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"Disc-o-Fever": getting down with *Giardia*'s groovy microtubule organelle

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Abstract

Protists have evolved a myriad of highly specialized cytoskeletal organelles that expand known functional capacities of microtubule polymers. One such innovation – the ventral disc— is a cup-shaped microtubule organelle that the parasite *Giardia* uses to attach to the host small intestine. The molecular mechanisms underlying the generation of suction-based forces by overall conformational changes of the disc remain unclear. The elaborate disc architecture is defined by novel proteins and complexes that decorate almost all disc microtubule protofilaments, and vary in composition and conformation along the length of the microtubules. Future genetic, biochemical, and functional analyses of DAPs will be central toward understanding not only disc architecture and assembly, but also the overall disc conformational dynamics that promote host attachment.

Keywords

Giardia; parasite; organelle; microtubule; evolution

Cytoskeletal innovations in protists expand the range of microtubule polymer functions

Paradigms of microtubule function, dynamics, assembly, and nucleation have been shaped by the study of the dynamic mitotic spindle and cilium in model systems. Cell biological models tend toward macroscopic eukaryotes, yet microbial eukaryotes, or protists, have a myriad of unique interphase cytoskeletal organelles that have been described for nearly 300 years [1]. These unique cytoskeletal innovations and novel organelles are not atypical adaptations found in only a handful of protists; rather, they are widespread in both free-living and parasitic eukaryotic lineages [2]. The diversity of cytoskeletal organelles in microbial eukaryotes mirrors their extreme evolutionary divergence that is, in part, responsible for their "extreme" cell biology [3]. Non-canonical cytoskeletal arrays confer unique and adaptive functions to eukaryotic cells – expanding the known functional

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capacities of microtubule polymers and challenging conventional notions of microtubule organellar dynamics.

Emerging microbial eukaryotic model systems offer a wealth of unexplored cytoskeletal organelles, structures, proteins, and mechanisms [4]. Microtubules (MTs) in protists can assemble into cytoskeletal arrays that adopt shapes, functions, or regulatory mechanisms that are not seen in other organisms. Diverse protistan cytoskeletal structures are often composed of proteins that lack homology to proteins in other eukaryotes [5–7], and thus may be an untapped reservoir of non-canonical MT-binding proteins governing MT assembly, nucleation, or dynamics [3]. For example, in the apicomplexan protist *Toxoplasma gondii*, the MT based apical complex acts as an invasion machine that is necessary for infection of host cells and parasite replication, and is constructed from canonical tubulins, non-canonical tubulins, and novel proteins [5, 8]. Parabasalid protists such as the human pathogen *Trichomonas vaginalis* possess a cone-shaped MT organelle termed the axostyle [9] which may participate in cellular movement, attachment, or cell division [10, 11]. The ubiquity and diversity of unique cytoskeletal organelles in microbial eukaryotes underscores the fact that cytoskeletal variation is the norm rather than the exception.

This review focuses on the evolution, architecture, function and conformational dynamics of one such enigmatic and complex microtubule organelle—the ventral disc of *Giardia lamblia* (see Glossary, FIGURE 1 and FIGURE 2). *Giardia*, a diplomonad protist, is one of the most common parasites of humans and causes significant diarrheal disease worldwide [12]. Diplomonads have been described as “double” or duplicated cells, as they are bilaterally symmetrical with two equivalent diploid nuclei and two spindles. *Giardia* and other diplomonads have eight flagella and basal bodies organized into four pairs [13]. The “suction-cup”-like ventral disc mediates reversible parasite attachment to the host intestinal microvilli [14] (FIGURE 3). Attachment via the ventral disc occurs in seconds and is a necessary process for infection as it allows *Giardia* to resist peristalsis and remain in the gut. It is unclear whether overall conformational changes of the ventral disc architecture directly generate forces for attachment, or whether disc conformational dynamics maintain attachment forces generated by some other mechanism [15, 16]. Despite recent studies of ventral disc architecture and composition, we are still in the very preliminary stages of understanding the contribution of specific structural elements in generating the forces for attachment and the mechanism that *Giardia* uses to rapidly assemble the ventral disc during cell division (see Outstanding Questions).

Novel protein complexes define the intricate cup-shaped architecture of the ventral disc

The ventral disc is a highly ordered and complex spiral microtubule (MT) array [17–22]. Parallel, uniformly spaced MTs spiral approximately one and a quarter turns into a domed structure. The disc spiral array has one region of overlap, termed the overlap zone, between the upper and lower portions of the disc (FIGURE 1). The majority of ventral disc microtubules terminate with their plus ends either on the periphery of the disc or in the overlap zone, with a small subset observed to terminate within the disc body itself In sum,

the entire disc contains more than 1.2 mm of tubulin forming roughly one hundred MTs that vary in length from 2 to 18 μm [23].

Associated with the entire length of the MT spiral are unique substructural elements – the trilaminar microribbons – that are found throughout the disc body and extend 150–400 nm dorsally into the cytoplasm [20, 21] (see FIGURE 1 ‘disc body’ and FIGURE 2). The microribbons consist of two sheets of globular subunits, separated by a fibrous inner core, forming a structure about 25 nm thick [21]. Regularly spaced crossbridge structures link adjacent microribbons [20] (see FIGURE 2). In the early 1980’s, Holberton successfully fractionated and identified low-molecular weight microribbon proteins that he termed giardins [20, 21]. Like microtubules, fractionated giardins can polymerize in solution. Microribbon polymers do not resemble canonical microtubules but they can form sheets, tactoids, and ribbons. The contribution of the microribbons to ventral disc stability, conformational dynamics, or to attachment is also unknown.

The overall architecture of the disc was first described by Cheissin over 50 years ago [24], and the first 3D high-resolution architecture of the ventral disc was obtained recently using cryo-electron tomography (cryo-ET) [25]. Cryo-ET of whole isolated ventral discs with volume averaging of repetitive structural elements provided details of the cytoskeletal architecture and revealed dense protein complexes coating nearly all protofilaments of the microtubule spiral array (FIGURE 2). Along with previously identified substructures (e.g., microribbons and crossbridges), this study also defined several new repetitive MT-associated substructures including: three *Giardia* MT-associated proteins (gMAPs 1–3) and three MT inner proteins (gMIPS 5, 7 and 8), each associated with specific protofilaments, as well as two other substructures termed sidearms and paddles. Repeating every 8 nm, the sidearms and paddles are spaced at the distance of a single alpha/beta tubulin dimer. Crossbridges repeat every 16 nm corresponding to the distance of two alpha/beta tubulin dimers (FIGURE 2). The disc is a “hyperstable” structure in that drugs that normally affect MT dynamic instability have no effect on ventral disc microtubules [26] and no turnover of any disc-associated protein has been reported [7]. The large number of unique microtubule associated proteins and other associated structural elements decorating the disc spiral may contribute to the observed hyperstability.

Additional structural elements are associated with the ventral disc (FIGURE 1). These include a highly ordered structure, the lateral crest, which surrounds the periphery of the ventral disc [27] and is proposed to have contractile functions [17]. A smaller left-handed MT spiral array, the supernumerary MT array, lies dorsal to the main ventral disc structure and has no known function. The composition and function of prominent substructural elements like the crossbridges, sidearms, and paddles is also unknown. Further dissection of the mechanism of disc conformational dynamics will first require an understanding of the functional roles of these unique disc substructural elements.

The disc is primarily composed of proteins lacking known MT-binding properties

Holberton originally coined the term “giardins” for proteins isolated from the disc [18]. We now use the term disc-associated proteins (or DAPs) to mirror the term microtubule-associated proteins, or MAPs. Our emerging view of disc composition indicates that the majority of DAPs lack homology to both known MT-binding proteins and to proteins outside of *Giardia* species (TABLE 1) – including other diplomonads like *Spironucleus* [28].

In a recent proteomic analysis of detergent-extracted, isolated ventral discs, over twenty new candidate DAPs were identified that specifically localize to regions of the ventral disc or lateral crest [7]. In an ongoing GFP tagging project associated with the GiardiaDB [29], the number of DAPs has increased to closer to ninety (see TABLE 1). Like gamma-giardin [30], twenty-six DAPs lack any homology to proteins in other eukaryotes. One non-homologous DAP, median body protein (MBP, DAP16343), is associated with the disc spiral MT array, particularly with the overlap zone. MBP has been shown to be necessary for proper ventral disc biogenesis and function [31]. Close to thirty DAPs simply contain ankyrin repeat domains. Disc-associated ankyrin repeat proteins may contribute to disc assembly or architecture, as ankyrin repeat proteins are known to mediate protein-protein interactions, protein folding, and protein stability [32].

Some DAPs share homology with members of conserved protein families, including: three members of the striated fiber (SF)–assemblins (beta-giardin, delta-giardin, and SALP-1 [33]); four annexin family members (e.g., alpha-giardins [34–37]), and at least twelve NEK kinases (TABLE 1). The SF-assemblin homologs beta-giardin, delta-giardin, and SALP-1 [33] likely form the structural basis of the microribbons upon which other microribbon-associated proteins assemble [27] (see TABLE 1). Beta-giardin does not turn over following photobleaching, consistent with the hyperstable state of disc microtubules [38]. *Giardia* has an expanded repertoire of over 70 NEK kinases [39], and NEK kinases have been associated with the cytoskeleton in other eukaryotes [40]. Nine of the twelve disc-associated NEKs are putative pseudokinases that lack conserved catalytic residues, however may still retain kinase activity [7].

Despite the fact that many well-known MAPs (EB1, XMAP215, and katanin) and motors (kinesins and dyneins) are present in the *Giardia* genome [41], these proteins localize to the *Giardia* flagella or spindle, but not to the ventral disc (TABLE 1). Of the over eighty DAPs identified to date, only DAP5374, a CAP-Gly protein, has a conserved microtubule binding motif [42] and thus could interact with tubulin monomers, dimers, and MT lattices. Only one of the twenty-four *Giardia* kinesins – kinesin-6a (DAP102455) – localizes to the ventral disc, in the disc margin region. DAP16263, a homolog of DIP13, also localizes to the disc. DIP13 belongs to a MT-associated protein family conserved in diverse protists, plants, and animals that have flagellated cell stages [43, 44]. DIP13 homologs contain a conserved “KREE” binding motif that directly binds MTs [43]; however, the *Giardia* DIP13 homolog lacks this motif. In *Chlamydomonas*, DIP13 localizes to the centrioles and to cytoplasmic and flagellar MTs, and may stabilize or connect MTs to other cellular structures [43].

DAPs are primarily uncharacterized with respect to their microtubule binding or biochemical properties. Many known DAPs are likely components of the disc substructures (e.g., microribbons, crossbridges, sidearms, or paddles), whereas other DAPs may directly influence ventral disc MT dynamics including MT nucleation, MT + end binding, MT stability, and MT curvature and structure. DAPs likely generate and stabilize the curved spiral array of the ventral disc microtubules [23, 25]. Furthermore, DAPs may be required for the overall disc conformational dynamics and domed shape hypothesized to be necessary for parasite attachment [31]. Whether conserved MAPs play a role in ventral disc biogenesis or whether ventral disc biogenesis is governed by novel DAPs must also be determined. Given a high-resolution structure and a growing list of upwards of ninety disc proteins, the next steps in understanding the functioning of the ventral disc should include assigning disc proteins to the various substructures [23].

The complex ventral disc spiral MT array and associated structures (e.g., lateral crest) have evolved only in *Giardia* species. Complex cytoskeletal organelles like the disc could evolve by cooption, modification and elaboration of existing proteins or structures like flagella, or through the invention of new MT-binding proteins or other components. The sheer number of non-homologous proteins in the disc suggests that much of the complexity of the ventral disc has evolved through the invention of novel cytoskeletal proteins. The microribbon component of the ventral disc may be derived from ancestral flagellar structures as SF-assemblins are known to be associated with flagellar root structures in other protists [45] including the *Toxoplasma* apical complex [46].

Regional variation in the structure and composition of the ventral disc

Recently, Brown et al. [23] defined specific regional variations in the ventral disc architecture that, in concert with subcellular localization of DAPs [7], articulate distinct structural regions of the ventral disc (FIGURE 1). These variations include differences in the size and spacing of the substructures, as well as variations in protein densities within individual substructures. For example, microribbons vary in height (from 55 to about 120 nm) and their angles relative to microtubules change throughout the disc architecture [23]. Microribbons are entirely absent in the dense bands and are partially formed in the supernumerary MT array. The lateral packing of microtubule–microribbon complexes also varies substantially (about 25 nm spacing in the dense bands to about 80 nm in the disc body), and lateral packing distance may be governed by crossbridge extension or contraction [23] [20]. At the disc margin, microtubule–microribbon complexes may function as outer, laterally contractile lids that aid the disc in clamping onto the intestinal microvilli [23]. In the ventral groove region, located at the posterior of the disc, disc MTs lose much of their curvature [23]. Due to regional variations, a single microtubule can be coated with different protein densities in different disc regions, beginning at the dense band nucleation zone and terminating at the disc margin (FIGURE 1). The structural variation in the disc defined by cryo-ET is consistent with the distinct localization patterns of DAPs observed in our GFP screen. These localizations delineate the disc body (43 DAPs), the disc margin or lateral crest (43 DAPs), the overlap zone (26 DAPs), the ventral groove (18 DAPs), and the dense bands or supernumerary MTs (15 DAPs) (see FIGURE 1 and TABLE 1).

The disc is a rapidly assembled composite structure nucleated in several regions

Following ingestion of cysts by a host, *Giardia* excysts into a multi-flagellated cell – or trophozoite – that swims and proliferates in the gut, eventually attaching to the intestinal microvilli via the ventral disc. Trophozoites have four pairs of flagella with basal bodies located dorsal to the center of the disc spiral (see FIGURE 1), with the anterior flagellar pair exiting through the disc before exiting the cell body as membrane bound flagella. *Giardia* also has a semi-organized MT structure termed the median body. Recent live imaging of dividing *Giardia* supports the idea that the somewhat disordered MTs of the median body contribute to disc biogenesis [47], serving perhaps as a reservoir of polymerized MTs for spindle assembly, disc biogenesis, and ciliogenesis [27, 47]. In contrast to the disc MT array, both the median body and flagella do exhibit microtubule dynamics during interphase [7, 13].

Giardia rapidly divides in laboratory culture. Mitosis occurs in 6.5 minutes and new daughter discs and new flagella are assembled in less than three minutes [47]. The amount of polymerized tubulin is nearly tripled in dividing cells [23]. During mitosis, trophozoites remain attached from the onset of cell division through the assembly of the new daughter discs [48, 49]. In late mitosis, the parental disc undergoes dramatic structural changes, leading to parental ventral disc disassembly and detachment prior to the late stages of cytokinesis. Before parental disc disassembly occurs, the two daughter discs are assembled *de novo* on the anterior dorsal side of the attached parent cell, with their ventral sides exposed on the parental cell surface [49]. Assembly of daughter discs is thought to terminate after the detachment of the dividing cell [49].

Following mitosis, the ventral disc appears to be rapidly nucleated in at least four ways. Recent cryo-ET studies indicated that about 59% of ventral disc microtubules are nucleated near the eight basal bodies [23]. Disc MT minus ends do not directly contact basal bodies but rather arise from a series of perpendicular bands termed the dense band (DB) nucleation zone [23]. The protein composition of these dense bands and the mechanism by which they support MT nucleation is undefined, although we have identified several proteins localizing to this region (FIGURE 1). *Giardia* lacks some components of the gamma-TuRC nucleation complex yet retains the two gamma-TuSC components and gamma tubulin [50]. Despite lacking an augmin homolog, about 39% of disc MTs nevertheless nucleate from the disc margin (DM) region, possibly via a branching nucleation-type mechanism [23]. A small subset of MTs (~2%) is nucleated within the disc MT array itself. Lastly, an additional subset of about 20 MTs nucleate from a distinct yet overlapping array of dense bands dorsal to the ventral disc, termed the supernumerary MTs (SN). This array is hypothesized to nucleate a new ventral disc during cell division, but this hypothesis fails to fully explain ventral disc biogenesis because two new discs are generated instead of one [49].

The mechanism underlying the synchronized bending of newly growing disc microtubules and the control of their length is also unknown. During dorsal daughter disc assembly, the MT spiral is nucleated first, with subsequent assembly and lengthening of the disc microribbons. The lateral crest is the last of the disc substructures to be assembled [49].

Assembling daughter discs appear to have varying levels of competence for attachment. As daughter discs assemble, the parental disc opens and the spiral MT array disassembles. This process is accompanied by the progressive shortening and loss of the microribbons and the degradation of crossbridges. The final release of the disc from the basal bodies coincides with parental disc disassembly, and results in parasite detachment [47, 49].

Dividing trophozoites not only need to build new daughter discs, but must also assemble other MT-based structures including two spindles and eight new flagella. Thus, the ventral disc MTs must be distinguished from the MTs of other MT arrays to properly recruit proteins required for the assembly of disc substructures. One obvious way that ventral disc MTs could be marked is by tubulin post-translational modifications (PTMs) [51], which could mediate the recruitment of DAPs to the nucleating disc during cell division. Disc substructures assemble sequentially on two daughter disc MT arrays in mitosis and excystation, yet the molecular details of this process are unclear [49, 52]. Several regulatory proteins localize to the disc during division, including the sole *Giardia* aurora kinase [53], two putatively cell cycle-specific NEK kinases [54], and an ERK1 kinase that localizes to the disc during encystation [55]. Understanding how the ventral disc is assembled and which substructures and associated DAPs are essential for functional competency is critical for selecting potential druggable disc targets that may disrupt attachment and parasite colonization.

During colonization of the host, key developmental cues cause trophozoites to develop into infectious cysts, and the disc is disassembled into fragments that have been observed to persist within mature cysts [49]. Cysts are eventually shed and persist in the environment to infect new hosts [56, 57]. The assembly of daughter discs also occurs during excystation [52], but the cytological details of disc assembly during this stage remain unknown.

How does the complex ventral disc mediate attachment?

Conflicting biophysical data [16, 58–64] and incomplete knowledge of ventral disc composition [14] have made the evaluation of any proposed attachment mechanism at the molecular and cellular levels problematic [14, 65]. The “hydrodynamic model” was the predominant proposal to explain how a negative pressure differential resulting in suction-based attachment might be produced under the disc [58]. This model invokes the continual beating of the ventral flagella to create a hydrodynamic suction for attachment. We recently demonstrated that flagellar motility is not directly required to maintain attachment forces. Thus ventral flagellar beating merely coincides with, rather than causes, attachment [15]. Flagellar motility is required, however, for early stages of attachment, including site recognition and orientation [15].

Attachment occurs in seconds via a stepwise process defined by the degree of *Giardia* cellular contact with an inert surface (FIGURE 3 and [15]). The stages of attachment were defined using TIRF microscopy of *Giardia* trophozoites stained with a fluorescent membrane marker [15]. The earliest attachment stages include skimming and mechanosensory contact with the surface via the ventrolateral flange, followed by the formation of a seal. Additional contacts of the plasma membrane with the surface occur

later, including lateral shield and bare area contacts (FIGURE 3). The bare area region, lacking MTs, is located in the center of the disc array, and contains numerous membrane-bound vacuoles [22]. Various conformational changes in the disc may be required for either early or late stage suction-based attachment [15] [66]. The rigid structure of the ventral disc could also indirectly contribute to attachment by maintaining a negative pressure differential created by some other unknown mechanism such as an osmotic pressure differential-based mechanism [16, 22, 59].

Seal formation during attachment is likely mediated by the lateral crest [15], a repetitive structure on the outer edge of the ventral disc that is composed of a network of fibers of up to 43 DAPs [17, 22] [7]. Lateral crest DAPs, like other DAPs, are primarily proteins that are unique to *Giardia* or possess ankyrin repeat or NEK kinase domains (e.g., DAP13981). Lateral crest contraction has not been observed *in vivo* [15]. Actin was initially reported to localize to the lateral crest and periphery of the disc using heterologous (anti-chicken) antibodies [17], yet this is likely an artefactual localization due to the divergence of the *Giardia* actin gene [14, 41]. The subsequent use of *Giardia*-specific actin antibodies [67] indicated that actin does not localize to the ventral disc or the lateral crest. Other actin-associated genes such as myosin or vinculin are not present in *Giardia*, yet these proteins were also initially reported to localize to the lateral crest using heterologous antibodies [41, 68, 69].

How does a microtubule structure lacking dynamic instability generate attachment forces? While the exogenous addition of ATP to isolated *Giardia* cytoskeletons is sufficient to drive flagellar beating, exogenous addition of ATP does not result in disc conformational dynamics [70]. Suction-based forces could theoretically be generated directly via an overall conformational change of the ventral disc from a flattened to a domed shape, resulting in a negative pressure differential relative to the outside medium [31]. If the disc substructures (e.g., microribbons, crossbridges, sidearms) are flexible, subtle substructure movements could be sufficient to generate the conformational changes required for the initiation and maintenance of attachment in the absence of canonical MT dynamics (FIGURE 3). For example, knockdown of MBP (DAP16343) results in cells with an open and flattened ventral disc conformation that are unable to proceed to later stages of attachment, supporting the notion that early disc conformational changes generate a negative pressure differential underneath the disc [59]. MBP associates specifically with the disc body, disc margin and overlap zone, as well as the median body, and the aberrant disc conformations observed after MBP knockdown are presumably the result of MBP depletion during disc biogenesis. A dome-shaped disc might also be required for proper lateral crest seal formation [15] in early stages of attachment.

Concluding remarks

Given the finite and relatively small number of known proteins that regulate microtubule dynamics and assembly, how do diverse eukaryotic cells create elaborate microtubule structures? We are at the very early stages of understanding the principles governing the extreme variation in cytoskeletal organelle assembly and function (see Outstanding Questions). The complex architecture and functional abilities of the ventral disc challenges

our conceptions of the capabilities of cytoskeletal polymers. At least with respect to the *Giardia* ventral disc, the intricate architecture is primarily composed of novel, non-homologous proteins. The function and mechanism by which regional variation in disc proteins is generated is unknown; however, the invention of novel microtubule binding or nucleation properties may facilitate the assembly of microtubule polymers into unique arrays and organelles with new functions. In this emerging model system, the ongoing development of molecular genetic and biochemical tools [26, 71, 72] will be central toward understanding not only disc architecture and assembly, but the overall disc conformational dynamics that promote attachment to the host.

Glossary

Ventral disc
the *Giardia* cup-like microtubule organelle that mediates attachment to the host epithelium or to inert surfaces

Microribbons
trilaminar sheets composed of SF-assemblins that that extend dorsally up to several hundred nanometers into the cytoplasm along the length of disc microtubules

Crossbridges
structures that laterally connect microribbons at regular 16 nm intervals, and maintain regular spacing of the MT spiral array

Side-arms
margin-facing repetitive protein densities associated with three of the disc MT protofilaments and attached to the paddles.

Paddles
margin-facing repetitive protein densities attached to the side-arms and one of the protofilaments of the disc MTs.

Disc body
the main microtubule array of the ventral disc with associated substructural elements

Disc margin (DM)
the outer edge of the ventral disc MT array characterized by MT plus ends

Overlap zone (OZ)
also characterized by MT plus ends, this overlapping region of the ventral disc spiral MT array has shortened microribbons.

Lateral crest (LC)
a repetitive fibrillar structure surrounding the periphery of the ventral disc margin that contacts and forms a seal with the attachment surface.

Ventral groove (VG)
a posterior region of the ventral disc with convex curvature under the exiting ventral flagella.

Ventrolateral flange (VLF)
membrane region at the anterior to the ventral disc that contacts the attachment surface and has proposed adhesive properties.

Lateral shield (LS)
cell body regions on opposite sides of the ventral flagella that contact the substrate during later stages of attachment.

Disc associated protein (DAP)
protein with localization to some region of the ventral disc

Median body
a semi-organized microtubule array in *Giardia* that is hypothesized to be a reservoir for the ventral disc microtubules

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Trends Box

- *Giardia*'s ventral disc is a complex microtubule-based organelle composed of many unique structural elements
- Most of the 85 disc-associated proteins (DAPS) lack homology to known microtubule-binding proteins
- The disc has a composite, varied architecture that is nucleated in several regions
- The disc is "hyperstable" and lacks interphase microtubule dynamics
- Disc-mediated attachment of *Giardia* to the host epithelium may be associated with conformational changes of the disc structure

Outstanding Questions

- What are the molecular identities of the unique disc substructures (e.g., crossbridges, sidearms, paddles)?
- How is the ventral disc nucleated and rapidly assembled during cell division?
- How is the disc “hyperstability” generated and maintained?
- What is the mechanism of disc-mediated attachment to surfaces?
- What do regional variations in substructure composition and size contribute to ventral disc functioning?
- Has the complex ventral disc organelle evolved independently or is it derived from existing organelles?

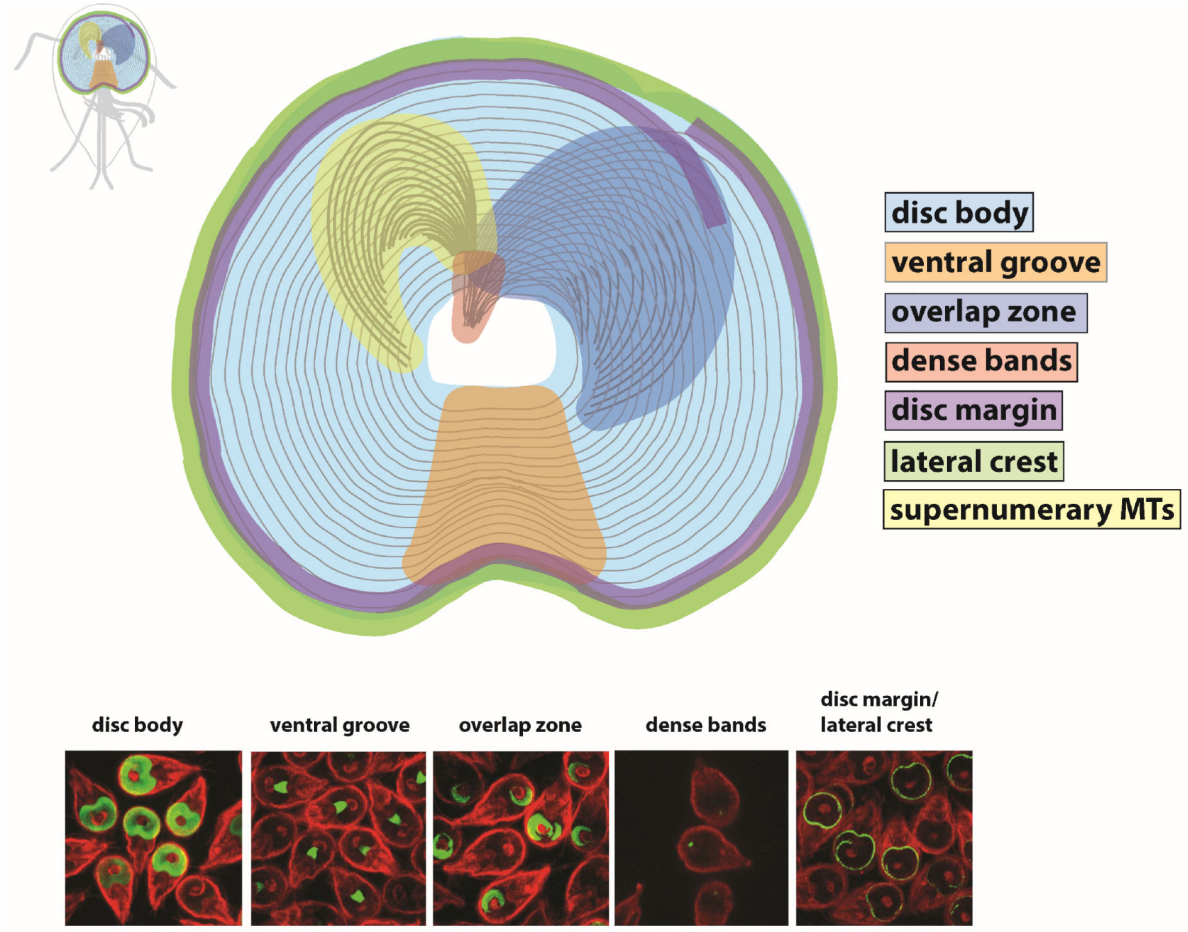

FIGURE 1. Regional variation in the structure and composition of the ventral disc.

Schematic representation of the ventral disc ultrastructure as determined using high-resolution cryo-ET. Compositionally varied regions of the disc are colored and include: the disc body, ventral groove, overlap zone, dense bands, disc margin, supernumerary microtubules and lateral crest. The overlap zone as defined here includes both the ventral and dorsal regions of the overlap zone [23]. Images show representative DAP-GFP fusions (DAP = green, membrane = red) for five regions (disc body (DAP86676), ventral groove (DAP11554), overlap zone (DAP40016), dense bands (DAP20688), and disc margin/lateral crest (DAP17096).

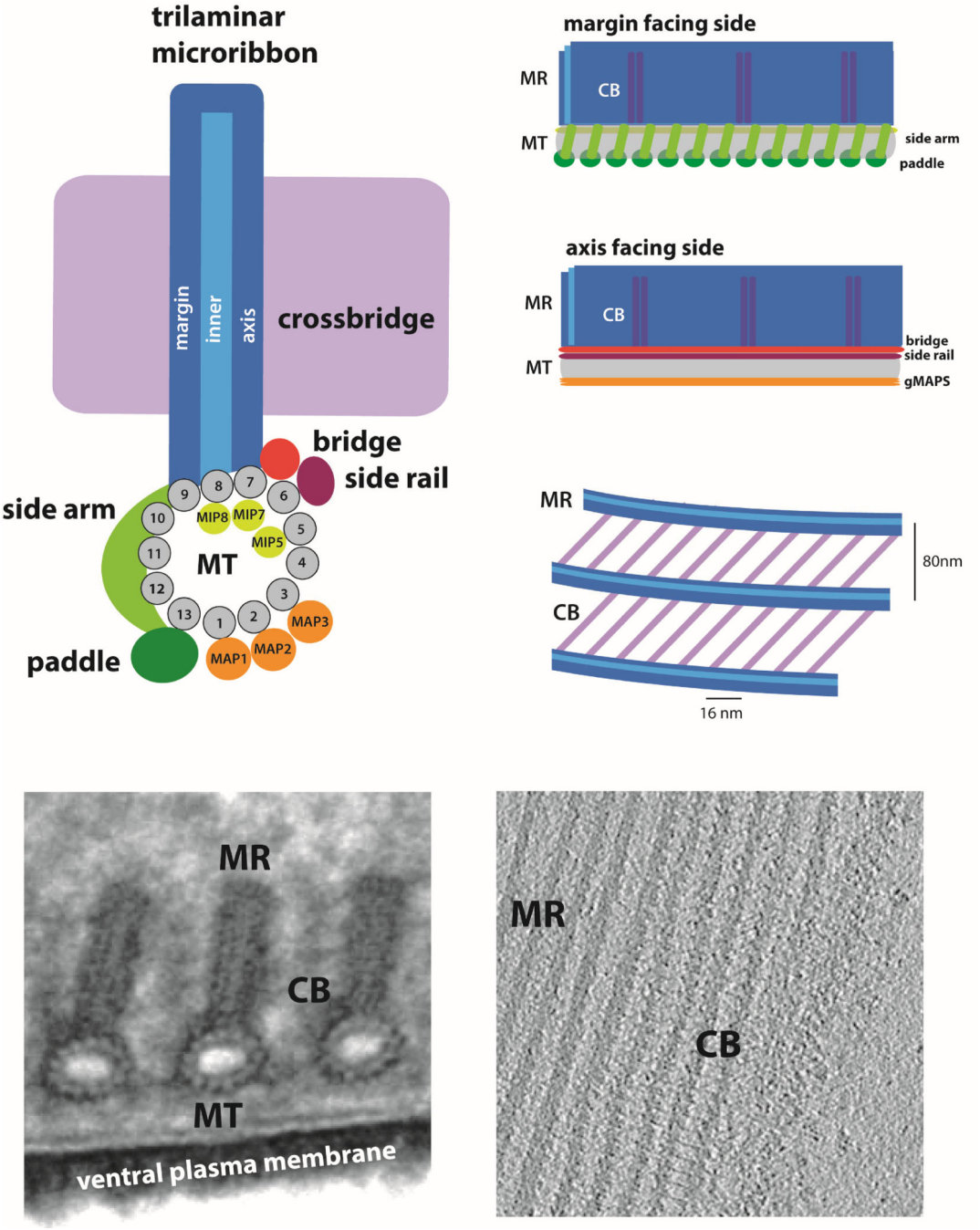

FIGURE 2. Dense protein complexes coat the protofilaments of the microtubule spiral forming the complex disc architecture

The dense protein complexes (or substructures) coating the disc MT protofilaments (numbered 1–13) are shown in the schematic (upper left). This slice through the ventral disc structure shows the conspicuous trilaminar microribbons (MR) that extend dorsally into the cytoplasm from three protofilaments (numbered 7, 8, 9). Microribbons are laterally connected by crossbridges (CB). Other protofilaments (1–3) are coated with MT binding proteins named gMAP1–3. The bridge, side rail, side arm and paddle complexes are associated with six other protofilaments [25]. The three MT inner proteins (gMIP5, 7, 8) are

also noted. The asymmetry of the substructures is seen in a comparison of the margin and axis-facing sides of the disc spiral (upper right). The lower panels show a slice through the ventral disc structure (TEM, lower left) and a top down view (cryo-ET, lower right) of the regularly spaced microribbons (MR) and repetitive crossbridges (CB).

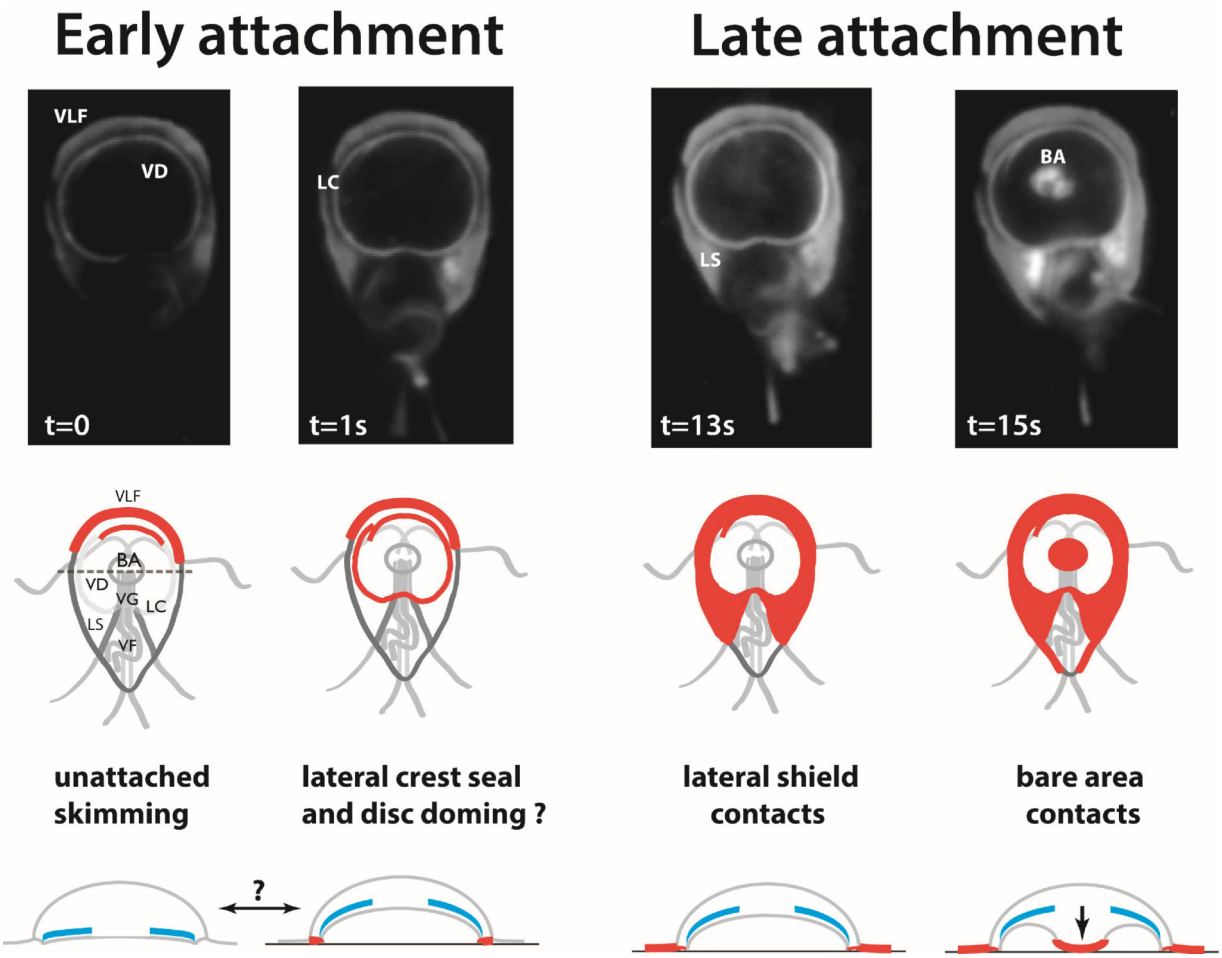

FIGURE 3: Attachment occurs in stages defined by surface contacts

TIRF time-lapse imaging of membrane-stained trophozoites contrasts the initiation of attachment (early, t=0–1s) with maintenance of attachment (late, t=13–15s). Stages of attachment are classified by surface contacts of the ventral disc (VD). Early attachment includes skimming and ventrolateral flange (VLF) contact, and the lateral crest (LC) seal. Late attachment is defined by surface contacts of the lateral shield (LS) and the bare area (BA). The schematics indicate ventral surface contacts (red) and potential conformational states and movements (arrows) of the ventral disc (blue) during early and late stages of attachment.

TABLE 1.

Confirmed *Giardia* ventral disc-associated proteins (DAPs)

GiardiaDB is a member of pathogen-databases that are housed under the NIAID-funded EuPathDB Bioinformatics Resource Center (BRC) umbrella.

GiardiaDB	Annotation	PFAM	Verified Substructure Localization	disc body	Disc Margin (DM) or Lateral Crest (LC)	Overlap Zone (OZ)	Ventral Groove (VG)	Dense Bands (DB) or Supernumerary Array (SA)	Localization	REF
GL50803_137684	ankyrin repeat protein	ankyrin	n/a	disc	DM	n/a	n/a	DB	C-term GFP	[29]
GL50803_5188	ankyrin repeat protein	ankyrin	n/a	disc	n/a	OZ	VG	n/a	C-term GFP	[29]
GL50803_103807	ankyrin repeat protein	ankyrin	n/a	disc	n/a	OZ	n/a	n/a	C-term GFP	[7]
GL50803_7268	ankyrin repeat protein	ankyrin	n/a	disc	n/a	OZ	n/a	n/a	C-term GFP	[29]
GL50803_13766	ankyrin repeat protein	ankyrin	n/a	disc	n/a	OZ	n/a	n/a	C-term GFP	[7]
GL50803_17053	ankyrin repeat protein	ankyrin	n/a	disc	n/a	OZ	n/a	n/a	C-term GFP	[7]
GL50803_14859	ankyrin repeat protein	ankyrin	n/a	disc	n/a	OZ	n/a	n/a	C-term GFP	[29]
GL50803_9515	ankyrin repeat protein	ankyrin	n/a	disc	n/a	OZ	n/a	n/a	C-term GFP	[29]
GL50803_40016	ankyrin repeat protein	ankyrin	n/a	disc	n/a	OZ	n/a	n/a	C-term GFP	[29]
GL50803_17551	ankyrin repeat protein	ankyrin	n/a	disc	n/a	OZ	n/a	n/a	C-term GFP	[29]
GL50803_112557	ankyrin repeat protein	ankyrin	n/a	disc	n/a	OZ	n/a	n/a	C-term GFP	[29]
GL50803_7414	ankyrin repeat protein	ankyrin	n/a	disc	n/a	n/a	VG	n/a	C-term GFP	[29]
GL50803_3760	ankyrin repeat protein	ankyrin	n/a	disc	n/a	n/a	n/a	n/a	C-term GFP	[29]
GL50803_12139	ankyrin repeat protein	ankyrin	n/a	n/a	DM	n/a	VG	n/a	C-term GFP	[29]
GL50803_14872	ankyrin repeat protein	ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[7]

GiardiaDB	Annotation	PFAM	Verified Substructure Localization	disc body	Disc Margin (DM) or Lateral Crest (LC)	Overlap Zone (OZ)	Ventral Groove (VG)	Dense Bands (DB) or Supernumerary Array (SA)	Localization	REF
GL50803_17096	ankyrin repeat protein	ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[7]
GL50803_17097	ankyrin repeat protein	ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[7]
GL50803_24194	ankyrin repeat protein	ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[7]
GL50803_10219	ankyrin repeat protein	ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_14800	ankyrin repeat protein	ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_8850	ankyrin repeat protein	ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_16843	ankyrin repeat protein	ankyrin	n/a	n/a	n/a	n/a	n/a	DB	C-term GFP	[29]
GL50803_13590	ankyrin repeat protein	ankyrin	n/a	n/a	n/a	n/a	n/a	DB	C-term GFP	[29]
GL50803_103810	ankyrin repeat protein	ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[7]
GL50803_15576	ankyrin repeat protein	ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[7]
GL50803_23492	ankyrin repeat protein	ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[7]
GL50803_14681	ankyrin repeat protein	ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_15410	Ser/Thr protein kinase	ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_11683	alpha3-giardin	annexin	n/a	disc	DM	n/a	n/a	n/a	AU1 tag	[34]
GL50803_15101	alpha17-giardin	annexin	n/a	disc	n/a	n/a	n/a	n/a	AU1 tag	[34]
GL50803_7797	alpha5-giardin	annexin	n/a	disc	n/a	n/a	n/a	n/a	AU1 tag	[34]
GL50803_7796	alpha2-giardin	annexin	n/a	disc	n/a	n/a	n/a	n/a	C-term GFP	[15]
GL50803_5010	Ser/Thr Phos PP2A	calcineurin-like phosphoesterase	n/a	disc	n/a	n/a	n/a	n/a	antibody	[73]

GiardiaDB	Annotation	PFAM	Verified Substructure Localization	disc body	Disc Margin (DM) or Lateral Crest (LC)	Overlap Zone (OZ)	Ventral Groove (VG)	Dense Bands (DB) or Supernumerary Array (SA)	Localization	REF
GL50803_5374	tubulin-specific chaperone B	CAP-GLY	n/a	disc	DM	OZ	VG	n/a	C-term GFP	[7]
GL50803_5568	DUF866 domain protein	DUF866	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_3256	epsin	ENTH	n/a	disc	DM	n/a	n/a	n/a	HA tag	[74]
GL50803_4912	Nek kinase GK265	kinase	n/a	disc	n/a	OZ	n/a	n/a	C-term GFP	[29]
GL50803_4977	Nek kinase GK282	kinase	n/a	disc	n/a	n/a	VG	n/a	C-term GFP	[29]
GL50803_24321	Nek kinase GK261	kinase	n/a	disc	n/a	n/a	n/a	n/a	C-term GFP	[7]
GL50803_5358	aurora kinase	kinase	n/a	disc	n/a	n/a	n/a	n/a	antibody	[53]
GL50803_16279	Nek kinase GK256	kinase	n/a	disc	n/a	n/a	n/a	n/a	AU1 tag	[75]
GL50803_92498	Nek kinase GK270	kinase	n/a	disc	n/a	n/a	n/a	n/a	AU1 tag	[75]
GL50803_17563	ERK1 kinase	kinase	n/a	n/a	DM	n/a	VG	n/a	antibody	[55]
GL50803_10893	Nek kinase GK193	kinase	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_11554	Nek kinase GK249	kinase	n/a	n/a	n/a	n/a	VG	n/a	C-term GFP	[29]
GL50803_13981	Nek kinase GK185	kinase, ankyrin	LC	n/a	DM	n/a	VG	n/a	C-term GFP	[7]
GL50803_16272	Nek kinase GK187	kinase, ankyrin	n/a	n/a	DM	OZ	VG	DB	C-term GFP	[29]
GL50803_3957	NEK kinase GK212	kinase, ankyrin	n/a	n/a	DM	n/a	VG	n/a	C-term GFP	[29]
GL50803_17231	Nek kinase GK186	kinase, ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[7]
GL50803_11775	Nek kinase GK301	kinase, ankyrin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_102455	kinesin 6 GiKIN6a	kinesin	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_16424	Mlf1IP domain protein	Mlf1IP	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[7]
GL50803_16343	median body protein	none	MT/OZ	disc	DM	OZ	VG	n/a	C-term GFP	[7]
GL50803_17230	gamma giardin	none	MR	disc	n/a	OZ	n/a	n/a	antibody	[30]
GL50803_24537	hypothetical protein	none	n/a	disc	DM	OZ	n/a	DB	C-term GFP	[29]

GiardiaDB	Annotation	PFAM	Verified Substructure Localization	disc body	Disc Margin (DM) or Lateral Crest (LC)	Overlap Zone (OZ)	Ventral Groove (VG)	Dense Bands (DB) or Supernumerary Array (SA)	Localization	REF
GL50803_4852	hypothetical protein	none	n/a	disc	DM	n/a	n/a	n/a	HA tag	[76]
GL50803_4239	hypothetical protein	none	n/a	disc	n/a	OZ	VG	n/a	C-term GFP	[29]
GL50803_5883	hypothetical protein	none	n/a	disc	n/a	OZ	n/a	DB	C-term GFP	[29]
GL50803_3934	hypothetical protein	none	n/a	disc	n/a	OZ	n/a	n/a	C-term GFP	[29]
GL50803_10524	hypothetical protein	none	n/a	disc	n/a	OZ	n/a	n/a	C-term GFP	[29]
GL50803_86815	hypothetical protein	none	n/a	disc	n/a	n/a	VG	DB	C-term GFP	[29]
GL50803_15918	hypothetical protein	none	n/a	disc	n/a	n/a	VG	n/a	C-term GFP	[29]
GL50803_10181	hypothetical protein	none	n/a	disc	n/a	n/a	n/a	n/a	C-term GFP	[29]
GL50803_2556	hypothetical protein	none	n/a	disc	n/a	n/a	n/a	n/a	C-term GFP	[29]
GL50803_17412	hypothetical protein	none	n/a	n/a	DM	OZ	VG	n/a	C-term GFP	[29]
GL50803_6751	hypothetical protein	none	n/a	n/a	DM	n/a	VG	n/a	C-term GFP	[29]
GL50803_6171	hypothetical protein	none	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_6709	hypothetical protein	none	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_7520	hypothetical protein	none	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_16263	conserved hypothetical protein	none	n/a	n/a	n/a	n/a	n/a	DB	C-term GFP	[7]
GL50803_15499	hypothetical protein	none	n/a	n/a	DM	n/a	n/a	DB	C-term GFP	[29]
GL50803_33866	hypothetical protein	none	n/a	n/a	n/a	n/a	n/a	DB	C-term GFP	[29]
GL50803_10232	hypothetical protein	none	n/a	n/a	n/a	n/a	n/a	DB	C-term GFP	[29]
GL50803_13651	hypothetical protein	none	n/a	n/a	n/a	n/a	n/a	DB	C-term GFP	[29]
GL50803_16342	hypothetical protein	none	n/a	n/a	n/a	n/a	n/a	DB	C-term GFP	[29]
GL50803_101326	hypothetical protein	none	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_16935	hypothetical protein	none	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_4410	SALP-1	SF assemblin	MR	disc	n/a	OZ	n/a	n/a	C-term GFP	[29]

GiardiaDB	Annotation	PFAM	Verified Substructure Localization	disc body	Disc Margin (DM) or Lateral Crest (LC)	Overlap Zone (OZ)	Ventral Groove (VG)	Dense Bands (DB) or Supernumerary Array (SA)	Localization	REF
GL50803_86676	delta-giardin	SF-assemblin	MR	disc	n/a	OZ	n/a	n/a	C-term GFP	[29]
GL50803_4812	beta giardin	SF-assemblin	MR	disc	n/a	OZ	n/a	n/a	antibody	[77]
GL50803_103164	SHIPPO repeat family protein	SHIPPO-repeat	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_9148	SHIPPO repeat family protein	SHIPPO-repeat	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]
GL50803_101291	beta-tubulin 1	tubulin	MT	disc	DM	OZ	VG	DB	antibody	[78]
GL50803_112079	alpha-tubulin 2	tubulin	MT	disc	DM	OZ	VG	DB	antibody	[78]
GL50803_15218	WD-40 repeat protein	WD40	n/a	n/a	DM	n/a	n/a	n/a	C-term GFP	[29]