorigenesis (89). Other specific MMR factors such as MSH4-MSH5, MLH1-PMS2, and MLH1-MLH3 heterodimers also participate in meiotic recombination (90–93).

Despite the fact that the MSH2 and MLH1 genes have no effect on VSG switching, disrupting the MMR system in *T. brucei* clearly increased the frequency of homologous recombination between either identical and divergent sequences. By assaying the efficiency of DNA transformation through different DNA constructs containing an increased amount of base mismatches (100 to 89% divergence), *Msh2* mutants were found to undergo recombination between nonidentical sequences more frequently than WT cells (77). More recently, the *in vitro* generation of *Leishmania* hybrids has been made possible by *Msh2* inactivation, which influences recombination between divergent sequences (78). The feasibility of whole-genome transformation in an *Msh2*-deficient background by transferring 45 kb of heterologous genomic DNA (gDNA) on a homologous locus between two species (*L. major* to *L. infantum*) undeniably illustrates the role of this gene in the parasite.

DNA DOUBLE-STRAND BREAK REPAIR

Detection of Double-Strand Breaks

The importance of fine-tuning in sensing and signaling double-strand breaks (DSBs) at critical stages termed DNA damage checkpoints is at the heart of the DNA damage response. In fact, to prevent genome rearrangements produced by an inappropriate DNA replication (G_1/S checkpoint) or segregation (G_2/M check-

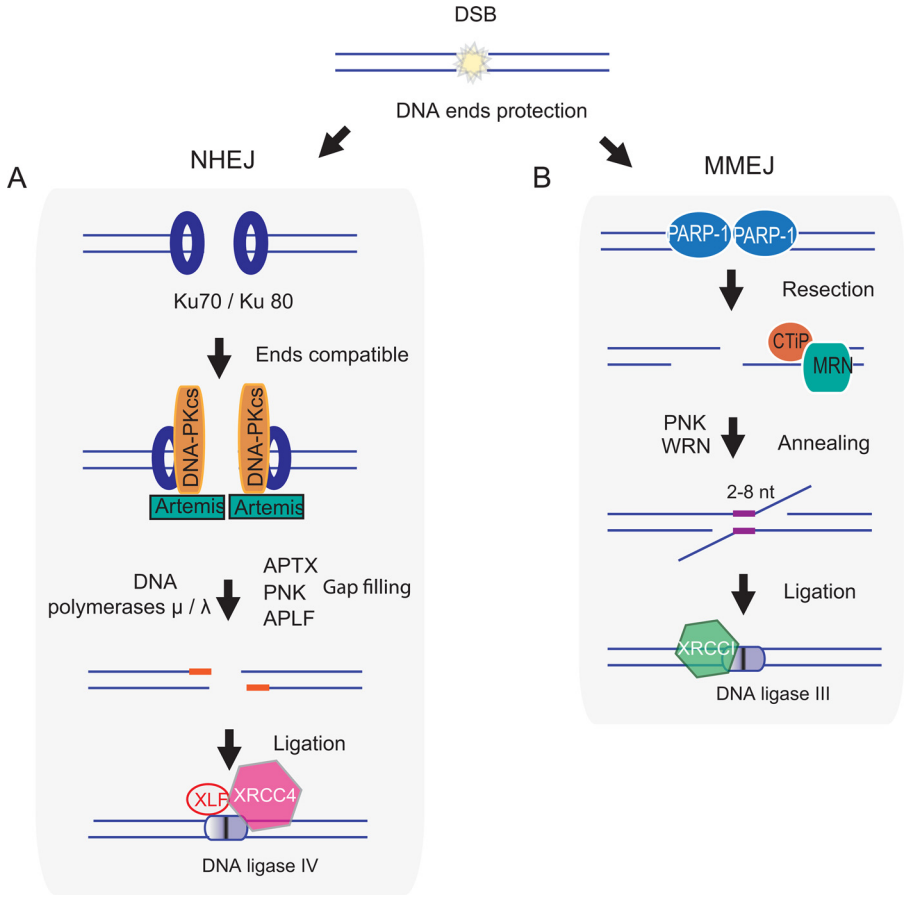

FIG 4 DNA double-strand break repair by nonhomologous end joining (A) or microhomology-mediated end joining (B). MMEJ is distinguished from NHEJ by its use of 2- to 8-bp microhomologous sequences to align the broken strands before DNA joining.

point), the activation of checkpoints temporarily halts the progression of the cell cycle to repair DSBs, one of the most cytotoxic DNA lesions. To activate the DNA damage signaling cascade, phosphatidylinositol 3 kinase-like protein kinases [ATM(tel1)/ATR(Mec1)/DNA-PK] perform phosphorylation of several proteins, including histones. This modification induces chromatin remodeling and gives access for DNA repair factors to the break site (94, 95). Identified in 1998 by Rogakou et al., phosphorylation of serine 139 at the C terminus of histone variant H2AX (γH2AX) represents a rapid event that begins at the break and extends beyond 2 Mb in higher eukaryotes (96, 97). Subsequently, a plethora of proteins orchestrates the dynamic of repair according to their functions as DNA damage sensors, transducers, mediators, and effectors (for a review, see reference 98). Used as a prominent marker of DNA damage in eukaryotes, the phosphorylation of Ser-139 in mammalian cells represents a helpful tool to dissect DNA repair pathways. However, this was not described for any trypanosomatids until recently, when Glover and Horn identified the phosphorylation of γH2A in *T. brucei* (99). Despite the fact that they failed to find any conserved SQ motif, they identified a threonine 130 residue on the C-terminal tail of all trypanosomal H2A sequences. Monitoring the repair processes underlying antigenic variation, the authors used the site-specific meganuclease I-SceI to generate targeted breaks. Using a specific phospho-antibody, in conjunction with an anti-RAD51 antibody, they showed that induction of a DSB led to an accumulation of γH2A/RAD51 foci, as well as a delay in the S and G_2 phases of the cell cycle. Consistent with this, the presence of trypanosomal kinases (ATM and ATR), as well as MDC1, TopBP1, BRCA1, and Chk2 (see Table S3 and Fig. S4 in the supplemental material), supports the existence of a DNA damage signaling network in trypanosomatids.

DSBs created randomly in the genome are one of the most deleterious types of damage, since a single unrepaired DSB can triggers aneuploidy, genetic defects, or apoptosis. It is salient to point out, however, that *Leishmania* has developed mechanisms to deal with considerable aneuploidy (33, 35). The two main strategies to cope with these cytotoxic lesions are nonhomologous end joining (NHEJ) and homologous recombination (HR). The former process, which is simpler but more mutagenic, proceeds by direct ligation of the broken ends and operates throughout the cell cycle and in G_1 in particular, making it the primary repair mechanism in higher eukaryotes. HR is limited to S and G_2, when the newly synthesized sister chromatid is available as a template, leading to accurate DNA repair.

Nonhomologous End Joining

Known as an error-prone mechanism, nonhomologous end joining (NHEJ) processes a number of different structures into a ligatable form, causing deletions or insertions at the break site (Fig. 4A

and B). Nevertheless, this erroneous repair is usually of little consequence, since most of the genome in higher eukaryotes is made of noncoding regions. NHEJ proteins were first described through their involvement in ionizing radiation resistance and V(D)J recombination (100). The initial step of this process is the recognition of DNA ends in a sequence-independent manner by the Ku70/Ku80 heterodimer (the subunits are named "Ku" for the initials of a scleroderma patient in whose cells the complex was discovered) (Fig. 4A). The ring structure (toroidal shape) of Ku encircles DNA ends (with a strong affinity of $\sim 10^{-9}$ M) and then recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) to bring DNA ends together and stimulate its kinase activity afterward (101, 102). If the extremities are not compatible for ligation (because of the absence of 3′ OH or 5′ P or presence of DNA loops, flaps, and gaps), the nuclease Artemis in complex with DNA-PKcs degrades DNA ends through its 5′-3′ exonuclease and endonuclease activities (103). Others accessory factors, such as polynucleotide kinase (PNK), Aprataxin (APTX), and Aprataxin-and-PNK-like Factor (APLF), can help in promoting this step. All these components interact with XRCC4 while PNK adds a phosphate to a 5′-OH extremity and remove 3′-phosphate groups. To avoid failed ligation, APTX plays an important role in deadenylation of the remaining AMP group at the 5′ end, and APLF acts as an endonuclease and a 3′-exonuclease (100, 104, 105). Likewise, the cleavage triggers loss of genetic information while the gaps are filled by PolX family polymerases μ and λ. Recruited by DNA-PKcs to the DSB site, the XLF/XRCC4/DNA ligase IV complex carries out the final end-joining step (for extensive reviews, see references 106 and 107). Individual knockouts for HR proteins (RAD51, BRCA2, or XRCC2) and NHEJ proteins (DNA ligase IV or XRCC4) are embryonic lethal, showing that both pathways are essential (108). Taken together, these findings suggest a necessary collaboration between NHEJ and HR, while competition for DSBs is also present (109).

Since only a few NHEJ factors (Mre11, Ku70/Ku80, and APTX) are conserved in trypanosomatids (Table 3; see Fig. S1B and C and S6 in the supplemental material), the NHEJ pathway might not occur in these parasites. Some work on the heterodimeric protein Ku has been done with the sleeping sickness parasite *T. brucei*, with the hypothesis that this key protein might be involved in differential expression of variant surface glycoprotein (VSG). As opposed to the case in other organisms, *Tb*Ku70 and *Tb*Ku80 homologs code for intriguing 81-kDa and 69-kDa proteins, respectively. All the structures (α helices/β strands) from the human crystal structure are conserved in the predicted *Tb*Ku70 except the part of the ring that encircles DNA. Likewise, in common with Ku70 from other species, a partial putative DNA-binding SAP domain is present (110, 111). Moreover, *Tb*Ku80 lacks at its C terminus a DNA-dependent protein kinase-binding sequence required for DNA-PK interaction (112). Generation of null mutants of *Ku70* or *Ku80* and growth analysis showed no detectable influence on either DNA double-strand break repair or VSG switching (113). The prevalence of HR in antigenic variation for the VSG gene might explain the evasion of host immunity. On the other hand, *T. brucei* nuclear extracts exhibit efficient and rapid DNA joining of linear DNA plasmids, resulting in dimer- and trimer-sized products. Using different types of restriction-digested substrates (with a 5′ or 3′ overhang or blunt), Burton et al. showed no relation between DNA end conformation and joining efficiency (in contrast to the case for mammalian nuclear extracts) (114). PCR amplification, cloning, and sequencing revealed that joins happen within microhomologous sequences ranging from 6 to 16 bp in the DNA molecule. Foremost, *TbKu70* or *TbRad51* (a key factor in HR) null mutants showed no difference in joining activity, indicating that this homology sequence end-joining reaction occurs in a Ku- and HR-independent manner (114).

In agreement with our bioinformatics analysis, Burton and colleagues detected a ligase I homolog encoded in the kinetoplastid parasite genome but no ligase IV/XRCC4, the complex responsible for resealing DNA during NHEJ (Table 3). The main feature that distinguishes eukaryotic ligase IV is the two BRCT motifs in the C terminus that are involved in the interaction with XRCC4 (115–117). The absence of this particular domain in all the ligases identified in kinetoplastids and the lack of a detectable homolog for the poorly conserved XRCC4 imply that end joining is presumably performed by microhomology-mediated end joining (MMEJ), a backup repair pathway when Ku-dependent NHEJ is absent.

Microhomology-Mediated End Joining

Another pathway to repair DSB is microhomology-mediated end joining (MMEJ), also called alternative end joining (Alt-NHEJ or A-EJ) or backup NHEJ (B-NHEJ), which is normally suppressed when NHEJ is present (Fig. 4B). In a Ku-independent way, DNA ends are locally resected (2 to 8 nt) to exhibit regions that contain homologous sequences for ligation. This mechanism shares with HR the initial step of resection, but there is no need for an extended resection and sequence homology to proceed. Following the DSB, unprotected DNA ends (without Ku70/Ku80) are recognized by PARP-1 and then resected by the complex MRN-Ctip to reveal complementary sequences for annealing and ligation by XRCC1/DNA ligase III (118–120).

Horn's group identified microhomology-mediated end joining in *T. brucei* by using a tetracycline-inducible I-SceI meganuclease system (121). They found that DSBs were repaired mostly by allelic HR but also by ectopic HR and MMEJ. To better characterize MMEJ in antigenic variation, they established a system to remove allelic HR survivors by replacing the gene Tb11.02.2110, which is adjacent to the cleavage site on the other allele, by a NEO selectable marker (121). After disruption of this gene, which is essential for growth, lesion association with undamaged homologous sequences cannot occur (no allelic HR). With this system, ~60% of cells survive via MMEJ, compared to ~5% when allelic HR is not lethal. They then presented evidence of intrachromosomal end joining mediated by two pathways of MMEJ: MMEJ-based deletions (RAD51 independent) and MMEJ-based gene conversion (RAD51 dependent) (121). Since Ku-deficient *T. brucei* parasites do not display a detectable difference in the rate of VSG switching, it seems plausible that VSG rearrangement is driven by MMEJ. In the absence of NHEJ, the prominence of MMEJ-mediated deletions (with a mean size of 284 bp) might explain the abundant synteny gaps found in the trypanosomatid genomes.

Homologous Recombination

In humans, the homologous recombination pathway functions primarily to guard genome integrity by ensuring faithful repair of DNA double-strand breaks. HR also intervenes in interstrand cross-link repair, recovery of stalled replication forks, and telomere maintenance, and it certifies proper chromosome segregation during meiosis (122). The error-free nature of HR is

TABLE 3 Trypanosomatid genes involved in nonhomologous end joining

Gene product designation (alternate designation)	Protein; function	Gene ID[a]						
		Human	*S. cerevisiae*	*S. pombe*	*L. infantum*	*L. major*	*T. brucei*	*T. cruzi*
MRE11 (Rad32)	MRN (MRX) complex protein; ssDNA endonuclease and 3′–5′ exonuclease	NM_005590	YMR224C	SPAC13C5.07 (B)	LinJ.27.1790 (B)	LmjF.27.1890 (B)	Tb927.2.4390 (B)	Tc00.1047053509099.70 (B)
XRCC1	X-ray repair cross-complementing protein 1; DNA ligase III factor	NM_006297	X	SPAC23C4.18c (H)	X	X	X	X
XRCC4 (Lif4)	X-ray repair cross-complementing protein 4; DNA ligase IV factor	NM_003401	YGL090W	SPAC20G4.05c (H)	X	X	X	X
XRCC5 (Ku80, Pku80, Yku80)	X-ray repair cross-complementing protein 5; heterodimeric (Ku70-Ku80) DNA end-binding protein	NM_021141	YMR106C	SPBC543.03c (B)	LinJ.30.0340 (B)	LmjF.30.0340 (B)	Tb927.6.1760 (B)	Tc00.1047053503643.10 (Y)
XRCC6 (Ku70, Pku70, Yku70)	X-ray repair cross-complementing protein 6; heterodimeric (Ku70-Ku80) DNA end-binding protein	NM_001469	YMR284W	SPCC126.02c (B)	LinJ.29.1140 (B)	LmjF.29.1050 (H)	Tb927.3.5030 (B)	Tc00.1047053503643.10 (B)
DNA-PK (XRCC7)	DNA-dependent protein kinase	NM_006904	YJR066W	SPBC30D10.10c (H)	LinJ.34.3750 (H)	LmjF.34.3940 (H)	Tb927.4.800 (H)	Tc00.1047053508997.20 (H)
Artemis (DCLRE1C)	Nuclease participating in DNA end processing	NM_022487	X	SPAC22A12.01c (H)	X	X	Tb927.4.1480 (H)	X
XLF (NHEJ1)	XRCC4-like factor; structural component of LIG4-XRCC4-XLF ligation complex	NM_024782	YLR265C	X	X	X	X	X
APTX	Aprataxin; processing of DNA single-strand interruptions	NM_175073	YOR258W	SPCC18.09c (B)	LinJ.29.0670 (B)	LmjF.29.0640 (B)	Tb927.3.3710 (B)	Tc00.1047053510149.100 (B)
APLF/PALF	Aprataxin and PNKP-like factor	NM_173545	X	X	X	LmjF.36.1350 (H)	Tb927.10.6550 (H)	X
Ctip (Sae2, RIM, RBBP8)	Carboxy-terminal BRCA1-interacting protein; MRN factor involved in Alt-NHEJ by promoting DNA end resection	NM_002894	YGL175C	X	X	X	X	X
LIG III	DNA ligase involved in Alt-NHEJ	NM_013975	X	SPAC20G8.01 (H)	LinJ.30.3490 (H)	LmjF.30.3440 (H)	Tb927.6.4780 (H)	Tc00.1047053506945.80 (H)
LIG IV (Lig4p)	DNA ligase involved in classis NHEJ	NM_002312	YOR005C	SPCC1183.05c (B)	X	X	X	X
Polλ	DNA polymerase; gap filling	NM_001174084	YCR014C	SPAC2F7.06c (H)	LinJ.08.0830 (H)	LmjF.08.0890 (H)	Tb927.5.2780 (H)	Tc00.1047053503955.20 (H)
Polμ	DNA polymerase; gap filling	NM_013284	YCR014C	SPAC2F7.06c (H)	LinJ.08.0830 (H)	LmjF.08.0890 (H)	Tb927.5.2780 (H)	Tc00.1047053503955.20 (H)

[a] H, found from human homolog only; Y, found from yeast homolog only; B, found from both human and yeast homologs; X, no homolog.

owed to the use of an intact homologous sequence, most likely the sister chromatid, as the repair template. Crucial to this process is a recombinase, RecA in prokaryotes and RAD51 in eukaryotes, which forms a nucleoprotein filament responsible for catalyzing the core steps that typify HR for homology search and strand exchange.

Conceptually, HR can be divided into three phases: presynapsis, synapsis, and postsynapsis (Fig. 5). In the presynaptic phase, DSBs are first recognized by the MRE11-RAD50-NBS1 complex and processed through resection by the concerted activities of nucleases and helicases involving MRE11, CtIP, BLM, EXO1, and DNA2 (123–125). Resection then exposes 3′ single-stranded overhangs that are rapidly coated by replication protein A (RPA) to protect against secondary structure formation and nuclease digestion (Fig. 5A). The resulting single-stranded DNA (ssDNA)-RPA intermediate then serves in checkpoint activation to slow or arrest cell cycle progression, thereby allowing time for proper repair, and in the formation of a RAD51 nucleoprotein filament, referred to as the presynaptic filament (126, 127) (Fig. 5B). In order for the presynaptic filament to assemble, however, RAD51 must remove RPA to access DNA-binding sites. This occurs with the help of recombination mediators, which by definition facilitate RAD51 nucleation onto ssDNA via RPA displacement (128, 129). Mediators can also act by increasing stabilization and protection of the RAD51 presynaptic filament needed for subsequent HR steps (130). One key mediator is the BRCA2 protein, whose interaction with RAD51 plays a crucial role in controlling nucleoprotein filament formation and function (131, 132). PALB2, RAD52, and the RAD51 paralogs are also known to display RAD51 mediator activity (133, 134). This activity is counterbalanced by the ATP-dependent action of antirecombinases, which dismantle the RAD51 filament, thereby preventing untimely or unwanted recombination that could lead to inappropriate genome rearrangements.

During the synaptic phase, the nucleoprotein filament catalyzes a strand exchange reaction by engaging sequence homology search and strand invasion into the intact sister chromatid, forming joint molecules (D-loops) (Fig. 5C) where the 3′ end of the invading strand serves as a primer for subsequent DNA synthesis (Fig. 5D). The chromatin remodeler RAD54 protein was found to exert a stabilizing effect on the RAD51-ssDNA filament, making it more competent for DNA strand exchange, and to be involved in multiple postsynaptic steps (135, 136).

The postsynaptic phase comprises the late steps of HR, from the extension of the 3′ invading strand by DNA synthesis to the elimination of recombination-mediated junction intermediates and recovery of lost information. RAD54 postsynaptic contributions include dissociation of RAD51 from heteroduplex DNA to allow extension of the invading 3′-OH end by DNA polymerase and processing of recombination intermediates (137–140). In humans, DNA synthesis is believed to be carried out by DNA polymerase eta (141). The cloning and characterization of *T. cruzi* DNA polymerase eta were reported (142). Purified polymerase eta promoted DNA synthesis in primer extension assays and bypassed oxidative DNA lesions such as 8-oxoguanine. In addition, polymerase eta localizes to the nucleus, and its overexpression in *T. cruzi* confers resistance to hydrogen peroxide but not to gamma irradiation. However, it complements the UV sensitivity of polymerase eta-deficient yeast cells. The low fidelity of polymerase eta might provide the parasite with adaptive mutations, allowing it to escape from host immune surveillance (142). It remains to be seen whether *T. cruzi* polymerase eta also extends D-loops.

Once DNA synthesis is initiated, different routes can be envisioned (Fig. 5E). According to the synthesis-dependent strand annealing (SDSA) model, the invading strand is displaced from the D-loop and anneals to its complementary sequence on the other side of the break. Gap-filling DNA synthesis and nick ligation complete DSB repair, forming noncrossover products (134, 143). Alternatively, as predicted by the classical double-strand break repair (DSBR) model, the second DSB end can be captured by the displaced strand of the D-loop to prime another round of DNA synthesis. Processing of the resulting precursor by Mus81-Eme1 generates crossover products, while ligation produces a joint molecule with two Holliday junctions (HJs) (144, 145). The so-called double Holliday junction intermediate can then be dissolved to noncrossover products via the BLM-TOPOIIIα-RMI1/RMI2 complex or resolved to either crossover or noncrossover products by the GEN1 or SLX1/SLX4 resolvase (146–150). When a DSB presents only one repairable end, as seen at eroded uncapped telomeres or collapsed replication forks, break-induced replication (BIR) establishes a replication fork at the D-loop, where the entire chromosome arm can be copied (Fig. 5) (130, 151, 152). In the case where the DSB is flanked by sequence repeats, cells may be directed toward an error-prone pathway, independent of RAD51, called single-strand annealing (SSA) (Fig. 5). This pathway is promoted by RAD52, and end resection occurs at sequence repeats to provide complementary single strands that anneal, leading to the deletion of the intervening sequence (153, 154).

In trypanosomatids such as *Trypanosoma brucei* and *Leishmania*, HR is used for antigenic variation and gene amplification, respectively, as a mechanism for survival in their hosts (34, 113). Essential components from each phase of HR have been identified in their genomes (Table 4; see Fig. S1B and C and S7A in the supplemental material). This is the case for the presynapsis proteins MRE11, RAD50, NBS1, and RPA, with the *T. brucei* MRE11 homolog, *Tb*MRE11, being the best characterized (25). MRE11, a member of the lambda phosphatase family of phosphoesterases, is a conserved protein with an N-terminal nuclease domain that has both 3′-to-5′ exonuclease and endonuclease activities (155, 156). *Tb*MRE11 was shown to share 37% identity with human MRE11 over the N-terminal region, with striking sequence conservation in phosphoesterase motifs that have been linked to nuclease activity (157, 158). The conservation of Asp16, Asp56, His125, and His213, which are crucial residues for endo- and exonuclease activities of budding yeast Mre11, in *Leishmania* and *Trypanosoma* is shown in Fig. 6A (159–163). A *Tb*MRE11 null mutation was shown to increase sensitivity to the DNA-damaging agent phleomycin, impair homologous recombination, and lead to chromosomal rearrangements, phenotypes suggesting conservation of functions between species (158, 164). We recently generated a *Leishmania infantum Mre11* null mutant and showed increased sensitivity to DNA-damaging agents (Laffitte et al., unpublished observations). We also showed that the formation of linear extrachromosomal amplicons, but not of circular amplicons, was decreased in an *Mre11* null mutant upon drug selection (Laffitte et al., unpublished observations). The *Leishmania infantum* MRE11 protein was purified and resected double-stranded DNA (dsDNA) (Laffite et al., unpublished observations). Only one subunit of the heterotrimer replication protein A (RPA), called RPA-1, is conserved in trypanosomatids. Lacking the N-terminal

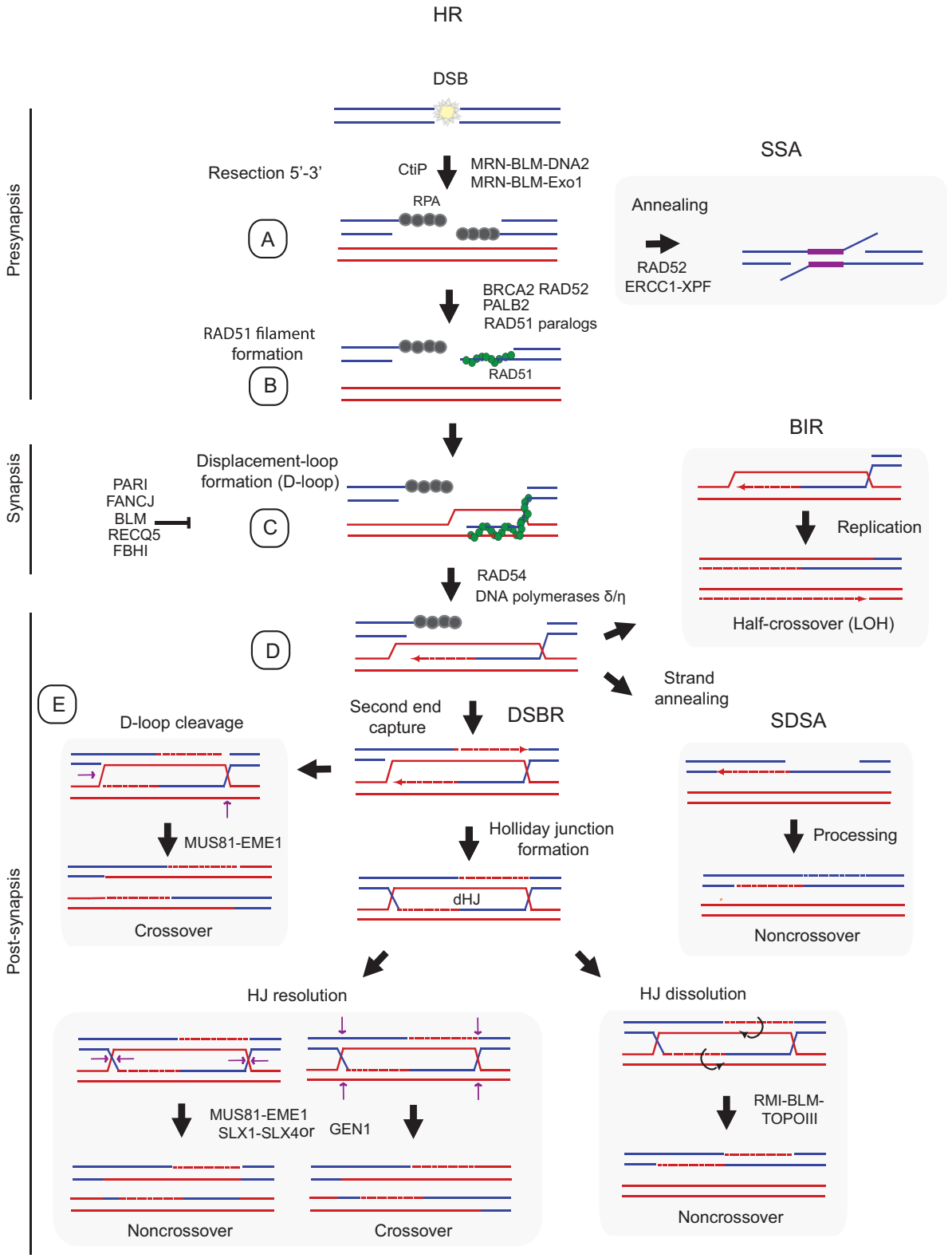

FIG 5 DNA double-strand break repair by homologous recombination. (A) DNA resection and formation of 3′-tailed DNA; (B) RAD51 filament formation; (C) invasion of the undamaged sister chromatid by RAD51; (D) DNA synthesis by polymerase eta or delta; (E) D-loop cleavage, Holliday junction resolution or dissolution, or synthesis-dependent strand annealing.

TABLE 4 Trypanosomatid genes involved in homologous recombination

Gene product designation (alternate designation)	Protein; function	Gene ID[a]						
		Human	*S. cerevisiae*	*S. pombe*	*L. infantum*	*L. major*	*T. brucei*	*T. cruzi*
Ctip (Sae2, RIM, RBBP8)	Carboxy-terminal BRCA1-interacting protein; MRN factor involved in Alt-NHEJ by promoting DNA end resection	NM_002894	YGL175C	X	X	X	X	X
MRE11 (Rad32)	MRN (MRX) complex protein; ssDNA endonuclease and 3′-5′ exonuclease	NM_005590	YMR224C	SPAC13C5.07 (B)	LinJ.27.1790 (B)	LmjF.27.1890 (B)	Tb927.2.4390 (B)	Tc00.1047053509099.70 (B)
RAD50	MRN (MRX) complex protein; DNA-binding and -tethering activity	NM_005732	YNL250W	SPAC1556.01c (B)	LinJ.28.0560 (B)	LmjF.28.0530 (B)	Tb11.01.0340 (B)	Tc00.1047053503799.10 (B)
NBS1 (Xrs2)	Nijmegen breakage syndrome 1; MRN (MRX) complex protein, nuclear localization, interacts with ATM	NM_002485	YDR369C	X	LinJ.16.0330 (H)	LmjF.16.0320 (H)	Tb927.8.5710 (H)	Tc00.1047053506743.180 (H)
BLM (Rqh1, Sgs1)	Bloom syndrome; 3′-5′ helicase member of the RecQ family	NM_000057	YMR190C	SPAC2G11.12 (B)	LinJ.24.1590 (B)	LmjF.24.1530 (B)	Tb927.8.6690 (B)	Tc00.1047053507433.9 (B)
RMI1	RecQ-mediated genome instability protein 1; processing of HR intermediates to limit DNA crossover formation, part of RMI complex	NM_024945	YCR028C-A	SPAC26A3.03c (H), SPAC2F3.04c (Y)	LinJ.23.0100 (H)	LmjF.23.0090 (H)	Tb927.8.2040 (H)	Tc00.1047053509497.9 (H)
RMI2	RecQ-mediated genome instability protein 2; processing of HR intermediates to limit DNA crossover formation, part of RMI complex	NM_152308	X	X	X	X	X	X
Top3α	DNA topoisomerase 3α; part of RMI complex that interacts with BLM	NM_004618	YLR234W	SPBC16G5.12c (B)	LinJ.36.3350 (B)	LmjF.36.3200 (B)	Tb11.01.1280 (B)	Tc00.1047053511589.120 (B)
RecQL5 (FBH1, Srs2)	Antirecombinase	NM_001003715	YMR190C	SPAC2G11.12 (H)	LinJ.24.1590 (H)	LmjF.24.1530 (H)	Tb927.8.6690 (H)	Tc00.1047053507433.9 (H)
DNA2	ssDNA endonuclease, helicase, and ATPase	NM_001080449	YHR164C	SPBC16D10.04c (B)	LinJ.09.1300 (Y)	LmjF.09.1240 (Y)	Tb927.5.2140 (Y)	Tc00.1047053506755.140 (Y)
EXO1 (HEX1)	Exonuclease 1; 5′-3′ exonuclease and flap endonuclease	NM_003686	YOR033C	SPBC29A10.05 (B)	LinJ.23.1530 (B)	LmjF.23.1270 (B)	Tb927.8.3220 (B)	Tc00.1047053510517.90 (B)
RPA1 (p70)	Replication protein A 1; ssDNA-binding heterotrimer	NM_002945	YAR007C	SPBC660.13c(B)	LinJ.28.1940 (B)	LmjF.28.1820 (B)	Tb11.01.0870 (B)	X
RPA2 (p32)	Replication protein A 2; ssDNA-binding heterotrimer	NM_002946	YNL312W	SPCC1753.01c (B)	LinJ.15.0310 (H)	LmjF15.0270 (H)	Tb927.5.1700 (B)	X
RPA3 (p11)	Replication protein A 3; ssDNA-binding heterotrimer	NM_002947	YJL173C	X	X	X	X	X
RAD51 (Rhp51)	RecA homolog; promotes homologous pairing and strand exchange	NM_002875	YER095W	SPAC644.14c (B)	LinJ.28.0580 (B)	LmjF.28.0550 (B)	Tb11.01.0360 (B)	Tc00.1047053503801.30 (B)
RAD51B (RAD51L1)	RAD51 paralog; recombination mediator	NM_002877	X	X	X	X	X	X
RAD51C (RAD51L2, FANCO)	RAD51 paralog; recombination mediator	NM_002876	X	X	LinJ.33.2620	LmjF.33.2490	Tb11.02.0150	Tc00.1047053504153.220
RAD51D (RAD51L3)	RAD51 paralog; recombination mediator	NM_002878	X	X	LinJ.29.0460	LmjF.29.0450	Tb927.3.5230	Tc00.1047053508075.20
RAD51-5	RAD51 paralog in *Trypanosoma*	X	X	X	X	X	Tb10.389.1770	Tc00.1047053510123.30
XRCC2	X-ray repair cross-complementing protein 2; RAD51 paralog	NM_005431	X	X	LinJ.11.0230	LmjF.11.0230	X	Tc00.1047053503613.30
XRCC3	X-ray repair cross-complementing protein 3; RAD51 paralog	NM_005432	X	X	X	X	X	X
Shu1	Component of Shu complex which promotes HR	X	YHL006C	X	X	X	X	X
Psy3	Component of Shu complex which promotes HR	X	YKR376C	X	X	X	X	X
Rad55	Rad51 paralog in *S. cerevisiae*	X	YDR076W	X	X	X	X	X
Rad57	Rad51 paralog in *S. cerevisiae*	X	YDR004W	X	X	X	X	X
RAD52	Recombination mediator; ssDNA binding, annealing	NM_134424	YML032C	SPAC30D11.10 (B)	X	X	X	X
RAD54L	RAD54-like; stimulates DNA recombination	NM_003579	YGL163C	SPAC15A10.03c (B)	LinJ.24.0770 (B)	LmjF.24.0760 (B)	Tb11.02.3110 (B)	Tc00.1047053508647.290 (B)
RAD54B (Rdh54)	RAD54 homolog B; DNA-dependent ATPase participating in strand transfer and in dissociation of RAD51 filament	NM_012415	YBR073W	SPAC15A10.03c (B)	LinJ.24.0770 (B)	LmjF.24.0760 (B)	Tb11.02.3110 (B)	Tc00.1047053508647.290 (B)

BRCA1	Breast cancer type 1; accessory factor for transcription and recombination, E3 ubiquitin ligase	NM_007294	X	SPCC548.05c (H)	LinJ.08.1130 (H)	LmjF.36.1930 (H)	Tb927.5.3070 (H)	X	
BRCA2 (FANCD1)	Breast cancer type 2; mediator of RAD51	NM_000059	X	X	LinJ.20.0070 (H)	LmjF.20.0060 (H)	Tb927.1.640 (H)	Tc00.1047053511417.120 (H)	
DSS1 (SHFM1)	Deleted in split hand/split foot 1; BRCA2 factor	NM_006304	YDR363W-A	SPAC3G6.02 (H)	X	X	X	X	
MUS81	Methyl methanesulfonate and UV sensitive 81	NM_025128	YDR386W	SPCC4G3.05c (B)	X	X	Tb927.8.6740 (H)	X	
EME1 (Mms4)	Essential meiotic endonuclease 1; MUS81 partner	NM_152463	YBR098W	SPAPB1E7.06c (H)	X	X	X	X	
SLX1 (GIYD1)	Synthetic lethal X 1; structure-specific endonuclease	NM_001014999	YBR228W	SPAP27G11.15 (B)	LinJ.25.1370 (B)	LmjF.25.1330 (B)	Tb927.3.1220 (B)	Tc00.1047053509453.60 (B)	
SLX4 (BTBD12, FANCP)	Synthetic lethal X 4; structure-specific endonuclease	NM_032444	YLR135W	X	X	X	X	X	
GEN1 (Yen1)	XPG-like endonuclease 1; nuclease cleaving Holliday junctions	NM_182625	YER041W	SPAC3G6.06c(H), SPBC3E7.08c (Y)	LinJ.27.0260 (H), LinJ.35.3640 (Y)	LmjF.27.0250 (H), LmjF.35.3590 (Y)	Tb927.3.830 (H), Tb09.211.2870 (Y)	Tc00.1047053511867.110 (H), Tc00.1047053510517.90 (Y)	
PARI (Srs2)	PCNA-associated recombination inhibitor; antirecombinase	NM_017915	YJL092W	SPAC4H3.05 (Y)	LinJ.09.0640 (Y)	LmjF.09.0590 (Y)	Tb11.01.4440 (Y)	Tc00.1047053508777.90 (Y)	
PALB2 (FANCN)	Partner and localizer of BRCA2	NM_024675	X	X	X	X	X	X	
WRN	Werner syndrome; helicase and 3′ exonuclease	NM_000553	YMR190C	SPAC2G11.12 (H)	LinJ.24.1590 (H)	LmjF.24.1530 (H)	Tb927.8.6690 (H)	Tc00.1047053507433.9 (H)	
Polα	DNA polymerase alpha	NM_002689	YNL102W	SPCC553.09c (H), SPAC3H5.06c (Y)	LinJ.32.1800 (H), LinJ.16.1640 (Y)	LmjF.32.1720 (H), LmjF.16.1540 (Y)	Tb11.01.6520 (H), Tb927.8.4880 (Y)	Tc00.1047053506221.60 (H), Tc00.1047053508837.180 (Y)	
Polδ	DNA polymerase delta	NM_002691	YDL102W	SPBC336.04 (B)	LinJ.33.1790 (B)	LmjF.33.1690 (B)	Tb927.2.1800 (B)	Tc00.1047053510259.6 (B)	
Polε	DNA polymerase epsilon	NM_006231	YNL262W	SPBC25H2.13c (B)	LinJ.35.4430 (B)	LmjF.35.4360 (B)	Tb09.211.1820 (B)	Tc00.1047053506147.180 (B)	
Polζ	DNA polymerase zeta	NM_002912	YPL167C	SPAC688.10 (B)	LinJ.23.1590 (B)	LmjF.23.1330 (B)	Tb927.8.3290 (B)	Tc00.1047053509769.130 (B)	

[a] H, found from human homolog only; Y, found from yeast homolog only; B, found from both human and yeast homologs; X, no homolog.

domain involved in RPA-protein interaction, RPA-1 (51 kDa) is smaller than the other homologs identified so far (70 kDa). In 2007, Neto and colleagues reported their work on RPA-1 from *Leishmania amazonensis*, showing a nuclear localization and association with telomeres *in vivo* (165). *La*RPA-1 is involved with the damage response and telomere protection, although it lacks the RPA1N domain involved in the binding to multiple checkpoint proteins (166). Da Silveira et al. recently proposed that *La*RPA-1 assumes a capping function at telomeres (166).

RAD51, whose key function in HR is to catalyze homology recognition and DNA strand exchange as helical protein filaments on DNA, belongs to the highly conserved RecA family of recombinases. Sequence homology between human RAD51 and RecA lies mainly within a core domain, often referred to as the RecA fold, containing Walker A and Walker B nucleotide-binding motifs that confer ATP binding and hydrolysis activity to the enzymes (Fig. 6B) (167, 168). The core domain is also composed of two disordered loops (denoted L1 and L2 motifs) for ssDNA binding and is preceded by a short polymerization motif (PM) responsible for helical filament assembly (169, 170). RAD51 has an N-terminal domain, lacking in RecA, that contains a helix-hairpin-helix (HhH) domain involved in dsDNA binding (171). Biochemical studies have shown that presynaptic filament assembly, turnover, and strand exchange activity are coupled to ATP binding and hydrolysis by RAD51 (172, 173). While ATP binding is associated with RAD51 presynaptic filament assembly, homologous DNA pairing, and strand exchange activity, ATP hydrolysis appears to be a prerequisite for filament disassembly (174–176). Biochemical studies also indicate significant differences between eukaryote and prokaryote recombinases. Unlike RecA, which shows a strong bias toward single-stranded DNA, the yeast and human proteins assemble helical filaments on both ssDNA and dsDNA (177). Furthermore, eukaryotic homologs are considerably less efficient in ATP hydrolysis and strand exchange than RecA (178).

The RAD51 gene has been identified and characterized in several trypanosomatid parasites, including *T. brucei*, *T. cruzi*, *L. major*, and *L. infantum*. Sequence alignments reveal that the parasite RAD51 homologs share between 60 to 80% sequence identity with human RAD51 and that sequence conservation appears to extend outside the RecA fold, as an HhH and a PM motif have been reported in *Tb*Rad51 (179–181). Genetic analysis in *T. brucei* revealed that *Rad51*$^{-/-}$ mutants display increased sensitivity to DNA-damaging agents, such as methyl methanesulfonate (MMS) and phleomycin, and reduced integration of transformed DNA, supporting a role in homologous recombination (113, 179, 182). *In vivo*, RAD51 is known to assemble at DSB sites, where it forms discrete nuclear foci after DNA damage (183, 184). Focus formation has also been described in *Tb*Rad51 upon phleomycin treatment or introduction of a site-specific DSB with the I-SceI endonuclease (121, 181). *In vitro*, purified *Lm*Rad51 was shown to bind DNA and possess DNA-stimulated ATPase activity (180). Furthermore, recent biochemical characterization in *L. infantum* has described *Li*Rad51 as a functional recombinase that binds both ssDNA and dsDNA and promotes strand invasion, similar to human RAD51 (185). Interestingly, *Leishmania* Rad51 exhibits significantly elevated ATPase activity, and, unlike hRAD51, its expression is induced after DNA damage, suggesting the existence of different RAD51 regulatory mechanisms between species (180, 185). When overexpressed, *Tc*Rad51 was shown to confer a more

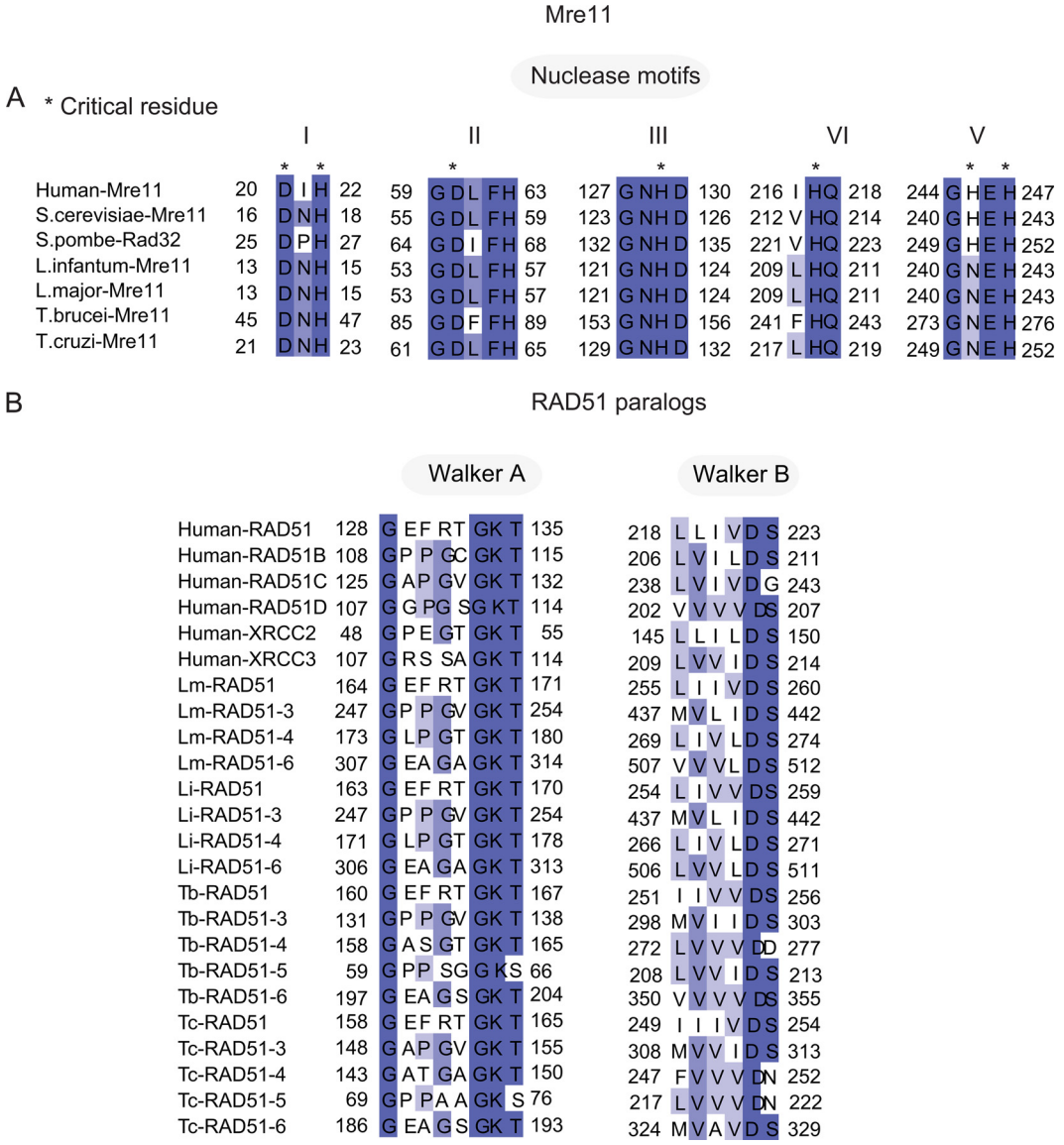

FIG 6 (A) Alignment of the nuclease motifs of MRE11 in the indicated species. (B) Alignment of the Walker A and B motifs of the RAD51 paralogs in human, *Leishmania major* (Lm), *Leishmania infantum* (Li), *Trypanosoma brucei* (Tb), and *Trypanosoma cruzi* (Tc).

efficient repair of DSBs after genotoxic treatment, which supports a role for the HR machinery (186). An *L. infantum Rad51*$^{-/-}$ mutant was recently generated, and it was shown to be more sensitive to DNA-damaging agents (Ubeda et al., submitted). In contrast to the case for the *Mre11* null mutant, we also showed that the formation of circular extrachromosomal DNA amplicons, but not linear amplicons, was decreased in a *Rad51* null mutant upon drug selection (Ubeda et al., submitted).

While RAD51 appears to be largely conserved among eukaryotes, its mediators vary in complexity and function between organisms. In humans, several proteins, including BRCA2, PALB2, RAD52, and the RAD51 paralogs, have been shown to fulfill mediator functions (133, 134). Orthologs of BRCA2 and RAD51 paralogs have been identified in *Trypanosoma* and *Leishmania*, but PALB2 and RAD52 are absent (181, 187). BRCA2 is thought to sustain the function of the latter in these species. In humans, the protein possesses eight BRC repeats through which it binds RAD51 and controls RAD51 nucleoprotein filament formation (188). Binding to RAD51 also occurs via a C-terminal unrelated motif, which appears to stabilize the filament (189). An oligonucleotide/oligosaccharide-binding (OB) fold has also been described and associated with the ssDNA-binding activity of the protein (190). Although generally smaller (approximately one-third the size of human BRCA2 for some), trypanosomatid orthologs all possess an OB fold and BRC repeats. Brca2 from *T. cruzi* and *Leishmania* has two BRC repeats, while *T. brucei* has evolved 15 such repeats (25, 185, 187, 191). In *T. brucei*, the BRC repeats are important for HR and *Tb*Rad51 localization, as shown by the impaired Rad51 localization and decreased HR efficiency of *Tb*Brca2 variants with reduced BRC repeat numbers (191, 192). In *L. infantum*, the BRC repeats of *Li*Brca2 were shown to be involved its interaction with *Li*Rad51 (185). The role of BRCA2 in RAD51

localization was also demonstrated in this species, where *Li*Rad51 is no longer found in the nucleus in the absence of *Li*Brca2 (185). This is consistent in that accumulation of RAD51 into nuclear foci is impaired in BRCA2-deficient cells and that RAD51 remains mostly cytoplasmic in CAPAN-1 cells, which contain a truncated version of BRCA2 (132, 193). The capacity of BRCA2 to mediate RAD51 activity also appears to be conserved, since *Li*Brca2 alleviates the inhibitory effect of hRPA, leading to binding of Rad51 on ssDNA, and stimulates Rad51 invasion *in vitro* (185). These results can account for the genomic instability and the reduced HR efficiency exhibited by the *Brca2* null mutants of *T. brucei* and *L. infantum* (185, 191, 192).

Although widespread among eukaryotes, RAD51 paralogs are probably the most poorly understood of the RAD51 mediators. In vertebrates, five RAD51 paralogs (RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3) are thought to play collaborative and nonredundant roles in HR (194). The crucial role for these paralogs in DNA repair and homologous recombination is evidenced by the sensitivity of hamster and chicken DT40 mutant cell lines to a variety of DNA-damaging agents and their propensity for reduced HR frequency and increased chromosomal instability (195, 196). On a structural level, these RAD51 paralogs share limited (20 to 30%) amino acid sequence identity with each other and with RAD51, with the conserved residues being concentrated primarily around the Walker A and B motifs (Fig. 6B) (197–199). In human cells, these are found in two primary complexes, RAD51B-RAD51C-RAD51D-XRCC2 and RAD51C-XRCC3, where RAD51C is the central component. Evidence for the existence of subcomplexes containing RAD51B-RAD51C and RAD51D-XRCC2 has been reported as well (200–204). A number of investigations in vertebrates are consistent with these paralogs acting as mediators of RAD51 presynaptic filament assembly and activity (Fig. 5). For instance, they are required for the formation of DNA damage-induced subnuclear RAD51 foci, and the RAD51B-RAD51C complex has been found to stimulate RAD51-mediated strand exchange (196, 201, 205–209). The paralogs have also been documented to interact with RAD51 (201, 202, 204, 206, 210). Moreover, increasing evidence points to functions for the RAD51 paralogs beyond the early stage of HR, as they have been implicated in the Holliday junction resolution process (Fig. 5). Data to support this include binding of CX3 and BCDX2 ring structures to Holliday junctions and Holliday junction branch migration and resolution activities by these complexes *in vitro* (211, 212). Evidence for BCDX2 acting upstream and CX3 downstream of RAD51 recruitment has been brought recently by RNA interference (RNAi) depletion studies (213).

Although no direct homologs of RAD51B-C-D, XRCC2, and XRCC3 exist in nonvertebrates, significant homologies exist between XRCC2 and XRCC3 and the *Saccharomyces cerevisiae* Rad55 and Rad57 proteins, respectively (198, 214). The yeast Rad51 paralogs form a tight heterodimer that is known to interact with Rad51 and aid its loading onto RPA-coated ssDNA, thereby promoting the strand exchange reaction (215). Recently, the Rad55-Rad57 heterodimer has been shown to protect Rad51-ssDNA filaments by counteracting the antirecombinase activity of the UvrD domain helicase Srs2 (216). This protection function is likely to be conserved in humans.

Sequence homology searches indicated that *Trypanosoma* species possess four Rad51 paralogs (Rad51-3, Rad51-4, Rad51-5, and Rad51-6), one of which (Rad51-5) is lacking in *Leishmania* (181). Like the human paralogs, they share the RecA core with RAD51, distinguishing themselves by unique C- and N-terminal extensions. Interestingly, however, the *T. brucei* paralogs are longer than other eukaryote homologs, with sizes ranging from 389 to 507 amino acids, versus 280 to 376 for the human polypeptides (179, 197). Nevertheless, protein alignments of RAD51 family members in tritryps proposed that Rad51-3 is closest to RAD51C (179), Rad51-4 to XRCC2, Rad51-5 to XRCC3, and Rad51-6 to RAD51D (see Fig. S7B in the supplemental material). Paralogs from tritryps present a number of features reminiscent of the human proteins. Importantly, they possess typical Walker A and Walker B motifs necessary for ATP hydrolysis (Fig. 6B). Furthermore, studies have shown that mutations in the conserved glycine and lysine residues of the Walker motif A of RAD51D affect DNA interstrand cross-link repair and that RAD51D K113R and K113A mutants no longer interact with RAD51C (217). Since this crucial lysine is conserved in all *Trypanosoma/Leishmania* species analyzed, it is likely that ATP hydrolysis is important for the regulation of the interaction between the *Leishmania* Rad51 paralogs. Genetic analyses have shown that mutants of each *T. brucei* paralog exhibit increased sensitivity to DNA damage induced by phleomycin and MMS, reduced recombination efficiency, and impaired RAD51 focus formation, consistent with a role in homologous recombination. The contribution of these paralogs to DNA repair appears to be nonequivalent, since *Rad51-4* $^{-/-}$ mutants are less sensitive to phleomycin-induced damage than the other mutants (179, 181). Yeast two-hybrid, glutathione *S*-transferase (GST) pulldown, and immunoprecipitation experiments have highlighted interactions between Rad51-3 and Rad51-4, Rad51-3 and Rad51-6, and Rad51-4 and Rad51-6, suggesting that they may act as complexes like their human counterparts. No evidence for interaction with Rad51 in *T. brucei* has been provided yet (179). However, the three *Leishmania infantum* Rad51 paralogs (*Li*Rad51-3, *Li*Rad51-4, and *Li*Rad51-6) were purified individually, and each protein bound *Li*Rad51 (M.-M. Genois et al., unpublished observations). We succeed in inactivating the *Li*Rad51-4 gene, leading to increased DNA damage sensitivity and growth delay (Genois et al., unpublished observations).

Furthermore, some evidence suggests that BIR might occur in *T. brucei* (218) and *Leishmania infantum* (219). TOPO3α, a member of the RecQ-Top3-Rmi1 (RTR) complex involved in suppression of crossovers, has been identified in *T. brucei* (Table 4). In fact, *Topo3*α-deficient cells show an increase in VSG switching, while unresolved intermediates would have been mostly repaired by BIR. Kim and Cross proposed that this protein might play a critical role in suppression of BIR-mediated VSG switching (recombinogenic structures), but extensive characterization of BIR still remains (218). Interestingly, Mukherjee et al. considered BIR as a mechanism for tandem duplication during their attempt to inactivate an essential telomeric gene (GSH1) in *Leishmania infantum* (219).

Meiosis and Meiotic Recombination

Meiosis is a crucial cell division for eukaryotes due to its importance in sexual reproduction and genetic diversity. The pairing of meiotic chromosomes, synaptonemal complex formation, recombination of homologous chromosomes in prophase I, and chromosome segregation are the steps unique to this cell division. In particular, programmed DNA DSBs can initiate meiotic recombination, which is 100- to 1,000-fold more frequent than mi-

totic recombination, to ensure physical exchanges between homologous chromosomes, thereby promoting genetic diversity (134). For many years, there has been evidence that genetic exchange occurs in *T. brucei* (220), *T. cruzi* (221, 222), and *L. major* (223). One major difficulty is that these organisms undergo many successive developmental forms. It was recently found that meiosis occurs in *T. brucei*. The localization of four meiosis genes (*Spo11*, *Dmc1*, *Hop1*, and *Mnd1*) fused to yellow fluorescent protein (YFP) was monitored in the salivary glands of the tsetse fly. The expression of YFP-MND1, YFP-DMC1, and YFP-HOP1 was restricted to a subset of trypanosome epimastigotes in the salivary glands (224). These three proteins are all involved in the meiotic recombination in prophase I: MND1 stabilizes heteroduplexes after double-strand break formation, DMC1 is a recombinase homologous to RAD51, and HOP1 is a component of the lateral elements of the synaptomenal complex. In addition, homologs of *spo11*, *dmc1*, *mnd1*, and *hop2* were identified in tritryp genomes (23, 26, 225).

Our bioinformatics analyses revealed a conservation ranging from 40 to 60% of the human/yeast meiosis pathway in *Trypanosoma* and *Leishmania* (Table 5; see Fig. S1B and C and S8 in the supplemental material). To initiate meiotic recombination, Spo11 creates a double-strand break in the early pachytene stage of prophase I in meiosis I (226). The initiation of meiotic recombination by Spo11 has been studied in yeast, mouse, worm (*Caenorhabditis elegans*), and other eukaryotes. It has been hypothesized that Spo11 binds double-stranded DNA as a dimer and cleaves DNA by a topoisomerase-like reaction to generate transient, covalent protein-DNA intermediates. On one hand, at least eight different proteins are involved during meiotic double-strand break formation and processes by Spo11 (Rec12). These proteins include Rec14, Rec6, Rec24, Rec7, Rec15, Mre11, Rad50, and Nbs1, but the specific roles of these proteins are still not clear. On the other hand, yeast Spo11 makes DSBs on specific chromosomal sites, named meiotic recombination hot spots, but recent genome-wide studies on budding yeast revealed that Spo11 can make DSBs not only on meiotic hot spots but also all over the chromosome (227). Our bioinformatic analyses revealed that the *Spo11* gene is conserved in trypanosomes and *Leishmania major* but not in *Leishmania infantum*. It has been reported (228, 229) that fission yeast Spo11 (Rec12) contains a distinct catalytic motif composed of amino acids R94, D95, Y97, and Y98 and a DNA-binding motif which is defined by glycine 202. Interestingly, these residues are conserved in *L. major*, *T. brucei*, and *T. cruzi* (Fig. 7A).

Following meiotic DSB formation, the MRE11-RAD50-NBS1 (MRN) complex removes Spo11 from the double-strand break site by the nuclease activity of MRE11 as Spo11 remains covalently bound to the 5′ end of the broken DNA. Following this, meiotic recombination can occur. In a genome-wide BLAST search for tritryps, MRN complex-coding genes were identified in *Leishmania* and *Trypanosoma* (Table 4). As mentioned above, crucial amino acids in the nuclease domain of MRE11 are conserved. Although this remains to be shown experimentally, these observations suggest that the processing of meiotic DSBs by Spo11 and removal in tritryps is a conserved mechanism.

In lower eukaryotes, Rad51 and Dmc1 are structural and functional homologs of *E. coli* strand exchange recombinase RecA. Rad51 is expressed abundantly in both mitotic and meiotic cells, but DMC1 is expressed specifically in meiotic cells, where it plays an important role in DNA strand exchange. A Dmc1 yeast mutant shows distinct phenotypes, such as accumulation of unrepaired DSBs, reduction of homolog pairing, delayed synapsis, and defective synaptonemal complex formation (230). For its ATPase catalytic activity, ATP binding, and ssDNA/dsDNA binding, human DMC1 is regulated through specific motifs. The N-terminal residues of DMC1 are highly flexible and help the interactions with the neighboring ATPase domain and the formation of helical filament structures (231). Several features of DMC1 are conserved in tritryps, including a helix-hairpin-helix motif involved in DNA binding, the Walker A and B motifs, and a polymerization motif involved in RAD51 nucleoprotein filament formation (232) (Fig. 7B). Localization studies using trypanosome transgenic cell lines expressing YFP fused to DMC1 showed a strong YFP-DMC1 nuclear signal in meiosis-specific cells and the formation of discrete nuclear foci, suggesting mechanistic similarities with yeast Dmc1 (224).

Genetic studies in yeast and mice have highlighted important roles for Hop2 and Mnd1 in meiotic recombination. Mnd1 stabilizes heteroduplex DNA and is bound to chromatin throughout the meiotic prophase, while Hop2 is required for the localization of Mnd1 on chromatin (233). *S. cerevisiae* Hop2 mutants arrest in meiotic prophase and show frequent synapsis with nonhomologous part of chromosome (234). On the other hand, Mnd1 mutants arrest before the first meiotic division, similar to the Dmc1 phenotype (235) (236). Using purified proteins from *S. cerevisiae*, the role of the Hop2-Mnd1 complex in HR was elucidated. In fact, this complex has the capacity to induce the activity of DMC1 to make DNA joints (236). In fission yeast, the Hop2-Mnd1 complex can physically interact with Dmc1 or Rad51 and stimulate strand invasion by these two recombinases (237). By bioinformatic analysis, we found that in contrast to the *Mnd1* gene, the *Hop2* gene is not well conserved through all tritryps. Structure-function analysis of Hop2 revealed that a region close to the C terminus of the protein is important for efficient DNA binding. Deletions of the last 36, 55, 67, or 74 amino acids in the C terminus of mouse Hop2 significantly decrease its DNA-binding ability (238). Surprisingly, only little homology is found at the C terminus of trypanosome Hop2 (Fig. 7C). As DNA-binding activity is required for Hop2 to promote D-loop formation and single-strand annealing, the weak conservation necessitates further analysis to decipher whether the *T. brucei* and *T. cruzi* sequences are homologs of Hop2.

POORLY CHARACTERIZED DNA REPAIR MECHANISMS IN TRITRYPS

Several DNA repair mechanisms are poorly defined at the molecular level in tritryps. In this section we review current knowledge on these pathways and possible homologs.

The Alkyltransferase Pathway

The abundant presence of alkylating agents in the environment as well as endogenous metabolic cellular products constantly threaten the DNA structure by generating species that react with the nitrogen (N) and oxygen (O) atoms of DNA bases. Among the variety of alkylation possibilities, the most toxic one is methylation at the O^6 position of guanine, called O^6-methylguanine (O^6meG), by S_N1-type agents. Even though the O^6meG is the most frequent O-alkyl lesion, it represents only ~5% of total alkyl DNA adducts, compared to 60 to 80% produced from N-alkyl lesions (239). The incorporation of O^6meG during replication is of

TABLE 5 Trypanosomatid genes involved in meiosis

Gene product designation (alternate designation)	Protein; function	Gene ID[a]						
		Human	*S. cerevisiae*	*S. pombe*	*L. infantum*	*L. major*	*T. brucei*	*T. cruzi*
Spo11 (Rec12)	Endonuclease; catalyzes formation of DSBs by topoisomerase-like transesterase reaction	NM_012444	YHL022C	SPAC17A5.11 (B)	X	LmjF.16.0630 (B)	Tb927.5.3760 (B)	Tc00.1047053503619.10 (B)
DMC1	RAD51 homolog	NM_007068	YER179W	SPAC8E11.03c (B)	LinJ.35.4950 (B)	LmjF.35.4890 (B)	Tb09.211.1210 (B)	Tc00.1047053506885.310 (B)
Hop2	Homologous-pairing protein 2; stimulates D-loop reaction, stabilizes presynaptic filament with Mnd1	NM_013290	YGL033W	SPAC222.15 (B)	X	X	Tb927.2.5190 (H)	Tc00.1047053511627.130 (H)
Mnd1	Meiotic nuclear division protein1; stimulates D-loop reaction, stabilizes presynaptic filament with Hop2	NM_032117	YGL183C	SPAC13A11.03 (B)	LinJ.24.1030 (H)	LmjF.24.1010 (H)	Tb11.02.3380 (B)	Tc00.1047053508647.10 (B)
Mei5 (Sfr1)	Recombination mediator in *S. cerevisiae* or *S. pombe* with Sae3	NM_001002759	YPL121C	X	X	X	X	X
Sae3 (Swi5)	Recombination mediator in *S. cerevisiae* or *S. pombe* with Mei5	NM_001040011	YHR079C-A	SPBC409.03 (B)	X	X	X	X
MSH4	MutS homolog 4; specialized for meiosis	NM_002440	YFL003C	SPAC8F11.03 (H), SPBC19G7.01c (Y)	LinJ.33.0420 (B)	LmjF.33.0410(B)	Tb927.10.11020 (H)	Tc00.1047053507711.320 (B)
MSH5	MutS homolog 5; specialized for meiosis	NM_002441	YDL154W	SPAC8F11.03 (H)	LinJ.33.0420 (H), LinJ.36.2050 (Y)	LmjF.33.0410(H), LmjF.36.1950(Y)	Tb927.3.4280 (B)	Tc00.1047053506237.10 (B)

[a] H, found from human homolog only; Y, found from yeast homolog only; B, found from both human and yeast homologs; X, no homolog.

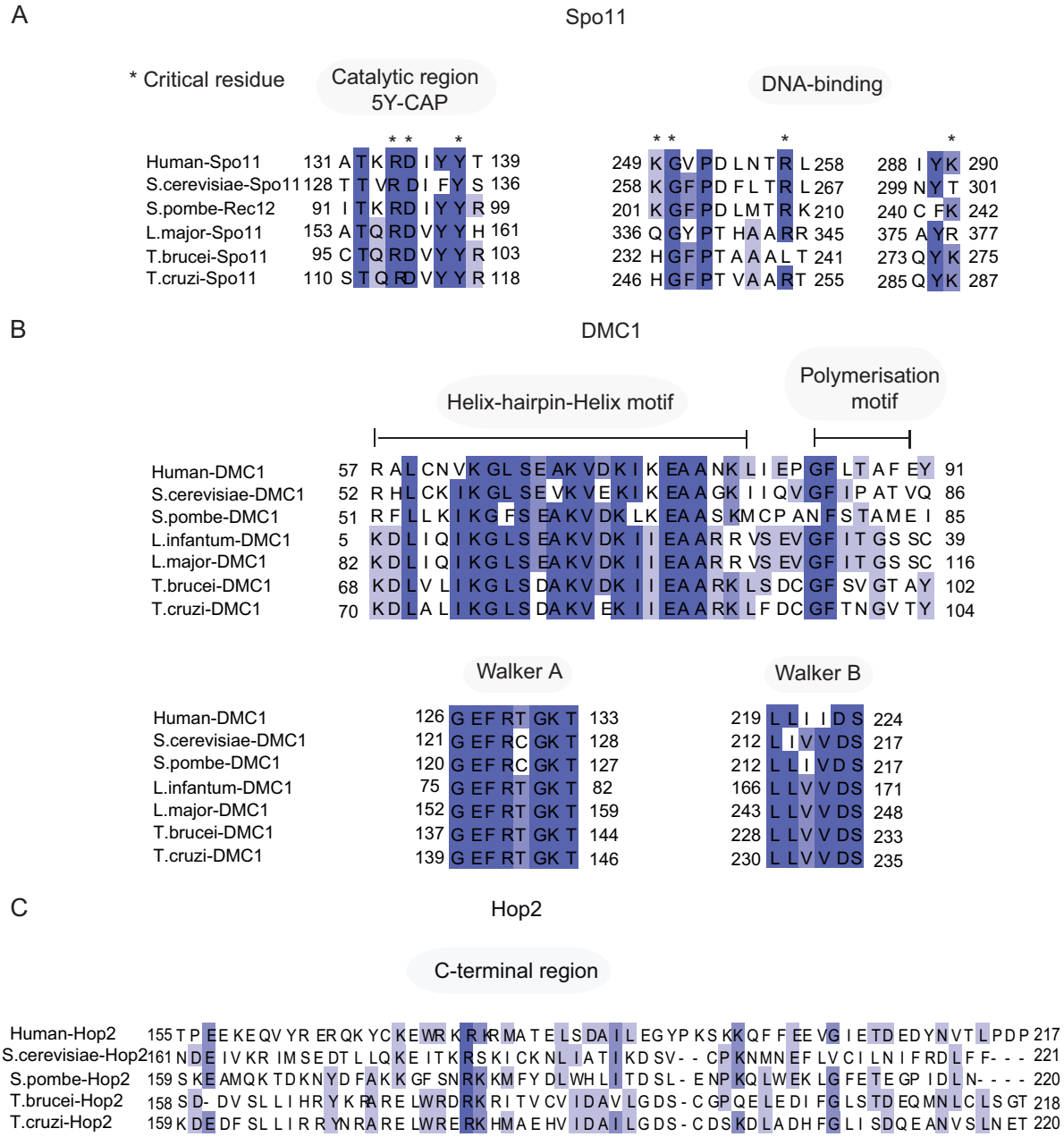

FIG 7 (A) Alignment of the catalytic and DNA-binding regions of Spo11 in the indicated species. (B) Alignment of the helix-hairpin-helix, polymerization motif, and Walker A and B motifs of DMC1 in the indicated species. (C) Alignment of the C terminus of Hop2 in the indicated species.

crucial importance, as it preferentially yields to misincorporation of thymine, leading to G·C-to-A·T transitions. Hence, O^6meG is the typical lesion (with 3-methyladenine) produced in chemotherapeutic alkylating drugs against cancer (239). Remarkably, a one-step DNA repair mechanism is executed by O^6-methylguanine DNA methyltransferase (Ada in *E. coli* or MGMT in humans, also known as ATase, AGT, or AGAT) (Fig. 8; see Fig. S9 and Table S1 in the supplemental material). Mechanistically, MGMT acts as a suicide enzyme, whereby transfer of the alkyl group from the guanine to its acceptor site (Cys-145) leads to irreversible inactivation of the protein; it is then ubiquitinated and targeted for proteasomal degradation (240, 241). Our bioinformatic analysis revealed that homologs of MGMT are present in *L. major*, *T. brucei*, and *T. cruzi* (see Table S1 in the supplemental material).

The Oxidative Demethylase Pathway

The biological consequences of DNA alkylation in causing clastogenic effects are often underscored due to efficient enzymatic removal. In fact, from a unique alkylating agent, different types of alkylated base lesions can be generated, and among them, cytotoxic N^1-methyladenine (N^1meA) and N^3-methylcytosine

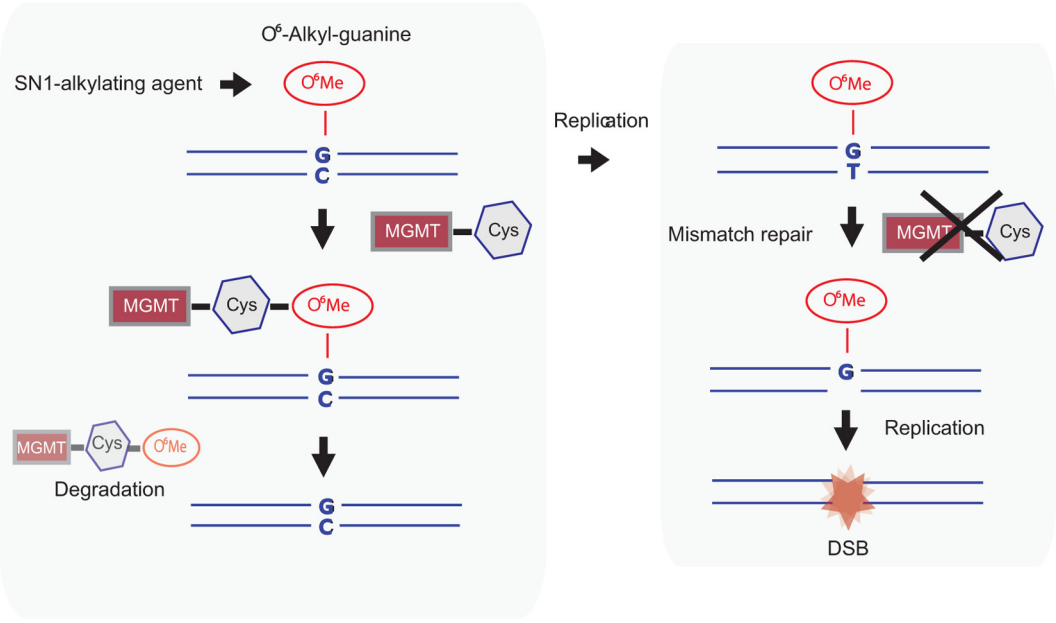

FIG 8 Repair of O^6-alkylguanine by direct reversal. A cysteine residue is used on the MGMT protein to remove the alkyl adducts on the O^6 position of guanine. If DNA replication occurs, O^6-alkylguanine mispairs with a thymine, which is removed by mismatch repair. This leads to a single-strand gap, which is converted in a DSB after a subsequent round of DNA replication.

(N^3meC) residues are directly repaired by oxidative demethylases (Fig. 9; see Fig. S9 and Table S1 in the supplemental material). Discovered in 1983 in *E. coli* (for a review, see reference 242), AlkB is a dioxygenase able to revert N-alkyl adducts. Basically, the enzyme requires two cofactors (α-ketoglutarate and Fe^{2+}) and releases the methyl group as formaldehyde to restore an unmethylated structure (243). Since then, eight human homologs (hABH1 to -8) have been discovered, where hABH2 and hABH3 have been shown to be functional homologs (244). Conceivably, the availability of crystal structures of AlkB, hABH2, and hABH3 could elucidate their role in DNA and RNA repair as demethylases (245, 246). No homologs have been found for this pathway.

The Photoreactivation Pathway

In contrast to the positive input as an energy source that sunlight can provide to Earth, UV light constantly jeopardizes the DNA backbone, triggering cyclobutane pyrimidine dimers or 6-4 photoproducts. Notably, these photolesions interfere with ongoing transcription and replication by distorting the DNA double helix. Photolyase, which does not exist in humans, binds and removes

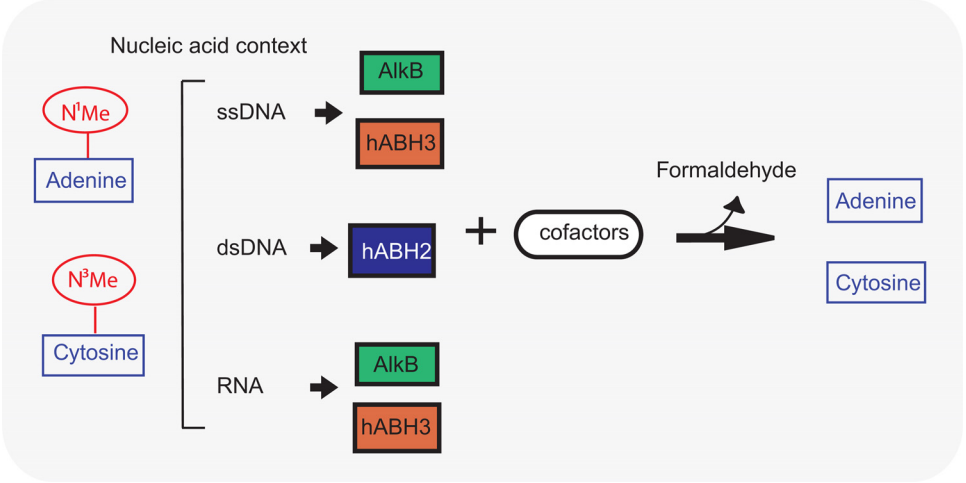

FIG 9 Repair of N^1-methyladenine (N^1meA) and N^3-methylcytosine (N^3meC) residues by oxidative demethylases. N^1meA and N^3meC occur in single- and double-stranded DNA and RNA. Depending on the context, the damage is repaired by the AlkB family of proteins, removing the alkyl group through oxidation and eliminating a methyl group as formaldehyde.

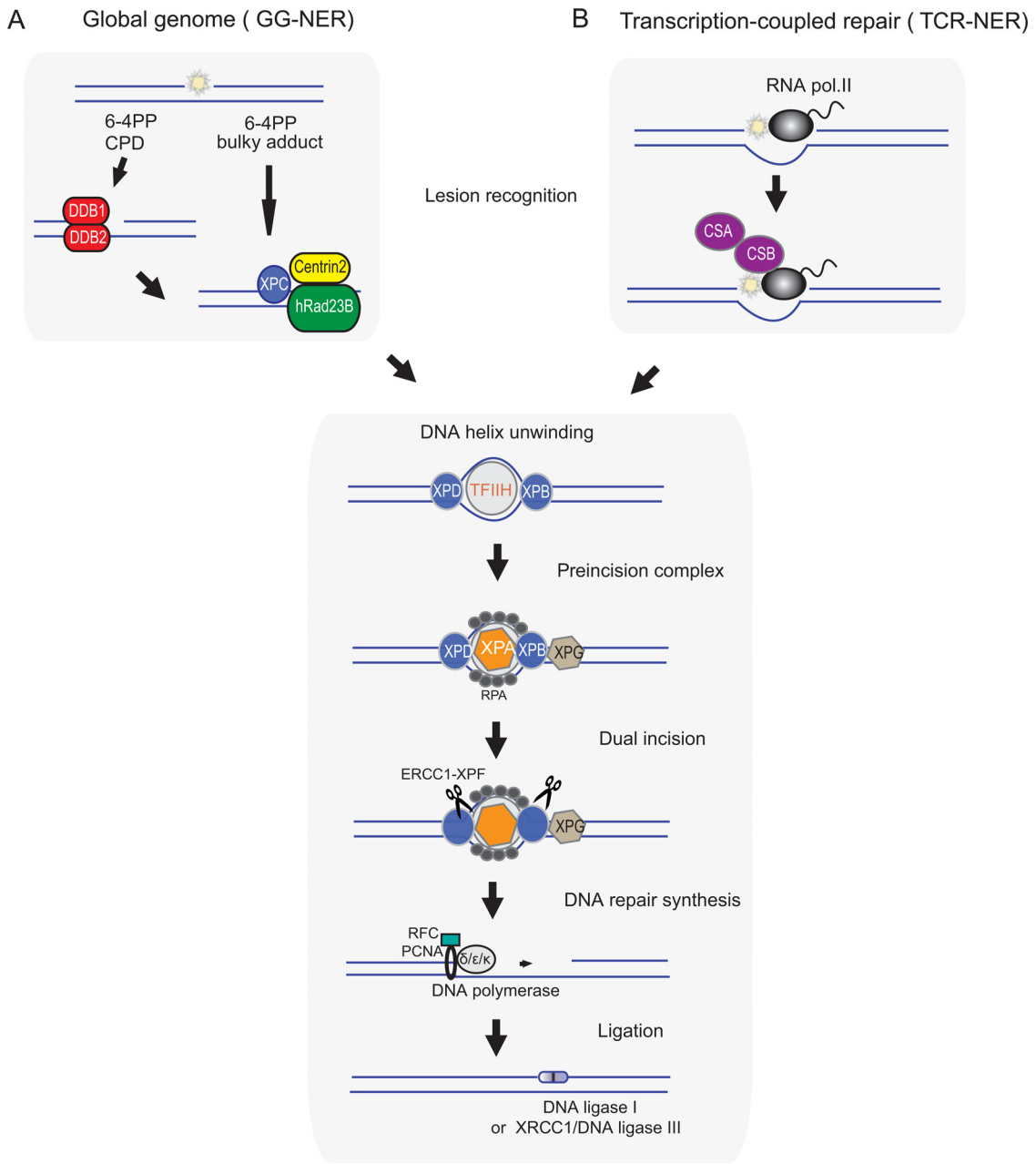

FIG 10 Global genome NER (A) and transcription-coupled NER (B). These two subpathways differ in how they recognize DNA damage, but they share the same process for lesion incision, repair, and ligation. Global genome NER repairs damage in both transcribed and untranscribed DNA strands in active and inactive genes, while transcription-coupled NER repairs transcriptionally active genes.

photoproducts by a process named photoreactivation (247). Species in all three kingdoms of life that lack this enzyme (for reviews, see references 248 and 249) may rely exclusively on the versatile nucleotide excision repair (NER), which is covered in the next section. More specifically, photolyase binds the cyclobutane pyrimidine dimers in a light-independent reaction by flipping them into the active-site pocket. Making specific contacts between dimers and flavin cofactors located within the core of the protein engages an efficient cyclic electron transfer in a light-dependent manner (250). Thus, this photorepair mechanism successfully splits the dimer into two pyrimidines to preserve genome integrity. We detected the presence of photolyase in several Trypanosomatidae species, although they remain to be fully characterized (see Fig. S9 and Table S1 in the supplemental material).

Nucleotide Excision Repair

First discovered in *E. coli* (251, 252) as a way to remove UV radiation products, nucleotide excision repair (NER) is an error-free repair pathway that recognizes an abnormal backbone conformation arising from either endogenous or exogenous DNA-damaging agents (Fig. 10). This process is known to be the most versatile DNA repair mechanism due to its wide substrate specificities

(253). The machinery termed "excinuclease" hydrolyzes the DNA phosphodiester backbone 3′ and 5′ of the DNA injury. A short DNA region is then excised, followed by DNA synthesis. In higher eukaryotic cells, over 30 proteins are involved in the elimination of UV-induced dipyrimidinic photolesions, cyclobutane pyrimidine dimers (CPDs), and pyrimidine-(6,4)-pyrimidone products (6-4PPs) as well as helix-distorting bulky adducts and intrastrand cross-links from chemicals or alkylating agents. Until now, 10 genes coding for the NER protein machinery have been associated with NER deficiency as genetic complementation groups (XP-A to -G, CS-A, CS-B, and TTD-A) (47). In humans, the absence of efficient NER leads to several autosomal recessive disorders such as UV sensitivity syndrome (UVSS), xeroderma pigmentosum (XP), Cockayne's syndrome (CS), and trichothiodystrophy (TTD) (240). There are two distinct NER subpathways that process similarly but that are initiated in different manners at the damage recognition step. Global genome NER (GG-NER) handles base lesions by scanning throughout the genome for DNA damage (Fig. 10A), whereas transcription-coupled NER (TC-NER) is specialized to deal with lesions that obstruct the transcription machinery (Fig. 10B). Because it searches lesions anywhere in the genome, GG-NER is considered to be a slow, transcription-independent process, less efficient than TC-NER. Nevertheless, 6-4PPs, which trigger helix distortions, are rapidly suppressed by GG-NER, while CPD removal is achieved efficiently by TC-NER on the transcribed strand (254).

DNA damage recognition. As mentioned above, there are different strategies in NER to perform DNA damage surveillance. Actors specific to the whole repair process (GG-NER) include the XPC-hRad23B complex (with centrin2), which recognizes UV-induced 6-4PPs with high affinity, and the auxiliary protein UV-damaged DNA binding complex (UV-DDB) formed by two subunits, DDB1/DDB2 (XPE), which facilitates the detection of lesions which are less recognized by XPC-hRad23B, such as CPDs. Initially identified as a complex that strongly binds UV-irradiated DNA (with a 500,000-fold preference over undamaged DNA), this heterodimer appears to play a role as a damage detector according to biochemical and X-ray crystallographic studies (255, 256). In striking contrast, XPC is considered a structure-specific DNA binding factor rather than a specialized protein that directly interacts with damaged bases (253). *In vitro* studies have shown that this essential player can initiate GG-NER alone but that in the presence of hRad23B and centrin2 (centrosomal protein), the complex is further stabilized, leading to an increase of XPC activity (257, 258). In addition, XPC also bears a binding domain for the multifunctional factor TFIIH, where a strong interaction has been observed *in vivo* and *in vitro*. Hence, previous findings suggested that DNA unwinding, the subsequent step, occurs in an XPC-dependent manner (259).

In the late 1970s, seminal findings described by Lehmann and colleagues suggested that cells from patients with the hereditary disorder Cockayne's syndrome (CS) that are exposed to UV light and RNA synthesis inhibition recover more slowly than normal cells (260). Further observations have shown that CS strains can repair normally pyrimidine dimers from the genome but not at the same rate as normal cells in transcriptionally active DNA. This genetic evidence underlies the presence of a special pathway coupled with transcription called TC-NER (261). This genetic defect is now associated with two proteins, CSA and CSB, which are involved in removing stalled RNA polymerase II at a lesion on the transcribed strands of active genes. To displace RNA PolIII, CSB interacts directly with it, while CSA is known to interact with CSB, XPA-binding protein 2 (XAB2), and the p44 subunit of the TFIIH complex. Here, CSA and CSB recruitment plays a crucial role in TC-NER, where cells from patients with Cockayne's syndrome exhibit normal GG-NER, deficient TC-NER, and UV light sensitivity (254, 262). In trypanosomatids, XPC, Rad23, and DDB1 (XPE) proteins involved in recognition of global genome repair have been identified, while only CSB (Rad26) is present for TC-NER (22) (see Fig. S10 and Table S2 in the supplemental material). Mechanistically, even if the lesion detection is divergent for the two processes, the following steps converge into the same route and components of proteins.

DNA helix unwinding. Normally known to initiate basal transcription, the multifunctional protein complex TFIIH also plays a key role in NER. It includes 10 subunits divided in two subcomplexes: the core complex formed of XPB, XPD, p62, p52, p44, p34, and p8, and the CDK-activating kinase (CAK) complex containing MAT1, CDK7, and cyclin H (254). In an ATP-dependent manner, this complex generates locally an open bubble structure by unwinding the DNA duplex around the lesion with its helicase activity conferred by XPB (3′ to 5′) and XPD (5′ to 3′) (263). After the formation of single-stranded DNA, the preincision complex (XPA, RPA, and XPG) stabilizes, protects, and facilitates the entry of endonucleases to the lesion site. Unlike in yeasts and humans, neither XPA nor RPA3 has been identified in tritryps (see Fig. S10 and Table S2 in the supplemental material), but tandem affinity purification and liquid chromatography-tandem mass spectrometry allowed identification of TFIIH core subunits XPB and XPD with their regulators TFB1 (p62), TFB2 (p52), TFB4 (p34), TFB5, and SSL1 (p44), suggesting formation of a complete TFIIH core complex (264, 265). However, the electron microscopy (EM) structure of *T. brucei* TFIIH lacks the cyclin-activating kinase (CAK) subcomplex, which is replaced by two essential trypanosomatid-specific subunits termed trypanosomatid-specific proteins 1 and 2 (TSP1/TSP2) (264). Thus, TSP sequences are not associated with a CAK subcomplex, since the highly conserved protein kinase domain and the N-terminal ring domain of MAT1 are missing.

Incision, DNA repair synthesis, and ligation. In concerted actions, a dual incision is performed at sites flanking the DNA injury by two structure-specific NER endonucleases: XPG (3′ incision) and ERCC1-XPF (5′ incision). The order of incision by XPG or ERCC1-XPF is not clear. In some studies, the 3′ activity or the presence of XPG was demonstrated to be a prerequisite for the 5′ incision, while in a "cut-patch-cut-patch" mechanism, the 5′ incision was proposed to precede the 3′ incision, without the presence of XPG (266, 267). In eukaryotes, a portion of 24 to 32 nucleotides is released from the damaged DNA strand. In order to fill the resulting gap that remains, DNA repair synthesis may be mediated by three DNA polymerases (δ, ε, and κ) with the aid of the elongation clamp (PCNA) and the clamp loader ATPase (RFC) (268). The final step, DNA ligation, is accomplished by DNA ligase I or XRCC1/DNA ligase III, which carry out gap filling in proliferating or nondividing cells, respectively (269). The identification of XPG and ERCC1-XPF in tritryps without recognizable ligase III or XRCC1 prompted us to conclude that the NER mechanism might occur with the help of ligase I only (see Fig. S10 and Table S2 in the supplemental material) (22).

RESISTANCE AND TREATMENT

Treatment of leishmaniasis and trypanosomiasis depends on a few chemotherapeutic agents (reviewed in references 270 and 271). Most of these drugs have limitations, such as cost, toxicity, and side effects, and with the emergence of resistance, several of these drugs are less efficient. In this section, we will recapitulate the link between DNA repair proteins and resistance mechanisms and highlight potential new drug strategies. Resistance mechanisms that may involve DNA repair proteins involve point mutations and modulation of parasitic gene expression by the amplification or deletion of key genes involved in resistance. This phenomenon of gene rearrangement is particularly prevalent in the genus *Leishmania* and is often observed in drug-resistant mutants (272).

Pentavalent antimonials are still the mainstay against most forms of leishmaniasis, and a frequent mechanism of antimony resistance is amplification of the ABC transporter *MRPA* gene, observed first in *in vitro* drug-resistant mutants (32, 37, 273, 274) but also in field isolates (275, 276). The MRPA protein sequesters antimony conjugated to thiols into an intracellular organelle (277), leading to antimony resistance. Amplification was found to be part of extrachromosomal circular or linear DNAs and rearrangements invariably happening at the level of homologous direct repeats for circular amplicons and at the level of inverted repeats for linear amplicons. One useful model drug that has been helpful in deciphering gene amplification mechanism in *Leishmania* is the antifolate methotrexate (MTX). Extrachromosomal circular DNA amplification of the *DHFR-TS* gene was also observed in cells resistant to MTX, while linear or circular amplification of the pteridine reductase *PTR1* gene was observed in many species (272, 278, 279). *DHFR-TS* is the target of MTX, and while the main function of PTR1 is to reduce pterins, it can also reduce folates, and hence their amplification and overexpression can lead to resistance. These amplification and deletion of DNA loci appear to occur via homologous recombination between homologous regions of the genomic DNA. It has been proved that many repeated DNA sequences are widespread throughout the *Leishmania* genome and can therefore recombine with each other in order to amplify or delete some DNA regions (43). Considering the importance of HR events in resistant *Leishmania* parasites, a discussion on the involvement of DNA repair protein implicated in HR is warranted. We have previously demonstrated that *L. infantum Brca2* null mutants display a decreased efficiency of HR (185). Furthermore, *Li*Brca2 was shown to be able to stimulate *Li*Rad51 DNA-binding and strand invasion activities, both of which are required in HR (185). Inactivation of these two enzymes or abolition of their interaction could then become a useful strategy to prevent DNA amplification and deletion through HR. In humans, some RAD51 inhibitors were identified by high-throughput screening of the NIH Small Molecule Repository (>200,000 compounds) (280). Specific RAD51 inhibitors for trypanosomatids could be found by this type of screening. To address this, we have generated a *Leishmania Rad51* null mutant which was then selected for drug resistance. The level of extrachromosomal circles formed by homologous recombination between direct repeated sequences was found to be lower in the *Rad51* null mutant (Ubeda et al., submitted). However, the level of linear amplicons formed by the annealing of inverted repeated sequences was found to be higher in the *Rad51* null mutant than in wild-type cells, suggesting that different recombination pathways may lead to different amplicons and that more that one pathway would need to be blocked to prevent the emergence of resistance by gene amplification in *Leishmania*. So far, no chemical component inhibiting Brca2 has been discovered. Nevertheless, a study performed in *T. brucei* took advantage of a unique and essential BRC sequence motifs whereby *Tb*Brca2 interacts with *Tb*Rad51 and showed that ectopic expression of a peptide aptamer mimicking the *Tb*Rad51-binding domain of *Tb*Brca2 inhibits DNA damage repair by HR (281). This property needs to be tested in *Leishmania*, where it may be employed to avoid HR between repeated DNA sequences and therefore suppress DNA amplification, which is responsible for drug resistance (Fig. 11). Knowing the important role of hMRE11 in HR, we can also hypothesize that this protein might play a role in the HR process occurring during DNA amplification and that its absence could alter the resistance. Inactivation of the *Mre11* gene in *Leishmania* was achieved, and when these cells were selected for drug resistance, we observed a marked decrease in the generation of linear amplicons (Laffitte et al., unpublished observations). Perhaps inhibition of both the Rad51 and Mre11 pathways might be necessary to reduce the emergence of drug resistance by gene amplification in *Leishmania*. With the aim of impairing Mre11 nuclease activity, a specific inhibitor called Mirin (282) can be helpful, although it also has activity against the human MRE11.

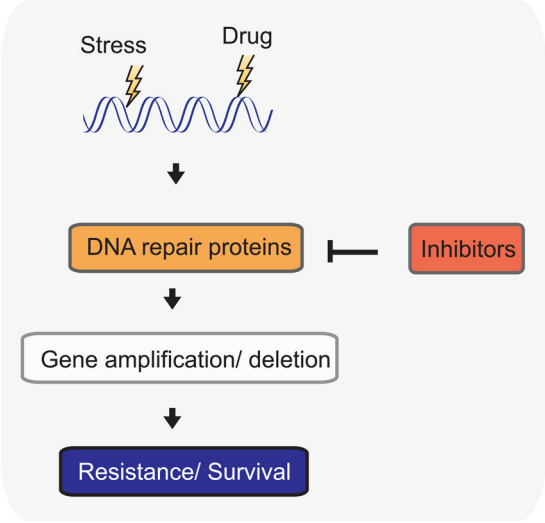

FIG 11 DNA repair proteins can be employed as a strategy to prevent or reduce resistance mechanisms in parasites. Given that stress or drugs elicit a DNA repair response leading to gene amplification/deletion and resistance, the use of DNA repair inhibitors can be a useful antiparasite strategy.

The HR pathway is also important in antigenic variation occurring in *T. brucei*. This mechanism allows the parasite to escape the immune system through variant surface glycoproteins (VSGs) by periodically switching the expression of the *VSG* genes (283). *Tb*Brca2 and *Tb*Rad51 play a major role in antigenic variation, where their disruption in *T. brucei* results in impaired VSG switching (182, 191). *Tb*Brca2 and *Tb*Rad51 appear to be the only DNA repair proteins involved in this phenomenon, since genetic inactivation of *TbMre11* or *TbKu70* and *TbKu80* does not affect antigenic variation (113, 164). The need to maintain genome integrity is crucial for parasites and could be targeted by new treatments strategies. In this regard, inactivation of DNA repair proteins might change genome stability. *T. brucei Mre11* and *Brca2* null

mutants developed considerable variation in chromosome size, indicating spontaneous gross chromosomal rearrangements (GCRs) associated with shortening of chromosomes (164, 191). However, in contrast to the case for *Ku70* and *Ku80* null mutants, loss of *Mre11* and *Brca2* in *T. brucei* did not result in telomere shortening (113). It should be noted that inactivation of *Mre11* and *Rad51* in *Trypanosoma* along with inactivation of *Brca2* in *Trypanosoma* and *Leishmania* was associated with a growth delay, a phenotype that can be useful in thinking about a strategy consisting of slowing parasite proliferation (158, 182, 185, 191). All these null mutants are more sensitive to DNA-damaging agents such as methyl methanesulfonate (MMS); however, using a strategy grounded on damaging the DNA should also alter the human cells.

Chemical agents are also known to induce stress features in trypanosomatids such as reactive oxygen species (ROS). Previous studies suggested that these features are markers of programmed cell death (284), but a recent review has provided a series of arguments questioning regulated cell death in protozoan parasites (285). Still, an increase of ROS production has been shown in sensitive *L. infantum* promastigotes under antimonial (SbIII), miltefosine, and amphotericin B treatments but was absent in resistant parasites, suggesting that, somehow, the resistant strains had succeeded in avoiding oxidative stress (28). ROS formation was also shown in *T. brucei* and in *T. cruzi* under dihydroquinoline derivative and nifurtimox treatment, respectively (286, 287). *Leishmania* parasites are known to be sensitive to ROS from macrophage (288). Parasites more sensitive to oxidative stress or unable to decrease ROS production could then become more sensitive to drug treatment. Some DNA repair proteins have been associated with oxidative stress and can be helpful in improving treatment efficiency. Inactivation of both *Msh2* alleles in *T. brucei* led to lower tolerance to H_2O_2, which induces oxidative stress (88). Surprisingly, *T. brucei Mlh1* null mutants were not sensitive to H_2O_2, suggesting a major role for *Tb*MSH2 in this event (180). Moreover, it was shown that upon H_2O_2 treatment, *Leishmania* promastigotes display a cleavage of a poly(ADP-ribose) polymerase (PARP)-like protein (289). Inhibiting the MSH2 protein or preventing the PARP-like protein cleavage could be a successful way of favoring oxidative stress in the cell. Another way of taking advantage of the major role of PARP is to inhibit the protein in order to impair DNA repair mechanisms and therefore cell proliferation and drug resistance. Some PARP inhibitors were tested in *T. cruzi*, where PARP inhibition was effective on the amastigote but not the trypomastigote forms in cell culture (64). Pretreatment of parasites with the PARP inhibitor Olaparib decreased the number of intracellular amastigotes in infected cells. Knocking down the human *parp* gene in infected cells also reduced the amount of intracellular parasites, suggesting that the human PARP protein seems to play a role as important as the *T. cruzi* one during infection. Furthermore, it was shown that inhibition of PARP in *Brca2*-deficient human cells affects cell survival and genomic stability (290). The lethality of PARP inhibition could be assayed in trypanosomatids if paired with BRCA2 inhibition using, for example, a BRC repeat. Targeting the PARP protein might be employed to diminish the infection rate and intracellular proliferation along with impairing genome integrity. However, DNA damage created in the infected human cells due to PARP inhibition could be harmful to the cells and should not be ignored in the use of a PARP inhibitor as a treatment.

According to their features, DNA repair proteins can be employed to prevent or reduce resistance mechanisms in parasites (Fig. 11). Moreover, some of these proteins might be used to affect infection and proliferation rates. Drug combinations for the treatment of leishmaniasis represent a promising area of research, and as such, targeting DNA repair in combination with other drugs should be considered. However, resistance should be avoided. For instance, miltefosine-paromomycin and SbIII-paromomycin combinations lead to resistance in *Leishmania donovani* (291). The combination of therapeutic arsenals to increase treatment efficiency as well as decrease cure duration and drug resistance is for the moment the most satisfactory strategy, since no vaccine against trypanosomatids has been discovered so far.

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