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REVIEW ARTICLE

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Nuclear DNA replication and repair in parasites of the genus *Leishmania*: Exploiting differences to develop innovative therapeutic approaches

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ABSTRACT

Leishmaniasis is a common tropical disease that affects mainly poor people in underdeveloped and developing countries. This largely neglected infection is caused by *Leishmania* spp, a parasite from the Trypanosomatidae family. This parasitic disease has different clinical manifestations, ranging from localized cutaneous to more harmful visceral forms. The main limitations of the current treatments are their high cost, toxicity, lack of specificity, and long duration. Efforts to improve treatments are necessary to deal with this infectious disease. Many approved drugs to combat diseases as diverse as cancer, bacterial, or viral infections take advantage of specific features of the causing agent or of the disease. Recent evidence indicates that the specific characteristics of the Trypanosomatidae replication and repair machineries could be used as possible targets for the development of new treatments. Here, we review in detail the molecular mechanisms of DNA replication and repair regulation in trypanosomatids of the genus *Leishmania* and the drugs that could be useful against this disease.

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Introduction

Transmission of the genome from one generation to the next is a process common to all organisms. This involves the faithful duplication of the genetic material and the transmission of one copy to each descendent. DNA replication, the mechanism used to copy the genome, is tightly coordinated with the cell cycle. It begins with the licensing of replication origins in G_1 and their activation throughout the entire S phase (Remus & Diffley, 2009; Zegerman & Diffley, 2009). Much work describing DNA replication and its regulation has been performed in eukaryotes, especially in model organisms, such as budding yeast, fission yeast, and *Xenopus laevis* (Costas et al., 2011; Putnam et al., 2009; Sabatinos & Forsburg, 2015; Sequeira-Mendes et al., 2009). However, much less is known on how this process is regulated in Trypanosomatidae. In this review we focus on *Leishmania* spp., a kinetoplastid protozoan parasite that belongs to the Trypanosomatidae family and that causes many zoonotic diseases. In humans, these sand fly-transmitted pathogens cause a spectrum of infections, ranging from localized cutaneous lesions to disseminated mucocutaneous and visceral forms. The World Health Organization reported in 2012 that 310 million people were at risk of *Leishmania* infection in 98 countries around the world, with approximately 1.3–2 million new cases per year (World Health Organization (WHO), 2010).

To combat these diseases, approaches that exploit the host immune response against *Leishmania* parasites have been developed and tested (Beiting, 2014; Singh & Sundar, 2014). Efforts are also focused on developing novel drugs by using different strategies, from high-throughput screening for the identification of new targets to the characterization of already marketed drugs

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that display activity not only against their specific target but also against *Leishmania* parasites (Benaim et al., 2013, 2014; De Menezes et al., 2015; Paniz Mondolfi et al., 2011).

Currently, many drugs are employed to block DNA replication in viruses and bacteria, as well as in cancer cells (Pommier, 2006; Pommier & Robert, 2001). However, the use of DNA replication and repair enzymes as clinical targets for leishmaniasis treatment has remained largely unexplored. Therefore, investigating in depth the mechanisms of *Leishmania* DNA replication could open the way to the identification of potential clinical targets and to the development of novel therapeutic approaches.

Although the *Leishmania* replication machinery resembles that of other eukaryotes, significant differences have also been reported. In this review article, we present and discuss the current knowledge on the molecular mechanisms and the different known factors involved in the regulation of DNA replication and repair in trypanosomatids of the genus *Leishmania*.

Leishmania cell cycle

Leishmania parasites have a complex life cycle, with a mainly asexual mode of reproduction. After inoculation into the bloodstream by a sand fly, *Leishmania* promastigotes are actively absorbed by circulating macrophages, where they differentiate into amastigotes. After reaching a critical number by active division, amastigotes disrupt the host cell, and invade other macrophages, thus repeating the cycle and causing the disease (Ivens et al., 2005). Promastigotes, intracellular amastigotes, and axenic amastigotes can be studied *in vitro* (De Almeida et al., 2010; Gupta et al., 2001). However, intracellular amastigote cultures require a complex experimental setup and a large supply of macrophages (De Almeida et al., 2010). Promastigote culture is limited by the fact that such parasites lose infectivity over time (Santarém et al., 2014). Therefore, their transmission into macrophages or hosts becomes essential to maintain the promastigote features, reflecting the high plasticity of these parasites (Sterkers et al., 2012). Some reports suggest that the medium composition also can influence their biology, implying that improved media could facilitate their *in vitro* culture (Santarém et al., 2014).

The cell cycle of *Leishmania mexicana* promastigotes has been well described (Wheeler et al., 2011). In controlled laboratory conditions, Gull et al. have reported a doubling time of 7.1 h for *L. mexicana* (Wheeler et al., 2011). Four markers can be used to study the *Leishmania* spp. cell cycle: the nucleus, the kinetoplast (the single, giant mitochondrion in kinetoplastid species), the flagellum and the cell body length. The nucleus (N), kinetoplast (K), and flagellum (F) are single organelles that duplicate once per cell cycle and in *L. mexicana* segregate in the following order: 1K1N1F, 1K1N2F, 1K2N2F, and finally 2K2N2F (Wheeler et al., 2011). In other species, such as *L. donovani,* nuclear division precedes kinetoplast segregation only in 80% of cells, while in the other 20%, the kinetoplast segregates first (Minocha et al., 2011a). In *L. major*, nuclear DNA replication precedes kinetoplast segregation, but nuclear division is accomplished after kinetoplast segregation (Ambit et al., 2011). A similar order of events has been described in *Trypanosoma brucei* and *Trypanosoma cruzi*, in which kinetoplast segregation precedes karyokinesis (Godoy et al., 2009). These differences suggest distinct molecular and signaling mechanisms.

Another useful cell-cycle marker is the cell body length, which is independent from the culture density (Wheeler et al., 2011). Cell body length increases slowly from 6 to 11 μm during G_1 and remains approximately constant during S phase. Then, it decreases rapidly after cytokinesis, returning to the initial length of 6 μm. Flagellum length and cell body width are not good cell-cycle markers, because variations of these parameters are dependent on the cell culture density. Indeed, flagella in daughter cells are shorter than in mother cells. The combination of these well-defined cell cycle markers provides a simple tool for identifying the cell cycle stage in single *Leishmania* cells by microscopy. This is essential for carrying out studies on DNA replication. In addition, labeling of DNA or proteins combined with flow cytometry, high-throughput sequencing, DNA combing, and bioinformatics are powerful techniques that allow addressing, in kinetoplastids, the poorly understood molecular basis of DNA replication, or the mechanism of action of potential drugs. However, one of the main limitations of cell-cycle studies is the need to synchronize the promastigote populations under study. Most of the drugs used to block the cell cycle are genotoxic and thus, cells are blocked in S phase. One interesting, alternative approach for cell synchronization is elutriation, which allows isolating G_1 cells from an asynchronous population without adding drugs. This technique has been successfully used by McCulloch et al. to describe the profile of DNA replication in *T. brucei* (Tiengwe et al., 2012a).

Genome organization

Around 30 species of *Leishmania* have been described. Old World species (for instance, *L. donovani* and *L. major*) have 36 chromosomes, while New World

Leishmania species have 34 or 35 chromosomes. Indeed, chromosomes 8 and 29 and chromosomes 20 and 36 are fused in *L. mexicana*; and chromosomes 20 and 34 are fused in *L. braziliensis* (Britto et al., 1998). *Leishmania* spp. and other kinetoplastids have a specific genome organization, not found in other eukaryotes. Genes lack introns and are organized in polycistronic units on both DNA strands, a characteristics reminiscent of prokaryotes. Polycistronic mRNAs are processed via trans-splicing and polyadenylation mechanisms (Kazemi, 2011).

In *L. major*, genes are arranged in approximately 133 directional gene clusters (DGCs) along its 36 chromosomes. Each cluster contains between ten and hundreds of protein-coding genes on the same DNA strand (Daniels et al., 2010; Ivens et al., 2005; Tiengwe et al., 2012a). Transcription by RNA polymerase II initiates between two divergent DGCs and terminates between convergent clusters, at regions that often contain tRNA, rRNA, and snRNA genes. These genes are transcribed by RNA polymerases I and III, but the mechanisms by which the promoters and termination sites are regulated are not well understood (Ivens et al., 2005; Martínez-calvillo et al., 2004; Worthey, 2003). This form of genomic organization seems to be conserved amongst other kinetoplastid species (Tiengwe et al., 2012a, 2014). Polycistronic gene organization, the abundance of proteins with zinc finger domains, which is characteristic of RNA binding proteins, and the expanded number of translation factors suggest that gene expression is mainly regulated through post-transcriptional mechanisms (Ivens et al., 2005), such as mRNA *trans*-splicing rather than *cis*-splicing events and mRNA degradation.

Nuclear DNA replication

Initiation of DNA replication and prevention of re-replication

In eukaryotes, DNA replication involves the firing of multiple origins throughout the S phase. This is preceded by origin licensing during the M/G_1 phase. Origins are licensed by the assembly of the pre-replication complex (pre-RC) (Figure 1(A)). Pre-RC formation is initiated by the binding of the six-subunit origin recognition complex (ORC) to origins of replication (Oehlmann et al., 2007). ORC recruits the minichromosome maintenance (MCM) complex, which is the eukaryotic replicative helicase and is composed of MCM 2–7, via interaction with CDC6 and CDT1. MCM helicase activity is required for origin licensing. This term indicates that origins are set to be fired, and this happens only when the MCM complex has been loaded. For this, CDT1 interacts directly with MCM and the CDT1–MCM complex binds to the origin through CDC6–ORC interaction. However, only a fraction of the licensed origins will be used during the S phase. Indeed, at S phase onset, once the CDT1–MCM complex has been loaded, cyclin-dependent kinases (CDKs) and CDC7/DBF4 kinases target CDC45 only to some pre-RCs, initiating DNA synthesis on these selected origins. In budding yeast, CDKs phosphorylate the essential initiation factors Sld2p and Sld3p, allowing their binding to Dpb11p. The association of these factors promotes origin firing by loading Cdc45p and DNA polymerase (Pospiech et al., 2010). In parallel, Cdc7p/Dbf4p activates the MCM complex by phosphorylation, also facilitating Cdc45p loading at origins and the formation of the pre-initiation (pre-IC) complex (Masai et al., 2006). Then, Dpb11p–Sld3p are displaced by the GINS complex (Sld5p and Psf1p, Psf2p and Psf3p), thus forming the CMG (Cdc45p–MCM–GINS) helicase complex (Dhingra et al., 2015; Takeda & Dutta, 2005). A single CMG complex travels with each replisome during elongation until DNA replication is complete. CMG complexes converge to termination sites, where unloading is performed (Lengronne & Pasero, 2014).

The molecular mechanisms that control DNA replication are highly conserved from yeasts to humans. However, they seem to be substantially different in Trypanosomatidae, because many eukaryote proteins are not conserved and some new ones have been identified (Table 1 and Figure 1(B)). For instance, homologs of ORC complex subunits, of CDT1, and of the limiting factor Treslin (Sld3p in budding yeast) and of CDC7/DBF4 kinases have not been identified (Dang & Li, 2011; Genois et al., 2014; Li, 2012).

One of the main differences concerning DNA replication in these early branched eukaryotes concerns the ORC complex. ORC involvement in replication initiation was first described in the budding yeast *Saccharomyces cerevisiae*. In yeast and metazoans, ORC is a six-subunit complex. Orthologues have been identified in many eukaryotes, such as *Schizosaccharomyces pombe*, *Drosophila melanogaster, Xenopus laevis,* and *Homo sapiens* (Bleichert et al., 2015; Kong et al., 2003; Kumar et al., 2012). ORC1–6 interacts with replication origins in an ATP-dependent manner (Bleichert et al., 2015; Zwerschke et al., 1994) and ORC-mediated ATP hydrolysis is essential for recruiting MCM proteins (Godoy et al., 2009).

Kinetoplastids seem to express a single ORC1/CDC6 protein, which is structurally most related to eukaryotic CDC6 (Table 1 and Figure 1(B)). This feature is reminiscent of archaea organization (Barry & Bell, 2006; Godoy et al., 2009; Kumar et al., 2008). Indeed, most archaea

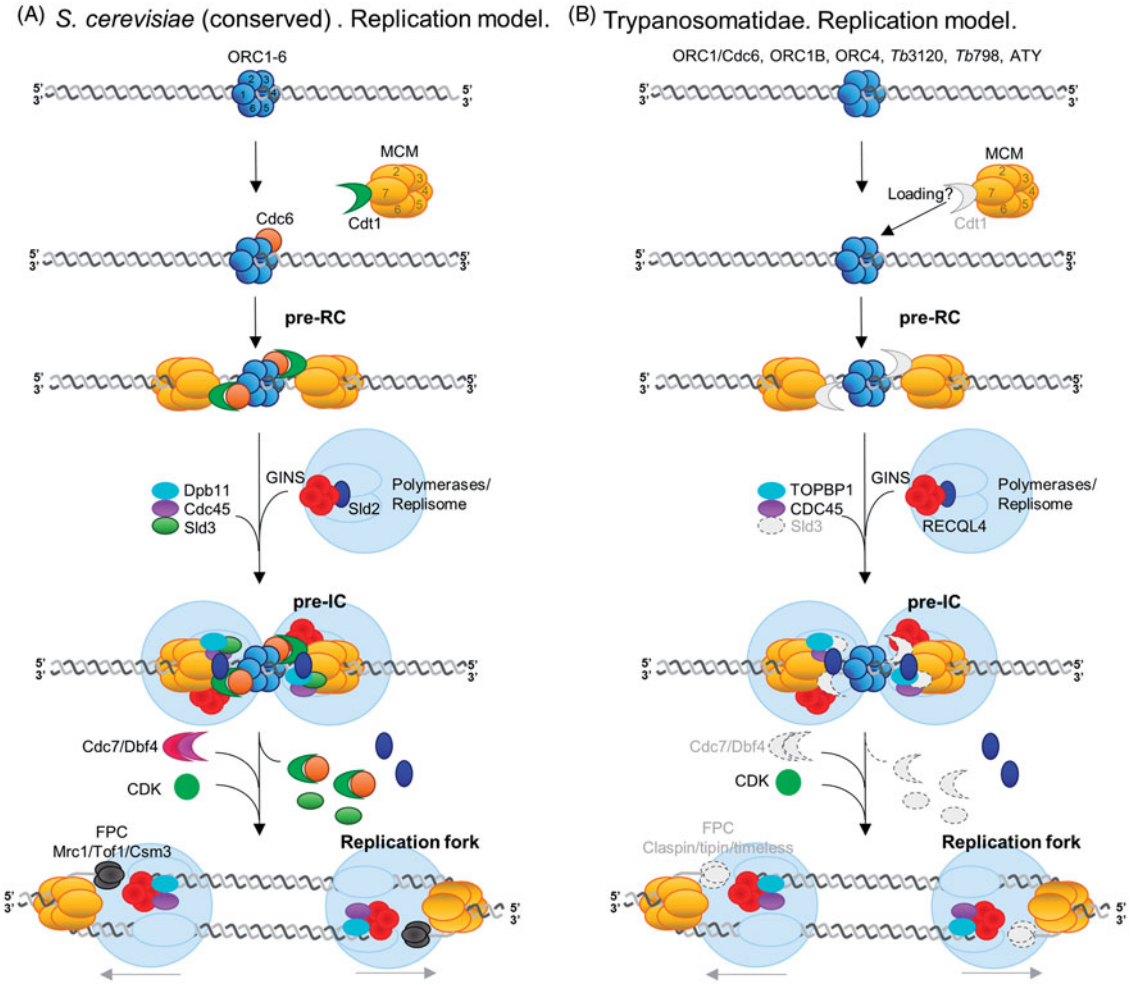

Figure 1. General models of the sequential recruitment of factors for DNA replication initiation to highlight the main differences identified between high eukaryotes (A. based in *S. cerevisiae*) and Trypanosomatidae (B). In most eukaryotes, origin recognition starts with the binding of the ORC complex. Then the loading of the replicative helicase MCM is mediated by Cdc6-Cdt1, forming two pre-replication complexes (pre-RC) (licensing reaction). Additional factors, such as DNA polymerases, GINS, and Cdc45, are then recruited to form two pre-initiation complexes (pre-IC). The activation of the replication fork is finally controlled by Cdc7/Dbf4 and CDK kinases. Gray symbols with dashed lines in (B) represent proteins involved in DNA replication in yeast/high eukaryotes, but not identified in Trypanosomatidae. See text for more details.

genomes contain at least one gene with similarity to both *ORC1* and *CDC6* genes, although exceptions are found in some methanogenic species (reviewed by Barry & Bell, 2006; Shen, 2013). In *T. brucei* and *T. cruzi*, ORC1/CDC6 is expressed throughout the cell cycle. It localizes to the nucleus and is constitutively associated with chromatin (Godoy et al., 2009; Tiengwe et al., 2012b). In budding yeast, ORC proteins are also present and associated with chromatin throughout the cell cycle. In other metazoans, this association is restricted to some cell cycle stages (reviewed in Duncker et al., 2009). Recombinant ORC1/CDC6 from *T. cruzi* (*Tc*ORC1/CDC6) and *T. brucei* (*Tb*ORC1/CDC6) shows conserved ATPase activity *in vitro*. Moreover, phenotypic complementation assays revealed that *Tc*ORC1/CDC6 and *Tb*ORC1/CDC6 can functionally replace yeast Cdc6p, but not Orc1p (Godoy et al., 2009). Interestingly, *ORC1/CDC6* knockdown in *T. brucei* results in enucleated cells, suggesting defects in DNA replication (Godoy et al., 2009; Tiengwe et al., 2012b). An *ORC1* ortholog has been identified in *L. major* and *L. donovani* (*LdORC1/CDC6*) (Kumar et al., 2008, 2012) and the protein is constitutively expressed in the nucleus, as described in trypanosomes. *Ld*ORC1/CDC6 has an N-terminus sequence essential for its nuclear import. This signaling sequence comprises residues 2–5 (KRSR), among which K2, R3, and R5 are crucial for *Ld*ORC1/CDC6 proper localization (Kumar et al., 2012). Importantly, ChIP-chip approaches revealed that ORC1/CDC6 co-localizes with fired replication origins in *T. brucei* (Tiengwe et al., 2012a), supporting the hypothesis that ORC1/CDC6 is the functional counterpart of the ORC complex.

In addition to ORC1/CDC6, ORC1b has been identified in *T. brucei* (Dang & Li, 2011) by protein sequence

Table 1. Components from replication fork and other components related with its assembly. The table show the gene symbol for *H. sapiens*, *S. cerevisiae,* and three *Leishmania* spp. (*L. infantum*, *L. braziliensis*, and *L. mexicana*). In parenthesis are indicated the systematic gene name for *Leishmania* spp.

H. sapiens	*S. cerevisiae*	*Leishmania* sp. (*infantum*, *braziliensis*, and *mexicana*)	Function[a]
TOPBP1	*DPB11*	*TOPBP1* (*LinJ.29.1910*; *LbrM.31.0180*; *LmxM.08_29.1790.1*)	DNA replication initiation. Loads DNA polymerase ε on pre-replication complexes at origins and acts as checkpoint sensor
CDT1	*CDT1*	Not found (Genois et al., 2014; Li, 2012)	Chromatin licensing and DNA replication factor 1 (CDT1) is required for pre-replication complex assembly by loading the minichromosome maintenance proteins (MCM) on the replication origin
GMNN (Geminin)	Not found	Not found	At the start of S-phase until late mitosis, geminin inhibits the replication factor CDT1, preventing the assembly of the pre-replicative complex and thus, preventing re-replication
MCM2	*MCM2*	*MCM2* (*Linj.28.0940*; *LbrM.28.0920*; *LmxM.28.0850*)	Replicative helicase, composed of six different proteins of the AAA + ATPase family. This helicase is recruited and loaded by ORC, CDC6 and CDT1 and forms a double hexamer that is topologically wrapped around DNA in the pre-replicative complex. After replication initiation, each MCM complex moves away from ORC in opposite directions, ahead of the replication fork
MCM3	*MCM3*	*MCM3* (*Linj.33.2840*; *LbrM.33.2980*; *LmxM.32.2700*)	
MCM4	*MCM4*	*MCM4* (*LinJ.09.0400*; *LbrM.09.0250*; *LmxM.09.0250*)	
MCM5	*MCM5*	*MCM5* (*LinJ.24.0930*; *LbrM.24.0920*; *LmxM.24.0910*)	
MCM6	*MCM6*	*MCM6* (*LinJ.28.2550*; *LbrM.28.2580*; *LmxM.28.2385*)	
MCM7	*MCM7*	*MCM7* (*LinJ.32.3120*; *LbrM.32.3220*; *LmxM.31.2960*)	
MCM10	*MCM10*	*MCM10* (*LinJ.26.2410*; *LbrM.26.2320*; *LmxM.26.2390*)	Required for the association of the MCM2-7 complex to origins and stabilization of DNA polymerase alpha
CDC6	*CDC6*		Essential ATP-binding protein required for DNA replication. Binds chromatin by association with ORC complex and is required for MCM2-7 loading. It is a component of the pre-replicative complex (pre-RC).
ORC1	*ORC1*	*ORC1/CDC6* (*LinJ.28.0030*; *LbrM.28.0040*; *LmxM.28.0030*)	Hetero-hexameric complex composed of ORC1–ORC6 proteins. Binds to DNA and assembles the MCM2–7 complex on chromatin together with CDC6 and CDT1
		ORC1B (*LinJ.26.2220*; *LbrM.26.2130*; *LmxM.26.2210*)	
ORC2	*ORC2*	Not found (Dang & Li, 2011; Tiengwe et al. 2012b)	
ORC3	*ORC3*	Not found (Dang & Li, 2011; Tiengwe et al. 2012b)	
ORC4	*ORC4*	*ORC4* (*LinJ.18.0720*; *LbrM.18.0810*; *LmxM.18.0720*)	
Not found	Not found	*LinJ.01.0680*; *LbrM.01.0640*; *LmxM.01.0660.1* (orthologues of *Tb3120* (*Tb927.9.4530*[b]))	Specific Trypanosomatidae genes, with a possible role in DNA replication initiation. They share low homology with ORC and have been purified by affinity IP (Shen, 2013; Tiengwe et al., 2012b)
Not found	Not found	*LinJ.36.7010*; *LbrM.35.7060*; *LmxM.36.6700.1* (orthologues of *Tb7980* (*Tb927.10.7980*))	
Not found	Not found	*ATY* (*LinJ.06.0750*; *LbrM.06.0710*; *LmxM.06.0720*) Orthologues of *LamATY* (Kelly et al., 2011)	Specific Trypanosomatidae gene, with a possible role in DNA replication. Contains an AT-hook domain that binds to DNA (Kelly et al., 2011)
CDC45	*CDC45*	CDC45 (*LinJ.33.2450*; *LbrM.33.2610*; *LmxM.32.2320*)	DNA replication initiator factor. Associates with GINS and MCM complexes, and travels with the replication fork
DDK (*CDC7/DBF4*)			
CDC7	*CDC7*	Not found (Jones et al., 2014; Parsons et al., 2005; Tiengwe et al., 2012a)	Catalytic subunit of DDK (CDC7/DBF4), required for origin firing and replication fork progression in mitotic S phase
DBF4	*DBF4*	Not found (Tiengwe et al., 2012a)	Regulatory subunit of DDK (CDC7/DBF4), required for origin firing and replication fork progression in mitotic S phase
TICRR (Treslin)	*SLD3*	Not found (Dang & Li, 2011; Genois et al., 2014; Li, 2012)	Protein involved in the initiation of DNA replication; required for proper assembly of replication proteins at origins of replication; interacts with CDC45
RECQL4	*SLD2*	*RECQL4* (*LinJ.30.2300*; *LbrM.30.2240*; *LmxM.29.2290*)	Single-stranded DNA origin-binding and annealing protein; required for initiation of DNA replication
GINS complex			
SLD5	*SLD5*	*SLD5* (*LinJ.29.2370*; *LbrM.29.2240*; *LmxM.08_29.2260*)	Complex essential for the establishment of DNA replication forks and replisome progression. It is one of the components of the eukaryotic replicative helicase: the CMG (CDC45–MCM–GINS) complex
PSF1	*PSF1*	*PSF1* (*LinJ.15.1580*; *LbrM.15.1510*; *LmxM.15.1510.1*)	
PSF2	*PSF2*	*PSF2* (*LinJ.36.4400*; *LbrM.35.4440*; *LmxM.36.4190.1*)	
PSF3	*PSF3*	*PSF3* (*LinJ.31.1680*; *LbrM.31.1890*; *LmxM.30.1660.1*)	
CDK1 (*CDC2*)	*CDC28*	*CRK1* (Mottram et al., 1993) (*LinJ.21.1320*; *LbrM.21.1310*; *LmxM.21.1080*)	

[a]Functional description from the Saccharomyces genome database and Genome UCSC.
[b]Tb09.160.3120 original identification number.
Genes nomenclature has been retrieved from HUGO gene nomenclature committee (http://www.genenames.org/) for *H. sapiens*; SGD: Saccharomyces genome database (http://www.yeastgenome.org/) for *S. cerevisiae*; and GeneDB (http://www.genedb.org). For Leishmania, genes names designation follows the guidelines for *Trypanosoma* and *Leishmania* genetic nomenclature (Clayton et al., 1998). These genes are identified by protein similarity obtained by BLAST search (http://www.ncbi.nlm.nih.gov/; http://www.genedb.org/blast).

homology searches, and ORC4, *Tb*3120 and *Tb*7980 (Shen, 2013; Tiengwe et al., 2012b) by affinity purification. Through additional phylogenetic analyses, McCulloch et al. (Tiengwe et al., 2012b) concluded that *Tb927.10.13380* from *T. brucei* encodes a divergent orthologue of the eukaryotic *ORC4* subunit. Homologs of ORC4, *Tb*3120 and *Tb*7980 have been also found in *Leishmania* spp. (Tiengwe et al., 2012b; Table 1).

In summary, in Trypanosomatidae, at least three divergent ORC subunit ortholog have been identified (*ORC1/CDC6*, *ORC1B,* and *ORC4*) and two additional orthologues (*Tb3120* and *Tb7980*). More studies are needed to determine whether these subunits are organized in a ring-shaped complex similar to those of other eukaryotes. These significant divergences could underlie differential mechanisms of origin activation in this poorly-studied family, but a recent report strongly supports the view that ORC1/CDC6, ORC1B, ORC4, and *Tb*3120 are directly involved in DNA replication (Marques et al., 2016).

The multi-subunit ORC complex in budding and fission yeast recognizes AT-rich DNA sequences (Chuang & Kelly, 1999; Kelly et al., 2011) (Table 1). AT-rich sequences have been associated with DNA replication, repair and transcription in many eukaryotes and are recognized by AT-hook motifs. These motifs can bind to the minor groove of AT-rich DNA, thus inducing a conformational change or recruiting other proteins (Kelly et al., 2011). In fission yeast, the Orc4 subunit contains nine AT-hooks, whereas *S. cerevisiae* Orc2 contains only one AT-hook (Chuang & Kelly, 1999; Kelly et al., 2011). HMGA1a is a classic AT-hook protein. Its three AT-hook domains are essential to form transcription enhanceosomes that are involved in the recruitment of DNA replication components to origins (Dragan et al., 2008; Norseen et al., 2008). Mammalian viruses also use AT-hook proteins to recruit ORC to the viral origin of replication (Kelly et al., 2011).

Aiyar et al. (Kelly et al., 2011) identified *LmjF*06.0720, a *L. major* protein that contains AT-hook domains and a nuclear localization sequence (NLS). They also found highly conserved *LmjF*06.0720 homologs in all sequenced *Leishmania* spp., *T. cruzi* and *T. brucei*, but not in the mammalian hosts. *Lam*ATY, the *L. amazonensis* homolog of *LmjF*06.0720, is expressed in both promastigotes and amastigotes and its AT-hook domains are functionally equivalent to the AT-hook domains of HMGA1a. Interestingly, these authors characterized a *Lam*ATY peptidomimetic that inhibits replication specifically in *L. amazonensis* promastigotes and intracellular amastigotes, without significant effects on mammalian cells (Kelly et al., 2011). Attempts to construct a knockout were infructuous, suggesting an essential role for *Lam*ATY. The molecular function of this protein is still unknown, but published results suggest that it could regulate a specific aspect of DNA replication in Trypanosomatidae.

In metazoan, the pre-RC assembles on ORC-bound origin DNA through the sequential association of CDC6, CDT1, MCM, and CDC45 (DePamphilis et al., 2006; Figure 1(A)). CDT1 is a central regulator of origin licensing in eukaryotes, from *S. cerevisiae* to mammals. CDT1 is essential for MCM complex recruitment and for origin licensing. CDT1 is a tightly regulated factor to prevent new origin firing before the accomplishment of the previous replication round (DePamphilis et al., 2006; Lutzmann et al., 2006; Nishitani et al., 2006; Oehlmann et al., 2007). Specifically, after origin firing, CDT1 is inactivated by different mechanisms. In yeasts, the Cullin4p–Ddb1p–Cdt2p complex promotes the degradation of Cdc6p and Cdt1p by the proteasome (Elsasser et al., 1999; Jallepalli et al., 1997). Cdt1p and MCM are also exported outside the nucleus during the S-G_2-M phases (Blow & Dutta, 2005; Labib et al., 1999). These mechanisms are also regulated by S-phase CDKs. In metazoans, the ORC complex is targeted for degradation during S-phase (Méndez et al., 2002). However, re-replication (i.e. the initiation of a new round of DNA replication before the completion of the previous one) is avoided primarily by inhibiting CDT1 function through two mechanisms: degradation in early S-phase and geminin-dependent inhibition (DePamphilis et al., 2006; Lutzmann et al., 2006; Nishitani et al., 2006; Oehlmann et al., 2007). CDT1 degradation has been described in human cells, *X. laevis* and *Caenorhabditis elegans* and occurs via a SCF complex-dependent pathway (Lutzmann et al., 2006; Thomer et al., 2004; Zhu et al., 2004). Geminin is a protein that binds to CDT1, thus inhibiting its recruitment to the ORC complex. This dual control prevents undesired re-replication. Intriguingly, no *CDT1* orthologue has been found in Trypanosomatidae (Table 1). Careful identity-based searches did not reveal any evident candidate. Further analyses should be done with more advanced algorithms to identify possible conserved domains using homology- or structure-based approaches (Damasceno et al., 2013; Nunes et al., 2011).

Nevertheless, this raises the question about the mechanism to prevent re-replication in Trypanosomatidae. Like in other protozoans, geminin homologous were not found in Trypanosomatidae (Table 1). Moreover, ORC1/CDC6 (Kumar et al., 2012), MCM2–7 (Dang & Li, 2011; Kumar et al., 2009; Minocha et al., 2011b), and GINS (Dang & Li, 2011) are expressed constitutively in the nucleus throughout the entire cell cycle, suggesting that the sub-localization of their

respective complexes is not a regulative mechanism in these organisms. Interestingly, Li et al. found that, in *T. brucei*, CDC45 is excluded from the nucleus after DNA replication (Dang & Li, 2011). This suggests the existence of a re-replication prevention mechanism that was not previously identified in other eukaryotes.

DDK is a protein kinase that plays an essential role in the initiation of DNA replication, from yeast to humans (Jiang et al., 1999). DDK is composed of the CDC7 kinase and a regulatory subunit called DBF4. CDK1 (Cdc28p in budding yeast) is another S-phase kinase that regulates the initiation of DNA replication when associated with S-phase cyclins. Interestingly, whereas a *CDK* orthologue was identified in Trypanosomatidae, we and others have failed to identify *CDC7* and *DBF4* orthologues in these species (Dang & Li, 2011; Tiengwe et al., 2014) (Table 1). This could be explained by extensive sequence divergence. Indeed, several kinases involved in replication have been described in trypanosomatids (Jones et al., 2014; Parsons et al., 2005). It is possible that one or more of these kinases is the functional homolog of CDC7. Alternatively, we cannot rule out the possibility that these genes have appeared after the separation of Trypanosomatidae from other eukaryotes or have been lost during evolution. In this case, CDK would be the only kinase regulating DNA replication initiation in Trypanosomatidae.

Mammalian Treslin (Sld3p in *S. cerevisiae*) and CDC45 associate simultaneously with replication origins at the time of replication initiation (Pospiech et al., 2010). In budding yeast, Sld3p has been identified as a limiting factor that works with Dbf4p to regulate the sequential firing of late origins (Lopez-mosqueda et al., 2010). As neither Sld3p nor Dbf4p are conserved in Trypanosomatidae (Figure 1(B)), origin firing must be regulated by different mechanisms in these parasites (Yoshida et al., 2013, 2014).

Unlike the ORC complex, the MCM complex, the GINS complex, and CDC45 are well conserved in Trypanosomatidae (Dang & Li, 2011). *MCM2–7* have been annotated in the *Leishmania* genome project (Table 1 and Figure 1). Moreover, sequences of domains associated with nucleoside-triphosphatase activity and with DNA binding have been identified in these *MCM2–7* sequences. Like in other eukaryotes, CDC45, MCM, and GINS components associate to constitute the CMG (CDC45–MCM–GINS) replicative helicase (Dang & Li, 2011; Takeda & Dutta, 2005).

Elongation

Synthesis of the two DNA strands occurs by two different mechanisms. One strand is synthesized continuously (leading strand) and the other discontinuously (lagging strand), with some mechanistic differences (Forsburg, 2004; Moldovan et al., 2007). The DNA polymerase cofactor PCNA (proliferating cell nuclear antigen) enhances significantly the processivity of replicative DNA polymerases (Forsburg, 2004; Moldovan et al., 2007). PCNA belongs to the family of DNA binding β clamps and is loaded onto the DNA by the RCF complex (Majka & Burgers, 2003). This clamp forms a trimeric ring around DNA, tethering not only replicative DNA polymerases but also many other proteins, such as error-prone translesion (TLS) polymerases, topoisomerase II, the licensing factor CDT1, base and nucleotide excision repair proteins, remodeling factors, and histone modifiers (reviewed in Moldovan et al., 2007). The association between these factors can be regulated by PCNA post-translational modifications. For instance, PCNA ubiquitination at lysine K164 is the signal to recruit TLS polymerases (Sebesta et al., 2013).

DNA elongation is also controlled by PI3KCB, a member of class I_A phosphatidylinositol 3-kinases (PI3KCs), in a PCNA-dependent manner. These PI3KCs include a p85 regulatory subunit and a p110 catalytic subunit, of which there are four isoforms (p110α, p110β, p110δ, and p100γ), thus forming PI3KCA, PI3KCB, PI3KCD, and PI3KCG, respectively (Jackson et al., 2005; Marqués et al., 2009). PI3KCB activity regulates the DNA replication rate by controlling PCNA binding to chromatin and to the lagging-strand replicative DNA polymerase δ (Marqués et al., 2009).

PCNA has been detected throughout the cell cycle in *L. donovani*, *T. brucei* and *T. cruzi* (Calderano et al., 2011; Kaufmann et al., 2012; Kumar et al., 2009; Valenciano et al., 2015) and its subnuclear expression pattern varies during the cell cycle. In the S phase, it is localized in subnuclear foci at the nucleus periphery, while in G_2/M, it is more widely distributed. This feature, which is conserved also in higher eukaryotes, indicates the presence of replication factories in specific sub-locations in *Leishmania* and related species (Calderano et al., 2011; Kumar et al., 2009; Schönenberger et al., 2015). Also two *PI3KC* paralogues are present in *Leishmania* spp (Table 3), but little is known about their *in vivo* roles.

During elongation, DNA is synthesized by replicative DNA polymerases belonging to the B family. Three DNA polymerase activities are conserved among all eukaryotes. DNA polymerase α contains primase activity and initiates replication by generating *de novo* RNA–DNA primers that are elongated by the polymerases δ and ε. Then, DNA polymerase δ replicates the lagging strand and polymerase ε the leading strand (Rudd et al., 2013, 2014). Replicative polymerases are characterized by high fidelity and high PCNA-regulated processivity.

Table 2. Homologous DNA polymerases from *H. sapiens*, *S. cerevisiae*, and *Leishmania* spp. (*L. infantum*, *L. braziliensis*, and *L. mexicana*).

	Family	*H. sapiens*	*S. cerevisiae*	*Leishmania* sp *(infantum, braziliensis and mexicana)*	Function[a]
DNA pol α	B-family	*POLA1* NP_058633.2	*POL1* *YNL102W*	*POLA1* (*LinJ.16.1640*; *LbrM.16.1600*; *LmxM.16.1540*)	Catalytic subunit of the DNA polymerase I α-primase complex; required for initiation of DNA replication during mitotic DNA synthesis
Primase	B-family (AEP-superfamily)	*PRIM1* NP_000937	*PRI1* 1 *YIR008C*	*PRIM1* (*LinJ.22.0270*; *LbrM.22.0370*; *LmxM.22.0390*)	Subunit of the DNA polymerase I α-primase o complex
DNA pol δ	B-family	*POLD1* NP_001243778.1	*POL3* *YDL102W*	*POLD1* (*LinJ.33.1790*; *LbrM.33.1960*; *LmxM.32.1690*)	Lagging-strand synthesis
DNA pol ε	B-family	*POLE* NP_006222.2	*POL2* *YNL262W*	*POLE* (*LinJ.35.4430*; *LbrM.34.4340*; *LmxM.34.4360*)	Leading-strand synthesis; checkpoint signal
Prim Pol	B-family (AEP-superfamily)	*PRIMPOL/* *CCDC111*	–	*PPL1* (*LinJ.33.0030*; *LbrM.33.0030*; *LmxM.32.0030*) *PPL2* (Rudd et al., 2013) (*LinJ.34.0110*; *LbrM.20.0070*; *LmxM.33.0100*)	Error-prone polymerase

[a]Functional description from the Saccharomyces genome database and Genome UCSC.
Genes nomenclature has been retrieved from HUGO gene nomenclature committee (http://www.genenames.org/) for *H. sapiens*; SGD: Saccharomyces genome database (http://www.yeastgenome.org/) for *S. cerevisiae*; and GeneDB (http://www.genedb.org). For Leishmania, genes names designation follows the guidelines for *Trypanosoma* and *Leishmania* genetic nomenclature (Clayton et al., 1998). Gene homologous are identified through an explicit heuristic phylogenetic analysis (not shown) optimized by maximum parsimony, using amino acid sequences available in existing public genetic databases.

These polymerases also display 3′-to-5′-exonuclease (proofreading) activity that strongly reduces nucleotide misincorporation. These replicative polymerases are conserved in *Leishmania* spp. (Table 2).

The Y-family (eta (η), iota (ι), kappa (κ), and REV1) and also the B-family (zeta (ζ)) of DNA polymerases have a role in TLS DNA synthesis. Members of the X-family (lambda (λ), mu (μ), beta (β)) and the A-family (nu (ν), theta (θ)) of DNA polymerases have special roles in DNA repair and are usually error-prone. TLS polymerases have low fidelity when replicating DNA, but play a key function in DNA lesion bypass. Indeed, although the vast majority of DNA lesions are rapidly repaired, some persist and block the progression of replicative polymerases. In this situation, TLS polymerases take the place of replicative polymerases, thus bypassing DNA damage but compromising sequence fidelity. Therefore, TLS is involved in an error-prone DNA damage tolerance mechanism that is associated with increased mutagenesis and is often related to carcinogenesis. However, in the long term, TLS also promotes genetic diversity and evolutionary fitness (Goodman & Woodgate, 2014). TLS polymerase orthologues have been found in Trypanosomatidae (Genois et al., 2014), suggesting a conserved role. However, little is known about these proteins and differences could exist. This is the case, for instance, of PrimPol-like polymerases. These proteins belong to the archaeo-eukaryotic primase (AEP) superfamily of the B-family of DNA polymerases. These polymerases can initiate *de novo* DNA/RNA synthesis and elongate the DNA chain at stalled replisomes, rather than catalyze TLS DNA synthesis. Human PRIM1 has primase activity and acts together with DNA polymerase α for DNA/RNA primer synthesis on the leading and lagging strands (Okazaki fragments). An orthologue of human *PRIM1* was identified in *S. cerevisiae* (*PRI1*) and in *L. braziliensis* (*LbrM.22.0370*) (Table 2). Human PRIMPOL is another member of the AEP superfamily and is recruited to DNA lesions via the RPA1 protein (Wan et al., 2013). Recently, Doherty et al. (Rudd et al., 2013) reported that Trypanosomatidae, including *Leishmania spp*., is the only group with two PRIMPOL-like proteins (PPL1 and PPL2) (Table 2). PPL1, like human PRIMPOL, is not essential for *T. brucei* viability. On the contrary, PPL2 seems to have a specialized role in finishing DNA replication properly, particularly in perturbed conditions. Therefore, it is essential for *T. brucei* viability. PrimPol-like proteins are not conserved among all eukaryotes. For instance, they are absent in *Drosophila*, *C. elegans,* and many fungi, including *S. cerevisiae*, suggesting an early horizontal origin. It has been proposed that PPL2 is required for bypassing endogenous DNA damage generated during S phase (Rudd et al., 2013, 2014).

Mechanisms involved in maintaining genome stability

DNA double-strand breaks (DSBs) are one of the most harmful cell lesions. Unsuccessful DSB repair can result in uncontrolled chromosome rearrangements, leading to cell damage and death. Cells have developed mechanisms to detect and properly repair DSBs, thus ensuring genome integrity. The DNA damage checkpoint is a specialized pathway that detects DNA damage in the G_1, S and G_2/M phases. Once this surveillance mechanism is activated, signal transduction results in activation of DNA repair genes, transient arrest of cell-cycle progression, and other protective cellular responses.

Overall, these signaling and repair proteins are highly conserved in eukaryotes, from yeast to mammalian species (Finn et al., 2012; Nnakwe et al., 2009). However, little is known about the involvement of these mechanisms in the stability of Trypanosomatidae genomes. A detailed review on DNA repair pathways in Trypanosomatidae has been written by Masson et al. (Genois et al., 2014).

DNA repair pathways

Two major repair pathways are activated in eukaryotic cells, in response to DSBs: non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ acts primarily during the G_1 phase, ligating the two broken ends with little or no processing (Genois et al., 2014; Gobbini et al., 2013). NHEJ is a very efficient pathway, but can result in uncontrolled chromosomal rearrangements. HR, which occurs mainly during the S and G_2 phases, is more precise because it uses undamaged homologous DNA templates for error-free repair (Genois et al., 2014; Longhese et al., 2010; Prado, 2014).

In mammalian cells when a DSB is repaired by NHEJ, the heterodimer Ku70/Ku80 and the kinase ATM are quickly recruited to the lesion. ATM marks the adjacent chromatin by phosphorylating H2AX (γ-H2AX), a histone variant frequently used as DNA damage marker. γ-H2AX is recognized by the BRCT domains of the MDC1 mediator (Rad9p *in S. cerevisiae*) that transduces the DNA damage response. Rad9 also has homology with human 53BP1, which has a role in inhibiting DSB resection in G_1 (Bothmer et al., 2010) and directs repair by NHEJ. In parallel, the Ku heterodimer recruits, among other factors, DNA-PKcs-Artemis, a nuclease that can process DNA ends, and XRCC4, a ligase IV that seals the DSB. Blast searches have only identified a few NHEJ factors in Trypanosomatidae (Genois et al., 2014), such as the KU heterodimer and DNA-PKcs. Conversely, orthologues of Artemis and XRCC4 were not found. Taken together, these observations suggest that this pathway might not be conserved in Trypanosomatidae (Conway et al., 2002a). However, other evidences suggest that NHEJ variants operate in Trypanosomatidae to join DSB ends, when HR is switched off. These include alt-NHEJ and microhomology-mediated end joining (MMEJ), which is DNA ligase IV-independent and requires a small resection of DNA ends (Burton et al., 2007; Glover et al., 2008).

When DSBs occur in the S or G_2 phase, DNA ends are extensively resected to generate protruding 3′ single-stranded DNA (ssDNA) tails. This structure inhibits NHEJ and targets DSB for repair by HR (Prado, 2014). In this context, the eukaryotic MRN (MRE11–RAD50–NBS1) complex and the CtIP protein (Sae2p in *S. cerevisiae*) induce the resection of DSB ends by EXO1 exonucleolytic activity and select HR for lesion repair (Genois et al., 2014, 2015; Nimonkar et al., 2011). CtIP is required also for the recruitment of replication protein A (RPA). Then, RPA-coated ssDNA recruits the ATR (Mec1p in *S. cerevisiae*) checkpoint kinase, triggering the signaling cascade. Subsequently, RPA is replaced by RAD51, a highly conserved recombinase. The RAD51–ssDNA nucleoprotein filament searches for DNA homology on the intact sister chromatid, generating the so-called D-loop. In humans, BRCA2 and four RAD51 homologs (reviewed in Genois et al., 2014, 2015) assist RAD51 in this process. Finally, the 3′-end is extended and the resulting DNA structure is resolved, generating two intact copies at their respective location. DNA extension in humans seems to be done by DNA polymerase δ (an error-free polymerase) as well as η and κ, but not ι (error-prone polymerases) (Sebesta et al., 2013).

Trypanosomatidae could use HR as a general strategy to generate genomic variation, especially when exposed to toxic stress. For instance, in *T. brucei,* HR generates antigenic variation on the variant surface glycoprotein (VSG) protein (Conway et al., 2002b). This could explain its ability to evade host immunity. A different strategy has been identified in *Leishmania* spp., where gene amplification is performed by generating linear and circular extrachromosomal amplicons, as a mechanism to escape drug exposure. To do this, the whole genome is constantly rearranged through inverted repeats, possibly explaining the high plasticity of this microorganism (Ubeda et al., 2008, 2014).

DNA damage response

Alongside the activation of the repair mechanism, DSBs also trigger the activation of the DNA damage checkpoint. In mammals, ATM and ATR (Tel1p and Mec1p in *S. cerevisiae*) are sensor proteins that belong to the phosphoinositide 3-kinase-related protein kinase (PIKK) family and play a central role in DNA damage checkpoint signaling. ATM and ATR are activated by different types of DNA damage. ATM is activated by DSBs, but the specific signals are still unclear. ATR responds to abnormal DNA structures, such as stalled replication forks or DSBs, by interacting with RPA-coated ssDNA during the S and G_2 phases (Ciccia & Elledge, 2010; Gobbini et al., 2013; Yoshida et al., 2013). Well-conserved homologs have been identified in *Leishmania* spp. (Genois et al., 2014) (Table 3), supporting the idea that the DNA damage response is functional in this microorganism. Nevertheless, few functional studies

have been performed so far. These are necessary to determine whether other mechanisms are also conserved because these proteins are potential therapeutic targets.

Once activated, the signal is transduced to the effector kinases CHK2 and CHK1 (Rad53p and Chk1p in *S. cerevisiae*) through the mediators MDC1 and 53BP1 (Finn et al., 2012; Nnakwe et al., 2009; Wang et al., 2012). The roles of MDC1 and 53BP1, the two homologs of *S. cerevisiae* Rad9p, are quite overlapping, although they also might have specific functions (Hable et al., 2012; Wilson & Stern, 2008). Downregulation of MDC1, the mediator of the DNA-damage checkpoint, induces different phenotypes, including hypersensitivity to DSB induction, defective checkpoint activation, and failure to maintain genome stability (Coster & Goldberg, 2010; Lou et al., 2006; Stewart et al., 2003; Xu & Stern, 2003). We could not identify *ScRAD9* and *Hs53BP1* orthologues in Trypanosomatidae, consistently with previously published studies (Genois et al., 2014) (Table 3, Figure 2). However, a *hsMDC1* orthologue has been identified (Genois et al., 2014). Further work is needed to determine whether this *hsMDC1* orthologue has a conserved role in Trypanosomatidae.

To fully activate ATR in response to DNA damage, the 9-1-1 ring complex (RAD9–RAD1–HUS1 in humans; Ddc1p–Rad17p–Mec3p in *S. cerevisiae*) is loaded at the junction between ssDNA and dsDNA. This complex stimulates ATR kinase activity, which probably recruits TOPBP1 (Dpb11p in *S. cerevisiae*) through interaction with RAD9 (Ddc1p in *S. cerevisiae*) (Gobbini et al., 2013). Differences in molecular mechanisms, depending on the DNA damage type and the cell cycle phase, have been described (Puddu et al., 2011). HUS1 (*Lm*HUS1) and RAD9 homologs have been identified in *L. major* (Damasceno et al., 2013; Nunes et al., 2011) (Table 3). Despite its low-sequence identity (11.7% with *H. sapiens HUS1* gene), *Lm*HUS1 secondary and tertiary structures are highly conserved. Analyses also revealed the functional conservation of *Lm*HUS1 function in the DNA damage response. *Lm*HUS1 co-localizes with RPA1 in the nucleus. When overexpressed, *Lm*HUS1 confers resistance to genotoxic agents (hydroxyurea, HU, and

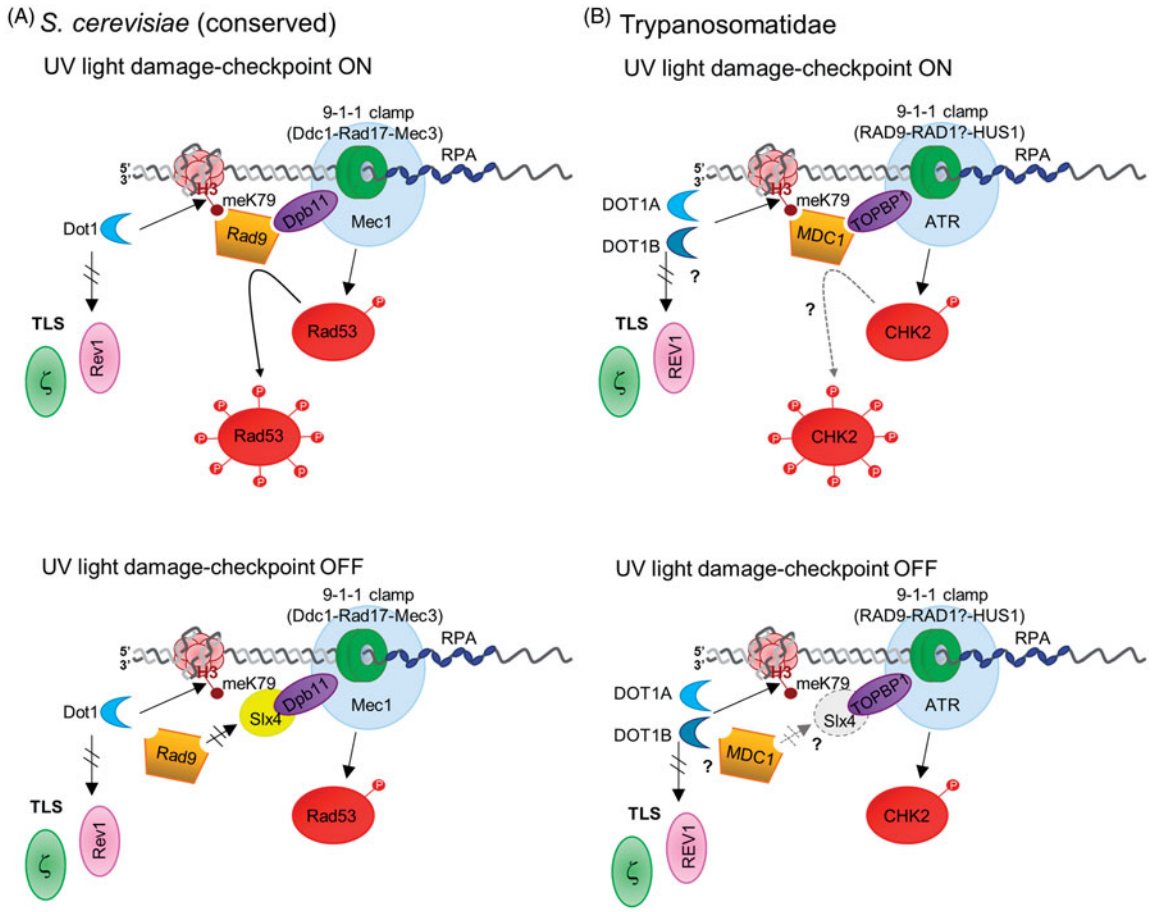

Figure 2. Representation of the main players involved in switching on/off the DNA damage response in high eukaryotes (A, based in *S. cerevisiae*) and Trypanosomatidae (B). Gray symbols with dashed lines represent proteins involved in DNA damage signaling in yeast/high eukaryotes, but not identified in Trypanosomatidae.

methyl methane sulfonate, MMS). In agreement, a *Lm*HUS1-deficient cell line is sensitive to HU, MMS, and camptothecin (CPT) (Damasceno et al., 2013; Nunes et al., 2011). These authors also found an orthologue of *RAD9* in *L. major* (*LmRAD9*), but not of *RAD1*. Like *Lm*HUS1, *Lm*RAD9 is involved in the DNA damage response (Damasceno et al., 2013; Figure 2). Nevertheless, not all Trypanosomatidae species have *HUS1* and *RAD9* orthologues (Genois et al., 2014). This indicates evolutionary divergences within this taxon that deserve further analysis. Finally, in the case of failure of searches based on sequence identity, it may be possible to identify many missing orthologues solely based on the conservation of their tertiary structure.

Post-translational modifications involved in the regulation of the DNA damage response

In *S. cerevisiae*, Rad9p-mediated activation of the DNA damage checkpoint depends on two sub-pathways mediated by Dot1p and Dpb11p (through interaction with the 9–1-1 ring complex), separately or simultaneously (Ohouo et al., 2013; Puddu et al., 2008). Dot1p is a histone methyltransferase that methylates histone H3 on lysine 79 (H3K79me), thus allowing Rad9p binding to methylated H3K79 and the transduction of the checkpoint signal. Dpb11p has two functions: one in checkpoint signal transduction via interaction with Rad9p, and the second one in Cdc45p loading during replication initiation (Finn et al., 2012; Tanaka et al., 2013; Wang et al., 2012).

A competition mechanism to regulate Mec1p-dependent checkpoint signal transduction has been described in yeast. *Saccharomyces cerevisiae* Rad9p is usually considered to work as an adaptor that senses Mec1p activation through Dpb11p and H3K79me interactions, channeling signal transduction to the effector kinase Rad53p (Ohouo et al., 2013). However, Slx4p competes with Rad9p for binding to Dpb11p, therefore, controlling the checkpoint status. No *SLX4* orthologue has been identified in Trypanosomatidae, and more studies are required to understand how this mechanism operates in these microorganisms (Genois et al., 2014; Figure 2). It should be noted that *MDC1* homologs have been identified in some species of the Trypanosomatidae family, such as *L. infantum, L. braziliensis*, *L. major,* and *T. congolense*, but not in *T. cruzi*, *T. brucei,* or *T. vivax* (Genois et al., 2014) (Table 3).

In budding yeast, Dot1p is involved in many activities, mostly DSB repair but also sister-chromatid cohesion and gene expression (Kim et al., 2014). Interestingly, it has been reported that *S. cerevisiae* Dot1p represses the TLS polymerases Rev3p and Rev1p (Conde et al., 2009; Lévesque et al., 2010) and, therefore, contributes to genome stability and sequence fidelity. Moreover, two *DOT1* orthologues (*DOT1A* and *DOT1B*) identified in *T. brucei* are also conserved in *Leishmania* spp. (Janzen et al., 2006) (Table 3) DOT1A dimethylates and DOT1B trimethylates H3K79. DOT1B seems to have a role in the transition to trypanosome procyclic forms, while DOT1A has an essential role in regulating DNA replication (Gassen et al., 2012). When DOT1A is overexpressed, cells show over-replication, and when it is suppressed by RNA interference, DNA replication is abolished. It would be interesting to determine whether DOT1 function as a TLS polymerase repressor is conserved in the Trypanosomatidae homologs DOT1A and DOT1B.

TLS polymerases are also regulated by PCNA mono-ubiquitination on K164. This modification increases PCNA binding affinity for the ubiquitin binding domains of TLS polymerases. PCNA is ubiquitinated on K164 by RAD6 and RAD18 (E2-ubiquitin conjugation and E3-ubiquitin ligase enzyme, respectively) (McIntyre & Woodgate, 2015). It was recently reported that the *L. donovani* histone acetyltransferase HAT3 can acetylate PCNA, a requirement for its subsequent ubiquitination (Kumar & Saha, 2015).

Ubiquitination is a general strategy to mark proteins for degradation. The $CUL4^{DDB1-MMS22L}$ complex ($Rtt101p^{Mms1p-Mms22p}$ complex in *S. cerevisiae*) is an E3-ubiquitin ligase involved in the DNA damage response activated by replication stress (Kaur et al., 2012; Piwko et al., 2010; Roberts et al., 2008; Zaidi et al., 2008). *CUL4* and *DDB1* orthologues are present in *Leishmania* spp. This suggests that this fork protection mechanism is conserved, although we have been unable to find an orthologue of *MMS22L* (Table 3).

Another important factor in DNA damage signaling is phosphorylation of the histone variant H2AX ($\gamma-$H2AX). This post-translational modification is highly conserved among eukaryotes. In humans, H2AX is a variant encoded by a gene distinct from the canonic histone H2A. H2AX is phosphorylated ($\gamma-$H2AX) on serine 139 in response to DNA damage (Rogakou et al., 1998). In budding yeast, two genes (*HTA1* and *HTA2*) encode a single canonic histone H2A. This histone can be phosphorylated on serine 129 upon stress (like for γH2AX) (Downs et al., 2000; Szilard et al., 2011). Similarly, Trypanosomatidae have a canonical histone H2A that is phosphorylated in response to DNA damage. Noteworthy, this phosphorylation occurs on threonine 130, a position that seems to be unique to the Trypanosomatidae family (Glover & Horn, 2012). It was proposed that H2AX phosphorylation leads to

Table 3. Components from checkpoint and repair pathways. The table show the gene symbol for *H. sapiens*, *S. cerevisiae*, and three *Leishmania* spp. (*L. infantum*, *L. braziliensis*, and *L. mexicana*). In parenthesis are indicated the systematic gene name for *Leishmania* spp.

H. sapiens		*S. cerevisiae*	*Leishmania* sp *(infantum, braziliensis and mexicana)*	Function[a]
ATR		*MEC1*	*ATR* (Genois et al., 2014) (*LinJ.32.1520*; *LbrM.32.1620*; *LmxM.31.1460*)	Genome integrity checkpoint protein and phosphoinositide 3-kinase superfamily member; sensor effector kinase for damaged or non-replicated DNA
ATM		*TEL1*	*ATM* (Genois et al., 2014) (*LinJ.02.0100*; *LbrM.02.0130*; *LmxM.02.0120*)	Protein kinase primarily involved in telomere length regulation; contributes to cell cycle checkpoint control in response to DNA damage
BLM		*SGS1*	*SGS1* (*LinJ.24.1590*; *LbrM.24.1330*; *LmxM.24.1530*)	RecQ family nucleolar DNA helicase; role in genome integrity maintenance, chromosome synapsis. Human BLM and WRN implicated in Bloom syndrome and in Werner syndrome, respectively
CHK1		*CHK1*	Not found (Genois et al., 2014)	Serine/threonine kinase and DNA damage checkpoint effector; phosphorylated by the checkpoint signal transducer ATR
CHEK2		*RAD53*	*CHK2* (Genois et al., 2014) (*LinJ.17.0070*; *LbrM.17.0070*; *LmxM.17.0060*)	DNA damage response protein kinase; required for cell-cycle arrest in response to DNA damage. In yeast, Rad53 activation occurs through auto-phosphorylation and direct phosphorylation by Mec1
PCNA		*POL30*	*PCNA* (Genois et al., 2014) (*LinJ.15.1500*; *LbrM.15.1440*; *LmxM.15.1450*)	Proliferating cell nuclear antigen (PCNA); functions as the sliding clamp for DNA polymerase delta; may function as a docking site for other proteins required for mitotic and meiotic chromosomal DNA replication and for DNA repair
UBE2A/*UBE2B* (ancient nomenclature *Rad6A*/*Rad6B*)		*RAD6*	*RAD6* (Genois et al., 2014) (*LinJ.22.0480*; *LbrM.22.0550*; LmxM.22.0610)	Ubiquitin-conjugating enzyme (E2); involved in post-replication repair as a heterodimer, in yeast, with Rad18. Role in PCNA ubiquitination at stalled replication forks
RAD18		*RAD18*	*RAD18* (Genois et al., 2014) (*LinJ.17.0340*; *LbrM.17.0270*; *LmxM.17.0290*)	Ubiquitin-conjugating enzyme (E2); involved in post-replication repair as a heterodimer, in yeast, with Rad6. Role in PCNA ubiquitination at stalled replication forks
DOT1		*DOT1*	*DOT1A* (Frederiks et al., 2010) (*LinJ.07.0030*; *LbrM.07.0030*; *LmxM.07.0025*) *DOT1B* (Frederiks et al., 2010) (*LinJ.20.0030*; *LbrM.20.4110*; *LmxM.20.0030*)	Nucleosomal histone H3-Lys79 methylase; methylation is required for telomeric silencing, meiotic checkpoint control, and DNA damage response. In Trypanosomatidae, DOT1A has a role in DNA replication and DOT1B in the transition to procyclic forms of the parasites
SLX4		*SLX4*	Not found	Endonuclease involved in DNA processing
Class I_A Phosphatidylinositol 3-kinase (PI3K) family	*PIK3CA* *PIK3CB* *PIK3CD* *PIK3CG*	*VPS34*	*PIK3C1* (*LinJ.14.0020*; *LbrM.14.0020*; *LmxM.14.0020.1*) *PIK3C2* (*LinJ.24.2090*; *LbrM.24.2090*; *LmxM.24.2010*)	Enzyme with phosphatidylinositol 3-kinase (PI3K) class IA activity, required for controlling cell-cycle entry
53BP1, *BRCA1*		*RAD9*	Not found (Genois et al., 2014)	Proteins involved in DNA damage repair and cell cycle progression
MDC1			*MDC1* (*LinJ.34.4070* (Genois et al., 2014); *LbrM.20.3870*; *LmxM.33.4240*)	Mediator of DNA-damage checkpoint 1
(9-1-1 clamp)	*RAD9*	*DDC1*	*RAD9* (*LinJ.15.1040*; *LbrM15.1020*; *LmxM.15.0980*) (Damasceno et al., 2013)	Heterotrimeric PCNA-like complex that encircles DNA specifically at damaged sites, where it acts as a platform for checkpoint and DNA repair proteins
	HUS1	*MEC3*	*HUS1* (*LinJ.23.0330*; *LbrM.23.0320*; *LmxM.23.0290*) (Damasceno et al., 2013; Nunes et al., 2011)	
	RAD1	*RAD17*	*RAD1* (*LinJ.20.0460*; *LbrM.20.4640*; *LmxM.20.0390*)	
Cul4 E3 Ubiquitin Ligase	*CUL4A*/*CUL4B*	*RTT101*	*CUL4* (*LinJ.24.2380*; *LbrM.24.2370*; *LmxM.24.2290.1*)	Cullin-Roc1-dependent E3 ubiquitin ligase complex; role in anaphase progression; implicated in DNA repair and stabilization of replication forks after DNA lesions
	DDB1	*MMS1*	*DDB1* (*LinJ.30.3770*; *LbrM.30.3750*; *LmxM.29.3710*)	
	MMS22L	*MMS22*	Not found	

[a]Functional description from the Saccharomyces genome database and Genome UCSC.

Genes nomenclature has been retrieved from HUGO gene nomenclature committee (http://www.genenames.org/) for *H. sapiens*; SGD: Saccharomyces genome database (http://www.yeastgenome.org/) for *S. cerevisiae*; and GeneDB (http://www.genedb.org). For Leishmania genes names designation follows the guidelines for *Trypanosoma* and *Leishmania* genetic nomenclature (Clayton et al., 1998). These gene are identified by protein similarity obtained by BLAST search (http://www.ncbi.nlm.nih.gov/; http://www.genedb.org/blast).

nucleosome remodeling to allow repair factors to access the DNA break, possibly facilitating its repair by the NHEJ and HR pathways (Heo et al., 2008).

Genome-wide replication on kinetoplastids

On one hand, few studies have investigated the global replicative program in kinetoplastids. Nevertheless, the detailed analysis by McCulloch et al. (Tiengwe et al., 2012a) highlighted the interdependence between the genome polycistronic arrangement and global DNA replication in *T. brucei*. They studied DNA replication in *T. brucei* by detecting BrdU incorporation using a marker frequency analysis (MFA) approach. They estimated approximately 100 origins per cell, which means one origin every 260 kbp. This density is much lower than in other organisms (budding yeast: one origin/46 kbp; *Arabidopsis thaliana*: one origin/77 kbp; mammalian cells: one origin/25–130 kbp) (Cayrou et al., 2011; Costas et al., 2011; Crabbé et al., 2010; Sequeira-Mendes et al., 2009). On the other hand, most kinetoplastid origins are found in the chromosome core (defined as the highly transcribed region containing housekeeping genes), like in other eukaryotes (Tiengwe et al., 2012a). Moreover, many studies in yeast reported that the transcription machinery can interfere with replication fork progression (Azvolinsky et al., 2009). In agreement, the orientation of the transcription machinery is correlated with that of the replication machinery (Tiengwe et al., 2012a). Indeed, in Trypanosomatidae, when an active origin is located between two divergent directional gene clusters (DGCs), the replication and transcription machineries move in the same direction, as indicated by symmetrical BrdU peaks. Asymmetrical peaks are found when the origin is located between two DGCs where transcription both initiates and terminates. Specifically, the slope is weaker when transcription and replication proceed in the same direction and sharper when they travel in the opposite ways. These data are consistent with the molecular mechanism called transcription-associated recombination (TAR), described by Aguilera et al. (Prado & Aguilera, 2005). TAR is activated in response to conflicts between replication and transcription to repair stalled replication forks by recombination (Poveda et al., 2010; Prado & Aguilera, 2005).

Intriguingly, BrdU incorporation sites in *T. brucei* colocalize with only a subset of ORC1/CDC6 binding sites. Conversely, ORC1/CDC6 binding sites located at the boundaries of transcriptional units are not associated with BrdU signals. Interestingly, ORC1/CDC6 silencing by RNAi results in general changes of mRNA abundance, increasing the expression of mRNA transcripts upstream and downstream of the transcription unit's start and stop points, but not of genes within the DGCs. Whether ORC1/CDC6 has a non-replicative function or acts as a silencer remains to be determined. As *Trypanosoma* and *Leishmania* are two closed species, it was expected that these genome organization features were conserved. But surprisingly, a recent work shows an atypical genome organization in *Leishmania major* (Marques et al., 2015), containing a single replication origin by chromosome. Even if authors provide evidence that the origin location is conserved between *Leishmania* spp. and *T. brucei*, this particularity of *Leishmania* spp. uncovers prominent variation in the underlying replication mechanism that deserves to be explored. *L. major* has more DGC (133, Ivens et al., 2005); 171 predicted, Marques et al., 2015) than replication origins (36, Marques et al., 2015), but the overlap between origins and DGC boundaries is 21%. These features are reminiscent of *T. brucei*, where an overlapping of 27% has been reported (Marques et al., 2015; Tiengwe et al., 2012a). Next, an interesting challenge is to define whether there is a correlation between both structures, and why.

On one hand, as the genomic organization in polycistronic DGCs is characteristic of kinetoplastids, these parasites provide a unique model for studying the interactions between replication and transcription. This genome organization suggests that specific mechanisms to avoid collisions and modulate interferences between the replication and transcription machineries could have been developed in these microorganisms in order to maintain genome stability. On other hand, these interferences could also be a source for genomic arrangements that could explains, at least in part, the high plasticity of kinetoplastids. More detailed studies are necessary to better understand the mechanism involved in genomic stability maintenance in kinetoplastids.

Drugs and potential targets

Commercial drugs for leishmaniasis are classified in five groups (reviewed in De Menezes et al., 2015; Table 4). These treatments have been developed many years ago and have serious limitations, such as high toxicity and severe adverse reactions. Pentavalent antimonials are the most frequently used drugs, but they have several adverse effects, such as cardiotoxicity, pancreatitis and nephrotoxicity. Similarly, amphotericin B is frequently administered despite its serious adverse effects (myocarditis and nephrotoxicity). Its liposomal formulation is less toxic, but the associated cost increment makes it unaffordable. Pentamidine is a less used alternative, because of its toxicity and low efficacy. In addition,

Table 4. Drugs currently used for leishmaniasis treatment and unexploited drugs with potential application.

Drugs	Mechanism of action[a]	References
Pentavalent antimonials	Inhibit TOPIB in trypanosomatids	Walker & Saravia (2004), Bakshi et al. (2009), and Balaña-Fouce et al. (2012)
Amphotericin B/liposomal amphotericin B	Alters cell membrane sterols, resulting in disruption of membrane integrity	Saha et al. (1986)
Pentamidine	The exact mechanism of action is unknown, but interferes with nuclear metabolism by inhibiting the synthesis of DNA, RNA, proteins and phospholipids	Imming et al. (2006)
Paromomycin	Inhibit protein synthesis by binding to ribosomal RNA	Salah et al. (2013)
Miltefosine	Induces apoptosis and disturbs lipid-dependent cell signaling pathways. Also alters Ca2+ homeostasis	Verma & Dey (2004) and Serrano-Martín et al. (2009)
Unexploited potential drugs	**Mechanism of action[a]**	**References**
PI3K inhibitors	Inhibitors of the catalytic subunit of class IA phosphoinositide 3-kinase (PI3K), an enzyme involved in controlling cell cycle entry. Unexplored in trypanosomatids	Jackson et al. (2005), Knight et al. (2006), and Marqués et al. (2009)
Nucleoside, peptidomimetics, and other analogs	Inhibit DNA replication, inhibit DOT1L, inhibit ATY1. Commonly used in cancer therapy or as antiviral drugs. Target unknown in trypanosomatids	Imming et al. (2006), Basavapathruni et al. (2007), Travesa et al. (2008), Araújo et al. (2011), Kelly et al. (2011), Martin et al. (2014), Carreras Puigvert et al. (2015), Freitas et al. (2015), and Stein & Tallman (2015)
Quinolones and derivatives, podophylotoxins, anthracyclins, flavonoids, aminocoumarins, acridines, triterpenoids	Inhibit bacterial TopII (gyrase; Topo IV) and trypanosomatid TOPII	Nenortas et al. (1999), Romero et al. (2005), Cortázar et al. (2007), and Carreras Puigvert et al. (2015)
Camptothecin, indocarbazoles, flavonoids poliheterocyclic, bis-benzimidazoles, triterpenoids, unsaturated fatty acids, lignan glycosides	Inhibit TOPIB in trypanosomatids	Deterding et al. (2005), Balaña-Fouce et al. (2012), Prada et al. (2013), Balaña-Fouce et al. (2014), and Carreras Puigvert et al. (2015)
Radiomimetics, DNA crosslinkers, alkylating agents, nitrogen mustards, intercalators	Interact with DNA and damage the DNA template	Raman et al. (2008a, b), Pommier (2009), Raman et al. (2013), and Carreras Puigvert et al. (2015)
Antiarrhythmic agents	Alter Ca2+ homeostasis	Benaim et al. (2013, 2014)
Triazole drugs	Interfere with ergosterol biosynthesis	Calvopina et al. (2004), Paniz Mondolfi et al. (2011), De Andrade et al. (2015), and Molina et al. (2015)

[a]Functional description from the DrugBank database.

cases of resistance development have been reported. Paromomycin also is associated with high toxicity. Finally, mitelfosine has the advantage of oral administration, but has a teratogenic effect and low overall efficacy. This, together with the long treatment duration, leads to high abandonment rates. In addition, these drugs are effective only against some *Leishmania* species (De Menezes et al., 2015) and many cases of resistances have been described (Calvopina et al., 2006; Leprohon et al., 2015; Ubeda et al., 2014). The remarkable genomic plasticity of these parasites seems to play a central role in drug resistance. *Leishmania* parasites can increase their gene copy number by gene amplification or expansion. Amplified DNA is usually extrachromosomal, arranged in linear or circular mini-chromosomes. Aneuploidy and single nucleotide polymorphisms are also widespread phenomena in *Leishmania* spp. (reviewed by Leprohon et al., 2015). All these events contribute to developing drug resistance. In this context, it is crucial to characterize alternative compounds with pharmacological activity against *Leishmania* (Table 4). Understanding the molecular mechanism of drugs and their targets is essential for developing new compounds with pharmacological activity against *Leishmania* spp. In particular, the use of drugs targeting DNA replication and damage proteins (and also other biological processes) for the treatment of leishmaniasis has remained largely unexplored. Moreover, some already commercially available drugs that target these factors may have leishmanicidal activity, but clinical studies need to be performed to determine their potential efficiency.

In mammals, p110β, one of the four of class I phosphatidylinositol 3-kinases (PI3K) catalytic subunit (Table 3), has a role in controlling S phase (Marqués et al., 2009). p110β is selectively inhibited by TGX-221 (Jackson et al., 2005). Moreover, NIH 3T3 human cells treated with TGX-221 *in vitro* show a defect in DNA replication fork progression (Marqués et al., 2009). In *Leishmania* spp., only two homologs have been founded, encoded by *PIK3C1* and *PIK3C2* (Table 3), already suggesting differential molecular mechanism.

Further studies are needed to determine whether this compound or its derivatives could also inhibit either PI3KCs identified in *Leishmania* spp.

Nucleosides analogs could also be used against *Leishmania* infections. For instance, the anti-retroviral prodrug zidovudine (3-azido-3-deoxythymidine, AZT) has leishmanicidal activity (Araújo et al., 2011; Timm et al., 2015). AZT is recognized and phosphorylated by *L. major* thymidine kinase, a critical step that allows its incorporation in DNA, leading to DNA elongation inhibition (Timm et al., 2015). Recently, it has been reported that immucillins (deazapurine nucleoside analogs) could potentially be used as specific *Leishmania* inhibitors without macrophage toxicity (Imming et al., 2006). The mechanism of action has not been established yet, but it may involve inhibition of DNA or RNA polymerases or other enzymes related to nucleoside metabolism or transport. Other possibility is that anti-retroviral weaken *Leismania* cells because it affects LRV (*Leishmania* RNA virus), a natural endosymbiont found in *L. guyanensis* (Ives et al., 2012; Zangger et al., 2013). If this or other similar viruses are associated to other *Leishmania* species is currently unknown.

Topoisomerases are also a promising target. Trypanosomatids have five topoisomerases [TOPIA, TOPIB (L, large and S, small), TOPII and TOPIII], and the mitochondrial topoisomerase mtTOPII. TOPIB and TOPII are validated targets for anti-neoplastic therapies. Silencing of the orthologous genes in trypanosomatids leads to kDNA degradation and parasite death, indicating that they have essential functions (reviewed in Balaña-Fouce et al., 2014). The effect on trypanosomatids of many TOPIB and TOPII inhibitors have been widely tested (Table 4) and good killing efficiency against *T. brucei*, *L. panamensis,* and *L. infantum* has been reported (Balaña-Fouce et al., 2014; Cortázar et al., 2007; Farca et al., 2012; Nenortas et al., 1999; Romero et al., 2005; Vouldoukis et al., 2006). In particular, many studies support the combination of fluoroquinolones with other drugs, as a promising alternative for leishmaniasis treatment (Farca et al., 2012; Romero et al., 2005; Vouldoukis et al., 2006).

Recent efforts have been focused on TOPIB. Indeed, the presence in trypanosomatids of two different TOPIB genes (*TOPIBL* and *TOPIBS*) (Annessa et al., 2015; Balaña-Fouce et al., 2014) indicates different structural properties compared with mammalian TOPIB, thus making of them good potential candidates for leishmaniasis treatment. Interestingly, pentavalent antimonials, the current first-line chemotherapy for patients with leishmaniasis, have TOPIB inhibitor activity (Bakshi et al., 2009; Walker & Saravia, 2004). In accordance, their high toxicity is probably explained by their effect against both trypanosomatid and human TOPIB (Pommier, 2003, 2009). Similarly, many TOPII inhibitors show also activity against trypanosomatid TOPII (Table 4).

Other types of drugs have been characterized for their ability in damaging the DNA template. Radiomimetic drugs, such as bleomycin, cisplatin, and its derivatives, alkylating agents, nitrogen mustards, and intercalators (e.g. Schiff bases) belong to this group (Pommier & Robert, 2001; Raman et al., 2013). More studies are needed to determine their potential as leishmanicidal agents.

Differential molecular mechanism on DNA replication and repair pathways between humans and trypanosomatids highlight the presence of divergent genes, which constitute interesting potential targets for dealing with this infection. A special attention should be paid to specific trypanosomatids genes (Table 1). *ATY* orthologues are present in *Leishmania* spp. In *L. amazonensis* ATY seems to have an essential role in DNA replication (Kelly et al., 2011). Using a peptidomimetic authors succeed to inhibit *L. amazonensis* promastigotes and amastigotes, with minimal macrophage death.

Also orthologues of *Tb3120* and *Tb7980* are found in *Leishmania* spp. (Table 1; Shen, 2013; Tiengwe et al., 2012b). These genes have been identified as divergent homologs of *ORC4*. Also *ORC1/CDC6* and *ORC1B* genes from trypanosomatids share a limited homology with other eukaryotic *ORC1* genes. Together, these findings strongly support that the establishment of competitive replication origins in trypanosomatids differs from other eukaryotes, where ORC1–6 complex is the responsible of this process. It is precisely these particularities that makes these proteins suitable targets for a potential therapy against *Leishmania* and/or *Trypanosoma* spp.

Other interesting approach is the possibility of using anti-cancer drugs for combating leishmaniasis and related diseases. Many efforts have been made in characterizing anticancer drugs and a subset of these drugs targets proteins implicated in DNA replication. The advantage of these anti-cancer drugs is that most of them are well characterized and clinical trial has been performed. Taken advantage of this understanding and considering some targets with specific differences in trypanosomatids, these drugs open encouraging opportunities to deal with these tropical diseases, which deserve to be explored. Promising targets include DOT1A and DOT1B (Table 3; Frederiks et al., 2010; Janzen et al., 2006). The corresponding genes are duplicated in trypanosomatids, while humans have only one homologous gene, *DOT1L*. Interestingly, in *T. brucei* DOT1A is essential for accomplishing DNA replication, while DOT1B seems to have a role in differentiation to midgut procyclic forms (Gassen et al., 2012; Janzen

et al., 2006). In humans, DOT1L seems to play an essential role in leukemic transformation, so many novel studies in the last years have focused on this protein (McLean et al., 2014). A specific and potent inhibitor based on the analogy with DOT1L substrate, S-adenosylmethionine (SAM), has been used in a phase 1 clinical trial for leukemia treatment (Basavapathruni et al., 2007; Stein & Tallman, 2015). If this compound also has the capability to inhibit either trypanosomatid DOT1A and/or DOT1B, this would open new applications as leishmanicidal treatment, besides its use in fighting leukemia.

Targeting the RAD51 recombinase is another promising option. RAD51 has a central role in repairing DNA damage by homologous recombination. In trypanosomatids, RAD51 is required for the amplification of extrachromosomal DNA circles involved in drug resistance (Genois et al., 2015). Masson et al. identified four *RAD51* paralogues in *Trypanosoma* spp. and three in *Leishmania* spp, while five *RAD51* paralogues have been described in humans and vertebrates (Genois et al., 2014, 2015), suggesting that different underlying regulation mechanism could be exploited to combat these parasitic infections. Similar to DOT1, emergent interest has been reported in identifying small molecules to inhibit RAD51 as an alternative for cancer therapies (Ward et al., 2015). These molecules deserve to be explored as potential treatment against trypanosomatid diseases.

Finally, another interesting potential target are PPL1/PPL2, the two trypanosomatids homologs to the human PRIMPOL polymerase (Table 2). In particular, the requirement for PPL2 to accomplish normal DNA replication (Rudd et al., 2013) makes it a desirable candidate for *Leishmania* treatment.

Besides drugs that perturb the normal DNA replication processes, other compounds that target different cellular processes have shown potential effects in leishmaniasis. This is the case of some drugs that alter the Ca2 + homeostasis. For instance, one well-characterized drug, mitelfosine, opens an uncharacterized plasma membrane Ca2 + channel in *Leishmania* (Benaim et al., 2013). Interestingly, the mechanism of action of some approved antiarrhythmic drugs also is based on altering Ca2 + homeostasis, and some of them are promising candidates for parasite disease treatment. Benaim et al. (2013, 2014) showed that amiodarone and dronedarone, two common antiarrhythmic drugs, are potentially effective against *L. mexicana* and *T. cruzi*. Another interesting drug is the antifungal triazole. Like in fungi, ergosterol is the major sterol in trypanosomatid parasites. Thus, antifungal agents that target ergosterol biosynthesis could be active also in infections caused by some members of the Trypanosomatidae family. Administration of high doses of itraconazole to patients with mucocutaneous leishmaniasis showed leishmanicidal activity during the first month of treatment. However, 1 year after the treatment only 23% of the patients showed complete resolution of the disease and in the others lesions re-appeared, suggesting the development of resistance (Calvopina et al., 2004). Posaconazole, another triazole drug, has been used in patients infected by *L. infantum* or with disease *T. cruzi* (Molina et al., 2015; Paniz Mondolfi et al., 2011). Posoconazole showed activity against trypanosomatids, but not enough for curative treatment on its own. These results suggest that drug combinations should be tested to obtain a synergic effect and to diminish the toxicity of high-dose treatments. Additionally, new triazole derivatives with improved activity are promising candidates for the treatment of trypanosomatid diseases (De Andrade et al., 2015).

Nevertheless, studies on potential anti-*Leishmania* drugs often remain at the preclinical research phase and their transfer to the clinic never materializes. This situation highlights the need of serious efforts to strengthen the link between laboratory research and clinical studies. New national policies may be required to encourage the completion of these studies, leading to real applications that benefit the population. Worldwide, much research is focused on the identification/development of new compounds; however, it is important also to draw attention on the fact that many drugs that have been already tested and approved by the Food and Drug Administration (FDA) could be useful in infections caused by *Leishmania* and other trypanosomatid parasites. Additional studies should be performed to determine which of these drugs have good leishmanicidal activity, or may improve the current treatments, when used in combination or sequential therapies. Finding low-cost drugs with decreased toxicity and shorter treatment duration remains a major challenge to fight this tropical disease.

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References

Ambit A, Woods KL, Cull B, et al. (2011). Morphological events during the cell cycle of *Leishmania major*. Eukaryot Cell 10:1429–38.

Annessa ID, Castelli S, Desideri A. (2015). Topoisomerase 1B as a target against leishmaniasis. Mini Rev Med Chem 15: 203–10.

Araújo CA, Araújo AA, Batista CL, et al. (2011). Morphological alterations and growth inhibition of *Leishmania* (*L.*) *amazonensis* promastigotes exposed to zidovudine (AZT). Parasitol Res 108:547–51.

Azvolinsky A, Giresi PG, Lieb JD, Zakian VA. (2009). Highly transcribed RNA polymerase II genes are impediments to replication fork progression in *Saccharomyces cerevisiae*. Mol Cell 34:722–34.

Bakshi RP, Sang D, Morrell A, et al. (2009). Activity of indenoisoquinolines against African trypanosomes. Antimicrob Agents Chemother 53:123–8.

Balaña-Fouce R, Álvarez-Velilla R, Fernández-Prada C, et al. (2014). Trypanosomatids topoisomerase re-visited. New structural findings and role in drug discovery. Int J Parasitol: Drugs Drug Resist 4:326–37.

Balanã-Fouce R, Prada CF, Requena JM, et al. (2012). Indotecan (LMP400) and AM13-55: two novel indenoisoquinolines show potential for treating visceral leishmaniasis. Antimicrob Agents Chemother 56:5264–70.

Barry ER, Bell SD. (2006). DNA replication in the archaea. Microbiol Mol Biol Rev 70:876–87.

Basavapathruni A, Olhava EJ, Daigle SR, et al. (2007). Nonclinical pharmacokinetics and metabolism of EPZ-5676, a novel DOT1L histone methyltransferase inhibitor. Biopharm Drug Dispos 28:135–43.

Beiting DP. (2014). Protozoan parasites and type I interferons: a cold case reopened. Trends Parasitol 30:491–8.

Benaim G, Casanova P, Hernandez-Rodriguez V, et al. (2014). Dronedarone, an amiodarone analog with improved anti-*Leishmania mexicana* efficacy. Antimicrob Agents Chemother 58:2295–303.

Benaim G, García-Marchán Y, Reyes C, et al. (2013). Identification of a sphingosine-sensitive Ca2+ channel in the plasma membrane of *Leishmania mexicana*. Biochem Biophys Res Commun 430:1091–6.

Bleichert F, Botchan MR, Berger JM. (2015). Crystal structure of the eukaryotic origin recognition complex. Nature 519:321–6.

Blow JJ, Dutta A. (2005). Preventing re-replication of chromosomal DNA. Nat Rev Mol Cell Biol 6:476–86.

Bothmer A, Robbiani DF, Feldhahn N, et al. (2010). 53BP1 regulates DNA resection and the choice between classical and alternative end joining during class switch recombination. J Exp Med 207:855–65.

Britto C, Ravel C, Bastien P, et al. (1998). Conserved linkage groups associated with large-scale chromosomal rearrangements between Old World and New World *Leishmania* genomes. Gene 222:107–17.

Burton P, McBride DJ, Wilkes JM, et al. (2007). Ku heterodimer-independent end joining in *Trypanosoma brucei* cell extracts relies upon sequence microhomology. Eukaryot Cell 6:1773–81.

Calderano SG, de Melo Godoy PD, Motta MCM, et al. (2011). *Trypanosoma cruzi* DNA replication includes the sequential recruitment of pre-replication and replication machineries close to nuclear periphery. Nucleus 2:136–45.

Calvopina M, Gomez EA, Sindermann H, et al. (2006). Relapse of new world diffuse cutaneous leishmaniasis caused by *Leishmania* (*Leishmania*) *mexicana* after miltefosine treatment. Am J Trop Med Hyg 75:1074–7.

Calvopina M, Guevara A, Armijos R, et al. (2004). Itraconazole in the treatment of New World mucocutaneous leishmaniasis. Int J Dermatol 43:659–63.

Carreras Puigvert J, Sanjiv K, Helleday T. (2015). Targeting DNA repair, DNA metabolism and replication stress as anti-cancer strategies. FEBS J 283:232–45.

Cayrou C, Coulombe P, Vigneron A, et al. (2011). Genome-scale analysis of metazoan replication origins reveals their organization in specific but flexible sites defined by conserved features. Genome Res 21:1438–49.

Chuang RY, Kelly TJ. (1999). The fission yeast homolog of Orc4p binds to replication origin DNA via multiple AT-hooks. Proc Natl Acad Sci USA 96:2656–61.

Ciccia A, Elledge SJ. (2010). The DNA damage response: making it safe to play with knives. Mol Cell 40:179–204.

Clayton C, Adams M, Almeida R, et al. (1998). Genetic nomenclature for *Trypanosoma* and *Leishmania*. Mol Biochem Parasitol 97:221–4.

Conde F, Refolio E, Cordón-Preciado V, et al. (2009). The Dot1 histone methyltransferase and the Rad9 checkpoint adaptor contribute to cohesin-dependent double-strand break repair by sister chromatid recombination in *Saccharomyces cerevisiae*. Genetics 182:437–46.

Conway C, McCulloch R, Ginger ML, et al. (2002a). Ku is important for telomere maintenance, but not for differential expression of telomeric VSG genes, in African trypanosomes. J Biol Chem 277:21269–77.

Conway C, Proudfoot C, Burton P, et al. (2002b). Two pathways of homologous recombination in *Trypanosoma brucei*. Mol Microbiol 45:1687–700.

Cortázar TM, Coombs GH, Walker J. (2007). *Leishmania panamensis*: comparative inhibition of nuclear DNA topoisomerase II enzymes from promastigotes and human

macrophages reveals anti-parasite selectivity of fluoroquinolones, flavonoids and pentamidine. Exp Parasitol 116:475–82.
Costas C, De M, Sanchez P, et al. (2011). Genome-wide mapping of *Arabidopsis* origins of DNA replication and their associated epigenetic marks. Nat Struct Mol Biol 18:395–400.
Coster G, Goldberg M. (2010). The cellular response to DNA damage: a focus on MDC1 and its interacting proteins. Nucleus 1:166–78.
Crabbé L, Thomas A, Pantesco V, et al. (2010). Analysis of replication profiles reveals key role of RFC-Ctf18 in yeast replication stress response. Nat Struct Mol Biol 17:1391–7.
Damasceno JD, Nunes VS, Tosi LRO. (2013). LmHus1 is required for the DNA damage response in *Leishmania major* and forms a complex with an unusual Rad9 homologue. Mol Microbiol 90:1074–87.
Dang HQ, Li Z. (2011). The Cdc45·Mcm2-7·GINS protein complex in trypanosomes regulates DNA replication and interacts with two Orc1-like proteins in the origin recognition complex. J Biol Chem 286:32424–35.
Daniels JP, Gull K, Wickstead B. (2010). Cell biology of the trypanosome genome. Microbiol Mol Biol Rev 74:552–69.
De Almeida I, Da Silva BA, Dos Santos ALS, et al. (2010). A new experimental culture medium for cultivation of *Leishmania amazonensis*: its efficacy for the continuous in vitro growth and differentiation of infective promastigote forms. Parasitol Res 106:1249–52.
De Andrade P, Galo OA, Carvalho MR, et al. (2015). 1,2,3-Triazole-based analog of benznidazole displays remarkable activity against *Trypanosoma cruzi*. Bioorg Med Chem 23:6815–26.
De Menezes J, Guedes CE, Petersen AL, et al. (2015). Advances in development of new treatment for leishmaniasis. BioMed Res Int 2015:1–11.
DePamphilis ML, Blow JJ, Ghosh S, et al. (2006). Regulating the licensing of DNA replication origins in metazoa. Curr Opin Cell Biol 18:231–9.
Deterding A, Dungey FA, Thompson KA, Steverding D. (2005). Anti-trypanosomal activities of DNA topoisomerase inhibitors. Acta Trop 93:311–16.
Dhingra N, Bruck I, Smith S, et al. (2015). Dpb11 protein helps control assembly of the Cdc45·MCM2–7·GINS replication fork helicase. J Biol Chem 290:7586–601.
Downs JA, Lowndes NF, Jackson SP. (2000). A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. Nature 408:1001–4.
Dragan AI, Carrillo R, Gerasimova TI, Privalov PL. (2008). Assembling the human IFN-beta enhanceosome in solution. J Mol Biol 384:335–48.
Duncker BP, Chesnokov IN, McConkey BJ. (2009). The origin recognition complex protein family. Genome Biol 10:214.
Elsasser S, Chi Y, Yang P, Campbell JL. (1999). Phosphorylation controls timing of Cdc6p destruction: a biochemical analysis. Mol Biol Cell 10:3263–77.
Farca AM, Miniscalco B, Badino P, et al. (2012). Canine leishmaniosis: in vitro efficacy of miltefosine and marbofloxacin alone or in combination with allopurinol against clinical strains of *Leishmania infantum*. Parasitol Res 110:2509–13.
Finn K, Lowndes NF, Grenon M. (2012). Eukaryotic DNA damage checkpoint activation in response to double-strand breaks. Cell Mol Life Sci 69:1447–73.
Forsburg SL. (2004). Eukaryotic MCM proteins: beyond replication initiation. Microbiol Mol Biol Rev 68:109–31.
Frederiks F, van Welsem T, Oudgenoeg G, et al. (2010). Heterologous expression reveals distinct enzymatic activities of two DOT1 histone methyltransferases of *Trypanosoma brucei*. J Cell Sci 123:4019–23.
Freitas EO, Nico D, Alves-Silva MV, et al. (2015). Immucillins ImmA and ImmH are effective and nontoxic in the treatment of experimental visceral leishmaniasis. PLoS Negl Trop Dis 9:e0004297.
Gassen A, Brechtefeld D, Schandry N, et al. (2012). DOT1A-dependent H3K76 methylation is required for replication regulation in *Trypanosoma brucei*. Nucleic Acids Res 40:10302–11.
Genois MM, Paquet ER, Laffitte MCN, et al. (2014). DNA repair pathways in trypanosomatids: from DNA repair to drug resistance. Microbiol Mol Biol Rev 78:40–73.
Genois MM, Plourde M, Ethier C, et al. (2015). Roles of Rad51 paralogs for promoting homologous recombination in *Leishmania infantum*. Nucleic Acids Res 43:28–34.
Glover L, Horn D. (2012). Trypanosomal histone γH2A and the DNA damage response. Mol Biochem Parasitol 183:78–83.
Glover L, McCulloch R, Horn D. (2008). Sequence homology and microhomology dominate chromosomal double-strand break repair in African trypanosomes. Nucleic Acids Res 36:2608–18.
Gobbini E, Cesena D, Galbiati A, et al. (2013). Interplays between ATM/Tel1 and ATR/Mec1 in sensing and signaling DNA double-strand breaks. DNA Repair 12:791–9.
Godoy PDDM, Nogueira-Junior LA, Paes LS, et al. (2009). Trypanosome prereplication machinery contains a single functional orc1/cdc6 protein, which is typical of archaea. Eukaryot Cell 8:1592–603.
Goodman MF, Woodgate R. (2014). Translesion DNA polymerases. Cold Spring Harbor Perspect Biol 5:a010363
Gupta N, Goyal N, Rastogi AK. (2001). *In vitro* cultivation and characterization of axenic amastigotes of *Leishmania*. Trends Parasitol 17:150–3.
Hable V, Drexler GA, Brüning T, et al. (2012). Recruitment kinetics of DNA repair proteins Mdc1 and Rad52 but not 53BP1 depend on damage complexity. PLoS One 7:1–11.
Heo K, Kim H, Choi SH, et al. (2008). FACT-mediated exchange of histone variant H2AX regulated by phosphorylation of H2AX and ADP-ribosylation of Spt16. Mol Cell 30:86–97.
Imming P, Sinning C, Meyer A. (2006). Drugs, their targets and the nature and number of drug targets. Nat Rev Drug Discov 5:821–34.
Ivens AC, Peacock CS, Worthey EA, et al. (2005). The genome of the kinetoplastid parasite, *Leishmania major*. Science 309:436–42.
Ives A, Ronet C, Prevel F, et al. (2012). *Leishmania* RNA virus controls the severity of mucocutaneous leishmaniasis. Science 331:775–8.
Jackson SP, Schoenwaelder SM, Goncalves I, et al. (2005). PI 3-kinase p110beta: a new target for antithrombotic therapy. Nat Med 11:507–14.
Jallepalli PV, Brown GW, Muzi-Falconi M, et al. (1997). Regulation of the replication initiator protein p65cdc18 by CDK phosphorylation. Genes Dev 11:2767–79.

Janzen CJ, Hake SB, Lowell JE, Cross GAM. (2006). Selective di-or trimethylation of histone H3 lysine 76 by two DOT1 homologs is important for cell cycle regulation in *Trypanosoma brucei*. Mol Cell 23:497–507.

Jiang W, McDonald D, Hope TJ, Hunter T. (1999). Mammalian Cdc7-Dbf4 protein kinase complex is essential for initiation of DNA replication. EMBO J 18:5703–13.

Jones NG, Thomas EB, Brown E, et al. (2014). Regulators of *Trypanosoma brucei* cell cycle progression and differentiation identified using a kinome-wide RNAi screen. PLoS Pathog 10:e1003886.

Kaufmann D, Gassen A, Maiser A, et al. (2012). Regulation and spatial organization of PCNA in *Trypanosoma brucei*. Biochem Biophys Res Commun 419:698–702.

Kaur M, Khan MM, Kar A, et al. (2012). CRL4-DDB1-VPRBP ubiquitin ligase mediates the stress triggered proteolysis of Mcm10. Nucleic Acids Res 40:7332–46.

Kazemi B. (2011). Genomic organization of *Leishmania* species. Iran J Parasitol 6:1–18.

Kelly BL, Singh G, Aiyar A. (2011). Molecular and cellular characterization of an AT-hook protein from *Leishmania*. PLoS One 6:e21412.

Kim W, Choi M, Kim JE. (2014). The histone methyltransferase Dot1/DOT1L as a critical regulator of the cell cycle. Cell Cycle 13:726–38.

Knight ZA, Gonzalez B, Feldman ME, et al. (2006). A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. Cell 125:733–47.

Kong D, Coleman TR, DePamphilis ML. (2003). *Xenopus* origin recognition complex (ORC) initiates DNA replication preferentially at sequences targeted by *Schizosaccharomyces pombe* ORC. EMBO J 22:3441–50.

Kumar D, Kumar D, Saha S. (2012). A highly basic sequence at the N-terminal region is essential for targeting the DNA replication protein ORC1 to the nucleus in *Leishmania donovani*. Microbiology (Reading, Engl.) 158:1775–82.

Kumar D, Minocha N, Rajanala K, Saha S. (2009). The distribution pattern of proliferating cell nuclear antigen in the nuclei of *Leishmania donovani*. Microbiology (Reading, Engl.) 155:3748–57.

Kumar D, Mukherji A, Saha S. (2008). Expression and subcellular localization of ORC1 in *Leishmania major*. Biochem Biophys Res Commun 375:74–9.

Kumar D, Saha S. (2015). HAT3-mediated acetylation of PCNA precedes PCNA monoubiquitination following exposure to UV radiation in *Leishmania donovani*. Nucleic Acids Res 43:5423–41.

Labib K, Diffley JF, Kearsey SE. (1999). G1-phase and B-type cyclins exclude the DNA-replication factor Mcm4 from the nucleus. Nat Cell Biol 1:415–22.

Lengronne A, Pasero P. (2014). Closing the MCM cycle at replication termination sites. EMBO Rep 15:1226–7.

Leprohon P, Fernandez-Prada C, Gazanion É, et al. (2015). Drug resistance analysis by next generation sequencing in *Leishmania*. Int J Parasitol: Drugs Drug Resist 5:26–35.

Lévesque N, Leung GP, Fok AK, et al. (2010). Loss of H3 K79 trimethylation leads to suppression of Rtt107-dependent DNA damage sensitivity through the translesion synthesis pathway. J Biol Chem 285:35113–22.

Li Z. (2012). Regulation of the cell division cycle in *Trypanosoma brucei*. Eukaryot Cell 11:1180–90.

Longhese MP, Bonetti D, Manfrini N, Clerici M. (2010). Mechanisms and regulation of DNA end resection. EMBO J 29:2864–74.

Lopez-mosqueda J, Maas NL, Jonsson ZO, et al. (2010). Damage-induced phosphorylation of Sld3 is important to block late origin firing. Nature 467:479–83.

Lou Z, Minter-Dykhouse K, Franco S, et al. (2006). MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals. Mol Cell 21:187–200.

Lutzmann M, Maiorano D, Méchali M. (2006). A Cdt1-geminin complex licenses chromatin for DNA replication and prevents rereplication during S phase in *Xenopus*. EMBO J 25:5764–74.

Majka J, Burgers PMJ. (2003). Yeast Rad17/Mec3/Ddc1: a sliding clamp for the DNA damage checkpoint. Proc Natl Acad Sci USA 100:2249–54.

Marques CA, Dickens NJ, Paape D, et al. (2015). Genome-wide mapping reveals single-origin chromosome replication in *Leishmania*, a eukaryotic microbe. Genome Biol 16:230.

Marques CA, Tiengwe C, Lemgruber L, et al. (2016). Diverged composition and regulation of the *Trypanosoma brucei* origin recognition complex that mediates DNA replication initiation. Nucl Acids Res 44:4763–84.

Marqués M, Kumar A, Poveda AM, et al. (2009). Specific function of phosphoinositide 3-kinase beta in the control of DNA replication. Proc Natl Acad Sci USA 106:7525–30.

Martin JL, Yates PA, Soysa R, et al. (2014). Metabolic reprograming during purine stress in the protozoan pathogen *Leishmania donovani*. PLoS Pathog 10:e1003938.

Martínez-Calvillo S, Nguyen D, Stuart K, Myler PJ. (2004). Transcription initiation and termination on *Leishmania major* chromosome 3. Eukaryot Cell 3:506–17.

Masai H, Taniyama C, Ogino K, et al. (2006). Phosphorylation of MCM4 by Cdc7 kinase facilitates its interaction with Cdc45 on the chromatin. J Biol Chem 281:39249–61.

McIntyre J, Woodgate R. (2015). Regulation of translesion DNA synthesis: posttranslational modification of lysine residues in key proteins. DNA Repair 29:166–79.

McLean CM, Karemaker ID, van Leeuwen F. (2014). The emerging roles of DOT1L in leukemia and normal development. Leukemia 28:2131–8.

Méndez J, Zou-Yang XH, Kim SY, et al. (2002). Human origin recognition complex large subunit is degraded by ubiquitin-mediated proteolysis after initiation of DNA replication. Mol Cell 9:481–91.

Minocha N, Kumar D, Rajanala K, Saha S. (2011a). Kinetoplast morphology and segregation pattern as a marker for cell cycle progression in *Leishmania donovani*. J Eukaryot Microbiol 58:249–53.

Minocha N, Kumar D, Rajanala K, Saha S. (2011b). Characterization of *Leishmania donovani* MCM4: expression patterns and interaction with PCNA. PLoS One 6:e23107.

Moldovan G, Pfander B, Jentsch S. (2007). Review PCNA, the maestro of the replication fork. Cell Biol Toxicol 129:665–79.

Molina I, Salvador F, Sá Nchez-Montalvaurpose AN. (2015). The use of posaconazole against Chagas disease. Curr Opin Infect Dis 28:397–407.

Mottram JC, Kinnaird JH, Shiels BR, et al. (1993). A novel CDC2-related protein kinase from *Leishmania mexicana*,

*Lmm*CRK1, is post-translationally regulated during the life cycle. J Biol Chem 268:21044–52.
Nenortas E, Burri C, Shapiro TA. (1999). Antitrypanosomal activity of fluoroquinolones. Antimicrob Agents Chemother 43:2066–8.
Nimonkar AV, Genschel J, Kinoshita E, et al. (2011). BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. Genes Genes Dev 25:350–62.
Nishitani H, Sugimoto N, Roukos V, et al. (2006). Two E3 ubiquitin ligases, SCF-Skp2 and DDB1-Cul4, target human Cdt1 for proteolysis. EMBO J 25:1126–36.
Nnakwe CC, Altaf M, Côté J, Kron SJ. (2009). Dissection of Rad9 BRCT domain function in the mitotic checkpoint response to telomere uncapping. DNA Repair 8:1452–61.
Norseen J, Thomae A, Sridharan V, et al. (2008). RNA-dependent recruitment of the origin recognition complex. EMBO J 27:3024–35.
Nunes VS, Damasceno JD, Freire R, Tosi LRO. (2011). The Hus1 homologue of Leishmania major encodes a nuclear protein that participates in DNA damage response. Mol Biochem Parasitol 177:65–9.
Oehlmann M, Mahon C, Nasheuer H. (2007). Comparison of DNA replication in *Xenopus laevis* and *Simian Virus 40*. Adv Exp Med Biol 604:3–16.
Ohouo PY, Bastos de Oliveira FM, Liu Y, et al. (2013). DNA-repair scaffolds dampen checkpoint signalling by counteracting the adaptor Rad9. Nature 493:120–4.
Paniz Mondolfi AE, Stavropoulos C, Gelanew T, et al. (2011). Successful treatment of Old World cutaneous leishmaniasis caused by *Leishmania infantum* with posaconazole. Antimicrob Agents Chemother 55:1774–6.
Parsons M, Worthey EA, Ward PN, Mottram JC. (2005). Comparative analysis of the kinomes of three pathogenic trypanosomatids: *Leishmania major*, *Trypanosoma brucei* and *Trypanosoma cruzi*. BMC Genomics 6:127.
Piwko W, Olma MH, Held M, et al. (2010). RNAi-based screening identifies the Mms22L-Nfkbil2 complex as a novel regulator of DNA replication in human cells. EMBO J 29:4210–22.
Pommier Y. (2003). Repair of and checkpoint response to topoisomerase I-mediated DNA damage. Mutat Res 532:173–203.
Pommier Y. (2006). Topoisomerase I inhibitors: camptothecins and beyond. Nat Rev Cancer 6:789–802.
Pommier Y. (2009). DNA topoisomerase I inhibitors: chemistry, biology, and interfacial inhibition. Chem Rev 109:2894–902.
Pommier Y, Robert D. (2001). Pharmacological agents that target DNA replication. In: DePamphilis ML, ed. DNA replication in eukaryotic cells. 2nd ed. Chap. 26. New York: Cold Spring Harbor Press, 1–28.
Pospiech H, Grosse F, Pisan FM. (2010). The initiation step of eukaryotic DNA replication. Sub-Cell Biochem 50:17–42.
Poveda AM, Le Clech M, Pasero P. (2010). Transcription and replication: breaking the rules of the road causes genomic instability. Transcription 1:99–102.
Prada CF, Alvarez-Velilla R, Balaña-Fouce R, et al. (2013). Gimatecan and other camptothecin derivatives poison *Leishmania* DNA-topoisomerase IB leading to a strong leishmanicidal effect. Biochem Pharmacol 85:1433–40.
Prado F. (2014). Genetic instability is prevented by Mrc1-dependent spatio-temporal separation of replicative and repair activities of homologous recombination: homologous recombination tolerates replicative stress by Mrc1-regulated replication and repair activities opera. BioEssays 36:451–62.
Prado F, Aguilera A. (2005). Impairment of replication fork progression mediates RNA polII transcription-associated recombination. EMBO J 24:1267–76.
Puddu F, Granata M, Di Nola L, et al. (2008). Phosphorylation of the budding yeast 9-1-1 complex is required for Dpb11 function in the full activation of the UV-induced DNA damage checkpoint. Mol Cell Biol 28:4782–93.
Puddu F, Piergiovanni G, Plevani P, Muzi-Falconi M. (2011). Sensing of replication stress and Mec1 activation act through two independent pathways involving the 9-1-1 complex and DNA polymerase epsilon. PLoS Genet 7:e1002022
Putnam CD, Jaehnig EJ, Kolodner RD. (2009). Perspectives on the DNA damage and replication checkpoint responses in *Saccharomyces cerevisiae*. DNA Repair 8:974–82.
Raman N, Ali S, Raja D. (2008a). Designing, synthesis and spectral characterization of Schiff base transition metal complexes: DNA cleavage and antimicrobial activity studies. J Serb Chem Soc 73:1063–71.
Raman N, Jeyamurugan R, Sudharsan S, et al. (2013). Metal based pharmacologically active agents: synthesis, structural elucidation, DNA interaction, in vitro antimicrobial and in vitro cytotoxic screening of copper(II) and zinc(II) complexes derived from amino acid based pyrazolone derivatives. Arab J Chem 6:235–47.
Raman N, Raja JD, Sakthivel A. (2008b). Design, synthesis, spectroscopic characterization, biological screening, and DNA nuclease activity of transition metal complexes derived from a tridentate Schiff base. Russ J Coord Chem 34:400–6.
Remus D, Diffley JFX. (2009). Eukaryotic DNA replication control: lock and load, then fire. Curr Opin Cell Biol 21:771–7.
Roberts TM, Zaidi IW, Vaisica JA, et al. (2008). Regulation of Rtt107 recruitment to stalled DNA replication forks by the cullin Rtt101 and the Rtt109 acetyltransferase. Mol Biol Evol 19:171–80.
Rogakou EP, Pilch DR, Orr AH, et al. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J BIol Chem 273:5858–68.
Romero IC, Saravia NG, Walker J. (2005). Selective action of fluoroquinolones against intracellular amastigotes of *Leishmania* (*Viannia*) *panamensis* in vitro. J Parasitol 91:1474–9.
Rudd S, Glover L, Jozwiakowski S, et al. (2013). PPL2 translesion polymerase is essential for the completion of chromosomal DNA replication in the African trypanosome. Mol Cell 52:554–65.
Rudd SG, Bianchi J, Doherty AJ. (2014). PrimPol – a new polymerase on the block. Mol Cell Oncol 1:1–10.
Sabatinos S, Forsburg S. (2015). Managing single-stranded DNA during replication stress in fission yeast. Biomolecules 5:2123–39.
Saha AK, Mukherjee T, Bhaduri A. (1986). Mechanism of action of amphotericin B on *Leishmania donovani* promastigotes. Mol Biochem Parasitol 19:195–200.

Salah AB, Ben Messaoud N, Guedri E, et al. (2013). Topical paromomycin with or without gentamicin for cutaneous leishmaniasis. N Engl J Med 368:524–32.

Santarém N, Cunha J, Silvestre R, et al. (2014). The impact of distinct culture media in *Leishmania infantum* biology and infectivity. Parasitology 141:192–205.

Schönenberger F, Deutzmann A, Ferrando-May E, Merhof D. (2015). Discrimination of cell cycle phases in PCNA-immunolabeled cells. BMC Bioinformatics 16:1–10.

Sebesta M, Burkovics P, Juhasz S, et al. (2013). Role of PCNA and TLS polymerases in D-loop extension during homologous recombination in humans. DNA Repair 12:691–8.

Sequeira-Mendes J, Díaz-Uriarte R, Apedaile A, et al. (2009). Transcription initiation activity sets replication origin efficiency in mammalian cells. PLoS Genet 5:e1000446.

Serrano-Martín X, Payares G, De Lucca M, et al. (2009). Amiodarone and miltefosine act synergistically against *Leishmania mexicana* and can induce parasitological cure in a murine model of cutaneous leishmaniasis. Antimicrob Agents Chemother 53:5108–13.

Shen Z. (2013). The origin recognition complex in human diseases. Biosci Rep 33:475–83.

Singh OP, Sundar S. (2014). Immunotherapy and targeted therapies in treatment of visceral leishmaniasis: current status and future prospects. Front Immunol 5:1–9.

Stein EM, Tallman MS. (2015). Mixed lineage rearranged leukaemia: pathogenesis and targeting DOT1L. Curr Opin Hematol 22:92–6.

Sterkers Y, Lachaud L, Bourgeois N, et al. (2012). Novel insights into genome plasticity in eukaryotes: mosaic aneuploidy in *Leishmania*. Mol Microbiol 86:15–23.

Stewart GS, Wang B, Bignell CR, et al. (2003). MDC1 is a mediator of the mammalian DNA damage checkpoint. Nature 421:961–6.

Szilard RK, Jacques PE, Laramée L, et al. (2011). Systematic identification of fragile sites via genome-wide location analysis of γ-H2AX. Nat Struct Mol Biol 17:299–305.

Takeda DY, Dutta A. (2005). DNA replication and progression through S phase. Oncogene 24:2827–43.

Tanaka S, Komeda Y, Umemori T, et al. (2013). Efficient initiation of DNA replication in eukaryotes requires Dpb11/TopBP1-GINS interaction. Mol Cell Biol 33:2614–22.

Thomer M, May NR, Aggarwal BD, et al. (2004). *Drosophila* double-parked is sufficient to induce re-replication during development and is regulated by cyclin E/CDK2. Development 131:4807–18.

Tiengwe C, Marcello L, Farr H, et al. (2012). Genome-wide analysis reveals extensive functional interaction between DNA replication initiation and transcription in the genome of *Trypanosoma brucei*. Cell Rep 2:185–97.

Tiengwe C, Marcello L, Farr H, et al. (2012). Identification of ORC1/CDC6-interacting factors in *Trypanosoma brucei* reveals critical features of origin recognition complex architecture. PLoS One 7:e32674.

Tiengwe C, Marques CA, McCulloch R. (2014). Nuclear DNA replication initiation in kinetoplastid parasites: new insights into an ancient process. Trends Parasitol 30:27–36.

Timm J, Bosch-navarrete C, Recio E, Nettleship JE. (2015). Structural and kinetic characterization of thymidine kinase from *Leishmania major*. PLoS Negl Trop Dis 1–19: e0003781.

Travesa A, Duch A, Quintana DG. (2008). Distinct phosphatases mediate the deactivation of the DNA damage checkpoint kinase Rad53. J Biol Chem 283:17123–30.

Ubeda JM, Légaré D, Raymond F, et al. (2008). Modulation of gene expression in drug resistant *Leishmania* is associated with gene amplification, gene deletion and chromosome aneuploidy. Genome Biol 9:R115.

Ubeda JM, Raymond F, Mukherjee A, et al. (2014). Genome-wide stochastic adaptive DNA amplification at direct and inverted DNA repeats in the parasite *Leishmania*. PLoS Biol 12:e1001868,

Valenciano AL, Ramsey AC, Mackey ZB. (2015). Deviating the level of proliferating cell nuclear antigen in *Trypanosoma brucei* elicits distinct mechanisms for inhibiting proliferation and cell cycle progression. Cell Cycle 14:674–88.

Verma NK, Dey CS. (2004). Possible mechanism of miltefosine-mediated death of *Leishmania donovani*. Antimicrob Agents Chemother 48:3010–15.

Vouldoukis I, Rougier S, Dugas B, et al. (2006). Canine visceral leishmaniasis: comparison of in vitro leishmanicidal activity of marbofloxacin, meglumine antimoniate and sodium stibogluconate. Vet Parasitol 135:137–46.

Walker J, Saravia NG. (2004). Inhibition of *Leishmania donovani* promastigote DNA topoisomerase I and human monocyte DNA topoisomerases I and II by antimonial drugs and classical antitopoisomerase agents. J Parasitol 90:1155–62.

Wan L, Lou J, Xia Y, et al. (2013). *h*Primpol1/CCDC111 is a human DNA primase-polymerase required for the maintenance of genome integrity. EMBO Rep 14:1104–12.

Wang G, Tong X, Weng S, Zhou H. (2012). Multiple phosphorylation of Rad9 by CDK is required for DNA damage checkpoint activation. Cell Cycle 11:3792–800.

Ward A, Khanna KK, Wiegmans AP. (2015). Targeting homologous recombination, new pre-clinical and clinical therapeutic combinations inhibiting RAD51. Cancer Treat Rev 41:35–45.

Wheeler RJ, Gluenz E, Gull K. (2011). The cell cycle of *Leishmania*: morphogenetic events and their implications for parasite biology. Mol Microbiol 79:647–62.

World Health Organization (WHO), Geneva, 2010. Control of the leishmaniasis [online]. Available from: http://apps.who.int/iris/bitstream/10665/44412/1/WHO_TRS_949_eng.pdf.

Wilson KA, Stern DF. (2008). NFBD1/MDC1, 53BP1 and BRCA1 have both redundant and unique roles in the ATM pathway. Cell Cycle 7:3584–94.

Worthey EA. (2003). *Leishmania major* chromosome 3 contains two long convergent polycistronic gene clusters separated by a tRNA gene. Nucleic Acids Res 31:4201–10.

Xu X, Stern D. (2003). NFBD1/MDC1 regulates ionizing radiation-induced focus formation by DNA checkpoint signaling and repair factors. FASEB J: Off Publ Feder Am Soc Exp Biol 17:1842–8.

Yoshida K, Bacal J, Desmarais D, et al. (2014). The histone deacetylases sir2 and rpd3 act on ribosomal DNA to control the replication program in budding yeast. Mol Cell 54:691–7.

Yoshida K, Poveda A, Pasero P. (2013). Time to be versatile: regulation of the replication timing program in budding yeast. J Mol Biol 425:4696–705.

Zaidi IW, Rabut G, Poveda A, et al. (2008). Rtt101 and Mms1 in budding yeast form a CUL4(DDB1)-like ubiquitin ligase that promotes replication through damaged DNA. EMBO Rep 9:1034–40.

Zangger H, Ronet C, Desponds C, et al. (2013). Detection of *Leishmania* RNA Virus in *Leishmania* parasites. PLoS Negl Trop Dis 7:1–11.

Zegerman P, Diffley JFX. (2009). DNA replication as a target of the DNA damage checkpoint. DNA Repair 8:1077–88.

Zhu W, Chen Y, Dutta A. (2004). Rereplication by depletion of geminin is seen regardless of p53 status and activates a G 2/M checkpoint rereplication by depletion of geminin is seen regardless of p53 status and activates a G2/M checkpoint. Mol Cell Biol 24:7140–50.

Zwerschke W, Rottjakob H, Kuntzels H. (1994). The *Saccharomyces cerevisiae* CDC6 gene is transcribed at late mitosis and encodes a ATP/GTPase controlling S phase initiation. J Biol Chem 269:23351–6.