An Evolutionary View of Trypanosoma Cruzi Telomeres

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Like in most eukaryotes, the linear chromosomes of Trypanosoma cruzi end in a nucleoprotein structure called the telomere, which is preceded by regions of variable length called subtelomeres. Together telomeres and subtelomeres are dynamic sites where DNA sequence rearrangements can occur without compromising essential interstitial genes or chromosomal synteny. Good examples of subtelomeres involvement are the expansion of human olfactory receptors genes, variant surface antigens in Trypanosoma brucei, and Saccharomyces cerevisiae mating types. T. cruzi telomeres are made of long stretches of the hexameric repeat 5'-TTAGGG-OH-3', and its subtelomeres are enriched in genes and pseudogenes from the large gene families RHS, TS and DGF1, DEAD/H-RNA helicase and N-acetyltransferase, intermingled with sequences of retrotransposons elements. In particular, members of the Trans-sialidase type II family appear to have played a role in shaping the current T. cruzi telomere structure. Although the structure and function of T. cruzi telomeric and subtelomeric regions have been documented, recent experiments are providing new insights into T. cruzi's telomere-subtelomere dynamics. In this review, I discuss the co-evolution of telomere, subtelomeres and the TS gene family, and the role that these regions may have played in shaping T. cruzi’s genome.

Keywords: Trypanosoma cruzi (T. cruzi), telomeres, evolutionary dynamics, genome, sialidases

INTRODUCTION

Trypanosome cruzi causes Chagas disease a debilitating and often lethal malaise affecting millions of people in Latin American countries. T. cruzi populations are very variable and this variability is due in part to a clonal population structure, genome plasticity and abundance of repeated sequences. Nearly 50% of the total genome is made of repeated sequences, some of which code for protein families such as the Transialidases (TS), mucins (MUC), mucins associated proteins (MASP), Disperse gene family 1 (DGF-1), Retrotransposon Hot Spot proteins, and retrotransposons elements (El-Sayed et al., 2005).

The Trans-sialidase family is divided into eight groups (Freitas et al., 2011), and the family name derives from enzymes included in the first group that can transfer host sialic acid onto the parasite's surface MUC. The rest of the TS groups have no enzymatic activity but code for surface glycoproteins involved in infectivity, adhesion, and evasion from the host immune response. MUC are not only receptors for sialic acid (Di Noia et al., 1995) but together with MASP proteins (Bartholomeu et al., 2009) provide a developmentally regulated shield that protects the parasite in hostile environments. DGF-1 are integrin-like proteins (Gonzalez et al., 2009; Kawashita et al., 2009; Lander et al., 2010) that are developmentally regulated, but whose role has not been deciphered. The Retrotransposon Hot Spot (RHS) family codes in T. brucei for nuclear and perinuclear proteins.
and their sequences are targets for the insertion of RIME/ingi non-LTR retrotransposons (Bringaud et al., 2002). In T. cruzi RHS genes are frequently interrupted by the non-LTR retrotransposon LTe1 (Bringaud et al., 2006).

Early experiments in Manning’s lab (Peterson et al., 1989; Ruef et al., 1994) revealed that two members of the Transialidase family type II (TSII) located in the vicinity of the telomere were highly expressed, whereas another member, in a more internal chromosomal location, showed a lower expression level (Peterson et al., 1989). After this original report, Freitas-Júnior et al. (1999) and Chiurillo et al. (1999) confirmed the presence of TSII gene members in the subtelomeres of several T. cruzi strains. The cloning of T. cruzi’s telomeric and subtelomeric regions using a vector-adaptor complementing the last nine nucleotides of the telomere (Chiurillo et al., 1999, 2002; Kim et al., 2005), allowed us to define the sequence of the telomeric repeat, and have a detailed view of a large segment of the subtelomere.

Subtelomeres are often described as regions of genomic instability (Baird, 2018), and this observation is supported by the abundance of retrotransposon fragments and pseudogenes in T. cruzi telomeric clones, but they also represent a buffer region to minimize chromosome damage when telomere attrition occurs. However, despite this unstable environment, complete gene copies of DGF-1, TS, and RHDEAD/H-RNA helicase and N-acetyltransferase in T. cruzi subtelomeres are expressed, indicating that a positive selection is preserving the integrity of these genes at these locations (Moraes Barros et al., 2012). At the end of subtelomeres, there is a 189 base pairs sequence with homology for the 3′ and 5′UTRs sequences of a gp85 gene (TSII) that we dubbed the 189 bp junction (Kim et al., 2005). A recapitulation of the possible events associated with the creation of this junction was proposed by Kim et al. (2005), Chiurillo et al. (2017) in which tandem repeats of TSII genes suffered breakages, excisions and rejoining to generate the junction. After this junction, the chromosomes are capped by runs of hexamer repeats of variable length ending in a single strand terminus 5′-TTAGGG-OH-3′.

THE EVOLUTION OF T. cruzi TELOMERES AND THE ROLE OF MEMBERS OF THE TSII GENE FAMILY IN SHAPING ITS GENOME

The intimate association between telomeres and TSII (gp85) sequences raises many questions about the creation of T. cruzi telomeres, such as how and when TS II family members were incorporated into the 189 bp junction? Were they part of transposon elements that eventually evolved into a primitive telomere? Did they come along with the telomeric repeats?

When we examine the structure of eukaryotic telomeres we observe a tremendous diversity in telomeric repeats and sequences associated with telomeres, the extreme case of Diptera where transposons assumed the role of telomeres. Telomeres arose during euakaryogenesis as a need to protect the ends of linear chromosomes from exonucleases degradation, prevent chromosome rearrangements, and facilitate the replication of the DNA lagging strand. This process may have started with the endosymbiosis of an ancestral phagotrophic host cell (Cavalier-Smith, 2002) and an α-proteobacterium carrying Type II introns.

Type II introns are Eubacterial mobile retroelements with ribozyme and reverse transcriptase activities. Eventually, Type II introns were transferred into the host cell genome and gave rise to the spliceosome, the non-LTR transposons and telomerase (Garavis et al., 2013; Podlevsky and Chen, 2016). According to this hypothesis, these non-LTR transposons were inserted at several genome locations, including the chromosome ends, originating the prototelmomeres.

On the other hand, although still debatable, based on the sequence homogeneity of the transialidases within the animal kingdom, and their sequence homology with their very variable bacterial counterparts (Roggentin et al., 1993) proposed that sialidases originated in ancestors of the Echinodermata and Deuterostomate animals, and later these genes were horizontally transferred to bacteria via viruses. However, Schwerdtfeger and Melzig (2010) argued that the irregular appearance of sialidases in invertebrates does not support a common evolution of this gene family. In agreement with this view, in the Trypanosomatidae family, a taxon that includes pathogenic flagellates, we also observe this kind of patchy inheritance, since of all genera within this family the TS genes are only present in the genus Trypanosoma.

It is assumed that the first trypanosome to acquire sialidase genes was T. brucei or a common ancestor of both T. brucei and T. cruzi. The separation Trypanosoma brucei from Trypanosoma cruzi occurred approximately 100 millions of years during the Gondwanaland breakage (Briones et al., 1995; Stevens et al., 1999), and since then, the TS family suffered an expansion exclusively in the T. cruzi clade (Chiurillo et al., 2016a). During this long separation, the two species developed different survival strategies within their vectors and hosts. T. brucei uses the telomeric-subtelomeric compartment as a specialized expression site for variable antigenic determinants. On the contrary, T. cruzi acts like a stealth invader jamming the vertebrate host immune system through a simultaneous expression of multiple surface antigens (Millar et al., 1999), and among these, members of the TS family play an important role.

How the expansion of TSII genes occurred in T. cruzi is unknown, but likely, several events of gene mutation, duplication, recombination, transposition, and genetic drift generated the current picture of the TS family. Vestiges of these events are the salad of sequences derived from retrotransposon elements and Retrotransposons Hot Spot (RHS) genes scattered through the genome, and particularly in T. cruzi subtelomeres (Figure 1). The abundance of these elements at the subtelomere suggests the active participation of these regions in shaping the parasite genome (Kim et al., 2005). Regarding the telomeric repeat 5′-TTAGGG-OH-3′, it is found in Plantae, Chromoalveolata, Excavata (where Trypanosomatids are included) and Rhizaria supergroups, a reason why some authors (Dressen et al., 2007; Fulneckova et al., 2013) have suggested that it was the repeat of the ancestral Eukaryotic telomeres. Thus, a safe assumption is that the TS sequences in the subtelomeric transition (189 bp junction) appeared after the hexamer repeats were already fixed, and perhaps the fixation of the 189 bp
FIGURE 1 | Schematic representation of T. cruzi telomeric recombinants and two recombinants containing retrotransposons associated sequences. Modules M1 and M2 are Ltc1 retrotransposon flanking sequences: M1, RS13TC+RS1Tc (upstream); M2, Seq3Tc + SIRE associated sequences S2 (downstream); TSA, transialidase genes (or pseudogenes); L1Tc, Ltc1 non-LTR retrotransposon (genes and pseudogenes); DGF1, Disperse gene family 1 genes (or pseudogenes); J, 189 bp junction; DSHX, double strand hexameric sequences; SSHX, single strand hexameric sequences. (A) Telomeric BAC6 recombinant containing T. cruzi subtelomere and telomere showing duplication of M2. (B) Telomeric cosmid C6 showing M2 duplications. (C) Recombinant pBAC62 showing multiple LTc1 retrotransposons inserted between M1 and M2. (D) Recombinant pBAC62 showing a TSA gene located downstream of 213 bp of a truncated Ltc1 (T) flanked upstream by the insertion sequence GTATCTTTG and a poly A track. Probably the TSA gene was transduced by the retrotransposon. Sizes are approximated; the flanking sequences are part of the Retrotransposon Hot Spot proteins. Adapted from Kim et al. (2005), Olivares et al. (2013).

junction has to do with its adaptation to the telomeric function, thus this sequence appears to be an integral part of T. cruzi telomere. Chiurillo et al. (2017) have proposed that the 189 bp junction may act as a seed to stabilize and/or create new telomeres via telomerase, but it is also possible that it was adopted as a recognition site for other proteins that contribute to the telomeric function.

GENE FAMILIES AND SUBTELOMERES

In T. cruzi apart from the TS family other gene families suffered big expansions, namely RHS, DFG-1, MASP, and MUC and these genes tend to form clusters at multiple locations in the genome, in some cases covering whole chromosomes (El-Sayed et al., 2005; Weatherly et al., 2009; Berna et al., 2018; Callejas-Hernández et al., 2018). Except for MUC and MASP families, these expanded families are often found at chromosomal terminal locations (El-Sayed et al., 2005; Weatherly et al., 2009; Moraes Barros et al., 2012; Callejas-Hernández et al., 2018). Therefore it is not surprising to detect members of these families when T. cruzi telomeres and subtelomeres are cloned (Chiurillo et al., 1999; Freitas-Junior et al., 1999). Thus, the T. cruzi genome is organized in blocks of syntenic non-repeated gene sequences at more interstitial locations, and non-syntenic blocks consisting of repeated genes that are intermingled with retrotransposons and other highly repeated elements, some of which are located at subtelomeres (El-Sayed et al., 2005). The presence of many repeated genes may propitiate recombination events that, although not desirable for housekeeping genes, favor the generation of variability for genes coding for surface proteins.

The uniqueness of terminal chromosomal locations (subtelomeres) as a ground for the generation of new capabilities has been well documented among other organisms in yeast (Haber, 1998), Plasmodium (Scherf et al., 2008), T. brucei (Horn, 2004), and the expansion of the human odor receptors (Mefford et al., 2001). Based on these observations and in the composition of T. cruzi telomeres and subtelomeres, we proposed that subtelomeres were places where the variability of some of these multigene families was generated, and in later events, the gene variants were mobilized to different locations in the genome (Kim et al., 2005).

HYPOTHETICAL MECHANISMS TO GENERATE GENE VARIABILITY

What are the mechanisms that may generate gene variability in T. cruzi, and how has the mobilization of gene variants occurred? Is the variability generated by gene conversion or unequal crossing over? Are these processes still occurring in T. cruzi populations?

Our first attempt to address these questions was through in silico simulation studies using as inputs real gene sequences generated by the T. cruzi genome project (Azuaje et al., 2007). The simulations evaluated the generation of variability by introducing different mutagenic pressures in housekeeping genes, and members of gene families with presence at subtelomeres. The premise was that mutation rates would be a trade-off between the generation of variability to expand
adaptive capabilities, and the need to keep important core functions. This study concluded that housekeeping genes were more robust against the introduction of random point mutations than genes coding for surface proteins and that the most effective mechanism to introduce variability was gene conversion. The energetic burden of keeping a large number of pseudogenes is an indication that they play an important role in the parasite, an observation that prompted us to include pseudogenes of RNS, TS, and DGF-1 in our simulations. The results confirmed the potential of pseudogenes to contribute to the generation of variability. This finding contradicts the idea that pseudogenes are merely relics of gene deterioration (Rogers et al., 2011) or pseudogenization.

An important piece of information came from experiments using T. cruzi artificial chromosomes (pTAC) carrying the 189 bp junction, hexameric repeats, and drug selection markers (Curto et al., 2014). When T. cruzi epimastigotes were transformed with these pTACs, they were able to replicate with surprising stability for 150 generations in the presence of the selection drug, or 60 generations without it. In other words, the pTACs showed non-detectable sequence exchange with the host chromosomes and replicated and segregated without the presence of centromeres, or perhaps the telomere was fulfilling this role. In a more recent experiment (Chiuirillo et al., 2016b), addressed the possibility of chromosomal sequence exchanges by introducing a cutting site for the rare meganuclease I-SceI within the RHS gene of one pTACs (pTAC-D6CISceI*) harboring larger portions of T. cruzi subtelomeres. After transforming this pTAC into T. cruzi cells expressing the meganuclease I-SceI, and confirming that double-strand breaks (DSBs) were produced, the probing pTAC was examined to check whether the DSBs were repaired. Out of seven clones studied, six showed repairs, as evidenced by the disappearance of the I-SceI site, and the reversion to the original pTAC (pTAC-D6C*). The most likely explanation is that the repair events used as template the host subtelomere homologous to the pTAC. The seventh clone was repaired losing the I-SceI site, but the sequences around the repair site shared homology with the subtelomere of another chromosome (ectopic recombination). From these experiments several conclusions can be derived: first chromosomal exchanges at the subtelomere can be promoted by the introduction DSBs; second the repair mechanism for these DSBs is homologous recombination (HR), or some version of it (Dressen et al., 2007); third, events involving exchanges with non-homologous chromosomes (ectopic recombination) can occur, with the possibility of generating gene variants. These experiments don’t rule out that similar exchanges can occur at more interstitial locations. The possibility of detectable recombination in earlier pTAC experiments was likely due to a lack of sufficient homology with any given chromosome subtelomere, and/or that DSBs are strictly necessary to induce recombination.

Contrary to most eukaryotes in T. brucei and T. cruzi the most important repair mechanism for DSBs repair is HR, with minor participation of microhomology repair (MHR). To detect MHR-DSB repair, it was necessary to abolish HR (Glover et al., 2008).

In T. cruzi out of the potential genes that participate in HR, RAD51 plays a central role in facilitating homologous strand invasion (Gomes Passos Silva et al., 2018). An interesting activity discovered after massive gamma radiation of T. cruzi cells was tyrosyl-DNA phosphodiesterase I (Tdr-I), an enzyme that plays an important role in Topoisomerase I mediated DNA DBS repair (Das et al., 2010). No genes for NHEJ have been found in T. cruzi (Gomes Passos Silva et al., 2018). MHR seems to occur in chromosomal rearrangements when single DSB is introduced by Cas9 endonuclease (Lander et al., 2015; Soares Medeiros et al., 2017).

An efficient HR repair mechanism in T. cruzi may explain the rapid karyotype and cell growth recovery after massive irradiation with 500 Gy of gamma radiation (Garcia et al., 2016), and the difficulty in generating mutations (indels) in CRISPR experiments involving a single DSB vs. gene replacement (Lander et al., 2015; Soares Medeiros et al., 2017). But also, the prevalence of a very stringent HR repair mechanism in genomes with a large number of repeated sequences, like T. cruzi, hampers (but does not eliminate) chromosomal-internal recombination events that can be detrimental to the organism.

Thus, we believe that an important source for the generation of variability in some T. cruzi surface proteins is subtelomeric recombination promoted by DSBs followed by a dispersion of these variants either by transpositions or ectopic recombination events. Further duplications events and genetic drift produced the current clusters that we see at several locations of the T. cruzi’s genome.

Along this line, experiments with meganuclease I-Sce-I in T. brucei (Dressen et al., 2007) revealed that the introduction of a DSB in telomeric VSG gene promoted antigenic switching via gene conversion. The break is resolved by a replication mechanism induced by this break (Break Induced Replication, BIR). Similar detailed studies to determine how DSBs repair occurs in T. cruzi are missing due in part to the lack of an RNAi machinery and/or inducible promoters coupled to CRISPR-Cas9 vectors. As mentioned before, despite this stringent HR mechanism interstitial chromosome rearrangement leading to karyotype changes can occur via ectopic recombination within multigene families through duplicated sites flanking these sequences (Figures 1A,B).

HOW DSBs CAN BE INTRODUCED IN T. cruzi SUBTELOMERES?

In T. cruzi retrotransposon elements occupy nearly 5% of its genome and among these elements, the L1Tc non-LTR retrotransposon has the necessary machinery for its mobilization (Macías et al., 2018). L1Tc genome codes for an AP endonuclease activity (NL1Tc) capable of introducing breaks for the insertion of the retrotransposon, but also plays a role in repairing DSBs produced by daunorubicin (Oliveiras et al., 2013). Other retrotransposon elements like SLAKS and CZAR code for site-specific endonucleases (Macías et al., 2018). So, some DSBs may be introduced in the subtelomeres by retrotransposon nucleases, and HR repairs the break using as templates homologous chromatids, or on occasion non-homologous...
chromatids (ectopic recombination). In T. cruzi there are active L1Tc transposons and no RNAi machinery to counteract their activities. Several observations about the organization of multigene families reveal close associations with retrotransposon sequences i.e., most DGF-1 and TSII copies are flanked by RHS (Olivares et al., 2000; Kim et al., 2005) or L1Tc retrotransposons sequences, the duplications of RHS and L1Tc genes at both sides of these genes suggest the occurrence of ectopic recombination events (Figure 1; Olivares et al., 2000). Although L1Tc appears to be randomly distributed in T. cruzi’s genome, 50% of its copies are associated with RHS genes flanked by the putative insertion site 5’-TGCAGACAT-OH-3’ (Olivares et al., 2000; Figures 1B,C). Also, they are found inserted downstream the sequence GA (x)2AxGa (x)5xTATG↑A(x) 11↑ where arrows mark the single strand cleavage sites (El-Sayed et al., 2005; Bringaud et al., 2006). How frequent DBSs leading to genetic recombination and gene variability occurs is difficult to assess, since as shown in the experiments with meganuclease I-Sce-I (Azuaje, F. J., Ramirez, J. L., and Franco Da Silveira, J. (2007).

In silico, biologically-inspired modeling of genomic variation generation in surface proteins of Trypanosoma cruzi. Kinetoplastid Biol. Dis. 6, 1–12. doi: 10.1186/1475-9292-6-6

Bartholomeu, D. C., Cerqueira, G. C., Leao, A. C. A., daRocha, W. D., Pais, F. S., Macedo, C., et al. (2009). Genomic organization and expression profile of the mucin-associated surface protein (masp) family of the human pathogen Trypanosoma cruzi. Nucleic Acids Res. 37, 3407–3417. doi: 10.1093/nar/gkp172

REFERENCES

CONCLUSIONS

Once T. cruzi telomeres were fixed, members of the TS II family positioned at the subtelomeres co-evolved to be part of the transition to the telomeric repeat. Although the reason for the fixation of this junction is still unknown, it suggests a potential telomeric function for this region. The variability of some surface proteins and their localization at the subtelomeres together with retrotransposon elements suggests that these regions are grounds for the generation genetic variability. We propose that DBSs introduced in the subtelomeres by retrotransposon nucleases are repaired by homologous recombination, and when the repair includes non-homologous chromatids there is a possibility to generate gene variants. These variants are mobilized elsewhere either by transposition or ectopic recombination. Gene families increased their numbers by gene duplication to achieve higher expression levels. MUC and MASP superfamilies likely evolved in later events not related to the subtelomeres.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

ACKNOWLEDGMENTS

The publication cost was partially covered by the Programme UNU-BIOLAC. Thanks to Don Humphreys and Sharon Sumpter for English revision.

Bartholomeu, D. C., Cerqueira, G. C., Leao, A. C. A., daRocha, W. D., Pais, F. S., Macedo, C., et al. (2009). Genomic organization and expression profile of the mucin-associated surface protein (masp) family of the human pathogen Trypanosoma cruzi. Nucleic Acids Res. 37, 3407–3417. doi: 10.1093/nar/gkp172

Berna, I., Rodriguez, M., Chiribao, M. L., Parodi-Talice, A., Pita, S., Rijo, G., et al. (2018). Expanding an expanded genome: long-read sequencing of Trypanosoma cruzi. Microbiol. Genom. 4:e00017. doi: 10.1099/mgen.0.000177
Briones, M. R., Egima, C. M., Eichinger, D., and Schenkman, S. (1995). Telomere biology in Trypanosomatids. 

Garcia, J. B. F., Vieira da Rocha, P., Costa-Silva, H. M., Alves, Ceres, C. L., and Machado, C. R., Cruz, A.K. (2016). Leishmania major and Trypanosoma cruzi present distinct DNA damage responses. Mol. Biochem. Parasit. 207, 23–32. doi: 10.1016/j.molbiopara.2016.05.004

Glover, L., McCulloch, R., and Horn, D. (2008). Sequence homology and microhomology dominate chromosomal double-strand break repair in African trypanosomes. Nucleic Acids Res. 36, 2608–2618. doi: 10.1093/nar/gkn104

Gomes Passos Silva, D., da Silva Santos, S., Nardelli, S. C., Mendes, T. C., Guimarães Freire, N. C., Marcal Repolés, B., et al. (2018). The in vivo and in vitro roles of Trypanosoma cruzi Rad51 in the repair of DNA double-strand breaks and oxidative lesions. PLOS Neg. Trop. Dis. 12:e0006875. doi: 10.1371/journal.pntd.0006875

Gonzalez, A. M., Azuaje, F. J., Ramirez, J. L., Da Silveira, J. F., and Dorronsoro, J. R. (2009). Machine learning techniques for automated classification of adhesine-like proteins in Trypanosoma cruzi. IEEE/ACM Trans. Comp. Biol. Bioinf. 6, 695–702. doi: 10.1109/TCBB.2008.125

Haber, J. E. (1998). Mating-Type Gene Switching in Saccharomyces cerevisiae. Annu. Rev. Genet. 32, 561–599. doi: 10.1146/annurev.genet.32.1.561

Horn, D. (2004). The molecular control of antigenic variation in Trypanosoma brucei. Curr. Mol. Med. 4, 563–574. doi: 10.1016/j.mcm.2004.10.014

Landers, N., Bernal, C., Díez, N., Añez, N., Docampo, R., and Ramirez, J. L. (2010). Localization and developmental regulation of a disperse gene family 1 protein in Trypanosoma cruzi. Infect. Immun. 78, 231–241. doi: 10.1128/IAI.00780-09

Lander, N., Li, Z.-H., Niyogi, S., and Docampo, R. (2015). CRISPR/Cas9-induced disruption of paraflagellar rod protein 1 and 2 genes in Trypanosoma cruzi reveals their role in flagellar attachment. MBio 6: e00115. doi: 10.1128/MBio.00115-12

Macias, F., Fonseca-Lehmann, R., Lopez, M. C., Gomez, L., and Thomas, M. C. (2018). Biology of Trypanosoma cruzi retrotransposons: from an enzymatic to a structural point of view. Curr. Genom. 19, 110–118. doi: 10.2174/1389209218661701510738

Mefford, H. C., Linardopoulos, E., Coil, D., van den Engh, G., and Trask, B. J. (2001). Comparative sequencing of a multicopy subtelomeric region containing olfactory receptor genes reveals multiple interactions between non-homologous chromosomes. Hum. Mol. Genet. 10, 2363–2372. doi: 10.1093/hmg/10.21.2363

Millar, A. E., Wildkins-Lee, M., and Kahn, S. J. (1999). The surface Protein superfamily of Trypanosoma cruzi stimulates a solarized Th1 response that becomes anergic. J. Immunol. 162, 6092–6099.

Moraes Barros, R. R., Marini, M. M., António, C. R., Cortez, D. R., Miyake, A. M., and Lima, F. M., et al. (2012). Anatomy and evolution of telomeric and subtelomeric regions in the human protozoan parasite Trypanosoma cruzi. BMC Genom. 13:229. doi: 10.1186/1471-2164-13-229

Nanavaty, V., Sandhu, R., Jehi, S. G., Pandya, U. M., and Li, B. (2017). Trypanosoma brucei RAPI maintains telomere and subtelomere integrity by suppressing TRF1 and telomeric RNA: DNA hybrids. Nucleic Acids Res. 45, 5785–5796. doi: 10.1093/nar/gkw184

Olives, M., Lopez, M. C., Garcia-Perez, J. L., Briones, P., Pulgar, M., and Thomas, M. C. (2013). The endonuclease NLR1Tc encoded by the LINE LITc...
from Trypanosoma cruzi protects parasites from daunorubicin DNA damage. Biochim. Biophys. Acta 1626, 25–32. doi: 10.1016/S0167-4781(03)00022-8

Olivares, M., Thomas, M. C., Lopez-Barajas, A., Requena, J. M., Garcia-Perez, J. L., and Angel, S. (2000). Genome clustering of the Trypanosoma cruzi nonlong terminal L1Tc retrotransposon with defined interspersed repeated DNA elements. Electrophoresis 21, 2973–2982. doi: 10.1002/1522-2683(20000801)21:14<2973::AID-ELPS2973>3.0.CO;2-4

Peterson, D. S., Fouts, D. L., and Manning, J. E. (1989). The 85-kd surface antigen gene of the Trypanosoma cruzi is telomeric and a member of a multigene family. EMBO J. 8, 3911–3916. doi: 10.1002/j.1460-2075.1989.tb08571.x

Podlevsky, J. D., and Chen, J.-L. (2016). Evolutionary perspectives of telomerase RNA structure and function. RNA Biol. 13, 720–732. doi: 10.1080/15476286.2016.1205768

Roggentin, P., Schauer, R., Hoyer, L. L., and Vimr, E. R. (1993). The sialidase superfamily and its spread by horizontal gene transfer. Mol. Microbiol. 9, 915–921. doi: 10.1111/j.1365-2958.1993.tb01221.x

Souza, R. T., Santos, M. R. M., Lima, F. M., El-Sayed, N. M., Myler, P. J., Ruiz, J. C., et al. (2007). New Trypanosoma cruzi repeated element that shows site specificity for insertion eukary. Cell 6, 1228–1238. doi: 10.1128/EC.00036-07

Stevens, J. R., Noyes, H. A., Dover, G. A., and Gibson, W. C. (1999). The ancient and divergent origins of the human pathogenic trypanosomes, Trypanosoma brucei and T. cruzi. Parasitology 118, 107–116. doi: 10.1017/S0031182098003473

Weatherly, D. B., Boelhke, C., and Tarleton, R. L. (2009). Chromosome level assembly of the hybrid Trypanosoma cruzi genome. BMC Genomics 10:255. doi: 10.1186/1471-2164-10-255

Xing, J., Tan, G., Belancio, V. P., Cordaux, R., Deininger, P. L., and Batzer, M. A. (2006). Emergence of primate genes by retrotransposon mediated sequence transduction PNAS 103, 17608–17613. doi: 10.1073/pnas.0603224103

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