

Review Article

Overview of DNA Repair in *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major*

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A wide variety of DNA lesions arise due to environmental agents, normal cellular metabolism or intrinsic weaknesses in the chemical bonds of DNA. Diverse cellular mechanisms have evolved to maintain genome stability, including mechanisms to repair damaged DNA, to avoid the incorporation of modified nucleotides and to tolerate lesions (translesion synthesis). Studies of the mechanisms related to DNA metabolism in trypanosomatids have been very limited. Together with recent experimental studies, the genome sequencing of *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major*, three related pathogens with different life cycles and disease pathology, has revealed interesting features of the DNA repair mechanism in these protozoan parasites, which will be reviewed here.

1. Introduction

The trypanosomatids *Trypanosoma cruzi*, *Trypanosoma brucei*, and *Leishmania major* are the causative agents of Chagas disease, African sleeping sickness and leishmaniasis, respectively. These protozoan pathogens affect over 27 million people, primarily in developing countries within tropical and subtropical regions. There are no vaccines for these diseases and only a few drugs, which are largely ineffective due to toxicity and resistance [1].

These three pathogens (herein collectively referred to as Trityps) share many general characteristics, especially the presence of the unique mitochondrion, which contains a dense region named as kinetoplast. This mitochondrial region is composed by a network of several thousand minicircles and a few dozen maxicircles that form the kinetoplast DNA (kDNA) [2]. Minicircles encode guide RNAs that modify maxicircle transcripts by RNA editing while maxicircles are correspondent to the mitochondrial DNA in higher eukaryotes that encodes rRNAs and the subunits of respiratory complexes [2]. The mitochondrion replicates its DNA, maintains its structural integrity, and

undergoes division. Actually, kDNA replication always takes place earlier than mitosis, indicating that the kDNA may be needed for cell division, either by signaling a successful replication or by affecting the structure [3]. Furthermore, the trypanosome mitochondrion may hold vital metabolic pathways besides a possible role in Ca²⁺ homeostasis, fatty acid metabolism, and apoptosis [3]. In fact, kDNA function and integrity may play a crucial role in the survival of some stages of Trityps lifecycles [3–5]. However, the kDNA is subjected to large amounts of endogenous oxidative damage generated by oxidative phosphorylation. Thus, an efficient kDNA maintenance mechanism is necessary to repair and avoid oxidative lesions in the mitochondrial DNA.

The draft genome sequences of the Trityps, released in 2005, have allowed a better understanding of the genetic and evolutionary characteristics of these parasites [6–9]. A comparison of gene content and genome architecture of *T. cruzi*, *T. brucei*, and *L. major* revealed large syntenic polycistronic gene clusters. In addition, many species-specific genes, such as large surface antigen families, occur at nonsyntenic chromosome-internal and subtelomeric regions. Syntenic discontinuities are associated with retroelements, structural

RNAs, and gene family expansion. Along with these factors, gene divergence, acquisition and loss, and rearrangement within the syntenic regions help to shape the genome of each parasite [8]. Expansion of gene families by tandem duplication is a potential mechanism by which parasites can increase expression levels to compensate for a general lack of transcriptional control due to polycistronic structure and the absence of general transcription factors [7].

Concerning the individual features of each parasite, which reflect differences in their lifecycles, *T. brucei* has large subtelomeric arrays that contain variant surface glycoprotein (VSG) genes used by the parasite to evade the mammalian immune system. Meanwhile, over 50% of the *T. cruzi* genome consists of repeated sequences, such as genes for large families of surface molecules, which might function in immune evasion and adaptation to an intracellular environment. *Leishmania* spp. has a simpler genome but also has the ability to amplify genomic regions. This genus contains genes for the synthesis of complex surface glycoconjugates that are likely to enhance survival in the macrophage phagolysosome [8].

Analyses of the Trityps genomes have identified differences in the DNA maintenance mechanisms (nuclear and mitochondrial) between Trityps and other eukaryotes. DNA repair systems are responsible for preserving the genome stability *via* correcting DNA lesions caused by damaging agents both from the environment and endogenous metabolic processes [10–14]. This system embraces several distinct pathways: (1) sanitization of the nucleotide pool, (2) direct reversal of the base modifications by demethylation processes, by the action of photolyases or dioxygenases, or (3) excision of (i) oxidized, methylated, or misincorporated bases by base excision repair (BER), (ii) bulky damage by nucleotide excision repair (NER), and (iii) misincorporated bases in the newly replicated DNA strand by mismatch repair (MMR). DNA is also susceptible to single-strand breaks (SSBs) and double-strand breaks (DSBs), which can be repaired by homologous recombination (HR) and nonhomologous end joining (NHEJ). Even though these mechanisms repair the majority of DNA lesions, some of the damage remains, leading to mutations or block of the DNA replication. Alternative DNA polymerases can bypass these lesions in an error-free or error-prone fashion using a tolerance process known as translesion synthesis (TLS) [14]. Basic knowledge of DNA damage repair and tolerance processes is crucial to understanding how and why the genome is affected during the organism lifespan and how the cells will deal with it.

T. cruzi, *T. brucei*, and *L. major* appear to be able to catalyze most of the DNA repair pathways [6–9]. Here, we briefly review the current information on DNA repair mechanisms in Trityps with an emphasis on experimentally characterized genes (Table 1). We highlight the main features of the major DNA repair pathways and report the presence or absence of key genes in Trityps. Most of the genes were previously identified by their genome projects [6–9, 15], and few of them were identified through similarity screening and domain analysis. The gene “absence” could truly represent a nonoccurrence of the gene (whose function

could be compensated or not by another gene), a large sequence divergence, or even an annotation error, which made the search for a homolog difficult.

2. Direct Repair

Two mechanisms of direct repair are present in Trityps: alkylation reversal and oxidative damage repair [6–9]. These pathways perform immediate chemical reversals of specific forms of DNA damage. Single homologs of O⁶-methylguanine alkyltransferase (MGMT) can be found in the three genomes. This enzyme catalyzes the repair of O⁶-meG, a critical mutagenic lesion that yields G:C to A:T transitions [41]. AlkB, an iron-dependent dioxygenase that reverses DNA lesions (1-meA and 3-meC) in single-strand DNA (ssDNA) or RNA [42], is also present in Trityps. The third mechanism of direct repair utilizes photolyases, which catalyze the splitting of pyrimidine dimers into the constituent monomers, a process called photoreactivation [43]. *T. cruzi* does not contain a clear photolyase homolog although *T. brucei* and *L. major* are thought to perform photoreactivation because they have a gene that contains an N-terminal photolyase domain [6–9]. The absence of photoreactivation as a repair mechanism for pyrimidine dimers in *T. cruzi* could be associated with the availability of transcription-coupled repair (TCR), which would efficiently deal with such lesions. This subject is discussed further in a later section.

3. Base Excision Repair

BER is the predominant pathway for dealing with a wide range of lesions that modify individual bases without large effects on the double helix structure. Such modifications on DNA bases can arise as a result of oxidation, alkylation, and/or deamination. The BER pathway consists of modified base recognition and removal by a DNA glycosylase, cleavage of the sugar-phosphate backbone, and excision of the abasic (apurinic-apyrimidinic, AP) site by a DNA AP endonuclease, followed by DNA synthesis and ligation steps [44].

The gapfilling and rejoining steps can occur by either of two subpathways: short-patch BER or long-patch BER. In the short-patch BER subpathway, only one nucleotide is replaced by DNA Pol β and the nick is sealed by LIG3, all steps being coordinated by XRCC1 [45]. In long-patch BER, 2–13 nucleotides are replaced with the involvement of the replicative polymerases δ (Pol δ) or ϵ (Pol ϵ) [46]. This polymerization gives rise to a “flap” structure that is removed by FEN1 through a single-stranded break for subsequent nick ligation by ligase 1 (LIG1) [47]. The long-patch mechanism also involves PCNA, which interacts and coordinates the enzymes involved, and poly ADP-ribose polymerase (PARP), that binds to DNA SSBs preventing DSBs and facilitates access for the long-patch machinery [48].

The primary components of the BER pathway have been identified in *T. cruzi*, *T. brucei* and *L. major* genomes [6–9] and are organized in the *TritypDB* database [15]. The Trityps possess the enzymes required to effectively

TABLE 1

Gene	Function	Organism (Gene ID)	Experimental data	Ref.		
<i>BER Genes</i>						
Uracyl-DNA glycosylase (UNG)	Excision of uracil in DNA	<i>T. cruzi</i> (Tc00.1047053511277.330)	(i) <i>In vitro</i> activity (enhanced by AP endonuclease) (ii) Heterologous complementation of <i>E. coli</i>	[16, 17]		
AP endonuclease1	Cleavage of the phosphodiester bond at the 5' side of AP site	<i>T. cruzi</i> (Tc00.1047053507083.30)	(i) Heterologous complementation of <i>E. coli</i>	[18]		
		<i>L. major</i> (LmjF16.0680)	(i) Heterologous complementation of <i>E. coli</i> (ii) Increment of H ₂ O ₂ and methotrexate resistance	[18–20]		
POL β	Polymerization of DNA Strand displacement (long-patch) Cleavage of the 5' - dRP	<i>T. cruzi</i> (Tc00.1047053503955.20)	(i) <i>In vitro</i> activity	[21]		
		<i>T. brucei</i> (Tb927.5.2780)	(ii) Kinetoplast localization	[22]		
PARP	Binding to ssDNA Stimulation of DNA synthesis and strand displacement	<i>T. cruzi</i> (Tc00.1047053509721.60)	(i) <i>In vitro</i> activity (enhanced by SSB)	[23]		
<i>NER Genes</i>						
TFIIH-TFB1 TFIIH-TFB2 TFIIH-TFB4 TFIIH-TFB5	Component of TFIIH	<i>T. brucei</i> (Tb11.01.1200) <i>T. brucei</i> (Tb927.10.5210) <i>T. brucei</i> (Tb11.01.7730) <i>T. brucei</i> (Tb10.61.2600)	(i) Essential for initiating synthesis of spliced leader RNA	[24]		
TFIIH-XPB TFIIH-XPB		Component of TFIIH (helicase)			<i>T. brucei</i> (Tb11.01.7950Tb927.3.5100) <i>T. brucei</i> (Tb927.8.5980)	(i) Interaction with TSP1 and TSP2 (i) Nuclear localization
TFIIH-TSP1 TFIIH-TSP2					Trypanosomatid-specific component of TFIIH	<i>T. brucei</i> (Tb927.1.1080) <i>T. brucei</i> (Tb11.01.5700)
XAB2*		May function as a scaffold for protein complex formation				<i>T. cruzi</i> (Tc00.1047053509767.40) <i>T. brucei</i> (Tb927.5.1340) <i>L. major</i> (LmjF23.1550)
<i>MMR Genes</i>						
MSH2	Repair of single base-base and IDL mismatches Heterodimers with MSH3 or MSH6	<i>T. cruzi</i> (Tc00.1047053507711.320)	(i) Three isoforms with different efficiencies (ii) Involvement in oxidative stress response (independently from MLH1)	[25, 26]		
		<i>T. brucei</i> (Tb927.10.11020)	(i) Involvement in oxidative stress response (independently from MLH1) (ii) Microsatellite instability and MNNG tolerance in <i>MSH2/MLH1</i> double mutants (iii) Regulatory role in HR	[26–28]		
MLH1	Heterodimers with MutL homologs Matchmaker for coordinating events from mismatch binding to DNA synthesis	<i>T. brucei</i> (Tb927.8.6840)	(i) Microsatellite instability and MNNG tolerance in <i>MSH2/MLH1</i> double mutants (ii)Regulatory role in HR	[27, 28]		

TABLE 1: Continued.

Gene	Function	Organism (Gene ID)	Experimental data	Ref.
<i>NHEJ Genes</i>				
Ku70	DSB recognition DSB bridging nucleolytic processing of the ends	<i>T. brucei</i> (Tb927.3.5030)	(i) Telomere maintenance	[29, 30]
Ku80	Telomere maintenance	<i>T. brucei</i> (Tb927.6.1760)		
<i>HR Genes</i>				
Mre11	DSB end resection Nuclease activities	<i>T. brucei</i> (Tb927.2.4390)	(i) Mre11 mutations cause impairment of HR and increased DNA damage sensitivity	[31, 32]
		<i>T. cruzi</i> (Tc00.1047053503801.30)	(i) Gene expression induced by DNA damaging agents (ii) Involved in DSBs and oxidative lesions repair	[33]
Rad51	Recombinases	<i>T. brucei</i> (Tb11.01.0360)	(i) Null mutants led to impairments in VSG switch and DNA transformation, besides a higher sensitivity to genotoxic agents	[34]
		<i>L. major</i> (LmjF28.0550)	(i) Gene expression induced by DNA-damaging agents	[35]
Dmc1	Recombinases	<i>T. brucei</i> (Tb09.211.1210)	(i) DMC1 mutation does not affect HR or VSG switching	[36]
BRCA2	ssDNA binding Recombination mediator	<i>T. brucei</i> (Tb927.1.640)	(i) Expansion in the number of BRC repeats (ii) BRCA2 mutants display antigenic variation impairment and genome instability	[37]
Rad51-3		<i>T. brucei</i> (Tb11.02.0150)	(i) Rad51-3 mutations resulted in reduced levels of VSG switching, altered RAD51 localization following DNA damage and DNA damage sensitized parasites	[38]
Rad51-5	ssDNA binding Recombination mediator activity	<i>T. brucei</i> (Tzb10.389.1770)	(i) Rad51-5 mutations caused altered RAD51 localization following DNA damage and DNA damage sensitized parasites	[38]
<i>TLS Genes</i>				
Pol η	Error-free bypass of cis-syn cyclobutane pyrimidine dimers (CPDs)	<i>T. cruzi</i> (Tc00.1047053511911.120)	(i) Heterologous complementation of <i>S. cerevisiae</i> (ii) <i>In vitro</i> bypass of 8-oxoG (iii) Overexpression increases H ₂ O ₂ resistance	[39]
Pol κ	Bypass of N2-adducted dG lesions Extension of mismatched primer termini	<i>T. cruzi</i> (Tc00.1047053503755.30)	(i) Mitochondrial localization (ii) <i>In vitro</i> bypass of 8-oxoG (iii) DNA synthesis within recombination intermediates (iv) Overexpression increases zeocin, gamma radiation, and H ₂ O ₂ resistance	[40]

perform BER of different base lesions. However, it is not clear whether they can perform short-patch and long-patch BER since the homologs of LIG3 and XRCC1, which are supposedly essential for the short-patch mechanism [46, 49, 50], have not yet been identified in the three organisms.

However, these BER components are also absent in plants, and Córdoba-Cañero et al. [51] recently demonstrated that BER of uracil and abasic sites occurs in *Arabidopsis thaliana* whole-cell extracts by both single-nucleotide insertion and long-patch DNA synthesis. In contrast to the other Trityps,

the *L. major* genome allegedly does not encode for the PARP enzyme, which could play a role in the long-patch subpathway [7].

Different DNA glycosylases involved in the removal of modified bases from DNA have been characterized in Trityps. The Uracyl-DNA glycosylase from *T. cruzi* (TcUNG) was the first one to be characterized by Fárez-Vidal and coworkers [16]. They demonstrated that the enzyme activity was enhanced by the addition of an AP endonuclease from *L. major*, suggesting that there could be a functional interaction between the two enzymes [16]. Recently, Peña-Dias and colleagues [17] reported that TcUNG is able to complement *E. coli ung* mutants, and that the trypanosome enzyme has a catalytic activity similar to human UNG. Surprisingly, their results indicated that TcUNG is able to excise uracil in DNA *via* short-patch BER using a polymerase that follows a Pol β -like pattern of inhibition. The characterization of the TcUNG protein sequence suggested that it has a probable PCNA-binding motif and could be directed either to the mitochondrion or nucleus [17].

Another glycosylase found in Trityps is 8-oxoG-DNA glycosylase (OGG1), an enzyme that removes the oxidative lesion 7,8-dihydro-8-oxoguanine (also known as 8-oxoguanine or 8-oxoG) when it is paired with cytosine. Among the DNA damage caused by reactive oxygen species (ROS), 8-oxoG is of outstanding interest because of its highly mutagenic potential and abundance [52]. This lesion has the ability to mimic thymine functionally, forming a stable 8-oxoG:A base pair. This conformation allows the replicative DNA polymerases to efficiently bypass 8-oxoG failing to detect this damaged DNA base [53]. A functional homolog of OGG1 in *T. cruzi* has been studied *in vivo* by Furtado and colleagues (unpublished data). This gene is able to complement yeast OGG1 mutants, reducing the mutation rate of these cells. The expression of OGG1-GFP fusion protein in *T. cruzi* revealed that the intracellular localization of OGG1 is both nuclear and mitochondrial. In fact, overexpression of the OGG1 in *T. cruzi* diminishes the levels of 8-oxoG within the nucleus and mitochondrion after hydrogen peroxide (H₂O₂) treatment. The unusual localization of OGG1 in the mitochondrion could indicate the importance of the maintenance of the kDNA integrity in this parasite.

In addition to OGG1 glycosylase, MutT and MutY also contribute to counteract the mutagenesis effects of 8-oxoG. These three enzymes constitute the so-called GO-system [54]. MutT degrades 8-oxo-dGTP from the nucleotide pool to 8-oxo-dGMP, preventing mutations that arise from the misincorporation of this oxidized form of dGTP. On the other hand, the DNA glycosylase MutY removes adenine from the 8oxoG:A pair [54]. When 8-oxo-dGTP is misincorporated opposite adenine in template DNA, MutY can fix an A:T to C:G mutation because it removes the correct adenine from the A:8oxoG pair. Therefore, when MutY is present, the action of MutT is crucial because oxidized nucleotides must be eliminated from the nucleotide pool [54]. At the time the genome sequence was released, homologs of 8-oxoguanine hydrolase MutT were not encountered in the Trityps genome [6–9]. A more

accurate search of the Trityps genomes revealed that MutT homologs are present in *T. brucei*, *L. major*, and possibly in *T. cruzi* [15]. This is not unexpected given that these parasites have putative MutY homologs [15]. Indeed, a *T. cruzi* MutY homolog has been characterized (Kunrath-Lima, unpublished data). This gene is able to complement MutY-deficient bacteria, diminishing its mutation rates. Moreover, the *T. cruzi* MutY recombinant protein removes the adenine paired with 8-oxoG *in vitro* from a 30 mer fluorescent substrate.

The AP endonucleases 1 from *T. cruzi* and *L. major* have also been characterized [18–20]. Both were able to efficiently complement AP endonuclease-deficient *E. coli*, conferring resistance to alkylating and oxidizing agents [18]. The *L. major* AP endonuclease was more extensively studied, and the purified protein exhibited endonuclease and high 3' phosphodiesterase activities on AP DNA *in vitro*. Moreover, *Leishmania* parasites overexpressing the AP endonuclease showed increased H₂O₂ and methotrexate resistance as well as reduced DNA fragmentation [19]. The structural characteristics of the *L. major* enzyme exhibited similarities with previously characterized homologs [20].

Among the polymerases, Pol β from *T. cruzi* and *T. brucei* have already been characterized [21, 22]. The TcPol β localizes to the parasite kinetoplast and exhibits DNA polymerization and 5' dRP lyase activity [21]. Similarly, the TbPol β characterization also showed that, in addition to a mitochondrial localization, it is active as a DNA polymerase and as a lyase [22]. The cellular localization of these polymerases highlights an important feature of the Trityps: the presence of kDNA. The kDNA structure is so complex that it requires an unusual replication mechanism, which differs from higher eukaryotes [55, 56]. This complexity is reflected in the DNA repair and replication machinery that can be localized to this organelle [7]. Pol β is an example of a polymerase that shows a nuclear localization in higher eukaryotes [57] but is addressed to the kinetoplast in the Trityps [21, 22]. The *L. major* Pol β has not yet been experimentally characterized; however, a Pol β from *L. infantum* was shown to have a nuclear localization [58], which could indicate that the *L. major* polymerase is also nuclear, as their primary protein sequences showed 100% identity. The possibility that *L. major* possesses a nuclear Pol β , combined with the fact that this parasite does not have the PARP enzyme [7, 15], suggests that short-patch BER could play an important role in nuclear DNA repair for this organism. As *Leishmania* proliferates inside macrophage phagolysosomes, a well-coordinated nuclear short-patch BER is essential to combat oxidative DNA damage during parasite nuclear DNA replication [58]. The Trityps genomes apparently do not encode for the other X-family polymerases, DNA polymerase lambda (Pol λ), and mu (Pol μ) [7, 15]; thus, *L. major* may be the only Trityps parasite that has an X-family polymerase in the nucleus, reinforcing the importance of short-patch BER in this organelle.

PARP from *T. cruzi*, another enzyme that is involved in long-patch BER pathway, has also been characterized. The activity of this enzyme has been shown to be dependent on the presence of DNA and was enhanced by SSB in DNA

in a concentration-dependent manner. Moreover, it was demonstrated that DNA-damaging agents, such as H₂O₂ and β -lapachone, induced PAR synthesis in the parasite nucleus, indicating that this enzyme could be involved in the signaling of this phenomenon [23].

4. Nucleotide Excision Repair

Nucleotide excision repair is one of the most versatile DNA repair mechanisms, responsible for repairing lesions that alter the tridimensional DNA conformation, such as cisplatin adducts [59] and UV-induced lesions (pyrimidine dimers and pyrimidine photoproducts [60]). This mechanism can be divided into two major pathways: global genome repair (GGR), which operates in the noncoding parts of the genome and in the nontranscribed strand of active genes, and TCR, which is activated when a lesion appears in a gene that is being transcribed, ensuring that the transcribed strand of active genes has a higher priority for being repaired than the rest of the genome [61].

The GGR-NER mechanism comprises several steps: (i) distortion detection, performed by XPC and HR23B [62] or alternatively by the complex DDB1/XPE-DDB2 [63]; (ii) double-strand opening by the TFIIH complex *via* its XPB and XPD helicase subunits [64]; (iii) recruitment of XPA complexed with the three heterotrimeric replication protein A (RPA) subunits [65]; (iv) DNA incision by the XPG endonuclease (3' side of lesion [66]) and by the XPF-ERRC1 heterodimer (5' side of the lesion [67]); (v) gap filling by the replicative polymerases δ and ϵ associated with PCNA [68, 69]; (vi) nick sealing by ligase III together with XRCC1 (in quiescent cells) or at a lower level by ligase I (in actively replicative cells) [70].

TCR-NER has a mechanism similar to GGR, but it differs in the initial steps because it lacks the XPC and DDB1 complexes. TCR-NER is triggered by the stalling of RNA polymerase II, which subsequently recruits CSA, CSB, and XAB2. The following steps are performed by the TFIIH complex as in GGR [71].

Although the entire NER mechanism is well conserved in nature, there is no sequence homology between the NER proteins from bacteria and eukaryotes. Despite the sequence conservation shared by the eukaryotic NER proteins, not all the genes that encode those proteins are found among distantly related phylogenetic groups. The most remarkable examples are the lack of XPA in *Arabidopsis thaliana* and the lack of XPA, XPC, and XPE in *Plasmodium falciparum* [72], which suggest that the NER mechanism can have slight variations between different taxons.

The Trityps genomes contain the majority of the NER components [7, 15], but the biochemical mechanisms of this pathway may present some minor differences from the higher eukaryotes. Some of the genes are duplicated. For example, Trityps have two copies of XPB and DDB1 appear duplicated in *T. cruzi*. However, others such as XPA could not be identified in Trityps. It is also possible that the Trityps ligation step is different from the ligation step from higher eukaryotes. The Trityps lack a recognizable

ligase III, which together with its partner XRCC1 plays a major role in this final step. However, because their genomes encode ligase I, it might be possible that the ligation step is performed exclusively by this protein in those parasites. DDB2, which interacts with DDB1 and recognizes UV-induced lesions, and RPA3, a component of the RPA heterotrimer, also could not be identified in the genomes of these trypanosomatids. The TFIIH complex shows some differences when compared to yeast and mammals because it does not contain the cyclin-activating kinase (CAK) sub-complexes. In addition to that, a recent study showed that *T. brucei* TFIIH contains two trypanosomatid-specific subunits of TFIIH (TSP1 and TSP2), which are indispensable for parasite viability and transcription of splice-leader gene [24]. These subunits are also present in the genomes of *T. cruzi* and *L. major*.

Protein-coding genes are constitutively transcribed in trypanosomatids [73]. This peculiarity implies that TCR could be one of the most crucial mechanisms involved in repairing DNA damage in those parasites. Surprisingly, the Trityps genomes apparently lack the gene that encodes CSA. Although the role of CSA in TCR is not clear, recent evidence indicates that CSA is involved in CSB ubiquitination and degradation following UV irradiation [74], which would restore transcription at a normal rate after the repair. The absence of an evident CSA in Trityps implies that the trypanosomatid TCR differs from the standard TCR mechanism, either by the lack or divergence of this component, or by the presence of an alternative protein to perform this step. This could be related to the peculiar constitutive transcription of Trityps. In fact, overexpression of *T. cruzi* DNA polymerase η (Pol η), involved in the translesion synthesis of pyrimidine dimers, and overexpression or haploinsufficiency of RAD51, a key protein in HR, do not confer any protection against UV irradiation, which could suggest that the UV-induced lesions are fully repaired before the cell enters the S-phase ([39], Passos-Silva et al., submitted). In addition, results obtained by our group show that *T. cruzi* repairs cisplatin-induced lesions at an extremely high rate, with total lesion removal in less than an hour (Rajão, unpublished data). Taken together, these results led us to hypothesize that, in *T. cruzi*, lesions that cause DNA distortions are readily detected and repaired by TCR because the great majority of the protein-coding genes are transcribed constitutively. Whether the CSA absence or the presence of an alternative CSA is an adaptation to this distinctive repair is a topic for future investigation. When compared to other taxons, GGR-NER in trypanosomatids seems to be similar to the GGR pathway encountered in plants, as both groups of organisms share peculiarities regarding the presence and absence of some NER genes. Although plants encode all the TFIIH subunits and CSA, the plant genome, like Trityps, does not possess an identifiable XPA, RPA3, or ligase 3. In addition, the plant genome also carries two copies of XPB [72]. Interestingly, these DNA repair similarities found in Trityps and plants can also be observed in the MMR pathway, which could suggest that both groups might share some commonalities in their DNA repair mechanisms.

5. Mismatch Repair

Postreplicative DNA mismatch repair promotes genetic stability by repairing DNA replication errors (single base-base mismatches and insertion or deletion loops, IDLs), inhibiting recombination between nonidentical DNA sequences and participating in responses to DNA damage induced by genotoxic agents, such as H₂O₂, cisplatin, and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) [75].

The fundamental aspects of the pathway have been highly conserved throughout evolution. In essence, postreplicative MMR operates through (i) DNA mismatch recognition by MutS α (MSH2-MSH6) or MutS β (MSH2-MSH3), (ii) excision of the damaged DNA section mainly by ExoI, and (iii) DNA resynthesis by DNA Pol δ and ligation. Steps after DNA mismatch recognition are coordinated by MLH heterodimers that bind to MSH proteins and probably recruit and assemble downstream repair complexes. Strand discontinuities associated with DNA replication can serve as entry points for strand excision, conferring strand specificity to MMR [75].

Each trypanosomatid encodes a set of MMR proteins, which suggests they are fully competent for mismatch recognition and repair [7, 15]. Components of the MMR pathway are major players in processes known to generate genetic diversity, such as mutagenesis and DNA recombination. Evidences suggest that differences in MMR efficiency could be an important source of genetic diversity in organisms [76–79].

T. cruzi has a highly heterogeneous population, composed of a pool of strains with distinct characteristics such as morphology, growth rate, virulence, and sensitivity to drugs [80]. Despite its broad genetic diversity, three major lineages, named *T. cruzi* I, II and III, have been identified in the parasite population [81]. Studies with a number of molecular markers revealed that parasites belonging to the *T. cruzi* I lineage have lower genetic variability compared to *T. cruzi* II, and III [82–84]. The great genetic diversity observed in *T. cruzi* (and more precisely, in *T. cruzi* II strains) may play an important role in pathogenesis and survival of the parasite within its different hosts.

It is conceivable that components of DNA repair pathways participate in processes that resulted in increasing genetic variability within the parasite population [85]. MSH2, the core eukaryotic mismatch repair gene, has been characterized in *T. cruzi* [25, 26, 86]. Sequence analyses of TcMSH2 showed the existence of three distinct isoforms, named TcMSH2A, B, and C, encoded in the genome of *T. cruzi* I, III, and II strains, respectively [25]. It is possible that these isoforms have distinct protein activity, leading to variations in the efficiency of MMR. In fact, parasites that have TcMSH2A show increased sensitivity to cisplatin and MNNG, increased microsatellite instability, and greater resistance to H₂O₂ when compared to parasites expressing TcMSH2B or TcMSH2C ([25], Campos et al., submitted). Further studies are needed to determine if these variations in MMR efficiency have a broader impact on genetic variation and behavior in *T. cruzi* strains. Attempts to generate TcMSH2-null mutants indicate that, in addition to its role

in MMR, TcMSH2 acts in the parasite response to oxidative DNA damage in an MMR independent manner [26].

In *T. brucei*, MSH2 has been studied along with MLH1 [27]. Mutations in both genes give rise to increased microsatellite instability and lead to increased tolerance to the alkylating agent MNNG [27]. Both phenotypes are consistent with an impairment of nuclear MMR activity [75]. These results indicate that MMR in trypanosomatids is active in repairing errors that arise during replication and in response to chemical damage.

MMR also plays a regulatory role in homologous recombination in *T. brucei* [28]. Double mutants of MSH2 and MLH1 show an increased frequency of homologous recombination, both between perfectly matched DNA molecules and between DNA molecules with divergent sequences. However, MMR has little influence on antigenic variation in this parasite [28]. This topic is discussed in detail in the “Double strand break section”.

T. brucei MSH2-null mutants are more sensitive to H₂O₂ than wild-type cells [26]. Because MLH1-null mutants do not show this phenotype, TbMSH2 seems to have an additional role in dealing with oxidative damage, which may occur independently of MMR [26]. Interestingly, the heterologous expression of MSH2 from *T. cruzi* was able to counteract the increased sensitivity to H₂O₂ in the *T. brucei* MSH2-null mutant. However, it did not affect the classical MMR-deficient phenotypes, such as microsatellite instability and resistance to MNNG [26]. This differential activity of MSH2 has also been reported in colon adenocarcinoma cell lines where MSH2, but not MLH1, has been implicated in the repair of 8-oxoG [87]. In addition, *Helicobacter pylori*, which is suggested to be MMR-defective due to the lack of MutH and MutL homologs, presents a MutS homolog that is involved in repairing oxidative damage [88].

Four additional MSH-like genes can be found in the trypanosomatids: MSH3, MSH4, MSH5, and MSH6 [7, 15, 27]. The predicted MSH6 polypeptides in Trityps have N-terminal truncations relative to eukaryotic orthologues [27]. In comparison, MSH7, unique to plants, bears similar truncations in the N-terminus along with the conserved mismatch interaction residues indicative of the MSH6 subgrouping [89]. MSH4 and MSH5 predicted proteins that appear to lack an N-terminal mismatch interaction, indicating an absence of function in the mismatch repair and a possible role in meiotic recombination [27].

Concerning MutL-related genes, Trityps contain PMS1 and MLH1 [27]. Other MutL homologs, such as PMS2, MLH2, and MLH3, appear to be absent. Trypanosomatid MMR is therefore likely to involve only an MLH1-PMS1 heterodimer whereas the functions performed by the dimers formed between MLH1 and its three other binding partners in yeast are either absent or fulfilled by MLH1-PMS1.

6. Repair of Double-Strand Breaks

DNA DSBs are a particularly dangerous type of lesion. DSBs can arise when replication forks encounter blocking lesions, which leads to fork collapse, or can be induced by ionizing

radiation and radiomimetic chemicals. Failure to accurately repair such damage can result in cell death or large-scale chromosome changes, including deletions, translocations, and chromosome fusions that enhance genome instability. Two distinct and evolutionarily conserved pathways for DSB repair exist: homologous recombination and nonhomologous end joining [90].

6.1. Nonhomologous End Joining. NHEJ is frequently imprecise. The two ends of the DSB are held together and religated, often following the loss of some sequence by nucleolytic degradation or addition by polymerization [90].

Eukaryotic NHEJ is a multistep pathway beginning with limited end processing by the MRE11/RAD50/NBS1 (MRN) complex and initial recognition of DSBs through end binding by Ku, a ring comprised of the Ku70, and Ku80 subunits. In higher eukaryotes, the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is also recruited. In the final step, DNA ligase IV with its binding partners XRCC4 (Lif1 in yeast) and XLF (also called Cernunnos) seals the break [90].

NHEJ seems to be absent in trypanosomatids. With the exception of Mre11, Rad50, KU70 and KU80, no other factors implicated in NHEJ could be identified in these organisms. KU70 and KU80 have been identified in *T. brucei*, *T. cruzi*, and *L. major*. Studies in *T. brucei* have shown that these genes act in telomere maintenance [29, 30], a function they provide in addition to NHEJ [91]. However, the mutants of KU70 and KU80 did not display higher sensitivity to DNA-damaging agents, suggesting that they play, at most, a minor role in DSBs repair possibly due to the absence of NHEJ in this organism. The most striking absences are DNA ligase IV and XRCC4/Lif1 [92]. These absences in Trityps suggest one of two possibilities: either NHEJ is absent from these organisms or its catalytic components have been modified beyond recognition, perhaps using a distinct DNA ligase. These possibilities should be further investigated.

6.2. Homologous Recombination. HR is required for DNA DSBs repair and provides critical support for DNA replication in the recovery of stalled or broken replication forks. In addition, HR is involved in the repair of incomplete telomeres and in the correct segregation of homologous chromosomes during meiosis. The broad reaction scheme [93, 94] can be considered in three steps: initiation (or presynapsis) when the nucleolytic resection of DSBs occurs, generating single-stranded tails with 3'-OH ends; strand exchange (synapsis), when the end(s) of the DSB invades the intact DNA molecule *via* regions of sequence homology; resolution (postsynapsis), when strand exchange intermediates are separated and the DSB is repaired.

Homologous recombination is the major pathway of DSB repair in lower eukaryotes [95]. Essential components of this mechanism have been identified in the genome of *T. cruzi*, *T. brucei*, and *L. major*. HR can contribute to different strategies evolved by trypanosomatids to create genetic variability that is needed for survival in their hosts. Antigenic variation is used by *T. brucei* to evade the host immune system through the switch of surface proteins (VSGs). This

mechanism is regulated by HR, allowing the switch of one VSG at time to the expression site [85]. Meanwhile, *T. cruzi* displays a wide range of surface molecules that are highly polymorphic and may represent a useful arsenal to evade immune systems [85]. Recent works have been suggesting that HR is responsible for creating mosaic genes of surface molecules through segmental gene conversion and for decreasing the divergence between duplicated regions such as surface multigenic families [83, 96]. In addition, experiments with genetic manipulation have shown that homologous recombination is the main mechanism for integration of transformed DNA in these organisms [97–100].

The complex of proteins involved in the presynapsis step of HR can be found in Trityps, such as MRE11, Rad50, NSB1, and RPA. However, only MRE11 from *T. brucei* has been fully characterized. Mutation of MRE11 causes impairment in *T. brucei* homologous recombination, increases DNA damage sensitivity, and leads to gross chromosomal rearrangements [31, 32]. MRE11 does not contribute to recombination during antigenic variation, an important mechanism used by *T. brucei* to escape host immune response as mentioned before [32].

The core step of HR is the search for homology, homologous DNA pairing, and strand exchange reaction that is mediated by recombinases, such as RAD51 and DMC1. Both enzymes are present in Trityps. DMC1, a putative meiosis-specific recombinase, has only been studied in *T. brucei*. The lack of DMC1 does not affect HR repair or VSG switching in this parasite [36]. The presence of genes involved in meiosis is an intriguing feature of Trityps because they reproduce primarily through clonal reproduction [101]. Even though the population structure of each parasite is largely clonal [101], evidence of genetic exchange in the wild-type populations of *T. brucei* [102], *T. cruzi* [103, 104], and *L. major* [105] has been presented. However, it is unclear whether or not the existence of meiotic recombination genes implies that the trypanosomatids use meiosis.

RAD51 has been characterized in the three trypanosomatids. The expression of RAD51 in *T. cruzi* and *L. major* is induced by DNA-damaging agents [33, 35]. Moreover, the overexpression of RAD51 in *T. cruzi* confers a faster recovery and a more efficient DNA repair of DSBs formed after genotoxic treatment [33]. In addition, *T. cruzi* RAD51 accumulates in the nucleus after exposure to gamma radiation (Passos-Silva et al., submitted). Besides, the levels of Rad51 in *T. cruzi* reflect its susceptibility to oxidative agents. The overexpression of TcRad51 confers a greater resistance to H₂O₂ whereas the deletion of one of the TcRad51 alleles increases the sensitivity when compared to wild-type parasites (Passos-Silva et al., submitted). Thus, Rad51 seems to be involved in a greater resistance to oxidative damage in *T. cruzi* DNA. An active response to oxidative stress is an important feature of *T. cruzi* and *L. major* because they have an intracellular stage in the host that is subjected to a rigorous oxidizing environment [106]. For *T. brucei*, RAD51, and consequently HR, is directly involved in antigenic variation. Null mutants of RAD51 led to impairments in VSG switch and DNA transformation and

a higher sensitivity to genotoxic agents [34]. However, an RAD51-independent recombination pathway is also present, as evidenced by two mechanisms detected in *T. brucei* RAD51 mutants: (i) antigenic variation by gene conversion [34] and (ii) integration of transformed DNA by homology-based recombination although the frequency of detection is low [29]. Interchromosomal HR is the major pathway used by *T. brucei* to repair DSBs, as demonstrated by Glover and colleagues [107]. After the generation of single DSB through SceI endonuclease, RAD51 accumulates into the foci and a G2M checkpoint is activated [107]. In addition, Tritryps show intriguing differences concerning gamma radiation treatment, which generates high levels of DSBs. *T. cruzi* and *L. major* are highly resistant to gamma radiation when compared to other eukaryotes [33, 108, 109]. However, this resistance is not seen in *T. brucei* (unpublished data). In fact, after gamma radiation treatment, the expression of RAD51 in *T. cruzi* and *L. major* are induced [33, 35] whereas the RAD51 levels in *T. brucei* do not increase [34]. As mentioned before, these intriguing differences concerning the efficiency of recombination in Tritryps could be due to the distinct mechanisms used by these organisms to create genetic variability and to evade the mammalian immune system.

The loading of recombinases in the ssDNA is a rate-limiting process that is enhanced by recombination mediators [93]. BRCA2, the RAD51 paralogs, and RAD54 are among the recombination mediators present in trypanosomatids. RAD52, however, seems to be absent in these organisms. Whether or not this has a significant impact on recombination is unclear. Unlike the yeast mutant, in which RAD52 is a key protein for HR, mouse RAD52 mutants display an exceedingly mild recombination defect and no ionizing radiation sensitivity [110]. It is unclear which proteins functionally replace the yeast RAD52 protein in mammalian cells or trypanosomatids. One candidate is BRCA2, which is not found in budding yeast. BRCA2 can interact with RAD51 through the BRC repeat motifs [111–113] and unrelated sequences. BRCA2 from *T. cruzi* and *Leishmania* have two nonidentical BRC repeats [37]. On the other hand, *T. brucei* BRCA2 has undergone an expansion in the number of BRC repeats (15 BRC repeats), and these elements are crucial for the efficiency of HR and RAD51 localization. In addition, *T. brucei* BRCA2 mutants display antigenic variation impairment and genome instability [37]. Four RAD51 paralogs appear to be encoded by *T. brucei* and *T. cruzi*, one of which appears to be missing in *L. major*. Two of the *T. brucei* RAD51-like proteins play a role in DNA repair and recombination [38].

Studies in *T. brucei* have been showing that HR in this organism is regulated by MMR through the rejection of HR between insufficiently homologous DNA sequences. This has been evidenced by experiments done with MSH2 mutants which are able to recombine mismatched substrates more efficiently than wild type cells. Around 100 bp of homology are required for an efficient Rad51-mediated recombination [28]. In contrast, the HR that occurs during VSG switching uses a short and divergent substrate such as the 70 bp repeats upstream of VSG genes. Thus, the VSG switching may

happen through a specific recombination pathway that is independent of MMR or the suppression of MMR would be necessary [100, 114].

7. Translesion Synthesis

Lesions in DNA can block replicative DNA polymerases (Pol δ and Pol ϵ), causing the stall of the replication fork. This halt leads to PCNA monoubiquitination by Rad6/Rad18 complex, promoting the switch from replicative DNA polymerase to TLS DNA polymerase, which catalyses nucleotide insertion opposite the lesion. Then, nucleotide extension is performed mostly by DNA polymerase zeta (Pol ζ). After the extension step, replicative DNA polymerases return to DNA synthesis [115]. TLS DNA polymerases contain a minimally stringent catalytic site, allowing for the accommodation of templates containing damaged bases. Moreover, this group of specialized DNA polymerases has lost 3'-5' proofreading activity, having a highly mutagenic character [116].

T. cruzi, *T. brucei*, and *L. major* genomes encode for a wide variety of translesion synthesis proteins. Pol κ , Pol η , Rev1, and Pol ζ homologs are found in these species. PCNA and Rad6 homologs are also present. These parasites show an expansion of Pol κ gene, present in two, ten, and three copies in *T. cruzi*, *T. brucei*, and *L. major* genomes, respectively [6–9]. The gene duplication/amplification displayed by Tritryps Pol κ gene could result in an increment of Pol κ gene expression level which would compensate the lack of pretranscriptional mechanisms in these organisms [9].

Pol η from *T. cruzi* has been characterized *in vitro* and *in vivo* [39]. TcPol η is able to complement yeast Rad30 mutant (Pol η -null mutant), increasing yeast resistance to UV radiation, which indicates that Pol η is able to bypass UV lesions. Parasites overexpressing TcPol η show a higher resistance to H₂O₂ treatment. This resistance could be associated with the ability of TcPol η to bypass 8-oxoG lesions *in vitro*, suggesting that this enzyme is able to incorporate nucleotides opposite oxidative lesions as well. In contrast to the result seen in yeast, parasites overexpressing this nuclear polymerase do not show a higher resistance to UV radiation. The lack of conferred resistance might be related to the number of lesions remaining during S phase because it is possible that the majority of UV lesions would be repaired by TCR-NER prior to DNA replication, as the majority of the protein-coding genes are constitutively transcribed in this organism [39].

T. cruzi DNA polymerase kappa has been studied by our group. One copy of TcPol κ localizes in the *T. cruzi* mitochondrion [40]. This result indicates that *T. cruzi* is the first organism described in the literature to contain one exclusively mitochondrial Pol κ . Mitochondrial TcPol κ bypasses 8-oxoG *in vitro*, which correlates with the increase in H₂O₂ resistance observed in parasites overexpressing this protein. This DNA polymerase could also participate in the homologous recombination pathway in *T. cruzi* because it synthesizes DNA within recombination intermediates. Reinforcing this hypothesis, TcPol κ overexpression confers higher resistance to gamma radiation and zeocin, which are

agents known to cause DSBs [40]. Recent results have shown that the other copy of TcPolk has nuclear localization (Rajão, unpublished results).

TLS deals with DNA damage that blocks the replication fork, thus rescuing the cell from death. This accounts for the survival increase displayed by TcPolk-overexpressing parasites when treated with agents that cause DSBs. In addition, TcPolk- and TcPol η -overexpressing cells also presented increased resistance to H₂O₂ treatment [39, 40]. The presence of TLS DNA polymerases that efficiently bypass oxidative lesions might be important during *T. cruzi* lifecycle, especially in the intracellular amastigote phase, when this organism deals with ROS generated by the infected host cell [117]. Moreover, because TLS can operate in an error-prone fashion, TLS can generate DNA punctual mutations in the parasite genome [116]. This can be correlated with the generation of genetic variability in Trityps, notably in surface molecules. In fact, mutation is considered one of the main driving forces that increase the divergence between genes from multigenic families in *T. cruzi*, in contrast to the genetic conversion, another main driving force that decreases this divergence [83, 96]. A variable repertoire of surface molecules is a key strategy for *T. cruzi* to achieve a successful rate of infection. These proteins interact with different molecules on the host cell membranes and the extracellular matrix, increasing its chance to adapt to distinct cell types and hosts [83, 96]. Besides, the polymorphism of *T. cruzi* surface proteins contributes to evade cellular immune response of the mammalian host through the presentation of a broad range of possible target epitopes to CD8+T cells. This can result in an inefficient activation of naïve CD8+T cells, leading to a delayed protective immune response [118]. Thus, TLS can affect the general diversity in the organism, which is important for acquiring evolutionary novelty and for adaptation to the parasitic lifestyle.

8. Conclusion

The genome sequencing of *T. cruzi*, *T. brucei*, and *L. major* has provided insight into the DNA maintenance mechanisms in these pathological protozoa [6–9]. Experimental data have revealed additional particular features of these systems in the Trityps, which presumably reflect the distinct aspects of the infectious cycle that shape the survival strategies of each protozoa pathogen. Among these particularities, the unexpected mitochondrial localization of some typically nuclear proteins (Furtado, unpublished data) [21, 22, 40] and the additional roles of a few proteins in response to oxidative treatment were highlighted in this paper (Passos-Silva, submitted) [26, 39, 40]. The former is important during the replicative stages of the parasites when the metabolic activity is higher and the unique mitochondrion is then exposed to higher amounts of ROS generated by oxidative phosphorylation [3–5]. The later feature is particularly critical during the intracellular stage of the parasites *L. major* and *T. cruzi* when they are subjected to the immune response of mammalian host cells through oxidative stress [106, 119].

The DNA damage repair and tolerance mechanisms of Trityps are also involved in the generation of genetic variability which raises the successful rate of infection through the increasing of surface molecules diversity in *T. cruzi* and the expanding of the repertoire of VSGs in *T. brucei* [85]. Indeed, strains of *T. cruzi* that have a higher genetic variability (possibly a reflection of a less efficient MMR—Campos et al., unpublished data) are frequently associated with human infection [120, 121]. On the other hand, HR regulates antigenic variation in *T. brucei*, the strategy used by this parasite to evade mammalian immune system [106]. A clear association between DNA repair in *L. major* and evasion of the mammalian immunological response has not been established yet possibly due to a relatively narrow range of studies that investigate DNA repair in this organism.

Essentially, with the exception of NHEJ, the major DNA repair pathways appear to be present in Trityps [7]. Further studies are necessary to clarify the information about DNA repair pathways in Trityps, specifically the differences in Trityps machinery from the typical eukaryotic machinery for DNA repair, which could provide potential points of attack against the parasites.

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References

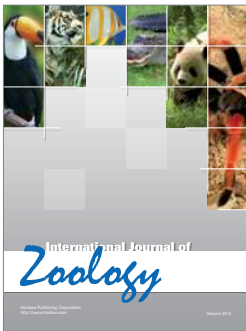
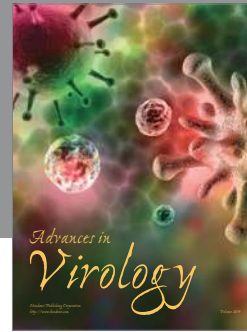
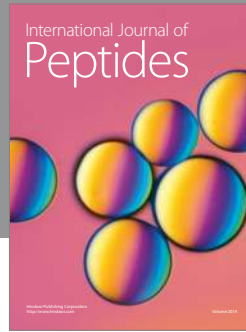
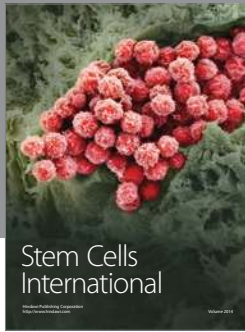
- [1] K. Nussbaum, J. Honek, C. M. Cadmus, and T. Efferth, “Trypanosomatid parasites causing neglected diseases,” *Current Medicinal Chemistry*, vol. 17, no. 15, pp. 1594–1617, 2010.
- [2] W. de Souza, “Structural organization of *Trypanosoma cruzi*,” *Memorias do Instituto Oswaldo Cruz*, vol. 104, no. 1, pp. 89–100, 2009.
- [3] A. Schnauffer, G. J. Domingo, and K. Stuart, “Natural and induced dyskinetoplastic trypanosomatids: how to live without mitochondrial DNA,” *International Journal for Parasitology*, vol. 32, no. 9, pp. 1071–1084, 2002.
- [4] A. O. M. Stoppani, R. Docampo, J. F. de Boiso, and A. C. C. Frasc, “Effect of inhibitors of electron transport and oxidative phosphorylation on *Trypanosoma cruzi* respiration and growth,” *Molecular and Biochemical Parasitology*, vol. 2, no. 1, pp. 3–21, 1980.
- [5] R. Dey, C. Meneses, P. Salotra, S. Kamhawi, H. L. Nakhasi, and R. Duncan, “Characterization of a Leishmania stage-specific mitochondrial membrane protein that enhances the activity of cytochrome c oxidase and its role in virulence,” *Molecular Microbiology*, vol. 77, no. 2, pp. 399–414, 2010.
- [6] M. Berriman, E. Ghedin, C. Hertz-Fowler et al., “The genome of the African trypanosome *Trypanosoma brucei*,” *Science*, vol. 309, no. 5733, pp. 416–422, 2005.
- [7] N. M. El-Sayed, P. J. Myler, D. C. Bartholomeu et al., “The genome sequence of *Trypanosoma cruzi*, etiologic agent of chagas disease,” *Science*, vol. 309, no. 5733, pp. 409–415, 2005.
- [8] N. M. El-Sayed, P. J. Myler, G. Blandin et al., “Comparative genomics of trypanosomatid parasitic protozoa,” *Science*, vol. 309, no. 5733, pp. 404–409, 2005.

- [9] A. C. Ivens, C. S. Peacock, E. A. Wortley et al., "The genome of the kinetoplastid parasite, *Leishmania major*," *Science*, vol. 309, no. 5733, pp. 436–442, 2005.
- [10] J. H. J. Hoeijmakers, "Genome maintenance mechanisms for preventing cancer," *Nature*, vol. 411, no. 6835, pp. 366–374, 2001.
- [11] D. Branzei and M. Foiani, "Maintaining genome stability at the replication fork," *Nature Reviews Molecular Cell Biology*, vol. 11, no. 3, pp. 208–219, 2010.
- [12] P. Huertas, "DNA resection in eukaryotes: deciding how to fix the break," *Nature Structural & Molecular Biology*, vol. 17, no. 1, pp. 11–16, 2010.
- [13] K. D. Arczewska and J. T. Kuśmierk, "Bacterial DNA repair genes and their eukaryotic homologues: 2. Role of bacterial mutator gene homologues in human disease. Overview of nucleotide pool sanitization and mismatch repair systems," *Acta Biochimica Polonica*, vol. 54, no. 3, pp. 435–457, 2007.
- [14] L. S. Waters, B. K. Minesinger, M. E. Wiltrout, S. D'Souza, R. V. Woodruff, and G. C. Walker, "Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance," *Microbiology and Molecular Biology Reviews*, vol. 73, no. 1, pp. 134–154, 2009.
- [15] M. Aslett, C. Aurrecochea, M. Berriman et al., "TriTrypDB: a functional genomic resource for the Trypanosomatidae," *Nucleic Acids Research*, vol. 38, supplement 1, pp. D457–D462, 2009.
- [16] M. E. Fárez-Vidal, C. Gallego, L. M. Ruiz-Pérez, and D. González-Pacanowska, "Characterization of uracil-DNA glycosylase activity from *Trypanosoma cruzi* and its stimulation by AP endonuclease," *Nucleic Acids Research*, vol. 29, no. 7, pp. 1549–1555, 2001.
- [17] J. Peña-Díaz, M. Akbari, O. Sundheim et al., "*Trypanosoma cruzi* contains a single detectable uracil-DNA glycosylase and repairs uracil exclusively via short patch base excision repair," *Journal of Molecular Biology*, vol. 342, no. 3, pp. 787–799, 2004.
- [18] J. Pérez, C. Gallego, V. Bernier-Villamor, A. Camacho, D. González-Pacanowska, and L. M. Ruiz-Pérez, "Apurinic/aprimidinic endonuclease genes from the trypanosomatidae *Leishmania major* and *Trypanosoma cruzi* confer resistance to oxidizing agents in DNA repair-deficient *Escherichia coli*," *Nucleic Acids Research*, vol. 27, no. 3, pp. 771–777, 1999.
- [19] C. Gallego, A. M. Estévez, E. Fárez, L. M. Ruiz-Pérez, and D. González-Pacanowska, "Overexpression of AP endonuclease protects *Leishmania major* cells against methotrexate induced DNA fragmentation and hydrogen peroxide," *Molecular and Biochemical Parasitology*, vol. 141, no. 2, pp. 191–197, 2005.
- [20] A. E. Vidal, M. Harkiolaki, C. Gallego et al., "Crystal Structure and DNA Repair Activities of the AP Endonuclease from *Leishmania major*," *Journal of Molecular Biology*, vol. 373, no. 4, pp. 827–838, 2007.
- [21] D. d. O. Lopes, B. L. F. Schamber-Reis, C. G. Regis-da-Silva et al., "Biochemical studies with DNA polymerase β and DNA polymerase β -PAK of *Trypanosoma cruzi* suggest the involvement of these proteins in mitochondrial DNA maintenance," *DNA Repair*, vol. 7, no. 11, pp. 1882–1892, 2008.
- [22] T. T. Saxowsky, G. Choudhary, M. M. Klingbeil, and P. T. Englund, "*Trypanosoma brucei* has two distinct mitochondrial DNA polymerase beta enzymes," *The Journal of biological chemistry*, vol. 278, no. 49, pp. 49095–49101, 2003.
- [23] S. H. Fernández Villamil, R. Baltanás, G. D. Alonso, S. C. Vilchez Larrea, H. N. Torres, and M. M. Flawiá, "TcPARP: a DNA damage-dependent poly(ADP-ribose) polymerase from *Trypanosoma cruzi*," *International Journal for Parasitology*, vol. 38, no. 3–4, pp. 277–287, 2008.
- [24] J. H. Lee, H. S. Jung, and A. Günzl, "Transcriptionally active TFIIH of the early-diverged eukaryote *Trypanosoma brucei* harbors two novel core subunits but not a cyclin-activating kinase complex," *Nucleic Acids Research*, vol. 37, no. 11, pp. 3811–3820, 2009.
- [25] L. Augusto-Pinto, S. M. R. Teixeira, S. D. J. Pena, and C. R. Machado, "Single-nucleotide polymorphisms of the *Trypanosoma cruzi* MSH2 gene support the existence of three phylogenetic lineages presenting differences in mismatch-repair efficiency," *Genetics*, vol. 164, no. 1, pp. 117–126, 2003.
- [26] A. Machado-Silva, S. M. R. Teixeira, G. R. Franco et al., "Mismatch repair in *Trypanosoma brucei*: heterologous expression of MSH2 from *Trypanosoma cruzi* provides new insights into the response to oxidative damage," *Gene*, vol. 411, no. 1–2, pp. 19–26, 2008.
- [27] J. S. Bell, T. I. Harvey, A.-M. Sims, and R. McCulloch, "Characterization of components of the mismatch repair machinery in *Trypanosoma brucei*," *Molecular Microbiology*, vol. 51, no. 1, pp. 159–173, 2004.
- [28] J. S. Bell and R. McCulloch, "Mismatch repair regulates homologous recombination, but has little influence on antigenic variation, in *Trypanosoma brucei*," *Journal of Biological Chemistry*, vol. 278, no. 46, pp. 45182–45188, 2003.
- [29] C. Conway, C. Proudfoot, P. Burton, J. D. Barry, and R. McCulloch, "Two pathways of homologous recombination in *Trypanosoma brucei*," *Molecular Microbiology*, vol. 45, no. 6, pp. 1687–1700, 2002.
- [30] C. J. Janzen, F. Lander, O. Dreesen, and G. A. M. Cross, "Telomere length regulation and transcriptional silencing in KU80-deficient *Trypanosoma brucei*," *Nucleic Acids Research*, vol. 32, no. 22, pp. 6575–6584, 2004.
- [31] K. S. W. Tan, S. T. G. Leal, and G. A. M. Cross, "*Trypanosoma brucei* MRE11 is non-essential but influences growth, homologous recombination and DNA double-strand break repair," *Molecular and Biochemical Parasitology*, vol. 125, no. 1–2, pp. 11–21, 2002.
- [32] N. P. Robinson, R. McCulloch, C. Conway, A. Browitt, and J. D. Barry, "Inactivation of Mre11 does not affect VSG gene duplication mediated by homologous recombination in *Trypanosoma brucei*," *Journal of Biological Chemistry*, vol. 277, no. 29, pp. 26185–26193, 2002.
- [33] C. G. Regis-da-Silva, J. M. Freitas, D. G. Passos-Silva et al., "Characterization of the *Trypanosoma cruzi* Rad51 gene and its role in recombination events associated with the parasite resistance to ionizing radiation," *Molecular and Biochemical Parasitology*, vol. 149, no. 2, pp. 191–200, 2006.
- [34] R. McCulloch and J. D. Barry, "A role for RAD51 and homologous recombination in *Trypanosoma brucei* antigenic variation," *Genes and Development*, vol. 13, no. 21, pp. 2875–2888, 1999.
- [35] P. G. McKean, J. K. Keen, D. F. Smith, and F. E. Benson, "Identification and characterisation of a RAD51 gene from *Leishmania major*," *Molecular and Biochemical Parasitology*, vol. 115, no. 2, pp. 209–216, 2001.
- [36] C. Proudfoot and R. McCulloch, "*Trypanosoma brucei* DMC1 does not act in DNA recombination, repair or antigenic variation in bloodstream stage cells," *Molecular and Biochemical Parasitology*, vol. 145, no. 2, pp. 245–253, 2006.

- [37] C. L. Hartley and R. McCulloch, "Trypanosoma brucei BRCA2 acts in antigenic variation and has undergone a recent expansion in BRC repeat number that is important during homologous recombination," *Molecular Microbiology*, vol. 68, no. 5, pp. 1237–1251, 2008.
- [38] C. Proudfoot and R. McCulloch, "Distinct roles for two RAD51-related genes in Trypanosoma brucei antigenic variation," *Nucleic Acids Research*, vol. 33, no. 21, pp. 6906–6919, 2005.
- [39] M. B. De Moura, B. L. F. Schamber-Reis, D. G. P. Silva et al., "Cloning and characterization of DNA polymerase η from Trypanosoma cruzi: roles for translesion bypass of oxidative damage," *Environmental and Molecular Mutagenesis*, vol. 50, no. 5, pp. 375–386, 2009.
- [40] M. A. Rajão, D. G. Passos-Silva, W. D. DaRocha et al., "DNA polymerase kappa from Trypanosoma cruzi localizes to the mitochondria, bypasses 8-oxoguanine lesions and performs DNA synthesis in a recombination intermediate," *Molecular Microbiology*, vol. 71, no. 1, pp. 185–197, 2009.
- [41] A. E. Pegg, "Repair of O6-alkylguanine by alkyltransferases," *Mutation Research*, vol. 462, no. 2-3, pp. 83–100, 2000.
- [42] L. Aravind and E. V. Koonin, "The DNA-repair protein AlkB, EGL-9, and leprecan define new families of 2-oxoglutarate- and iron-dependent dioxygenases," *Genome Biology*, vol. 2, no. 3, p. RESEARCH0007, 2001.
- [43] A. Sancar, "Structure and function of photolyase and in vivo enzymology: 50th anniversary," *Journal of Biological Chemistry*, vol. 283, no. 47, pp. 32153–32157, 2008.
- [44] A. B. Robertson, A. Klungland, T. Rognes, and I. Leiros, "Base excision repair: the long and short of it," *Cellular and Molecular Life Sciences*, vol. 66, no. 6, pp. 981–993, 2009.
- [45] Y. Kubota, R. A. Nash, A. Klungland, P. Schär, D. E. Barnes, and T. Lindahl, "Reconstitution of DNA base excision-repair with purified human proteins: interaction between DNA polymerase β and the XRCC1 protein," *EMBO Journal*, vol. 15, no. 23, pp. 6662–6670, 1996.
- [46] G. Frosina, P. Fortini, O. Rossi et al., "Two pathways for base excision repair in mammalian cells," *Journal of Biological Chemistry*, vol. 271, no. 16, pp. 9573–9578, 1996.
- [47] K. Kim, S. Biade, and Y. Matsumoto, "Involvement of flap endonuclease 1 in base excision DNA repair," *Journal of Biological Chemistry*, vol. 273, no. 15, pp. 8842–8848, 1998.
- [48] D. D'Amours, S. Desnoyers, I. D'Silva, and G. G. Poirier, "Poly(ADP-ribosylation) reactions in the regulation of nuclear functions," *Biochemical Journal*, vol. 342, no. 2, pp. 249–268, 1999.
- [49] I. I. Dianova, K. M. Sleeth, S. L. Allinson et al., "XRCC1-DNA polymerase β interaction is required for efficient base excision repair," *Nucleic Acids Research*, vol. 32, no. 8, pp. 2550–2555, 2004.
- [50] Y. Uchiyama, R. Takeuchi, H. Kodera, and K. Sakaguchi, "Distribution and roles of X-family DNA polymerases in eukaryotes," *Biochimie*, vol. 91, no. 2, pp. 165–170, 2009.
- [51] D. Córdoba-Cañero, T. Morales-Ruiz, T. Roldán-Arjona, and R. R. Ariza, "Single-nucleotide and long-patch base excision repair of DNA damage in plants," *Plant Journal*, vol. 60, no. 4, pp. 716–728, 2009.
- [52] S. Boiteux and J. P. Radicella, "The human OGG1 gene: structure, functions, and its implication in the process of carcinogenesis," *Archives of Biochemistry and Biophysics*, vol. 377, no. 1, pp. 1–8, 2000.
- [53] G. W. Hsu, M. Ober, T. Carell, and L. S. Beese, "Error-prone replication of oxidatively damaged DNA by a high-fidelity DNA polymerase," *Nature*, vol. 431, no. 7005, pp. 217–221, 2004.
- [54] T. Tsuzuki, Y. Nakatsu, and Y. Nakabeppu, "Significance of error-avoiding mechanisms for oxidative DNA damage in carcinogenesis," *Cancer Science*, vol. 98, no. 4, pp. 465–470, 2007.
- [55] R. Woodward and K. Gull, "Timing of nuclear and kinetoplast DNA replication and early morphological events in the cell cycle of Trypanosoma brucei," *Journal of Cell Science*, vol. 95, no. 1, pp. 49–57, 1990.
- [56] M. M. Klingbeil, S. A. Motyka, and P. T. Englund, "Multiple mitochondrial DNA polymerases in Trypanosoma brucei," *Molecular Cell*, vol. 10, no. 1, pp. 175–186, 2002.
- [57] D. N. Foster and T. Gurney Jr., "Nuclear location of mammalian DNA polymerase activities," *Journal of Biological Chemistry*, vol. 251, no. 24, pp. 7893–7898, 1976.
- [58] S. Taladriz, T. Hanke, M. J. Ramiro et al., "Nuclear DNA polymerase beta from Leishmania infantum. Cloning, molecular analysis and developmental regulation," *Nucleic Acids Research*, vol. 29, no. 18, pp. 3822–3834, 2001.
- [59] E. E. Trimmer and J. M. Essigmann, "Cisplatin," *Essays in Biochemistry*, vol. 34, pp. 191–211, 1999.
- [60] M. Tijsterman, R. De Pril, J. G. Tasseront-De Jong, and J. Brouwer, "RNA polymerase II transcription suppresses nucleosomal modulation of UV- induced (6-4) photoproduct and cyclobutane pyrimidine dimer repair in yeast," *Molecular and Cellular Biology*, vol. 19, no. 1, pp. 934–940, 1999.
- [61] T. Nospikel, "Nucleotide excision repair: variations on versatility," *Cellular and Molecular Life Sciences*, vol. 66, no. 6, pp. 994–1009, 2009.
- [62] M. Araki, C. Masutani, M. Takemura et al., "Centrosome protein centrin 2/caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair," *Journal of Biological Chemistry*, vol. 276, no. 22, pp. 18665–18672, 2001.
- [63] J. Tang and G. Chu, "Xeroderma pigmentosum complementation group E and UV-damaged DNA-binding protein," *DNA Repair*, vol. 1, no. 8, pp. 601–616, 2002.
- [64] F. Coin, V. Oksenyh, and J.-M. Egly, "Distinct roles for the XPB/p52 and XPD/p44 subcomplexes of TFIIH in damaged DNA opening during nucleotide excision repair," *Molecular Cell*, vol. 26, no. 2, pp. 245–256, 2007.
- [65] K. Sugawara, J. M. Y. Ng, C. Masutani et al., "Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair," *Molecular Cell*, vol. 2, no. 2, pp. 223–232, 1998.
- [66] A. O'Donovan, A. A. Davies, J. G. Moggs, S. C. West, and R. D. Wood, "XPG endonuclease makes the 3' incision in human DNA nucleotide excision repair," *Nature*, vol. 371, no. 6496, pp. 432–435, 1994.
- [67] D. Mu, D. S. Hsu, and A. Sancar, "Reaction mechanism of human DNA repair excision nuclease," *Journal of Biological Chemistry*, vol. 271, no. 14, pp. 8285–8294, 1996.
- [68] O. Popanda and H. W. Thielmann, "The function of DNA polymerases in DNA repair synthesis of ultraviolet-irradiated human fibroblasts," *Biochimica et Biophysica Acta*, vol. 1129, no. 2, pp. 155–160, 1992.
- [69] M. K. K. Shivji, M. K. Kenny, and R. D. Wood, "Proliferating cell nuclear antigen is required for DNA excision repair," *Cell*, vol. 69, no. 2, pp. 367–374, 1992.

- [70] J. Moser, H. Kool, I. Giakzidis, K. Caldecott, L. H. F. Mullenders, and M. I. Fousteri, "Sealing of chromosomal DNA nicks during nucleotide excision repair requires XRCC1 and DNA ligase III α in a cell-cycle-specific manner," *Molecular Cell*, vol. 27, no. 2, pp. 311–323, 2007.
- [71] S. Tornaletti, "DNA repair in mammalian cells: transcription-coupled DNA repair: directing your effort where it's most needed," *Cellular and Molecular Life Sciences*, vol. 66, no. 6, pp. 1010–1020, 2009.
- [72] R. M. A. Costa, V. Chiganças, R. D. S. Galhardo, H. Carvalho, and C. F. M. Menck, "The eukaryotic nucleotide excision repair pathway," *Biochimie*, vol. 85, no. 11, pp. 1083–1099, 2003.
- [73] S. Martínez-Calvillo, J. C. Vizuet-de-Rueda, L. E. Florencio-Martínez, R. G. Manning-Cela, and E. E. Figueroa-Angulo, "Gene expression in trypanosomatid parasites," *Journal of Biomedicine & Biotechnology*, vol. 2010, Article ID 525241, 15 pages, 2010.
- [74] R. Groisman, I. Kuraoka, O. Chevallier et al., "CSA-dependent degradation of CSB by the ubiquitin-proteasome pathway establishes a link between complementation factors of the Cockayne syndrome," *Genes and Development*, vol. 20, no. 11, pp. 1429–1434, 2006.
- [75] M. J. Schofield and P. Hsieh, "Dna mismatch repair: molecular mechanisms and biological function," *Annual Review of Microbiology*, vol. 57, pp. 579–608, 2003.
- [76] K. Drotschmann, P. V. Shcherbakova, and T. A. Kunkel, "Mutator phenotype due to loss of heterozygosity in diploid yeast strains with mutations in MSH2 and MLH1," *Toxicology Letters*, vol. 112–113, pp. 239–244, 2000.
- [77] J. E. LeClerc, B. Li, W. L. Payne, and T. A. Cebula, "High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens," *Science*, vol. 274, no. 5290, pp. 1208–1211, 1996.
- [78] O. Tenaillon, F. Taddei, M. Radman, and I. Matic, "Second-order selection in bacterial evolution: selection acting on mutation and recombination rates in the course of adaptation," *Research in Microbiology*, vol. 152, no. 1, pp. 11–16, 2001.
- [79] O. Tenaillon, B. Toupance, H. L. Nagard, F. Taddei, and B. Godelle, "Mutators, population size, adaptive landscape and the adaptation of asexual populations of bacteria," *Genetics*, vol. 152, no. 2, pp. 485–493, 1999.
- [80] A. M. Macedo, C. R. Machado, R. P. Oliveira, and S. D. J. Pena, "*Trypanosoma cruzi*: genetic structure of populations and relevance of genetic variability to the pathogenesis of chagas disease," *Memorias do Instituto Oswaldo Cruz*, vol. 99, no. 1, pp. 1–12, 2004.
- [81] J. M. de Freitas, L. Augusto-Pinto, J. R. Pimenta et al., "Ancestral genomes, sex, and the population structure of *Trypanosoma cruzi*," *PLoS Pathogens*, vol. 2, no. 3, p. e24, 2006.
- [82] C. A. Buscaglia and J. M. Di Noia, "*Trypanosoma cruzi* clonal diversity and the epidemiology of Chagas' disease," *Microbes and Infection*, vol. 5, no. 5, pp. 419–427, 2003.
- [83] G. C. Cerqueira, D. C. Bartholomeu, W. D. DaRocha et al., "Sequence diversity and evolution of multigene families in *Trypanosoma cruzi*," *Molecular and Biochemical Parasitology*, vol. 157, no. 1, pp. 65–72, 2008.
- [84] F. Mathieu-Daudé, M.-F. Bosseno, E. Garzon et al., "Sequence diversity and differential expression of Tc52 immuno-regulatory protein in *Trypanosoma cruzi*: potential implications in the biological variability of strains," *Parasitology Research*, vol. 101, no. 5, pp. 1355–1363, 2007.
- [85] C. R. Machado, L. Augusto-Pinto, R. McCulloch, and S. M. R. Teixeira, "DNA metabolism and genetic diversity in *Trypanosomes*," *Mutation Research*, vol. 612, no. 1, pp. 40–57, 2006.
- [86] L. Augusto-Pinto, D. C. Bartholomeu, S. M. R. Teixeira, S. D. J. Pena, and C. R. Machado, "Molecular cloning and characterization of the DNA mismatch repair gene class 2 from the *Trypanosoma cruzi*," *Gene*, vol. 272, no. 1–2, pp. 323–333, 2001.
- [87] P. Pitsikas, D. Lee, and A. J. Rainbow, "Reduced host cell reactivation of oxidative DNA damage in human cells deficient in the mismatch repair gene hMSH2," *Mutagenesis*, vol. 22, no. 3, pp. 235–243, 2007.
- [88] G. Wang, P. Alamuri, M. Z. Humayun, D. E. Taylor, and R. J. Maier, "The *Helicobacter pylori* MutS protein confers protection from oxidative DNA damage," *Molecular Microbiology*, vol. 58, no. 1, pp. 166–176, 2005.
- [89] K. M. Culligan and J. B. Hays, "*Arabidopsis* MutS homologs - AtMSH2, AtMSH3, AtMSH6, and a novel AtMSH7—form three distinct protein heterodimers with different specificities for mismatched DNA," *Plant Cell*, vol. 12, no. 6, pp. 991–1002, 2000.
- [90] M. Shrivastav, L. P. De Haro, and J. A. Nickoloff, "Regulation of DNA double-strand break repair pathway choice," *Cell Research*, vol. 18, no. 1, pp. 134–147, 2008.
- [91] B. Williams and A. J. Lustig, "The paradoxical relationship between NHEJ and telomeric fusion," *Molecular Cell*, vol. 11, no. 5, pp. 1125–1126, 2003.
- [92] P. Burton, D. J. McBride, J. M. Wilkes, J. D. Barry, and R. McCulloch, "Ku heterodimer-independent end joining in *Trypanosoma brucei* cell extracts relies upon sequence microhomology," *Eukaryotic Cell*, vol. 6, no. 10, pp. 1773–1781, 2007.
- [93] C. Wyman, D. Ristic, and R. Kanaar, "Homologous recombination-mediated double-strand break repair," *DNA Repair*, vol. 3, no. 8–9, pp. 827–833, 2004.
- [94] B. O. Krogh and L. S. Symington, "Recombination proteins in yeast," *Annual Review of Genetics*, vol. 38, pp. 233–271, 2004.
- [95] M. K. Bhattacharyya, D. E. Norris, and N. Kumar, "Molecular players of homologous recombination in protozoan parasites: implications for generating antigenic variation," *Infection, Genetics and Evolution*, vol. 4, no. 2, pp. 91–98, 2004.
- [96] D. C. Bartholomeu, G. C. Cerqueira, A. C. A. Leão et al., "Genomic organization and expression profile of the mucin-associated surface protein (masp) family of the human pathogen *Trypanosoma cruzi*," *Nucleic Acids Research*, vol. 37, no. 10, pp. 3407–3417, 2009.
- [97] C. E. Clayton, "Genetic manipulation of kinetoplastida," *Parasitology Today*, vol. 15, no. 9, pp. 372–378, 1999.
- [98] S. M. Beverley, "Protozomics: trypanosomatid parasite genetics comes of age," *Nature Reviews Genetics*, vol. 4, no. 1, pp. 11–19, 2003.
- [99] R. McCulloch, E. Vassella, P. Burton, M. Boshart, and J. D. Barry, "Transformation of monomorphic and pleomorphic *Trypanosoma brucei*," *Methods in Molecular Biology*, vol. 262, pp. 53–86, 2004.
- [100] R. L. Barnes and R. McCulloch, "*Trypanosoma brucei* homologous recombination is dependent on substrate length and homology, though displays a differential dependence on mismatch repair as substrate length decreases," *Nucleic Acids Research*, vol. 35, no. 10, pp. 3478–3493, 2007.

- [101] M. Tibayrenc and F. J. Ayala, "The clonal theory of parasitic protozoa: 12 years on," *Trends in Parasitology*, vol. 18, no. 9, pp. 405–410, 2002.
- [102] A. Tait, "Evidence for diploidy and mating in trypanosomes," *Nature*, vol. 287, no. 5782, pp. 536–538, 1980.
- [103] A. R. Bogliolo, L. Lauria-Pires, and W. C. Gibson, "Polymorphisms in *Trypanosoma cruzi*: evidence of genetic recombination," *Acta Tropica*, vol. 61, no. 1, pp. 31–40, 1996.
- [104] H. J. Carrasco, I. A. Frame, S. A. Valente, and M. A. Miles, "Genetic exchange as a possible source of genomic diversity in sylvatic populations of *Trypanosoma cruzi*," *American Journal of Tropical Medicine and Hygiene*, vol. 54, no. 4, pp. 418–424, 1996.
- [105] J. M. Kelly, J. M. Law, C. J. Chapman, G. J. J. M. Van Eys, and D. A. Evans, "Evidence of genetic recombination in *Leishmania*," *Molecular and Biochemical Parasitology*, vol. 46, no. 2, pp. 253–264, 1991.
- [106] C. Bogdan and M. Röillinghoff, "How do protozoan parasites survive inside macrophages?" *Parasitology Today*, vol. 15, no. 1, pp. 22–28, 1999.
- [107] L. Glover, R. McCulloch, and D. Horn, "Sequence homology and microhomology dominate chromosomal double-strand break repair in African trypanosomes," *Nucleic Acids Research*, vol. 36, no. 8, pp. 2608–2618, 2008.
- [108] G. K. Takeda, R. Campos, J. Kieffer et al., "Effect of gamma rays on blood forms of *Trypanosoma cruzi*. Experimental study in mice," *Revista do Instituto de Medicina Tropical de Sao Paulo*, vol. 28, no. 1, pp. 15–18, 1986.
- [109] M. Seo, D. K. Chun, S. T. Hong, and S. H. Lee, "Influence of heat shock, drugs, and radiation on karyotype of *Leishmania major*," *Korean Journal of Parasitology*, vol. 31, no. 3, pp. 277–283, 1993.
- [110] T. Rijkers, J. Van Den Ouweland, B. Morolli et al., "Targeted inactivation of mouse RAD52 reduces homologous recombination but not resistance to ionizing radiation," *Molecular and Cellular Biology*, vol. 18, no. 11, pp. 6423–6429, 1998.
- [111] A. K. C. Wong, R. Pero, P. A. Ormonde, S. V. Tavtigian, and P. L. Bartel, "RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene *brca2*," *Journal of Biological Chemistry*, vol. 272, no. 51, pp. 31941–31944, 1997.
- [112] P.-L. Chen, C.-F. Chen, Y. Chen, J. Xiao, Z. D. Sharp, and W.-H. Lee, "The BRC repeats in BRCA2 are critical for RAD51 binding and resistance to methyl methanesulfonate treatment," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 9, pp. 5287–5292, 1998.
- [113] L. Y. Marmorstein, T. Ouchi, and S. A. Aaronson, "The BRCA2 gene product functionally interacts with p53 and RAD51," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 23, pp. 13869–13874, 1998.
- [114] P. A. Blundell, G. Rudenko, and P. Borst, "Targeting of exogenous DNA into *Trypanosoma brucei* requires a high degree of homology between donor and target DNA," *Molecular and Biochemical Parasitology*, vol. 76, no. 1-2, pp. 215–229, 1996.
- [115] A. R. Lehmann, A. Niimi, T. Ogi et al., "Translesion synthesis: Y-family polymerases and the polymerase switch," *DNA Repair*, vol. 6, no. 7, pp. 891–899, 2007.
- [116] S. Prakash, R. E. Johnson, and L. Prakash, "Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function," *Annual Review of Biochemistry*, vol. 74, pp. 317–353, 2005.
- [117] S. Gupta, V. Bhatia, J.-J. Wen, Y. Wu, M.-H. Huang, and N. J. Garg, "*Trypanosoma cruzi* infection disturbs mitochondrial membrane potential and ROS production rate in cardiomyocytes," *Free Radical Biology and Medicine*, vol. 47, no. 10, pp. 1414–1421, 2009.
- [118] R. L. Tarleton, "Immune system recognition of *Trypanosoma cruzi*," *Current Opinion in Immunology*, vol. 19, no. 4, pp. 430–434, 2007.
- [119] S. Gupta, J. J. Wen, and N. J. Garg, "Oxidative Stress in Chagas Disease," *Interdisciplinary Perspectives on Infectious Diseases*, vol. 2009, Article ID 190354, 8 pages, 2009.
- [120] J. M. Freitas, E. Lages-Silva, E. Crema, S. D. J. Pena, and A. M. Macedo, "Real time PCR strategy for the identification of major lineages of *Trypanosoma cruzi* directly in chronically infected human tissues," *International Journal for Parasitology*, vol. 35, no. 4, pp. 411–417, 2005.
- [121] O. Fernandes, R. H. Mangia, C. V. Lisboa et al., "The complexity of the sylvatic cycle of *Trypanosoma cruzi* in Rio de Janeiro state (Brazil) revealed by the non-transcribed spacer of the mini-exon gene," *Parasitology*, vol. 118, no. 2, pp. 161–166, 1999.



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