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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 Tritryps) 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 Ca+2 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 Tritryps 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 Tritryps, 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 Tritryps genomes have identified differences in the DNA maintenance mechanisms (nuclear and mitochondrial) between Tritryps 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 dioxigenases, 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 Tritryps 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 Tritryps. 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 nonoccurence 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 Tritryps: alkylation reversal and oxidative damage repair [6–9]. These pathways perform immediate chemical reversals of specific forms of DNA damage. Single homologs of O-6 methylguanine alkyltransferase (MGMT) can be found in the three genomes. This enzyme catalyzes the repair of O^6-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 Tritryps. 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 pirimidine 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 Polβ 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 (Polδ) or ε (Polε) [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 *TritrypDB* database [15]. The Tritryps possess the enzymes required to effectively

Table 1

Gene	Function	Organism (Gene ID)	Experimental data	Ref.
BER Genes				
Uracyl-DNA glycosylase (UNG)	Excision of uracil in DNA	*T. cruzi* (Tc00.1047053511277.330)	(i) *In vitro activity* (enhanced by AP endonuclease) (ii) Heterologous complementation of *E. coli*	[16, 17]
AP endonuclease1	Cleavage of the phosphodiester bond at the 5′ side of AP site	*T. cruzi* (Tc00.1047053507083.30)	(i) Heterologous complementation of *E. coli*	[18]
		L. major (LmjF16.0680)	(i) Heterologous complementation of *E. coli* (ii) Increment of H_2O_2 and methotrexate resistance	[18–20]
POLβ	Polymerization of DNA Strand displacement (long-patch) Cleavage of the 5′- dRP	*T. cruzi* (Tc00.1047053503955.20)	(i) *In vitro* activity (ii) Kinetoplast localization	[21]
		T. brucei (Tb927.5.2780)		[22]
PARP	Binding to ssDNA Stimulation of DNA synthesis and strand displacement	*T. cruzi* (Tc00.1047053509721.60)	(i) *In vitro* activity (enhanced by SSB)	[23]
NER Genes				
TFIIH-TFB1	Component of TFIIH	*T. brucei* (Tb11.01.1200)	(i) Essential for initiating synthesis of spliced leader RNA	[24]
TFIIH-TFB2		*T. brucei* (Tb927.10.5210)		
TFIIH-TFB4		*T. brucei* (Tb11.01.7730)		
TFIIH-TFB5		*T. brucei* (Tb10.61.2600)		
TFIIH-XPB	Component of TFIIH (helicase)	*T. brucei* (Tb11.01.7950Tb927.3.5100)	(i) Interaction with TSP1 and TSP2	[24]
TFIIH-XPD		*T. brucei* (Tb927.8.5980)	(i) Nuclear localization	
TFIIH-TSP1	Trypanosomatid-specific component of TFIIH	*T. brucei* (Tb927.1.1080)	(i) Essential for initiating synthesis of spliced leader RNA	
TFIIH-TSP2		*T. brucei* (Tb11.01.5700)	(i) Nuclear localization (ii) Essential for initiating synthesis of spliced leader RNA	
XAB2*	May function as a scaffold for protein complex formation	*T. cruzi* (Tc00.1047053509767.40)	(i) Putative	—
		T. brucei (Tb927.5.1340)	(i) Putative	—
		L. major (LmjF23.1550)	(i) Putative	—
MMR Genes				
MSH2	Repair of single base-base and IDL mismatches Heterodimers with MSH3 or MSH6	*T. cruzi* (Tc00.1047053507711.320)	(i) Three isoforms with different efficiencies (ii) Involvement in oxidative stress response (independently from MLH1)	[25, 26]
		T. brucei (Tb927.10.11020)	(i) Involvement in oxidative stress response (independently from MLH1) (ii) Microsatellite instability and MNNG tolerance in *MSH2/MLH1* double mutants (iii) Regulatory role in HR	[26–28]
MLH1	Heterodimers with MutL homologs Matchmaker for coordinating eventes from mismatch binding to DNA synthesis	*T. brucei* (Tb927.8.6840)	(i) Microsatellite instability and MNNG tolerance in *MSH2/MLH1* double mutants (ii)Regulatory role in HR	[27, 28]

Table 1: Continued.

Gene	Function	Organism (Gene ID)	Experimental data	Ref.
NHEJ Genes				
Ku70	DSB recognition DSB bridging nucleolytic processing of the ends	*T. brucei* (Tb927.3.5030)	(i) Telomere maintenance	[29, 30]
Ku80	Telomere maintenance	*T. brucei* (Tb927.6.1760)		
HR Genes				
Mre11	DSB end resection Nuclease activities	*T. brucei* (Tb927.2.4390)	(i) Mre11 mutations cause impairment of HR and increased DNA damage sensitivity	[31, 32]
Rad51	Recombinases	*T. cruzi* (Tc00.1047053503801.30)	(i) Gene expression induced by DNA damaging agents (ii) Involved in DSBs and oxidative lesions repair	[33]
		T. brucei (Tb11.01.0360)	(i) Null mutants led to impairments in VSG switch and DNA transformation, besides a higher sensitivity to genotoxic agents	[34]
		L. major (LmjF28.0550)	(i) Gene expression induced by DNA-damaging agents	[35]
Dmc1	Recombinases	*T. brucei* (Tb09.211.1210)	(i) DMC1 mutation does not affect HR or VSG switching	[36]
BRCA2	ssDNA binding Recombination mediator	*T. brucei* (Tb927.1.640)	(i) Expansion in the number of BRC repeats (ii) BRCA2 mutants display antigenic variation impairment and genome instability	[37]
Rad51-3	ssDNA binding Recombination mediator activity	*T. brucei* (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		*T. brucei* (Tzb10.389.1770)	(i) Rad51-5 mutations caused altered RAD51 localization following DNA damage and DNA damage sensitized parasites	[38]
TLS Genes				
Polη	Error-free bypass of cis-syn cyclobutane pyrimidine dimers (CPDs)	*T. cruzi* (Tc00.1047053511911.120)	(i) Heterologous complementation of *S. cerevisae* (ii) *In vitro* bypass of 8-oxoG (iii) Overexpression increases H_2O_2 resistance	[39]
Polκ	Bypass of N2-adducted dG lesions Extension of mismatched primer termini	*T. cruzi* (Tc00.1047053503755.30)	(i) Mitochondrial localization (ii) *In vitro* bypass of8-oxoG (iii) DNA synthesis within recombination intermediates (iv) Overexpression increases zeocin, gamma radiation, and H_2O_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 Tritryps,

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 Tritryps. 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 Polβ-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 Tritryps 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 (H_2O_2) 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 Tritryps genome [6–9]. A more accurate search of the Tritryps 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 H_2O_2 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, Polβ from *T. cruzi* and *T. brucei* have already been characterized [21, 22]. The TcPolβ localizes to the parasite kinetoplast and exhibits DNA polymerization and 5′dRP lyase activity [21]. Similarly, the TbPolβ 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 Tritryps: 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]. Polβ is an example of a polymerase that shows a nuclear localization in higher eukaryotes [57] but is addressed to the kinetoplast in the Tritryps [21, 22]. The *L. major* Polβ has not yet been experimentally characterized; however, a Polβ 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 Polβ, 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 Tritryps genomes apparently do not encode for the other X-family polymerases, DNA polymerase lambda (Polλ), and mu (Polμ)[7; 15]; thus, *L. major* may be the only Tritryps 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 H_2O_2 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 Tritryps 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, Tritryps have two copies of XPB and DDB1 appear duplicated in *T. cruzi*. However, others such as XPA could not be identified in Tritryps. It is also possible that the Tritryps ligation step is different from the ligation step from higher eukaryotes. The Tritryps 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) subcomplexes. 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 Tritryps 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 Tritryps 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 Tritryps. In fact, overexpression of *T. cruzi* DNA polymerase η (Polη), involved in the translesion synthesis of pirimidine 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 Tritryps, 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 Tritryps 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 H_2O_2, 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 MutSα (MSH2-MSH6) or MutSβ (MSH2-MSH3), (ii) excision of the damaged DNA section mainly by ExoI, and (iii) DNA resynthesis by DNA Polδ and ligation. Steps after DNA mismatch recognition are coordinated by MLH heterodimers that bind to MSH proteins and probably recruit and assembly 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 H_2O_2 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 H_2O_2 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 H_2O_2 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 Tritryps 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, Tritryps 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 (LifI 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 Tritryps 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 Tritryps, 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 Tritryps. 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 Tritryps 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 H_2O_2 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 (Polδ and Polε), 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 (Polξ). 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. Polκ, Polη, Rev1, and Polζ homologs are found in these species. PCNA and Rad6 homologs are also present. These parasites show an expansion of Polκ 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 Polκ gene could result in an increment of Polκ gene expression level which would compensate the lack of pretranscriptional mechanisms in these organisms [9].

Polη from *T. cruzi* has been characterized *in vitro* and *in vivo* [39]. TcPolη is able to complement yeast Rad30 mutant (Polη-null mutant), increasing yeast resistance to UV radiation, which indicates that Polη is able to bypass UV lesions. Parasites overexpressing TcPolη show a higher resistance to H_2O_2 treatment. This resistance could be associated with the ability of TcPolη 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 TcPolκ 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 Polκ. Mitochondrial TcPolκ bypasses 8-oxoG *in vitro*, which correlates with the increase in H_2O_2 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, TcPolκ 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 TcPolκ 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 TcPolκ-overexpressing parasites when treated with agents that cause DSBs. In addition, TcPolκ- and TcPolη-overexpressing cells also presented increased resistance to H_2O_2 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 Tritryps, 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 Tritryps, 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 Tritryps 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 Tritryps [7]. Further studies are necessary to clarify the information about DNA repair pathways in Tritryps, specifically the differences in Tritryps machinery from the typical eukaryotic machinery for DNA repair, which could provide potential points of attack against the parasites.

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