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Chapter 3

Morphological and Functional Aspects of Cytoskeleton of Trypanosomatids

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http://dx.doi.org/10.5772/66859

Abstract

Trypanosomatidae are protozoans that include monogenetic parasites, such as the *Blastocrithidia* and *Herpetomonas* genera, as well as digenetic parasites, such as the *Trypanosoma* and *Leishmania* genera. Their life cycles alternate between insect vectors and mammalian hosts. The parasite's life cycle involves symmetrical division and different transitional developmental stages. In trypanosomatids, the cytoskeleton is composed of subpellicular microtubules organized in a highly ordered array of stable microtubules located beneath the plasma membrane, the paraflagellar rod, which is a lattice-like structure attached alongside the flagellar axoneme and a cytostome-cytopharynx. The complex life cycle, the extremely precise cytoskeletal organization and the single copy structures present in trypanosomatids provide interesting models for cell biology studies. The introduction of molecular biology, FIB/SEM (focused ion beam scanning electron microscopy) and electron microscopy tomography approaches and classical methods, such as negative staining, chemical fixation and ultrafast cryofixation have led to the determination of the three-dimensional (3D) structural organization of the cells. In this chapter, we highlight the recent findings on Trypanosomatidae cytoskeleton emphasizing their structural organization and the functional role of proteins involved in the biogenesis and duplication of cytoskeletal structures. The principal finding of this review is that all approaches listed above enhance our knowledge of trypanosomatids biology showing that cytoskeleton elements are essential to several important events throughout the protozoan life cycle.

Keywords: trypanosomatids, cytoskeleton, ultrastructure, microscopy, three-dimensional reconstruction

1. Introduction

Trypanosomatids are uniflagellated protozoan parasites belonging to the Kinetoplastid order. They are the etiological agents of several diseases [1] and exhibit particular features that

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differentiate them significantly from their mammalian host [2]. First, they have subpellicular microtubules (SPMT), which are a network of organized stable microtubules that are closely associated with the plasma membrane and to each other forming a corset that confers rigidity to the cell body and help to determine the shape of the cell. Second, trypanosomatids have the microtubule quartet (MtQ) of the flagellar pocket (FP) that encircles the flagellar pocket in a helicoidal pattern [3]. Third, they have microtubule sets of cytostome-cytopharynx that forms a gutter in this funnel invagination [4]. Fourth, trypanosomatids have a flagellum attachment zone (FAZ) where the flagellum emerges from the flagellar pocket and remains attached to the cell body [5]. Finally, they have a paraflagellar rod (PFR), a lattice-like structure that runs parallel to the axoneme from the flagellar pocket to the flagellar tip [6].

Recent studies using techniques, such as electron tomography and focused ion beam-scanning electron microscopy, that allow three-dimensional reconstruction of whole protozoan, allowed for the accurate understanding of their subcellular morphology. High-resolution microscopy studies have provided detailed cellular information regarding protein localization and phenotype after cytoskeleton-protein depletion aiming at the elucidation of protein function during life cycle of trypanosomatids. Using FIB/SEM, one can examine a large number of whole cells at the same time, which enables qualitative and quantitative studies about different morphological aspects of cytoskeleton elements during the life cycle of trypanosomatids. This chapter aims to provide an overview of the topographical relationship among the cytoskeletal elements throughout the protozoan life cycle.

2. Subpellicular microtubules

The shape of cells in trypanosomatids is defined by SPMT. SPMT are a cage of stable microtubules located underneath the plasma membrane and composed of α/β tubulin [7]. High-resolution field emission scanning electron microscopy (FESEM) revealed a helicoidal pattern of SPMT in the promastigote form of nonpathogenic *Herpetomonas megaseliae*; whereas in the procyclic trypomastigote form of *Trypanosoma brucei*, it appears as a straight pattern [8] (**Figure 1A–C**).

In contrast to the microtubules of mammalian cells, SPMT are resistant to low temperatures and drugs that usually promote microtubule depolymerization [10]; thus, SPMT contribute to the stabilization of the trypanosomatids shape. Transversal ultrathin sections of *Leptomonas samueli* fixed with glutaraldehyde and tannic acid showed that trypanosomatids' SPMT are formed by 13 typical protofilaments [11]. Immunolabeling studies using *T. brucei* revealed that the region of tubulin polymerization is localized at the posterior region of the procyclic forms, which is consistent with the identification of the plus end of microtubules at this region [12].

These SPMT are cross-linked to each other by short 6 nm thick filaments and to the plasma membrane [9, 13] (**Figure 1D**). Molecular studies using *T. brucei* indicated the presence of microtubule-associated proteins (MAPs) linking SPMT to each other [14]. These links are particularly strong and are not disrupted by cell lysis. It is likely that they play a role in the rigidity of the trypanosomes' cell bodies [15]. Moreover, a connection between SPMT and the endoplasmic reticulum was observed in *Leishmania amazonensis* [16]. The transmission electron microscopy image of thin sections showed that the distance between the SPMT of

trypanosomatids is 44 nm and approximately 15 nm between SPMT and the plasma membrane. The number of microtubules varies by the region of the cell body. In *Trypanosoma cruzi* trypomastigotes, the posterior region where the Golgi apparatus is located has approximately 120 microtubules. At the cell extremities, approximately 40 microtubules were counted. In the intracellular dividing amastigote, the highest number of microtubules, 222, was observed. These data suggest that newly formed microtubules are inserted between preexisting microtubules during the cell division, because the number of microtubules remained constant [17]. A study on the biogenesis of subpellicular microtubules in *T. brucei* indicated that new microtubules are incorporated primarily in a region adjacent to the new FAZ and between the new and the old FAZs during cell growth [18]. The addition of new microtubules into the FAZ region facilitates the segregation of the basal bodies and consequently, mitochondrial genome segregation. Furthermore, this incorporation promotes the formation of the membrane fold in preparation for cell division [12, 19, 20].

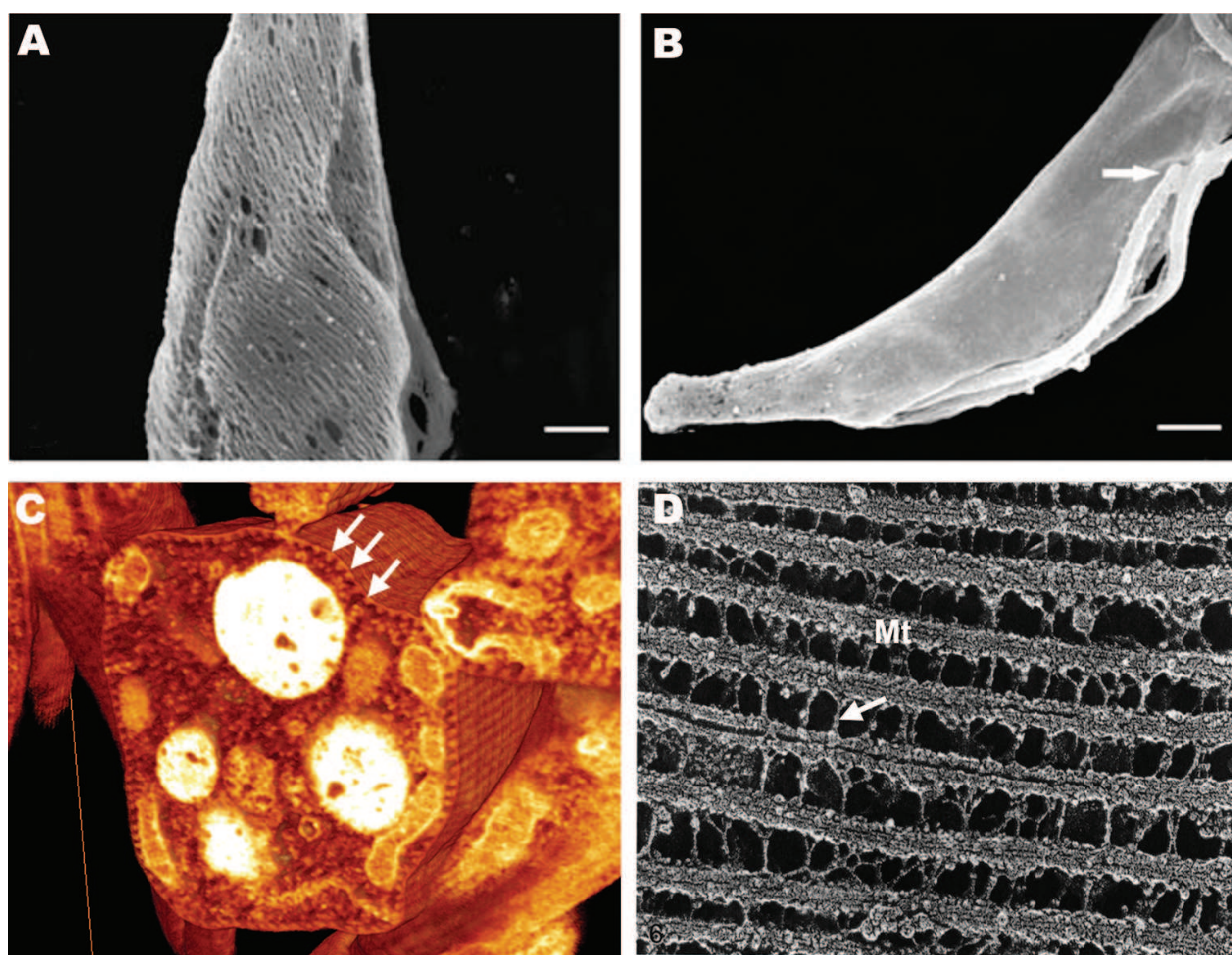

Figure 1. Ultrastructural pattern of subpellicular microtubules (SPMT). (A–B) Field emission scanning electron micrographs of whole-extracted *H. megaseliae* and *T. brucei*, respectively, showing the SPMT. (A) The helical pattern of *H. megaseliae* SPMT and (B) The straight pattern of *T. brucei* SPMT [8]. (C) Several images of *T. cruzi* epimastigotes obtained by focused ion beam scanning electron microscopy (FIB-SEM) were joined in order to emphasizing the SPMT cage (arrows) underneath the plasma membrane [unpublished data from Wanderley de Souza]. (D) Promastigotes of *H. megaseliae* were extracted using Triton-X-100, revealing the parallel array of SPMT. Additionally, the fibrils connecting SPMT to each other were observed (arrow) [9]. Scale bars: (A) 3.5 μm and (B) 8 μm.

Due to the rigidity of the SPMT cage beneath the cell membrane, endocytic events are limited to sites where these microtubules are absent: the flagellar pocket and the complex cytostome-cytopharynx.

3. Cytoskeletal elements associated with endocytic entry sites

3.1. Microtubules

The FP is an invagination close to the basal body and surrounds the site of flagellum exit. It is found at the anterior and posterior regions of the cell body of *T. cruzi* epimastigotes and the procyclic form of *T. brucei*, respectively. It is involved in cell polarity and cell division. The FP membrane is compositionally and functionally distinct from both the cell body and flagellar membranes, despite being continuous with them [21]. The absence of SPMT at the FP site allows budding of endocytic and exocytic vesicles. It is also essential for defense against the innate and acquired immune responses of the host [22, 23]. Trypanosomatids' FP is precisely positioned and it is closely related to cytoskeletal elements which are highly conserved throughout trypanosomatids. Flagellar pocket architecture and composition have been described in several studies; however, it was best analyzed in the African trypanosome, *T. brucei* [22, 24–29]. Electron microscopy tomography revealed a set of MtQ that are nucleated adjacent to the basal bodies [3] (**Figure 2A** and **C**). Their polarity is opposite (plus end at the anterior of the cell) that of the subpellicular microtubules. MtQ encircles the FP and forms a membrane dislocation, as a deep and longitudinal channel through which nutrients achieve the lumen of the flagellar pocket [30] (**Figure 2A**). These microtubules are different from the SPMT because they do not depolymerize in media with high salt concentrations [31]. The same MtQ, with a helicoidal pattern located close to the FP, was observed in a 3D reconstruction of *T. cruzi* epimastigotes [4] (**Figure 2B** and **C**).

Several studies with *T. brucei* have shown that FP is divided into several domains, such as the neck of the FP. In the neck, MtQ joins SPMT and is associated with FAZ; thus, MtQ defines the flagellum and flagellar pocket axes [32]. The flagellar collar is a horseshoe-shaped, electron-dense cytoskeletal structure located in the flagellar neck; the first protein characterized at this domain was BILBO1. Using RNA interference (RNAi)-mediated ablation of this protein in the procyclic form of *T. brucei*, it was shown that BILBO1 is essential for flagellar pocket biogenesis and cell survival [29].

T. brucei also possesses a cytoskeletal feature of unknown function, named bilobe. TbMORN1 was the first protein described as an exclusive component of the bilobe [33]. The bilobe is located near the Golgi apparatus; it is thought to function in Golgi apparatus replication and as an adaptor during cytokinesis [34]. Recently, TbMORN1 depletion experiments suggested that this protein allows access of endocytic tracers into the flagellar pocket [35].

Although *T. cruzi* epimastigotes and amastigotes uptake nutrients by endocytosis via the flagellar pocket they primarily obtain nutrients from the cytostome-cytopharynx complex [36–38]. The latter was also found in a free-living kinetoplastid, *Bodo sp*. [39] as well as in

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Trypanosoma vespertilionis and *Trypanosoma dionisii* [15]. The cytostome is an opening in the anterior region of the cell surface that is followed by a membrane invagination called cytopharynx. Recently, using serial electron microscopy tomography and FIB-SEM to reconstruct the entire length of the cytostome-cytopharynx present in *T. cruzi*, it was shown that this invagination is supported by seven microtubules. A triplet of these microtubules was located underneath the cytostome aperture membrane. A quartet of microtubules originated underneath the flagellar pocket membrane in a staggered formation and followed the preoral ridge before reaching the cytopharynx invagination [4] (**Figure 3A**). This quartet was different from the MtQ described in *T. brucei*. These two sets of microtubules assisted the cytopharynx in forming a "gutter" and creating a microtubule-free side where vesicles can bud or fuse.

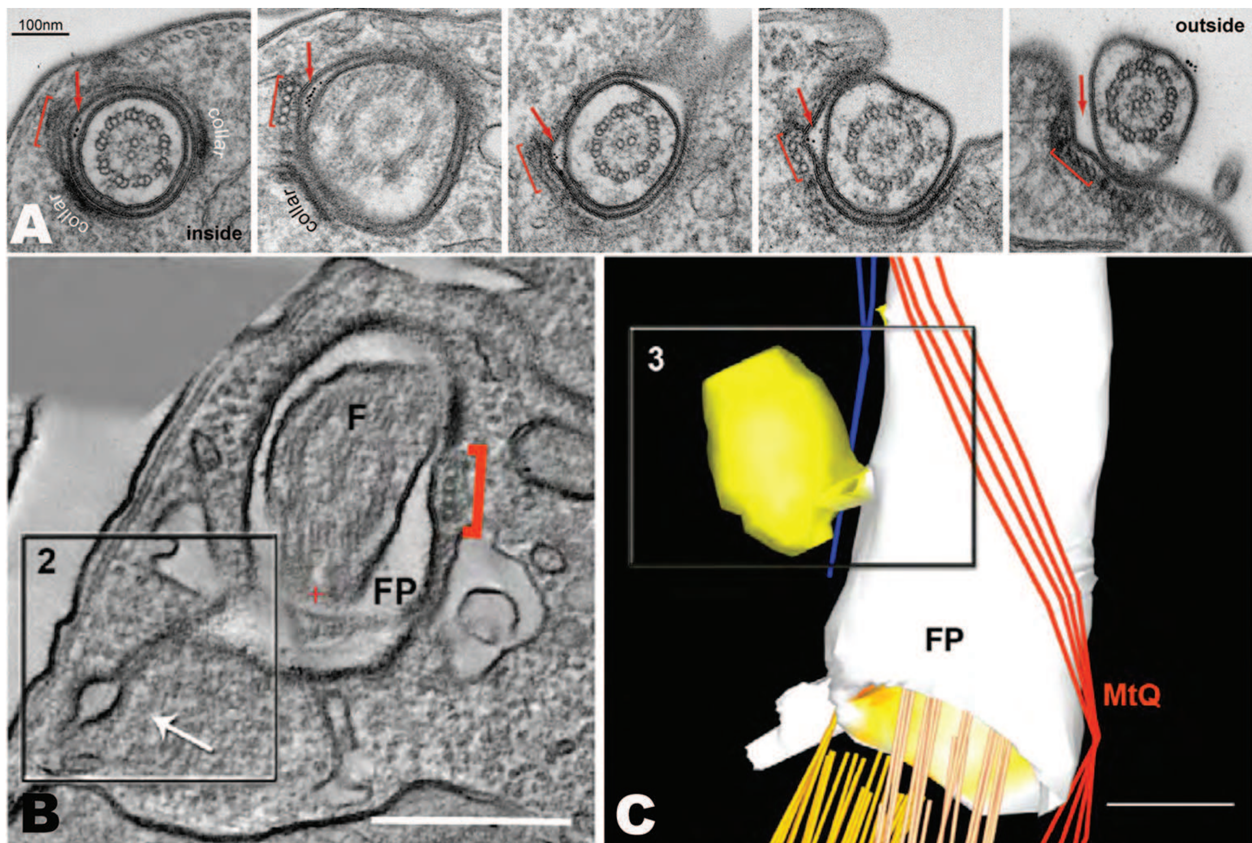

Figure 2. Microtubule quartet (MtQ) is associated with the FP of *Trypanosoma brucei* and *T. cruzi*. (A) Electron micrographs showing the MtQ (brackets) forming a channel that permits endocytic tracers (red arrows) to enter into the *T. brucei* FP [30]; (B–C) the MtQ is nucleated near the basal bodies and encircles the FP (brackets) *T. cruzi* epimastigotes [4]. Scale bars: 200 nm.

Electron microscopy tomography and dual beam scanning electron microscopy are used to observe epimastigotes of *T. cruzi* that were synchronized using hydroxyurea, a compound that induces G1/S cell cycle arrest, we observed that the cytostome-cytopharynx complex is completely disassembled during cell division. This complex is formed *de novo* from the flagellar pocket membrane during cytokinesis. This experiment elucidated the biogenesis of this structure [40] (**Figure 3B1–B3**).

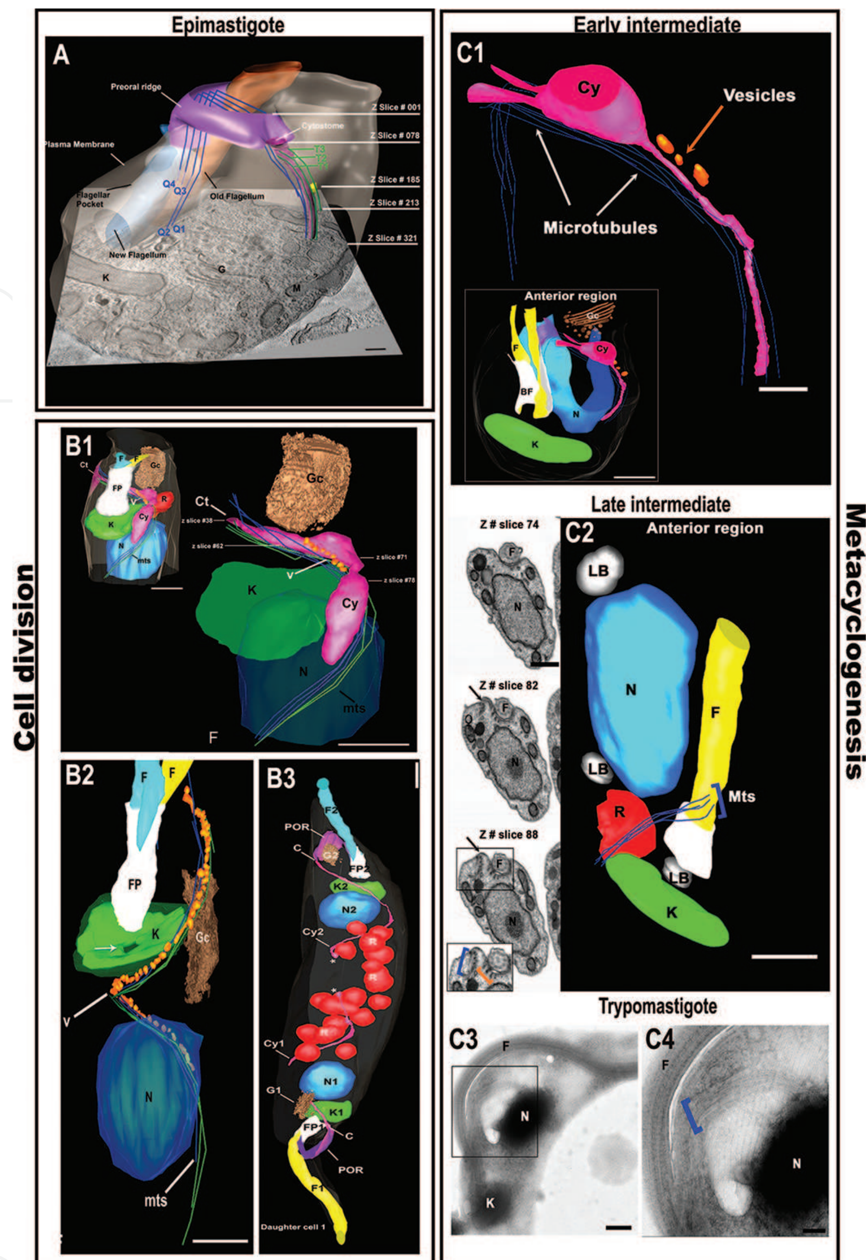

Figure 3. Ultrastructural changes of the cytostome-cytopharynx complex during the *T. cruzi* life cycle. (A-C1) Tomographic reconstruction of the cytostome-cytopharynx complex. (A) The cytostome-cytopharynx complex of epimastigotes is sustained by a microtubule triplet (green) that extends from underneath the cytostome membrane and a microtubule quartet (blue) that appears to start close to the flagellar pocket. It continues underneath the preoral ridge membrane [4]. (B) Cell division: (B1) in early G2, the cytopharynx was shorter. (B2) After this, the cytostome-cytopharynx complex becomes fragmented during the G2 phase. The microtubules maintained their helical arrangement. The vesicles were aligned with the microtubules, following the path of the cytopharynx. (B3) At the end of cytokinesis, both daughter cells possess a complete cytostome-cytopharynx complex (3D model of the cell) [40]; (C) The metacyclogenesis process. (C1) Some structures were omitted in order to highlight the structure of the cytopharynx (inset square). The reconstruction revealed the enlarged proximal portion of cytopharynx. Despite this alteration, this invagination still has the two microtubule sets and adjacent vesicles that were observed in the epimastigotes. Tomogram representing the intermediate forms; (C2) sequence of cell images showing different portions of the cytostome-cytopharynx complex present in a late intermediate form; the reconstruction shows the absence of the cytopharynx membrane; the microtubule triplet is still present; (C3–4) negative staining of a metacyclic trypomastigote shows the presence of the microtubule triplet. The cytostome-cytopharynx complex was not detected [41]. Flagellar pocket (FP-white), preoral ridge (POR-purple), cytostome-cytopharynx (Cy-pink), vesicles (V-orange), flagella (F-yellow and light blue), kinetoplast (K-green), nucleus (N-blue), and the Golgi complex (G-gold). Scale bars: 200 nm.

The same methodology was used to obtain 3D reconstructions of the ultrastructural changes present in the intermediate forms of *T. cruzi* during the metacyclogenesis process (differentiation of epimastigotes into metacyclic trypomastigotes). It was shown that the migration of the kinetoplast/flagellar pocket to the posterior region drags the cytostome aperture. Thus, the invagination shortens from the end to the beginning. The late intermediate forms did not have a cytopharynx membrane; only the accompanying microtubules remained in this form. In the trypomastigote form, shorter microtubules were still observed [41] (**Figure 3C1–C4**).

3.2. Microfilaments

The presence of actin has been confirmed in some trypanosomatids species. Nevertheless, little is known about microfilament function or localization [7, 42]. In trypanosomatids, it does not appear to polymerize into highly structured cytoskeletal microfilaments [43].

The trypanosomatids genome contains putative actin and actin-binding protein sequences [44]. However, to date, few studies have successful visualized microfilaments in trypanosomatids. Sahasrabuddhe et al. visualized actin filaments in *Leishmania spp*. promastigotes using immunogold and immunofluorescence approaches. They detected actin in the flagellar pocket, flagellum, nucleus and kinetoplast (mitochondrial DNA, termed kDNA). The presence of actin in the flagellar pocket strongly suggests that it participates in endocytosis. Interesting, in *Leishmania*, actin also colocalized with SPMT. The study speculates that actin may cooperate with SPMT to help maintain the shape of the cell. This study suggested that the actin present in *Leishmania* is a new isoform which may differ functionally and structurally from that of eukaryotes [45]. Immunofluorescence microscopy observations showed that actin of *T. brucei* colocalized with the endocytic pathway; however, in procyclic forms, actin was distributed throughout the entire cell. The endocytic pathway and cell division are arrested in the actin-depleted, bloodstream stage of *T. brucei* [46] (**Figure 4A–B**). These observations suggest different roles for actin during the life cycle of *T. brucei*.

Molecular methods confirmed the expression of actin in trypanosomes, although the role of actin in *T. cruzi* has not been fully elucidated. Immunoblotting of parasite extracts with polyclonal serum raised against a recombinant version of the *T. cruzi* actin protein confirmed the presence of actin variants; the pattern of expression was different for each stage of the parasite life cycle [43], as previously observed with *T. brucei* [46].

Using cytochalasin to depolymerize actin resulted in morphological changes to the cytoskeletal elements associated with the cytostome-cytopharynx of *T. cruzi* epimastigotes; cytochalasin treatment also impaired transferrin uptake [48]. Comparative genomic analyses suggested that the actin-myosin system might function at the cytostome-cytopharynx during endocytosis [44]. The first detailed characterization of actin (TcActin) and actin-binding proteins in *T. cruzi* was carried out by De Melo et al.. The authors showed that TcActin was distributed in patch-like cytoplasmic structures during all stages of the *T. cruzi* life cycle (**Figure 4C–F**) [47]. Recently, confocal microscopy analyses of several trypanosomatids (*T. cruzi*, *T. brucei*, *L. major*, *Angomonas deanei*, *Crithidia fasciculata*, *Herpetomonas samuelpessoai*, *Strigomonas culicis* and *Phytomonas serpens*) showed that these protozoa express actin, which is present diffusely throughout the cytoplasm. In *T. cruzi* epimastigotes, actin seems to be localized at the

cytopharynx [49], which may explain the disruption of endocytosis when epimastigotes are treated with cytochalasin.

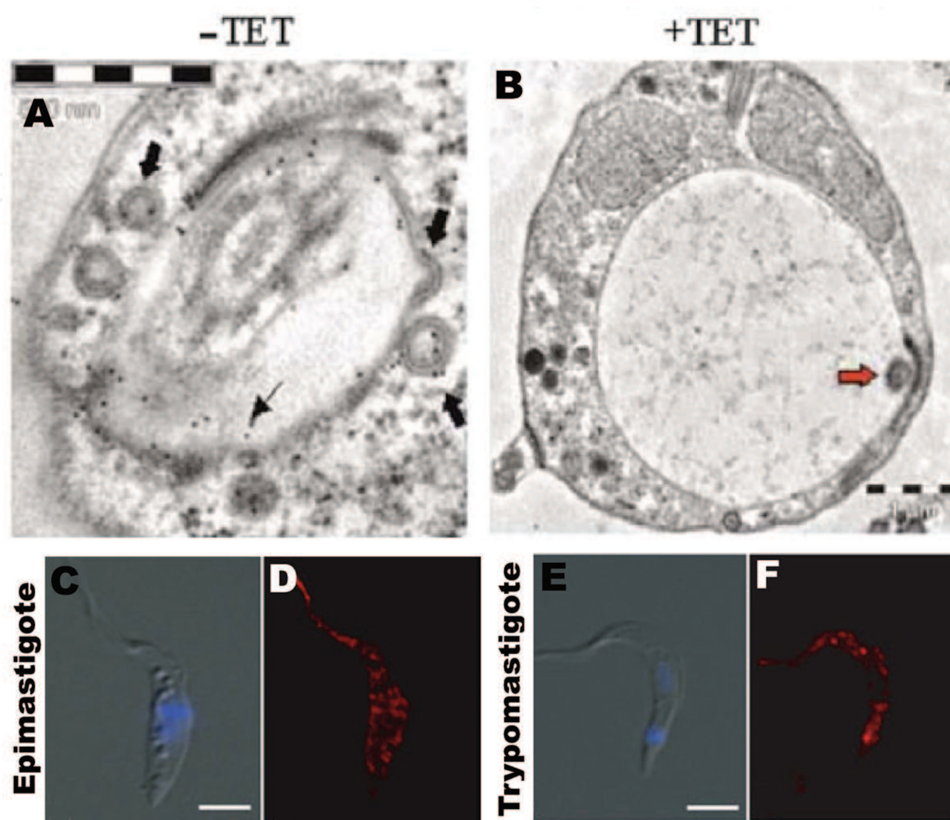

Figure 4. Effect of actin RNAi on the bloodstream form of *T. brucei* (A–B) and the subcellular distribution of actin in *T. cruzi* (C–H). (A) Electron micrograph of control bloodstream *T. brucei* (−TET). The flagellar pocket has budding vesicles. (B) Electron micrograph of RNAi-induced cells (+TET). These cells show loss of vesicles budding from the flagellar pocket membrane [46]; (C–F) Immunofluorescence confocal microscopy images of *T. cruzi* epimastigote and trypomastigote forms showing actin patches along the flagellum, and also spread all over the parasitic body [47].

4. Paraflagellar structure and flagellum attachment zone

The trypanosomatid flagellum consists of an evolutionarily conserved axoneme. It is also composed of peculiar elements such as the FAZ and the paraflagellar rod (PFR), a structure that is attached to the flagellum. FAZ is a specialized cytoskeletal region that links most of the flagellar membrane to the membrane of the cell body [5] (**Figure 5A–C**). The FAZ filament, the MtQ and the bilobe structure also help to link the flagellum to the cell surface [34, 50] (**Figure 2A**).

This complex has a left-handed, helical conformation around the cell body and within the microtubule array [51]. This region is considered a junctional complex and formed by lined apposed macular structures. The FAZ is an essential structure; disruption of FAZ assembly can lead to dramatic changes in morphogenesis.

The flagellum adhesion glycoprotein 1 (FLA1) was identified in *T. brucei*. FLA1 is critical for flagellar attachment and RNAi depletion of FLA1 results in flagellum detachment, cytokinesis

defects and cell death [52]. Studies in *T. cruzi* indicate that the glycoprotein GP72 (homologous of FLA1) is an important molecule for flagellum attachment. When its gene is deleted, the flagellum is no more attached to the protozoan body [53].

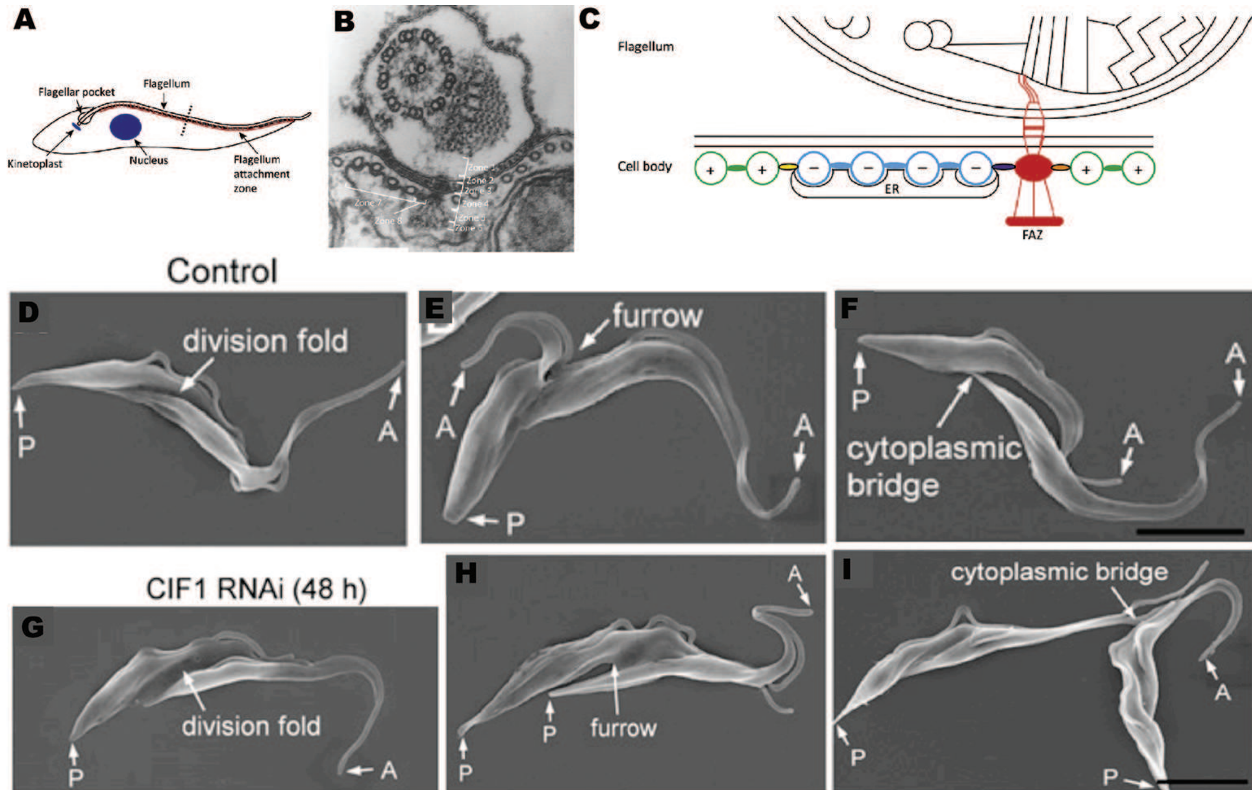

Figure 5. (A) Drawing of the main components of the *T. brucei* trypomastigote. The dashed diagonal line indicates the region seen in (B) and (C) [60]. (B) Electron micrograph showing a transversal section of the FAZ and its relative position to the microtubule quartet (MtQ-zone 7)) [60]. (C) Cartoon of the transversal section shown in (B) [60]. The connections among the paraflagellar rod, subpellicular microtubules (green), and FAZ (red) are emphasized. The drawing also shows the connection between the subpellicular microtubules, the microtubule quartet (MtQ-blue), and endoplasmic reticulum (ER). (D–I) Scanning electron micrographs of a cleavage furrow in control (D–F) and CIF2 RNAi treated cells (G–I); (D–F) control cells demonstrate a different direction for cytokinesis (arrows). It occurs from the posterior towards the anterior regions [58]. Scale bars: 5 μm.

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The first molecular component of the *T. brucei* FAZ filament, FAZ1, was identified by screening an expression library with a monoclonal antibody. The knockdown of this protein resulted in disrupted FAZ and defects in cytokinesis. To date, FAZ1 is considered an essential protein needed to correctly assemble the FAZ in *T. brucei* [54]. Recently, immunoprecipitation assays detected a FLA-1 binding protein (FLA1BP). An RNAi approach targeting FLA1BP showed that this protein is involved in anchorage assembly of the new flagellum. Also, cell growth of these cultures was not disturbed [55]. RNAi knockdown of a coiled-coil-rich protein that contains a C2-domain (CC2D/a protein associated with the flagellum cytoskeleton) impairs the assembly of the FAZ filament; however, the formation of the four microtubules was not affected [56]. These results confirmed the participation of the FAZ filament in both flagellum attachment and cell morphogenesis.

Recently, depletion of ClpGM6, a calpain-like protein localized to the FAZ of *T. brucei* trypomastigotes, resulted in cells with a shorter FAZ. Consequently, these treated organisms were also missing portions of the basal body, the kinetoplast, the Golgi complex and the flagellar pocket. Cells produced long free flagellum, a characteristic phenotype of epimastigote-like cells [57]. Despite a normal growth rate, this phenotype highlights FAZ as an important structure in orchestrating basal body positions and determining the plane of cytokinesis. Recently, during *T. brucei* cytokinesis, a novel signaling pathway was described; it is composed of Polo-like kinase, CIF1, CIF2 and Aurora B kinase [58]. These signaling molecules behave as cytokinesis initiation regulators. *In vivo* studies show CIF2 interacting with CIF1. Furthermore, both of them colocalize at the new FAZ tip during early cell cycle stages [58, 59]. By inhibiting typical anterior-to-posterior cytokinesis, these studies characterized a backup cytokinesis pathway located at the posterior end of the cell [59] (**Figure 5D–I**).

In live parasites, flagellar beating produces a wave that gives the appearance of an "undulating membrane" on the sides of the cell body linked to flagellum as consequence of the FAZ arrangement. A detailed study using high-resolution microscopy to compare the swimming behavior of several trypanosome species that infect livestock showed that the waveforms are distinctive for each trypanosome species. This is due to variations in the microenvironment, such as differences in viscosity [61]. Inside the flagellar membrane of *T. brucei*, it was possible to observe a filamentous structure connecting the membrane associated with the FAZ to the proximal domain of the paraflagellar rod [31]. The paraflagellar rod is an extra-axoneme structure unique to kinetoplastids, euglenoids and dinoflagellates [62–64]. Transmission electron microscopy observations showed the paraflagellar rod as a lattice-like structure that is localized along the entire length of the axoneme once it exits from the flagellar pocket; it is linked to the FAZ. The PFR has three distinct portions: proximal, intermediate and distal. These portions are defined by their location relative to the axoneme (**Figure 6A**) [65].

Replicas of quick-frozen, freeze-fractured, deep-etched and rotary-replicated *T. cruzi* epimastigotes examined by transmission electron microscopy (TEM) provided detailed observations of PFR in straight and bent flagella. Based on these observations, an animated model for the PFR structure during flagellar beating was proposed [66] (**Figure 6B–G**). The PFR structure is absent in the amastigote forms of *Leishmania* and other trypanosomatids [7]. Studies of the biogenesis of *L. amazonensis* flagellum during amastigote-promastigote differentiation show different stages of differentiation (**Figure 6H–L**), initial stages of the process, the early intermediate forms presented an expansion of the flagellar tip and the duplication of flagellum was observed (**Figure 6H**). Interestingly, PFR formation was observed inside the flagellar pocket (**Figure 6J**). In later stages of differentiation, intermediate cells display a longer flagellum (even if shorter than in promastigotes) that contains a PFR (**Figure 6K–L**) [67]. Only after this stage typical flagellar beating was observed, suggesting that the presence of PFR is a prerequisite for flagellum motility [67].

The paraflagellar major proteins are PFR1 and PFR2; null mutant and RNAi ablation of PFR2 demonstrated that this protein is required for efficient flagellar beating in *L. mexicana* and *T. brucei* [68]. These parasites displayed an incomplete paraflagellar structure, showing that this protein is essential for the correct assembly of PFR. Paraflagellar rod components can be divided into four groups: (a) those involved in the formation of the lattice-like pattern; (b)

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those with a role in metabolism and in adenine nucleotide signaling; (c) those that participate in calcium signaling; and (d) those with unknown function [69]. Some studies showed the presence of calmodulin (CaM) within PFR; depletion of CaM resulted in catastrophic failure of the PFR architecture and disruption of the links between PFR and axoneme [70].

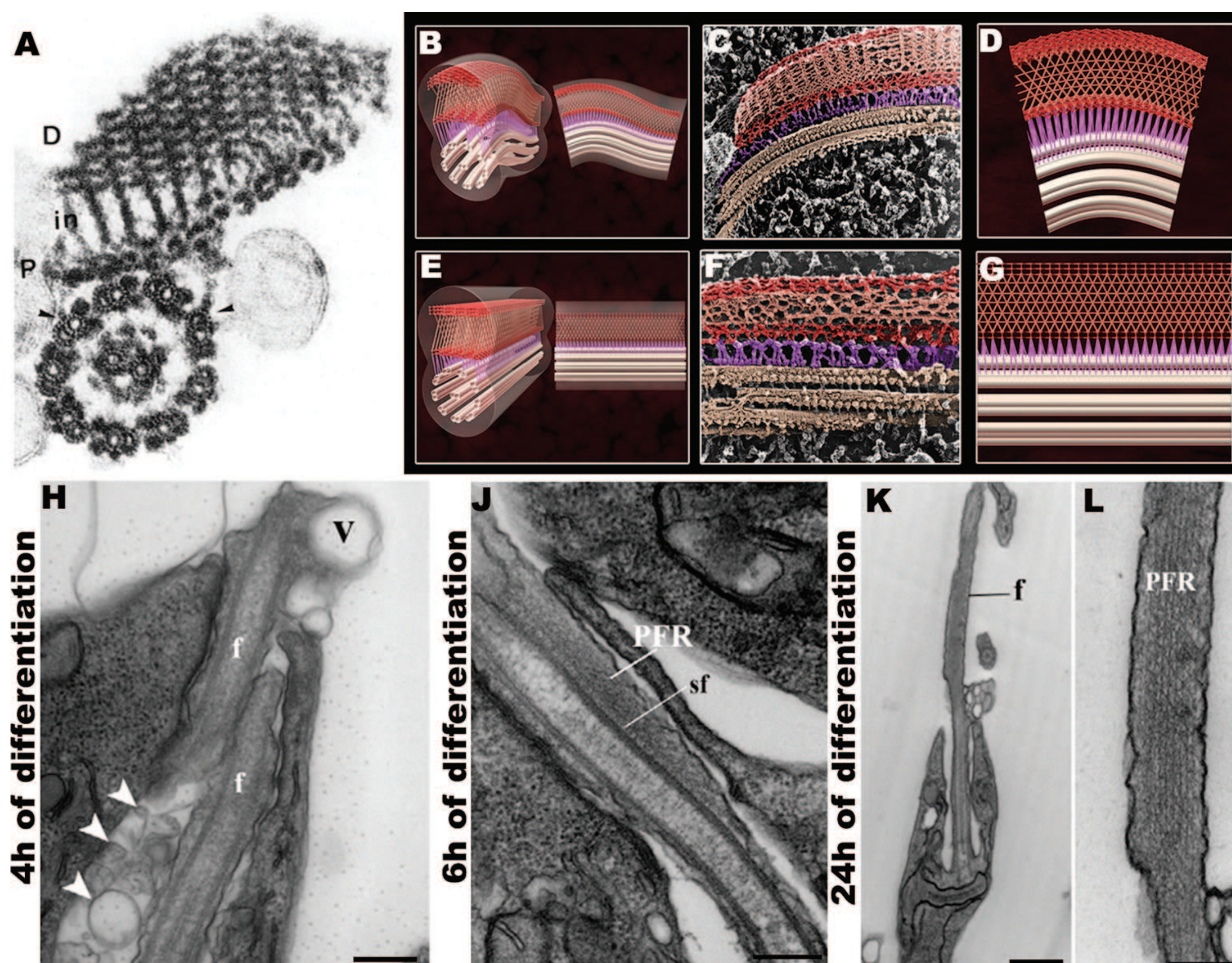

Figure 6. (A) Transmission electron microscopy images of a thin section showing the *T. cruzi* paraflagellar portions relative to the axoneme: proximal (p), intermediary (in) and distal (d) [65]. (B) One frame of an animation showing the flagellum in a bent state; (C) deep-etching replica image of the flagellum revealing the different portions of PFR in a bent flagellum; (D) one frame of an animation based on the deep-etching replica of a curved flagellum; (E) one frame of an animation showing a straight flagellum; (F) deep-etching replica image of the flagellum revealing the different portions of PFR in a straight flagellum; (G) an animation frame based on the deep-etching replica of a straight flagellum. (H–L) Steps of *Leishmania amazonensis* amastigote-promastigote differentiation; (B–G) [66]; (H) after 4 h of differentiation, the duplication of flagellum was observed with vesicles inside the flagellar pocket (arrowhead) and in flagellar tip (v). Scale bar: 200 nm; (J) at 6 h of differentiation, the PFR is observed inside the flagellar pocket. Scale bar: 200 nm; (K) at 24 h of differentiation, the flagellum presented axoneme and paraflagellar rod. Scale bar: 500 nm; (L) higher magnification of H showing the PFR [67].

The PFR in *T. cruzi* is composed of four proteins: PAR1–4 [71]. An important study indicated PAR4 as the target of *T. cruzi*-specific CD8+ T cell responses. Over expression of PAR4 improved PAR4-specific CD8+ T cell responses and provided significantly enhanced protection from infection; this chapter speculated that flagellar proteins can be used as antigens in potential vaccines against *T. cruzi* [72].

5. Conclusions

All the cytoskeletal structures and related proteins covered here are essential for the biology of trypanosomatids. Advances in high-resolution microscopies and molecular biology have provided more information regarding protein localization and function in these protozoa. This chapter provided an overview of unique and essentials cytoskeleton elements and proteins in trypanosomatids that may provide alternative targets in the future for chemotherapeutic drugs.

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