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Functions of myosin motors tailored for parasitism

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Myosin motors are one of the largest protein families in eukaryotes that exhibit divergent cellular functions. Their roles in protozoans, a diverse group of anciently diverged, single celled organisms with many prominent members known to be parasitic and to cause diseases in human and livestock, are largely unknown. In the recent years many different approaches, among them whole genome sequencing, phylogenetic analyses and functional studies have increased our understanding on the distribution, protein architecture and function of unconventional myosin motors in protozoan parasites. In Apicomplexa, myosins turn out to be highly specialized and to exhibit unique functions tailored to accommodate the lifestyle of these parasites.

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Introduction

Protozoa form a highly diverse assemblage of unicellular organisms, found throughout the eukaryotic tree of life [1]. Some of them are classified as human or animal pathogens and have received much attention due to their medical importance. These include the amoeba *Entamoeba histolytica* (Amoebiasis), the excavates *Trichomonas vaginalis* (Trichomoniasis), *Giardia lamblia* (Giardiasis), *Leishmania spp.* (Leishmaniasis including 'Kala-azar'), *Trypanosoma brucei* (Sleeping sickness and Nagana) and *Trypanosoma cruzi* (Chagas disease), as well as the well-known parasites within the phylogenetic group of Alveolata including *Plasmodium falciparum* (Malaria), *Toxoplasma gondii* (Toxoplasmosis), *Eimeria tenella* (Coccidiosis) and *Cryptosporidium parvum* (Cryptosporidiosis).

Myosins are actin-dependent motors that convert adenosine triphosphate (ATP) into mechanical energy (reviewed by [2]). They are composed of a myosin heavy chain (MHC) that consists in a conserved N-terminal globular head or motor domain that contains the actin and ATP binding sites and is responsible for the ATPase activity. The following neck region contains a varying number of IQ motifs to which myosin light chains (MLCs), that is, calmodulin (CaM) or CaM-like proteins bind. MLCs stabilize the lever arm and amplify the movement generated during the ATP hydrolysis cycle. The neck is followed by a carboxy-terminal tail region of variable length, which often contains an α-helical stretch responsible for MHC dimerization and a globular domain that features functional motifs and is responsible for cargo binding and myosin localization.

Myosin motors are among the largest and best studied protein families in eukaryotes that exhibit a broad range of cellular functions. Several phylogenetic analyses of myosin motors have led to their classification and the reconstruction of their evolutionary diversification [3–5]. The apicomplexan myosins were placed into several distinct classes encompassing myosins from other systematic lineages (classes VI, XXII, XXIII, XXIV). In addition, class XIV was found to no longer accommodate only apicomplexan myosins but also myosins from the ciliate *Tetrahymena thermophila* belonging to Alveolates. Characterization of this broader repertoire of motors led to the identification of intriguing protein domains not previously associated with myosins and their functions [3].

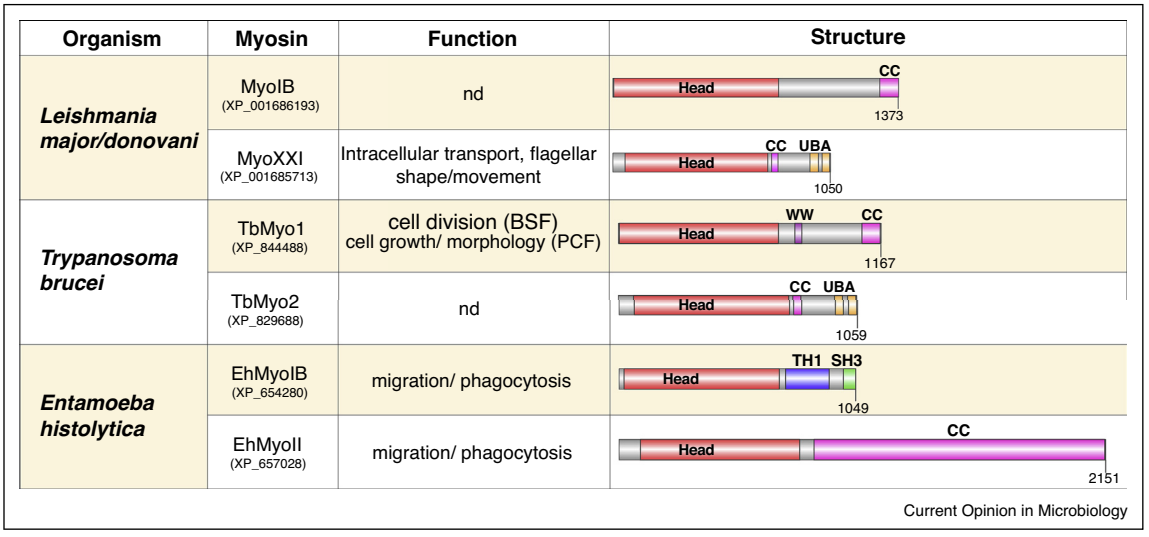
In the recent years, numerous studies have uncovered the biological function of these unconventional myosins in protozoan parasites. This review aims to provide an integrated view on the functional roles of these myosins. It focuses primarily on the Apicompexans for which the myosin phylogenetic tree that now also includes two closely related photosynthetic chromerids *Chromera velia* and *Vitrella brassicaformis*, was updated [6••] (Table S1).

Main body

Some protozan parasites rely on no or only a limited set of myosins

Unintuitively, myosin motors are not a prerequisite for eukaryotic life. A few species, among them the protozoan

Figure 1



Schematic representation of myosins from Entamoeba and Trypanosomatids. The scheme describes myosins of protozoan parasites that are discussed in the text and do not belong to the phylum Apicomplexa: *Leishmania major* and *Leishmania donovani* (Lm/LdMyoIB, Lm/LdMyoXXI), *T. brucei* (TbMyo1, TbMyo2), and *E. histolytica* (EhMyoIB, EhMyoII). The function and domain architecture of the different myosins is also described. BSF (bloodstream form), PCF (procyclic form), CC (coiled-coil), UBA (ubiquitin-associated domains), WW (WW domain), TH1 (Motor and Tail Homology 1 Domain), SH3 (SRC Homology 3 Domain), nd (not defined). Accession numbers are between brackets.

parasites *G. lamblia* and *T. vaginalis*, do not contain any myosin coding gene [4]. Other pathogenic eukaryotes possess very few myosins, such as *Naegleria fowleri* (Excavata) that causes primary amoebic meningoencephalitis in humans and for which only one MHC with unknown function has been discovered.

The amoeba *E. histolytica* is a human pathogen that possesses only a myosin II and an unconventional myosin IB that exhibits the same domain architecture as other class I myosins (Figure 1) [7,8]. EhMyoIB is an intriguing motor also able to cross-link actin filaments [9]. EhMLC1 (or calcium binding protein CaBP20) has been shown to colocalize and interact with EhMyoII [10,11]. In addition, *E. histolytica* encodes 27 CaBPs and at least two of them, EhCaBP3 and EhCaBP5, have been shown to interact with EhMyoIB [12,13].

Leishmania species possess two myosins that are assigned to class IB and to the kinetoplastid specific class XXI, respectively. While no expression of myosin IB could be detected, *Leishmania major* MyoXXI has been localized to the proximal region of the flagellum in the promastigote (insect form) [14]. LmMyoXXI can adopt a monomeric or dimeric state, and is crucially required for flagellar assembly and elongation and for intracellular trafficking [15,16].

T. brucei possesses two myosins belonging to classes I (TbMyo1) and XXI (TbMyo2). TbMyo1 is an unusual class I myosin that localizes to the polarized endocytic pathway in the bloodstream form and is evenly distributed throughout the procyclic form (insect from) [17]. Knockdown of TbMyo1 impacts on cell growth and morphology in the bloodstream but not in the procyclic form (Figure 1) [17,18].

Functional data on myosins of *T. cruzi* are missing. It contains one class I, six class XXI and one non-classified myosin [3,19].

Importantly, the absence or the limited number of myosins in these eukaryotes is compensated by alternative mechanisms based on flagellar functions that ensure fundamental processes such as cell motility and cytokinesis [20,21••].

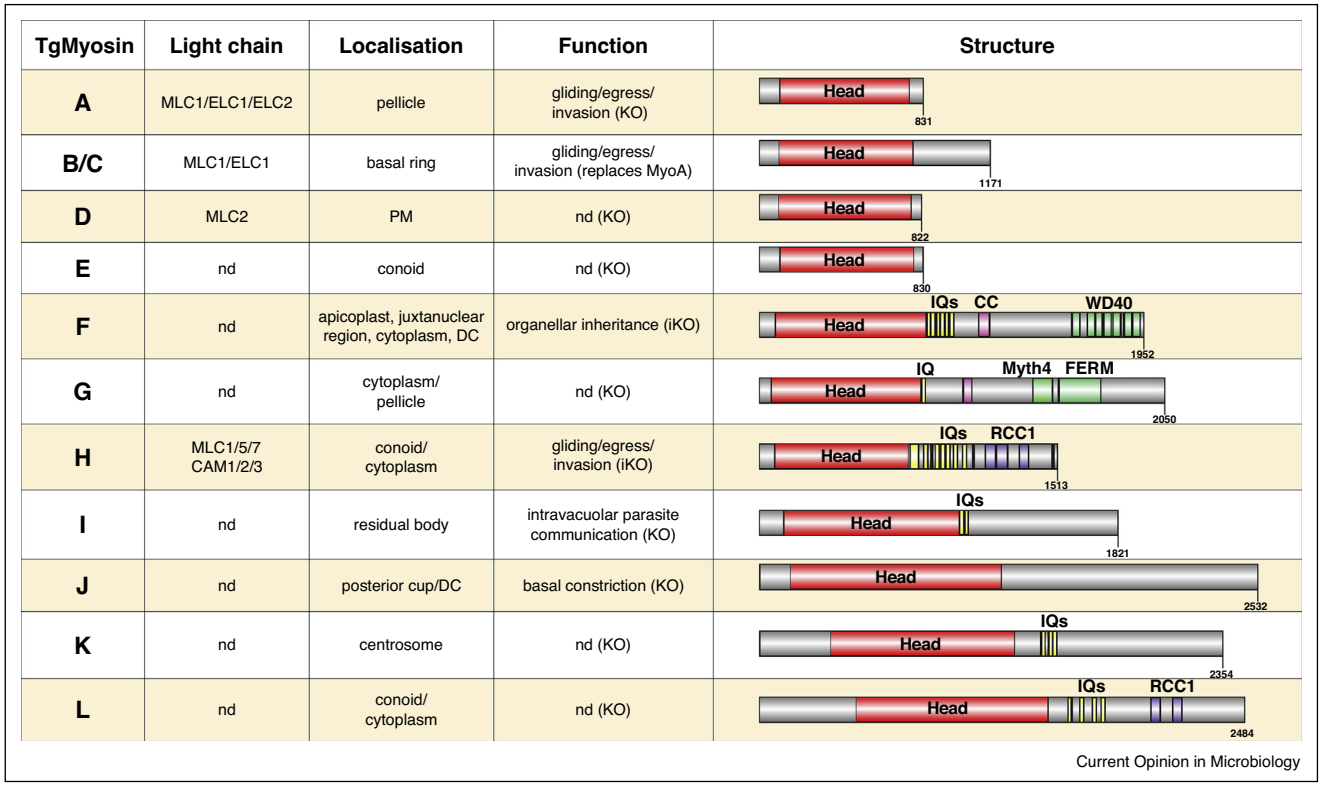
The Apicomplexans possess several classes of divergent myosin motors

Among the Apicomplexans, *T. gondii* possesses the largest repertoire of 11 myosins that are implicated in distinct biological processes: motility, positioning, trafficking and inheritance of organelles, basal pole constriction and cell–cell communication (Figure 2).

Glideosome-associated myosins of Apicomplexa

The Apicomplexans exhibit a substrate dependent mode of locomotion, which is a prerequiste for traversing biological barriers, host cell invasion and egress. The class XIV myosin A (TgMyoA) is conserved across the phylum and responsible for gliding motility (Figures 2–4) [22–25]. TgMyoA is a fast, nonprocessive, single-headed motor

Figure 2

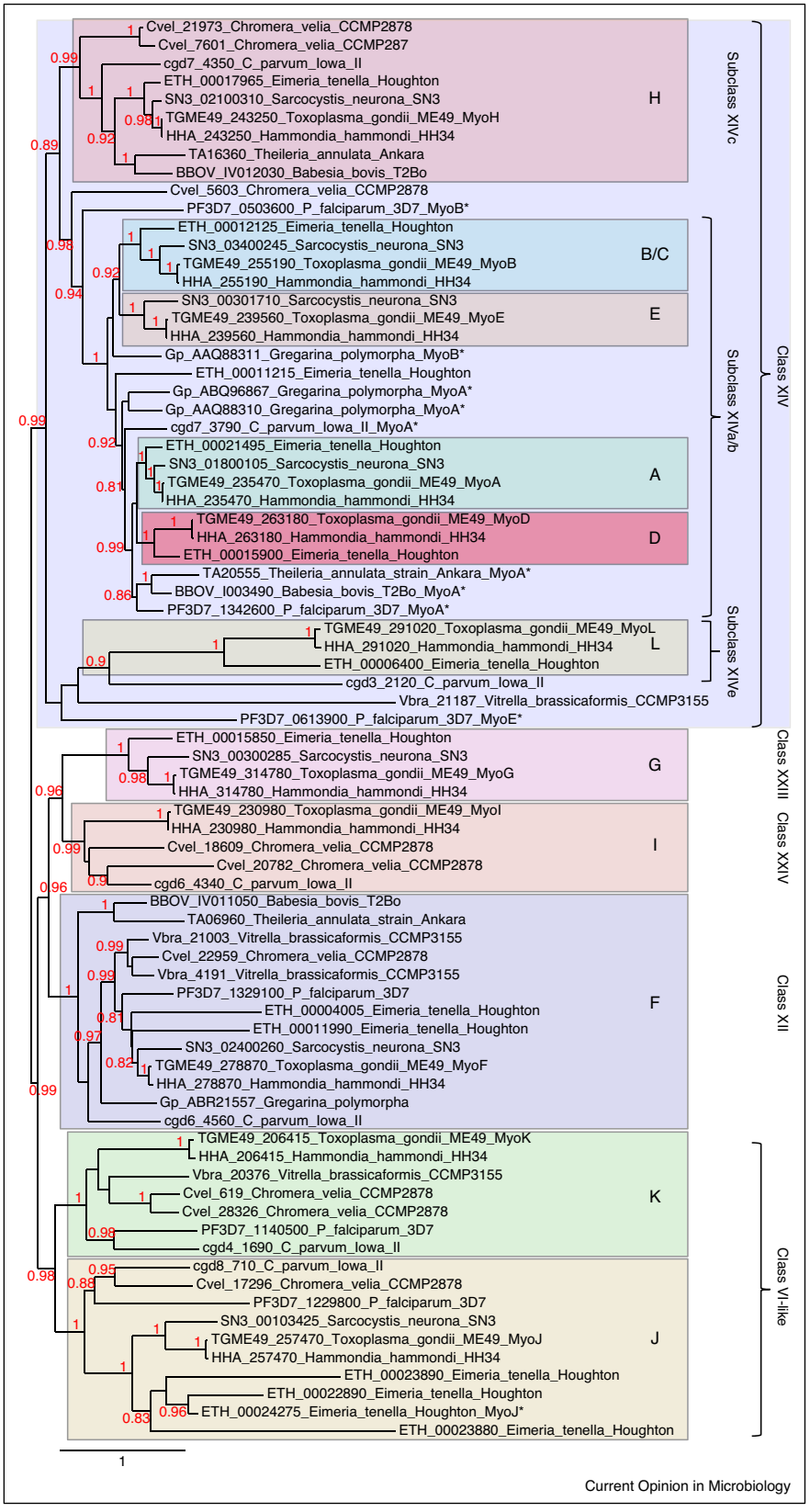


Overview of the repertoire of *T. gondii* myosins. The scheme represents the eleven myosin motor proteins present in *T. gondii*, their light chains, localization, function and structural arrangement. MLC (myosin light chain), ELC (essential light chain), CaM (calmodulin-like protein), IMC (innermembrane complex), PM (plasma membrane), DC (daughter cells), nd (not defined), KO (knockout), iKO (conditional knockdown), IQ (IQ refers to the two first amino acids of the motif), CC (coiled-coil), WD40 (also known as WD or beta-transducin repeat that often terminates in a tryptophane-aspartic acid (W-D) dipeptide), MyTH4 (Myosin Tail Homology 4), FERM (four-point-one, ezrin, radixin, moesin), ATS1 (α-tubulin suppressor 1), RCC1 (regulator of chromosome condensation 1).

that moves towards the barbed end of actin filaments and functions in the context of large motor arrays [24,26]. TgMyoA associates with three regulatory light chains; TgMLC1, and the two functionally redundant essential light chains TgELC1 and TgELC2 [27,28•]. Although TgELC1 and TgELC2 interact with TgMyoA in a mutually exclusive manner and share the same MyoA binding site, actin displacement was shown to be much faster when purified TgMyoA was bound to TgMLC1 and TgELC1 instead of TgMLC1 only [28•,29]. Similarly, *P. falciparum* myosin A (PfMyoA) associates with PfMTIP (myosin tail domain interacting protein) and a newly identified essential-type light chain (PfELC), which also enhances actin filament displacement *in vitro* [30•,31,32]. Both MTIP and PfELC bind to the C-terminus of the PfMyoA neck region and the binding regions were narrowed down to 786-803 for PfELC, and 801-818 for MITP [32]. An assembly model for the ternary complex TgMyoA-TgMLC1-TgELC1 as well as the precise binding sites of TgMLC1 and TgELC1 to TgMyoA have also recently been mapped using a combination of solution binding and structural studies [33•]. Both TgMLC1 and PfMTIP are unusual light chains since they contain degenerated EF-hands unable to bind calcium. However, Ca^{2+} is important for the maintenance and stability of the intermolecular interactions between TgELC1/TgELC2 and TgMLC1, and impacts on the quality, speed, and displacement of gliding motility [28•,34].

MyoA belongs to a larger complex referred to as the 'glideosome'. A comprehensive review (including several illustrations) on gliding motility has recently been published [35]. The MyoA-glideosome is firmly anchored in the parasite pellicle (composed of the PM and the inner membrane complex (IMC) formed by flattened vesicles). The MyoA-glideosome is composed of TgMyoA, TgMLC1, TgELC1/TgELC2, the gliding-associated protein 45 (TgGAP45), which is anchored to the PM via N-terminal acylation and to the IMC via its C-terminus and two integral membrane proteins of the IMC: TgGAP50 and TgGAP40 [26,28•,36,37]. Analogues to the glideosome in *T. gondii*, its different components have also been identified and characterized in *Plasmodium* spp. [32,38–41].

Figure 3


Cvel_21973_Chromera_velia_CCMP2878
Cvel_7601_Chromera_velia_CCMP287
cgd7_4350_C_parvum_Iowa_II
ETH_00017965_Eimeria_tenella_Houghton
SN3_02100310_Sarcocystis_neurona_SN3
TGME49_243250_Toxoplasma_gondii_ME49_MyoH
HHA_243250_Hammondia_hammondi_HH34
TA16360_Theileria_annulata_Ankara
BBOV_IV012030_Babesia_bovis_T2Bo
H
Cvel_5603_Chromera_velia_CCMP2878
PF3D7_0503600_P_falciparum_3D7_MyoB*
ETH_00012125_Eimeria_tenella_Houghton
SN3_03400245_Sarcocystis_neurona_SN3
TGME49_255190_Toxoplasma_gondii_ME49_MyoB
HHA_255190_Hammondia_hammondi_HH34
B/C
SN3_00301710_Sarcocystis_neurona_SN3
TGME49_239560_Toxoplasma_gondii_ME49_MyoE
HHA_239560_Hammondia_hammondi_HH34
E
Gp_AAQ88311_Gregarina_polymorpha_MyoB*
ETH_00011215_Eimeria_tenella_Houghton
Gp_ABQ96867_Gregarina_polymorpha_MyoA*
Gp_AAQ88310_Gregarina_polymorpha_MyoA*
cgd7_3790_C_parvum_Iowa_II_MyoA*
ETH_00021495_Eimeria_tenella_Houghton
SN3_01800105_Sarcocystis_neurona_SN3
TGME49_235470_Toxoplasma_gondii_ME49_MyoA
HHA_235470_Hammondia_hammondi_HH34
A
TGME49_263180_Toxoplasma_gondii_ME49_MyoD
HHA_263180_Hammondia_hammondi_HH34
ETH_00015900_Eimeria_tenella_Houghton
D
TA20555_Theileria_annulata_strain_Ankara_MyoA*
BBOV_I003490_Babesia_bovis_T2Bo_MyoA*
PF3D7_1342600_P_falciparum_3D7_MyoA*
TGME49_291020_Toxoplasma_gondii_ME49_MyoL
HHA_291020_Hammondia_hammondi_HH34
ETH_00006400_Eimeria_tenella_Houghton
L
cgd3_2120_C_parvum_Iowa_II
Vbra_21187_Vitrella_brassicaformis_CCMP3155
PF3D7_0613900_P_falciparum_3D7_MyoE*
Subclass XIVc
Subclass XIVa/b
Subclass XIVe
Class XIV
ETH_00015850_Eimeria_tenella_Houghton
SN3_00300285_Sarcocystis_neurona_SN3
TGME49_314780_Toxoplasma_gondii_ME49_MyoG
HHA_314780_Hammondia_hammondi_HH34
G
Class XXIII
TGME49_230980_Toxoplasma_gondii_ME49_MyoI
HHA_230980_Hammondia_hammondi_HH34
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Cvel_20782_Chromera_velia_CCMP2878
cgd6_4340_C_parvum_Iowa_II
I
Class XXIV
BBOV_IV011050_Babesia_bovis_T2Bo
TA06960_Theileria_annulata_strain_Ankara
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Cvel_22959_Chromera_velia_CCMP2878
Vbra_4191_Vitrella_brassicaformis_CCMP3155
PF3D7_1329100_P_falciparum_3D7
ETH_00004005_Eimeria_tenella_Houghton
ETH_00011990_Eimeria_tenella_Houghton
SN3_02400260_Sarcocystis_neurona_SN3
TGME49_278870_Toxoplasma_gondii_ME49_MyoF
HHA_278870_Hammondia_hammondi_HH34
Gp_ABR21557_Gregarina_polymorpha
cgd6_4560_C_parvum_Iowa_II
F
Class XII
TGME49_206415_Toxoplasma_gondii_ME49_MyoK
HHA_206415_Hammondia_hammondi_HH34
Vbra_20376_Vitrella_brassicaformis_CCMP3155
Cvel_619_Chromera_velia_CCMP2878
Cvel_28326_Chromera_velia_CCMP2878
PF3D7_1140500_P_falciparum_3D7
cgd4_1690_C_parvum_Iowa_II
K
cgd8_710_C_parvum_Iowa_II
Cvel_17296_Chromera_velia_CCMP2878
PF3D7_1229800_P_falciparum_3D7
SN3_00103425_Sarcocystis_neurona_SN3
TGME49_257470_Toxoplasma_gondii_ME49_MyoJ
HHA_257470_Hammondia_hammondi_HH34
ETH_00023890_Eimeria_tenella_Houghton
ETH_00022890_Eimeria_tenella_Houghton
ETH_00024275_Eimeria_tenella_Houghton_MyoJ*
ETH_00023880_Eimeria_tenella_Houghton
J
Class VI-like
1
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The role of TgMyoA phosphorylation has also been addressed. Multiple phosphorylation sites have been identified for TgMyoA and the calcium-dependent protein kinase 3 (TgCDPK3) was shown to be responsible for TgMyoA phosphorylation, thereby facilitating parasite motility and host cell egress [42]. The small molecule enhancer compound 130038 was found to cause a calcium-dependent increase in TgMyoA phosphorylation, and upon mutation of the major TgMyoA phospho-sites calcium-induced host cell egress was delayed [43]. Moreover the small-molecule inhibitors tachypleginA and its analogues were shown to directly and covalently bind C58 of TgMLC1, thereby inhibiting motility and invasion [44].

Conditional DiCre-dependent excision of the genes coding for the core components of the glideosome, such as *TgMyoA*, *TgGAP40*, *TgGAP45*, *TgGAP50*, *TgMLC1* and *TgACT1* have been generated using notably the gene-swap strategy [45,46]. From this list only parasites lacking *TgMyoA* have been cloned, suggesting that other myosins might contribute and compensate for the absence of this motor in the glideosome function or that a myosin-independent process sustains residual invasion including the participation of host cell membrane dynamics [46,47•]. Noteworthy a combined deletion of *TgMyoA* and *TgMyoB/C* could not be isolated [46] and TgMyoC assembles into a glideosome that shares several components with the TgMyoA-glideosome, that is, the myosin light chains, TgGAP50 and TgGAP40 (Figures 2–4) [48]. Instead of TgGAP45, TgGAP80 recruits TgMyoC to the basal polar ring and assembles the TgMyoC-glideosome [48]. Another member of the TgMyoC-glideosome, the IMC-associated protein 1 (TgIAP1) was proven to be a key determinant in restricting the TgMyoC motor complex to the posterior polar ring. Importantly, deletion of specific components of the TgMyoC-glideosome leads to functional compensatory mechanisms by TgMyoA-glideosome components and *vice versa*. This redundancy between the two glideosomes delineates a fine example of plasticity that ensures parasite survival [49]. TgMyoC is encoded by the *TgMyoB/C* gene that produces two isoforms by an alternative splicing event, TgMyoB and the longer one, TgMyoC [50].

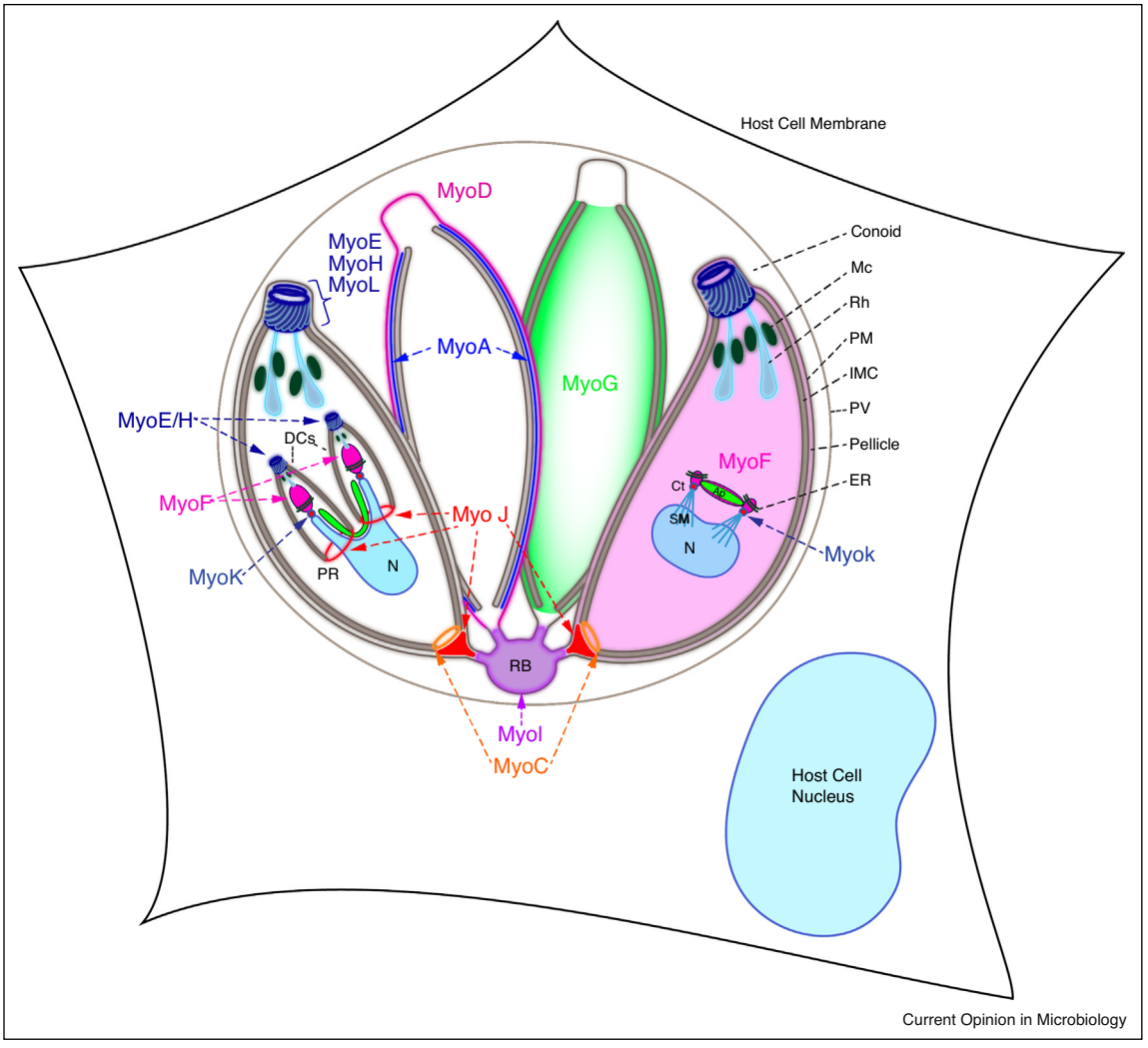
More recently, the properties of *Plasmodium* MyoB, which like MyoA belongs to class XIV myosins, has been studied in *P. falciparum*, *P. berghei* and *P. knowlesi* [51]. This tailless motor does not associate with the glideosome and PfMTIP. MyoB is expressed in all invasive stages of the life cycle and localizes to the very apical end of the parasites. It interacts with a putative myosin light chain, MLC-B, which is well conserved in *Plasmodium* species and is the largest MLC to be discovered in any species. Its unusual structure is composed of an EF-hand containing CaM-like carboxy-terminal domain (CTD) that assemble into a complex with PfMyoB [51].

TgMyoH was the first myosin to be localized to the conoid, close to the pre-conoidal rings (Figure 4) [52•]. TgMyoH belongs to the class XIVc and besides being conserved in coccidians, it is also present in *C. velia* (Figure 3). Its extended neck region comprises eight IQ motifs that are presumably bound by MLC1 and by the two dispensable and to the conoid localizing MLCs TgMLC5 and TgMLC7 [52•]. The association of TgMyoH with the conoid is dependent on three ATS1/RCC1 domains that are present in the tail region. These domains anchor TgMyoH directly or indirectly to the conoid tubulin fibers, thus establishing a link between the tubulin-based and actin-based cytoskeletons. TgMyoH is indispensable for parasite survival and its conditional depletion completely blocks the glideosome-associated functions, namely gliding motility, invasion and egress, in spite of the presence of an intact MyoA-glideosome. Conoid protrusion, which was thought to be actin-dependent is not affected in absence of TgMyoH. The TgMyoH-dependent phenotypes were recently reported to be phenocopied upon the combined loss of the CaM-like proteins TgCaM1 and TgCaM2 or upon loss of TgCaM3 [53•]. These three proteins localize to the conoid, in close proximity to TgMyoH and disruption of TgMyoH resulted in cytosolic TgCaM1 and TgCaM2 and degradation of TgCaM3. The current model suggests that these proteins act as regulatory light chains for TgMyoH and control this myosin activity in a calcium-dependent manner [53•]. Taken together, TgMyoH appears to be a central component of a conoidal glideosome that acts as the initiator of gliding motility, which is then relayed by the TgMyoA-glideosome at the level of the IMC [52•].

Other myosins that have recently been localized to the conoid of *T. gondii* tachyzoites include TgMyoE, which belongs to the class XIV and localizes to the conoid of daughter and mature parasites, and TgMyoL, which is distributed both to the conoid and in the cytoplasm (Figure 4) [54••]. Although TgMyoL has not been classified yet, the phylogenetic analysis suggests that it belongs to the newly defined subclass XIVe and is closely related to PfMyoE (Figure 3). Both myosins were found to be dispensable for parasite survival and thus, are individually

(Figure 3 Legend) Maximum likelihood phylogenetic trees based on Alveolate myosin head protein sequences. The tree was constructed using PhyML and WAG model of amino acids substitution with NNI topology search, based on the amino acid alignment computed by MUSCLE (Supplementary Fig. 1). Only SH-like aLRT branch support values >0.80 were indicated and values >0.90 were considered as significant. The scale bar at the base of the phylogenetic tree represents the branch length values. *Represents proteins that have already been named but do not necessarily group together with their *T. gondii* homologues.

Figure 4



Schematic representation of *Toxoplasma gondii* myosin localizations during parasite division. MyoA localizes at the pellicle. MyoC localizes at the basal ring, MyoD at the plasma membrane, MyoE/H/L at the conoid. MyoF localizes in the cytosol and along the apicoplast, it accumulates at the extremities of the organelle and, after apicoplast fission, MyoF localizes inside the growing daughter cells. MyoG localizes in the cytosol and accumulates at the pellicle. MyoI localizes in the residual body, MyoJ at the posterior cup. MyoK localizes close to the centrosome. For space reasons myosins and organelles are not represented in all parasites. Mitochondria are not represented. Ap, apicoplast; Ct, centrosomes; DCs, daughter cells; ER, endoplasmatic reticulum; IMC, inner membrane complex; N, nucleus; Mc, micronemes; PM, plasma membrane; PR, posterior ring; PV parasitophorous vacuole; RB, residual body, Rh, rhoptries; SM, spindle microtubules.

not crucial for conoid protrusion or motility [54••]. Surprisingly though, a recently performed high-throughput CRISPR-Cas9 mediated gene disruption approach led to the assignment of a fitness score to each individual gene in *T. gondii*, and TgMyoL was identified as a gene contributing to parasite welfare [55••].

Like TgMyoA and TgMyoD, TgMyoE contains a single putative degenerated IQ motif and lacks a tail domain [3]. Whether TgMLC5 and TgMLC7 that appear to belong to the TgMyoH motor complex, also bind to TgMyoE and/or TgMyoL is currently not known [52•].

T. gondii myosin F functions in organelle positioning and inheritance

Beside MyoA, the class XXII MyoF is the only other myosin broadly conserved across the Apicomplexa phylum (Figure 3, Table S1) [3]. TgMyoF harbours a coiled-coil domain and probably functions as a dimer [56]. The neck region contains six putative IQ motifs, however the associated MLCs have not been identified yet. MyoF possesses four to six WD40 repeats in the tail domain that adopt a beta-propellar fold that has never been associated with myosins before [3,57]. In dividing parasites, TgMyoF is found in close proximity to the apicoplast

(Figure 4), a relict plastid-like organelle present in Apicomplexans (except *Cryptosporidium* spp.) [58]. In non-dividing parasites however, TgMyoF localizes diffusely and does not change upon short-time treatment with actin perturbators [56]. TgMyoF is essential for parasite survival and participates in centrosome positioning and apicoplast inheritance. Depletion of TgMyoF leads to an accumulation of secretory organelles along with the apicoplast into enlarged residual bodies (RBs) [56] indicative of its importance in organellar trafficking within the cell [59]. Concordantly, TgMyoF has been associated to the apical positioning of the rhoptries, a process strictly dependent on TgARO, an acylated protein at the surface of these organelles and to the trafficking of the dense granules [60,61].

T. gondii Myosin J participates in parasite basal pole constriction

TgMyoJ belongs to the class XXIII (VI-like) and is present in all Apicomplexans except *Theileria* and *Babesia* (Figure 3) [3]. TgMyoJ localizes to the basal pole of mature and developing daughter parasites and is dispensable (Figure 4). Parasites lacking this motor display an enlarged posterior pole and an overall reduced fitness with a loss of virulence in the mouse model of infection [54••]. In consequence, TgMyoJ functions in the constriction of the basal complex and co-localizes with the small, EF-hand containing protein centrin 2 (TgCEN2), a protein displaying a complex distribution pattern including the tip of the parasite, the anulli and the basal pole [54••]. Remarkably, depletion of TgCEN2 affects TgMyoJ localization and impacts on the constriction of the basal pole. Taken together, it is plausible that TgCEN2 acts as a MLC for TgMyoJ although a direct association could not be demonstrated [54••].

T. gondii Myosin I promotes cell–cell communication between intravacuolar parasites

TgMyoI belongs to the class XXIV, which is present in a few coccidians including *T. gondii* and *C. parvum* and interestingly also in the chromerids (Figure 3). TgMyoI comprises two IQ motifs, a coiled-coil domain, which might be implicated in myosin dimerization, and a long tail without any recognisable protein domains [62]. TgMyoI is mainly detected in the RB (Figure 4). Deletion of TgMyoI showed no defect in the parasite lytic cycle, however the parasites failed to develop into organized rosettes. Most strikingly, the mutant parasites divide asynchronously within a given vacuole. Fluorescent recovery after photobleaching (FRAP) on parasites expressing cytosolic or nuclear GFP demonstrated that soluble proteins are redistributed between parasites residing in the same vacuole. TgMyoI establishes or maintains this connection between the parasites within a vacuole, thus ensuring cell–cell communication and synchorinzed cell division. Relevantly, this connection is absent in the asynchronously dividing cyst forming bradyzoites. Asynchronous parasite division and lack of cell–cell communication were also observed upon deletion of *TgMyoI*. Presumably, basal pole constriction indirectly participates in the formation or maintenance of the tubes connecting parasites following division. This connection between parasites is in continuity with the parasites plasma membrane and composes the RB [63]. More work is needed to define if MyoI is implicated in the formation of the connection and/or also in the active transport of material between parasites along the actin filaments visualized at the same location by using actin chromobodies [64••].

Unknown functions for MyoD, MyoG, MyoK

The unusual class XIV of Apicomplexa is subdivided into five subclasses (XIVa to e) (Figure 3). TgMyoD is the smallest motor (91 kDa) and belongs to the XIVa subclass along with the structurally closely related TgMyoA and presumably behaves as a fast, single-headed and non-processive type myosin. *TgMyoD* is not essential for the tachyzoites [65] but might be important in other stages.

The class XXIII MyoG was only found in *T. gondii*, *Sarcocystis neurona* and *E. tenella*. The neck region includes one IQ motif and the tail possesses a single MyTH4 but no FERM domain as typically found in other myosins [3]. The MyTH4/FERM tandem domain is mainly described as a single functional complex with actin- and tubulin-based roles. Whether the single MyTH4 domain of TgMyoG is sufficient to interact with the cytoskeleton is unknown. TgMyoG localizes to the parasite periphery (Figure 4) and gene disruption showed no alteration of parasite fitness [54••].

The class XXIII (VI-like) myosin, TgMyoK showed a striking cell cycle dependent localization with a pronounced staining at the centrosome at the onset of division (Figure 4) [54••]. Deletion of *TgMyoK* did not affect the lytic cycle. Initially, TgMyoJ and TgMyoK were grouped in the class of retrograde myosin VI [3]. A more recent phylogenetic analysis has reclassified these two proteins into class XXIII [5]. However this finding is not supported by the phylogenetic analysis presented here, in which class VI clearly groups outside of the class XXIII/XXIV cluster (Figure 3). Biochemical investigations will be necessary to establish if these apicomplexan moysins act as retrograde motors.

Myosins in less genetically and biochemically tractable apicomplexan parasites

The genomes of *Eimeria*, *Babesia* and *Cryptosporidium* as well as the more deep-branching Apicomplexans, the *Gregarines*, reveal the existence of myosin genes. However functional data about these isoforms is lacking, despite the discovery of a class XXII myosin in the eugregarine *G. polymorpha* called GpMyoF (class XXII) that shows the same domain architecture as TgMyoF and was localized to the so-called annular myonemes of the parasite cortex [66].

Chromerids and colpodellids are seen as a sister group to Apicomplexans, however from a phylogenetic point of view it is not understood how these photosynthetic and predatory algae relate to the phylum [67]. The prevailing hypothesis states that apicomplexan parasites originated from free-living photosynthetic algae. C*hromera* and *Vitrella* have a broader repertoire of myosins compared to the apicomplexan species who underwent a combination of lineage-specific losses and gains [6••]. Recently, 14 and 8 myosin-related proteins have been identified in *C. velia* and *V. brassicaformis*, respectively [6••]. On the basis of the presence of a myosin head domain (e.g. ATPase and actin binding domain) 9 *C. velia* and 4 *V. brassicaformis* myosins were integrated in the phylogenetic analysis (Figure 3). The global distribution of myosin classes is heterogeneous between these two organisms but both harbour at least one class XIV and one class XXII myosin. MyoF that belongs to this latter class is anticipated to participate in the inheritance of the photosynthetic plastid organelle in chromerids.

Concluding remarks

The availability of genome sequencing data from protozoan organisms uncovered the repertoire of myosins and also crucially contributed in placing these motors into an evolutionary context. Several novel classes of unconventional myosins have been discovered in the past years. These motors display protein architectures not being described for myosins in higher eukaryotes. Continuous research efforts will not only increase the knowledge on myosin function but also provide an integrated view on the interactions between myosin motors and accessory proteins including myosin chaperones, actins and actin binding proteins that together govern fundamental cellular processes in protozoan parasites.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.mib.2017.11.003.

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- • of special interest
- •• of outstanding interest

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