Microreview

An insider’s guide to the microtubule cytoskeleton of Giardia

Scott C. Dawson*
Department of Microbiology, One Shields Avenue, UC Davis, Davis, CA 95616, USA.

Summary

*Giardia intestinalis* is a zoonotic, parasitic protist with a complex microtubule cytoskeleton critical for motility, attachment, intracellular transport, cell division and transitioning between its two life cycle stages – the cyst and the trophozoite. This review focuses on the structures of the primary elements of the microtubule cytoskeleton and cytoskeletal dynamics throughout this complex giardial life cycle. The giardial cytoskeleton has both highly dynamic elements and more stable MT structures, including several novel structures like the ventral disc that change conformation via unknown mechanisms. While our knowledge of the giardial cytoskeleton is primarily cytological, the completed *Giardia* genome and recently developed reverse genetic tools affords an opportunity to uncover the mechanisms of *Giardia*s cytoskeletal dynamics. Fundamental areas of giardial cytoskeletal biology remain to be explored, including high resolution imaging and compositional characterization of cytoskeletal structures required for elucidating the molecular mechanisms of cytoskeletal functioning.

Introduction

*Giardia intestinalis* is a zoonotic, parasitic protist with a complex microtubule (MT) cytoskeleton that is of critical importance during its two life cycle stages (Adam, 2001; Elmendorf et al., 2003). *Giardia cysts* persist in the environment and are ingested from contaminated water or food. Cysts can *excyst* in the small intestine of the animal host to become flagellated *trophozoites* that attach to the intestinal microvilli using a specialized microtubule structure, the ventral disc. Attachment is critical to avoid peristalsis. Trophozoites later undergo morphological changes, encyst, and are released to infect new hosts (Roxstrom-Lindquist et al., 2006). Acute giardiasis is the most prevalent protozoan intestinal infection in the USA and worldwide, and chronic giardiasis is common in developing countries (Savioli et al., 2006; Troeger et al., 2007). Beyond its clinical relevance, study of the giardial cytoskeleton also informs basic cell biology, molecular biology and cellular evolution (Elmendorf et al., 2003).

The giardial cell is a flattened teardrop shape (roughly 15 μm long by 5 μm wide and 5 μm thick) and orients into hydrodynamic currents with the rounded, anterior end. Despite its small size, *Giardia* has a complex three-dimensional ultrastructure and novel cytoskeletal elements of unknown function and composition. This elaborate cytoskeleton is essential for motility, attachment, intracellular transport, cell division and encystation/excystation. The MT cytoskeleton has both highly dynamic elements that generate cellular movements via unknown mechanisms, and more stable MT structures, including several that are unique to *Giardia*. Most efforts to study the *Giardia* cytoskeleton have been cytological, and future work should focus on refining the details of its elaborate MT-based structures and elucidating the molecular mechanisms of *Giardia*s dynamic cytoskeletal movements. Genomic and/or proteomic-based approaches, combined with new reverse genetic tools to generate dominant negative mutants (Dawson et al., 2007b; Gaechter et al., 2008), or antisense (Touz et al., 2005) and morpholino-based knockdowns (Carpenter and Cande, 2009), will allow the identification of novel structural components and uncover the mechanisms of *Giardia*s cytoskeletal dynamics.

This review serves as a cytological guide to the major MT-based cytoskeletal elements and examines the dynamic movements and functions of the cytoskeleton. It focuses primarily on cytoskeletal structure and secondarily on cytoskeletal dynamics and pathogenesis. The focus on the MT cytoskeleton, rather than the actin cytoskeleton, is intentional. While the actin cytoskeleton...
undoubtedly plays essential roles in giardial biology, polymerized actin filaments have not been identified cytologically and are only inferred from the binding of TRITC-phalloidin (Castillo-Romero et al., 2009). Proteins that modulate actin dynamics appear to be missing in the genome (Morrison et al., 2007); thus the giardial actin cytoskeleton should receive more research attention.

The primary elements of the giardial microtubule cytoskeleton

The *Giardia* MT cytoskeleton is comprised of both unique structures (the median body, ventral disc, funis and axoneme-associated elements) and elements commonly found in flagellated protists (eight flagella and two mitotic spindles) (see Fig. 1). The MT cytoskeleton creates a...
stable scaffold for cell shape, cell polarization and intracellular trafficking (Elmendorf et al., 2003). The primary structural MT-based cytoskeletal elements in Giardia are the:

Ventral disc

This unique, highly organized MT structure is critical to virulence as it promotes giardial attachment to the intestinal microvilli (Elmendorf et al., 2003), allowing parasites to colonize and resist peristalsis. Using the ventral disc, trophozoites orient ventral side ‘down’ to either biological or inert substrates via an undefined mechanism (Holberton, 1973b; 1974) that might involve suction (Hansen et al., 2006). The ventral disc (VD) is composed of three main elements: (i) a concave spiral array of microtubules, nucleated by the caudal flagellar basal bodies; (ii) microribbons, trilaminar structures that attach perpendicularly along the length of the MT spiral and extend into the cytoplasm; and (iii) crossbridges, structures that horizontally link the microribbons (Holberton, 1973a; Holberton, 1981; Holberton and Ward, 1981; Feely et al., 1982; Crossley and Holberton, 1983; 1985) (see Fig. 2). Microribbons are about 25 nm thick, and extend 150–400 nm into the cytoplasm (Holberton, 1973a; 1981). The microribbons purportedly consist of two sheets of globular subunits, separated by a fibrous inner core (Holberton, 1981). The spacing between the MT/microribbons of the disc spiral is uniform (~250–300 nm). MTs of the spiral are physically linked to the ventral plasma membrane by ‘sidearms’, electron dense structures of unknown composition (Holberton, 1973a). A ‘bare area’, central to the region where the disc spiral MTs overlap, contains numerous membrane-bound vacuoles (Elmendorf et al., 2003). The marginal plate regions of the anterior axonemes are sandwiched within the disc spiral arrays (Kulda and Nohynkova, 1995). Finally, the VD is surrounded by the lateral crest, a fibrillar structure of unknown composition with proposed contractile functions (Kulda and Nohynkova, 1995). Actin has been reported to localize to the microribbons, and no proteins have been localized to the ventral disc. Few proteins have been reported to localize to the microribbons, and no proteins are known to localize to the crossbridges or the MT-associated structures such as the ‘sidearms’. Ultimately, molecular genetic analysis of both structural and regulatory disc-associated proteins and visualization of live ventral disc dynamics will be pivotal in assessing the mechanism of giardial attachment.

Median body

The enigmatic median body (MB) is a non-membrane-bound, semi-organized MT array of unknown function (Piva and Benchimol, 2004) present on the dorsal side of Giardia trophozoites, slightly posterior to the ventral disc (Elmendorf et al., 2003). It is absent in other diplomonads (Feely et al., 1990). The MB is commonly described as a ‘haystack’ of disordered MTs, roughly perpendicular to the caudal axonemes, that forms the crooked giardial ‘smile’. MB MTs appear to be dynamic during interphase as they are sensitive to both MT stabilizing and depolymerizing drugs (Sagolla et al., 2006; Dawson et al., 2007a), and their dynamics are regulated by the depolymerizing kinesin motor protein, kinesin-13 (Dawson et al., 2007b). While there is no evidence that ventral disc MTs undergo dynamic growth and shrinkage during attachment, MB MTs may still have a critical function in the giardial life cycle (Kabnick and Peattie, 1990). The MB varies in form and presence during the cell cycle, disappearing altogether following mitosis, prior to disc division (Sagolla et al., 2006).

The MB is a reservoir of tubulin subunits for duplicating MT structures, such as daughter ventral discs, which form just prior to cytokinesis (Brugerolle, 1975) (Feely et al., 1990). The presence of such a reservoir could allow rapid assembly of structures like the ventral disc, and permit the maintenance of infection by minimizing the removal of dividing trophozoites by peristalsis. Brugerolle identified small ‘appendages’
similar to the disc microribbons on MB MTs (Brugerolle, 1975), and Crossley et al. (1986) showed that polyclonal antibodies against β-giardin also localized to the MB. An alternative proposal is that the MB is necessary for *Giardia* detachment, but no experiments have been done to test the ‘reservoir’ hypothesis or this alternative ‘detachment’ hypothesis (Piva and Benchimol, 2004).

**Flagella and axoneme-associated structures**

*Giardia* has eight flagella organized into bilaterally symmetrical flagellar pairs: the anterior, the caudal, the posteriolateral and the ventral (see Fig. 1). The anterior axonemes cross over and curve around the ventral disc spiral before exiting at the anterior regions of the tropho-
zoite. Caudal axonemes run longitudinally through the cell midline and exit at the extreme posterior tip. The ventral axonemes exit at the posterior end of the ventral disc in the 'ventrolateral flange' region. Finally, the posteriolateral axonemes are angled outward and exit at the lower third of the cell, posterior to the MB and ventral disc. The eight basal bodies are positioned mainly between the two nuclei towards the cell interior, with the anterior basal bodies at the anterior end of the nuclei. Each axoneme has radial spokes, dynein arms and the central microtubule pair (Clark and Holberton, 1988; Elmendorf et al., 2003, Carvalho and Monteiro-Leal, 2004). Electron-dense 'collars' occur at the regions where each flagellum exits the cell body (Hoeng et al., 2008).

*Giardia*'s flagella are intriguing in that each axoneme has long, cytoplasmic regions that are not elongated basal body 'transition zones'. Each axoneme also has a membrane-bound portion, and the ratio of the length of the cytoplasmic region to the length of the membrane-bound portion varies between each flagellar pair. How the cytoplasmic portions and associated structures are assembled during cell division is unclear (Hoeng et al., 2008). Different pairs of axonemes have specific proteins that localize exclusively to either the cytoplasmic or membrane-bound regions, including GASP-180, a member of a novel family of coiled-coil proteins (Elmendorf et al., 2005), and several α-giardins (Weiland et al., 2005; Szkodowska et al., 2002). Each pair also has specific structures associated with the cytoplasmic or membrane-bound portions, such as the 'marginal plates' associated with anterior axonemes, the fin-like structures extending off the ventral axonemes (Kulda and Nohynkova, 1995), the electron dense material on the posteriolateral axonemes, and the 'caudal complex' — a jacket of microtubules around the cytoplasmic region of the caudal axonemes. These ancillary structures are compositionally and functionally uncharacterized (Elmendorf et al., 2003), and confer on each flagellum a unique structural identity and likely, unique functional roles (Campanati et al., 2002). The structure of giardial axonemes is evolutionarily conserved, and the membrane-bound portions are assembled by the conserved mechanism of intraflagellar transport (IFT).

**Funis**

The funis has no known function, yet has been suggested to have either a structural role in maintaining the giardial cell shape or a potential role in the flexion of the posterior 'tail' region during detachment (Benchimol et al., 2004). It is composed of sheets of MTs associated with the caudal axonemes, and is thought to be nucleated in the area between the nuclei as bands of linked MTs that then laterally fan out at the point of emergence of the ventral axonemes (Benchimol et al., 2004). Microtubule ends of the funis appear to be anchored in the cytoplasmic regions of the posteriolateral axonemes. Filamentous links to the underlying plasma membrane have also been reported (Benchimol et al., 2004). A specific type of movement termed 'dorsal tail flexion' (Fig. 3) has been attributed to the funis (Ghosh et al., 2001), the caudal complex (Carvalho and Monteiro-Leal, 2004) or the caudal flagella (Campanati et al., 2002).

**Mitotic spindles**

Until recently, there was no evidence of mitotic spindles or of the precise MT-based mechanism of chromosome segregation in *Giardia*. In 2006, however, a semi-open mitosis (Raikov, 1994) with two extranuclear spindles was observed in *Giardia* (Sagolla et al., 2006), similar to a description in a related diplomonad, *Hexamita inflata* (Brugerolle, 1975). Chromosomes are segregated along the left-right (L-R) axis, and cytokinesis occurs along the longitudinal axis, perpendicular to the spindle (Sagolla et al., 2006). In anaphase B, each nucleus elongates as the spindle elongates during pole-pole separation. Daughter cells inherit one copy of each parent nucleus.

The giardial spindle MTs radiate from one of the flagellar basal bodies near each spindle pole, forming a sheath around the nuclear envelope. Each spindle pole is associated with at least one axoneme. The nuclear envelope remains, forming a barrier between cytoplasmic microtubule arrays and chromatin; there is no evidence of mixing of the chromatin between nuclei (Sagolla et al., 2006). Presumptive kinetochore microtubules penetrate at the spindle poles through large polar openings in the nuclear membrane (Sagolla et al., 2006). Likely, more than one microtubule is attached per kinetochore in *Giardia*. The internal (presumably kinetochore) microtubules extend only a few microns into the nucleus near the chromatin in late stage (anaphase B) nuclei.

**Dynamic cytoskeletal movements during the giardial life cycle**

In colonizing the small intestine, *Giardia* first excysts, then trophozoites find suitable sites for attachment, attach to the intestinal villi, divide and eventually encyst before passing into the environment. Each of these important stages in the giardial life cycle is facilitated by dynamics of the microtubule cytoskeleton. Some types of microtubule dynamics, such as flagellar assembly and mitosis, are well characterized in other organisms. Cellular movement involves both intrinsic MT dynamics and active regulation of assembly/disassembly, sliding or other mechanisms of force production by microtubule motors like kinesins or dyneins. The molecular mechanisms of some *Giardia*...
specific microtubule dynamics – such as attachment, cell division and encystation/excystation – are essentially uncharacterized at the molecular level, and this remains a fruitful area of Giardia cell biology. Specific events in the giardial life cycle likely to involve dynamic movement and force production by the MT cytoskeleton include:

Ventral disc dynamics and attachment

Giardia must remain attached to the intestinal villi to proliferate and avoid peristalsis. Parasites can attach to both biological substrates (in vivo attachment) and inert substrates like plastic or glass (in vitro attachment). Unique strategies for attachment to surfaces are known in microbes, including focal adhesions and podosomes in amoebae and fibroblasts (Décavé et al., 2002; Evans and Matsudaira, 2006; Pellegrin and Mellor, 2007), and holdfasts in bacteria (Tsang et al., 2006). The giardial mechanism of attachment via the ventral disc, however, appears to be without precedent, as it likely involves conformational changes in the overall VD structure to generate a negative pressure beneath the disc.

The mechanism of giardial attachment is currently unknown and is somewhat controversial. Proposed models of giardial attachment to surfaces can be broadly categorized as: ligand-independent interactions (electrostatic or van der Waals forces) (Hansen et al., 2006), ligand-specific interactions (Nash et al., 1983; Inge et al., 1988; Magne et al., 1991; Ortega-Barria et al., 1994; Sousa et al., 2001), clutching mechanisms (Holberton, 1973a,b; Feely and Erlandsen, 1981; Inge et al., 1988; Elmendorf et al., 2003), or suction-mediated mechanisms (Holberton, 1973a,b; 1974; Feely and Erlandsen, 1981; Elmendorf et al., 2003; Hansen et al., 2006; Hansen and Fletcher, 2008) (reviewed in Elmendorf et al., 2003). When considering attachment in vivo, each of the proposed models is not necessarily mutually exclusive; several types of mechanisms may contribute to in vivo attachment, whereas suction is likely sufficient for in vitro attachment (Hansen et al., 2006; Hansen and Fletcher, 2008).

Do intrinsic or regulated MT dynamics contribute to attachment? If disc MT dynamics are involved in generating cytoskeletal movements necessary for attachment, anti-cytoskeletal drugs should negatively impact the attachment process. Several studies using antimicrotubule drugs have sought to determine whether the actin or MT cytoskeleton is required for attachment (Feely...
and Erlandsen, 1982; Mariante et al., 2005). Anti-microtubule drugs, however, affect dynamic populations of MTs (not polymerized MTs) by either sequestering cellular tubulin pools and inhibiting polymerization (nocodazole, colchicine) or by stabilizing growing MTs (Taxol) (Long, 1994; Pellegrini and Budman, 2005; Bhattacharyya et al., 2008). The MTs of the eight flagella, the MB and the mitotic spindles are dynamic as they are sensitive to these drugs; however, the ventral disc MTs are unaffected (Sagolla et al., 2006). Indeed, drugs that limit MT polymerization, such as albendazole, have a minimal effect on attachment unless there is severe deformation of the disc after multiple rounds of cell division (Chavez et al., 1992; Oxberry et al., 1994). This does not imply that the disc structure itself is not dynamic; rather it implies that the MTs of the disc do not undergo rapid polymerization/depolymerization.

Giardial attachment models are based primarily on light or electron microscopic descriptions rather than on detailed molecular genetic studies (Holberton, 1973a; 1974; Piva and Benchimol, 2004; Benchimol, 2004b; Mariante et al., 2005). Based on such observations, two possible mechanisms involving dynamic movements of the disc or flagella have been proposed to produce suction and thus generate a negative pressure differential under the VD. Because the VD undergoes a conformational change upon attachment to become less concave (Sousa et al., 2001), a compelling model suggests that conformational changes of the VD could be sufficient to produce suction-based attachment by generating suction in vitro, or alternatively, ‘clutching’ in vivo (Elmendorf et al., 2003). In this model, the principal structural components of the disc (MTs, microribbons, crossbridges and/or motor proteins) would generate and maintain attachment forces. No known disc-associated protein has been shown to modulate disc conformational changes, however.

Early attachment models invoked a hydrodynamic force generated by ventral flagellar beating (Holberton, 1974) to create suction. These models are based primarily on an early report that the ventral flagella beat when cells are attached, and stop beating when cells detach. Whether these observations reflect causality (ventral flagellar beating causes attachment) or correlation (ventral flagella beat at the same time cells attach) remains to be determined; nonetheless, theoretical and observational arguments have been put forth that hydrodynamic currents generated by the ventral flagella (Holberton, 1974; Ghosh et al., 2001) can cause attachment. In a molecular genetic sense, there has been little evaluation of whether the ventral (or any) flagella are necessary and/or sufficient for giardial attachment. Flagellar motility, however, is doubtless important for positioning the cell parallel to the substrate and for movement towards suitable areas for attachment and colonization. Future mechanistic studies of attachment should consider both disc conformational dynamics and flagellar motility.

Flagellar motility and flagellar assembly

Flagellar motility is required for Giardia to find suitable sites for attachment and for detachment from surfaces, including the intestinal villi (Campanati et al., 2002). The beating of the four flagellar pairs produces complex movements that are involved in these processes (Fig. 3). The anterior and posteriolateral flagellar pairs beat asynchronously, while the ventral flagella beat synchronously. The anterior and ventral flagellar pairs are primarily involved in forward and downward directional movements (Fig. 3) (Campanati et al., 2002). The beating of the ventral flagella has also been proposed to mediate suction-based attachment via the ‘hydrodynamic model’ (Holberton, 1974). Rotational or tumbling movements have been associated with the beating of the anterior and/or posteriolateral flagella (Fig. 3). The caudal flagella do not beat, but rather appear to flex. The lack of beating of the caudal flagella has been attributed to the presence of a MT sheet, termed the caudal complex, that surrounds the cytoplasmic portions of the caudal axonemes. The giardial ‘tail’ region can bend dorsally as well as laterally and this movement, termed ‘dorsal tail flexion’ (Carvalho and Monteiro-Leal, 2004), is proposed to result from the flexing of the caudal complex, caudal axonemes, or funis (Fig. 3). Dorsal tail flexion has been associated with detachment (Owen, 1980). Dynein may be involved in the interaction between this MT sheet and the caudal axonemes, as they appear to slide over one another during dorsal tail flexion (Campanati et al., 2002).

Flagellar motility is also required for Giardia to complete cell division and cytokinesis (Nohynkova et al., 2006; Tumova et al., 2007). During giardial division, daughter flagella are proposed to undergo a maturation process in which the parent flagella migrate and transform to different flagellar types and new flagella are built (Nohynkova et al., 2006); however, specific molecular markers have not been used to track each flagellar pair. During encystation, the flagella are internalized within the cyst, but do not completely resorb. The length of giardial flagella must also be maintained during interphase. Growth and maintenance of flagellar length are dependent upon IFT to provide building blocks to the site of flagellar assembly at the distal flagellar tip (Kozinski et al., 1995). In Giardia, IFT particles likely dock on cytoplasmic portions of axonemes, and accumulate at flagellar collar regions and at the distal flagellar tips, making these areas the respective start and end-points of
the IFT pathway (Hoeng et al., 2008). Both IFT raft proteins and the heterotrimeric kinesin-2 motor complex are present in Giardia and have conserved functions in assembly of the external regions of axonemes (Hoeng et al., 2008). An open question, however, is whether IFT is required for assembly of the cytoplasmic portions of axonemes. In two studies that investigated the function of kinesin-2 in flagellar assembly, cytoplasmic axoneme length was unaffected by either interfering morpholinos or by the overexpression of a dominant negative kinesin-2 (Hoeng et al., 2008; Carpenter and Cande, 2009). Cytoplasmic regions of giardial axonemes are thus likely not to require kinesin-2 for their assembly. Both IFT-mediated and non-IFT mediated assembly of axonemes have been shown to occur simultaneously in the same cell in other organisms (Han et al., 2003; Briggs et al., 2004).

In addition to kinesin-2, another kinesin has been shown to be involved in flagellar length regulation in Giardia. Overexpression of a dominant negative kinesin-13 resulted in long flagella, indicating a role of kinesin-13 in flagellar disassembly (Dawson et al., 2007b). This observation is complemented by the finding that Giardia’s flagellar length is sensitive to both MT stabilizing and destabilizing drugs (Dawson et al., 2007b). Thus hierarchical levels of flagellar length regulation exist in Giardia, and these involve both intrinsic microtubule dynamics, and active assembly (by IFT) and active axonemal MT disassembly by kinesin-13.

The cytoskeleton and cell division

Despite some initial claims of novel non-spindle based mechanisms of chromosome segregation (Benchimol, 2004a,b), chromosome segregation in Giardia is achieved by the mitotic spindle as in other eukaryotes (Sagolla et al., 2006). Three lines of evidence strongly support the role of kinetochore MTs in chromosome segregation rather than an alternative mechanism. First, intra-nuclear kinetochore MTs were identified via TEM (Sagolla et al., 2006). Second, the conserved centromere protein cenH3 (Dawson et al., 2007a) localizes to the leading edge of chromosomes during mitosis. Finally, Taxol treatment, which inhibits MT depolymerization, causes the loss of interzonal microtubules, and results in the formation of two half spindles and chromosome missegregation (Sagolla et al., 2006). These insights into giardial mitosis may inform studies of spindle organization and dynamics in other protists and/or in more commonly studied experimental model organisms.

Kinesins and dyneins are known to have essential roles in spindle assembly and dynamics (Mcintosh and Pfarr, 1991). The depolymerizing kinesin motors, including kinesin-13 family members, regulate microtubule dynamics in mitotic arrays, and aid in establishing proper kinetochore-microtubule attachments and mitotic progression (Kline-Smith and Walczak, 2002). Recently, the depolymerizing motor, kinesin-13, was shown to localize to giardial kinetochores and function in giardial mitosis (Dawson et al., 2007b). Overexpression of a dominant negative kinesin-13 resulted in a high number of cells (>50%) with visible mitotic defects, including spindle defects and lagging anaphase chromosomes. As in other organisms, kinesin-13 at the giardial kinetochores likely has a role in the maintenance of spindle length (Goshima et al., 2005). This was the first time a conserved function was found for kinesin-13 in a eukaryote with semi-open mitosis (spindle poles external to the nuclear envelope and kinetochore microtubules that penetrate the nuclei at polar openings) (Sagolla et al., 2006).

During cell division, the major giardial cytoskeletal elements (e.g. flagella, MB and disc) undergo dramatic rearrangements prior to their partitioning to the daughter cells (Nohynkova et al., 2006). Beyond cytological descriptions of mitosis (Nohynkova et al., 2006; Sagolla et al., 2006), flagellar division (Nohynkova et al., 2006) and disc division (Tumova et al., 2007), there is little understanding of the molecular mechanisms of cytoskeletal movements during giardial cell division. Microtubule dynamics are critical during cell division in Giardia wherein the two nuclei (Kabnick and Peattie, 1990) undergo mitosis and the two extranuclear spindles penetrate at polar nuclear openings (Sagolla et al., 2006). Chromosome segregation in the two nuclei and nuclear partitioning are followed by the duplication and repositioning of eight flagella in each of the daughter cells (Tumova et al., 2007). This process has not been investigated at the molecular level, but likely involves microtubule motors such as kinesins and dyneins to generate forces for repositioning of organelles during cell division.

Before mitosis is completed, flagellar duplication occurs (Nohynkova et al., 2006; Sagolla et al., 2006). In late telophase, two new daughter discs are assembled on the dorsal side of the cell, and the parental disc is reorganized and disassembled (Tumova et al., 2007), presumably to maintain attachment during division. Thus, the daughter discs are formed de novo, and are neither templated nor built from components of the parental disc. A complete understanding of disc assembly and disassembly in Giardia has been hampered by the lack of cytological descriptions of intermediate stages and a lack of knowledge of the specific functions of proteins involved in disc biogenesis. Parent disc disassembly or MB disassembly could also contribute to the total cellular MT pools during cell division. We currently have no information on how the parental disc is disassembled, yet this also likely involves MT severing and disassembly proteins like katanin and kinesin-13 respectively.
The cytoskeleton and encystation/excystation

Morphological changes resulting from cytoskeletal movements transform trophozoites into the environmentally resistant cyst form. During encystation, trophozoites are remodelled from the flattened teardrop shape to a more rounded ovoid shape as the cyst wall is being assembled (Midlej and Benchimol, 2009). The ventral disc structure rearranges as the MT spiral gradually opens into a horseshoe-shaped morphology before eventual fragmentation and partial disassembly by unknown mechanisms. Flagella are internalized during cyst formation, and are known to continue to beat inside the newly formed cyst (Midlej and Benchimol, 2009).

Morphological aspects of excystation have been investigated using scanning electron microscopy (Feely, 1986; Buchel et al., 1987). Flagellar motility is suggested to play a mechanical role in the initial opening of the cyst; however, other contractile or MT-mediated forces have not been excluded. Trophozoites appear to partially divide before excystation, although it is unclear at what point in the excystation process this occurs and whether meiosis occurs (Poxleitner et al., 2008). A slightly rounded trophozoite then exits the cyst and progressively elongates, flattens and undergoes cytokinesis. The subsequent assembly of fully functional ventral disc allows the trophozoite to attach to surfaces. Encystation and excystation also require cytoskeletal disassembly and assembly; however, the details or stages of cytoskeletal dynamics during these important transitions in the giardial life cycle have not been described at the molecular level.

Conclusions

Fundamental areas of giardial cytoskeletal biology remain to be explored, including defining cytoskeletal structures, identifying their protein composition and assessing the role of microtubule-associated proteins in dynamic cellular processes. Initial cytological and ultrastructural studies need to be updated and revisited at higher resolution using current state of the art fixation techniques and microscopy. Previous technical limitations have made studying giardial protein function difficult at the molecular level (Davis-Hayman and Nash, 2002; Elmendorf et al., 2003), but recently developed molecular tools to study protein function will permit detailed analysis of cytoskeletal mechanisms (Carpenter and Cande, 2009). Further investigations of cytoskeletal mechanisms should include: (i) attachment in vitro and in vivo; (ii) the function of the enigmatic MB; (iii) biogenesis of the disc; and (iv) morphogenesis during encystation/excystation. With advances in molecular tools and techniques for microscopic investigations, these are exciting times for giardial cytoskeletal biology – an area of giardial biology virtually unexplored at the molecular or mechanistic level.

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