Chapter

Life Cycle of *Trypanosoma cruzi* in the Invertebrate and the Vertebrate Hosts

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Abstract

Trypanosoma cruzi (*T. cruzi*) is a protozoan parasite that causes Chagas disease, a zoonotic disease that can be transmitted to humans by blood-sucking triatomine bugs. *T. cruzi* is a single-celled eukaryote with a complex life cycle alternating between reduviid bug invertebrate vectors and vertebrate hosts. This article will look at the developmental stages of *T. cruzi* in the invertebrate vector and the vertebrate hosts, the different surface membrane proteins involved in different life cycle stages of *T. cruzi*, roles of different amino acids in the life cycle, carbon and energy sources and gene expression in the life cycle of *T. cruzi*. The author will also look at extracellular vesicles (EV) and its role in the dissemination and survival of *T. cruzi* in mammalian host.

Keywords: *Trypanosoma cruzi*, metacyclogenesis, trypanosomatid, epimastigote, metacyclic trypomastigotes, extracellular vesicles

1. Introduction

The genus *Trypanosoma* has many species of protozoans but only *Trypanosoma cruzi*, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* cause disease in humans. *Trypanosoma cruzi,* a protozoan parasite hemoflagellate is the etiologic agent of Chagas disease [1] while *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* causes African trypanosomiasis. Chagas disease also known as American trypanosomiasis affects millions of people throughout the Americas [2]. In 1909, Carlos Chagas first described this disease when he discovered the parasite in the blood of a Brazilian child with lymphadenopathy, anemia and fever [3, 4].

T. cruzi is a member of the family trypanosomatidae in the order Kinetoplastida and belongs to a subspecie called stercoraria (**Figure 1**). The development of stercoraria parasites takes place in the intestinal track of the invertebrate vector and the infection to the vertebrate occur through the feces. *T. cruzi* is carried in the guts of hematophagous triatomine bugs (kissing bugs) and transmission occurs when infected bug feces contaminate the bite site or intact mucous membranes. During feeding, the infected triatomine insect receives a significant amount of blood in its digestive system which forces the elimination of the bulk of accumulated excreta (consisting of feces and urine) which is normally deposited on the skin surface. The released feces contain the metacyclic trypomastigotes, which by

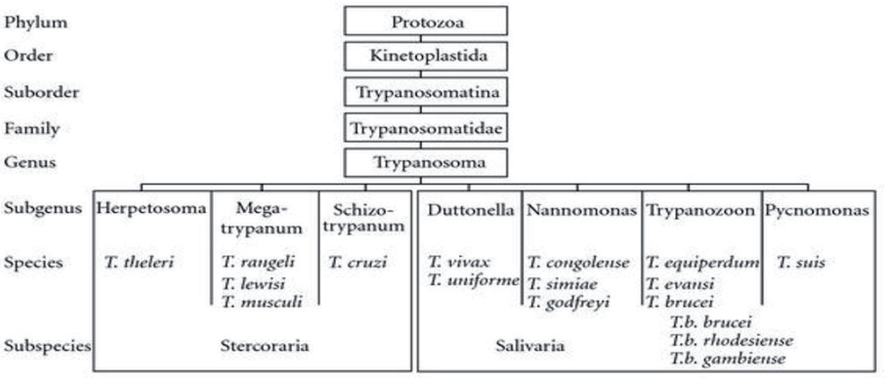

Figure 1.
Classification of trypanosomes. Source: Baral [7].

active movement and release of histolytic enzymes, actively penetrate the skin. Other modes of *T. cruzi* transmission includes through organ transplant, through transfusion and congenitally [5]. The mechanism of transmission of *T. cruzi* contrasts with that of the three subspecies of African trypanosomes that causes human and animal African trypanosomiasis disease, *Trypanosoma brucei gambiense*, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei brucei*, which are transmitted via the saliva of their vectors (salivarian) and with the mechanism by which the nonpathogenic trypanosome found in the Americas *Trypanosoma rangeli* is transmitted to its mammalian hosts. In addition to colonizing the stomach of their invertebrate vector, salivarian parasites migrate towards the salivary gland of the vector where the infectious form for the vertebrate develops but never pass to the intestinal track. In the process of obtaining a blood meal by the vector, infection of the vertebrate occurs through saliva. Like tsetse fly (the vector of human and animal African trypanosomiasis), the triatomine vector ingests circulating trypomastigotes when it takes a blood meal from an infected mammalian host. *T. cruzi* infects vertebrate and invertebrate hosts during defined stages in its life cycle [6].

2. The life cycle of *Trypanosoma cruzi*

The life cycle of *Trypanosoma cruzi* involves two intermediate hosts: the invertebrate vector (triatomine insects) and the vertebrate host (humans) and has three developmental stages namely, trypomastigotes, amastigotes and epimastigotes [8].

The general view of the life cycle of *Trypanosoma cruzi* is as shown in **Figure 2**. The cycle started with the insect sucking of bloodstream trypomastigotes of the infected vertebrates. Most of the ingested trypomastigotes are broken down in the stomach of the insect while the surviving trypomastigotes transform into either spheromastigotes (spherical stage) or into epimastigote stage few days later [9]. Epimastigotes move to the intestine where they divide intensely and attach to the perimicrovillar membranes which are secreted by intestinal cells of posterior midgut [10, 11]. Attachment to perimicrovillar membranes (PMM) in the insect midgut is an essential step for parasite division and is important in the process of metacyclogenesis which involves the transformation of the non-infective epimastigotes into highly infective trypomastigotes known as metacyclic trypomastigotes [12].

Metacyclic parasite forms express a set of surface glycoproteins that interact with mammalian cells [13, 14]. One of the glycoproteins, a metacyclic-stage-specific 82-kDa glycoprotein (gp82), has been implicated in host cell invasion [15]. The gp82 glycoprotein is an adhesion molecule that binds to host cells in a receptor-mediated manner and triggers Ca^{2+} mobilization [16] which is essential for parasite

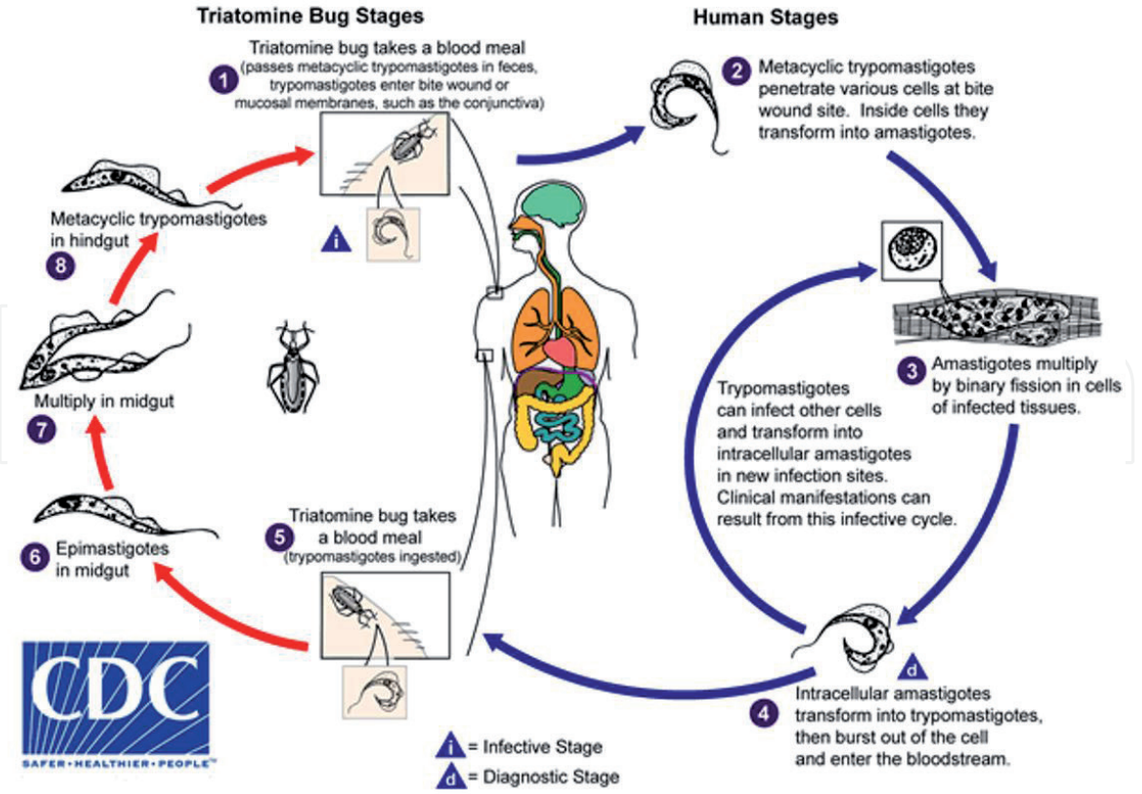

Figure 2.
Life cycle of T. cruzi showing the various forms of the protozoan in the invertebrate (triatomines) and vertebrate (mammals) hosts. Adapted from the Center of Control Diseases homepage.

penetration [17–20]. The gp82 also induces the activation of metacyclic trypomastigote protein tyrosine kinase [21] and an increase in the parasite intracellular Ca^{2+} concentration [22]. Other glycoproteins which are expressed in bloodstream or tissue-culture derived trypomastigotes and which are implicated in mammalian cell adhesion/invasion are gp83 [23], gp85 [24], and Tc-85 [25]. The gp83 has been reported to signal through the mitogen-activated protein kinase pathway to up regulate *T. cruzi* entry in macrophages [26]. Also, surface glycoinositolphospholipids (GIPLs) of the parasite have been shown to be involved in the attachment process [10].

3. Metacyclogenesis

Metacyclogenesis is the fundamental step in the life cycle of *T. cruzi* which involves the differentiation of epimastigotes into metacyclic trypomastigotes and occurs in the midgut of triatomine vector. During metacyclogenesis, bloodstream trypomastigotes differentiate into replicative epimastigotes in the triatomine insect's stomach, which divide in the midgut, migrate to the rectum and adheres to the epithelium through a flagellum prior to differentiation into a non-replicative, infective metacyclic trypomastigote form which are then released into the excreta of the triatomine insect while taking a blood meal on the vertebrate [27, 28]. The factors that trigger metacyclogenesis are still unknown, but might be stimulated by nutritional starvation, cyclic AMP and adenylate cyclase [29]. For instance, while in the midgut of triatomine vector, *T. cruzi* epimastigotes multiply in the nutrient rich environment after obtaining blood meal and as the meal is digested, the parasite density increases, the environment becomes nutrient poor making the epimastigotes become more elongate. On the epimastigotes reaching the insect

rectum, it undergoes metacyclogenesis into human infective trypomastigote forms. Metacyclogenesis occurs when epimastigotes from the nutrient poor hindgut attach to the waxy cuticle of the triatomine vector rectum, initiating a dramatic morphological change. Once formed, metacyclics detach from the waxy cuticle and are excreted. Contamination of the triatomine vector bite wound of the mammalian host with these excreta leads to infection, completing the life cycle.

Description of metacyclogenesis can be in two parts, the first leading to the second. Firstly, the trypanosome senses loss of sugars from its environment and responds by elongating its cell body and flagellum and by activating its mitochondrion which leads to the lengthening of the trypanosome flagellar membrane that is rich in sterol and more hydrophobic than the somatic membrane. Secondly, the lengthening of the flagellar permits the trypanosomes to adhere to a hydrophobic surface and it is this interaction that triggers metacyclogenesis. This trigger for metacyclogenesis is cyclic adenosine monophosphate (cAMP) mediated.

Cyclic AMP plays an important role in the control of lower eukaryotes differentiation [30–32]. The relative amounts of cyclic AMP can change according to the surrounding environment, enabling the organisms to adapt quickly to new conditions. The differential balance of cAMP may result in activation of protein kinases [33, 34], transcription of specific genes [35–37] and changes in the cytoskeleton structure [38], which ultimately lead to morphogenetic cell alterations. Cyclic AMP balance could vary as a response to a changing environment leading to differential gene expression and morphological changes allowing the parasite to go through its life cycle. Calmodulin is known to play a direct role in controlling the levels of cAMP in eukaryotic cells [39] and in the case of *T. cruzi*, it has been shown to activate the cAMP phosphodiesterase [40].

4. The developmental stages of *T. cruzi* in vertebrate and invertebrate

The *T. cruzi* developmental stages alternates between infective and noninfective forms. Amastigote and epimastigote are noninfective but replicative stages inside the mammalian host (vertebrate) and in the gut of the insect vector (invertebrate) respectively (**Figure 3**). The bloodstream trypomastigotes found in the blood of the

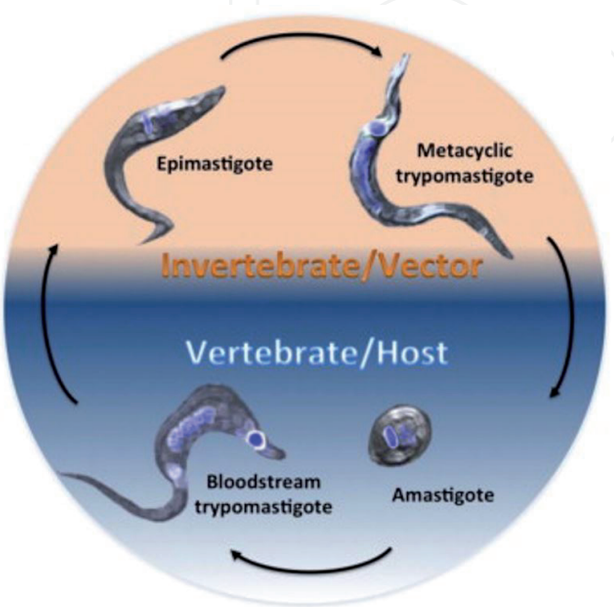

Figure 3.
Developmental stages of T. cruzi in vertebrate and invertebrate. Adapted from: Jimenez [96].

vertebrate host and the metacyclic trypomastigotes found in the rectum of the insect invertebrate vector are considered as the two different trypomastigote infective but nonreplicative developmental stages. The sucking of the blood of vertebrate mammalian host infected with the bloodstream trypomastigotes by the insect started the cycle and inside the stomach of the insect, the ingested trypomastigotes transform into epimastigotes which replicates intensely in the midgut. The epimastigotes transform into metacyclic trypomastigotes in the hindgut of the insect host which are eliminated through feces when the insect vector takes a blood meal from an uninfected host. The excreted metacyclic trypomastigote in the lesioned skin caused by the insect bite leads to *T. cruzi* infection. Once the metacyclic trypomastigote is inside the mammalian host, it invades the host cells at the inoculation site and transform into the replicative amastigote form which transform back into bloodstream trypomastigote form upon completion of a replicative cycle as intracellular amastigotes.

5. Surface membrane proteins

Membrane proteins play an important role in the biology of *T. cruzi*, including the interaction between parasite and host [41–45]. Two thousand seven hundred and eighty four (2784) proteins belonging to 1168 protein groups were identified in the proteomic analyses of different stages in the life cycle of *T. cruzi*. The *T. cruzi* proteins is in relative abundance throughout its life cycle since about 30% of the identified proteins were found at all life cycle stages and at least 248 proteins were only expressed at one stage of the life cycle. The families of surface membrane proteins from *T. cruzi* which are the most abundant and/or relevant during its life cycle are: mucin, trans-sialidase, TcGP63, amastin, TcTASV, mucin-associated surface proteins (MASP) and cruzipain.

5.1 The mucins family

Trypanosoma cruzi is covered by a dense layer of mucin-type molecules which are the major *T. cruzi* surface glycoproteins [46]. These proteins are widely distributed over the cell body, flagellar pocket and flagellum of the different developmental forms [47] and play a key role in the parasite protection as well as in the infectivity and modulation of the host immune response throughout the life cycle of *T. cruzi* [48–51]. The mucins of *trypanosoma cruzi* is divided into two gene families, namely TcMUC and TcSMUG [48, 52] and these proteins are divided into groups based on their central domains: TcMUC is divided into TcMUC I, TcMUC II and TcMUC III [52, 53] . TcMUC I protein is distributed on the amastigote and the bloodstream trypomastigote surface and is the major component in the amastigote form. This protein show internal tandem repeats on their structure with a T_8KP_2 amino acid consensus sequence which are suitable targets for the O-glycosylation pathway in *T. cruzi*, flanked by an N-terminal signal peptide and a C-terminal glycosylphosphatidylinositol anchor signal [48, 55]. TcMUC II protein is also distributed on the amastigote and the bloodstream trypomastigote surface and is found more in membrane lipid rafts of the trypomastigote stage [54]. Like TcMUC I, TcMUC II genes encode proteins that share similar N- and C-terminal but without the T_8KP_2 motifs [56, 57]. The single gene product of the TcMUC III group is termed trypomastigote small surface antigen (TSSA) and has been identified as a mucin-like glycoprotein [58]. TSSA are displayed on the surface of the trypomastigote forms of *Trypanosoma cruzi* and are expressed in vivo as a 20-kDa protein during the mammal-derived stages [59–61].

The second mucin protein family TcSMUG family is divided into two groups: TcSMUG S (small) and TcSMUG L (large) according to their encoded mRNA size

[58, 62] and encodes for very small open reading frame containing a putative signal peptide at the N-terminus and a GPI-anchor signal in the C-terminus. The TcSMUG S group is found in the epimastigote and metacyclic trypomastigote forms and encodes for 35–50 kDa mucins N-glycosylated (Gp35/50 mucins) and they are the major acceptors of sialic acid on the parasite surface by parasite trans-sialidases in *T. cruzi* [63, 64]. In contrast, TcSMUG L group encodes for mucin-type glycoconjugates which are not sialic acid acceptors and only present in the surface of the epimastigote stage [65, 66] and contains one or two additional N-glycosylation signals between the N-terminal region and the threonine-rich region depending on the origin of the encoding allele [65].

5.2 The trans-sialidases (TS) protein family

The trans-sialidases (TS) protein family of *trypanosoma cruzi* are a large superfamily, which includes 1430 gene members, including 693 pseudogenes [67]. Trans-sialidase shares certain characteristics with mucin protein family as they are distributed along the cell body, flagellum, and flagellar pocket of *T. cruzi* [68]. The trans-sialidase superfamily is classified into four groups based on their sequence similarity and functional properties namely: TS 1, TS II, TS III and TS IV [69]. The TS activity involves the transfer of sialic acid from host glycoconjugates to mainly the parasite mucins present in the plasma membrane of trypomastigotes [70, 71]. Trypanosomes are unable to synthesize the monosaccharide sialic acid; therefore they need to scavenge it from the infected host using these TS activities. The sialylation process in *T. cruzi* is crucial for its viability and propagation into the host [72, 73].

The TS I comprises of proteins with trans-sialidase (TS) and/or neuraminidase activities [74]. Neuraminidase activity occurs when nonsuitable acceptor molecules for sialic acid are present and then sialic acid is transferred to water [75]. Neuraminidase activity is involved in the removal of sialic acid from parasites and/or host-cell molecules which is required for parasite internalization [76]. The TS I members incudes: TCNA (neuraminidase), SAPA (shed acute-phase antigen), and TS-epi. SAPA and TCNA proteins are closely related with 84% homology at the amino acid level and have active trans-sialidase and neuraminidase activities and are expressed during bloodstream trypomastigote stage [77]. SAPA and TCNA have two main regions: an N-terminal catalytic region and a C terminal extension, which repeats 12 amino acids (SAPA repeats) in tandem with the consensus sequence. SAPA has only 14 tandem repeats compared to 44 for TCNA and the presence of SAPA repeats increases the half-life of the protein in the blood [78]. Both proteins are attached by glycosylphosphatidylinositol to the parasite plasma membrane and can be found in the serum of deeply infected mammals.

TS-epi, the third member of group TS I is an active trans-sialidase expressed in the insect dwelling epimastigote form at the stationary phase and is different from the trans-sialidase expressed of the blood trypomastigotes. Unlike other members of the group, TS-epi lacks SAPA repeats and is not attached to the membrane by glycosylphosphatidylinositol.

Members of TS group II includes: ASP-1, ASP-2, TSA-1, Tc85, SA85, GP82, and GP90 and they all have been implicated in host-cell attachment and invasion. ASP-1, ASP-2 (both are amastigote surface proteins) and TSA-1 (trypomastigote surface antigen) are targets of *T. cruzi*-specific $CD8^+$ cytotoxic T lymphocytes and they induce strong antibody responses in infected mice and humans. The SA85 glycoproteins are expressed by amastigote and bloodstream trypomastigote forms but only the amastigote form expresses the mannose-binding protein ligand which seems to be involved in the opsonization of the parasite enhancing its infection

capability. The Tc85 molecule is an 85 kDa glycoprotein which is found abundantly in bloodstream trypomastigotes and is identified as a ligand capable of binding to different host receptor molecules located on the cell surface of either monocytes, neutrophils, or fibroblasts. The GP82 and GP90 members of TSII are glycoproteins expressed on the surface of the metacyclic trypomastigote form and they are mainly found at the plasma membrane with opposite roles in mammalian cell invasion. GP82 is able to activate a Ca^{2+} signaling pathway in host cells following parasite adhesion, which is required for *T. cruzi* internalization and is also the signaling receptor that mediates protein tyrosine phosphorylation, which is necessary for host-cell invasion. As defined by its reactivity with monoclonal antibodies, GP90 is a metacyclic stage-specific glycoprotein and expressed by metacyclic forms but lacks any enzymatic activity. GP90 is present in the mammalian stages of *T. cruzi* life cycle and has the antiphagocytic effect mediated by the removal of sugar residues necessary for parasite internalization.

TS Group III which is formed by surface proteins present in mammal bloodstream trypomastigotes includes: complement regulatory protein (CRP), surface flagellar protein (FL-160), chronic exoantigen (CEA), and trypomastigote excretory-secretory antigens (TESA) [79]. These surface proteins are recognized by sera from patients infected with Chagas' disease and they are able to inhibit the classical and the alternative pathways of complement activation, which could be a protection from lysis by the host in the trypomastigote form [80, 81]. TESA is distributed on the cell surface membrane of *T. cruzi* [80] whereas CRP, FL160, and CEA are flagellum associated membrane proteins [80, 82, 83].

The TS Group IV is included in the trans-sialidase superfamily because it contains the conserved motif VTVxNVxLYNR, which is shared by all known TS members and is composed of genes encoding trypomastigote surface antigens whose biological function is still unknown [84, 85]. The TsTc13 protein, a member of TS Group IV has been shown to be highly antigenic and is present in the infective metacyclic trypomastigote form [86].

5.3 TcGP63 family

This protein is expressed by trypanosomes and *Leishmania* species and is a family of cell surface-localized, zinc-dependent metalloproteases also known as GP63 proteins, major surface proteases or leishmanolysins. They serve as ligands for host cell attachment and protect the parasite from intraphagolysosomal degradation in *Leishmania* while they function to release variant surface glycoproteins from the cell surface during antigenic variation in the bloodstream form of the African trypanosome (*Trypanosoma brucei)*. *Trypanosoma cruzi* possesses GP63-like genes (TcGP63) which are differentially regulated, suggesting its functional importance at multiple stages in the parasite life cycle [87]. The TcGP63 family is made up of two groups of proteins namely: TcGP63-I and TcGP63-II [88]. The *TcGP63-I* has low gene copies of 5–10, whereas *TcGP63-II* has 62 gene copies [87]. The TcGP63-I group is present in all the life-stages/cycles of *T. cruzi* and possess metallopeptidase activity and are bound to the protozoan's membrane by a C-terminal glycosylphosphatidylinositol- (GPI-) anchor signal. In *T. cruzi*, TcGP63-I exist in two isoforms: the glycosylated and the nonglycosylated isoforms. The 61 kDa glycosylated isoform is present in similar levels in both epimastigote and amastigote forms and is irregularly expressed on the surface membranes of the epimastigote while the 55 kDa nonglycosylated isoform is present in the metacyclic trypomastigote and is located intracellularly near the kinetoplast and the flagellar pocket [88]. Members of TcGP63-II are two transcripts of 2.6 and 2.8 kb protein.

5.4 Amastin family

The amastin family which is a group of transmembrane glycoproteins consists of small proteins of about 180 amino acids. The genome of *trypanosoma cruzi* has two groups of amastin family: the β-amastin and the δ-amastins. The β-amastin group has two members namely: β1-amastin and β2-amastin. Genes encoding for the β1-amastin and β2-amastin are localized in the chromosome 32 of *T. cruzi*. The δ-amastins group has δ-amastin and δ-ama40/50 as members and are found on chromosomes 34 and 26, respectively. β1-amastin and δ-amastins are located at the cell surface. In addition to their surface localization, β2 amastin shows a disperse distribution within the cytoplasm [89]. Even though the exact biological function of amastin is still unknown, as a transmembrane proteins, amastins could play a role in proton or ion traffic across the membrane [90]. The β-amastin transcripts are more abundant in epimastigotes than in amastigotes or trypomastigotes while transcript levels of δ-amastins are upregulated in amastigotes from different *T. cruzi* strains and β-amastins may be involved in the parasite adaptation to the insect vector [91].

5.5 *T. cruzi* trypomastigote alanine, serine and valine (TcTASV) family

The TcTASV protein family is conserved among all the *T. cruzi* lineages analyzed so far and has no orthologues in other species, including the closely-related trypanosomatids *T. brucei*, *T. rangeli* and *Leishmania sp* and belong to a medium-size multigene family of ~40 members that was identified from a library of trypomastigote-enriched mRNAs [92]. TcTASV proteins are expressed mainly in the trypomastigote stage and its function is still unknown. In TcTASV proteins, the N- and C-terminal regions respectively possess a signal peptide and a consensus for a glycosylphosphatidylinositol (GPI) anchor addition, and display the highest level of conservation, while the central region presents more variability. The TcTASV protein family is divided into 4 groups: TcTASV-A, TcTASV-B, TcTASV-C and TcTASV-W, with each group defined by the primary amino acid sequence and length of polypeptides. TcTASV protein family can be distinguished by the common amino acid motif *tasv_all* that starts approximately at amino acid 42 ($Vx_1x_2x_3$[CES]x_4x_5TDGx_6Lx_7W$x_8x_9x_{10}x_{11}$E$x_{12}x_{13}$W$x_{14}x_{15}$C$x_{16}x_{17}x_{18}$P). Each group has certain amino acid in-between the *tasv_all* motif. The TcTASV-B contains serine and arginine, and TcTASV-W has alanine at X_4 and glutamic acid at X_5 while TcTASV-C and TcTASV-A both have proline and glycine at positions X_4 and X_5.

5.6 Mucin-associated surface proteins (MASPs) family

The MASP family is characterized by having highly conserved N and C-terminal domains and a variable and repetitive central region, with a maximum expression in the human infective stages of the parasite. MASP are expressed simultaneously in bloodstream trypomastigotes as well as in amastigotes and epimastigotes and MASP molecules are the most abundant antigens found on the surface of the infective trypomastigote stage of *T. cruzi*. MASP family plays an important role in the invasion of the mammalian host cell, but could also be crucial for the survival and the establishment of the parasite in the invertebrate host. The overexpression of MASPs in the intracellular parasites prior to the division of the amastigotes located in the plasma membrane suggests that some of the proteins of this extensive family play a major biological role in the survival and multiplication of intracellular amastigotes.

5.7 Cruzipain family

This glycoprotein is synthesized as a zymogen that is activated by cleavage of the N-terminal pro-domain to generate the mature protease and belongs to the mammalian papain superfamily but contains, as other cysteine proteases (CPs) from trypanosomatids, an unusual C-terminal extension. Cruzipain family has many groups which include: native-cruzipain (N-cruzipain), recombinant-cruzipain 1 (R-cruzipain 1) and recombinant cruzipain 2 (R-cruzipain 2). Cruzipains are expressed on all the body surface of epimastigotes and amastigotes forms while in the trypomastigote form, cruzipain is expressed only in the flagellar pocket region. Cruzipain plays a role in the process of *T. cruzi* internalization into mammalian cells. Cruzipain is not only essential for parasite survival but also generates a strong immune response in infected individuals.

6. Extracellular vesicles in the life cycle of *T. cruzi*

Extracellular vesicles (EVs) typically consist of a lipid bilayer membrane containing integral membrane proteins and a luminal cavity that is loaded with a variety of soluble proteins and nucleic acids. *T. cruzi* parasites, like many other cells, release extracellular vesicles (EV) that are involved in cell-cell communication or in the modulation of the host immune responses to promote the establishment of an infection [93]. In *T. cruzi* parasites, two classes of extracellular vesicles have been characterized based on size. These include exovesicles also referred to as ectosomes and exosomes. Ectosomes which bud directly from the plasma membrane have a size of 100–1000 nm while exosomes which are vesicles that are secreted into the extracellular environment following the fusion of multivesicular endosomes with the plasma membrane, typically occurring at the flagellar pocket membrane have a size of 30–100 nm. Analysis of extracellular vesicles released by epimastigotes and metacyclic trypomastigotes in culture demonstrated the presence of two populations of extracellular vesicles containing plasma membrane and intracellular proteins, and also nucleic acids. The *T. cruzi* small membrane proteins (TcSMP), a family of proteins or phosphatases detected on *T. cruzi* EVs has been shown to trigger Ca^{2+} signaling and lysosome mobilization/exocytosis, events that promote formation of parasitophorous vacuoles and parasite invasion. In the early stages of *T. cruzi* infection, parasites promote the release of plasma membrane vesicles from the host cell, which may contribute to parasite survival in the circulatory system, an event thought to help mediate host cell invasion.

Immune cells are one of the main targets of extracellular vesicles. Extracellular vesicles secreted during acute and/or chronic *T. cruzi* infection should play a role in the dissemination and survival of this parasite in the vertebrate mammalian host since the released extracellular vesicles from virus-infected cells, bacteria, fungi or parasites have been demonstrated to play a pivotal role in the modulation of the immune system. Several types of extracellular vesicles are promoters of the innate and acquired immune response and defined as types of pathogen-associated molecular patterns (PAMPs) which could be formed by a wide range of macro-molecules such as lipids, proteins, carbohydrates, or nucleic acids and are recognized by pattern recognition receptors (PPRs) such as toll-like receptors (TLRs) present in leukocytes and various non-immune cells, which will in turn initiate a signaling cascade that leads to the activation of an immune response against the pathogen [94]. In *T. cruzi*, several PAMPs have already been described, for instance, parasite cytidine-phosphate-guanosine (CpG)-DNA released from lysed intracellular parasites stimulates TLR7 and TLR9 activation and production of T helper type 1 (Th1) proinflammatory cytokines or parasite α-Gal-containing glycoconjugates such as

mucins or gp85/trans-sialidase recognized by TLR2/6 leading to tumor necrosis factor (TNF-α) production in macrophages and inhibition of IL-12 in dendritic cells. Studies on *T. cruzi* extracellular vesicles have shown that these vesicles could act as an agonist of TLR2 signaling, which leads to the secretion of proinflammatory cytokines (TNF-α and IL-6) and nitric oxide which could be explained by the presence of GPI-anchored molecules like mucins, mucin-associated surface proteins (MASPs), or trans-sialidases (TSs) on the extracellular vesicles surface.

7. Carbon and energy sources in *T. cruzi* life cycle

The life cycle of *T. cruzi* alternates between glucose-rich and glucose-poor environments having to adapt to different sources of energy and carbon. The differentiation of epimastigotes, the non-infective dividing forms found in the digestive tract of the invertebrate host into metacyclic trypomastigotes (metacyclogenesis) occur in an amino-acid-rich and carbohydrate-poor medium. In vertebrate, the trypomastigotes differentiate into the dividing forms called amastigotes occur in a medium poor in free glucose.

Trypanosomatids can use either glucose or amino acids as main carbon and energy source, although one cannot rule out the use of fatty acids as well. Amino acids, especially L-proline and L-glutamine which are abundant in the hemolymph and tissue fluids of the blood sucking vector are the main source of carbon and energy in the insect stages. L-Proline seems to be involved in several mechanisms of resistance to oxidative, nutritional and thermal stress and is important in metacyclogenesis for the differentiation of intracellular epimastigotes to trypomastigotes in *T. cruzi*. Several amino acids such as proline, aspartate and glutamate are actively transported and oxidized by *T. cruzi*. The catabolism of aromatic amino acids appears to be related to the cytosolic $NADH^+$ reoxidation. Also, the presence of at least 60 genes belonging to a single family of amino acid transporter in *T. cruzi* further reinforces the relevance of amino acids in the biology of these organisms. While some trypanosomatids metabolically prefer glucose to amino acid when grown in a medium rich in glucose and amino acid, as seen in proline and glucose metabolism in *T. brucei*, other trypanosomatids like *Crithidia deanei*, a monoxenic and non-pathogenic trypanosomatid living in the insect gut preferentially metabolize amino acids irrespective of the glucose content of the culture medium [95].

Amino acids are crucial nutrients during the *T. cruzi* life cycle; apart from their use as carbon and energy sources, they participate in several biological processes that help the parasite adjust to their changing environment. Arginine metabolism is linked to *T. cruzi* growth and is involved in the management of cell energy under nutritional stress condition. Certain amino acids such as proline, glutamate, and aspartate are essentials in the process of metacyclogenesis. Apart from being involved in the process of metacyclogenesis, proline and glutamate seems to have a broad variety of functions. While proline is involved in fulfilling the energy requirements for host cell invasion, differentiation from the intracellular transient epimastigote-like stage to trypomastigote forms and resistance to oxidative stress, glutamate is directly involved in osmoregulation and cell volume control.

8. Changes in gene expression during the life cycle of *T. cruzi*

As a result of changing environments during the life cycle, *T. cruzi* undergoes rapid and significant changes in gene expression, which are achieved essentially

at the post-transcriptional level through modulation of messenger RNA (mRNA) stability and translational control mechanisms. In order to adapt to the different environment they find within one or the other host species, *T. cruzi* also undergoes drastic morphological and biochemical changes and are also able to differentiate from proliferative to nonproliferative cells within the same host. All these changes are orchestrated by the differential expression of stage-specific genes.

Cellular differentiation is controlled at multiple levels including, for most eukaryotic cells, initiation of gene transcription. The discriminatory mechanisms for the initiation of transcription at individual loci is largely absent in trypanosomatids and most protein-coding genes lack promoters and are transcribed as long polycistronic units that are processed into individual mRNAs. Consequently, trypanosomes rely on post-transcriptional processes such as translational efficiency, mRNA stability and post-translational modification to coordinate developmental transitions and other adaptive responses encountered throughout their complex life cycles.

In eukaryotes, protein-coding genes are transcribed into monocistronic pre-mRNA transcripts containing coding sequences (exons) and non-coding sequences (introns) that are processed into mature mRNAs through *cis*-splicing reactions. RNA polymerase II is the enzyme responsible for the transcription of protein-coding genes while RNA polymerase I transcribes ribosomal RNA. In trypanosomatids, however, transcription is polycistronic, there are no introns and, therefore, no *cis*-splicing reactions. Processing of pre-mRNA into single gene units is effected by *trans*-splicing reactions, a process that has been found to operate only in trypanosomatids and other organisms like *Euglena*, nematode and trematode worms.

Granules of mRNA such as processing bodies (P bodies) and stress granules (SGs) are involved in post-transcriptional regulation of gene expression. P bodies are constitutively present in the cell and can grow in size and number when cells are perturbed while SGs only arise under cellular stress. P bodies contain mRNA and proteins involved in translational repression, mRNA decapping, 5′ → 3′ mRNA decay, nonsense-mediated decay (NMD) and the miRNA (microRNA) pathway. P bodies were initially thought to be the place where mRNA was recruited to be degraded and recently, a function as mRNA storage depots has been assigned to P bodies. By contrast, SGs are stalled 43S translation pre-initiation complexes, mainly composed of mRNA, translation initiation factors and 40S ribosomal proteins. SGs are thought to function as mRNA triage centers during stress.

9. Conclusion

Trypanosoma cruzi, the parasite responsible of Chagas disease has a complex life cycle including intracellular and extracellular forms, which alternate between invertebrate insect vectors and vertebrate mammalian hosts. *T. cruzi* replicate extracellularly within the insect, but have to infect cells to multiply within the mammal which contrasts with the African trypanosome which is extracellular in both hosts. During their life cycles, they alternate between a mammalian host and an insect vector and undergo profound biochemical and morphological transformations in order to adapt to the different environments changes orchestrated by precise gene regulation programs.

Conflict of interest

I have no conflict of interest to declare.

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