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Gliding motility powers invasion and egress in Apicomplexa

Karine Frénal[1], Jean-François Dubremetz[2], Maryse Lebrun[2] and Dominique Soldati-Favre[1]

Abstract | Protozoan parasites have developed elaborate motility systems that facilitate infection and dissemination. For example, amoebae use actin-rich membrane extensions called pseudopodia, whereas Kinetoplastida are propelled by microtubule-containing flagella. By contrast, the motile and invasive stages of the Apicomplexa — a phylum that contains the important human pathogens *Plasmodium falciparum* (which causes malaria) and *Toxoplasma gondii* (which causes toxoplasmosis) — have a unique machinery called the glideosome, which is composed of an actomyosin system that underlies the plasma membrane. The glideosome promotes substrate-dependent gliding motility, which powers migration across biological barriers, as well as active host cell entry and egress from infected cells. In this Review, we discuss the discovery of the principles that govern gliding motility, the characterization of the molecular machinery involved, and its impact on parasite invasion and egress from infected cells.

Apicomplexa
A phylum of diverse, single-celled, eukaryotic, obligate intracellular parasites.

Alveolata
A group of protists within the kingdom Eukarya that contains the phyla Dinoflagellata, Ciliophora and Apicomplexa.

[1]*Department of Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva, CMU, 1 Rue Michel-Servet, 1211 Geneva 4, Switzerland.*
[2]*Unité Mixte de Recherche 5235 – Dynamique des Interactions Membranaires Normales et Pathologiques, Université Montpellier, Place Eugène Bataillon, 34095 Montpellier cedex 5, France.*

Correspondence to K.F. and D.S.-F.
karine.frenal@unige.ch; dominique.soldati-favre@unige.ch

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The Apicomplexa phylum belongs to the Alveolata group and encompasses a broad range of obligate intracellular parasites that can infect most animal species. Apicomplexans cause severe diseases in humans; for example, *Plasmodium* spp. cause malaria, which is responsible for half a million deaths each year; *Toxoplasma gondii* is responsible for toxoplasmosis, which affects up to one-third of the human population; and *Cryptosporidium* spp. are a major cause of enteric infection in the developing world[1]. Other apicomplexan species are of substantial veterinary importance and are responsible for considerable economic losses: for example, *Theileria* spp. infect livestock in tropical and subtropical regions; and *Eimeria* spp. are responsible for the poultry disease coccidiosis worldwide[1].

The life cycle of these parasites is complex, alternating between asexual and sexual stages in intermediate and definitive hosts, respectively (FIG. 1). *T. gondii* carries out the sexual portion of its life cycle in the intestines of cats (the definitive host). Infected animals shed oocysts (encysted zygotes) that undergo meiosis to become infectious sporozoites, which can infect a warm-blooded intermediate host when ingested. In the intermediate host, sporozoites differentiate into tachyzoites, and these eventually differentiate into encysted bradyzoites that persist for the lifetime of the host. By contrast, the sporozoites of *Plasmodium* spp. are injected into an intermediate host through a mosquito bite. The sporozoites migrate to the liver and invade hepatocytes, where they divide to produce thousands of merozoites, which are released into the bloodstream to infect red blood cells and undergo asexual replication.

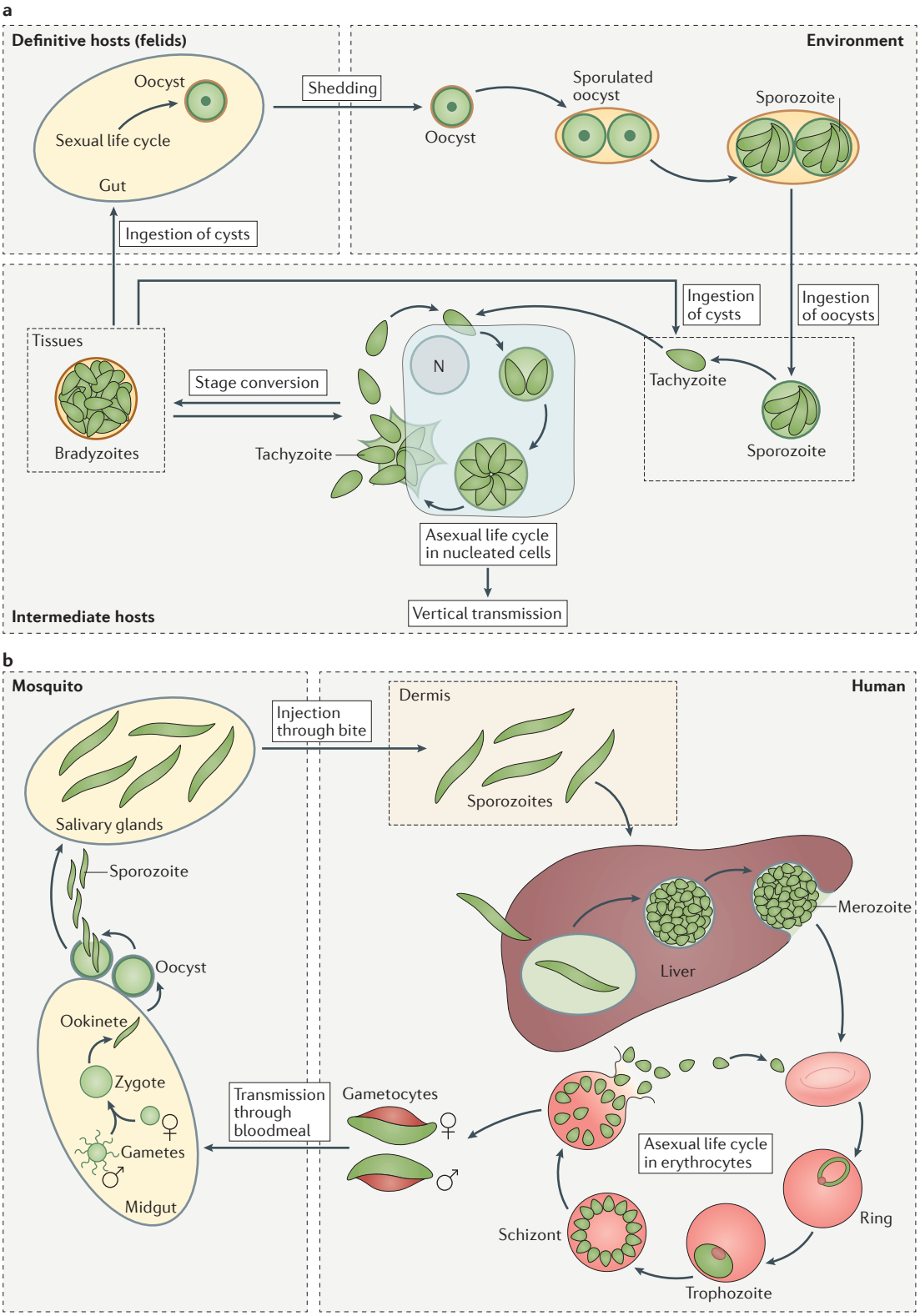
The motile and/or invasive stages of the apicomplexan life cycle (sporozoites, tachyzoites, merozoites and ookinetes), generally called zoites, have largely been studied in the best genetically tractable parasites of the phylum: namely, *T. gondii*, *Plasmodium falciparum* and the rodent model of malaria, *Plasmodium berghei* (BOX 1). The zoites rely on a unique mode of substrate-dependent locomotion known as gliding motility; this process is powered by the parasite actomyosin system, which is located underneath its plasma membrane. This gliding machinery, termed the glideosome[2], enables the zoite to actively propel itself across non-permissive biological barriers (migration), rapidly penetrate various cell types (invasion)[3–5] and exit from infected cells (egress)[6]. During host cell entry, the parasites induce the formation of a parasitophorous vacuole, which is derived from the invagination of the host cell plasma membrane. The non-fusogenic parasitophorous vacuole membrane (PVM) is secluded from endosomal compartments and ensures safe replication[7]. Following replication, the parasites exit an infected cell by rupturing the PVM and the host cell plasma membrane to rapidly invade neighbouring cells; thus, motility contributes to the dissemination of infection.

This Review recapitulates the historical steps that led to our current molecular understanding of gliding motility in Apicomplexa, and discusses how the composition, assembly and regulation of the glideosome affect parasite

motility, invasion and egress. In this Review, we focus primarily on the *T. gondii* tachyzoite, for which functional studies were first carried out, and we compare and contrast this with the motile stages of malaria parasites — the sporozoite, merozoite and ookinete.

Emergence of the capping model

The motility of Apicomplexa (historically named Sporozoa) has caught the attention of scientists for more than a century[8] and was named gliding motility early on, on the basis of microscopic observations



Toxoplasmosis
A food-borne infection caused by the parasite *Toxoplasma gondii*. The infection is usually mild or even asymptomatic, but can have serious consequences in patients who are immunocompromised and for the fetus in the case of primary infection during pregnancy.

Sporozoites
The infectious and motile stage produced in oocysts and transmitted by the definitive host.

Tachyzoites
The motile and fast-replicative stage of *Toxoplasma gondii* that is able to invade any nucleated cell in the host.

Merozoites
The stage of *Plasmodium* spp. that infects erythrocytes, in which it initiates a new asexual life cycle.

Actomyosin system
A complex that comprises actin filaments, myosin and associated proteins, and that is involved in movement.

Glideosome
A molecular complex that powers gliding motility in apicomplexan parasites.

Circumsporozoite precipitation reaction
A reaction in which sporozoites, incubated in immune serum, eject a tail-like precipitate in a process that corresponds to the shedding of the glycosylphosphatidylinositol-anchored circumsporozoite protein crosslinked by antibodies.

Cytochalasin
A type of fungal metabolite that inhibits actin polymerization. Cytochalasin A and cytochalasin B can have pleiotropic effects, which include the inhibition of monosaccharide uptake and transport.

Coccidia
A subclass of Apicomplexa comprising parasites that infect the intestinal tracts of animals, such as *Toxoplasma gondii*, *Neospora* spp., *Eimeria* spp. and *Sarcocystis* spp. These parasites harbour an apical structure, termed a conoid, that consists of anticlockwise spiralling fibres.

of live specimens (FIG. 2; Supplementary information S1–S5 (movies)). This mode of motility differs substantially to amoeboid motion involving pseudopods, and from the ciliary and flagellar motion used by most unicellular organisms. The main hypothesis to explain gliding motility in the early days was slime secretion, as seen in slugs; although this may now seem simplistic, it is not so far from the present concept of gliding motility in Apicomplexa (FIG. 3a). New insights into the process of gliding motility came from the study of *Plasmodium* spp. sporozoites and the discovery of the circumsporozoite precipitation reaction[4], which showed the posterior capping and shedding of antibodies bound to the parasite surface, implying that a parasite surface protein was translocated rearwards. The term 'capping' was first used to describe the redistribution of immune complexes on the surface of lymphocytes[9] and has been extended to refer to any polarized translocation of surface protein on a cell. Next, it was demonstrated that cytochalasin B blocks the motility of *Eimeria magna* sporozoites and also inhibits host cell invasion[10]. This suggested a possible role for actin in invasion, and a link between motility and invasion, both of which were eventually developed as paradigms for gliding motility in Apicomplexa.

Further demonstrations of links between surface ligand capping, motility and microfilaments came from investigations in the Coccidia subgroup of Apicomplexa and Gregarina[11–13]. The observation of diverse apicomplexan parasites during invasion by electron microscopy established that invasion starts with the apical pole of the parasite facing the host cell plasma membrane, followed by the formation of an intimate junction between the parasite and the host cell plasma membrane that enables the entry of the parasite[5]. This junction is referred to as the moving junction, tight junction or circular junction. Subsequently, motility and invasion have been understood as the capping of parasite proteins that interact with the host cell surface, accompanied by the shedding of these surface molecules.

Gliding motility involves cell-surface contacts with substrates (FIG. 3b), and, remarkably, the pellicle of apicomplexan zoites (as for all members of the group Alveolata) is visible by electron microscopy as a three-layered structure that encompasses the plasma membrane and the underlying inner membrane complex (IMC), which is composed of one or more vesicular sacs[14] (BOX 1). Freeze-fracture analyses of coccidians has demonstrated a remarkable degree of organization within the IMC, revealing the existence of intramembrane particles aligned on the anteroposterior axis of the cell, thus along the direction of gliding, and suggesting that these structures could function as a template for the mechanism of gliding motility[15]. It took another 20 years of work on the characterization of secretory organelle content to begin to understand the complex mechanism of gliding motility. The Apicomplexa-specific organelles termed rhoptries and micronemes, which are confined to the anterior part of the zoites (BOX 1), were first described in the 1950s and were suggested to be secretory[16]. Later, a secretory function was demonstrated by experiments that showed apical microneme exocytosis in *Sarcocystis muris*[17]. The link between the translocation of microneme proteins on the parasite surface and motility was then established in *P. berghei* sporozoites, in which the suppression of the gene that encodes a major microneme protein, thrombospondin-related anonymous protein (TRAP), led to the abolition of motility[18]. Furthermore, it was concurrently established that motility supports invasion, which is an active process powered by the parasite[19–21].

A few years later, the major players — the pellicle, the actomyosin system and the micronemes — were set on the stage. A large amount of data generated by many groups led to the concept of the glideosome[2]. The capping model of the basis of motility is now understood as a host–parasite interacting complex (ligand–receptor) that is translocated from the front to the rear of the parasite by the action of the actomyosin system (FIG. 3c). Rhoptry neck proteins, proteases and a connector were identified as additional key players of motility and invasion that act in a concerted manner in a process that is orchestrated in time and space through signalling cascades of regulatory molecules.

Actin polymerization and motility

Actin is one of the most abundant proteins in eukaryotic cells. It exists in a monomeric globular form (globular actin (G-actin)) that usually polymerizes into filaments (filamentous actin (F-actin)) through a mechanism that involves a slow nucleation phase followed by a fast elongation phase. The inhibition of parasite motility by

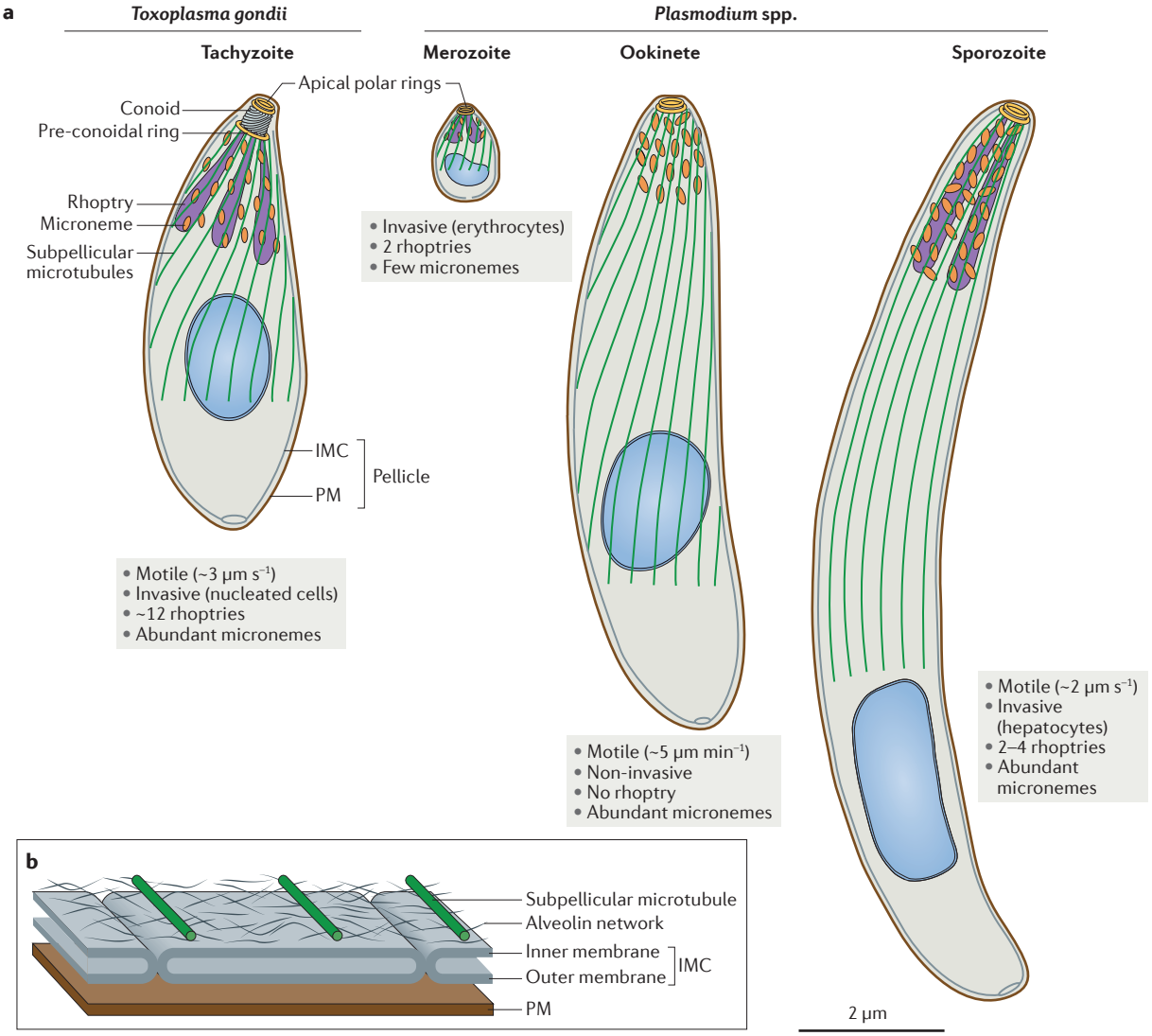
◀ Figure 1 | **The life cycles of *Toxoplasma gondii* and *Plasmodium falciparum*.** **a** | The life cycle of *Toxoplasma gondii*. The sexual life cycle of *T. gondii* occurs in the intestines of felids. Male and female gametes fuse to produce oocysts that are shed into the environment. These diploid unsporulated oocysts undergo meiosis and produce eight infectious haploid sporozoites. Sporulated oocysts can then contaminate food and water, and be ingested by an intermediate host, in which the asexual life cycle of the parasite takes place. In these hosts, the sporozoites differentiate into the fast-replicative tachyzoites, which are capable of entering almost any type of nucleated cell. As a result of pressure from the immune system, tachyzoites differentiate into encysted, slow-growing bradyzoites, which remain in smooth muscle and in the brain for the lifespan of the host. Humans are infected through the ingestion of oocyst-contaminated food or water, or of undercooked cyst-containing meat. The lytic cycle of the tachyzoite is the best studied because this stage is easily propagated in tissue culture. The parasite first attaches to the host cell surface, reorientates itself and penetrates the cell. As it enters the host cell, it creates a parasitophorous vacuole in which it replicates. After several rounds of division, the parasites egress from the infected cells and invade neighbouring cells. **b** | The life cycle of *Plasmodium* spp. Infection of the intermediate host starts with the bite of an infected female *Anopheles* spp. mosquito, which injects sporozoites into the dermis. The sporozoites travel to the blood vessels and migrate to the liver, where they migrate along the sinusoidal cells, enter and traverse Kupffer cells, and finally traverse and invade hepatocytes. The sporozoites divide and produce thousands of merozoites that are released into the bloodstream and infect erythrocytes. The parasites can undergo repeated asexual cycles within erythrocytes, during which they progress from the ring stage to the trophozoite stage, schizonts and eventually released merozoites. Some parasites will develop into sexual forms, termed gametocytes, that circulate in the bloodstream before being taken up as a blood meal by a mosquito. The sexual cycle takes place within this definitive host, and leads to the formation of motile but non-invasive ookinetes that migrate through the mosquito midgut and develop into oocysts in the epithelium, ultimately producing sporozoites that migrate to the salivary glands, where they are ready to infect subsequent hosts.

cytochalasins (which are inhibitors of actin polymerization), indicated a key contribution of parasite actin filaments in motility[10,22] (FIG. 4a). The conditional excision of *T. gondii* actin (TgACT1) severely compromised motility, invasion and egress[23,24], although the parasites could still be propagated for several days, which raised the possibility of the coexistence of an actin-independent mechanism of motility[25,26].

Box 1 | **The importance of secretory organelles and the cytoskeleton in the motility and invasion of zoites**

Substrate-dependent motility is a hallmark of most invasive zoites. It has been predominantly and extensively studied in cultured *Toxoplasma gondii* tachyzoites, in *Plasmodium berghei* sporozoites isolated from mosquito salivary glands and in ookinetes produced *in vitro* (which glide approximately 100-times slower than *T. gondii* tachyzoites and *P. berghei* sporozoites at 5 μm min^{-1})[72]. By contrast, *Plasmodium falciparum* merozoites, which infect erythrocytes, have either very limited or no gliding motility, and rosetting (that is, the adherence of infected red blood cells to uninfected ones) ensures the direct reinvasion of erythrocytes. Zoites are highly polarized cells that contain secretory organelles at their apical pole: namely, the micronemes and the rhoptries. The content of the micronemes is exocytosed at the apical pole in a calcium-dependent manner and through a membrane fusion process[161]. Motility can be correlated with the abundance of micronemes; *Plasmodium* spp. sporozoites have the most micronemes and glide for tens of minutes, whereas the merozoite form has few micronemes and does not glide[80]. The neck of the rhoptries is inserted into the apical polar rings (see the figure, part **a**), and the content of the rhoptry neck and bulb is successively secreted at the apical tip of the parasites. Invasion is correlated with the presence of rhoptry organelles, as several rhoptry neck proteins are involved in the moving junction, which is built by the parasite to support the traction forces generated during its penetration into the host cell[112,123]. Ookinetes are able to migrate through the epithelium of the mosquito midgut but do not invade.

The pellicle of Apicomplexa is composed of the plasma membrane (PM) and an inner membrane complex (IMC) formed of one or more flattened vesicular sacs (see the figure, part **b**). A network of intermediate filament-like proteins, termed alveolins, connects the IMC to the subpellicular microtubules. The crescent shape of the parasite is determined by the left-handed spiral arrangement of its subpellicular microtubules emanating from the apical polar ring and by the underlying alveolin network[164]. This cytoskeleton maintains the shape of the parasites and, during motility and invasion, influences their trajectory, which is described as a clockwise corkscrew trajectory[99–101].



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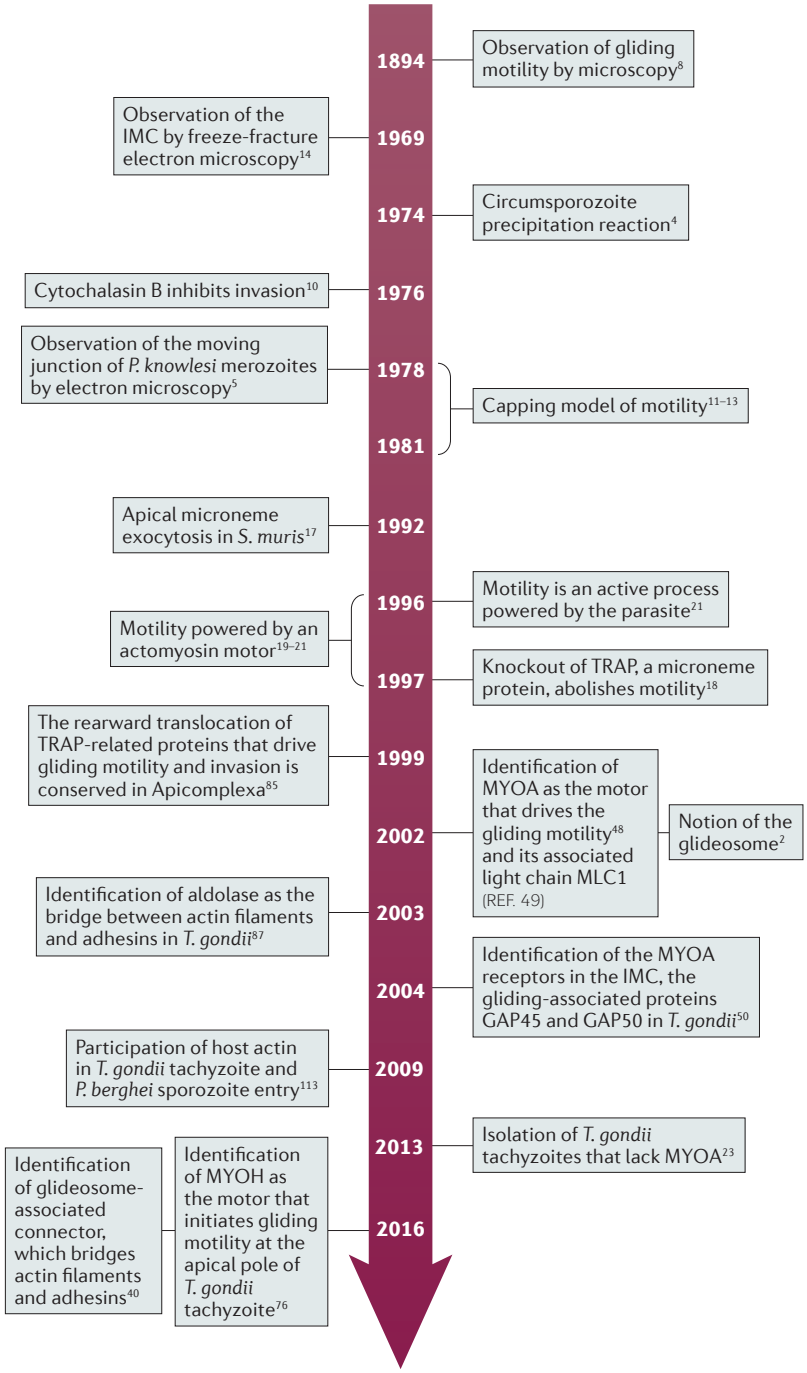


Figure 2 | **Major developments in the history of the capping model.** IMC, inner membrane complex; MLC1, myosin light chain 1; MYOA, myosin A; MYOH, myosin H; *P. berghei*, *Plasmodium berghei*; *P. knowlesi*, *Plasmodium knowlesi*; *S. muris*, *Sarcocystis muris*; *T. gondii*, *Toxoplasma gondii*; TRAP, thrombospondin-related anonymous protein.

Despite ample functional evidence such as this, actin filaments were found to be unusually short and heterogeneous by electron microscopy and ultracentrifugation, and they were not readily detectable in parasites using microscopy approaches[27]. Recently, the use of chromobodies has enabled the visualization of F-actin in *T. gondii*[28] for the first time, whereas F-actin in invading merozoites was described in an earlier study[29].

Gregarina
Large apicomplexan parasites that are ~0.5 mm in size and are capable of infecting terrestrial and marine invertebrates.

A pool of actin maintained in its globular form. In apicomplexan parasites, several factors contribute to maintaining a low concentration of polymerized actin. First, the apicomplexan F-actin seems to be intrinsically unstable, as it maintains substitutions in residues that usually stabilize the interaction between actin monomers in other organisms[30,31]. Second, the apicomplexans exhibit a smaller repertoire of actin-binding proteins (ABPs) than many lower eukaryotes, and they lack one of the major actin-nucleating machineries, the actin-related protein 2/3 (ARP2/3) complex[32]. Last, two ABPs — actin-depolymerizing factor (ADF) and profilin — unexpectedly contribute to the high monomer content by sequestering free G-actin[33,34] (FIG. 4b). Therefore, parasite actin is maintained as a heterogeneous mixture of sequestered actin monomers and dynamic filaments of variable length that might originate from unusual polymerization dynamics. In contrast to the classical nucleation–elongation model (in which a critical concentration is reached before a rapid polymerization phase), the polymerization of TgACT1 *in vitro* and in the apparent absence of nucleators is slow, has no lag phase and increases proportionally to the number of actin molecules[35]. This accommodates an isodesmic polymerization model, in which each monomer has the same assembly and/or disassembly rate in the polymer. Thus, apicomplexans have F-actin that is different from the actin in other organisms; how this contributes to gliding motility is discussed below.

A fast dynamic of actin polymerization is required for motility. Formins (FRMs) bind to actin and facilitate polymerization (FIG. 4b); in apicomplexans they are the only inducers of actin polymerization identified to date. *P. falciparum* and *T. gondii* FRM1 and FRM2 act as potent nucleators of rabbit actin *in vitro* through their FRM-homology 2 (FH2) domain[36,37]. *T. gondii* FRM1 (TgFRM1) and TgFRM2 also augment the polymerization of TgACT1, although they seem to increase the number of growing filaments rather than their length[34]. In invading merozoites, *P. falciparum* FRM1 (PfFRM1) localizes to the apical pole and follows the moving junction[36]. In addition, F-actin is highly concentrated posterior to the moving junction, where the parasite is attached to the host cell surface receptors to sustain its progression into the cell, which is powered by the myosin motor[29,38,39]. TgFRM1 was also recently shown to be located exclusively at the tip of the tachyzoites, but it does not follow the moving junction in invading parasites[40]. Regulators of actin dynamics clearly affect the motility and invasion of apicomplexan zoites, which suggests a crucial role for actin in the molecular machinery that powers their locomotion. For example, profilin and ADF proved to crucially contribute to F-actin turnover and motility in *T. gondii*[41,42] and *Plasmodium* spp.[43,44] (Supplementary information S6 (table)). Moreover, in *P. berghei*, disruption of the αβ-heterodimer capping protein that regulates the growth of F-actin critically impairs the motility of sporozoites and the propagation of infection[45]. Finally, the F-actin-binding protein coronin contributes to the organization of the filaments for productive motility in

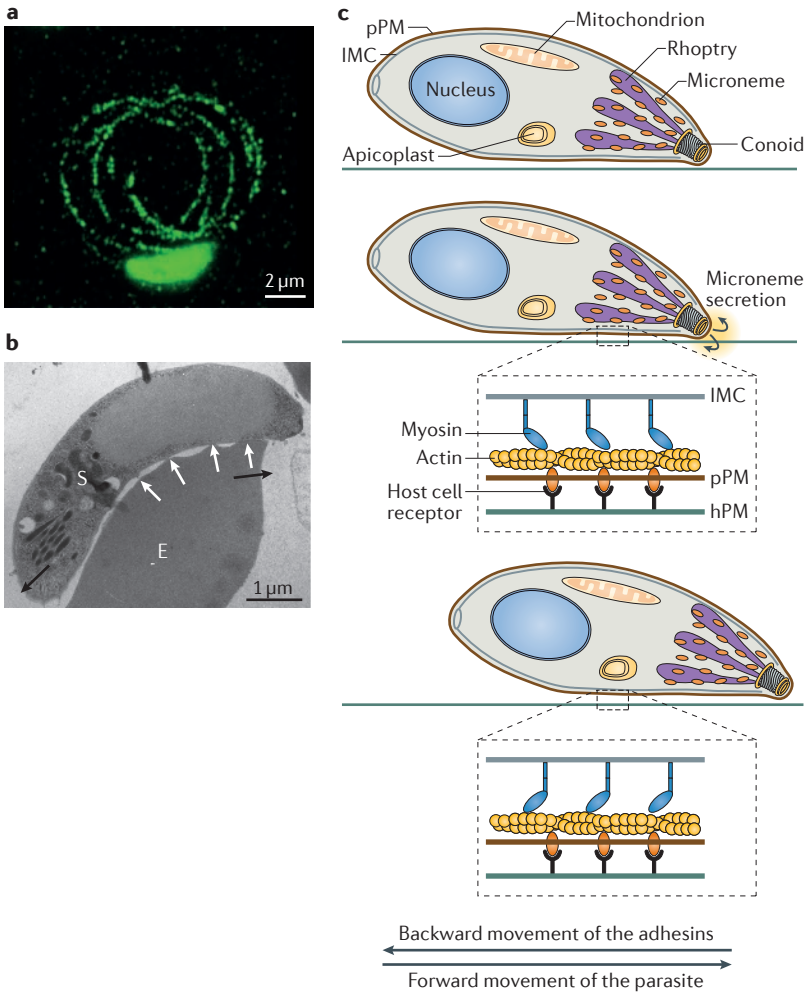


Figure 3 | **Gliding motility in Apicomplexa. a** | The motility of *Toxoplasma* spp. tachyzoites can be visualized by the trails (shown in green) deposited on a coated surface, which demonstrate circular gliding. The circular motion on a 2D surface is translated into a helical movement in tissues or in a 3D matrigel, and this helical movement can be described as a clockwise corkscrew trajectory that is linked to the left-handed twisted microtubules of the zoite cytoskeleton[99–101]. **b** | An *Eimeria nieschulzi* sporozoite (S) gliding along a rat erythrocyte (E) *in vitro*. Several sites of interaction are visible (indicated by the white arrows). The black arrows indicate the forward gliding of the sporozoite and the stretching of the erythrocyte. **c** | A generalized model of gliding motility in Apicomplexa. The exocytosis of secretory microneme organelles (orange) occurs at the apical pole of the parasite. During this process, transmembrane adhesive proteins that are embedded into the microneme membrane become inserted into the parasite plasma membrane (pPM), where they interact with extracellular receptors on the host cell (shown in black). Gliding motility results from the rearward translocation of these adhesin–receptor complexes, which is powered by the myosin motors (blue) along actin filaments (yellow) in the space between the inner membrane complex (IMC) and the pPM. hPM, host cell plasma membrane.

Moving junction
An intimate junction made at the point of apposition between the parasite and host cell plasma membranes. It is also referred to as a tight junction or circular junction in the literature.

P. berghei sporozoites[46], whereas in *T. gondii* coronin relocalizes to the posterior pole of the parasite concomitantly to microneme secretion but does not affect motility[47]. Thus, actin polymerization is clearly important for the process of gliding motility in Apicomplexa.

The glideosome machinery

The involvement of a myosin motor in the glideosome machinery, which powers motility and invasion, was initially postulated on the basis of experiments that used the myosin ATPase inhibitor 2,3-butanedione monoxime[20] (FIG. 4a). The conserved, short single-headed myosin heavy chain myosin A (MYOA), was then identified in *T. gondii* as the motor that was able to generate the rearward traction force necessary for motility, entry into, and egress from, host cells[48]. The composition and molecular details of the glideosome have been elucidated in more detail and discovered, respectively[49–52], and the importance of the glideosome in motility and invasion has been assessed further[23,25,26,53,54] (Supplementary information S6 (table)).

Glideosome composition, assembly and anchoring to the cytoskeleton. The glideosome is confined to the space between the plasma membrane and the IMC, and is conserved throughout the species in the Apicomplexa phylum[2]. It is composed of MYOA and the associated protein myosin light chain 1 (MLC1 (REF. 49); named MYOA tail domain-interacting protein (MTIP) in *Plasmodium* spp.[55]) and the three gliding-associated proteins GAP45, GAP50 (REFS 50,56) and GAP40 (REF. 51) (FIG. 4c). MYOA is a small myosin that lacks a proper tail domain, which is usually responsible for the localization and function of the motor through its interaction with cargo molecules[57]. Despite this, MYOA is a fast motor in both *T. gondii* and *P. falciparum*, and has kinetic and mechanical properties that are similar to those of fast skeletal muscle myosin and a velocity that is compatible with the speed of gliding tachyzoites and sporozoites (~3 µm s^{-1})[49,58]. The head domain of MYOA converts the chemical energy released by ATP hydrolysis into directed movement along F-actin. To do so, the myosin light chain (MLC1 or MTIP) binds to the degenerated IQ motif of the short MYOA neck domain, which acts as a lever arm to transduce the energy into movement[58–60] (FIG. 4d). In Coccidia parasites, two additional 'essential light chains' (ELC1 and ELC2) compete for a second binding site in the MYOA neck domain and contribute to the speed of the motor, probably by increasing the length of the lever arm and its rigidity[60,61]. In *T. gondii*, the conserved amino-terminal extension of MLC1 brings MYOA to its site of action within the pellicle, anchoring it to the IMC through its association with GAP45 (REF. 51) (FIG. 4c).

The glideosome component GAP45 is produced in the cytosol of all apicomplexans and is targeted to the plasma membrane by N-terminal acylation, whereas its conserved carboxy-terminal domain associates with the IMC[51,62]. GAP45 recruits MYOA to the IMC and ensures the integrity of the pellicle during motility by forming a bridge that is fluidly anchored into the plasma membrane and that maintains the appropriate spacing between the IMC and the plasma membrane[51]. *T. gondii* expresses two additional orthologues of GAP45, TgGAP70 and TgGAP80, which assemble into the glideosome containing TgMYOA at the apical cap and the glideosome containing TgMYOC (another myosin motor protein), respectively, at the basal pole of the parasite[53].

Interestingly, the glideosome is abundantly modified by phosphorylation and acylation in both *Toxoplasma gondii* and *Plasmodium* spp.[63], but site-directed

Pellicle
In apicomplexans, the three-layered structure comprising the plasma membrane and the underlying inner membrane complex.

Inner membrane complex
(IMC). In apicomplexans, one or more flattened vesicular sacs, also named alveoli, that are visible as double-membranous structures underneath the plasma membrane. The IMC is composed of only one alveolus in *Plasmodium* spp. or of a patchwork of alveoli in *Toxoplasma gondii* or *Eimeria* spp.

Rhoptries
Club-shaped secretory organelles that are located at the apical pole of parasites and are composed of two subcompartments: the neck and the bulb.

Micronemes
Elliptic secretory organelles that are located at the apical pole of parasites.

Actin-binding proteins
(ABPs). Proteins that bind to globular and/or filamentous actin and influence, for example, monomer sequestration or delivery, filament nucleation, polymerization, depolarization, stability and capping.

Myosin
A molecular motor that binds to cargo and converts chemical energy released by ATP hydrolysis into directed movement along tracks of actin filaments.

Acylation
The co-translational or post-translational addition of a lipid onto protein residues; examples include myristoylation (in which a 14-carbon saturated fatty acid is added onto a glycine residue at position 2) and palmitoylation (in which a 16-carbon saturated fatty acid is added onto a cysteine residue).

Conoid
A cone-shaped, apical structure that is present in coccidian parasites and is made of spirally arranged tubulin fibres. The conoid protrudes in a calcium-dependent manner during motility, invasion and egress.

mutagenesis has failed to identify phosphorylation sites that have an effect on the assembly of the motor complex[62,64]. By contrast, the calcium-dependent phosphorylation of TgMYOA affects its motor function[65,66]. In addition, acylation has a crucial role in the positioning of the glideosome within the pellicle. Mutations in the N-terminal acylation sites of TgGAP45 cause the protein to fail to anchor the glideosome to the plasma membrane and impair the integrity of the pellicle during motility and parasite invasion[51]. It remains to be determined whether the glideosome undergoes a disassembly process following motility, invasion or egress.

To generate traction, MYOA must be firmly anchored to the parasite cytoskeleton[67], possibly through GAP50 (REF. 50) and GAP40 (REF. 51) on the parasite IMC side and to extracellular host cell substrates on the other side (FIG. 4c). The indirect link with the underlying alveolin network and the subpellicular microtubules is made by a family of glideosome-associated proteins with multiple-membrane spans (GAPMs) that are present in the IMC of *T. gondii* and *P. falciparum*, and have been shown to interact with both alveolins and GAP50 (REF. 68) (FIG. 4c). GAP40, GAP50 and GAPMs also seemed to be crucial for the biogenesis of the IMC during intracellular replication[69]. These findings suggest a connection between the IMC and the basket of subpellicular microtubules, a role previously associated with the intramembrane particles visualized by electron microscopy in *Eimeria* spp. and *T. gondii*[15,70].

Disruption of components of the glideosome supports their role in gliding. Consistent with a role for these molecules in gliding, the conditional depletion of MYOA, myosin light chain (MLC1 or MTIP), the essential light chains (ELC1 and ELC2) or GAP45 had a substantial effect on the motility of *T. gondii* tachyzoites or *P. berghei* ookinetes, compromising their survival[25,48,51,61,71,72] (Supplementary information S6 (table)). In addition, the covalent modification of MLC1 by the small-molecule inhibitor tachyplegin A has been shown to inhibit the motility of *T. gondii* by affecting the activity of TgMYOA[73,74]. Remarkably, tachyzoites that lack TgMYOA could still be propagated in culture, although their lytic cycle was severely impaired[23]. A model based on retrograde membrane flow has been proposed to account for the residual motility[25,26]. However, a probable explanation is the functional redundancy and parasite adaptation conferred by the presence of a second myosin heavy chain, TgMYOC[53]. Indeed, it has not been possible to delete both TgMYOA and TgMYOC[25], and an artificially expressed second copy of TgMYOC immediately relocalizes to the periphery of the parasites and interacts with GAP45 in the absence of TgMYOA[53], a phenomenon also observed with endogenous TgMYOC[75].

In *T. gondii*, another myosin motor protein, TgMYOH, was recently identified as an indispensable motor acting upstream of TgMYOA and TgMYOC[76]. TgMYOH is present at the tip of the parasite and initiates gliding motility concomitantly with conoid protrusion (FIG. 4e). By contrast, *Plasmodium* spp. genomes encode only six myosin heavy chains, with no evident homologue of TgMYOC or TgMYOH[77]. Therefore, MYOA is anticipated to be crucial for the motile and invasive stages of *Plasmodium* spp., and concordantly, the depletion of *P. berghei* MYOA (PbMYOA) completely abolishes gliding motility in ookinetes, resulting in a failure to form oocysts in the mosquito midgut[71]. *Plasmodium* spp. do not possess a conoid but have an apical polar ring (BOX 1), where the predicted essential PfMYOB has recently been localized in the three motile stages[78].

Secretory organelles deliver adhesins

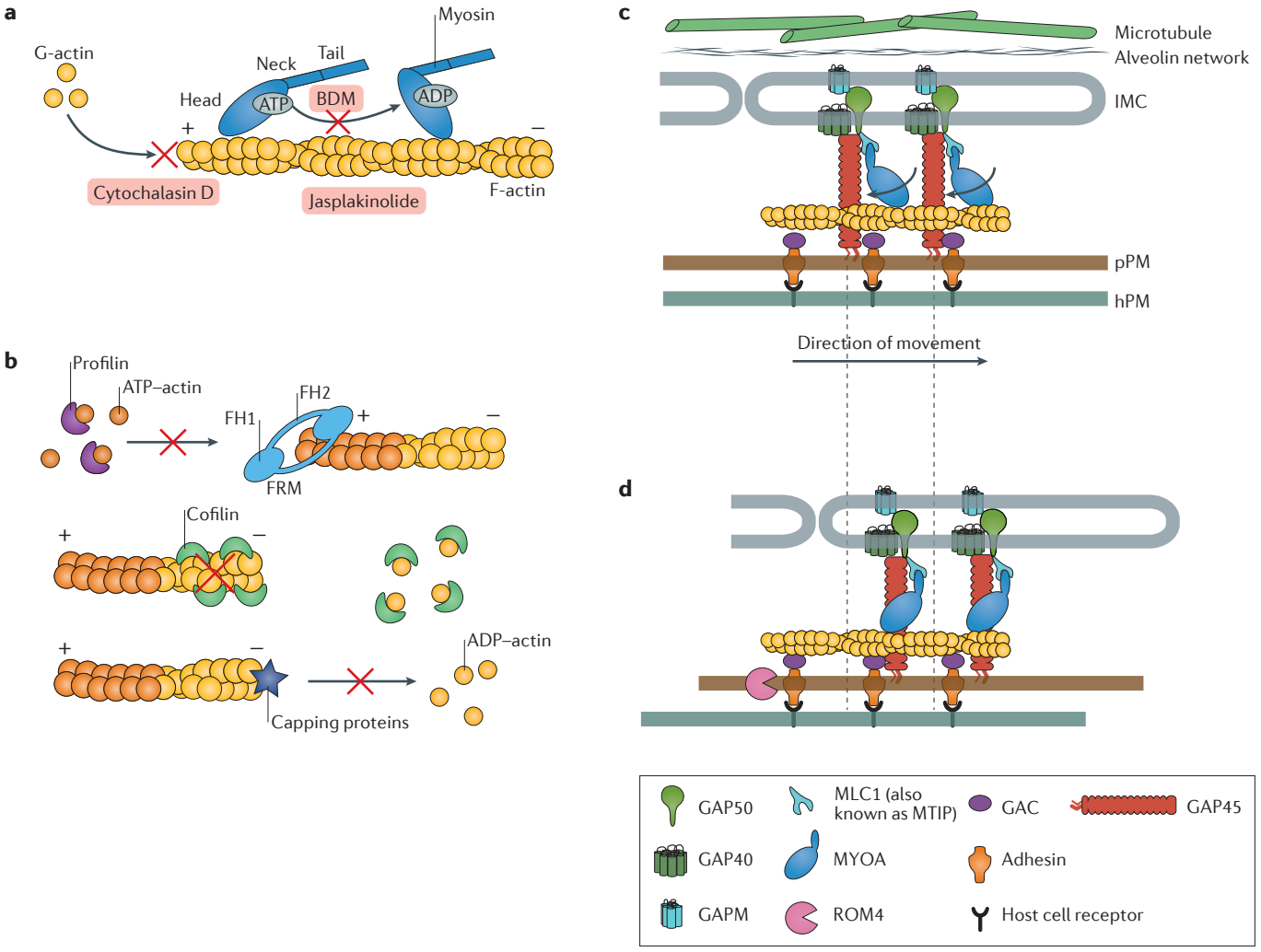
The glideosome is firmly attached to the cytoskeleton and must interact with extracellular host cell receptors to propel the parasite forwards. This is achieved by adhesins, which are inserted into the parasite plasma membrane following the apical exocytosis of micronemes (FIG. 4e) and act as specific ligands for host cell receptors[79].

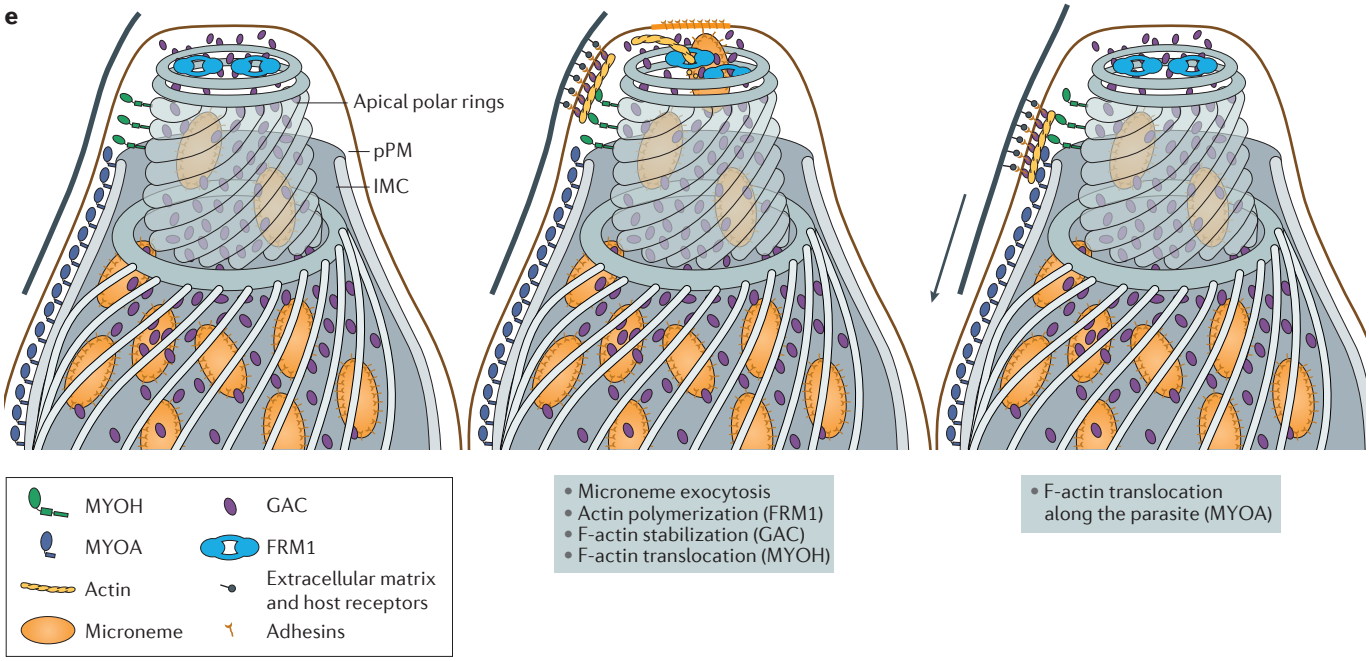
In *T. gondii*, at least three adhesin complexes are released from the micronemes: *T. gondii* micronemal protein 6 (TgMIC6)–TgMIC1–TgMIC4, TgMIC2–*T. gondii* MIC2-associate protein (TgM2AP) and TgMIC8–TgMIC3 (REF. 80). TgMIC1 binds to sialic acid on the surface of host cells, which functions as major determinant of invasion by coccidians[81]. In *Plasmodium* spp. merozoites, many ligands have been identified and classified into two families of proteins, the erythrocyte-binding antigens (EBAs) and the reticulocyte binding-like homologues (RHs), which are released from the micronemes and rhoptries, respectively. Their expression fluctuates to adapt to polymorphisms of erythrocyte receptors and also to evade the immune system[82]. An exception to this is RH5, which is non-redundant and thus an attractive target for intervention.

In *P. berghei* sporozoites, the adhesin PbTRAP has been demonstrated to be essential for gliding motility[18]. Subsequently, TRAP-like and TRAP-related proteins have been linked to the motility of other stages of *P. berghei*[83], as well as the motility of *T. gondii*, in which the TRAP homologue is named TgMIC2 (REF. 84) (BOX 2). Mutations in the tail domain of MIC2 and TRAP that are facing the glideosome complex impair the motility of sporozoites and tachyzoites, respectively[85]. However, the deletion of TgMIC2 does not completely abolish motility[23,86], which suggests that other microneme proteins may participate in gliding.

Adhesive complexes and the glideosome

For the past decade, the connection between the glideosome and adhesins was assumed to be mediated by the homotetrameric glycolytic enzyme aldolase (ALD) through its F-actin-binding activity[87]. Compellingly, the cytoplasmic tail moieties of several conserved adhesins, such as the micronemal proteins MIC2, TRAP and apical membrane antigen 1 (AMA1), as well as PfRH family members, were shown to bind to ALD, mainly *in vitro*[88]. In addition, TgALD-depleted *T. gondii* parasites showed a defect in motility and invasion, and a substantial loss of fitness[89]. However, another report showed that this fitness loss could be due to an accumulation of toxic metabolites caused by





incomplete glycolysis, which cast some doubt on the role of TgALD as a connector[90]. Finally, TgALD was proven dispensable for gliding in parasites that are grown in the absence of glucose[90]. The physiologically relevant glideosome-associated connector (GAC) was discovered following the functional characterization of a large armadillo repeats-containing protein that accumulates at the apical tip of *T. gondii* tachyzoites and in the invasive stages of malaria parasites[40]. *T. gondii* GAC (TgGAC) binds to F-actin, translocates along the parasite during motility and invasion, and binds to the tail of the adhesin TgMIC2. Concordantly, the tip of the parasite is also the site at which the FRM1 produces F-actin in the narrow space of the pellicle to fuel gliding motility[36,40] (FIG. 4e).

Disengagement of adhesive complexes

During host cell entry, parasites shed some of their surface adhesins. The concentration gradient of adhesive molecules generated as a consequence of this shedding presumably contributes to the reorientation of the parasite to face the cell with its apex, which is a necessary step for invasion. In *T. gondii*, the intramembrane serine protease of the rhomboid family rhomboid-like protease 4 (TgROM4) cleaves adhesins (for example, TgMIC2 and TgAMA1) in their transmembrane domain (FIG. 4d), and this leads to the disengagement of host receptor–parasite ligand interactions. Interestingly, the deletion of TgROM4 has no effect on parasite survival *in vitro* and is not compensated for by other rhomboid proteases[86,91,92]. However, the non-cleaved ligand–receptor complexes are translocated rearwards and accumulate at the posterior pole of the parasite, leading to tachyzoites exhibiting stationary twirling motility (visualized as upright parasites twirling on their posterior pole; Supplementary information S4 (movie)) instead of productive helical motility (Supplementary information S2 (movie))[86,92]. The non-cleaved, secreted microneme proteins accumulate on the surface of the parasites, and this results in increased attachment and a defect in apical reorientation. The function of ROM4 is conserved in *Plasmodium* spp., and mutations of the rhomboid cleavage site of the adhesin molecule PbTRAP in *P. berghei* sporozoites leads to a defect in motility and infectivity[93], whereas mutations of the rhomboid cleavage site of the glycophorin A-binding protein PfEBA-175 in *P. falciparum* merozoites seem to be deleterious to parasite survival[94]. By contrast, in the erythrocyte stage of the *P. falciparum* life cycle, the vsubtilisin-like serine proteases PfSUB1 and PfSUB2 seem to have a more pronounced contribution than ROM4 in the cleavage of surface adhesins[95,96].

◀ Figure 4 | **Gliding motility is initiated at the apical pole and depends on the glideosome complex. a** | The action of the drugs that have been used to identify the involvement of actin and myosin in motility and invasion. Cytochalasin D binds to the barbed end (+) of actin, inhibiting polymerization, and jasplakinolide can bind to and stabilize actin filaments (F-actin). These toxins inhibit the motility of *Toxoplasma gondii* tachyzoites and consequently their invasion. 2,3-butanedione monoxime (BDM), which is known to block myosin heavy chain II ATPase activity, also inhibits parasite invasion. **b** | Formins (FRMs) are the only known actin nucleators and actin-polymerizing factors in apicomplexans. The FRM homology 2 (FH2) domain remains associated with the barbed end during actin polymerization. Profilin binds to ATP–actin monomers and promotes the polymerization of F-actin by delivering globular actin (G-actin) to the nascent filament or by augmenting FRM activity. In *Toxoplasma gondii*, profilin does not augment FRM activity but contributes to maintaining the pool of G-actin and not actin depolymerization (red cross). Cofilin (also known as actin-depolymerizing factor (ADF)) also contributes to the sequestration of G-actin. Conversely, capping proteins prevent the depolymerization of F-actin by binding to its pointed end (–). **c** | The core motility complex is composed of the myosin A (MYOA) motor and its myosin light chain (myosin light chain 1 (MLC1) in *Toxoplasma gondii*; MYOA tail domain-interacting protein (MTIP) in *Plasmodium* spp.) linked to the inner membrane complex (IMC) proteins gliding-associated protein 40 (GAP40) and GAP50 through an interaction with GAP45. In addition, GAP45 maintains the spacing of the parasite plasma membrane (pPM) and the IMC. Glideosome-associated connector (GAC) bridges F-actin and adhesins. The glideosome is also linked to the underlying cytoskeleton, probably through members of the GAPs with multiple-membrane spans (GAPM) family, thus enabling the generation of traction forces. **d** | The conformational change of the myosin head domain on the F-actin following ATP hydrolysis drives the forward movement of the parasite. Surface-expressed rhomboid-like protease 4 (ROM4) cleaves the transmembrane domain of adhesins to disengage their interaction with host receptors. **e** | A schematic showing a magnified view of the apical pole of a *T. gondii* tachyzoite; motility is initiated at this site. Following calcium signalling, microneme exocytosis occurs at the apex of the parasite. Adhesins secreted by micronemes are inserted into the pPM and interact with host cell receptors or the extracellular matrix. At the tip, FRM1 polymerizes F-actin that is stabilized by GAC. GAC also interacts with the cytoplasmic tails of the adhesins and, together with F-actin and the adhesin–receptor complexes, is translocated backwards by the action of myosin heavy chains MYOH (at the level of the conoid) and MYOA (along the IMC). hPM, host cell plasma membrane. Parts **a**–**d** are adapted with permission from REF. 168, EDP Sciences. Part **e** is adapted with permission from REF. 40, Elsevier.

Gliding motility *in vitro*

Substrate-dependent motility has been predominantly studied in cultured *T. gondii* tachyzoites[3] (Supplementary information S2–S4 (movies)), in *P. berghei* sporozoites isolated from mosquito salivary glands (Supplementary information S1 (movie)) and in ookinetes produced *in vitro*[72] (Supplementary information S5 (movie)). Freshly isolated sporozoites can glide on a surface for tens of minutes[97], whereas ookinetes are able to glide for tens of minutes on a mosquito midgut surface but at a speed 100-times slower than that of sporozoites[98]. All of these zoites are crescent shaped and move in a circular motion on 2D surfaces that is translated into a helical movement in tissues or 3D matrigels. In a 3D substrate, this movement can be described as a clockwise corkscrew trajectory, which is linked to the left-handed twisted microtubules of the parasite cytoskeleton[99–101]. The force generated at the surface of tachyzoites has been measured using a laser trap and shown to become directional after a short lag phase, which might correspond to the signalling cascade that leads to microneme secretion and/or actin polymerization[102]. The directionality of the force observed along the anteroposterior axis of the parasite probably correlates with the direction of F-actin and follows the subpellicular microtubules[102]. In *P. berghei*, sporozoite motility has been investigated using reflection interference contrast and traction force microscopy, which has provided a deeper insight into the spatiotemporal dynamics of gliding motility[103]. This technique has revealed that the circular locomotion observed on a substrate is characterized by a continuous sequence of 'stick-and-slip' steps, and that the connected transient forces correlated with the

association and dissociation of substrate contact sites that were dependent on actin dynamics[103]. In addition, the retrograde flow of adhesin proteins is faster than the speed of parasite migration and was dependent on the presence of F-actin, which indicates that force production is not linearly coupled to the rearward translocation of adhesins[104]. In the absence of TRAP-like protein (TLP), this retrograde flow was even faster but less force was measured, which suggests that TLP is involved in coupling these two processes.

The glideosome and egress and invasion

Egress from infected cells is a key event for the dissemination of the parasites[105] and involves the rupture the PVM and the host cell plasma membrane. In *T. gondii*, this process is assisted by the release of the micronemal perforin-like protein TgPLP1, which forms pores in the PVM and the host cell plasma membrane[106,107], and by host calpain 1, which is a cysteine protease that remodels the host cytoskeleton[108]. In addition, tachyzoite motility imposes a mechanical force that ensures the rupture of the PVM and the host plasma membrane (FIG. 5a). Consequently, mutants with defective actin dynamics or glideosome components fail to exit perforated host cells[25,40–42,48,51,53,76]. In *P. falciparum* merozoites, egress is a tightly regulated process that is initiated 15 hours before exit from the infected erythrocyte[109]. The schizonts activate a series of parasite and host proteases that induce considerable changes in the erythrocyte cytoskeleton, leading to the curling and eversion of the plasma membrane, thus ejecting the parasites into the bloodstream[6,110] (Supplementary information S7 (movie)). It is still unclear whether motility contributes to the rupture of the membranes or whether the osmotic pressure is sufficient to induce merozoite release[111].

Invasion by *T. gondii* is a fast process that occurs with limited apparent changes to the host cell membrane. Tachyzoites initiate a pivoting movement, followed by a brief decrease in motion that coincides with moving junction formation on the host cell membrane, before entering the host cell by rotational progression[112] using a screw-like motion[13] (FIG. 5b). During invasion, a ring-shaped complex of F-actin, ARP2/3 and cortactin is observed in the host cell at the site of the moving junction[113] (FIG. 5c). This remodelling of the cortical actin cytoskeleton is thought to be important for anchoring the parasite to the host cell and for initiating the deformation of the host cell membrane during invasion[113,114]. The remodelling seems to be even more crucial for parasites that have substantially impaired gliding motility; for example, when TgMYOA is deleted[54]. Likewise, the attachment and invasion of red blood cells by *Plasmodium* spp. merozoites are completed in approximately 20 seconds, but accompanied by substantial deformations of the erythrocyte membrane[115,116]. Interestingly, when PfEBA-175 binds to its host receptor glycophorin A, it elicits a phosphorylation cascade in the host cell that includes components of the erythrocyte cytoskeleton and leads to the mechanical destabilization of the cell membrane[117] (FIG. 5d). Although erythrocyte actin is more dynamic than previously assumed[118], merozoite entry does not involve host actin reorganization at the moving junction[119]. During invasion, the concentration of parasite actin has been visualized as a ring posterior to the moving junction, which supports a role for gliding during invasion[29,38,39].

Invasion crucially depends on the discharge of rhoptries[120], and concordantly, *Plasmodium* spp. ookinetes that only traverse the epithelial midgut of the mosquito do not have rhoptries. The content of the rhoptry neck contributes to the formation of the moving junction, which is visible as a ring-like structure by immunofluorescence (FIG. 5b) and forms a solid platform[112] with the host cell plasma membrane, through which the motile zoite glides (FIG. 5c,d).

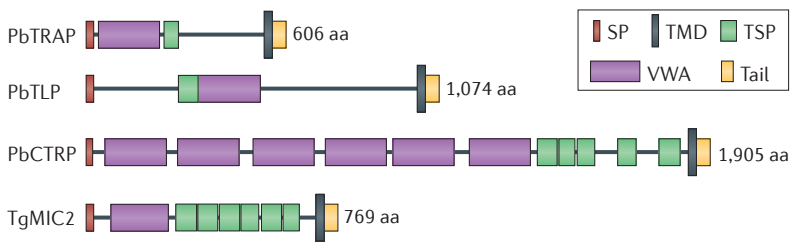
Box 2 | Transmembrane adhesive proteins are crucial for gliding and invasion

Adhesins of the thrombospondin-related anonymous protein (TRAP) family are transmembrane proteins that are stored in micronemes and discharged apically onto the surface of the parasite during gliding motility. These ligands bind to host cell receptors or the extracellular matrix, and are crucial for generating the forces necessary to propel the parasite forwards[103]. They are translocated rearwards on the surface of the parasite and have been associated with gliding in all motile stages. They are composed of a signal peptide (SP), von Willebrand factor type A (VWA) and thrombospondin type 1 repeat (TSP) domains (which are usually involved in multiprotein complexes[79]), a transmembrane domain (TMD) and a cytoplasmic tail (see the figure).

In *Plasmodium berghei* sporozoites, two non-redundant adhesins have an important role in motility and invasion: PbTRAP and PbS6. PbTRAP is secreted from the micronemes, binds to the heparin sulfate proteoglycans expressed on the surface of hepatocytes and is translocated to the rear of the parasites during motility. The consequences of deleting PbTRAP show that this protein is crucial for the motility of sporozoites, the invasion of the mosquito salivary glands and the invasion of rodent hepatocytes[18]. The PbTRAP-related protein PbS6 has a similar role in gliding motility and is required for efficient malaria transmission[166]. In addition, *P. berghei* TRAP-like protein (PbTLP) probably acts together with PbTRAP and PbS6 to regulate motility in sporozoites, and it is particularly involved in regulating the attachment sites during gliding to achieve continuous movement[167].

The gliding and migration of the *P. berghei* ookinete through the mosquito midgut epithelium has also been shown to rely on the TRAP family member circumsporozoite- and TRAP-related protein (PbCTRP). PbCTRP is a microneme protein that is secreted on the surface of the ookinete, and its disruption in *P. berghei* has been shown to result in defective ookinete motility and infectivity such that no ookinetes are found in the mosquito midgut epithelium[83].

In *Toxoplasma gondii* tachyzoites, the TRAP family member microneme protein 2 (TgMIC2) forms a complex with MIC2-associated protein (TgM2AP). The carboxy-terminal domain of TgMIC2, which is located downstream of the TMD, binds to gliding-associated connector (GAC) and therefore bridges the glideosome complex. The inducible depletion of TgMIC2 does not impair microneme secretion but severely affects the gliding motility of tachyzoites, especially the helical movement, thus leading to a marked defect in invasion and egress. In addition, TgMIC2-depleted parasites have been shown to be avirulent in a mouse model and to confer protection against a new challenge[84], which demonstrates the importance of adhesins for efficient infection by apicomplexan parasites.



aa, amino acid.

In *T. gondii*, rhoptry discharge follows microneme secretion and crucially depends on the presence of the microneme protein TgMIC8 (REF. 121), although the mechanistic contribution of TgMIC8 is not understood. Similarly, the glycophorin A-binding protein PfEBA-175 is reported to be a prerequisite for rhoptry secretion[122]. The moving junction was first observed more than 30 years ago by electron microscopy, following erythrocyte invasion of *P. knowlesi* merozoites[5], and the molecular details of the moving junction in *T. gondii* emerged recently in a study that identified an assembly of proteins originating from micronemes and rhoptries[123]. AMA1, which is secreted from the micronemes, associates with the pre-formed complex of four rhoptry neck proteins (RON2, RON4, RON5 and RON8) secreted into the host cell[123] (FIG. 5c). Exposed on both sides of the junction, AMA1 and RON2 form a tight complex that maintains the close apposition of parasite and host cell membranes[124–126].

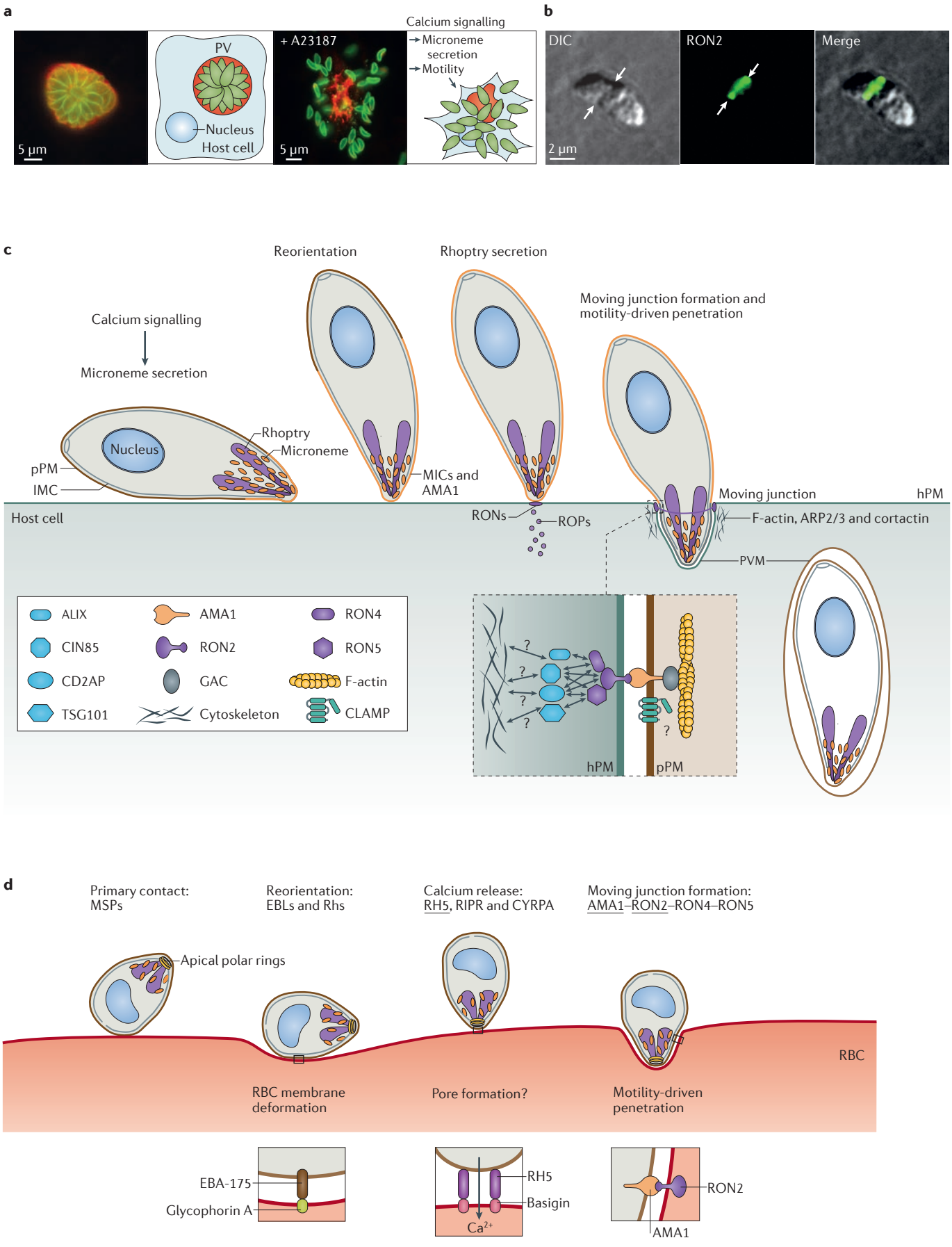
The solved structure of AMA1–RON2 complexes in *T. gondii* and *P. falciparum* highlights the strong affinity required to withstand the mechanical sheer forces enacted by the moving junction during invasion[127,128]. Remarkably, when TgAMA1 is actively engaged with TgRON2 in host cell penetration, TgAMA1 becomes less susceptible to cleavage by rhomboid proteases. This enables the two proteins to maintain intact the connection between the two cells during the entire invasion process[129]. Only a short ectodomain of RON2 is bound to AMA1; the majority of RON2 is localized to the cytosolic face of the host cell membrane as part of a complex with RON4, RON5 and RON8 (REFS 124,130,131). This complex delineates the moving junction in *T. gondii*, and recruits the cytosolic host proteins ALG2-interacting protein X (ALIX; also known as programmed cell death 6-interacting protein), tumour susceptibility gene 101 protein (TSG101), CBL-interacting protein of 85 kDa (CIN85; also known as SH3 domain-containing kinase-binding protein 1) and CD2-associated protein (CD2AP), which comprise a set of adaptor proteins known to connect membrane proteins to the underlying cytoskeleton[132]. Similarly, in *P. falciparum*, the localization of PfAMA1 during invasion is visible as a ring shape within a ring of PfRON4 (REFS 38,39). The AMA1–RONs complex may provide a physical link between the cortical cytoskeletons of the parasite and the host cell, and thus act as a stable structure on which the zoite applies traction during invasion. Concordantly, genetic approaches in *T. gondii* have validated the contribution of the AMA1–RONs complex to invasion[133–136], although parasite motility was not substantially affected (Supplementary information S6 (table)). Although a TgAMA1-null mutant could be obtained *in vitro*[137], the upregulation of TgAMA1 homologues that sustain residual invasion confirmed the crucial role of the moving junction in host cell penetration[134,138].

In *Plasmodium* spp., it was not possible to knockout AMA1 and RON4; however, the conditional depletion of *P. berghei* AMA1 (PbAMA1) and PfAMA1 caused a defect in merozoite invasion[139,140] and in the sealing of the parasitophorous vacuole at the end of invasion[140]. By contrast, the conditional depletion of PbAMA1 does not affect the entry of sporozoites[137,139], but blocking the interaction of AMA1–RON2 with a 20-residue peptide — 'R1 peptide', which specifically binds AMA1 — inhibits its hepatocyte invasion by *P. falciparum* sporozoites[141]. Moreover, the deletion of a distant PfAMA1 homologue, PfMAEBL, severely impairs hepatocyte invasion by *P. falciparum* sporozoites without having an effect on gliding motility[141]. This suggests that PfAMA1 and the homologue PfMAEBL are not fully redundant, but further work is required to determine whether they act together.

Other studies suggest that AMA1 has functions in signal transduction. In support of this, the phosphorylation of S610 on the tail of PfAMA1 by *P. falciparum* cAMP-dependent protein kinase (PfPKA) is important for efficient erythrocyte invasion by malaria parasites[142,143]. In *T. gondii*, the binding of the ectodomain of TgAMA1 to TgRON2 generates an outside-in signal that leads to the dephosphorylation of S527 on the cytosolic tail of TgAMA1, thus increasing invasion efficiency[129].

Signalling and gliding motility

It remains unclear what specific signalling pathways act to regulate the activity of the glideosome. However, a decrease in potassium concentration, which occurs when the integrity of the infected host cell is compromised, increases intracellular calcium levels in both *T. gondii* and *Plasmodium* spp.[122,144]. This spike in calcium concentration can mediate signalling by activating specific kinases and coordinating microneme secretion, and hence has an effect on gliding motility, invasion and egress[145,146]. Fluctuations in intracellular calcium concentrations have been monitored during the motility of both tachyzoites[147] and sporozoites[148] using the calcium indicator Fluo-4 and the Förster resonance energy transfer (FRET)-based sensor TN-XXL, respectively. Apicomplexans lack calmodulin-dependent kinases, which are part of the calcium signalling apparatus in higher animals, but instead have several calcium-dependent protein kinases (CDPKs) that are usually found in plants[149]. TgCDPK1 is essential for microneme exocytosis[150], whereas TgCDPK3 is only important for egress[151–153] (Supplementary information S6 (table)). Furthermore, TgCDPK3 is required for the initiation of motility, as it phosphorylates TgMYOA[66]. In *P. falciparum*, a peptide that specifically inhibits PfCDPK1 (which is an orthologue of TgCDPK3) causes a defect in merozoite microneme secretion and blocks invasion[154]. Moreover, parasites that are depleted of PfCDPK5 develop to the segmented schizont stage but are unable to rupture the PVM; however, following mechanical release, merozoites are fully competent to invade new red blood cells[155]. Together, these findings suggest that calcium signalling is important for parasite microneme secretion, motility and invasion. In addition to responding to changes in extracellular potassium concentration, parasites also react to low pH, which triggers the calcium-dependent secretion of micronemes and activates gliding motility[156].



Several other proteins and molecules have been linked to signalling pathways that regulate the glideosome. cGMP-dependent protein kinase (PKG) triggers microneme secretion and parasite egress in a calcium-independent manner[153,157–159]. Recently, phosphatidic acid has been identified as an important lipid mediator of microneme secretion that acts in parallel to calcium[160]. The production of inositol-1,4,5-triphosphate by phosphatidylinositol phospholipase C (PI-PLC) and the concurrent calcium mobilization that occurs before microneme secretion are linked to the generation of diacylglycerol (DAG). DAG can be interconverted to phosphatidic acid through the action of diacylglycerol kinase 1 (DGK1)[160]. The relevance of phosphatidic acid is linked to acylated pleckstrin homology domain-containing protein (APH), which is present at the surface of micronemes, conserved across the Apicomplexa phylum and acts as phosphatidic acid-detecting protein. Notably, the conditional depletion of TgAPH leads to a severe block in microneme secretion and consequently to an abrogation of the function of the glideosome[160]. In addition, a C2 domains-containing protein DOC2.1, which is also conserved across the apicomplexans, mediates apical microneme exocytosis in a calcium-dependent manner[161]. Last, a large protein localized at the apical polar ring of tachyzoites, and therefore named *T. gondii* RNG2 (TgRNG2), has been shown to affect microneme secretion in a poorly understood manner[162].

The apical tip of parasites is a strategic position from which organelles are secreted, FRM1-dependent actin nucleation and polymerization occurs, and motility is initiated (FIG. 4e). In *T. gondii*, apical lysine methyltransferase (TgAKMT) is concentrated at the apical polar ring of intracellular tachyzoites and quickly relocalizes to the cytosol in motile parasites[163]. The deletion of TgAKMT leads to severe defects in motility, invasion and egress without having an effect on microneme secretion (Supplementary information S6 (table)). Remarkably, TgAKMT is a prerequisite for the accumulation of TgGAC at the tip of the parasite[40], which indicates the importance of protein methylation in glideosome function. Interestingly, TgGAC also has a pleckstrin homology domain that binds to phosphatidic acid, and this binding further supports the role of phosphatidic acid signalling in the regulation of gliding motility[40].

Conclusions and unresolved issues

Although the capping model of gliding motility in Apicomplexa was conceptually formulated more than a century ago, the actual composition of the glideosome and the molecular details of its assembly and function have only emerged in the past 10 years. On the basis of the available data and in the absence of an alternative model, the glideosome seems to account for three crucial steps of the lytic cycle of Apicomplexa — motility, invasion and egress. Given the essential nature of invasion for an obligate intracellular parasite, functional redundancy and compensatory mechanisms exist as backup systems. In agreement with this, the deletion of TgMYOA or TgAMA1 in *T. gondii* is a clear example of parasite adaptation to deleterious conditions, whereby a reliance on a less powerful homologue sustains a vital function.

Recently, considerable methodological advances have led to the precise positioning of key effector molecules, such as F-actin motors and adhesins, and to the elucidation of the signalling pathways that are involved in coordinating invasion and egress through organelle discharge. Future challenges and endeavours reside in the identification of the kinase substrates that ensure parasite dissemination and in understanding how the key players that power motility are orchestrated in time and space to achieve a concerted action. A non-exhaustive list of unresolved questions to be addressed includes the following: what is the mechanism that controls the ATPase activity of myosins? What is the contribution of post-translational modifications to glideosome assembly and function? Is the glideosome disassembled and recycled? Where and how are actin dynamics regulated to control gliding? What are the molecular bases for the force generation and propulsion of parasites during motility and invasion? What is the machinery and triggering factor that govern rhoptry discharge? Future work should aim to address these questions as technological advances move the field forwards.

◀ Figure 5 | **Motility drives egress and invasion. a** | The two panels on the left show an immunofluorescence image and a schematic representation of intracellular tachyzoites residing in a parasitophorous vacuole (PV). The two images on the right show an immunofluorescence image and a schematic representation of parasites freshly egressed from the PV and host cell after induction by the calcium ionophore A23187. The PV is stained using a dense granule marker (dense granule protein 3; red) whereas anti-SAG1 antibodies were used to detect the pellicle of the parasites (green). **b** | Immunofluorescence images of an invading *Toxoplasma gondii* tachyzoite; the moving junction, labelled with anti-rhoptry neck protein 2 (anti-RON2) antibodies, is visible as a ring (green). The white arrows indicate the constriction of the moving junction. **c** | The invasion process of extracellular *T. gondii* tachyzoites, beginning with host cell adhesion, followed by the secretion of the content of micronemes (microneme proteins (MICs) and apical membrane antigen 1 (AMA1)), and the subsequent reorientation of the parasite, which places its apical pole in juxtaposition with the host cell plasma membrane (hPM). Subsequently, the secretion of RONs enables the formation of the moving junction between RON2–RON4–RON5 and AMA1, thus supporting the motility-driven progression of the parasite into the target cell. The magnified view (inset) highlights the interaction between the glideosome and the moving junction, and the interactions between the moving junction and host proteins (ALG2-interacting protein X (ALIX), CBL-interacting protein of 85 kDa (CIN85), CD2-associated protein (CD2AP) and tumour susceptibility gene 101 protein (TSG101)). Claudin-like apicomplexan microneme protein (CLAMP) also follows the moving junction during invasion, but its role is unknown[169]. After invasion, the parasite is enclosed in the parasitophorous vacuole membrane (PVM). **d** | After the egress of *Plasmodium* spp. merozoites, invasion starts with weak interactions between merozoite surface proteins (MSPs) and the surface of the red blood cell (RBC). Parasite reorientation occurs following tight interactions of erythrocyte-binding antigens (EBAs) and reticulocyte binding-like homologues (RHs) with host cell receptors. The binding of EBA-175 to glycophorin A mediates marked deformability of the RBC membrane, which is required for invasion[117]. At the tip of the merozoite, the binding of RH5 to basigin triggers calcium release[170] and the formation of the moving junction that supports the motility-dependent host cell invasion of the parasite. The underlined proteins are transmembrane proteins inserted in the plasma membrane. ARP2/3, actin-related protein 2/3; CYRPA, cysteine-rich protective antigen; DIC, differential interference contrast; F-actin, filamentous actin; GAC, glideosome-associated connector; pPM, parasite plasma membrane; RIPR, RH5-interacting protein; ROP, rhoptry proteins. Part **c** is adapted with permission from REF. 168, EDP Sciences. Part **d** is adapted from REF. 171; in this study, time-lapse imaging was used in combination with different methods of preventing merozoite–RBC interactions to provide the precise sequence of receptor–ligand interactions that occurs during the invasion process.

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Competing interests statement
The authors declare no competing interests.

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