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Toxoplasma as a novel system for motility[☆]

Dominique Soldati¹ and Markus Meissner

Motility is a characteristic of most living organisms and often requires specialized structures like cilia or flagella. An alternative is amoeboid movement, where the polymerization/depolymerization of actin leads to the formation of pseudopodia, filopodia and/or lamellipodia that enable the cell to crawl along a surface. Despite their lack of locomotive organelles and in absence of cell deformation, members of the apicomplexan parasites employ a unique form of locomotion called gliding motility to promote their migration across biological barriers and to power host-cell invasion and egress. Detailed studies in *Toxoplasma gondii* and *Plasmodium* species have revealed that this unique mode of movement is dependent on a myosin of class XIV and necessitates actin dynamics and the concerted discharge and processing of adhesive proteins. Gliding is essential for the survival and infectivity of these obligate intracellular parasites, which cause severe disease in humans and animals.

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Abbreviations

ARP	actin-related protein
CDPK	calmodulin-domain protein kinase
GFP	green fluorescent protein
IMC	inner membrane complex
IMP	intramembranous particles
JAS	jasplakinolide
MLC	myosin light chain
MPP	micronemal protein protease
MTIP	myosin-tail-interacting protein
MyoA	myosin A
NPF	nucleation-promoting factors
Pf	<i>Plasmodium falciparum</i>
PVM	parasitophorous vacuole membrane
Tg	<i>Toxoplasma gondii</i>
TRAP	thrombospondin-related anonymous protein

Introduction

The phylum Apicomplexa includes over five thousand parasitic species, many of which are important pathogens in humans and animals. *Plasmodium falciparum* is the most deadly member of this group, responsible for a significant fraction of the million malarial deaths each year, predominantly in young children. *Toxoplasma gondii* is the causative agent of toxoplasmosis, a life-threatening disease in immunocompromised patients and a potentially severe congenital disease, leading to blindness or cognitive impairment in infected infants. In the absence of any locomotive organelles such as flagella, invasive apicomplexans are unable to swim but exhibit instead a unique form of substrate-dependent motion called gliding motility. In contrast to crawling motion, gliding motility occurs without pseudopod extension or any other major changes in cell morphology. In *T. gondii*, locomotion was elegantly demonstrated to require an intact parasite actin cytoskeleton [1] and to involve three forms of movement: circular gliding, upright twirling and helical rotation [2]. More recent data support the view that the motility is also controlled by the regulation of actin filament polymerization [3[•]]. The involvement of a myosin in gliding motion was suggested by the use of a myosin ATPase inhibitor [4] and it was subsequently established that the class XIV *T. gondii* myosin A (TgMyoA) is necessary [5^{••}]. In addition, studies conducted in *Plasmodium berghei* demonstrated that the secretion of adhesive proteins at the apical surface of the parasite and their presumed interaction with the actomyosin system led to posterior relocalization, a critical event for gliding [6]. Interestingly, a similar form of gliding motility has been characterized in diatoms. These protists employ the actomyosin system to glide along a surface [7]. Like the apicomplexans, they leave behind them a trail of adhesive molecules (micronemal proteins in apicomplexans, proteoglycans in diatoms). By definition, gliding denotes a smooth and continuous movement and some bacteria also characteristically exhibit such movement, via mechanisms that do not involve pili but rely on a well-developed cytoskeleton and surface adhesion molecules (for review see [8]).

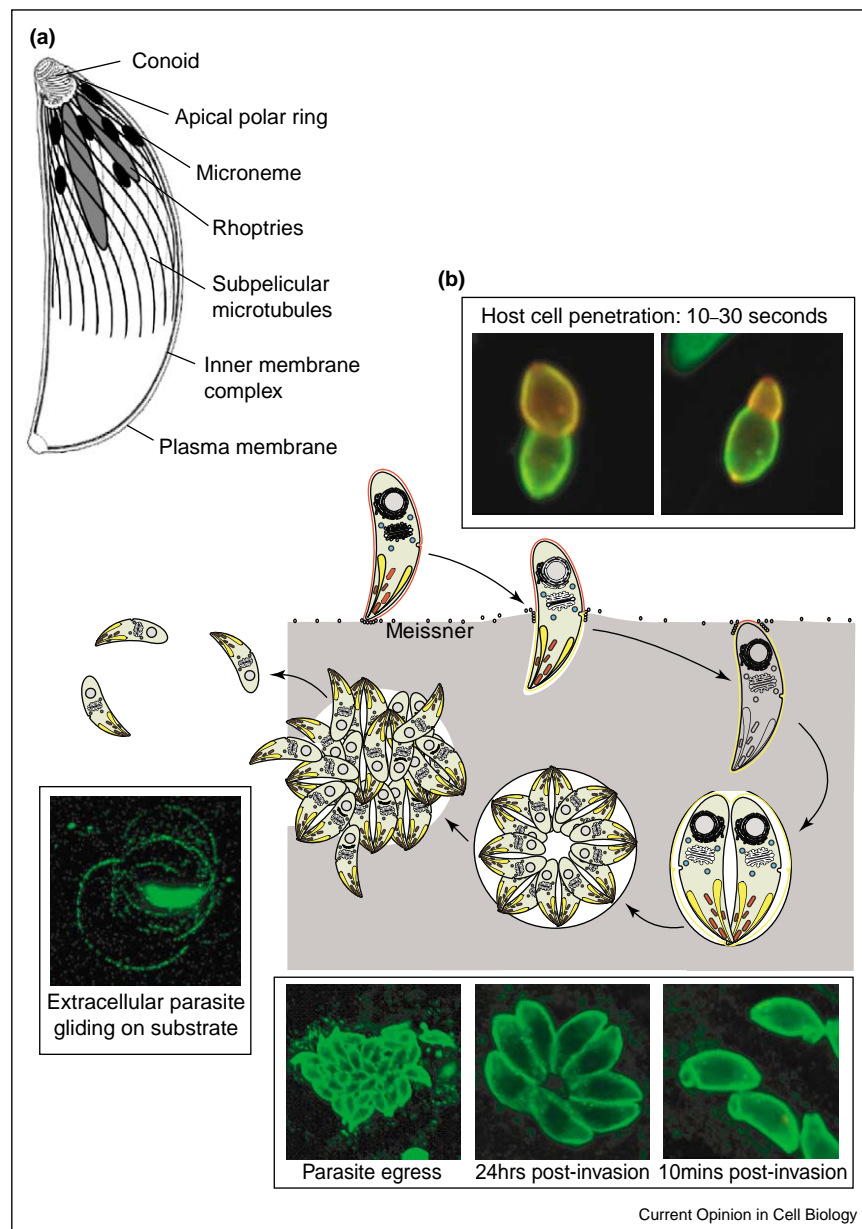
This review focuses on the molecular elements that orchestrate gliding motility in apicomplexans. This process is essential for the survival and infectivity of these obligate intracellular parasites, which cause severe disease in humans and animals.

The apical complex: an apparatus for gliding motility and invasion

The Apicomplexa are unified structurally and functionally in that they share a sophisticated apical apparatus

[☆]Supplementary data associated with this article can be found at doi:10.1016/j.ceb.2003.11.013

Figure 1



The lytic cycle of *Toxoplasma gondii*. **(a)** Schematic representation of *T. gondii* tachyzoite and the subcellular structures involved in gliding motility and host cell invasion. **(b)** Cycle of host cell invasion and egress by *T. gondii*. This multi-step process includes the attachment to host cells, the discharge by the micronemes (red), the discharge by the rhoptries (yellow), the formation and sealing of the parasitophorous vacuole, intracellular parasite replication, lysis of the PVM and parasite egress.

necessary for gliding and invasion (Figure 1a). The apical complex of *T. gondii* is composed of cytoskeletal elements and specialized regulated secretory organelles called rhoptries and micronemes. The apical polar ring serves as a microtubule-organizing center for the subpellicular microtubules, which form a basket and confer a crescent shape to the parasite. Not present in all apicomplexans, the conoid is a small cone-shaped structure, which is composed of a novel form of tubulin polymer [9^{*}] and

protrudes during invasion. Apicomplexans are also characterized by a three-layered pellicle, composed of the plasma membrane closely associated to the inner membrane complex (IMC), which is formed by one or more flattened vesicles aligned in longitudinal rows connected by sutures. The membranes of the IMC are characterized by the presence of a two-dimensional particle lattice composed of aligned intramembranous particles (IMPs) (recently reviewed in [10^{••}]). The lines of IMPs are

organized as single rows interspersed with double rows and the latter are aligned with the underlying subpellicular microtubules [11]. The subpellicular microtubules are surrounded by a meshwork of intermediate-sized filaments (10 nm diameter) of which TgIMC1 is a major component [12].

Gliding motility assists with three vital functions of the parasite: migration, host cell invasion and egress

Mechanical barriers represent the first line of protection against microbial infection and the apicomplexans are capable of crossing non-permissive biological barriers such as the intestine, the placenta and the blood–brain barrier, relying on several mechanisms including trafficking within leukocytes (i.e. the ‘Trojan horse’ mechanism), transcytosis and paracellular migration.

In *Plasmodium* sporozoites, transcellular migration through hepatocytes has been described in detail [13•] and occurs by a process that causes damage to some of the host cells [14]. Apparently, breaching of the host plasma membrane is a critical to induce the exocytosis of apical organelles, which is required for an ultimate successful infection of hepatocytes [13•]. *T. gondii* appears to disseminate very rapidly into the host by transmigration, using a paracellular route that bypasses the onset of intracellular replication and also avoids damage to the host cell that would otherwise initiate an inflammatory response [15]. Although the migratory machinery in both parasites is poorly understood, it certainly requires active parasite motility [14,16].

Unlike other intracellular viral, bacterial and parasitic pathogens that take advantage of natural or induced host-cell endocytosis or phagocytosis, apicomplexans do not exploit these host processes to establish infection. *T. gondii* invades a wide variety of vertebrate cells other than professional phagocytes by an active mechanism, which takes only 10–30 s and is dependent on the ability of the parasite to glide. Gliding involves the discharge of adhesive proteins at the parasite’s apical end by the micronemes; when bound to host-cell receptors these adhesive proteins are translocated along the parasite surface by an actin-dependent process and shed from the posterior pole. This shedding results in the formation of characteristic spiral trails, as first demonstrated for the circumsporozoite protein in *P. falciparum* [17].

Upon host-cell invasion, a parasitophorous vacuole is formed in the host cytoplasm, inside which the parasite replicates safely. The parasitophorous vacuole membrane (PVM) serves as a critical transport interface between the parasite and the host-cell cytoplasm. It is derived from the host plasma membrane and is extensively modified by the fusion of rhoptry-derived secretory organelles, which contribute to the function and the fate of the PVM

[18]. The recent identification of stomatin, a raft protein in *Plasmodium*, suggests that detergent-resistant membrane rafts are contributing to invasion and PVM formation [19]. The PVM is permeable to molecules smaller than 1.3–1.9 kDa [20], making the ionic composition within the vacuole equivalent to that of the host-cell cytoplasm. The active route of entry determines the fate of the PVM, which unlike other endocytic vacuoles is devoid of the regulatory molecules that govern vesicular fusion. Consequently the parasitophorous vacuole remains completely resistant to acidification and lysosomal fusion. The rapid, multi-step process of host invasion is depicted in Figure 1b.

The phenomenon of egress shares common features with the invasion process [21]. Like invasion, *T. gondii* egress is dependent on gliding motility and is regulated by a calcium signal transduction pathway. Intracellular parasites appear to possess the ability to monitor host-cell viability and, if fitness becomes compromised, the parasite will egress and invade neighboring cells. Upon rupture of the plasma membrane, a drop in host cytoplasmic K^+ level activates a parasite phospholipase C (PLC), leading to an increase in parasite cytoplasmic calcium [22,23]. One of the critical effects of intracellular elevation of Ca^{2+} is the extrusion of the conoid and microneme secretion in *T. gondii*. A Ca^{2+} -dependent calmodulin-domain protein kinase, TgCDPK1, is potentially implicated in the signal transduction leading to organelle discharge [24]. Interestingly, the parasite intracellular calcium stores, including the ER, the mitochondrion and the acidocalcisome (reviewed in [25]), have been shown to regulate microneme secretion and to initiate motility in *T. gondii* [26]. Additional factors have been recently proposed to play a role in microneme exocytosis. The parafusin-related protein 1 (PRP1) is a bifunctional protein acting as a phosphoglucomutase and as a Ca^{2+} -sensitive component of the cytoplasmic scaffold enveloping microneme vesicles [27]. Evidence based on cholesterol depletion suggests that the cholesterol content of the host cell, but not the parasite, critically influences the efficiency with which parasite organelles, including the micronemes, discharge [28].

Repertoire of myosins in apicomplexans

Myosins form an ever-increasing superfamily of distinct classes of actin-based motors [29]. The Apicomplexa appear to possess only a limited number of myosins restricted to a few exotic classes. The sequencing of the *P. falciparum* genome has revealed the presence of six genes coding for putative myosins, some of which are still awaiting experimental confirmation of their annotation. A recent sequence analysis of these genes revealed that four of these myosins, including the *P. falciparum* myosin A (PfMyoA), belong to the unusual class XIV, which is restricted to this phylum [30]; one is an unclassified myosin; and the last one belongs to the class V [31].

This latter motor, which has been named PfMyoC, (AF222717 and *Plasmodium falciparum*/CHR 13/MAL13P1.148) has a predicted size of 250 kDa and contains five conserved IQ motifs as well as three WD40 domains in the tail. Homologues of this myosin are found in other apicomplexans including *Cryptosporidium parvum* (CAD98272) and *T. gondii*.

TgMyoA and its homologues in other apicomplexans are usually small (91–93 kDa) and, although the motor domain is conserved, the neck and tail domains cannot be defined easily. When compared with the larger members of the class XIV myosins, TgMyoB and TgMyoC, TgMyoA appears to possess a very divergent neck with one degenerate IQ motif and virtually no tail. A similar divergent neck domain is present on TgMyoB and C, which additionally contain distinct tails generated by alternative splicing. The degenerate IQ motif, present at the extreme C terminus of MyoA, associates with a calmodulin-like protein TgMLC1 (where MLC stands for myosin light chain) [32] and a homologous protein in *P. falciparum* called myosin-tail-interacting protein (PfMTIP) [33^{*}]. TgMyoA and PfMyoA localize permanently beneath the plasma membrane of the parasite [34–37] and are likely, on the basis of new evidence, to be anchored in the inner membrane complex [33^{*}]. At this point, it is unclear if the ATPase activity of TgMyoA and other members of the class XIV can be modulated. Indeed, these myosins do not follow the TEDS (Thr, Glu, Asp, Ser) rule [38,39] according to which the amino acid positioned 16 residues upstream of the highly conserved DALAK sequence should be either a negatively charged amino acid or a phosphorylation site [40]. Functional TgMyoA purified from parasites was demonstrated to have biophysical and biochemical properties that theoretically would drive gliding motility at a speed of 3–5 $\mu\text{m s}^{-1}$, which is in the observed range of speed of *T. gondii* tachyzoites. Transient kinetics proved that MyoA is a fast, single-headed motor that moves with a step size of 5.3 nm and a velocity of 5.2 $\mu\text{m s}^{-1}$ towards the plus end of actin filaments [32].

The role of TgMyoA in invasion, egress and virulence *in vivo* has been unambiguously established by the generation of a tetracycline-inducible conditional knockout of this gene in *T. gondii* [5^{**}]. Depletion of MyoA was shown to completely abrogate gliding motility of these parasites, impairing both host-cell invasion and egress, whereas no effect on intracellular replication and secretion of micronemal proteins was observed. In *T. gondii*, four other members of the class XIV (TgMyoB, C, D and E) are expressed stage-specifically and some of these motors may fulfil a similar role to TgMyoA in the other invasive stages. Intriguingly, TgMyoC is concentrated as a ring at the posterior pole of the parasite. Even though overexpression of the motor leads to a significant defect in parasite division [41], the possible role of TgMyoB/C in

motility remains to be determined. Whether, as in other eukaryotes, some of the apicomplexan myosins are involved in other cellular functions including cytokinesis, endocytosis or transport of mRNAs [42] remains to be established.

Myosin-A-associated proteins

TgMyoA is associated with a calmodulin-like myosin light chain of 30 kDa, TgMLC1 and a 25 kDa myristoylated protein (previously named MADP) identified and characterized by the group of C Beckers. Recently, an additional component of the motor complex has been identified and characterized. This integral membrane protein is likely to serve as a receptor, anchoring the motor complex in the IMC (C Beckers, International Toxoplasmosis meeting New York 2003). TgMLC1/PfMTIP bind precisely to the membrane-targeting determinant previously mapped on TgMyoA [33^{*},39].

Role of actin cytoskeleton dynamics in gliding motility

Early studies using cytochalasin D indicated the importance of intact actin cytoskeleton on parasite motility and host-cell invasion, but the exclusive contribution of the parasite's actin was established using host and parasite mutants that are resistant to the drug [1]. Despite this clear dependence, formal demonstration of the presence of filamentous actin has been very difficult in apicomplexans, apparently because of the inherent instability of the filaments. Indeed, biochemical approaches also established that the vast majority of actin in *Toxoplasma* and *Plasmodium* is monomeric. Using the cyclopeptide jasplakinolide (JAS), a potent inducer of actin polymerization, it was possible to stimulate the formation of a prominent acronemal protrusion at the apical pole of the parasite [43]. JAS was also shown in static-endpoint assays to block gliding motility and host-cell invasion [43,44]. A subsequent study using lower doses of the drug revealed that actin polymerization increased the speed and controlled the duration and direction of gliding motility in *T. gondii* [3^{*}].

Actin-binding proteins in apicomplexans and actin nucleation and polymerization

The susceptibility of apicomplexans to actin-polymerizing and -depolymerizing drugs implies the existence of a machinery that promotes actin nucleation and polymerization. Very few actin-regulatory proteins have been described to date in these parasites. An actin-depolymerizing factor is present and abundantly expressed in many apicomplexans. This ADF/cofilin homologue is likely to be involved in filament severing [45] and might explain why actin filaments are highly destabilized. In addition, an actin-sequestering protein named toxofilin, for which no homologue can be found in the *Plasmodium* genome, may provide a mechanism for maintaining a large pool of globular actin in *T. gondii* [46]. When overexpressed in

mammalian cells, a GFP-tagged toxofilin readily disrupts the stress fibers and lowers the level of actin filaments. The actin-sequestering properties of toxofilin depend on its phosphorylation state, which is modulated by a parasite type-2C phosphatase and by casein-kinase-II-like activity [47]. Finally, another actin-binding protein belonging to the coronin family was previously described in *P. falciparum* [48]. This coronin-like protein is also present in other apicomplexans and contains five WD domains and a large C-terminal coiled coil. Recently, yeast coronin was reported to regulate the activity of the actin-related protein 2/3 (ARP2/3) complex [49].

The ARP2/3 complex is a well-characterized universal machine conserved across all eukaryotic phyla that orchestrates the formation of actin filaments. This complex, which is composed of seven proteins (ARP2, ARP3 and ARPC1–5), is activated by nucleation-promoting factors (NPFs). ActA was the first NPF to be identified in *Listeria* and since then many more activators of ARP2/3 have been characterized in diverse organisms [50,51]. In *T. gondii*, actin is encoded by a single-copy gene [52], whereas in *P. falciparum* two genes encoding actin were previously described [53]. Actin I is constitutively expressed whereas actin II is transcribed only in the sexual stages and exhibits an amino-acid sequence divergent from that of previously characterized actins. The availability of the parasite genome sequences revealed the presence of genes encoding additional actin and actin-related proteins in both *Plasmodium* species and *T. gondii*, some of which may represent functional equivalents of ARP2 and ARP3; however, the accuracy of current annotations of these genes and their functions will await experimental confirmation. The sequence alignments of some of these actin and actin-related proteins are included in the online [supplementary material](#). Some genes apparently coding for larger proteins containing actin interspaced by large loops are also present in both parasites, but it is not clear at this point if they are genes or pseudogenes. Additionally, a 40 kDa WD40 β -propeller protein with significant homology to the ARPC1 is present in the *P. falciparum* genome. By contrast, none of the other subunits normally associated with the complex (ARPC2–5) and no obvious NPFs can be identified in the apicomplexan genomes. Finally, two genes with significant homologies to the actin-capping subunits α and β are present in apicomplexans, suggesting that, like in other eukaryotes, an ubiquitous α/β heterodimer tightly caps the barbed ends of actin filaments and thus might contribute to the assembly and stabilization of short filaments [54].

Role of secretory organelles in gliding motility and host-cell invasion

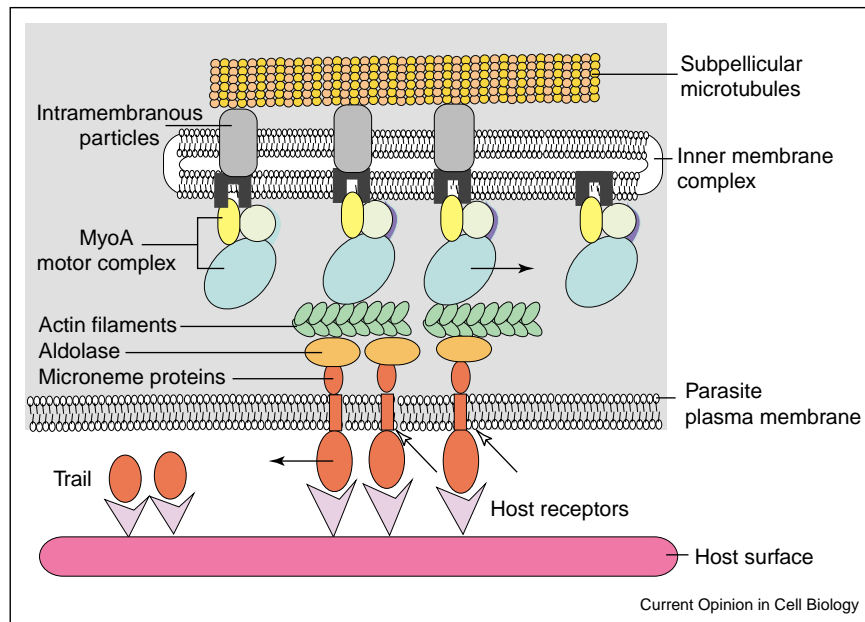
Micronemes and rhoptries are unique secretory organelles that contain products required for motility and

host cell attachment and for establishment of the parasitophorous vacuole, respectively. The micronemes discharge proteins containing diverse adhesive domains implicated in host-cell attachment. The transmembrane adhesins participate actively in motility and invasion by binding to components of the extracellular matrix or to specific host-cell receptors and by bridging them to the cytoplasmic actomyosin system of the parasite via their cytoplasmic tails. Several micronemal proteins have been shown to translocate towards the posterior end of the parasite and to be shed by proteolytic cleavage before entry of the parasite into the forming vacuole. It is the backwards movement of the adhesin molecules, interacting with their substrates, that propels the parasite forward. Importantly, the family of thrombospondin-related anonymous proteins (TRAPs) was proven in *P. berghei* to be essential for gliding motility and for invasion of liver cells [55]. Other homologues of TRAP in *Plasmodium* and MIC2 in *T. gondii* also play an instrumental role in invasion and can be found in other invasive stages of *Plasmodium* and in other apicomplexans [56,57]. The C-terminal cytoplasmic domain of TRAP contains a sub-terminal tryptophan residue preceded by an acidic stretch. In a series of mutational-analysis and domain-swap experiments, it was demonstrated that the cytoplasmic tail is essential for gliding motility and that at least part of the gliding machinery is conserved across the apicomplexans [58]. In addition to TRAP, apicomplexans possess other families of adhesive proteins, sharing similar structural features and containing diverse types of motifs including EGF-like, lectin-like and apple domains. These proteins tend to associate into complexes [57,59, 60], and might actually form large macro-complexes during invasion.

Proteolytic processing of micronemal proteins is linked to the invasion process

During the invasion process, several transmembrane micronemal proteins are subjected to proteolytic processing, which can release them from the parasite membrane. The micronemal protein protease 1 (MPP1) activity was initially shown to cleave the C terminus of TgMIC2 [61^{*}] and was more recently demonstrated to cleave within the transmembrane-spanning domain of several micronemal proteins [62^{*}]. This cleavage, which releases adhesins from parasite surface, is a prerequisite for successful invasion [63] and might constitute a significant mechanistic step in the process of gliding motility, for example by constituting a potential zipper mechanism by which the host cell receptor–parasite complexes are built up and dissociated as the parasite enters and the PMV forms. A recent study indicated that TgMIC2 serves as substrate for the human and *Drosophila* rhomboid intramembrane proteases. The presence of genes coding for rhomboid-like proteases in apicomplexans suggest that MPP1 could possibly correspond to a seven-transmembrane serine protease [64].

Figure 2



Model for the interaction between the myosin A motor complex and the micronemal protein–host receptor complex via aldolase/F-actin at the parasite pellicule. A potential link between the actomyosin system and the subpellicular microtubules via the intramembranous particles is postulated. The black arrows indicate the movement of the complexes; the open arrows indicate the proteolytic cleavage by MPP1.

Table 1

List of the proteins linked to gliding motility and host cell invasion in *Plasmodium falciparum* and/or *Toxoplasma gondii*.

Factors	Biological function	Reference/accession number
Actomyosin system		
Myosin A (class XIV)	Powers gliding motility and host-cell invasion	[40]
TgMLC1/PfMTIP	Myosin light chain/myoA tail interacting protein?	[31,32]
MyoA associated proteins, P25, P50	Receptor for the anchoring of Myo A to the IMC	C Beckers, unpublished
Actin	Intact filaments essential for invasion polymerization is rate-limiting for motility	[1,3*]
Actin-related proteins	Actin polymerization	NP_703397; NP_704647; NP_702107; NP_700976
Putative actin-β-propeller-related protein	ARPC1?	NP_701055
Toxofilin	Sequesters actin monomers	[44]
Coronin	?	[48]
ADF/cofilin	Depolymerizes actin filaments	[45]
α/β capping proteins	Regulator of actin dynamics?	NP_703626; NP_703520
Aldolase	F-actin binding protein and binds to C terminus of TRAP members	[65**,66]
Parasite ligands and protease		
TRAP family members	Host cell adhesion and motility C-terminal tail binds to aldolase	[55,58] [65**,66]
MPP1 (rhomboid-like protease?)	Cleavage of transmembrane microneme proteins	[61*,62*,63]
Signaling and regulatory pathway molecules		
PLC	Activates parasite egress	[23]
TgCDPK1, calcineurin, calmodulin	Activation of parasite motility and/or microneme secretion	[23,24]
Casein kinase II and phospholipase 2C	Modulate toxofilin activity	[47]

The missing link: a bridge between micronemal proteins and the actomyosin system

The acidic domain and conserved tryptophan residue at the C terminus of TRAPs show significant similarity with the functional motifs found in NPFs including WASP/Scar proteins, but no actin nucleation function has been reported for the parasite proteins [50]. However, recently a novel property of this domain has revealed a rather unpredictable link to the actomyosin system. Aldolase, a protein previously shown to bind to filamentous actin [65**], has now been reported to interact specifically with the cytoplasmic tail of TgMIC2 and PfTRAP, and these interactions have proved to be dependent on the presence of the conserved tryptophan residue essential for parasite motility [65**]. The aldolase substrate fructose-1,6-bisphosphate and its product, fructose-1-phosphate, appear to interfere with the association of aldolase with the C-terminal domain of TRAP [66]. Interestingly, these metabolites were also shown in previous studies to inhibit the interaction of aldolase with F-actin [65**]. Thus, TRAP- and actin-binding sites on aldolase may overlap with the catalytic pocket of the enzyme. This represents the first evidence of a direct link between the actomyosin complex and the complexes of micronemal ligands and host receptors (Figure 2).

Conclusions

Gliding motility and host cell invasion by the Apicomplexa require perfectly coordinated interactions between the actin skeleton, the myosin motor, adhesins and proteases, which are orchestrated by signal-transduction-pathway molecules. Currently, a broad range of factors are either known to be directly involved in gliding motility or presumed to participate in this complex process (Table 1).

Studies using JAS suggest that not only intact actin cytoskeleton but also actin polymerization plays a crucial role in the phenomenon of gliding. Stabilization of actin filaments unexpectedly reverses the orientation and alters the speed of the movements. A detailed understanding of the mechanism of gliding motility will await the elucidation of two major issues. First, what is the nature of the molecular elements governing and maintaining the directionality of gliding, which seems to be dictated by the polarized organization of the actin filaments and the helical arrangement of subpellicular microtubules? Second, what are the mechanisms controlling actin polymerization/depolymerization in the Apicomplexa?

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