# The Actomyosin Systems in Apicomplexa

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**Abstract**

The phylum of Apicomplexa groups obligate intracellular parasites that exhibit unique classes of unconventional myosin motors. These parasites also encode a limited repertoire of actins, actin-like proteins, actin-binding proteins and nucleators of filamentous actin (F-actin) that display atypical properties. In the last decade, significant progress has been made to visualize F-actin and to unravel the functional contribution of actomyosin systems in the biology of *Toxoplasma* and *Plasmodium*, the most genetically-tractable members of the phylum. In addition to assigning specific roles to each myosin, recent biochemical and structural studies have begun to uncover mechanistic insights into myosin function at the atomic level. In several instances, the myosin light chains associated with the myosin heavy chains have been identified, helping to understand the composition of the motor complexes and their mode of regulation. Moreover, the considerable advance in proteomic methodologies and especially in assignment of posttranslational modifications is offering a new dimension to our understanding of the regulation of actin dynamics and myosin function. Remarkably, the actomyosin system contributes to three major processes in *Toxoplasma gondii*: (i) organelle trafficking, positioning and inheritance, (ii) basal pole constriction and intravacuolar cell-cell communication and (iii) motility, invasion, and egress from infected cells. In this chapter, we summarize how the actomyosin system harnesses these key events to ensure successful completion of the parasite life cycle.

**Keywords**

Toxoplasma · Plasmodium · Actomyosin system · Motility · Invasion · Organelle inheritance · Basal pole constriction · Cell-cell communication

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L. M. Coluccio (ed.), *Myosins*, Advances in Experimental Medicine and Biology 1239,
https://doi.org/10.1007/978-3-030-38062-5_14

## 14.1 Introduction

### 14.1.1 The Phylum of Apicomplexa

The phylum Apicomplexa is composed of obligate intracellular parasites and comprises several pathogens of medical and veterinary significance such as *Plasmodium*, responsible for malaria, *Toxoplasma*, the agent of opportunistic toxoplasmosis, or *Cryptosporidium*, responsible for the diarrheal cryptosporidiosis. Apicomplexans are single-celled eukaryotes of the infrakingdom Alveolate, which also includes ciliates and dinoflagellate algae (Gould et al. 2008). Although these protists are very diverse in their shape and lifestyle, they are unified by a common structural feature: the presence of a pellicle composed of the external plasma membrane (PM) under which lies membranous sacs termed alveoli or inner membrane complex (IMC) (Fig. 14.1a). On the cytoplasmic face of the IMC, a meshwork of intermediate filament-like proteins connects the pellicle to the cortical microtubules that constitute the cytoskeleton of the parasite (Harding and Meissner 2014). Most Apicomplexans possess a non-photosynthetic relic plastid named the "apicoplast" that originates from secondary endosymbiosis of a red alga (van Dooren and Striepen 2013) (Fig. 14.1a). This organelle fulfils metabolic functions that are critical for parasite survival. Apicomplexans are further characterized by the presence of an apical complex composed of cytoskeletal elements, the apical polar rings, and two sets of secretory organelles, the micronemes and rhoptries, which play a critical role in gliding motility and during the invasion process and egress from infected cells (Hu et al. 2006; Frénal et al. 2017a). In the subclass of Coccidia, which comprises, among others, *Toxoplasma gondii*, *Eimeria,* and *Cryptosporidium* species, the conoid and the pre-conoidal ring are additional cytoskeletal elements of the apical complex (Fig. 14.1a). The conoid is an organelle found at the apex of these parasites and composed of α-tubulin-rich spiraling fibers named conoid fibers (Hu et al. 2002). The conoid is retracted in intracellular parasites and protrudes beyond the IMC in extracellular parasites upon an increase in intracellular calcium, although its function remains unknown (Monteiro et al. 2001).

In this chapter, we will focus on the two most studied and tractable parasites of the phylum, *Toxoplasma* and *Plasmodium.* Studies on *T. gondii* are mainly carried out on the fast-replicative stage, the tachyzoite, whose lytic cycle is depicted in Fig. 14.1b. Studies on human malaria parasite *P. falciparum* predominantly focus on erythrocytic stages, whereas *P. berghei*, a rodent model of malaria offers the possibility to investigate the full life cycle of the parasite taking place between the murine intermediate host and the *Anopheles* mosquito vector, the definitive host (Fig. 14.1c).

### 14.1.2 Parasite Lifestyle

The apicomplexan motile stages, also called zoites, exhibit a unique form of substrate-dependent locomotion. In contrast to other protozoans, they do not rely on specific attributes such as flagella or amoeboid movement. Instead, they use gliding motility powered by an actomyosin system, termed glideosome, to actively penetrate into their host cell (invasion), exit from the infected cell (egress), and cross biological barriers. This molecular machine is located within the pellicle in the space between the plasma membrane and the IMC (Frénal et al. 2017a). Following an increase in intracellular calcium, exocytosis of microneme content occurs at the apical pole of the parasite leading to the insertion of micronemal adhesins into the parasite plasma membrane. During motility, these adhesins can bind to host receptors at the surface of target cells and the rearward translocation of these adhesin-receptor complexes by the glideosome propels the parasite forward. During invasion, apical microneme secretion induces the reorientation of the parasite, placing the apex of the parasite in juxtaposition with the host cell plasma membrane (Fig. 14.1b). Subsequently, rhoptry discharge occurs, releasing rhoptry neck (RONs) and rhoptry bulb proteins (ROPs) into the host cells. A complex of RONs (RON2/4/5) inserted within the host cell plasma membrane and inter-

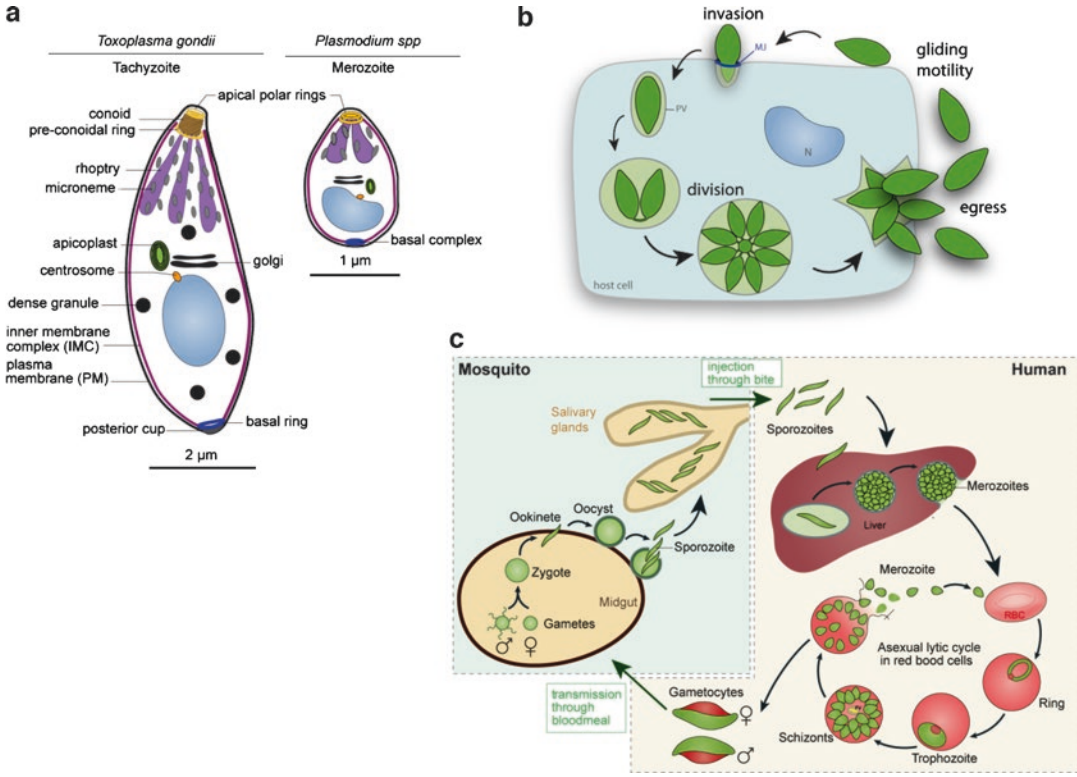


**Fig. 14.1** Example of zoites in *Toxoplasma gondii* and *Plasmodium species* and their life cycle. (**a**) Schematic representation of *Toxoplasma gondii* tachyzoite (left panel), a member of the coccidian sub-group of Apicomplexa and *Plasmodium* merozoite, belonging to the haemosporida sub-group (right panel). The main difference resides in the presence of the conoid, a structure composed of tubulin, located at the apex of the Coccidians. The zoites harbor three types of secretory organelles, the micronemes, rhoptries, and dense granules and a non-photosynthetic plastic-like organelle, the apicoplast. Mitochondrion and endoplasmic reticulum are not represented but are present in these zoites. (**b**) Lytic cycle of *Toxoplasma gondii*. The fast replicative tachyzoite is capable of entering virtually any nucleated cell. Within the host cell, the parasite divides by endodyogeny within a parasitophorous vacuole and after several rounds of replication, will eventually egress from the infected cell, lysing it, and glide to invade a neighboring cell. *N* nucleus, *PV* parasitophorous vacuole, *MJ* moving junction. (**c**). Life cycle of *Plasmodium falciparum* taking place between the definitive host, the *Anopheles* mosquito, and the intermediate host, the human. Infection of the human starts through the bite of an infected female mosquito, which injects sporozoites into the dermis. The sporozoites migrate to the liver and invade hepatocytes where they divide and produce thousands of merozoites that are released into the bloodstream. There, the merozoites infect erythrocytes (*RBC*, red blood cells). The parasites undergo repeated asexual cycles within the RBC, where they progress from rings to trophozoites and schizonts and are eventually released as merozoites. Some parasites will develop into gametocytes, the sexual forms that circulate in the bloodstream, prior to being taken up by the mosquito. The sexual cycle takes place within its midgut, leading to the formation of motile but non-invasive ookinetes, which migrate through the midgut and develop into oocysts in the epithelium. Maturation of the oocyst produce sporozoites that migrate to the salivary glands where they are ready to infect subsequent hosts through the mosquito bite

acting with the microneme protein AMA1 (apical membrane antigen 1) at the parasite plasma membrane forms the moving junction that supports the motility-driven progression of the parasite into the target cell (Harvey et al. 2014; Bichet et al. 2014). At the same time, the parasite induces the formation of a non-fusogenic parasitophorous vacuole membrane (PVM), which is derived from the invagination of the host-cell plasma membrane (Mordue et al. 1999). Once intracellular, tachyzoites secrete effectors from the secretory organelles named dense granules that ensure

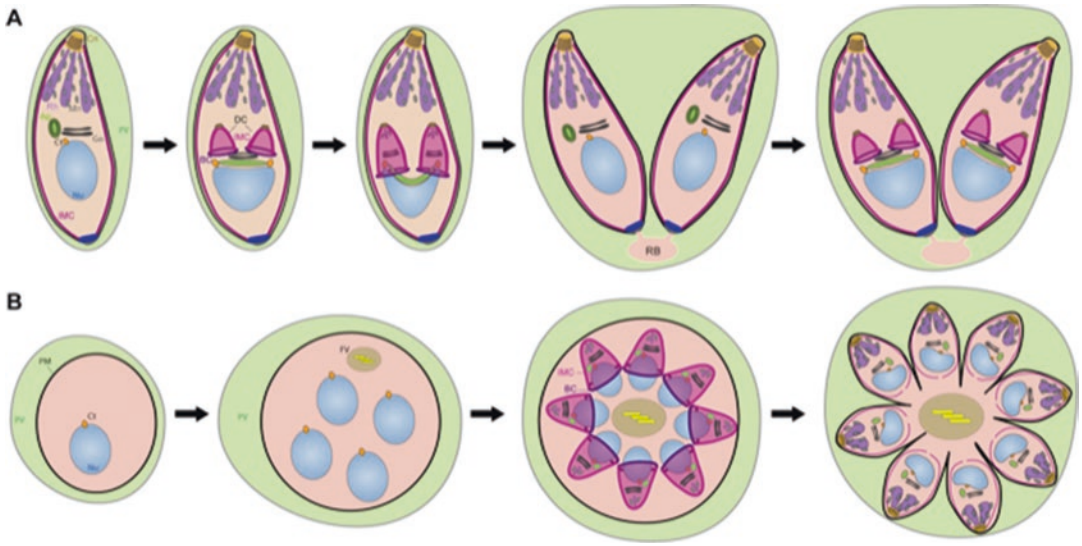


**Fig. 14.2** Endodyogeny of *Toxoplasma gondii* tachyzoite and schizogony of *Plasmodium*. (**a**) Scheme of tachyzoites dividing by endodyogeny during which two daughter cells grow inside the mother cell. The scheme highlights particularly the division of the apicoplast that is associated to centrosomes during its inheritance into the daughter cell. The apicoplast is first elongated, then forms a U-shape before being incorporated in the two developing progenies. *Cn* conoid, *Rh* rhoptries, *Mn* micronemes, *Ap* apicoplast, *Go* Golgi apparatus, *Ct* centrosome, *Nu* nucleus, *IMC* inner membrane complex, *BC* basal complex, *DC* daughter cells, *PV* parasitophorous vacuole, *RB* residual body. (**b**) Scheme of the schizogony process, the mode of division of *Plasmodium* species, taking place in the erythrocytes. During this process, the nucleus divides several times and the other organelles are also dividing and/or synthesized *de novo*. All the organelles are then packaged into the IMC at the same time before the segmentation of the individual merozoites. *PM* plasma membrane, *PV* parasitophorous vacuole, *FV* food vacuole

their safe replication by participating in the modification of the parasitophorous vacuole (PV), recruitment of host endoplasmic reticulum and mitochondria, and subversion of host cellular functions (Mercier and Cesbron-Delauw 2015; Hakimi et al. 2017).

Within the PV, *T. gondii* tachyzoites divide synchronously by endodyogeny, a process during which two daughter cells develop within the mother cell and consume it (Francia and Striepen 2014) (Fig. 14.2a). During this replication, some organelles are made *de novo* such as the IMC, micronemes, and rhoptries, whereas others, including the mitochondrion and the apicoplast, are inherited (Nishi et al. 2008). For the segregation of the apicoplast, the positioning of the centrosome is crucial since the ends of the apicoplast have been shown to be tightly associated to the centrosomes during its elongation and incorporation into the daughter cells (Striepen et al. 2000). At each round of division, the basal pole of the parasites constricts and the parasites remain associated through the residual body and adopt an organization in rosettes. After several rounds of replication, the tachyzoites actively egress from the infected cell using the gliding motility and lyse the PVM and host-cell PM through the action of at least one perforin (PLP1) secreted by the micronemes (Kafsack et al. 2009).

In contrast to *Toxoplasma*, *Plasmodium* undergoes schizogony in the erythrocytic stage, a process wherein the components of the daughter cells (such as mitochondrion, apicoplast, nucleus, and secretory organelles) are produced in the same cytoplasm before being encapsulated simultaneously within the forming daughter cells (Fig. 14.2b). Cytokinesis then occurs and produces individual invasive merozoites released in the bloodstream that are able to infect new erythrocytes (Francia and Striepen 2014).

Use of drugs, such as the actin-depolymerizing agent cytochalasin D, revealed that actin does not play a crucial cytoskeletal role during apicomplexan division (Jacot et al. 2013; Frénal et al. 2017b). In contrast, actin polymerization and the connected myosin functions are critical for several steps of the parasite lytic cycle. In the following sections, we will review the role of the actomyosin system for organelle positioning and inheritance, basal complex constriction and intravacuolar cell-cell connection, and gliding motility.

## 14.2 Myosin Heavy Chain and Actin Features in Apicomplexa

### 14.2.1 Overview of the Evolution and Classification of Protozoan Myosin Motors

Myosin motors, one of the largest protein families in eukaryotes, are involved in a multitude of cellular functions. Several comprehensive phylogenetic analyses of myosin heavy chains, progressively updated with newly sequenced genomes, have led to the classification and reconstruction of the evolutionary history of these proteins (Richards and Cavalier-Smith 2005; Foth et al. 2006; Odronitz and Kollmar 2007; Sebé-Pedrós et al. 2014). Foth et al. established the first phylogenetic analysis including myosin sequences from numerous protozoa, such as seven members of Apicomplexa, the ciliate *Tetrahymena thermophila* as well as five members of the Kinetoplastida phylum (*Leishmania* and *Trypanosoma* species), and those from metazoans, fungi, and plants (Foth et al. 2006) (Table 14.1). This analysis led to the discovery of six novel classes of unconventional myosins, three of them restricted to the alveolates (classes XXII, XXIII, XXIV), one of which is found only in trypanosomatids (class XXI). Interestingly, characterization of this broader repertoire of myosin heavy chains identified protein domains, such as FYVE, WW, UBA, ATS1-like and WD40, within the tails that were not previously associated with myosins. In addition, apicomplexan myosins, previously restricted to class XIV, were placed into several classes encompassing myosins from other systematic lineages (classes VI, XXII, XXIII, XXIV), while the class XIV was found to no longer accommodate only apicomplexan myosins but also myosins of *T. thermophila*.

Subsequent phylogenetic analyses have extended and modified the myosin classification described above. Odronitz et al. used 2269 myosin motor domains from 328 organisms to build a new eukaryotic tree of life (Odronitz and Kollmar 2007). This resulted in the definition of 35 myosin classes and some re-classifications. Of relevance concerning the protists, a new class XIII was attributed to myosins specific to the kinetoplastida and exhibiting SH3-like, coiled-coil, and UBA domains, and finally, five new classes were composed solely of apicomplexan myosins.

A more recent study used an expanded taxon sampling in which all major eukaryotic supergroups were represented to define 31 myosin classes (Sebé-Pedrós et al. 2014). With regard to the protozoan myosins, some interesting aspects of this study need to be mentioned. Some alveolate sequences that were previously grouped within myosin class VI (Foth et al. 2006) are now accommodated within myosin class XXIII. Eighteen myosins from the alveolate *T. thermophila* and *Paramecium tetraurelia* are grouped again within the alveolate-specific myosin class XIV (Sebé-Pedrós et al. 2014), and interestingly, several of them contain the protein domain combination MyTH4/FERM. They constitute the only example of bikonts harboring these domains. Recently, the classification from Foth et al. has been updated to include sequences of two recently sequenced Alveolates, the related photosynthetic chromerids, *Chromera velia* and *Vitrella brassicaformis* (Mueller et al. 2017) (Table 14.1). We have chosen to use this latest phylogeny to describe the apicomplexan myosin heavy chains in the following sections.

**Table 14.1** Overview of the repertoire of myosin heavy chains in Apicomplexa

| Class | Gene name | Gene ID | № of IQ motifs | MW (kDa) | Localization | Cellular function | Fitness | Coccidia | Cryptosporidia | Haemosporida | Piroplasmida | Gregarines | Chromerids |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| XIV a | MyoA | TGME49_235470 | 1 | 93 | Inner membrane complex | Gliding/ Invasion/ Egress | -3.09 | ● | ● | ● | ● | ● | ○ |
| | MyoD | TGME49_263180 | 0 | 91 | Plasma membrane | n.d. | 1.56 | ● | ○ | ○ | ○ | ○ | ○ |
| XIV b | MyoB/C | TGME49_255190 | 0–1 | 133 | Basal ring | Gliding/ Invasion/ Egress | 2.25 | ● | ○ | ○ | ○ | ● | ○ |
| | MyoE | TGME49_239560 | 1 | 93 | Conoid | n.d. | 0.11 | ◐ | ○ | ○ | ○ | ○ | ○ |
| XIV c | MyoH | TGME49_243250 | 6–8 | 170 | Conoid/ Cytoplasm | Gliding/ Invasion/ Egress | -3.94 | ● | ● | ○ | ● | ○ | ○ |
| XIV e | MyoL | TGME49_291020 | 4 | 278 | Conoid/ Cytoplasm | n.d. | -1.83 | ● | ○ | ○ | ○ | ○ | ○ |
| XXII | MyoF | TGME49_278870 | 3–6 | 216 | Apicoplast, juxanuclear region, cytoplasm, daughter cells | Organellar inheritance | -3.55 | ● | ● | ● | ● | ● | ● |
| XXIII | MyoG | TGME49_314780 | 1 | 227 | Cytoplasm/ Pellicle | n.d. | 0.54 | ● | ○ | ○ | ○ | ○ | ○ |
| XXIV | MyoI | TGME49_230980 | 2 | 201 | Residual body | Intravacuolar parasite communication | 0.21 | ◐ | ○ | ○ | ○ | ○ | ● |
| VI-like | MyoJ | TGME49_257470 | 0 | 274 | Posterior cup/ daughter cells | Basal constriction | -3.01 | ● | ● | ● | ○ | ○ | ● |
| | MyoK | TGME49_206415 | 2–3 | 263 | Centrosome | n.d. | -2.78 | ◐ | ● | ● | ○ | ○ | ● |
| XIV c | MyoB | PBANKA_1103300 | 1 | 93 | Apical ring | n.d. | D | ○ | ○ | ● | ○ | ○ | ○ |
| | MyoE | PBANKA_0613900 | 0 | 255 | Basal 'cap' | n.d. | n.d. | ○ | ○ | ● | ○ | ○ | ○ |

● *Present*
○ *Absent*
D : *dispensable*
n.d. : *no data*

The myosin classification is based on the review from Mueller et al. (2017). The GeneID corresponds to the accession numbers of EuPathDB. The number of IQ motifs has been predicted by SMART (Letunic and Bork 2018). The references for the localization and cellular functions are cited in the text. The fitness scores are from the CRISPR-Cas9 genome wide screen performed on *T. gondii* (Sidik et al. 2016) and the PlasmoGEM database established for the erythrocytic stages of *Plasmodium berghei* in mice (Bushell et al. 2017). Conservation within the Apicomplexa phylum was performed by a BLAST search. The genes written in purple are specific to *Plasmodium* species

### 14.2.2 Repertoire of Myosin Heavy Chains in Apicomplexa

Among the apicomplexans, *T. gondii* has the largest repertoire of myosin heavy chains with 11 isoforms, while *P. falciparum* genome encodes six, two of them, PfMyoB and PfMyoE, being specific to the malaria parasites (Table 14.1). All apicomplexan myosin heavy chains are unconventional and two of them are conserved across the phylum, namely the class XIV MyoA and the class XXVII MyoF, with the latter having also orthologs in the chromerids (Mueller et al. 2017). All the myosin heavy chains have been localized in *T. gondii* tachyzoites (Fig. 14.3) and *P. berghei* blood stages and their essentiality evaluated by the generation of knockout, when possible (Wall et al. 2019; Herm-Götz et al. 2006; Andenmatten et al. 2013; Frénal et al. 2014, 2017b), or knockdown (Meissner et al. 2002; Siden-Kiamos et al. 2011; Jacot et al. 2013; Graindorge et al. 2016) cell lines. Out of the 11 myosin heavy chains expressed by the tachyzoite, only two, TgMyoF and TgMyoH, have been completely refractory to deletion and thus are considered indispensable for parasite survival. The other nine motors have been individually deleted with no or very mild impact on tachyzoite fitness, except for TgMyoA. Interestingly, although the class VI-like TgMyoJ and the class XXIV TgMyoI are dispensable for tachyzoite growth *in vitro*, their deletion uncovered their respective function in basal complex constriction and in intravacuolar parasite connection allowing diffusion of soluble molecules and synchronized division (Frénal et al. 2017b). Deletion of the highly conserved TgMyoA showed a strong defect on the parasite lytic cycle being critical for gliding motility (Andenmatten et al. 2013). However, it was impossible to delete *TgMyoA* in a background lacking *TgMyoC* indicating that these two motors fulfil overlapping functions (Egarter et al. 2014).

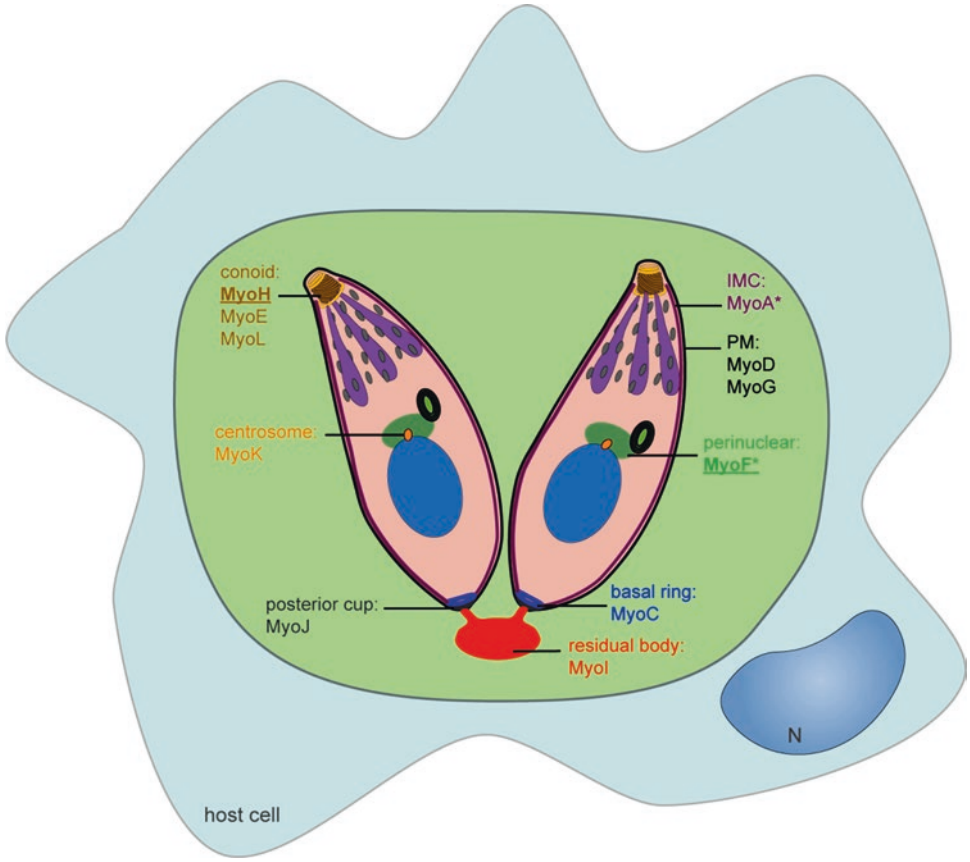


**Fig. 14.3** Localization of the *Toxoplasma gondii* myosins. Scheme of a parasitophorous vacuole containing two tachyzoites connected by the residual body showing the localization of the 11 myosins expressed by this parasite. The dense granule, mitochondria and endoplasmic reticulum are not represented but are present in these zoites. The bold and underlined myosins are the ones found essential for the survival of the tachyzoite. The asterisks indicate the myosins conserved across the Apicomplexa

In *Plasmodium* species that lack an ortholog of TgMyoC, MyoA is likely essential for all motile stages of the life cycle as is the case for the *P. berghei* ookinete (Siden-Kiamos et al. 2011). Moreover, in *P. berghei*, out of the five other myosin heavy chains, PbMyoF and PbMyoK are likely essential for the blood stages since their deletion was unsuccessful (Wall et al. 2019). However, PbMyoB, PbMyoE, and PbMyoJ were shown to be dispensable for these asexual stages while the class XIV PbMyoE appeared critical for motility of the mosquito salivary gland sporozoites (Wall et al. 2019).

For *Toxoplasma,* the experimental results of the individual deletions (Frénal et al. 2017b) are in accordance with the recently-published genome-wide loss-of-function screen performed on *T. gondii* using the CRISPR/Cas9 technology (Sidik et al. 2016), except for the class VI-like TgMyoK, which presents a fitness defect not detected in the phenotyping of the knockout strain (Frénal et al. 2017b) (Table 14.1). Likewise, the fitness data collected for *P. berghei* are in agreement for PbMyoA and PbMyoB, but a discrepancy exists for PbMyoK, which appears dispensable in the large *in vivo* genetic screen performed in the mouse model (Bushell et al. 2017) (Table 14.1). The individual role of each myosin heavy chain will be discussed in detail below.

### 14.2.3 Unusual Features of Apicomplexan Actin

Actin, one of the most abundant and conserved proteins in eukaryotic cells, exists in a monomeric globular state (G-actin) and a polymerized filamentous state (F-actin). It plays fundamental roles in many cellular processes such as muscle contraction, cell division, and cell motility (Dominguez and Holmes 2011; Pollard 2016).

The apicomplexans possess a single gene coding for actin (ACT1), except for the *Plasmodium* species, which also encode a second isoform (ACT2) mainly expressed in the gametocytes and mosquito stages (Deligianni et al. 2011) (Table 14.2). PfACT2 plays a critical role in gametogenesis and its deletion cannot be complemented by PfACT1 (Deligianni et al. 2011; Vahokoski et al. 2014). Actin filaments cannot be readily observed in the parasites. It appears to be mainly globular (Dobrowolski et al. 1997; Schmitz et al. 2005), and the generated filaments tend to be short and unstable (Schmitz et al. 2005; Sahoo et al. 2005). The inherent instability of apicomplexan F-actin resides both in its amino acid sequence, which is distant from other eukaryotic actins (Schüler et al. 2005a; Skillman et al. 2011; Pospich et al. 2017; Douglas et al. 2018), and in its regulation by a limited and divergent set of actin-binding proteins (ABPs) compared to most eukaryotic cells (Baum et al. 2006; Schüler and Matuschewski 2006) (Table 14.2). Yet, actin filaments are essential for parasite growth (Skillman et al. 2011) and especially for motility (Egarter et al. 2014; Drewry and Sibley 2015), suggesting a tight spatial and temporal regulation of the polymerization process within the parasites.

Actin dynamics have been carefully examined *in vitro* with recombinant TgACT1 and PfACT1 purified from the baculovirus insect cell expression system. TgACT1 polymerization was reported to follow an unconventional isodesmic model in which each monomer has the same assembly/disassembly rate in the polymer. Consequently, the polymerization rate is slow without a lag phase or critical concentration, and increases proportionally to the number of actin molecules (Skillman et al. 2013). In contrast, PfACT1 polymerization is reported to follow a classical nucleation-elongation model in which a slow nucleation step precedes a more rapid polymerization phase after reaching a critical concentration (Kumpula et al. 2017). Although the critical concentration and the polymerization rate of PfACT1 are similar to the canonical actin, structural and biochemical studies demonstrated that its depolymerization is faster, likely due to the inherent instability of the filaments (Vahokoski et al. 2014; Pospich et al. 2017). Indeed, despite a conserved location of the monomer interface, substitution of several residues in PfACT1 compared to rabbit actin weaken the interaction within the filament (Pospich et al. 2017; Kumpula et al. 2019). Interestingly, point mutations generated in both *Toxoplasma* and *Plasmodium* to stabilize F-actin revealed that filament instability is in fact essential for the survival of these parasites (Skillman et al. 2011; Douglas et al. 2018).

### 14.2.4 A Large Pool of Globular Actin

Actin turnover is fine-tuned by actin-binding proteins (ABPs). Searches across the sequenced genomes revealed that the apicomplexans have a limited repertoire of actin regulators, ten times smaller than most eukaryotes (Gordon and Sibley 2005; Schüler and Matuschewski 2006). These ABPs include a monomer-binding profilin (PRF), a F-actin-binding coronin (COR), filament-severing actin-depolymerizing factors (ADFs), and F-actin-capping proteins (CPs) (Table 14.2). The apicomplexans lack one of the main regulators of actin dynamics, the actin-related protein-2/3 (ARP2/3) complex, which mediates nucleation and branching of actin filaments, and possess instead two formins (FRM1 and FRM2). A third formin (FRM3) is found in the parasites of the coccidian sub-group of Apicomplexa.

As discussed above, the vast majority of actin is not incorporated into filaments but rather maintained in its globular form. Unexpectedly, ADF and PRF have been identified as the main contributors of the maintenance of this actin pool. Indeed, in contrast to most eukaryotic ADF, api-

**Table 14.2** Actin, actin-related and actin-binding proteins in *Toxoplasma gondii* and *Plasmodium berghei*

| | Class | Gene name | Gene ID | MW (kDa) | Localization | FS (Tg) | Ess (Pb) | Seq Sim. (Tg) | Seq Sim. (Pb) | PDB entry |
|---|---|---|---|---|---|---|---|---|---|---|
| Filament forming | Actin | ACT1 | TGME49_209030 PBANKA_1459300 | 42 | Cytoplasm | –4.50 | E | 84% | 82% | 4CBU 4CBW |
| | | ACT2 | PBANKA_1030100 | 43 | cytoplasm (gametocyte) | | D | NA | 76% | 4CBX |
| | Actin-like | ALP1 | TGME49_219280 PBANKA_0936900 | 46 | Cytoplasm | –2.53 | S | 39% | 49% | |
| | | ALP2a | TGME49_248890 PBANKA_0943300 | 61 | NA | –4.54 | S | 27% | 20% | |
| | | ALP2b | PBANKA_1104800 | 65 | NA | | n.d. | NA | n.d. | |
| | | ALP3 | TGME49_221410 PBANKA_0212300 | 52 | NA | –1.50 | D | 23% | 36% | |
| | | ALP5a | PBANKA_0811800 | 68 | NA | | n.d. | NA | n.d. | |
| | | ALP5b | PBANKA_1007500 | 48 | NA | | n.d. | NA | n.d. | |
| | | ALP8 | TGME49_294850 | 49 | NA | 0.59 | | 25% | NA | |
| | | ALP9a | TGME49_234670 | 72 | NA | –2.59 | | 24% | NA | |
| | | ALP9b | TGME49_269240 | 58 | NA | –0.37 | | n.d. | NA | |
| | Actin-related | ARP1 | TGME49_248630 PBANKA_0209300 | 42 | NA | –1.82 | n.d. | 53% | 63% | |
| | | ARP4a | TGME49_253040 PBANKA_1020600 | 77 | NA | –4.16 | E | 34% | 36% | |
| | | ARP4b | TGME49_258050 | 60 | NA | –4.44 | | 27% | NA | |
| | | ARP6 | TGME49_257710 PBANKA_0616800 | 67 | NA | –5.04 | D | 20% | 40% | |
| F-actin binding | Coronin | COR | TGME49_216970 PBANKA_1464100 | 68 | Cytoplasm | 0.71 | n.d. | 24% | 21% | 2AQ5 |
| F-actin capping | Capping protein | CPa | TGME49_208390 PBANKA_1233900 | 47 | NA | 0.09 | E | 15% | 19% | 2B0R |
| | | CPb | TGME49_219290 PBANKA_1232400 | 36 | NA | –1.30 | D | 29% | 25% | |
| G-actin sequestering | Profilin | PRF | TGME49_293690 PBANKA_0833000 | 18 | Cytoplasm | –4.58 | S | 16% | 18% | 2JKF 2KJG 3NEC |
| | Cofilin/ ADF | ADF1 | TGME49_220400 PBANKA_1137500 | 13 | Cytoplasm | –4.23 | n.d. | 27% | 21% | 3Q2B 2XF1 2L72 |
| | | ADF2 | PBANKA_1103100 | 14 | NA | | E | NA | 25% | 2XFA |
| | Cyclase-associated protein | CAP | TGME49_310030 PBANKA_0208000 | 225 | NA | 1.17 | n.d. | NA | NA | |
| F-actin nucleators | Formins | FRM1 | TGME49_206430 PBANKA_1245300 | 547 | Apical pole | –3.24 | n.d. | n.d. | n.d. | |
| | | FRM2 | TGME49_206580 PBANKA_1434600 | 492 | Cytoplasm, juxtanuclear region | –1.12 | n.d. | n.d. | n.d. | |
| | | FRM3 | TGME49_213370 | 299 | Residual body | –2.79 | | n.d. | NA | |

*E: Essential*
*S: slow*
*D: dispensable*
*n.d.: no data*

*Similarity with (BLAST-pairwise)*
*Oryctolagus cuniculus (rabbit)*
*TgACT1*

The GeneID corresponds to the accession numbers of EuPathDB. The references for the localization are cited in the text. The fitness scores are from the CRISPR-Cas9 genome wide screen performed on *T. gondii* (Sidik et al. 2016) and the PlasmoGEM database established for the erythrocytic stages of *Plasmodium berghei* in mice (Bushell et al. 2017). The sequence similarity (Seq. Sim.) is denoted as a percentage relative to the counterpart in the rabbit *Oryctolagus cuniculus (grey) or TgACT1 (blue)*. The solved structures of the proteins can be accessed via the RCSB Protein Data Bank (PDB). The genes in purple are specific to *Plasmodium* species

complexan ADF (or ADF1 in *Plasmodia*) does not contain the typical F-actin-binding site and therefore prevents actin polymerization by sequestering G-actin (Schüler et al. 2005b; Mehta and Sibley 2010; Singh et al. 2011; Yadav et al. 2011; Baroni et al. 2018). Likewise, PRF appeared to sequester actin monomers and prevent their incorporation into filaments (Skillman et al. 2012), while in other eukaryotes, the presence of PRF usually enhances the polymerization rate of the formin nucleators by increasing the local concentration of ATP-actin in the vicinity of the growing filaments (Pollard 2016). Despite divergence in the amino acids sequences, the apicomplexan PRF shares an overall common structure with mammalian PRFs with the exception of an additional region composed of an acidic loop and a β-hairpin (Kursula et al. 2008; Kucera et al. 2010). This flexible region is a key determinant for interaction with actin but also serves as a recognition pattern in TgPRF to elicit the Toll-like receptor 11 (TLR11) innate immune response

(Kucera et al. 2010; Moreau et al. 2017; Kadirvel and Anishetty 2018).

Another protein that has the capacity to bind G-actin and to regulate F-actin disassembly is the cyclase-associated protein or CAP (Table 14.2). In Apicomplexa, this protein harbors only a CARP domain able to interact and sequester G-actin and to promote the nucleotide exchange from ADP to ATP (Hliscs et al. 2010; Makkonen et al. 2013). CAP thus contributes to the abundant pool of G-actin, is important for the growth of tachyzoites, and appeared dispensable for the erythrocytic stages of malaria parasites but essential for oocyst development in the mosquito midgut (Hliscs et al. 2010; Hunt et al. 2019).

In the absence of ARP2/3 complex, the formins are the sole identified nucleators of F-actin in Apicomplexa. Formins are very large multidomain proteins (>300 kDa) that interact with the barbed end of an actin filament, wherein actin nucleation activity is achieved by the formin homology 2 (FH2) domain (Pollard 2016). It was indeed confirmed, in both *Toxoplasma* and *Plasmodium*, that all the formins expressed were able to bind ACT1 and were potent nucleators of filamentous actin through their FH2 domain (Baum et al. 2008; Daher et al. 2010, 2012; Skillman et al. 2012). In both parasites, formins have been localized in subcellular locations where actomyosin systems participate for critical functions: PfFRM1 and TgFRM1 at the apical pole for motility, TgFRM2 in the cytoplasm at the apical juxtanuclear region involved in organelle positioning and inheritance, and TgFRM3 in the residual body implicated in cell-cell communication (Baum et al. 2008; Jacot et al. 2016; Stortz et al. 2018; Tosetti et al. 2019).

Growth of actin filaments is regulated by their polymerization but also by F-actin-binding proteins such as CPs, a family of proteins that binds the barbed end of F-actin and prevents the exchange with new subunits (Cooper and Sept 2008) and COR, a protein known to stabilize and bundle newly-formed filaments (de Hostos 1999). CPs have been investigated in *P. berghei,* and although their sequences differ from the mammalian counterparts, their folding and biochemical properties are preserved. Remarkably, the length of rabbit actin filaments was significantly decreased by the addition of the heterodimer PbCPα/β (Ganter et al. 2009). *In vivo*, PbCPα/β disruption has a strong impact on the malaria life cycle blocking the transmission of the parasites to a new host as described below (Ganter et al. 2009). In contrast, COR was shown to bind F-actin and increase its polymerization and cross-linking in Apicomplexa (Salamun et al. 2014; Olshina et al. 2015).

Overall, the intrinsic features of apicomplexan actin and ABPs contribute to the maintenance of a heterogeneous mixture of sequestered free G-actin and short filaments in these parasites. A fine-tuned coordination and regulation between the actin polymerization process and the action of ABPs is therefore needed to achieve specific and vital functions. The process is tightly regulated in time and space during parasite division but also during motility when actin filaments are crucial for the survival of the obligate intracellular parasites. All the ABPs have a critical role in the parasites' life cycle and their specific contribution to the regulation of the different actomyosin systems will be discussed below.

## 14.3 Actomyosin Systems in Apicomplexa

### 14.3.1 Actin Polymerization Occurs at Specific Locations

After decades of great limitation in visualization of actin filaments, unexpectedly, antibodies raised against *Plasmodium* ACT1 that preferentially recognized F-actin allowed for the observation of actin concentrated near the nucleus, at the periphery of motile parasites (ookinetes, sporozoites and merozoites), and at the moving junction during merozoite invasion (Siden-Kiamos et al. 2012; Angrisano et al. 2012). In gametocytes, the sexual stages of the malaria parasite, super-resolution and immuno-electron microscopy revealed the presence of an actin cytoskeleton underneath the IMC, along the

microtubules with accumulation at the two poles of the parasite (Hliscs et al. 2015). Gametocytes express both ACT1 and ACT2, so it remains to be determined which one constitutes the observed actin cytoskeleton. More recently, actin chromobodies designed to visualize eukaryotic F-actin have been expressed in *T. gondii* and *P. falciparum* and revealed that polymerized actin is located in distinct subcellular compartments and at specific time points of the lytic cycle. In *P. falciparum*, they confirmed all the previous observations (Stortz et al. 2018). In *T. gondii* intracellular tachyzoites, F-actin was found at the apical perinuclear region, close to the apicoplast. In addition, an extensive F-actin network was stained in the residual body, which connects the basal pole of intravacuolar tachyzoites and organizes them into a rosette within the parasitophorous vacuole (Periz et al. 2017; Tosetti et al. 2019) (Fig. 14.2a, c). Remarkably, in extracellular tachyzoites, a ring of F-actin appears to be produced apically and to translocate to the rear of the parasite, co-localizing with the moving junction. Consequently, an accumulation of F-actin is observed as a dot at the basal pole of the parasites immediately after activation of motility (Tosetti et al. 2019).

Conditional knockout of TgACT1 and PfACT1 has recently been generated in *T. gondii* tachyzoites and *P. falciparum* erythrocytic stages, respectively (Andenmatten et al. 2013; Egarter et al. 2014; Drewry and Sibley 2015; Periz et al. 2017). As expected, absence of actin in both parasites is lethal, but the establishment of a dimerizable Cre-recombinase strategy that efficiently excises the loxP sites flanking the actin gene allowed scrutinizing all aspects of the parasite life cycle and dissecting the cellular functions for which actin is required. The localization of F-actin in both *T. gondii* and *P. falciparum* are in accordance with the location of the formins expressed by these parasites. The composition and role of the corresponding actomyosin systems are discussed below.

### 14.3.2 Contribution of Actomyosin Systems to Cell Division Processes

#### 14.3.2.1 Intravacuolar Connection and Cell-Cell Communication

While *in vitro* cultures of *T. gondii* are asynchronous, the division of tachyzoites within a given parasitophorous vacuole is highly synchronized. The class XXIV TgMyoI, a fairly large myosin (approximately 200 kDa) with two predicted IQ motifs in its neck and no domain identified in its tail, was shown to be responsible for this phenomenon. TgMyoI localizes to the residual body and seems to be associated with the F-actin network, and importantly, TgFRM3 is present at the same location (Frénal et al. 2017b; Periz et al. 2017; Das et al. 2017; Tosetti et al. 2019). Knockout of TgMyoI or TgFRM3 had no impact on the fitness of the tachyzoites. Yet, the parasites appeared disorganized within the PV, failed to organize in rosettes, and divided in an asynchronous manner (Frénal et al. 2017b; Tosetti et al. 2019). The same phenotype was also observed in actin-depleted tachyzoites (TgACT1-cKO) (Periz et al. 2017). It was therefore hypothesized that an actomyosin system could be involved in the formation and/or maintenance of a basal connection between the parasites within the vacuole. Fluorescence recovery after photobleaching (FRAP) experiments performed on wild-type, TgMyoI-KO, TgFRM3-KO and TgACT1-cKO parasites demonstrated that soluble proteins from the cytoplasm but also from the nucleus were able to diffuse between the parasites of the same vacuole only when TgMyoI, TgFRM3 or TgACT1 were expressed (Frénal et al. 2017b; Periz et al. 2017; Tosetti et al. 2019).

These experiments identified the presence of an actomyosin system in the residual body, composed of TgMyoI that likely moves along actin filaments assembled by TgFRM3. This complex is involved in the formation and maintenance of a cytoplasmic connection between intravacuolar parasites allowing the diffusion of soluble proteins and metabolites to ensure a tightly synchro-

nized division. Electron microscopy and 3D reconstruction revealed the presence of a tubular mitochondrion within the connection, passing through the basal complex and shared between intracellular parasites (Frénal et al. 2017b). This observation raises the possibility that diffusion or exchanges could occur between parasites through the mitochondrion as well. In addition, it has been shown that vesicles are also exchanged between the parasites along the actin filaments (Periz et al. 2017). It remains to be determined whether this transport is TgMyoI-dependent. Considering the extensive filamentous network present in the residual body, it is likely that ABPs influence the dynamics and structure of the network. Deletion of TgCAP, which modulates actin turnover through its G-actin sequestering activity, has a strong impact on the structure of the intravacuolar cell-cell connection and the arrangement in rosette of the parasites (Hunt et al. 2019). In this mutant, although the parasites are still connected and able to divide synchronously, the connections are really long, tubular, and not organized around a residual body. The endoplasmic reticulum was also observed within the connections suggesting a possible exchange of material through this organelle, just like the mitochondrion. However, diffusion of reporter protein was observed only between parasites in close proximity. These results open the question on the nature of the material that ensures synchronicity within the PV.

Of relevance, MyoI and FRM3 are only present in the genome of a few coccidians that divide by endodyogeny ensuring the communication that naturally exists in the other apicomplexans that divide by schizogony in the cytoplasm of the same cell (Tables 14.1 and Fig. 14.2c, d). Interestingly, this connection is not maintained in the bradyzoite stage, a latent and encysted form of *T. gondii* that grows slowly and asynchronously (Frénal et al. 2017b).

#### 14.3.2.2 Basal Pole Constriction and Cytokinesis

At the end of cell division, the basal pole of the daughter cells constricts. In *T. gondii*, the class VI-like TgMyoJ has been associated with this process (Frénal et al. 2017b). MyoJ is a large myosin (approximately 270 kDa), but no IQ motif or other domains have been identified (Table 14.1). The protein is found in most apicomplexans and the chromerids (Foth et al. 2006; Mueller et al. 2017). It localizes at a ring-shaped structure at the basal end of the IMC in the developing daughter cells (Frénal et al. 2017b). At the end of division, this structure will constrict and form the posterior cup of the mature parasite (Hu 2008) (Fig. 14.2c). TgMyoJ colocalizes with the CaM-like protein centrin 2 (TgCEN2) at the posterior cup. In the absence of TgMyoJ, tachyzoites display an enlarged posterior pole and a loss of TgCEN2 staining. However, no problem in cytokinesis was observed in these parasites, which only exhibit a modest fitness defect in *in vitro* culture but in contrast a clear loss of virulence in the mouse model of infection (Frénal et al. 2017b). Conversely, depletion of TgCEN2 impacts the basal pole constriction although TgMyoJ remains associated with the enlarged basal cup. These results indicate that TgMyoJ and TgCEN2 play a role in the constriction of the tachyzoite basal pole, but it is not clear yet if they are directly associated with one another (Frénal et al. 2017b). One possibility is that TgCEN2 could act as a light chain for TgMyoJ.

Intriguingly, although loss of basal pole constriction has been observed in TgACT1-depleted tachyzoites demonstrating that it is an actin-dependent process (Periz et al. 2017), none of the three formins expressed by the parasites seems to be involved in this process (Tosetti et al. 2019). This suggests that either an unknown nucleator acts at the basal cup or that some F-actin can be formed in the absence of polymerizing factors possibly following the isodesmic model identified *in vitro* with recombinant TgACT1 (Skillman et al. 2013). This hypothesis is yet to be tested.

In *T. gondii*, depletion of TgACT1 (or TgMyoJ) does not affect the cytokinesis process that occurs naturally, releasing individual parasites during egress. In contrast, in *P. falciparum*, PfACT1 depletion leads to a cytokinesis defect with conjoined merozoites that egress from the infected erythrocytes (Das et al. 2017). The same phenotype was also observed in PfFRM2 depleted

parasites, indicating that PfACT1 and PfFRM2 participate in cytokinesis of malaria schizonts (Das et al. 2017; Stortz et al. 2018). A defect in proper parasite segmentation has also been reported with the depletion of the newly identified basal complex protein PfCINCH (coordinator of nascent cell detachment). Interestingly, co-immunoprecipitation experiments performed on *P. falciparum* schizonts with PfCINCH identified several new proteins of the basal complex and pulled-down PfMyoJ suggesting that in malaria parasites, a basal actomyosin system is responsible for constriction of the schizont basal pole, but also cytokinesis prior egress (Rudlaff et al. 2019). As in *T. gondii*, PbMyoJ was successfully deleted in *P. berghei* without noticeable impact on its life cycle (Wall et al. 2019). Its endogenous tagging allowed its detection only in mature oocysts when sporozoites are formed. PbMyoJ localizes at the junction between the sporozoites and the residual bodies, consistent with a basal localization but remains associated with the oocyst body upon egress of the sporozoites (Wall et al. 2019). So far, it remains enigmatic how these different components contribute to the segregation of the schizonts, but these studies pave the way to a better understanding of the contractile ring, and the constriction and cytokinesis processes in Apicomplexa.

### 14.3.3 Contribution of Actomyosin to Organelle Positioning and Inheritance

Besides MyoA, the second myosin strictly conserved across the Apicomplexa is the class XXII MyoF, which interestingly also has orthologs in the chromerids *C. velia* and *V. brassicaformis* (Foth et al. 2006; Mueller et al. 2017). The function of MyoF has been characterized in *T. gondii* tachyzoites. TgMyoF possesses six predicted IQ motifs and a tail domain with seven WD40 and a coiled-coil domain suggesting that the protein might function as a dimer. Homology was found between TgMyoF and class V myosins that function as cargo transporters, moving organelles within the organism (Hammer and Sellers 2012; Heaslip et al. 2016). No myosin light chain has been found associated with TgMyoF so far (Table 14.3). TgMyoF localizes in the cytoplasm of the parasites, particularly concentrated in the juxtanuclear region and in the vicinity of the dividing apicoplast (Jacot et al. 2013), where the actin nucleator TgFRM2 is also located (Tosetti et al. 2019). Taking advantage of the possibility that TgMyoF could form a dimer, its function was tackled by disrupting this dimer *via* overexpression of the tail of TgMyoF, with the aim to generate a non-functional heterodimer having a dominant-negative effect. TgMyoF was thus found to be essential for the survival of the tachyzoites and a severe defect in apicoplast inheritance was detected as well as a loss of the close positioning of the centrosomes on one side of the nucleus (Jacot et al. 2013). The same phenotype was also observed with the inducible knockout and knockdown of TgMyoF subsequently generated (Jacot et al. 2013; Heaslip et al. 2016). Concordantly, a defect in apicoplast inheritance was observed in TgACT1-depleted parasites as well as in the TgFRM2-KO strain (Periz et al. 2017; Tosetti et al. 2019). The same phenotype is also observed in the intra-erythrocytic stages of *P. falciparum* depleted in PfACT1 or in PfFRM2 confirming the conservation of the machinery in Apicomplexa (Das et al. 2017; Stortz et al. 2018). In addition, PbMyoF is also likely essential in *P. berghei* since the attempts to delete the gene have been unsuccessful so far (Wall et al. 2019).

The primary function of MyoF is likely to maintain the positioning of the centrosomes during cell division. As a consequence of their mispositioning in TgMyoF mutants, the daughter cells grow in opposite directions within the mother parasite instead of growing side-by-side, and the recruitment and association of the apicoplast by the centrosomes are lost (Striepen et al. 2000; Jacot et al. 2013). Some rhoptry organelles also fail to be encapsulated in the progenies and enlarged residual bodies containing apicoplasts and rhoptries were observed. However, enough rhoptries are still accurately targeted to the apical pole and no defect in invasion was recorded in TgMyoF mutants (Jacot et al. 2013). TgMyoF

**Table 14.3** Myosin light chains in *Toxoplasma gondii*

| Gene name | Associated mysoin | Gene ID | FS (Tg) | MW (kDa) | Localization |
|---|---|---|---|---|---|
| MLC1 | MyoA – MyoB/C MyoH | TGME49_257680 | -2.70 | 24 | Inner membrane complex |
| MLC2 | MyoD | TGME49_297470 | 0.78 | 41 | Plasma membrane |
| MLC3 | - | TGME49_250840 | -1.91 | 100 | Conoid |
| MLC4 | - | TGME49_294390 | 0.38 | 19 | Endoplasmic reticulum |
| MLC5 | MyoH | TGME49_311260 | -0.33 | 14 | Conoid |
| MLC7 | MyoH | TGME49_315780 | -0.12 | 23 | Conoid |
| ELC1a | MyoA – MyoB/C | TGME49_269438 | 1.09 | 8 | Inner membrane complex |
| ELC1b | MyoA – MyoB/C | TGME49_269442 | 0.11 | 10 | Inner membrane complex |
| ELC2 | MyoA | TGME49_305050 | 1.57 | 15 | Inner membrane complex |
| CAM1 | MyoH | TGME49_246930 | 1.09 | 19 | Conoid |
| CAM2 | MyoH | TGME49_262010 | -0.81 | 15 | Conoid |
| CAM3 | MyoH | TGME49_226040 | -3.25 | 19 | Conoid |

The GeneID corresponds to the accession numbers of EuPathDB. The fitness scores are from the CRISPR-Cas9 genome wide screen performed on *T. gondii* (Sidik et al. 2016). The references for the localization are cited in the text

was also identified as the motor responsible for the directed movement of the dense granules, in agreement with the fact that this movement is an actin-dependent process (Heaslip et al. 2016). TgCAP has been shown to regulate the trafficking of the dense granules, given that the deletion of this ABP causes the organelles to move further distances and at higher speeds (Hunt et al. 2019). These findings support the fact that TgMyoF might function as class V myosin motors transporting organelles inside cells (Hammer and Sellers 2012). TgMyoF has been also found associated with the acylated protein ARO (armadillo-repeat only) anchored at the surface of the rhoptries and whose depletion leads to the dispersion of mature rhoptry organelles in the cytoplasm, preventing the invasion process to occur (Mueller et al. 2013). Yet, apical rhoptry positioning does not appear to be an actin-dependent process since these organelles are not impacted in the absence of TgACT1 (Egarter et al. 2014; Drewry and Sibley 2015). TgMyoF might play a role in tethering the newly-made rhoptries to the apical part of the developing daughter cells, where they are actually found to accumulate, in addition to the vicinity of the apicoplast (Jacot et al. 2013). Such a tethering function of myosin class Va has been observed for melanosomes in mouse melanocytes for their long-distance transport on microtubules (Hammer and Sellers 2012). We might hypothesize that rhoptries made *de novo* are first tethered by TgMyoF and then transported longer distances by microtubule-binding motor(s), explaining why some rhoptries are found lost in the residual body while most of them are still accurately transported and anchored to the apical pole (Lentini et al. 2019). Alternatively, the rhoptries observed in the residual body might be the ones of the mother that were not properly recycled during the cell division process.

Interestingly, TgMyoF is also found in chromerids, which possess a photosynthetic plastid. It is likely that the function of TgMyoF in positioning of the centrosomes and inheritance of the plastid is conserved in these organisms.

### 14.3.4 Vital Contribution of the Actomyosin System to Motility

MyoA has been identified in *T. gondii* as the motor responsible for the motility of the tachyzoite, powering the movement within the glideosome complex (Meissner et al. 2002). Conditional depletion of this motor clearly demonstrated its critical role for invasion of and egress from the host cell as well as virulence in mice. In *P. berghei*, a promoter-swap strategy has been used to down-regulate PbMyoA in the motile ookinete stage (Siden-Kiamos et al. 2011). This demonstrated the essential role of the protein for motility since no sign of productive gliding locomotion was recorded. As a consequence, the formation of oocysts was completely abolished and no sporozoite were found to be transmitted from the mosquito to a new host.

MyoA belongs to the class XIV myosins and is conserved across the phylum Apicomplexa (Table 14.1). It is one of the smallest myosins (93 kDa), exhibiting a short neck domain with degenerated IQ motifs and no tail (Heintzelman and Schwartzman 1997; Herm-Götz et al. 2002). Moreover, MyoA lacks two key conserved residues in the motor domain, one in the actin-binding surface loop and one in the pivot-point for the motion of the lever arm.

TgMyoA was the first apicomplexan motor that has been directly purified from the parasites for biochemical and biophysical characterization (Herm-Götz et al. 2002). TgMyoA is a monomeric and plus-end-directed motor and despite its unusual features, it exhibits the kinetic properties and velocity of a fast myosin such as the conventional skeletal muscle myosins designed to generate movement rather than force. TgMyoA is non-processive and likely functions in the context of large motor arrays. MyoA localizes to the periphery of the tachyzoites and all motile stages of *Plasmodium*, in tight association with the membrane, a localization dependent on a dibasic motif (two arginines) conserved across the phylum (Hettmann et al. 2000; Green et al. 2017). One myosin light chain (MLC) and one essential light chain (ELC) have been associated with TgMyoA and PfMyoA (Nebl et al. 2011; Williams et al. 2015; Bookwalter et al. 2017; Green et al. 2017) (Table 14.3). TgMLC1, named MyoA-tail interacting protein (MTIP) in *Plasmodia*, is conserved throughout the phylum and is unusual, presenting a long N-terminal extension of 70 residues preceding the CaM-like domain composed of degenerated EF-hands (Herm-Götz et al. 2002; Bergman et al. 2003). The solved structures of the CaM-like domain of TgMLC1 and PfMTIP bound to the neck of MyoA revealed a conserved clamping conformation of the EF-hands around the posterior region of the neck and a buried electrostatic surface between the two proteins as found for other myosin heavy/light chain interaction previously determined (Bosch et al. 2007; Powell et al. 2017). However, this binding is not influenced by the presence of calcium (Green et al. 2006; Bookwalter et al. 2014). In addition, dissection of TgMLC1 mutants in *T. gondii* identified the N-terminal extension as responsible for the localization of TgMyoA at the IMC, thus substituting for the role of the myosin heavy chain tail that TgMyoA lacks (Frénal et al. 2010). Upstream of the MLC1/MTIP binding site, one essential light chain has been identified first in *T. gondii* and very recently in *Plasmodium* species (Nebl et al. 2011; Bookwalter et al. 2017; Green et al. 2017). Surprisingly, the sequence identity between TgELC and PfELC is low (approximately 20%) and prevented the identification of PfELC based on homology search (Bookwalter et al. 2017; Green et al. 2017). In fact, in *T. gondii*, two ELCs, named TgELC1 and TgELC2, compete for the same binding site on the neck of TgMyoA, but TgELC1 is likely and predominantly bound since it was the only one identified by mass spectrometry following co-immunoprecipitation of the glideosome (Nebl et al. 2011; Williams et al. 2015). TgELC1 and TgELC2 are individually

dispensable but cannot be deleted at the same time. Indeed, their contribution to the glideosome complex is essential since their disruption totally destabilizes TgMyoA (Williams et al. 2015). The same is true for the myosin light chain. When TgMLC1 is depleted in tachyzoites or when PbMTIP is depleted in *P. berghei* ookinetes, TgMyoA and PbMyoA are also fully depleted (Sebastian et al. 2012; Egarter et al. 2014). Therefore, the myosin light chain MTIP/MLC1 is essential in both species.

Until recently, it had not been possible to produce soluble and functional apicomplexan myosins from heterologous expression systems, which hampered structural assessment of the unusual features of MyoA and its bound light chains. Remarkably, in 2014, Bookwalter et al. identified the ortholog of the striated muscle myosin-specific co-chaperone of the UCS protein family, Unc45b, in the *T. gondii* genome (Bookwalter et al. 2014). They succeeded in producing soluble and functional TgMyoA/TgMLC1 and TgMyoA/TgMLC1/TgELC1 complexes by co-expressing these proteins in the presence of the co-chaperone TgUNC. This not only led to the determination of the kinetics of the motor in the presence of its light chains in both *T. gondii* (Bookwalter et al. 2014) and *P. falciparum* (Bookwalter et al. 2017) but also to structural insight of TgMyoA bound to its light chains (Powell et al. 2017, 2018). In both parasites, it was elegantly demonstrated with the *in vitro* motility assay that the binding of ELC in addition to MLC/MTIP doubles the speed of the actin movement (Bookwalter et al. 2014, 2017). ELC binds upstream of MLC to the neck domain of MyoA but also to the converter of the head domain (Powell et al. 2017). With the two bound light chains, the length and stability of the lever arm is increased and optimized for force transduction. Interestingly, binding of ELC and MLC is cooperative and requires first binding of MLC to MyoA (Bookwalter et al. 2017; Powell et al. 2017). This could be due to the fact that TgMyoA needs TgMLC1 for its localization *in vivo* (Frénal et al. 2010), a possible pre-requisite for the binding of TgELC1.

The crystal structure of TgMyoA solved in complex with the CaM-like domain of TgMLC1 was an important step forward. It revealed that mutations of the key residues implicated in binding to actin and in the function of the motor domain are a clear adaptation to the divergent TgACT1 and create new interactions that maintain and possibly enhance the transduction of the force from the active site to the lever arm (Powell et al. 2018). These results unravel the mystery of MyoA being a *bona fide* fast motor despite its unusual features.

It remains to be determined how the function of the motor is regulated. Calcium seems to regulate the assembly of the motor complex since the cooperative binding of the light chains to MyoA is further increased in the presence of calcium (Powell et al. 2017). Phosphorylation of TgMyoA and PfMyoA appear to modulate rather than activate the motor since phospho-mimetic mutants enhanced the affinity for actin filaments and the speed of their displacement *in vitro* (Bookwalter et al. 2017; Powell et al. 2018). *In vivo*, some phosphorylation sites on TgMyoA seem to be important for the activation of the motility while no site on TgMLC1 was found to be critical to modulate gliding (Jacot et al. 2014; Gaji et al. 2015).

Unexpectedly, the gene coding for *TgMyoA* has been successfully deleted in *T. gondii* tachyzoites (Andenmatten et al. 2013). Parasites could be maintained in culture although their lytic cycle was severely impacted with a strong defect in motility, invasion, and egress. The isolation of this mutant raised the question of how some motility can still be achieved without TgMyoA. The identification of a second glideosome complex similar to the initial one but located at the basal ring of the parasite, which includes the class XIV TgMyoC instead of TgMyoA, raised the possibility of a functional complementation between the complexes, especially because TgMyoC shares the two light chains TgMLC1 and TgELC1 with TgMyoA (Frénal et al. 2014). Indeed, in the absence of TgMyoA, a relocalization of TgMyoC along the pellicle was observed, highlighting the plasticity and adaptation of the parasite to ensure comple-

tion of the vital step of host cell invasion (Frénal et al. 2014, 2017b). Accordingly, parasite clones with a simultaneous deletion of *MyoA* and *MyoB/C* could not be isolated (Egarter et al. 2014). In contrast to MyoA, MyoC is not conserved across the Apicomplexa, especially in *Plasmodium* species (Table 14.1). In these parasites, MyoA is therefore likely essential for motility as in *P. berghei* ookinete wherein down-regulation of PbMyoA completely abrogated gliding and blocked the life-cycle progression of the parasite into the mosquito vector (Siden-Kiamos et al. 2011).

A third motor from the class XIV, TgMyoH, has been found to be crucially involved in motility of the tachyzoites (Graindorge et al. 2016). TgMyoH is located in the conoid, at the apex of the parasites, and its conditional depletion led to a severe defect in motility, invasion, and egress from the infected cells. Invasion experiments demonstrated that, in contrast to wild-type parasites that completed invasion, TgMyoH-depleted parasites were attached to the surface of the host cell by their tip, forming a moving junction that cannot be translocated along the parasite body. In the same experiment, TgMyoA-depleted parasites were engaged in the penetration into the host cell, but most of them stopped at the level where the IMC starts. These results indicate that a relay of myosins is involved in the translocation of the adhesins along the parasites with TgMyoH initiating the process at the apex until the edge of the IMC, then TgMyoA along the pellicle and finally TgMyoC at the basal ring (Fig. 14.3). Noteworthy in this context, in the absence of TgMyoC, TgMyoA is additionally found at the basal ring (Frénal et al. 2014). The precise role of TgMyoC is therefore unknown, but it might be involved in the parasite twisting motion observed at the very end of the invasion process that enables sealing of the PV (Pavlou et al. 2018).

TgMyoH belongs to class XIV and orthologs are found in other coccidians such as *Eimeria* and *Cryptosporidium* that harbor a conoid but also in piroplasma such as *Theileria* and *Babesia* that do not (Table 14.1) (Mueller et al. 2017). However, no direct ortholog has been identified in *Plasmodium* species. Its sequence predicts a neck region with eight IQ motifs and a tail harboring three α-tubulin suppressor 1 (ATS1) or RCC1 (Regulator of chromosome condensation 1) domains. These domains target TgMyoH to the apex of the parasite, linking directly or not TgMyoH to the conoid fibers (Graindorge et al. 2016). Six CaM-containing domain proteins have been found associated with TgMyoH (Graindorge et al. 2016; Long et al. 2017). Four of them, TgMLC5, TgCaM1, TgCaM2, and TgCaM3, are only associated with TgMyoH, since their apical localization is disrupted when TgMyoH is down-regulated, but no difference in the localization of the two others, TgMLC1 and TgMLC7, was observed. In addition to TgMLC1, only TgCaM3 appears essential for tachyzoite survival (Long et al. 2017) (Table 14.3). The function of this protein is not dependent on the presence of calcium, suggesting a structural role of TgCaM3 in the neck domain of TgMyoH, in contrast to TgCaM1 and TgCaM2, which might have a regulatory role since their function is calcium-dependent (Long et al. 2017).

While no direct ortholog of TgMyoH has been found in *Plasmodium* species, PbMyoB, which was characterized in *P. berghei*, might be a functional homolog (Yusuf et al. 2015). PbMyoB, like TgMyoH, belongs to myosin class XIVc, but it is significantly smaller since it exhibits a shorter neck with one predicted IQ motif and no tail (Foth et al. 2006). PbMyoB has been localized at the extreme apical pole, likely at the apical polar rings, of all the motile forms of the parasite (merozoite, ookinete and sporozoite). Its associated light chain, PbMLC-B, is quite atypical being very long (652 aa) and possessing two coiled-coil domains, similar to TgMLC3, which is also found at the apex of the tachyzoite (Graindorge et al. 2016). Functional studies would be needed to determine the role of this complex in *Plasmodium,* although PbMyoB appeared dispensable both in the high throughput knockout screen of *P. berghei* genes (Bushell et al. 2017) as well as a specific reverse genetics approach (Wall et al. 2019). In contrast, PbMyoE is found at the basal pole of the motile ookinete and sporozoite (Table 14.1), and its deletion severely impacts the motility of the sporozoite (Wall et al. 2019).

In line with the role of myosin motors, TgACT1 and PfACT1 have been shown to be essential for gliding motility. *T. gondii* tachyzoites are severely impaired in their motility, invasion capacity and exit from the infected cell (Egarter et al. 2014; Drewry and Sibley 2015). In contrast, although invasion of the erythrocyte by *P. falciparum* merozoites is completely abolished in the absence of actin, egress is not (Das et al. 2017). For both parasites, egress involves the rupture the parasitophorous vacuole membrane and the host cell plasma membrane, which is assisted by the release of perforins from the micronemes (Kafsack et al. 2009; Garg et al. 2013). While the tachyzoite needs to impose an additional mechanical pressure on the membranes applied by its motility to exit the cell (Meissner et al. 2002; Plattner et al. 2008; Frénal et al. 2010; Mehta and Sibley 2011; Graindorge et al. 2016; Jacot et al. 2016), the schizonts activate parasite and host proteases that destabilize the cytoskeleton of the red blood cell, and it is not clear that motility is required (Millholland et al. 2011). The actin depletion performed in *Plasmodium* indicates that motility is absolutely required during invasion for the moving junction to progress backward along the parasite but not for egress, for which the destabilization of the erythrocyte cytoskeleton and then the curling of the red blood cell membrane might be sufficient to eject the merozoites into the bloodstream (Das et al. 2017).

## 14.4 ABPs Regulate the Function of the Actomyosin Systems

ABPs that influence actin turnover have obvious impacts on the function of the actomyosin systems. The two main actin monomer-sequestering proteins, ADF and PRF, have been shown to have a strong impact on motility, invasion, and egress of *T. gondii* and *P. berghei* (Plattner et al. 2008; Mehta and Sibley 2011; Moreau et al. 2017). In addition, depletion of TgADF and TgPRF also revealed a defect in the inheritance of the plastid-like apicoplast in daughter cells (Jacot et al. 2013). Contrastingly, the G-actin-sequestering protein TgCAP, which is also critical for gliding motility, invasion, and egress of *T. gondii*, as well as for dense-granule trafficking and parasite connection, has no influence on apicoplast inheritance (Hunt et al. 2019). These observations reflect the different spatial requirements for actin turnover within the parasite. In *Plasmodium*, PbCAP has an essential role only in the oocyst stage within the mosquito (Hliscs et al. 2010). In the absence of PbCAP, maturation of the oocysts is compromised and no sporozoites are formed, preventing transmission of parasites from the mosquito to a new host.

The growth of actin filaments is regulated by capping proteins. The heterodimer PbCPα/β has the ability to decrease the length of rabbit actin filaments *in vitro* (Ganter et al. 2009). *In vivo*, disruption of PbCPβ had no impact on the erythrocytic cycle of the parasite, but in the *Anopheles* mosquito vector, the sporozoites displayed a strong defect in motility preventing them from colonizing the salivary glands and therefore blocking transmission of the parasites to a new host (Ganter et al. 2009). Unexpectedly, PbCPα appears to be essential alone in the erythrocytic cycle likely working as a homodimer, but its precise role has not been assessed yet (Ganter et al. 2015). One hypothesis for the split role of these dimers is the environment-dependent function with PbCPα/β working at ambient temperature in the mosquito while the PbCPα homodimer could be adapted to the warm-blooded host.

Actin polymerization is also regulated by coronin (COR). Both TgCOR and PfCOR are able to bind F-actin and increase its polymerization and bundling *in vitro* (Salamun et al. 2014; Olshina et al. 2015). Deletion of TgCOR revealed that this protein is not essential in tachyzoites with only a modest effect on the invasion and egress steps of the lytic cycle (Salamun et al. 2014). Yet, interestingly, TgCOR is cytosolic but strikingly relocalizes to the posterior pole in motile parasites, independently of actin dynamics but concomitantly to microneme secretion (Salamun et al. 2014). This suggests that TgCOR could play a role in endocytosis and membrane

recycling linked to the microneme exocytosis to eliminate excessive accumulation of membranes at the posterior pole and preserve the pellicle integrity. A relocalization from the periphery to the rear of the parasite has also been observed in the sporozoite of *P. berghei* during motility, but this time in an actin-dependent manner occurring downstream of the calcium-signaling cascade leading to microneme secretion (Bane et al. 2016). Sporozoites lacking PbCOR are impaired in motility and in their ability to colonize the salivary glands of the *Anopheles* mosquito (Bane et al. 2016). Recently, PfCOR has also been associated with artemisinin resistance. Indeed, long-term *in vitro* culture of *P. falciparum* cell lines under dihydroartemisinin selection allowed the isolation of mutants presenting several point mutations in the beta-propeller region of PfCOR (Demas et al. 2018). After *PfKelch13*, *PfCOR* is the second gene demonstrated to confer reduced artemisinin susceptibility and the conservation of the beta-propeller motif in the two proteins suggests a common mechanism of resistance and the possible emergence of PfCOR mutants in nature.

## 14.5 Conclusion

Over the last decade, a big step forward has been made in elucidating the role of myosin heavy chains, visualizing actin filaments, and characterizing regulatory ABPs in *Toxoplasma* and *Plasmodium*. This led to the identification of actomyosin systems serving three major processes in these parasites; (i) organelle trafficking, positioning and inheritance, (ii) basal pole constriction and intravacuolar cell-cell connection and (iii) motility, invasion, and egress. Until recently, it was really challenging to purify soluble and functional apicomplexan myosin heavy chains from heterologous systems. The identification of the *T. gondii* homolog of a myosin-specific co-chaperone of the UCS (UNC-45/CRO1/She4p) family changed this and has been a key factor in producing TgMyoA and its light chains from the baculovirus/*Sf9* insect cell expression system (Bookwalter et al. 2014). This allowed in-depth biochemical and structural characterization of TgMyoA/TgMLC1 and TgMyoA/TgMLC1/TgELC1 complexes uncovering mechanistic and regulatory aspects of these interactions (Bookwalter et al. 2014; Powell et al. 2017, 2018). Functional study of the chaperone TgUNC in *T. gondii* tachyzoites demonstrated that it is indeed a *bona fide* myosin chaperone in the parasite (Frénal et al. 2017b). All 11 myosin heavy chains expressed were destabilized upon depletion of TgUNC, and the phenotype of its down-regulation is a combination of the phenotypes of each myosin heavy chain described previously.

The repertoire of myosin heavy chains has been localized in both *Toxoplasma* and *Plasmodium,* and all the essential motors have been functionally characterized in *T. gondii* (Wall et al. 2019; Jacot et al. 2013; Graindorge et al. 2016; Frénal et al. 2017b). It remains now to assess the function of the myosin heavy chains in *Plasmodium* and to identify the components of the different actomyosin systems, especially the proteins of the basal complex that could be involved in the constriction and cytokinesis of the parasites, a process shared by all the parasites of the phylum. To understand the function and regulation of the myosin heavy chains, it also remains to identify the associated myosin light chains (MLCs). Several of them have been localized in *T. gondii* (Polonais et al. 2011) (Table 14.3) but not yet demonstrated as being associated with a motor. The limited repertoire of classical MLCs suggests that some other EF-hand-containing proteins could act as myosin light chains. Three calmodulins (TgCAM1–3) have been identified as light chains for TgMyoH (Long et al. 2017), and it might be worth exploring the possibility that some centrins could also play this role, especially TgCEN2, which shares the same function as TgMyoJ in the basal complex constriction.

A better understanding of the dynamics of the actomyosin system has been provided by the biochemical and functional characterization of ABPs that unraveled unusual features of these proteins compared to other eukaryotes. The genome of *Toxoplasma* and *Plasmodium* further encodes a

large repertoire of actin-like and actin-related proteins that has not yet been explored, with several of them being likely critical for the survival of the parasites as suggested by the genome wide screens (Table 14.2). Characterization of these proteins should provide new insights on the contribution of actomyosin systems to the diverse cellular functions of the parasite.

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