Chapter 5

The Cytoskeleton of *Giardia intestinalis*

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Additional information is available at the end of the chapter

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Abstract

Giardia intestinalis is a pathogenic protozoan, which is the causative agent of giardiasis. The *Giardia* trophozoite presents a cytoskeleton formed by specialized microtubular structures such as the ventral disk, four pairs of flagella, the median body, and the *funis* that are involved in cell division and differentiation. Because trophozoite motility and adhesion to the host intestinal cells are important processes mediated by the parasite cytoskeleton, the fine regulation of these elements may be directly related to the mechanisms that underlie infection. The organization of *Giardia* cytoskeleton at the ultrastructural level has been analyzed by different classical microscopy methods, including negative stain and chemical fixation for scanning and transmission electron microscopy. In this chapter, we provide an overview of the *G. intestinalis* cytoskeleton, emphasizing its structural organization and proteins involved in the maintenance of the structures as well as their functional role. These structures have been recently analyzed in some detail using techniques such as electron microscopy tomography, cryoelectron microscopy, ultra-high resolution scanning electron microscopy (UHRSEM), and helium ion microscopy (HIM). In addition, genome survey and phylogenetic analysis as well as proteomic analysis have revealed the presence of several new and not yet well-characterized proteins.

Keywords: *Giardia intestinalis*, cytoskeleton, ventral disk, flagella, median body, *funis*, microfilaments

1. Introduction

Giardia intestinalis (also known as *Giardia lamblia* and *Giardia duodenalis*) is a flagellated protist described for the first time in 1681 by Antony Van Leeuwenhoek, after a discovery he made while examining his own feces using a primitive light microscope. This species is the causative agent of a parasitic disease known as giardiasis, an intestinal illness characterized by chronic diarrhea and undernutrition [1]. Giardiasis is a waterborne disease with a worldwide distribution [2].

Approximately, 200 million people are currently infected with *G. intestinalis.* The prevalence of giardiasis is higher in areas where sanitation conditions are inadequate. The illness mainly affects children and immunocompromised individuals. The life cycle of *G. intestinalis* comprises of two developmental stages: the trophozoite, in which it inhabits the host's small intestine, and the cyst, in which it is immobile and resistant to stress conditions of the environmental milieu [3]. A more detailed description of basic biological aspects of this protozoan is presented in another chapter of this book. Host infection begins after ingestion of cysts present in contaminated water or food. When a cyst is subjected to the acidic pH and gastric enzymes of the stomach, a reorganization of the cyst wall takes place initiating the encystment process. Each cyst will differentiate into two trophozoites [4]. This process ends in the duodenum through the proteolytic action of pancreatic enzymes (specifically, chymotrypsin and trypsin) and alkalinization on the cyst wall [5]. When released into the small intestine, the trophozoites penetrate the intestinal mucus layer and attach to the epithelium of the duodenum and the upper jejunum. After division by binary fission, they form a monolayer that covers the entire intestinal surface. Some researchers have suggested that the physical attachment of *Giardia* trophozoites to the host intestinal epithelium may contribute to structural and functional changes in the host intestinal cells [6, 7]. Analysis of the *Giardia*-host cell interactions *in vitro* shows that this parasite is responsible for an increase in intestinal permeability due to the rearrangement of proteins of the tight adherens and desmosomal junctions [8–10]. Here, we will focus on the cytoskeleton of the trophozoite, which presents a half-pear shape with a bilateral symmetry and exhibits several unusual cytoskeleton structures such as the ventral disk, the median body, the *funis*, and the lateral crest, in addition to four pairs of flagella (**Figure 1a–c**).

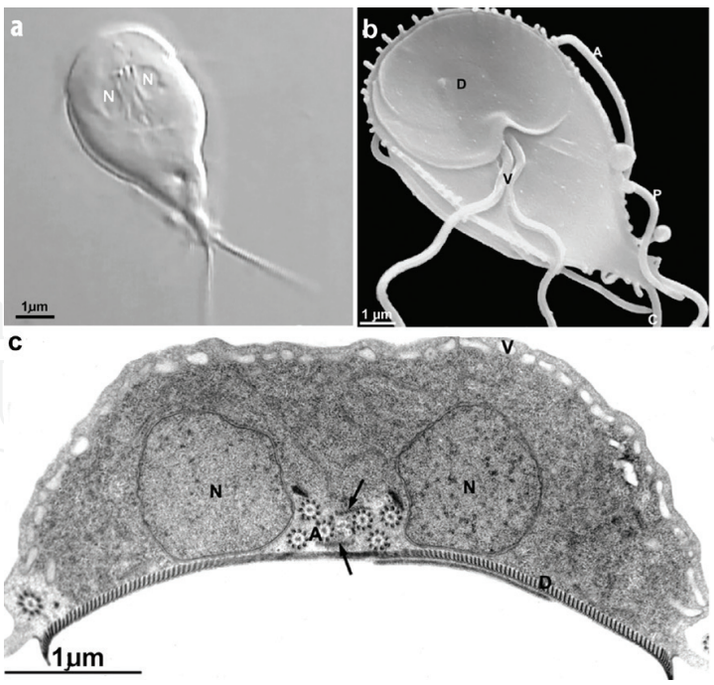

Figure 1. A general view of *G. intestinalis* trophozoites by light and electron microscopy. (a) Dorsal side of the trophozoite as observed by differential interference contrast (DIC). The two nuclei (N) are observed in the anterior region of the cell. (b) Scanning electron microscopy of the ventral side of the trophozoites. Note that the parasite displays the pairs of flagella (anterior flagella—A, posterior flagella—P, ventral flagella—V, caudal flagella—C), the ventral disk (D), and the ventro-lateral flange. (c) Routine preparation for transmission electron microscopy (TEM) of the trophozoite showing the ventral disk (D), the two nuclei (N), peripheral vesicles (V), flagellar axonemes (A) and funis (arrows) [78]. Bars = 1 µm.

The maintenance and establishment of cell shape are fundamental roles of the cytoskeleton. Since the classic work by Elmendorf et al. [11], the cytoskeleton of *Giardia* has been considered to play an essential role in the development and maintenance of the infection, mainly because its main component, the ventral disk, is indispensable for the attachment of the protozoan to the intestinal epithelial cells.

2. Cytoskeleton structures

2.1. Ventral disk

2.1.1. Structural organization

The attachment of *Giardia* trophozoites to the host intestinal epithelium is associated with a structure called ventral disk (**Figure 2a**), which is located on the ventral side and occupies two-third of the anterior region of the cell [12]. This structure is formed by a clockwise spiral layer of microtubules (**Figure 2a**) and is adjacent to the plasma membrane lining the ventral portion of the protozoan to which the microtubules are connected by small and thin filaments [13]. Observations by electron tomography show that the disk is composed of approximately 95 microtubules [14]. Trilaminar structures, known as microribbons, extend dorsally from the microtubule wall toward the cytoplasm [15] (**Figure 2b, c**). They are connected to each other by crossbridges that present a periodicity of 15–16 nm [13, 16]. Disk microtubules originate from dense bands in a region near the caudal and posterior-lateral basal bodies [16–18]. Capped microtubule ends found in this region show that these comprise minus-end areas, whereas other microtubules ending at the margin of the ventral disk are blunt and open (plus-end), suggesting a microtubule polarity [16]. Using cryo-electron tomography, Schwartz et al. [16] demonstrated that the spiral array of the ventral disk consists of microtubules ending within the ventral disk and new microtubules inserted at the inner edge near the bare area.

Observation made by several microscopy techniques shows clearly that the disk is not a homogeneous structure displaying several regions [14, 18]. High-resolution micrographs obtained by ultra-high resolution scanning electron microscope (SEM), helium ion microscopy (HIM), and cryo-electron microscopy tomography show the existence of different domains of the ventral disk [14, 18]. In a recent work, Brown et al. [14] suggested the presence of six regions. We will briefly describe each one, comparing results obtained by several groups, then add additional two regions.

The first region, designated as the dense band microtubule nucleation zone (**Figure 2a**), comprises an area of microtubules nucleation where the microtubules which continue into the disk body are assembled and another area containing a bundle of approximately 20 short microtubules [14], known as supernumerary microtubules [13], which curve slightly opposite to the main disk spiral [18] (**Figure 2a**). It is important to point out that microribbons have not been associated with the microtubules found in the dense zone [14].

The second region is the central one, also known as the "bare area" where microtubules-microribbons complexes have not been seen [19] (**Figures 2a** and **3a**). In this zone, the protrusion of the ventral disk, a projection of the ventral plasma membrane, is clearly observed [20]. When trophozoite cell membrane is extracted with detergents, the banded collars and the

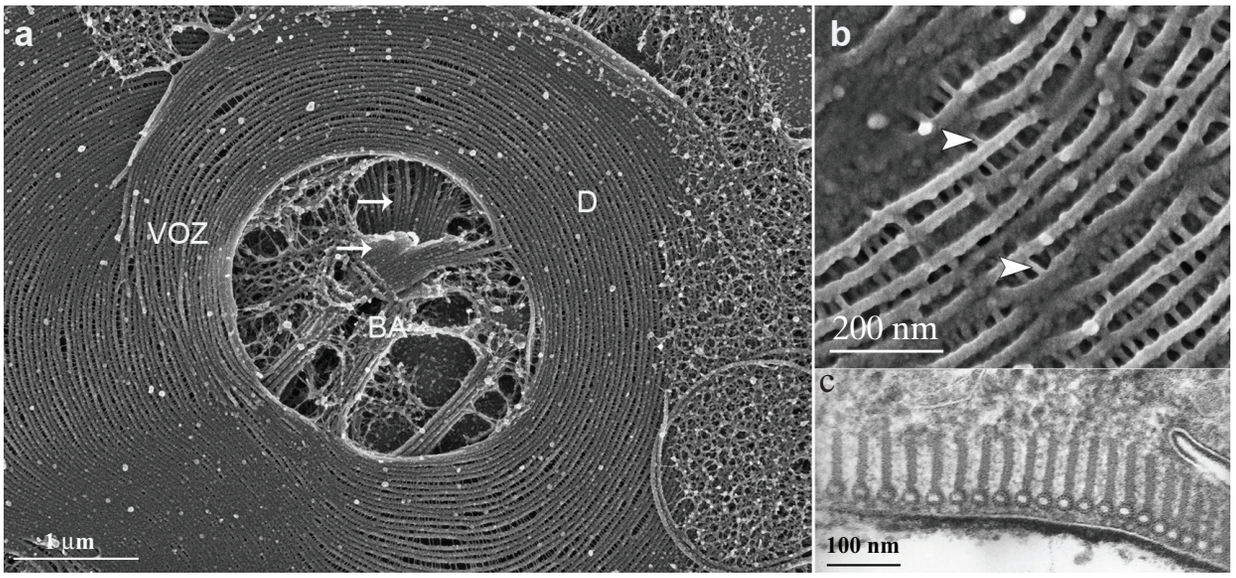

Figure 2. Cytoskeleton of *Giardia intestinalis* as observed in UHRSEM. (a) The spiral of ventral disk (D) and bare area (*) can be observed. Notice the microtubule nucleation zone with two sets of microtubules (arrows) that form the body ventral disk (D) and the ventral overlap zone (VOZ). (b, c) High magnification showing the microribbons that connect the disk microtubules (arrowheads) as seen by UHRSEM (b) and electron transmission microscopy (c) [15,18]. Bars = 1 μm.

basal bodies are observed in this region [18, 21] (**Figures 2a** and **3a**). Using ultra-high resolution scanning electron microscopy (UHRSEM) and HIM, Gadelha et al. [18] showed that there were two types of banded collars. Previously named as BC1 and BC2 [21] (**Figure 3a**), the collars were repeated on both sides of the cell. BC1 appeared as a belt-shaped structure with a thickness of 275 nm. It was associated with the basal bodies of the right caudal/posterior-lateral flagella, when cells were observed dorsally, yet associated with the left caudal/ventral flagella when the cells were observed ventrally [18] (**Figure 3b**). The BC2 was seen as a rope-shaped structure, presenting horizontal segments connected by short bridges. BC2 was continuous with the basal bodies of the left caudal/posterior-lateral flagella (dorsal view) and the right caudal/ventral flagella (ventral view) [18] (**Figure 3c**). Using electron tomography, Brown et al. [14] described this region (BC2) as a dense band composed of three distinct bands. As pointed out by Gadelha et al. [18], each BC2 presented a set of microtubules: the disk microtubules originated from the basal bodies associated with the left BC2, and the previously described supernumerary microtubules originated from the basal bodies associated with the right BC2 (**Figures 2a** and **3a**). It is not yet clear if the banded collars alone or in combination with the basal bodies could work as microtubule organizing centers that would drive the formation of a new ventral disk. Feely et al. [22] observed that isolated banded collars would have the capacity to nucleate new microtubules. Using electron tomography, Brown et al. [14] demonstrated that microtubules emerged from dense bands of two or three layers of densely packed microtubules end.

The third and fourth disk regions are the dorsal and ventral overlap zones (**Figures 2a** and **3a**). Short microribbons (30–40 nm) are connected to the microtubules found in the dorsal overlap zones and the space between each microtubule is reduced (about 25 nm) [14]. In the ventral overlap zone, the microribbons are longer (50–60 nm) and the distance between the

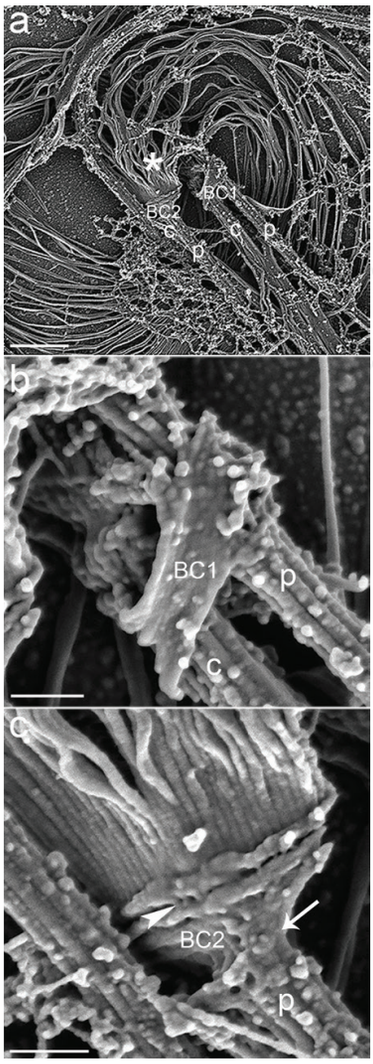

Figure 3. Dorsal view of the "bare area" of the ventral disk. (a) Axonemes of the posterior (p) and caudal (c) flagella and two banded collars (BC1 and BC2) are observed in this region. The disk microtubule (*) emerged from banded collar 2. (b) Banded collar 1 (BC1) displays a flat sheet appearance. (c) Banded collar 2 (BC2) shows horizontal segments connected by small bridges (arrowhead). These segments are continuous with the axonemes (arrow) of the left lateral-posterior (p) and caudal flagella [18]. Bars = 1 μm (a); 200 nm (b, c).

microtubules is larger (60 nm) [14]. A greater amount of microtubule-associated proteins' density, previously known as side-arms and paddles [16], is observed in the ventral zone than in the dorsal zone.

The fifth region is the disk body considered as the region where no microtubules overlap (**Figures 2a** and **3a**). At this region, the inter microtubular space is of about 70 nm, microribbons have a length of 100 nm, and the cross-bridges connecting the microtubules-microribbons complexes present a periodicity of 16 nm [14].

The sixth region is the ventral groove, which is an area located underneath the "bare area". In this region, the disk bridges are shorter and more resistant to breakage after detergent treatment, suggesting that they could be more rigid structures than those of the disk body (authors' unpublished data). As observed previously by transmission electron microscopy

[13], in the central region of the disk, overlying the slightly flattened roof of the ventral chamber, the lateral separation of the microtubules transform abruptly displaying a shorter interval between them. It is possible that the microtubules of this region are kept more closely packed due to the friction of the ventral flagella that emerges near this region and whose beating contributed to cell adhesion and motility [11, 13, 15]. The seventh region is the margin where the microtubules that nucleate at the dense band microtubule nucleation zone end. Microribbons of the marginal region of the disk are shortened and bent toward the disk center as they approach the plus-end and the margin [14]. The cross-bridges, which connect microribbons laterally, form a 16 nm axial repeats in the same way as those observed in the disk body. Volume averaging of microtubule–microribbon complexes reveals that microtubule-associated protein density and distribution in the margin are similar to the dorsal overlap zone, but much lower than in the disk body or the ventral overlap zone [14].

The eighth region is the lateral crest (**Figure 4a**), which has been described as a dense fibrous material in the periphery of ventral disk [11, 23]. As pointed by Gadelha et al. [18], this region was interconnected with the ventral disk and presented small filaments (**Figure 4b**). The low levels of cholesterol and intramembrane proteins found in this region may be associated with a great flexibility of this structure, facilitating the contraction of the outer part of the ventral disk [24]. Previous papers reported labeling for actin, myosin, α-actinin, and tropomyosin in the periphery of the ventral disk in an area that corresponded to the lateral crest [25]. Based on these observations, it was proposed that contractile activity of this region occurred during *Giardia* attachment [25]. However, recent data failed to indicate the presence of contractile proteins in the lateral crest [18, 26, 27] and demonstrated the presence of ankyrin repeat and Nek kinase domain-containing proteins [26].

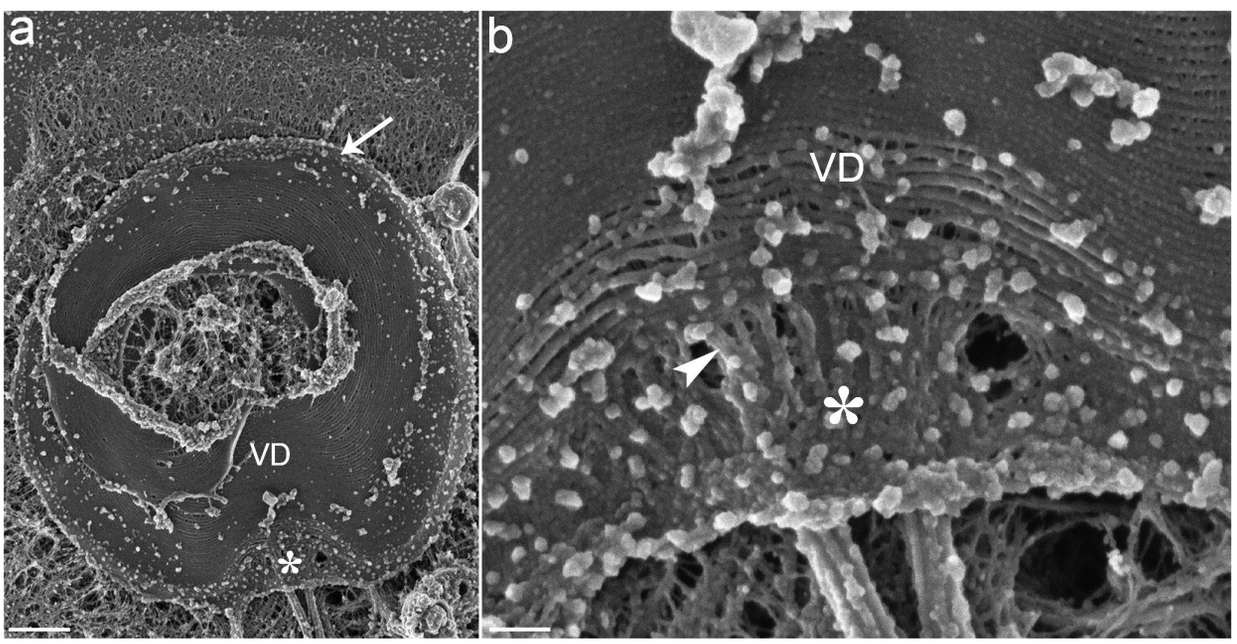

Figure 4. Lateral crest by UHRSEM. (a) The lateral crest (*) was located around the ventral disk (arrow). (b) Small filaments (arrowhead) were seen interconnecting the lateral crest (*) with the ventral disk (VD) [18]. Bars = 1 μm (a); 200 nm (b).

2.1.2. Composition

Several approaches have been used to identify the main components of the ventral disk. Since the first studies by transmission electron microscopy (TEM), it was clear that microtubules represented the major structural component of the disk. TEM studies also revealed the presence of the microribbons, another important structure. Several proteins designated as giardins have been associated with this structure. Molecular analysis demonstrated that *Giardia* has two genes for α-tubulin and three genes for β-tubulin [11, 28, 29]. *Giardia* tubulin has been described as highly modified, possibly playing a role in the stability of cytoskeleton elements. These post-translational modifications include acetylation, tyrosination, polyglycation, and polyglutamylation [30–33]. Giardin has been described as a 30 kDa protein found in microribbons and accounts for about 20% of total ventral disk protein content [34]. Several giardins have already been characterized: α-giardin, β-giardin, δ-giardin, and γ-giardin. Based on amino acid sequencing studies, α-giardins were identified as belonging to the annexin family and found in the dorsal plasma membrane of *G. intestinalis* [11, 35, 36]. One of these proteins, α-1 giardin, is a annexin with glycosaminoglycan-binding activity and is calcium-regulated [37]. However, β-giardin and δ-giardin are analogs of SF-assemblin and presumed to be present in ventral disk microribbons [11, 38, 39]. All these proteins have been shown to be associated with the various cytoskeleton structures by electrophoresis as well as by immunoblotting and immunofluorescence microscopy using specific antibodies to whole cells or to cytoskeleton preparations.

Palm et al. [40] carried out a proteomic analysis of the cytoskeleton preparation and reported the presence of a family of giardins (α-1, β, γ, and δ) and two isoforms of tubulin and a new protein, SALP-1, which is homologous to proteins that participate in the aggregation of striate fibers. Subsequently, a proteomic analysis was carried out by Lourenço et al. [41] using a cell fractionation approach. They obtained a highly enriched disk fraction that by SDS-PAGE showed the presence of five predominant bands, ranging from 25 to 58 kDa, as well as some light bands with higher molecular weight. Two-dimensional electrophoresis of the fraction revealed the presence of 18 spots. Mass spectrometry analysis of the major bands found by SDS-PAGE and of the spots identified in 2D gels revealed the presence of several additional proteins. More recently, in a seminal work Hagen et al. [26] also isolated an enriched disk fraction and used shotgun proteomics to identify its protein composition. They found 102 proteins potentially associated with the disk. In addition, several of these proteins were GFP-tagged and localized, using immunofluorescence microscopy. Six of the novel disk-associated proteins (DAPs) were localized in the whole disk in addition to those 18 previously identified [26]. Ten of the new proteins were localized in the lateral crest or along the outside edge of the ventral disk, including the Nek kinase DAP13981, a putatively contractile repetitive structure. Two novel proteins were localized in the supernumerary microtubules, which emerge from the central "bare zone" close to the flagellar basal bodies. Using the fluorescence recovery after photobleaching (FRAP) technique, evidence obtained showed that most of the identified proteins are associated with stable structures [26].

Microtubule inner proteins were also described in the ventral disk and were associated with the inner wall of the protofilaments associated with the interface microribbon-microtubules [16].

Microtubule outer proteins associated with protofilaments, localized opposite to microribbons, were also observed. A dense protein coat (previously named side-arms and paddle) of unknown composition is also observed on the margin-facing side of the microtubules [16]. In recent years, proteomic approaches combined with microscope localization technique were carried out and new disk-associated proteins were identified such as the NIMA-related kinases (Neks), ankyrin repeat domain-containing protein, median body protein, and fungal cell wall protein Mp1p. These proteins have specific sites involved in cell adhesion and TTHERM, a hypothetical protein associated with the microtubule formation in the ciliate *Tetrahymena thermophila* [26, 41]. However, despite the effort that has been made to characterize these structures by several research groups, the specific function of each of these proteins is not yet fully understood.

2.1.3. Function

The ventral disk has been considered the main structure associated with the parasite attachment to the host cell. The exact mechanism by which this occurs is still under study. In this context, several hypotheses have been raised to explain this process. Holberton's observations of the movement of the ventral flagella during cell adhesion led to the proposal of the hydrodynamic model [13, 42]. According to this theory, the suction pressure developed by the disk takes place due to the beat of the ventral flagella and the fluid flow generated by this beat through the ventro-lateral flange and the ventral groove. The authors suggested that the ventral disk would be responsible for maintaining the proper shape for creating both the suction pressure and the distance between the flange and the substrate [13, 42]. The adhesive activity of the flange was demonstrated by Hagen et al. [43]. Using interference reflection microscopy and field emission electron microscopy, these authors observed the establishment of focal contacts between the flange and the substrate [43]. Lenaghan et al. [44] showed that the ventral flagella presented a propulsive velocity of 9.4 μm/s and proposed, based on the hydrodynamic model described by [13], a suction pressure of 20.8 Pa. The main functional role of this flagella pair would then be related to the downward force required for the adhesion to the epithelium.

In contrast to the above-mentioned reports, Campanati et al. [45] suggested that the ventral flagella play a secondary role in the adhesion process. This was demonstrated with experiments where the viscosity of the medium was increased with a gradient concentration of Percoll, thereby decreasing the frequency of the flagella and checking the adhesion of the parasites. These authors found that even though the frequency of the ventral flagella decreased to about 2 Hz, many trophozoites remained adhered. They also observed contractions of the ventral disk, which consequently caused the detachment of the parasite [45]. Based on these observations, they suggested that the adhesion is not only associated with the flagellar movements; this process might also rely on other factors such as tubulin-associated movements within the ventral disk itself [45]. Using total internal reflection microscopy (TIRF) of trophozoites labeled with a fluorescent plasma membrane dye, House et al. [46] defined distinct stages of attachment: (1) skim and contact of the surface with the anterior region of the ventro-lateral flange, (2) the ventral disk periphery touches the surface, forming a continuous contact at the area of the lateral crest, (3) the lateral shield then presses the substrate, and (4) then presses the bare area region within the ventral disk. Defects in flagellar motility do not affect later

stages of the attachment (steps 2–4). This was demonstrated by the generation of a strain with defects in flagellar beating by a morpholino-based knockdown of the axonemal central pair protein PF16 as well as by construction of a strain with specific defects for the ventral flagellar waveform by overexpressing a dominant negative gene. House et al. [46] observed a slower attachment during earlier stages when motility is required for positioning the ventral disk against the substrate surface (step 1). They proposed that the ventral flagellar beating might contribute to the positioning of the cell during early stages of attachment [46]. Interestingly, Woessner and Dawson [47] demonstrated that the depletion of the median body protein, a ventral disk protein, altered the domed disk conformation, and consequently, the attachment.

In addition to the mechanical mode of adhesion of this parasite to intestinal cells, as described above, other studies suggest that biochemical mechanisms involving molecular lectin-sugar interactions on the surface of *Giardia* also play an important role in this process [48]. This hypothesis was first supported by the evidence that pre-treatment of parasites with trypsin or periodate could decrease adhesion to intestinal cells. In addition, the presence of a known concentration of glucose, fucose, galactose, mannose, mannose-6-phosphate, N-acetyl-glucosamine, and N-acetyl-galactosamine in the interaction medium inhibited the attachment of the parasites to the epithelial cells [48–50]. Although these results show that lectins mediate interactions between *Giardia* and the host cell, there is a clear evidence that the cytoskeleton is sufficient to allow adhesion, as pointed out by Elmendorf et al. [11]. This conclusion is supported by the finding that trophozoites can efficiently adhere to glass, plastic, and a wide variety of mammalian cell lineages. It has been suggested that interaction of *Giardia* lectin and the carbohydrate of the intestinal cells' surface, could be important for the recognition of host duodenal cells [48].

Transmission electron microscopy analysis also showed that during cell division, the ventral disk contacts the nucleus, suggesting that this structure could cause nuclear constriction, participating in the karyokinesis process [51].

2.2. Flagella

2.2.1. Structural organization

The flagella structure of *G. intestinalis* follows the canonical 9:2+2 microtubular axoneme. The eight flagella found in the parasite are organized in pairs and are named according to their position: (1) anterior, (2) lateral-posterior, (3) ventral, and (4) caudal (**Figure 1b**). They originate from the basal bodies, which are localized between two nuclei in the anterior region of the cell [11, 17, 21].

G. intestinalis flagella present components that are associated with each pair of flagella. The axonemes of the anterior flagella extend toward the anterior region of the cell, cross to the center, and then bend running back to the posterior region where they emerge at the lateral portion of the cell. Dense fibers, named paraflagellar or paraxial rods, are associated with the intracellular portion of these axonemes [13, 18, 52] (**Figure 5a**). As observed by 3D negative staining electron tomography, striated fibers, which form a regular brush-like border, are also connected to anterior flagella [14]. The marginal plates, which have been described

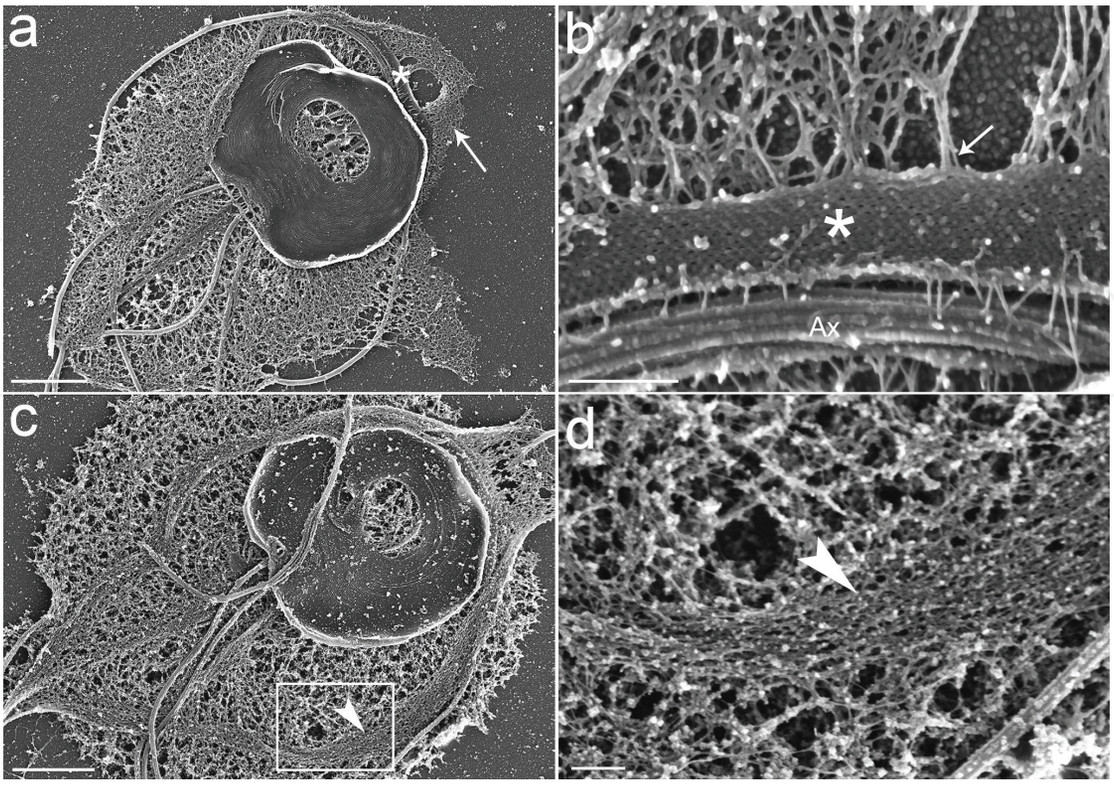

Figure 5. UHRSEM images of ventral (a–d) and dorsal surfaces of *Giardia.* (a) The marginal plates (*) are associated with a network of filaments (arrows). (b) High magnification of (a) showing the connections of the marginal plates (*) with the axoneme (Ax) and the network of filaments (arrow). (c, d) The network of filaments is continuous with the set of filaments in the periphery of the trophozoites (arrowhead). The square indicates the area in high magnification in d. (d) Note the filaments (arrowhead) parallel to the main cell axis [18]. Bars = 2 μm (a, c); 100 nm (b); 200 nm (d).

as part of the ventro-lateral flange, are also associated with the axonemes of the anterior flagella [53]. Using UHRSEM with detergent-extracted trophozoites, Maia-Brigagão et al. [52] described the fine organization of this structure. Images of the marginal plates show that they have a "boomerang-like" shape that forms an interlaced or web structure connected to the axonemes of the anterior flagella by small and apparently flexible filaments (**Figure 5b**). It has also been observed that the upper portions of the marginal plates are associated with a filamentous network in continuity with filaments that are parallel to the main cell axis [18] (**Figure 5c, d**). Together, these structures correspond to the ventro-lateral flange, which has been described as a fibrous structure of paracrystalline regularity [13, 14]. Despite the lack of biochemical information to support its functional role, the adhesive activity of the ventro-lateral flange has been suggested previously [43; see Section 2.1.3]. Dense rods are also localized just below the lateral-posterior flagella. They are shorter and associated with the inner portion of the axoneme in the region where they run along of the ventral disk [11, 54]. On the other hand, the ventral flagella are differentiated from the others by presenting a membrane projection that is filled by a dense material of unknown composition [13, 53]. A 30 kDa polypeptide was identified as the main constituent of this structure [17]. The axonemes of the caudal flagella are accompanied by two sheets of microtubules, which were called *funis* by Kulda and Nohýnková [55]. This structure will be described in more detail below.

Previous studies reported that *Giardia* flagella basal bodies were arranged in tetrads [22, 56, 57]. When the trophozoite is viewed dorsally, the left tetrad consists of anterior/ventral and caudal/posterolateral basal bodies, while the right tetrad consists of caudal/ventral and anterior/posterolateral basal bodies [56, 57]. During mitosis, association of microtubules from basal bodies with the spindle poles are thought to determine polarity of each daughter cell [58; see Section 2.6].

2.2.2. Composition

A number of proteins other than tubulin are found in the *G. intestinalis* flagella. Among them are the α-giardins, which have homology with human annexins and appear to have a function in trophozoite motility, adhesion, and membrane stability [59]. Interestingly, 21 α-giardin encoding genes were found in the *G. intestinalis* genome. Some members of this annexin-like protein family have been associated with the flagella. Immunocytochemical studies showed that α-2 and -19 giardin are localized in the caudal and ventral flagella, respectively [60]. An α-14 giardin that exhibits a calcium-dependent phospholipid-binding site resides at local slubs near the proximal part and the ends of the flagella, and associates with tubulin [61, 62]. Ankyrin repeats (such as GSP-180) have also been described and shown to contain coiled-coil domains and are found in the axoneme of the anterior flagellum [63]. Kinesin-2 GFP fusions (*GiKIN2a* and *GiKIN2b*) and IFT complex A and B raft homologues (IFT140 and IFT81) localize to the eight axonemes, as well as to external regions, including flagellar tips and flagellar pores [64]. Using shotgun proteomic approaches combined with GFP-tagging of microtubule-associated proteins, Hagen et al. [64] identified a homologue of DIP13, a *Chlamydomonas* protein that binds microtubules localized in the caudal and ventral flagella. γ-tubulin and centrin are located in the basal bodies of the flagella, indicating that this region could be a microtubule organizing center [65, 66] (**Figure 6**). Genomic and proteomic analysis showed that there are around 49–75 proteins localized to *Giardia* basal bodies [57, 67]. Although these proteins have been shown to be present in the flagella, it is not clear how they influence flagellar dynamics.

2.2.3. Functions

The *Giardia* flagella are required for motility and may be necessary for adhesion [13, 44, 45]. They participate in the emergence of the trophozoites during the excystation process [68].

Regarding the *G. intestinalis* motility, studies using video-microscopy suggest that the ventral pair beats continuously from base to the tip in a sinusoidal waveform parallel to the longitudinal axis of the cell [44, 45, 69]. The beat frequency observed in this pair of flagella was around 10 Hz, with mean amplitude of the waveform of 2.0 mm [45, 69]. Active beating is also observed in the anterior flagella, which present a beat frequency of approximately 18 Hz. Although Campanti et al. [45] have shown that the beat pattern of this pair is helical, Lenaghan et al. [44] using high-contrast and high-speed microscopic imaging (>800 fps), observed that the anterior flagella beat with a power stroke similar to ciliary motion. This same pattern was also observed during the beat of the posterior flagella [44], which was in contrast to previous analysis that suggested that the motion of the posterior flagella might be consequence of the simultaneous propagation of the waves produced by the ventral pair of the flagella [45]. The

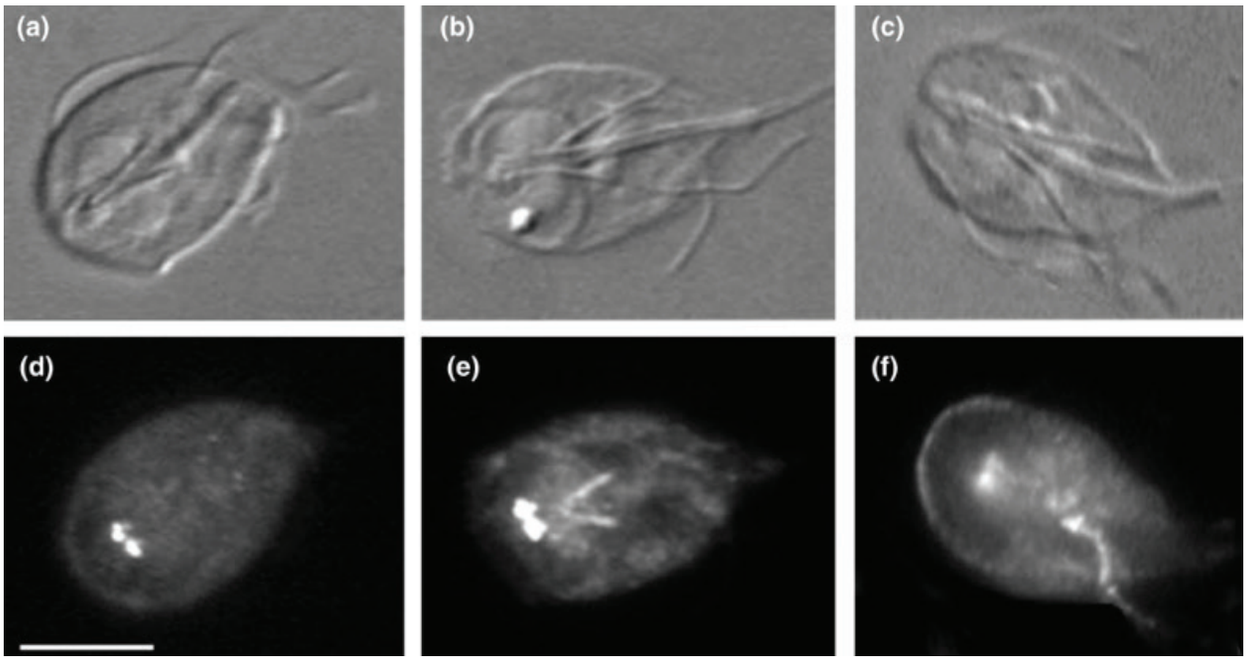

Figure 6. Presence of centrin in *G. intestinalis.* Immunostaining using anti-centrin antibodies 17E10, 2.4, and 20H5. All antibodies yielded the same labeling pattern. (a–f) Different cells are shown in differential interference contrast – DIC (a–c) and in fluorescence microscopy (d–f). Labeling is shown in the basal body, the dense rods of the posterior flagella, axonemes, flagella and disk [66]. Bars = 1 μm.

caudal pair of flagella, which emerges from the posterior end of the parasite, does not present active beating [44, 45], although motion of its intracellular portion has been observed [70].

In relation to the displacement of the trophozoite, the results found are complex. Video-microscopy observations done by Campanati et al. [45] showed that forward movement with a rocking motion was due to the beat of the anterior flagella of the cell. A change in the position of this pair of the flagella led to the rotation movement during swimming [45]. Subsequently, Lenaghan et al. [44] suggested that the fast swimming of the parasite was not the result of flagella beating, but was due to the wave-like motion of the caudal region of the cell. This movement could be the result of the active beating of the intracellular portion of the caudal flagella, which would be responsible for the dorsal-ventral flexion [44]. Another movement related to the caudal portion of the cell is lateral bending [45, 70]. This motion was observed in an early stage of attachment and was responsible for the circling swimming pattern [44]. Following the lateral flexion, a change in the direction of swimming occurs, which could be consequence of either the beating of anterior and/or ventral pair beating [44, 70].

Besides participation in the cell displacement, the *Giardia* flagella have been associated with such other cellular processes as adhesion and cell differentiation and division. Although it has been proposed that the ventral flagella beat might be essential for parasite attachment by generating a hydrodynamic force that would result in a suction-based adhesion, later studies show that this flagella pair is important in the early stages of adhesion, specifically in the positioning of the trophozoites near the substrate [45, 46; see Section 2.1.3].

The role of flagellar motility during cell differentiation and division is not yet clear. During the encystment, the flagella are not completely disarranged and flagellar movement has been

observed within cysts [68]. During excystation, the flagellar motion appears to be crucial for the rupture of the cystic wall and release of the trophozoites [71]. Flagellar motility seems to be essential for the separation of daughter cells during cell division. Tumová et al. [72] showed that in the final steps of this process, the cell detaches from the substrate. During this phase, the cells are seen joined by their posterior region and swim freely in the medium while the ventral disk is assembled. Interestingly, studies using kinesin-2 mutants showed that these parasites were unable to complete cell division due to flagellar defects [73].

2.3. Median body

2.3.1. Structural organization, composition, and function

The median body is another microtubular element of the *G. intestinalis* cytoskeleton, but it is not present in all cells [74]. Its presence in cysts is still under discussion. It is located dorsally to the ventral disk and is between 0.2 and 1.8 μm in thickness [74]. Since its shape and position vary among species of *Giardia*, it can be used as a taxonomic tool. Previous studies have shown that the median body consists of a variable number of layers containing stable and nonstable microtubules [33, 74] (**Figure 7a–d**). Acetylated tubulin, mono and polyglycylated tubulin, and tyrosinated tubulin were detected in this structure [32, 33]. Microtubules of the median body may be associated with caudal axonemes, *funis*, plasma membrane, and occasionally, ventral disk [74] (**Figure 7a–d**). The localization of β-giardin and the presence of small bridges in these microtubules indicated that microribbons, similar to those found in the ventral disk, could be present in this structure [74, 75] (**Figure 8**). Other proteins were also described in the median body as actin and α-actinin [25]. The localization of centrin led to the suggestion that this structure could be another MTOC in *Giardia* [65, 76], although γ-tubulin has not been detected. Using green fluorescent protein-tagged microtubule-associated proteins, Dawson et al. [77] showed that kinesin-13 and EB1 localized in the median body and could play a role in the microtubular dynamic. The median body protein (MBP), a 101-kDa protein with coiled-coil domains, was initially identified in this structure. However, MBP was later identified as an abundant protein in the ventral disk, being localized in the median body of the trophozoites during prophase [47]. In this context, it was suggested that ventral disk components could assemble on the median body microtubules prior to dorsal disk biogenesis. Taken together, several hypotheses have been made for the function of the median body such as (1) microtubule reserve for rapid mobilization during mitosis and ventral disk formation; (2) microtubule organizing center; and (3) participation in the movements of the caudal region of the trophozoite [11, 74, 76].

2.4. *Funis*

2.4.1. Structural organization, composition, and function

The axonemes of the caudal flagella are accompanied by two sheets of microtubules, which were called *funis* by [55]. One sheet is positioned ventrally and the other dorsally [70, 78]. This microtubular complex, which contains acetylated α-tubulin [30], emanates from between the nuclei, near the region of the basal bodies, and follows until the emergence of the caudal flagella. The *funis* microtubules partially surround the axonemes of the caudal flagella and spread

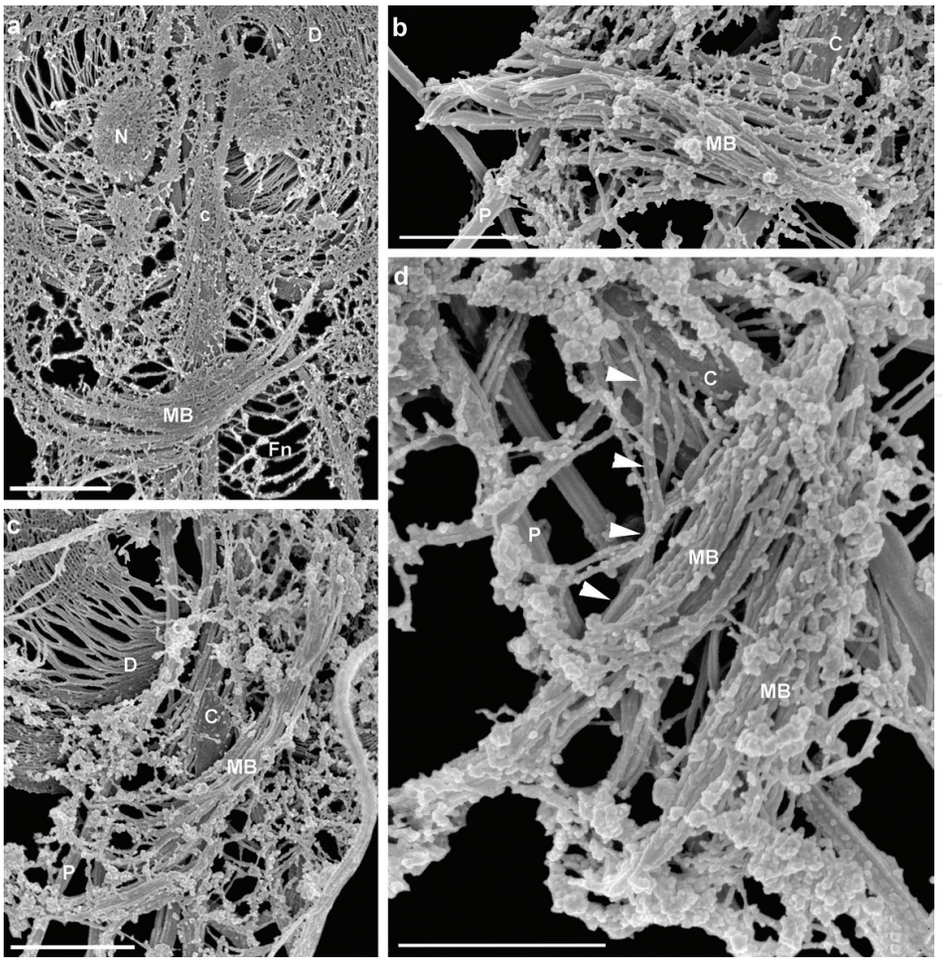

Figure 7. The FESEM of *Giardia* after detergent extraction. The plasma membrane was partially removed, allowing observation of the cytoskeleton, the MB included. The ventral disk (D), the two nuclei (N), MB, anterior (A), caudal (C) and posterior-lateral flagella (P) are seen. (a–b) Every fascicle that constitutes the MB is observed. (c–d) The median bodies are seen curved, and toward the cells' anterior region. The fascicles number is variable as well as their disposition and location. Posterior-lateral axoneme, P; caudal axoneme, C; [74]. Bars = 1 μm.

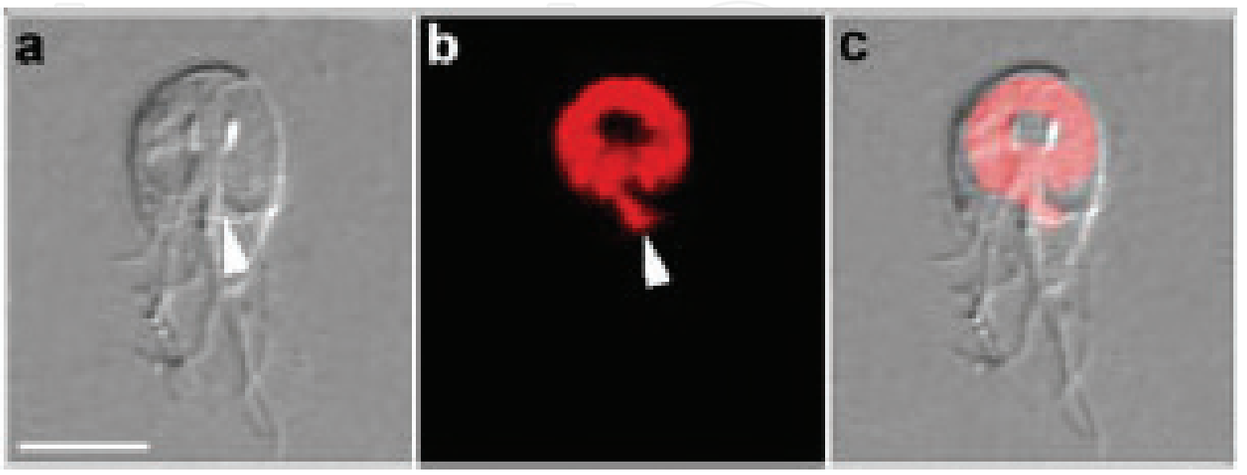

Figure 8. Immunofluorescence localization of β-giardin in *Giardia*, using the monoclonal antibody 7G9. The disk presents positive labeling as well the MB (arrowheads). (a) DIC visualization; (b) immunofluorescence; (c) overlay [74]. Bar = 5 μm.

out in the direction of the v rods associated with the lateral-posterior flagella (**Figure 9a–d**). Bridges of different lengths interconnect the *funis* microtubules [78] (**Figure 9a–d**). The microtubular sheets of this structure are covered by a lattice-like array of unidentified material, as revealed by HIM analysis [18]. In a previous work, an actin helix was shown to bundle the axonemes of the caudal flagella. In the final portion of the caudal complex, an association of the microtubules with cytoplasmic filaments was found [27]. It is proposed that the funis, together with the other axonemes, could work as a flexible cord and be responsible for the caudal movement of the cells such as lateral bending and dorsal-ventral flexion [70, 78; see Section 2.2.3].

2.5. Filament network

It has been shown that *G. intestinalis* genome contains a single divergent actin gene with an identity that is 58% similar to actin from other cells [79]. In addition, no genes coding for actin-binding proteins, formin, and myosin were found [11]. Further characterization of the *Giardia* actin showed that it is localized in several regions of the cell (cortex, axonemes, nuclei) and polymerizes *in vitro*, forming true microfilaments [27]. Some authors observed that drugs, which interfere with actin dynamic such as cytochalasin D and jasplakinolide, inhibited cytokinesis, and induced fragmentation of ventral disk and ventro-lateral flange [70, 80, 81]. Knockdown of the *Giardia* actin gene (giActin) interferes with clathrin-mediated endocytosis, membrane trafficking of CWP, and cytokinesis [27]. Subsequently, it was

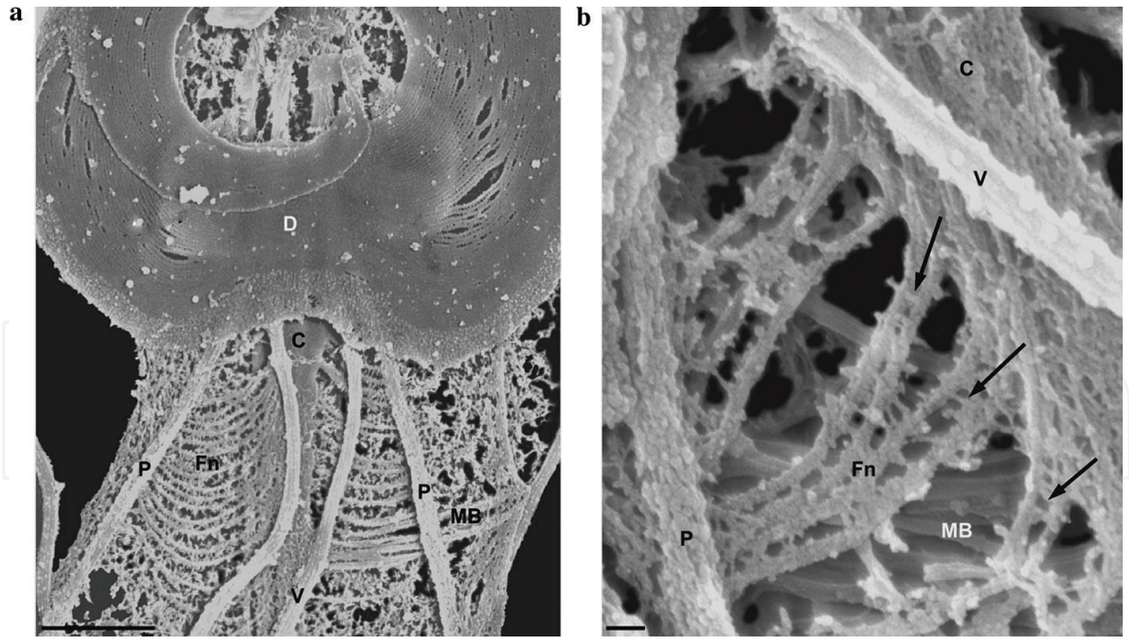

Figure 9. SEM of *G. intestinalis* in a ventral view. (a) Note that the microtubules of the *funis* (Fn) emanate from the caudal flagella (C) and are anchored to the posterior-lateral flagella (P). The median body (MB) is also appears as bundles of microtubules close to the *funis*. The arrows point to nuclei prints which are located dorsally to the ventral disk (D). (b) FESEM of *G. intestinalis* in a close view. The *funis* microtubules (Fn) are observed emanating from the caudal flagella (C) toward the posterior-lateral flagella (P). Notice that the microtubules present links (arrows), many of which were disrupted by the extraction treatment. The median body (MB), also formed by microtubules, is seen in a dorsal position in relation to the *funis*. Ventral flagella (V) [78]. Bar = 1 μm (a) and 100 nm (b).

shown that *Giardia* contains around 80 putative actin-binding proteins that may constitute a set of evolutionarily indispensable, actin-interacting proteins [82]. Using immunofluorescence approaches with specific antibodies combined with 3D-structured illumination light microscopy, Paredez et al. [27] showed that *Giardia* actin could form C-shaped filaments and helix-structures. A looser meshwork of filaments with a mean diameter of 9 nm was recently observed in the cytoskeleton of *G. intestinalis* using HIM [18]. The filaments spread out along the dorsal region and formed ring array structures [18] (**Figure 10a, b**). Immunogold labeling associated with UHRSEM coupled to backscattered electron detectors showed a labeling for actin in this region. These filaments therefore may correspond to microfilaments, which could act as a scaffold and provide support for the dorsal surface and cellular components [18]. Interestingly, Weiland et al. [59] observed that *Giardia* present several annexins, which are proteins known to interact with the F-actin in other cell models. Because annexins are associated with the trophozoite membrane, the Weiland team suggested that annexins could stabilize the cytoskeleton by cross-linking the plasma membrane to the underlying microtubules/microfilaments.

2.6. Behavior of the cytoskeleton during differentiation and cell division

The differentiation of trophozoites into cysts is an important process that allows the survival of the parasite under stress conditions of the environmental milieu. Morphological analyses using scanning and transmission electron microscopy show that during trophozoite-cyst transformation, several modifications occur [68, 83, 84]. Midlej and Benchimol [68] showed that in the early stages of encystment, the trophozoite gradually changes from its flattened form to an oval/rounded shape. This is accomplished by an increase in the membranous structure of the flange, which curves, causing cell folding and the formation

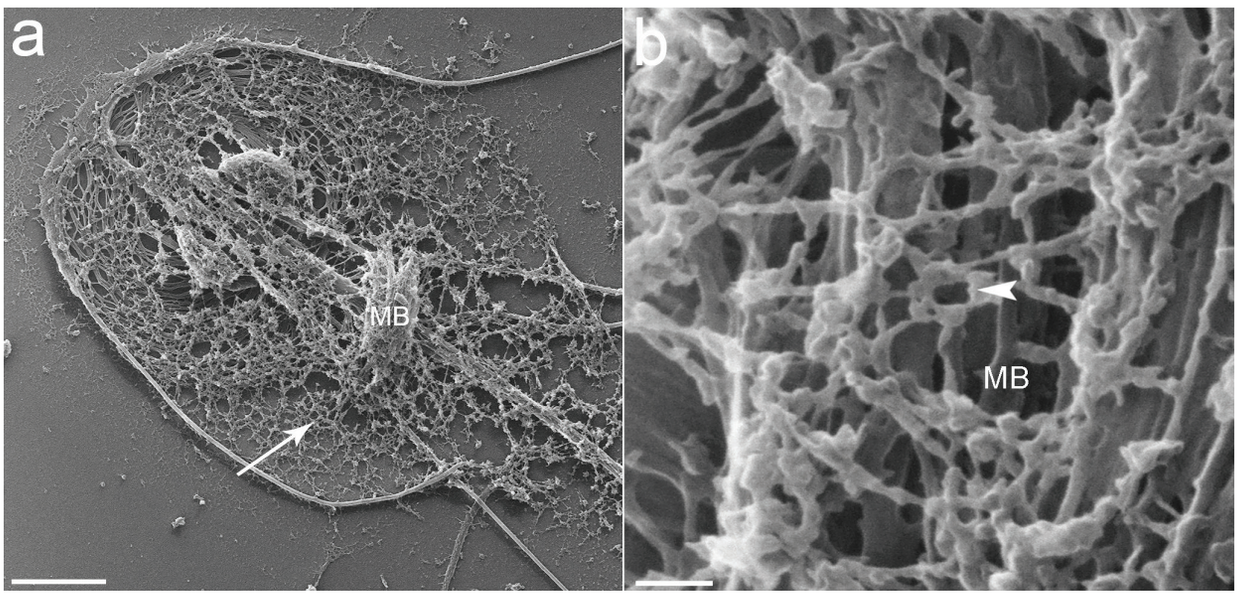

Figure 10. Dorsal view of Giardia by HIM. (a) Cytoplasmic filaments (arrow) contacting cytoskeletal structures such as the median body (MB). (b) High magnification of (a). It is possible to observe a ring array (arrowhead) [18]. Bars: 2 μm (a); 100 nm (b).

of the concave depression in the ventral region. In addition, the fibrillar material is deposited gradually on the encysting cells forming the cystic wall. At the same time, alterations also occur in the ventral disk spiral, which opens up and then assumed a horse-shaped structure. In the later stages of encystment, this structure fragments into four parts. These authors also observed that during differentiation of the trophozoite-cyst, the flagella are gradually internalized and kept in vacuoles. The ventral flagella are enclosed firstly by folding of the flange membrane. The last flagella to be internalized are the caudal that form a tail that persists until the last stages of the process. The flagella beating are still observed inside the cell. Midlej and Benchimol [68] demonstrated also the presence of an operculum in the final stage of the encystment, before the complete closing of the cyst. They suggested that this opening could be a weak region of the cyst, which would facilitate the exit of the trophozoite observed during encystment. During differentiation of the cyst into trophozoites, the flagella protrude through a small opening in the cyst wall, which is enlarged by flagella motion. The trophozoites emerged from cyst are oval in shape and quickly become flattened and elongate [71, 85].

The reorganization of the *Giardia* cytoskeleton also occurs during cell division [58]. Sagolla et al. demonstrated that *Giardi*a has a semi-open mitosis with two extranuclear spindles responsible for chromosome segregation. Using time-lapsed, confocal, and electron microscopy, Tumová et al. [72] described the different steps of *Giardia* cell division. According to these authors, the division begins in adhered cells by the detachment of the microtubule of the ventral disk from basal bodies. The overlapping region of the disk loosens and the central bare area increases. These alterations are accomplished by shortening and subsequent disappearance of the microribbons. At end of this stage, the nuclei are duplicated.

The rearrangement of the flagellar axonemes seems to take place in prophase when nuclear migration occurs in the cell midline [58]. Using light and electron microscopy and immunofluorescence methods, Nohýnková et al. [56] demonstrated that *Giardia* reorganize the flagellar apparatus during semi-conservative cell division. Each daughter *Giardia* receives four flagella from the parent cell, while the other flagella are assembled *de novo* in each cell. During this process, basal bodies/flagella migrate, assume different positions, and transform to different flagellar types in progeny until their maturation is completed [56]. As observed by Tumová et al. [72], after reorientation of the anterior flagella, the daughter cell disks are organized on the anterior dorsal side and positioned laterally. During this phase, lateral crest and ventro-lateral flange are not visualized. The Tumová team observed that in the final steps of cell division, the trophozoites are seen joined by their posterior region and swim freely in the medium, while the new disks are assembled. The daughter cells with fully developed disks (i.e., with the presence of lateral crest and flange) attach to a substrate but are still connected tail to tail by a cytoplasmic bridge. The cell division ends by a process resembling adhesion-dependent cytokinesis. Although these authors have suggested that the splitting of the diving organism occurs in ventral-ventral axial symmetry in the plane of the daughter disks, previous studies show that other types of cytokinesis (dorsal-dorsal or ventral-dorsal axial symmetry) may occur in *Giardia* [86].

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References

[1] Buret AG. Pathophysiology of enteric infections with *Giardia duodenalis*. Parasite. 2008;**15**:261-265. DOI: 10.1051/parasite/2008153261

[2] Karanis P, Kourenti C, Smith H. Waterborne transmission of protozoan parasites: A worldwide review of outbreaks and lessons learnt. Journal of Water and Health. 2007;**5**:1-38

[3] Adam RD. Biology of *Giardia lamblia*. Clinical Microbiology Reviews. 2001;**14**:447-475. DOI: 10.1128/CMR.14.3.447-475.2001

[4] Bingham AK, Meyer EA. *Giardia* excystation can be induced *in vitro* in acidic solution. Nature. 1979;**227**:301-302

[5] Boucher SE, Gillin FD. Excystation of in vitro-derived *Giardia lamblia* cysts. Infection and Immunity. 1990;**58**:3516-3522

[6] Ankarklev J, Jerlström-Hultqvist J, Ringqvist E, Troell K, Svärd SG. Behind the smile: Cell biology and disease mechanisms of *Giardia* species. Nature Reviews Microbiology. 2010;**8**:413-422. DOI: 10.1038/nrmicro2317

[7] Halliez MC, Buret AG. Extra-intestinal and long term consequences of *Giardia duodenalis* infections. World Journal of Gastroenterology. 2013;**19**:8974-8985. DOI: 10.3748/wjg.v19.i47.8974

[8] Teoh DA, Kamieeniecki D, Pang G, Buret AG. *Giardia lamblia* rearranges F-actin and alpha-actinin in human colonic and duodenal monolayers and reduces transepithelial electrical resistance. Journal of Parasitology. 2000;**86**:800-806. DOI: 10.1645/00223395(2000)086[0800:GLRFAA]2.0.CO;2

[9] Scott KG, Meddings JB, Kirk DR, Lees-Miller SP, Buret AG. Intestinal infection with *Giardia* spp. reduces epithelial barrier function in a myosin light chain kinase-dependent fashion. Gastroenterology. 2002;**123**:1179-1190

[10] Maia-Brigagão C, Morgado-Díaz JA, de Souza W. *Giardia* disrupts the arrangement of tight, adherens and desmosomal junction proteins of intestinal cells. Parasitology International. 2012;**61**:280-287. DOI: 10.1016/j.parint.2011.11.002

[11] Elmendorf HG, Dawson SC, McCaffery JM. The cytoskeleton of *Giardia lamblia.* International Journal of Parasitology. 2003;**33**:3-28

[12] Cheissin EM. Ultrastructure of *L. duodenalis*. I. Body surface, sucking disk and median bodies. Journal of Protozoology. 1964;**11**:19-98

[13] Holberton DV. Fine structure of the ventral disc apparatus and the mechanism of attachment in the flagellate *Giardia muris*. Journal of Cell Science. 1973;**13**:11-41

[14] Brown JR, Schwartz CL, Heumann JM, Dawson SC, Hoenger A. A detailed look at the cytoskeletal architecture of the *Giardia lamblia* ventral disc. Journal of Structural Biology. 2016;**194**:38-48. DOI: 10.1016/j.jsb.2016.01.01

[15] Campanati L, de Souza W. The cytoskeleton of *Giardia lamblia*. Trends in Cell & Molecular Biology. 2009;**4**:49-61

[16] Schwartz CL, Heumann JM, Dawson SC, Hoenger A. A detailed, hierarchical study of *Giardia lamblia*'s ventral disc reveals novel microtubule-associated protein complexes. PLoS One. 2012;**7**:e43783. DOI: 10.1371/journal.pone.0043783

[17] Crossley R, Marshall J, Clark JT, Holberton DV. Immunocytochemical differentiation of microtubules in the cytoskeleton of *Giardia lamblia* using monoclonal antibodies to alpha-tubulin and polyclonal antibodies to associated low molecular weight proteins. Journal of Cell Science. 1986;**180**:233-252

[18] Gadelha AP, Benchimol M, de Souza W. Helium ion microscopy and ultra-high-resolution scanning electron microscopy analysis of membrane-extracted cells reveals novel characteristics of the cytoskeleton of *Giardia intestinalis*. Journal of Structural Biology. 2015;**190**:271-278

[19] Kattenbach WM, Pimenta PF, de Souza W, Pinto da Silva P. *Giardia duodenalis*: A freeze-fracture, fracture-flip and cytochemistry study. Parasitology Research. 1991;**77**:651-658

[20] Lanfredi-Rangel A, Diniz JA, de Souza W. Presence of a protrusion on the ventral disk of adhered trophozoites of *Giardia lamblia*. Parasitology Research. 1999;**85**:951-955

[21] Campanati L, Sant'Anna C, Gadelha C, Lourenço D, Labati-Terra L, Bittencourt-Silvestre J, Benchimol M, Cunha-e-Silva NL, De Souza W. Improvement on the visualization of cytoskeletal structures of protozoan parasites using high-resolution field emission scanning electron microscopy (FESEM). Histochemistry and Cell Biology. 2005;**124**:87-95. DOI: 10.1007/s00418-005-0786-1

[22] Feely DE, Erlandsen SL, Chase DG, Holberton DV, Erlandsen SL. The biology of *Giardia*. In: Meyer EA, editor. Giardiasis. New York: Elsevier; 1990. pp. 11-49

[23] Holberton DV, Ward AP. Isolation of the cytoskeleton from *Giardia*: tubulin and a low-molecular-weight protein associated with microribbon structure. Journal of Cell Science. 1981;**47**:139-166

[24] Chavez B, Martinez-Palomo A. *Giardia lamblia*: Freeze-fracture ultrastructure of the ventral disc plasma membrane. Journal of Eukaryotic Microbiology. 1995;**42**:136-141

[25] Feely DE, Schollmeyer JV, Erlandsen SL. *Giardia spp*: Distribution of contractile proteins in the attachment organelle. Experimental Parasitology. 1982;**53**:145-154

[26] Hagen KD, Hirakawa MP, House SA, Schwartz CL, Pham JK, Cipriano MJ, De La Torre MJ, Sek AC, Du G, Forsythe BM, Dawson SC. Novel structural components of the ventral disc and lateral crest in *Giardia intestinalis*. PLoS Neglected Tropical Diseases. 2011;**5**:e1442. DOI: 10.1371/journal.pntd.0001442

[27] Paredez AR, Assaf ZJ, Sept D, Timofejeva L, Dawson SC, Wang CJ, Cande WZ. An actin cytoskeleton with evolutionarily conserved functions in the absence of canonical actin-binding proteins. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**:6151-6156. DOI: 10.1073/pnas.1018593108

[28] Kirk-Mason KE, Turner MJ, Chakraborty PR. Cloning and sequence of beta tubulin cDNA from *Giardia lamblia*. Nucleic Acids Research. 1988;**16**:2733

[29] Kirk-Mason KE, Turner MJ, Chakraborty PR. Evidence for unusually short tubulin mRNA leaders and characterization of tubulin genes in *Giardia lamblia*. Molecular and Biochemical Parasitology. 1989;**36**:87-99

[30] Soltys BJ, Gupta RS. Immunoelectron microscopy of *Giardia lamblia* cytoskeleton using antibody to acetylated alpha-tubulin. Journal of Eukaryotic Microbiology. 1994;**41**:625-632

[31] Weber K, Schneider A, Westermann S, Muller N, Plessmann U. Posttranslational modifications of alpha-and beta-tubulin in *Giardia lamblia*, an ancient eukaryote. FEBS Letters. 1997;**419**:87-91

[32] Campanati L, Bré MH, Levilliers N, de Souza W. Expression of tubulin polyglycylation in *Giardia lamblia*. Biology of the Cell. 1999;**91**:499-506

[33] Campanati L, Troester H, Monteiro-Leal LH, Spring H, Trendelenburg MF, de Souza W. Tubulin diversity in trophozoites of *Giardia lamblia*. Histochemistry and Cell Biology. 2003;**119**:323-331. DOI: 10.1007/s00418-003-0517-4

[34] Crossley R, Holberton D. Assembly of 2.5 nm filaments from giardin, a protein associated with cytoskeletal microtubules in *Giardia*. Journal of Cell Science. 1985;**78**:205-231

[35] Morgan RO, Fernandez MP. Molecular phylogeny of annexins and identification of a primitive homologue in *Giardia lamblia*. Molecular Biology and Evolution. 1995;**12**:967-979

[36] Bauer B, Engelbrecht S, Bakker-Grunwald T, Scholze H. Functional identification of alpha 1-giardin as an annexin of *Giardia lamblia*. FEMS Microbiology Letters. 1999;**173**: 147-153

[37] Weeratunga SK, Osman A, Hu NJ, Wang CK, Mason L, Svärd S, Hope G, Jones MK, Hofmann A. Alpha-1 giardin is an annexin with highly unusual calcium-regulated mechanisms. Journal of Molecular Biology. 2012;**423**:169-181

[38] Weber K, Geisler N, Plessmann U, Bremerich A, Lechtreck KF, Melkonian M. SF-assemblin, the structural protein of the 2-nm filaments from striated microtubule associated fibers of algal flagellar roots, forms a segmented coiled coil. Journal of Cell Science. 1993;**12**:837-845

[39] Macarisin D, O'Brien C, Fayer R, Bauchan G, Jenkins M. Immunolocalization of β-and δ-giardin within the ventral disk in trophozoites of *Giardia duodenalis* using multiplex laser scanning confocal microscopy. Parasitology Research. 2012;**111**:241-248. DOI: 10.1016/j.jmb.2012.06.041

[40] Palm JE, Weiland ME, Griffiths WJ, Ljungström I, Svärd SG. Identification of immunoreactive proteins during acute human giardiasis. Journal of Infectious Diseases. 2003;**187**:1849-1859. DOI: 10.1086/375356

[41] Lourenço D, Andrade IS, Terra LL, Guimarães PR, Zingali RB, de Souza W. Proteomic analysis of the ventral disc of *Giardia lamblia*. BMC Research Notes. 2012 Jan 19;**5**:41. DOI: 10.1186/1756-0500-5-41

[42] Holberton DV. Attachment of *Giardia*: hydrodynamic model based on flagellar activity. Journal of Experimental Biology. 1974;**60**:207-221

[43] Erlandsen SL, Russo AP, Turner JN. Evidence for adhesive activity of the ventrolateral flange in *Giardia lamblia*. Journal of Eukaryotic Microbiology. 2004;**51**:73-80

[44] Lenaghan SC, Davis CA, Henson WR, Zhang Z, Zhang M. High-speed microscopic imaging of flagella motility and swimming in *Giardia lamblia* trophozoites. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**:E550-E558. DOI: 10.1073/pnas.1106904108

[45] Campanati L, Holloschi A, Troster H, Spring H, de Souza W, Monteiro-Leal LH. Videomicroscopy observations of fast dynamic processes in the protozoon *Giardia lamblia*. Cell Motility and the Cytoskeleton. 2002;**51**:213-224. DOI: 10.1002/cm.10026

[46] House SA, Richter DJ, Pham JK, Dawson SC. *Giardia* flagellar motility is not directly required to maintain attachment to surfaces. PLoS Pathogens. 2011;**7**:e1002167. DOI: 10.1371/journal.ppat.1002167

[47] Woessner DJ, Dawson SC. The *Giardia* median body protein is a ventral disc protein that is critical for maintaining a domed disc conformation during attachment. Eukaryotic Cell. 2012;**11**:292-301. DOI: 10.1128/EC.05262-11

[48] Pegado MG, de Souza W. Role of surface components in the interaction of process of *Giardia duodenalis* with epithelial cells in vitro. Parasitology Research. 1994;**80**:320-326

[49] Magne D, Favennce L, Chochillon C, Gorenflot A, Meillet D, Kapel N, Raichvarg D, Savel J, Gobert JG. Role of cytoskeleton and surface lectins in *Giardia duodenalis* attachament to Caco 2 cells. Parasitology Research. 1991;**77**:659-662

[50] Katelaris PH, Naeem A, Farthing MJ. Attachment of *Giardia lamblia* trophozoites to a cultured human intestinal cell line. Gut. 1995;**37**:512-518

[51] Benchimol M. Participation of the adhesive disc during karyokinesis in *Giardia lamblia*. Biology of the Cell. 2004;**96**:291-301. DOI: 10.1016/j.biolcel.2004.01.007

[52] Maia-Brigagão C, Gadelha AP, de Souza W. New associated structures of the anterior flagella of *Giardia duodenalis*. Microscopy and Microanalysis. 2013;**19**:1374-1376. DOI: 10.1017/S1431927613013275

[53] Friend DS. The fine structure of *Giardia muris.* Journal of Cell Biology. 1966;**29**:317-332

[54] de Souza W, Campanati L, Attias M. Strategies and results of field emission scanning electron microscopy (FE-SEM) in the study of parasitic protozoa. Micron. 2008;**39**:77-87

[55] Kulda J, Nohýnková E. Flagellates of the human intestine and of intestines of other species. In: Kreier JP, editor. Parasitic Protozoa. New York: Academic Press; 1978. pp. 69-138

[56] Nohynková E, Tumová P, Kulda J. Cell division of *Giardia intestinalis*: Flagellar developmental cycle involves transformation and exchange of flagella between mastigonts of a diplomonad cell. Eukaryotic Cell. 2006;**5**:753-761. DOI: 10.1128/EC.5.4.753-761.2006

[57] McInally SG, Dawson SC. Eight unique basal bodies in the multi-flagellated diplomonad *Giardia lamblia*. Cilia. 2016; **4**:5-21. DOI: 10.1186/s13630-016-0042-4

[58] Sagolla MS, Dawson SC, Mancuso JJ, Cande WZ. Three-dimensional analysis of mitosis and cytokinesis in the binucleate parasite *Giardia intestinalis*. Journal of Cell Science. 2006;**119**:4889-4900. DOI: 10.1242/jcs.03276

[59] Weiland ME, McArthur AG, Morrison HG, Sogin ML, Svärd SG. Annexin-like alpha giardins: A new cytoskeletal gene family in *Giardia lamblia*. International Journal for Parasitology. 2005;**35**:617-626. DOI: 10.1016/j.ijpara.2004.12.009

[60] Steuart RF, O'Handley R, Lipscombe RJ, Lock RA, Thompson RC. Alpha 2 giardin is an assemblage A-specific protein of human infective *Giardia duodenalis*. Parasitology. 2008;**135**:1621-1627. DOI: 10.1017/S0031182008004988

[61] Vahrmann A, Sarić M, Koebsch I, Scholze H. Alpha14-Giardin (annexin E1) is associated with tubulin in trophozoites of *Giardia lamblia* and forms local slubs in the flagella. Parasitology Research. 2008;**102**:321-326. DOI: 10.1007/s00436-007-0758-6

[62] Pathuri P, Nguyen ET, Ozorowski G, Svärd SG, Luecke H. Apo and calcium-bound crystal structures of cytoskeletal protein alpha-14 giardin (annexin E1) from the intestinal protozoan parasite *Giardia lamblia*. Journal of Molecular Biology. 2009;**385**:1098-1112. DOI: 10.1016/j.jmb.2008.11.012

[63] Elmendorf HG, Rohrer SC, Khoury RS, Bouttenot RE, Nash TE. Examination of a novel head-stalk protein family in *Giardia lamblia* characterised by the pairing of ankyrin repeats and coiled-coil domains. International Journal of Parasitology. 2005;**35**:1001-1011. DOI: 10.1016/j.ijpara.2005.03.009

[64] Hoeng JC, Dawson SC, House SA, Sagolla MS, Pham JK, Mancuso JJ, Löwe J, Cande WZ. High-resolution crystal structure and *in vivo* function of a kinesin-2 homologue in *Giardia intestinalis*. Molecular Biology of the Cell. 2008;**19**:3124-3137. DOI: 10.1091/mbc.E07-11-1156

[65] Nohýnkova E, Dráber P, Reischig J, Kulda J. Localization of gamma-tubulin in interphase and mitotic cells of a unicellular eukaryote *Giardia intestinalis*. European Journal of Cell Biology. 2000;**79**:438-445. DOI: 10.1078/0171-9335-00066

[66] Corrêa G, Morgado-Diaz JA, Benchimol M. Centrin in *Giardia lamblia*–ultrastructural localization. FEMS Microbiology Letters. 2004;**233**:91-96. DOI: 10.1016/j.femsle.2004.01.043

[67] Lauwaet T, Smith AJ, Reiner DS, Romijn EP, Wong CC, Davids BJ, Shah SA, Yates JR, Gillin FD. Mining the *Giardia* genome and proteome for conserved and unique basal body proteins. International Journal for Parasitology. 2011;**41**:1079-1092. DOI: 10.1016/j.ijpara.2011.06.001

[68] Midlej V, Benchimol M. *Giardia lamblia* behavior during encystment: How morphological changes in shape occur. Parasitology International. 2009;**58**:72-80. DOI: 10.1016/j.parint.2008.11.002

[69] Ghosh S, Frisardi M, Rogers R, Samuelson J. How *Giardia* swim and divide. Infection and Immunity. 2001;**69**:7866-7872. DOI: 10.1128/IAI.69.12.7866-7872.2001

[70] Carvalho KP, Monteiro-Leal LH. The caudal complex of *Giardia lamblia* and its relation to motility. Experimental Parasitology. 2004;**108**:154-162. DOI: 10.1016/j.exppara.2004.08.007

[71] Coggins JR, Schaefer FW. *Giardia muris:* Scanning electron microscopy of *in vitro* excystation. Experimental Parasitology. 1984;**57**:62-67

[72] Tumova P, Kulda J, Nohynkova E. Cell division of *Giardia intestinalis*: Assembly and disassembly of the adhesive disc and the cytokinesis. Cell Motility and Cytoskeleton. 2007;**64**:288-298. DOI: 10.1002/cm.20183

[73] Nosala C, Dawson SC. The critical role of the cytoskeleton in the pathogenesis of *Giardia*. Current Clinical Microbiology Reports. 2015;**2**:155-162. DOI: 10.1007/s40588-015-0026-y

[74] Piva B, Benchimol M. The median body of *Giardia lamblia*: An ultrastructural study. Biology of the Cell. 2004;**96**:735-746. DOI: 10.1016/j.biolcel.2004.05.006

[75] Brugerolle G. Contribuition a l'etude cytologique e phyletique des diplozoaires (Zoomastigophorea, Diplozoa Dangeard 1910). V. Nouvelle interpretation de l'organisation cellulaire de *Giardia*. Protistologica. 1975;**11**:99-109

[76] Meng TC, Aley SB, Svard SG, Smith MW, Huang B, Kim J, Gillin FD. Immunolocalization and sequence of caltractin/centrin from the early branching eukaryote *Giardia lamblia*. Molecular and Biochemical Parasitology. 1996;**79**:103-108

[77] Dawson SC, Sagolla MS, Mancuso JJ, Woessner DJ, House SA, Fritz-Laylin L, Cande WZ. Kinesin-13 regulates flagellar, interphase, and mitotic microtubule dynamics in *Giardia intestinalis*. Eukaryotic Cell. 2007;**6**:2354-2364. DOI: 10.1128/EC.00128-07

[78] Benchimol M, Piva B, Campanati L, de Souza W. Visualization of the funis of *Giardia lamblia* by high-resolution field emission scanning electron microscopy–New insights. Journal of Structural Biology. 2004;**147**:102-115. DOI: 10.1016/j.jsb.2004.01.017

[79] Morrison HG, McArthur AG, Gillin FD, Aley SB, Adam RD, Olsen GJ, Best AA, Cande WZ, Chen F, Cipriano MJ, Davids BJ, Dawson SC, Elmendorf HG, Hehl AB, Holder ME, Huse SM, Kim UU, Lasek-Nesselquist E, Manning G, Nigam A, Nixon JE, Palm D, Passamaneck NE, Prabhu A, Reich CI, Reiner DS, Samuelson J, Svard SG, Sogin ML. Genomic minimalism in the early diverging intestinal parasite *Giardia lamblia*. Science. 2007;**317**:1921-1926. DOI: 10.1126/science.1143837

[80] Corrêa G, Benchimol M. *Giardia lamblia* behavior under cytochalasins treatment. Parasitology Research. 2006;**98**:250-256. DOI: 10.1007/s00436-005-0065-z

[81] Castillo-Romero A, Leon-Avila G, Perez Rangel A, Cortes Zarate R, Garcia Tovar C, Hernandez JM. Participation of actin on *Giardia lamblia* growth and encystation. PLoS One. 2009;**4**:e7156. DOI: 10.1371/journal.pone.0007156

[82] Paredez AR, Nayeri A, Xu JW, Krtková J, Cande WZ. Identification of obscure yet conserved actin-associated proteins in *Giardia lamblia*. Eukaryotic Cell. 2014;**13**:776-784. DOI: 10.1128/EC.00041-14

[83] Benchimol M. The release of secretory vesicle in encysting *Giardia lamblia*. FEMS Microbiology Letters. 2004;**235**:81-87. DOI: 10.1016/j.femsle.2004.04.014

[84] Bittencourt-Silvestre J, Lemgruber L, de Souza W. Encystation process of *Giardia lamblia*: Morphological and regulatory aspects. Archives of Microbiology. 2010;**192**:259-265. DOI: 10.1007/s00203-010-0554-z

[85] Buchel LA, Gorenflot A, Chochillon C, Savel J, Gobert JG. *In vitro* excystation of *Giardia* from humans: A scanning electron microscopy study. Journal of Parasitology. 1987;**73**:487-493

[86] Benchimol M. Mitosis in *Giardia lamblia*: Multiple modes of cytokinesis. Protist. 2004;**155**: 33-44. DOI: 10.1078/1434461000162