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Mechanisms controlling glideosome function in apicomplexans

Wassim Daher and Dominique Soldati-Favre

The glideosome is a unique attribute of the *Apicomplexa* phylum. This myosin-based machine powers parasite motility, migration across biological barriers, host cell invasion and egress from infected cells. The timing, duration and orientation of gliding motility are tightly regulated to assure establishment of infection. Control of glideosome function occurs at several levels. The assembly of the molecular motor complex is governed by posttranslational modifications resulting from a calcium-dependent signalling cascade. The spatially controlled polymerization of actin filaments crucially impacts motility. The relocation of glycolytic enzymes in close proximity of the glideosome may enhance the local production of energy to sustain movement.

Address
Department of Microbiology and Molecular Medicine, CMU, University of Geneva, 1 rue Michel-Servet, 1211 Geneva 4, Switzerland

Corresponding author: Daher, Wassim (wassim.daher@unige.ch)

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Introduction

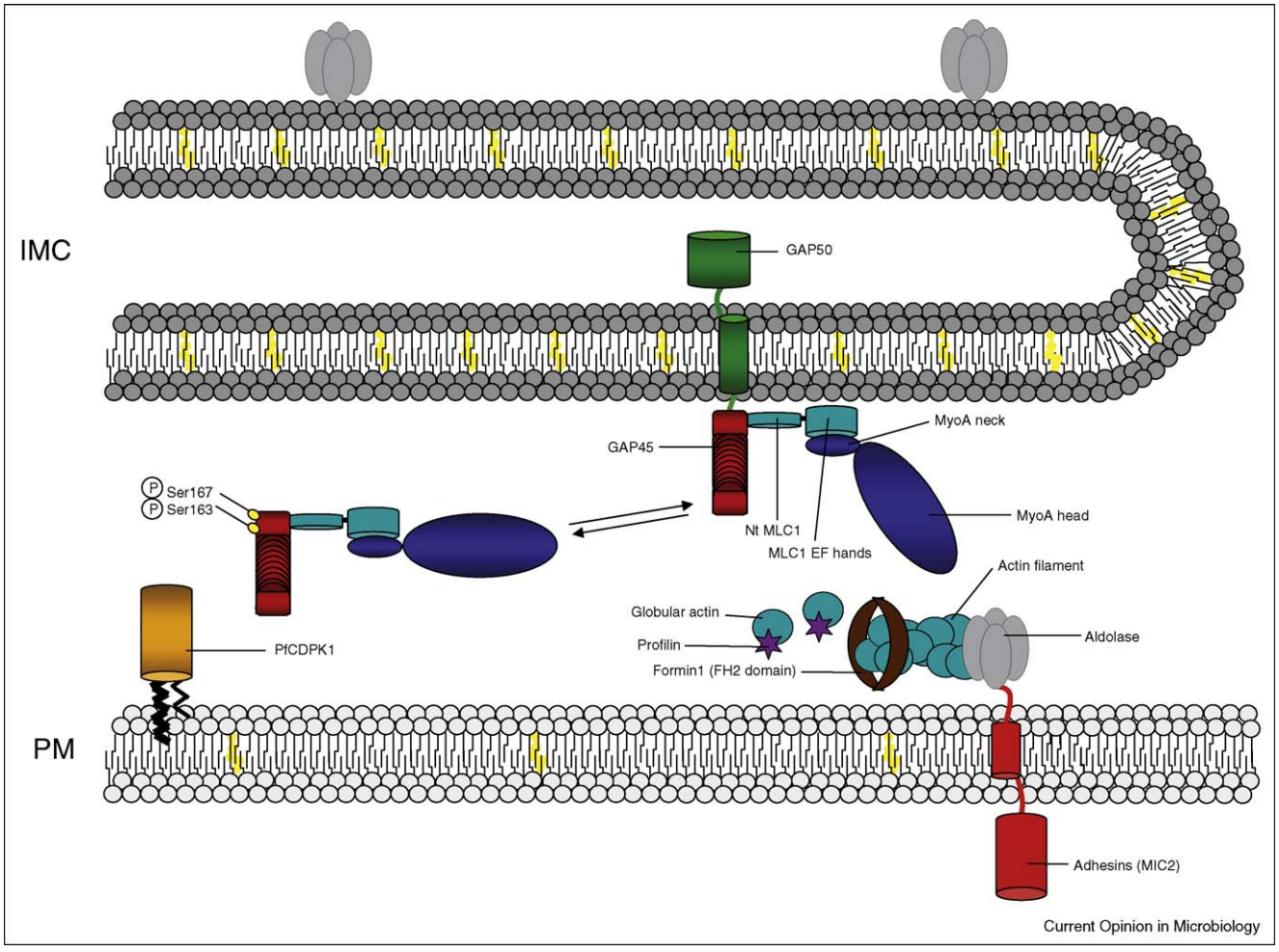
The phylum of *Apicomplexa* includes a large group of obligate intracellular parasites that cause severe diseases in humans and animals. Members of this phylum are distinguishable by the presence of an apical complex composed of the specialized secretory organelles named rhoptries and micronemes, an apical polar ring and in some species a conoid. Apicomplexans belong to the group of alveolates that possess a pellicle, which is composed of the plasma membrane and a closely apposed inner membrane complex (IMC) formed by membranous sacs. These sacs, known as alveoli are associated with specific proteins named alveolins [1–3].

Many pathogens take advantage of the phagocytic activity of the host cells to access the intracellular niche. By contrast, the apicomplexans enter host cells by an active, energy-dependent process leading to the formation of a parasitophorous vacuole. In *Toxoplasma gondii*, an intact parasite actin cytoskeleton and controlled polymerization of actin filaments have proven to be essential for gliding motility and host cell invasion [4,5]. The glideosome refers to the molecular machinery powering motility and includes the essential class XIV myosin A heavy chain (MyoA), the myosin light chain (TgMLC1 or PfMTIP) and the two glideosome associated proteins GAP45 and GAP50 that anchor the complex to the IMC [6]. The motor is assembled as a soluble precomplex MyoA–MLC1–GAP45 in the cytosol [7] before being firmly anchored in the cholesterol rich membrane of the IMC by association to the integral membrane glycoprotein, GAP50 [8] (Figure 1). Lasting only few seconds, the host cell penetration and egress processes are extremely fast and depend on signalling events that tightly control the glideosome function. The tuning of the engine, the shape of the road and the fuel supply are among the key modulating parameters in any movement. In this review we will discuss recent advances that shed light on how posttranslational modifications of the motor complex, regulators of actin dynamics and access to energy supplies influence the glideosome function. These multiple levels of control are likely to operate simultaneously and hence begin to explain how the parasite is so superbly in command of its motility.

Control of the myosin motor complex assembly and function

The function of myosin motors can be modulated either directly by phosphorylation of the myosin heavy chain, such as at the TEDS site or via the myosin light chain. The TEDS rule site is in a region of the myosin head that is believed to make a significant contact with actin when the site is negatively charged [9]. Given the fact that MyoA shows unusual features, it has been challenging to predict the potential modes of regulation. MyoA does not follow the TEDS rule, lacks a tail domain and possesses a single degenerated IQ motif (a calmodulin-binding motif (FILV)Qxxx(RK)Gxxx(RK)xx(FILVWY)) in the neck, which recruits an unusual myosin light chain, TgMLC1 or PfMTIP [10,11]. MLC1 contains four atypical calcium-binding motifs (EF hands) that interact with MyoA and a N-terminal extension implicated in targeting of the motor to the IMC [10] (Frenal and Soldati, unpublished). While GAP50 appears to contribute essentially in the anchoring of the precomplex in the IMC, the role of GAP45 is less obvious. GAP45 is myristoylated and palmitoylated and associates with the precomplex in the cytosol, and as hetero-tetramer with GAP50 in the outer membrane of the IMC [8,12]. Myristoylation is a co-translational modification, which promotes membrane interaction but is insufficient to offer a stable anchorage to the IMC. Once

Figure 1



Controlled glideosome function. Schematic representation of the checkpoints controlling the glideosome function. Myosin A is assembled as a precomplex together with MLC1 and GAP45 in the cytosol. The motor complex is anchored to the inner membrane complex (IMC) through the interaction of MyoA–MLC1–GAP45 with GAP50. The disassembly of the motor complex can occur by the action of kinase(s) on GAP45. In Plasmodium, PfCDPK1 is anchored in the plasma membrane can phosphorylate GAP45 and PfMTIP/MLC1. Formins and profilin are presumed to nucleate actin polymerization beneath the plasma membrane hence providing the scaffold for adhesin (TgMIC2) receptor complex translocation to the rear of the parasite by the action of MyoA. The progressive movement of F-actin/aldolase/adhesins complexes propels the parasite forward. Disengagement of the entire motor complex involves the fast depolymerization of actin filaments. The tetrameric aldolase binds to F-actin and to TgMIC2 tail and thus can act as a bridge between the motor and adhesin/receptors complexes [45••]. In an alternative model, aldolase as well as the other glycolytic enzymes are translocated to the cytosolic side of the IMC and contribute locally to the production of ATP that fuels and sustains motility [46••]. The lipid composition of the IMC and plasma membrane are likely to be significantly different and the content in cholesterol is selectively enriched in the IMC [8].

associated with the target membrane, GAP45 is palmitoylated and the motor associates with GAP50 to form the glideosome [12]. In contrast to the irreversible myristoylation, the palmitoylation is a reversible modification, which could play a role in regulating motor function by affecting its anchorage in the IMC.

The stability and the functional conformation of the motor complex are governed by additional posttranslational modifications and most prominently by phosphorylation as part of the signalling cascade leading to motility. Calcium signalling and phosphorylation have been known for some time to regulate erythrocyte invasion by *Plasmodium falciparum* [13–15]. In *T. gondii*, parasite egress is triggered by a drop in host intracellular potassium, resulting in the activation of a parasite phospholipase C and an increase in parasite cytosolic calcium which leads to microneme secretion [16]. Using specific kinase inhibitors, both calcium-dependent protein kinases and cGMP-dependent protein kinases, appear to contribute to parasite motility and invasion [16,17]. PfCDPK1 is a primary candidate as modulator of the

motor function during invasion. The expression of PfCDPK1 peaks in mature schizonts and the N-terminal acylation brings the protein to the parasite plasma membrane and into close proximity to the glideosome [18•]. Biochemical analyses have suggested that PfGAP45 and PfMTIP are phosphorylated in late schizonts and can be phosphorylated *in vitro* by CDPK1 [18•]. PfCDPK1 is also expressed in sporozoites and small molecules related to 2,6,9-trisubstituted purines target this kinase and affect parasite invasion and egress [19]. A distinct CDPK gene appears to control motility in ookinetes, on the basis of the disruption of *Plasmodium berghei CDPK3* gene [20]. Interruption of PbCDPK3 reduces ookinete motility and their capability to infect the mosquito midgut [21]. Another CDPK protein was recently described to be important for sporozoite invasion in *P. berghei* [22]. Disruption of PbCDPK6 reveals that this kinase plays a role in transducing the signal resulting in a switch from the migration to invasion mode [22]. However, the signalling pathway(s) as well as PbCDPK6 substrate(s) require(s) further investigation. Together these results highlight the importance of CDPKs regulated by calcium in controlling the glideosome function. Notably, the complex mechanism of regulation of these kinases has been resolved into five distinct steps by studying PfCDPK4 in great detail [23].

CDPKs are not the only signalling molecules that are regulated by calcium. PfPKB (protein kinase B) localizes at the apical end of merozoites, binds to PfGAP45 and is activated by a direct interaction with the calcium/calmodulin molecules [24]. The role of PfPKB was studied using either a specific chemical inhibitor or a peptide competing with its activator (calmodulin-like protein) and in both cases the invasion process was impaired [24]. In summary, PfGAP45 is targeted for phosphorylation by two different kinases regulated by the calcium/calmodulin complex however, the impact of these modifications on the protein, and the rest of the motor complex still await elucidation. In *T. gondii*, the final assembly step of the motor complex is controlled by phosphorylation of TgGAP45 [25•]. Mutation of serine residues 163 and 167 to glutamate, which mimics phosphorylated serine, alters the final assembly step of MyoA–MLC1–GAP45 complex with TgGAP50 without affecting the peripheral localization of TgGAP45 [25•]. According to this study, dephosphorylation of the TgGAP45 by a phosphatase could trigger the final assembly of the motor complex with TgGAP50 and this assembly has the potential to be reversed by phosphorylation of TgGAP45 at the Ser163 and Ser167. Unlike the situation in *P. falciparum*, TgGAP45 does not appear to be target for TgCDPK1 [25•].

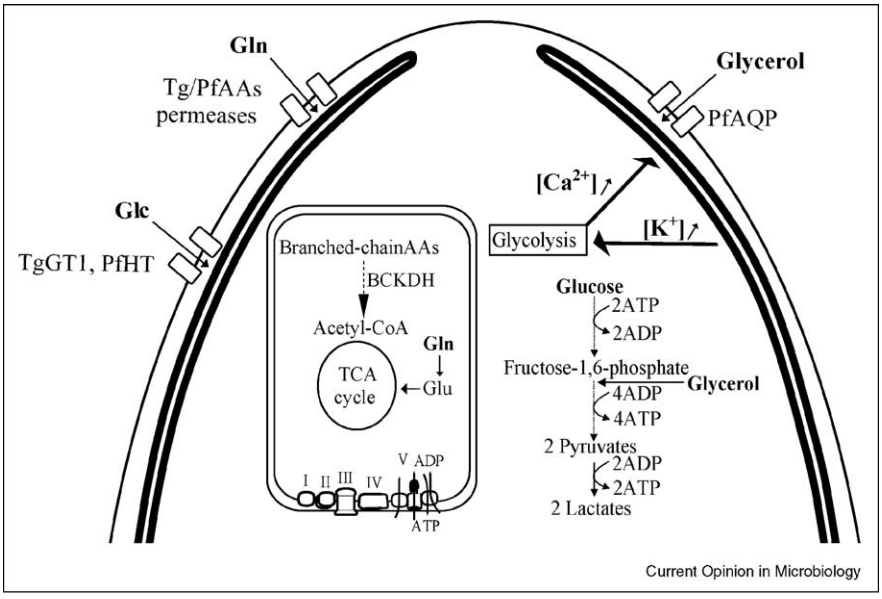
In conclusion, the assembly/disassembly of the motor complex appears to be controlled by phosphorylation and potentially by reverse palmitoylation however the functional significance for fine-tuning motility and invasion awaits further investigation.

Controlled actin polymerization regulates timing and orientation of gliding motility

Actin filaments cannot be readily visualized under physiological conditions in apicomplexans, nevertheless there is compelling evidence that the controlled polymerization of actin filaments in time and space governs the directionality and duration of gliding motility [5].

A number of actin-binding proteins have the capacity to orchestrate actin dynamics by nucleating, sequestering, capping, bundling, and disassembling the filaments. *In vitro* studies on purified or recombinant apicomplexan actins revealed the formation of unusually short filaments (100 nm) that are highly dynamic and fragmented [26–28]. One other atypical property of *T. gondii* actin is that polymerization occurs at a critical concentration three to four-fold lower than classical actins [26]. The repertoire of actin-binding proteins is relatively small in apicomplexans compared with that of other eukaryotes [29]. While the Arp2/3 complex is absent, two formins known to facilitate rapid assembly of filaments are conserved in these parasites [29]. The ability of these formins to nucleate chicken actin *in vitro* was confirmed by biochemical analysis of the FH2 (formin homology 2 domain) domains of the Plasmodium formins [30•]. PfFRM1 has been localized to the point of contact between the parasite and host plasma membranes, however functional studies are still needed to establish its contribution to gliding motility. Formins are known to harness the ability of profilin to couple ATP hydrolysis to filament assembly, in order to catalyze a fast assembly at the barbed end [31]. Conditional disruption of the unique gene coding for profilin in *T. gondii* revealed it is essential for parasite motility and host cell invasion [32]. On the contrary, the rapid disassembly of actin filaments in apicomplexans is likely to be provoked by the action of an actin depolymerizing factor (ADF) or cofilin. ADF is a widely conserved, low molecular weight, actin monomer sequestering protein, with filament severing activity [33,34]. TgADF is enriched under the plasma membrane and binds to actin monomers and increases the turnover of rabbit actin filaments *in vitro* [33]. PfADF1 is abundantly expressed in all motile stages and binds to globular actin with a preference for ADP-bound monomers, but does not appear to facilitate the turnover of bovine actin filaments *in vitro* [35]. PfADF1 instead stimulates nucleotide exchange on both Plasmodium and mammalian monomeric actins and might stimulate polymerization [35]. While most of the key players seem to be identified, their mode of action and regulation remain to be unravelled. In this context, the successful recombinant expression of PfACT1 and TgACT1 may help uncover some of the mysteries surrounding actin dynamics during motility and invasion.

Figure 2



Energy sources powering gliding motility metabolic pathways producing energy in apicomplexans. Plasmodium species and *T. gondii* possess transporters for the uptake of glucose, amino acids and glycerol as the potential sources of energy. Pathways leading to the production of ATP from glucose, glutamine or glutamate (Toxoplasma and Plasmodium), branched chain amino acids (Toxoplasma) and glycerol (Plasmodium) via glycolysis and TCA cycle are represented succinctly. In absence of a pyruvate dehydrogenase complex, the acetyl–CoA is possibly produced by the degradation of branched chain amino acids in *T. gondii*. Glutamine (Gln) and glutamate (Glu) can produce alpha-ketoglutarate and hence enter the tricarboxylic acid cycle. Plasmodium species possess the enzyme that converts glycerol to glyceraldehyde 3-phosphate and feed into glycolysis. The translocation of glycolytic enzymes from the cytosol to the IMC (upon increase in intracellular calcium concentration) and vice versa (upon rise in intracellular potassium concentration) are represented by arrows.

Optimized accessibility to energy sources powering the glideosome

ATP is required in high levels to locally fuel the myosin ATPase and the active rebuilding of the actin cytoskeleton. It is well established that ATP hydrolysis and release of P_i, drive actin filament dynamics by controlling their stability and determining nucleotide-dependent filament conformation and interaction(s) with regulatory actin-binding proteins [36,37].

Under aerobic conditions eukaryotic cells can produce energy by converting glucose into pyruvate via glycolysis and by oxidative phosphorylation of acetyl–CoA obtained from pyruvate, degradation of several amino acids, or beta-oxidation of fatty acids. Although the apicomplexans possess a complete glycolytic pathway and all the enzymes required for the tricarboxylic acid (TCA) cycle [38,39], the absence of a pyruvate dehydrogenase (PDH) complex in the mitochondrion hampers the oxidative decarboxylation of pyruvate into acetyl–CoA and hence complete oxidation to CO_2 by the TCA cycle [40]. Alternatively, these parasites can use glutamate or glutamine to enter the TCA cycle and produce energy. Unlike the Plasmodium species, *T. gondii* possesses the mitochondrial pathways for the degradation of branched chain amino acids (BCAA) and so is capable of producing acetyl–CoA to fuel the TCA cycle [38]. Both glucose and amino acids constitute energy sources that can be rapidly mobilized to power the ATP-demanding invasion process (Figure 2).

Fortuitously or not, the tetrameric glycolytic enzyme aldolase acts not only as a metabolic enzyme but also as a bridge connecting the actomyosin system to the host cell receptors through an interaction with transmembrane adhesins such as TgMIC2 and other members of TRAP [41,42]. Although the enzyme substrate, TRAP-binding sites and F-actin-binding sites of aldolase seem to overlap, its multimeric nature might assist the enzyme in fulfilling multiple tasks [43,44].

In a recent study, the dual role of aldolase was challenged experimentally by the creation of a conditional knockout for this gene in *T. gondii* followed by the functional complementation with a series of site-specific aldolase mutants that were defective in glycolytic activity, or in the ability to bind to TgMIC2 tail. The aldolase mutation D33A, which is defective in glycolysis but is still binding to TgMIC2 tail was unable to complement replication, gliding motility and invasion defects. By contrast, parasites expressing an aldolase double mutant K41E–R42G, which is severely impaired in MIC2 tail binding and only

partially affected in its enzymatic activity showed normal cellular ATP content, no significant intracellular growth defect but a 50% reduction in invasion. Unexpectedly, these parasites are not altered in gliding motility, which would be expected for a role of aldolase in bridging TgMIC2 to F-actin [45••]. While this elegant approach has revealed interesting results, owing to experimental limitations it did not definitively establish the essential role of aldolase as bridge for gliding motility.

It is well established that increased glucose metabolism and elevated production of ATP can be achieved by microcompartmentation and metabolic channelling. In this context, the fate of the parasite glycolytic enzymes including aldolase was investigated in invading parasites and a rapid translocation of glycolytic enzymes to the pellicle was observed, presumably for local generation of ATP to empower motility [46••]. The mechanism that overcomes molecular crowding and allows such spatial rearrangement of the enzymes to a close proximity of IMC remains to be uncovered. Interestingly, the translocation of the glycolytic enzymes appears to respond to changes in intracellular concentrations of calcium and potassium that are known to serve as sensors in controlling motility. In further support of the importance of glycolysis in gliding, the authors demonstrate that parasite gliding *in vitro* in minimal media was dependent on the presence of glucose. In these experiments treatment of parasites with atovaquone did not affect motility suggesting that oxidative phosphorylation is not providing energy for gliding [46••]. The translocation of aldolase was not observed in the study reported by Starnes *et al*., who instead saw this cytosolic enzyme accumulating at the anterior end of extracellular tachyzoites [45••]. Despite this discrepancy in term of localization both studies highlight the importance of glycolysis as energy source to power gliding motility [45••,46••].

To determine the importance of glycolysis and the level of versatility toward other energy sources, the repertoire and function of the glucose transporter(s) were recently examined in *T. gondii* tachyzoites. Only one *bonafide* sugar transporter, TgGT1 is expressed at the plasma membrane of tachyzoites [46••]. Unexpectedly, disruption of the *TgGT1* gene led only to a modest impairment in intracellular growth (30% decrease) and no alteration in gliding motility, invasion and virulence in mice [47]. Host-derived glucose uptake is however dramatically altered in *Tggt1ko* mutant suggesting that TgGT1 is the main transporter implicated in glucose import. In accordance with this observation, *Tggt1ko* mutant is defective in gliding motility in minimal media with or without glucose. By contrast, the addition of glutamine efficiently restored motility indicating that *T. gondii* can used another source of energy via the krebs cycle to power motility [47]. It remains to be determined if Plasmodium species exhibit the same flexibility as they have additionally the potential to take up glycerol via the aquaglyceroporin (AQP) localized at the plasma membrane [48,49]. PfAQP and the enzymes of glycerol metabolism are induced in parasites exhibiting a metabolic shift reflecting glucose starvation when isolated from malaria-infected patients [50••]. Glycolysis is an obvious pathway to be mobilized for the energy production during motility however, along with the potential for metabolic versatility, the importance of local rearrangement of the glycolytic enzymes remains to be demonstrated.

Perspectives and conclusions

Recent advances have uncovered the multiple levels and complexity with which the apicomplexans are in command of their motility. Assembly/disassembly of the motor complex, polymerization of short actin filaments and enhanced local supply in energy are at the heart of the control of gliding motility. Unravelling these key checkpoints and regulatory mechanisms will enrich our understanding regarding the fundamental needs of these parasites and may have a considerable impact on development of new avenues of intervention against deadly parasites.

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