Molecular and functional aspects of parasite invasion

Soldati-Favre, Dominique; Foth, Bernardo Javier; Cowman, Alan F

How to cite

SOLDATI-FAVRE, Dominique, FOTH, Bernardo Javier, COWMAN, Alan F. Molecular and functional aspects of parasite invasion. In: Trends in parasitology, 2004, vol. 20, n° 12, p. 567–574. doi: 10.1016/j.pt.2004.09.009

This publication URL: https://archive-ouverte.unige.ch/unige:78032
Publication DOI: 10.1016/j.pt.2004.09.009

Review TRENDS in Parasitology Vol.20 No.12 December 2004

Molecular and functional aspects of parasite invasion

Dominique Soldati[1], Bernardo J. Foth[1] and Alan F. Cowman[2]

[1]Department of Microbiology and Molecular Medicine, C.M.U. Faculty of Medicine, University of Geneva, 1 rue Michel-Servet, 1211 Geneva 4, Switzerland
[2]The Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Melbourne 3050, Australia

Apicomplexan parasites have evolved an efficient mechanism to gain entry into non-phagocytic cells, hence challenging their hosts by the establishment of infection in immuno-privileged tissues. Gliding motility is a prerequisite for the invasive stage of most apicomplexans, allowing them to migrate across tissues, and actively invade and egress host cells. In the late 1960s, detailed morphological studies revealed that motile apicomplexans share an elaborate architecture comprising a subpellicular cytoskeleton and apical organelles. Since 1993, the development of technologies for transient and stable transfection have provided powerful tools with which to identify gene products associated with these structures and organelles, as well as to understand their functions. In combination with access to several parasite genomes, it is now possible to compare and contrast the strategies and molecular machines that have been selectively designed by distinct life stages within a species, or by different apicomplexan species, to optimize infection.

Despite their morphological resemblance and close phylogenetic relationships, apicomplexans differ significantly with respect to host range, the niches they occupy and their mode of transmission. The transmission of *Plasmodium* species by the *Anopheles* female mosquito vector occurs by an obligatory two-host life cycle that alternates between the definitive mosquito host and the intermediate human host. By contrast, *Toxoplasma gondii, Eimeria tenella* and *Cryptosporidium parvum* are cyst-forming parasites that are transmitted orally by infective oocysts shed in the feces of the infected host. Most apicomplexans typically infect a limited range of host organisms, whereas *T. gondii* can infect virtually all warm-blooded animals and almost any cell type. Despite sexual reproduction occurring exclusively in felids (definitive hosts), the broad range of *T. gondii* has recently been explained by evidence for a significant change in its life cycle, allowing direct oral transmission between many different intermediate hosts [1].

Corresponding author: Dominique Soldati (dominique.soldati-favre@medecine.unige.ch).

Host cell invasion, parasitophorous vacuole formation and egress

The invasive stages (zoites) of most apicomplexans are motile cells that are extremely polarized and use their ability to glide on substrate to migrate across biological barriers, and to invade and escape from host cells (e.g. see Ref. [2]). Exit of these invasive forms from host cells, usually after parasite replication, is also known as egress. The zoites are characterized by the presence of an apical complex composed of specialized secretory organelles (including rhoptries, micronemes and dense granules) and an inner membrane complex formed of flattened vesicles associated with the subpellicular microtubules. The conoid, present in some parasites such as *Toxoplasma* and *Eimeria* but absent in *Plasmodium* and *Cryptosporidium*, is a motile apical organelle containing spiral fibers, composed of a novel polymer of tubulin [3], that protrude during host cell invasion. Some apicomplexans, such as *Theileria* sporozoites, lack several elements (e.g. the conoid and micronemes) of the apical complex apparatus. *Theileria* sporozoites and merozoites are non-motile and enter the host cell via a passive 'zippering' process that allows the parasites to invade in any orientation, suggesting that the organelles of the reduced apical complex are not directly involved in the invasion process and might serve a different function [4]. Nevertheless, the genes encoding proteins of the gliding machinery are present in the *Theileria parva* genome. Indeed, the kinetes egress the gut epithelial cells and invade the salivary glands in the tick using gliding motion.

Host cell invasion normally leads to the formation of a parasitophorous vacuole. The properties and morphological features of a parasitophorous vacuole differ significantly between apicomplexans. Although the protein and lipid composition of the vacuole membrane is largely uncharacterized, host-derived proteins are noticeably excluded [5] and the content of rhoptries appears to be important in forming the vacuole during invasion. The malaria parasite elaborates a tubulovesicular network that can protrude into the erythrocyte cytoplasm and these extensions may be important for transport of solutes [6]. Other membranous structures such as Maurer's clefts are formed in the host cell cytoplasm and are important in protein trafficking [7]. By contrast, the tubular network associated with the parasitophorous vacuole of *T. gondii* tachyzoites is not exposed to the host cytoplasm, but

 1471-4922/$ - see front matter doi:10.1016/j.pt.2004.09.009

remains confined within the vacuolar space. Infection of intestinal epithelial cells by *C. parvum* leads to the formation of an intracellular, but extracytoplasmic, vacuole. The zone of contact created at the parasite and the host cell interface is called the feeding organelle. Despite such a different invasion outcome, *C. parvum* is using the same components of the gliding machinery as other apicomplexans. In addition, *C. parvum* dramatically alters the host cell membrane and actin cytoskeleton, and causes the formation of a structural barrier that separates the intracellular parasite from the host cell cytoplasm, therefore creating its own intracellular niche just beneath the host plasma membrane. The remodeling of the host cell actin cytoskeleton by the parasite has been shown to involve the subversion of signaling through host phosphoinositide 3-kinase and frabin, which leads to the activation of the Cdc42 pathway [8,9]. The intracellular stages of *Theileria* and *Babesia* are again an exception as they do not appear to develop inside a parasitophorous vacuole but are free in the cytoplasm. Table 1 summarizes the mode of invasion and the ultrastructural differences between apicomplexan species and different stages of the same parasites.

The mechanism leading to egress of parasites from infected cells is still poorly understood, but it does share some common features with the process of invasion [10]. In *P. falciparum*, the process of merozoite release was recently examined using parasite lines expressing green fluorescent protein (GFP) fused to proteins targeted to the parasitophorous vacuole and the host erythrocyte cytosol. This study revealed that merozoite release involves a primary rupture of the parasitophorous vacuole membrane followed by a secondary rupture of the erythrocyte plasma membrane, both steps involving the action of distinct proteases [11]. A contribution of falcipain-2 in this release from red blood cells was postulated [12], but the disruption of the gene encoding falcipain 2 failed to confirm this prediction. However, these experiments substantiated a role of this protease in hemoglobin degradation [13]. Tachyzoites of *T. gondii* are able to sense the loss in host cell fitness and can be triggered to egress by the loss of the host plasma membrane integrity, which causes a major reduction in cytoplasmic potassium concentration [14].

Migration by transcellular and paracellular pathways

The invasive life stages of *Plasmodium* are morphologically shaped and functionally adapted for either migration or invasion, or both (Figure 1). The merozoite is distinctively specialized for invasion and parasitophorous vacuole formation, which are essential for further differentiation of the parasite inside the host erythrocyte. Consistent with such a function, late schizonts transcribe the genes necessary to differentiate into an invasive parasite [15]. In sharp contrast, the ookinete is selectively designed for locomotion. Ookinetes migrate through the midgut epithelium of the mosquito by a process that results in considerable damage to host cells. This damage includes induction of nitric oxide synthase (NOS) expression and remodeling of the actin cytoskeleton, and ultimately leads to apoptosis. Consequently, the ookinete rapidly escapes from the invaded cells without forming a parasitophorous vacuole. Eventually, the damaged host cells are expelled from the epithelium, hence preserving its integrity [16]. After reaching the midgut basal lamina, the ookinete develops into an oocyst. A soluble secreted ookinete adhesive protein (SOAP) stored in the micronemes has recently been shown to play a crucial role in both mosquito midgut invasion and oocyst formation [17].

The sporozoite is undoubtedly the most versatile invasive form of *Plasmodium* and is capable of gliding, migration, host cell invasion and egress. Entry into hepatocytes might occur either by disruption of the plasma membrane and parasite migration through cells, or by vacuole formation followed by parasite division [18]. Recent data suggest that migration through cells has an effect on both sporozoite infectivity (by inducing the exocytosis of sporozoite apical organelles) [19], and on the permissiveness of the surrounding hepatocytes through the secretion of hepatocyte growth factor (HGF), which increases their susceptibility to infection [20]. The recent discovery of a sporozoite microneme protein essential for cell traversal (SPECT) sheds new light on migration into the liver. Interestingly, this novel protein helps sporozoites gain access to hepatocytes by crossing the liver sinusoidal cell at the level of the Kupffer cells [21].

Two recent studies have carefully dissected the motile behavior of the ookinete and sporozoite stages in the living mosquito, taking advantage of the generation of

Table 1. Ultrastructural comparison between apicomplexan species and stages within species[a]

	CD	MN	RH	IMC	SM	Motile	Invasion	Migration	Replication in a PV
Plasmodium SPZ	−	++	+	+	+	+	Active	+	+
Plasmodium MZ	−	+	+	+	2–3	+	Active	−	+
Plasmodium OOK	−	+	−	+	60	+	Active	+	−
Toxoplasma TZ	+	+	+	+	22	+	Active	+	+
Toxoplasma BZ	+	++	+	+	+	+	Active	+	+
Toxoplasma SPZ	+	++	+	+	+	+	Active	+	+
Theileria SPZ	−	−	+	Reduced	−	−	Passive	−	−
Theileria MZ	−	−?	+	Reduced	−	−	Passive	−	−
Theileria kinete stage (tick)	−	+	+	+	+	+	Active	+	−
Cryptosporidium SPZ and MZ	−	+	+	+	+	+	Active	−?	+
Eimeria SPZ and MZ	+	+++	+	+	+	+	Active	+	+

[a]The presence or absence of organelles are indicated by +?or −, respectively. The different numbers of + provide an indication of the relative abundance of organelles. Abbreviations: ?, absence or presence of organelle is not certain; BZ, bradyzoite; CD, conoid; IMC, inner membrane complexes; MN, micronemes; MZ, merozoite; OOK, ookinete; PV, parasitophorous vacuole; RH, rhoptries; SM, subpellicular microtubules; SPZ, sporozoite; TZ, tachyzoite.

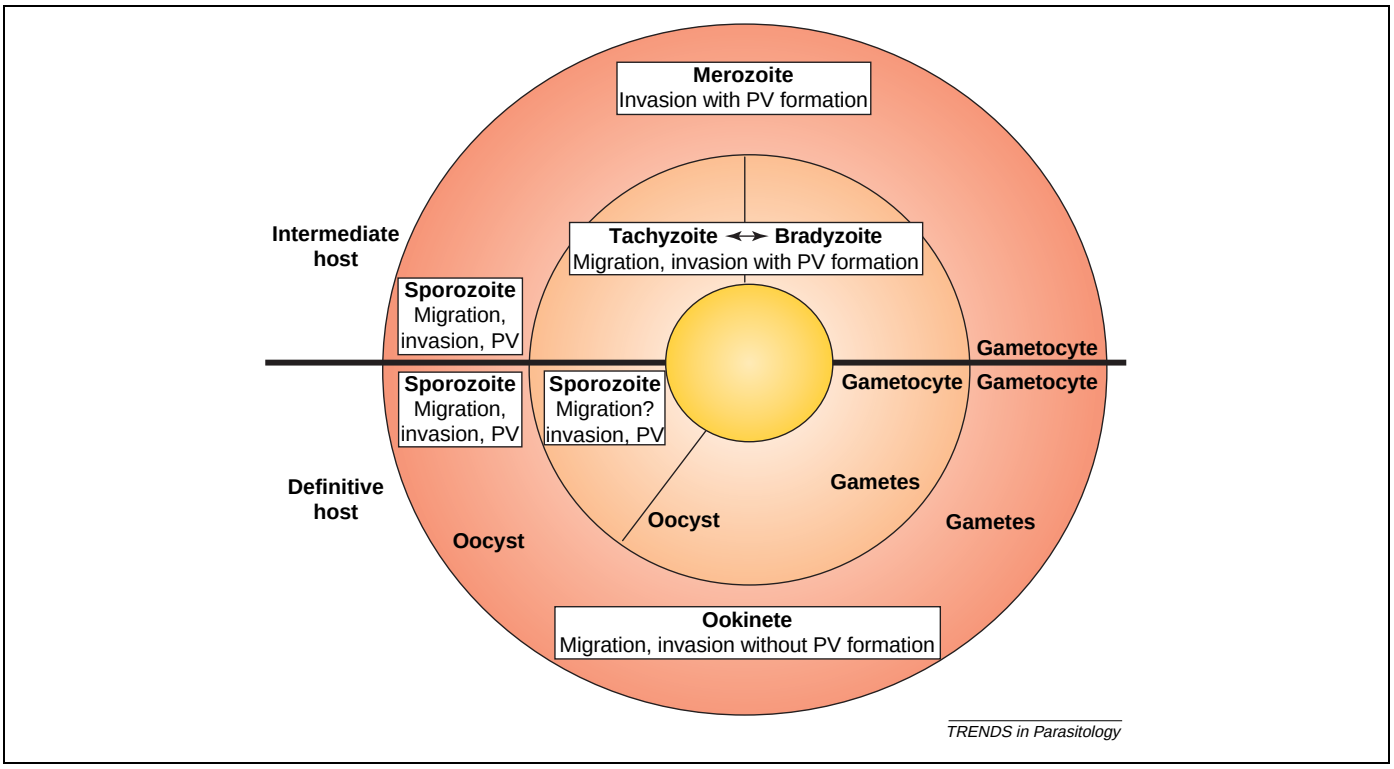

Figure 1. Life cycles of *Plasmodium falciparum* and *Toxoplasma gondii*. Comparison of *Toxoplasma gondii* (orange, inner circle) and *Plasmodium falciparum* (red, outer circle) life cycles in intermediate and definitive hosts. The ability of each invasive form to glide, migrate and invade host cells, with or without the formation of a parasitophorous vacuole (PV), is indicated.

fluorescent *Plasmodium berghei* transgenic lines. The ookinete displays distinct modes of motility described as stationary rotation, translocational spiraling and straight-segment motility [23], which are reminiscent of the three movements previously observed in *T. gondii* tachyzoites [22]. The ookinete also undergoes shape changes and local constrictions during its passage through several midgut epithelial cells [23]. Observations on sporozoite locomotion underscore the necessity of this life stage to glide for host cell invasion to occur, as well as the necessity for their journey inside the salivary cavities and ducts of the mosquito that is ultimately required for transmission [24]. The phenomenon of migration has not been studied in comparable details in any other apicomplexans. In *T. gondii*, only the tachyzoite stage has been studied, and these appear to use a paracellular route for transmigration, avoiding damage to the host cell and the concomitant inflammatory response [25]. Migration across epithelial cells is dependent on parasite motility and is linked to the acute virulence trait of type I parasite strains in mice [26].

The ability of individual invasive stages to migrate and/or invade correlates with the presence and abundance of microneme and rhoptry organelles at the apical end of the parasite, and with the sequential exocytosis of their contents, which contribute to attachment/invasion and parasitophorous vacuole formation, respectively [27]. Consistent with this notion, *T. gondii* tachyzoites and *Plasmodium* sporozoites contain rhoptries and micronemes. By contrast, ookinetes appear to contain only micronemes, whereas merozoites possess fewer micronemes and a pair of rhoptries. The relative expression of secretory proteins based on mass spectrometry analysis in malaria parasites of rodents also fit this pattern of specialized organelle functions in the respective invasive stages (J.D. Raine and R.E. Sinden, pers. commun.).

The molecular machinery powering motility and invasion

Studies using microarrays and proteomics on the infective stages of the malaria parasites have predicted several novel genes involved in motility and invasion. These include proteins involved in signaling cascades, adhesive molecules involved in recognition and attachment to host cells, proteases acting on parasite or host proteins, components of the myosin motor complex and molecules governing actin polymerization/depolymerization. Strikingly, most of the proteins implicated in the gliding machinery (adhesins and components of the motor complex) are extremely conserved throughout the members of the phylum, showing 30–60% amino acid sequence identity. A recent high-throughput screen of small molecules that inhibit host cell invasion by *T. gondii* led to the identification of novel inhibitors that perturb different aspects of invasion, including gliding motility, micronemal secretion and conoid extension. Intriguingly, several of these inhibitors are also effective against *Plasmodium* invasion, illustrating the intimate functional similarities between the processes used by both parasites [28].

Signaling and the role of calcium

Calcium (Ca^{2+}) is used as a major signaling molecule in all eukaryotic cells including protozoan parasites [29]. The

initial steps of host cell entry and egress by *T. gondii* are accompanied by morphological changes and secretion of micronemal proteins that coincide with a sharp rise in cytoplasmic Ca^{2+}. Such Ca^{2+} transients are achieved through the action of several transporters present in the plasma membrane, endoplasmic reticulum, mitochondria and acidocalcisomes [30,31]. Recent findings have established that host cell Ca^{2+} is not essential but that intracellular stores of Ca^{2+} in *T. gondii* are both necessary and sufficient to support microneme secretion and to initiate motility and host cell entry [32]. The intracellular channels that mediate Ca^{2+} release in apicomplexans are not yet characterized; however, ryanodine and caffeine have been demonstrated to stimulate release of Ca^{2+} from parasite intracellular stores. In addition, ethanol, which triggers efficient microneme secretion, causes an increase in parasite inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3], which is probably generated through the hydrolysis of phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] by phospholipase C (PLC) [33]. Collectively, these data suggest that an unknown receptor senses host cell stimuli and activates PLC to produce Ins(1,4,5)P_3, which then operates as a second messenger and mediates intracellular Ca^{2+} release by opening a specific Ca^{2+} channel of the Ins(1,4,5)P_3/ryanodine receptor superfamily. The recent isolation of a *Toxoplasma* mutant that is resistant to ionophore-induced egress, obtained by insertional mutagenesis, has led to the identification of a plasma membrane sodium/hydrogen exchanger, TgNHE1, which appears to play an important role in the regulation of Ca^{2+} homeostasis [34]. In *P. falciparum*, the food vacuole is an important storage compartment for Ca^{2+}, and the use of Ca^{2+} indicator dyes has revealed elevated Ca^{2+} concentrations in the parasitophorous vacuole of infected erythrocytes. The latter source of Ca^{2+} is also likely to contribute to the loading of parasite intracellular Ca^{2+} stores, a prerequisite for the use of a Ca^{2+}-based signaling mechanism [35]. The signaling cascade that operates downstream of Ca^{2+} transients, and which leads to invasion, has not been resolved but probably involves one of the calmodulin-like domain protein kinases (CDPK), previously described in *T. gondii* [36] and *P. falciparum* [37].

F-actin dynamics and myosin motor complex

Actin polymerization is apparently tightly controlled in apicomplexan zoites. Although polymerized actin is crucial for motility and invasion, actin filaments could not be easily visualized in apicomplexans [38]. The class XIV myosin A (MyoA) is restricted to the apicomplexans and has been demonstrated to power gliding motility, invasion and egress [39]. MyoA is associated with a myosin light chain [40] and has recently been shown to be anchored to the inner membrane complex [41], which is associated with the subpellicular microtubules by intramembranous particles [42] (Figure 2). The recent characterization of two novel proteins, the gliding-associated protein (GAP) 45 and GAP50 [43], has provided crucial information regarding the composition and biogenesis of the motor complex, as well as new insights into how movement is generated. Gliding motility requires the coordinated interactions between cell-surface adhesins and the parasite cytoskeleton (Figure 2, movie 2). The identification of aldolase as an actin-binding protein has provided the first link between the actomyosin system and the adhesin molecules of the thrombospondin-related anonymous protein (TRAP) family both in *Toxoplasma* and *Plasmodium* [44,45]. These studies provide a generic model linking adhesion with motility in apicomplexan parasites. At the present time, the pathway controlling the apicomplexan dynamics of F-actin remains unresolved. Nevertheless, several molecules known to regulate actin polymerization in other eukaryotic cells are present in the apicomplexan genomes, and some that are specifically expressed during the invasive stages, such as a profilin-like protein, will be interesting candidates to study in detail.

Reorientation and initiation of a junctional contact

Parasite reorientation on the host cell surface allows the anterior pole of the parasite to be in direct contact with the host plasma membrane and constitutes the first and obligatory step before host cell penetration. In *Plasmodium*, the apical membrane antigen (AMA)-1 is an important target of antibodies that inhibit invasion and is an attractive candidate for a malaria vaccine [46]. AMA-1 has been newly assigned as a micronemal protein and was used to follow microneme biogenesis during merozoite formation. The micronemes emerge from the Golgi, translocate along the subpellicular microtubles and eventually dock with the rhoptry tips [47]. Inhibitory antibodies blocking *Plasmodium knowlesi* AMA-1 strongly suggest that the protein is involved in the reorientation process of merozoites on the erythrocyte surface and also in the initiation of contact at the moving junction [48]. AMA-1 might possibly fulfill the same function in the sporozoites as suggested by a recent report showing that this protein is expressed in this stage, is released by the micronemes and is cleaved during invasion of hepatocytes [49]. Homologs of AMA-1 are expressed and conserved in several other apicomplexans; for instance, this micronemal protein also appears to play an essential role in invasion in *T. gondii* and *P. falciparum* (T. Triglia *et al.*, unpublished). A conditional knockout for the *TgAMA1* gene has been generated and the recombinant tachyzoites are not affected in their ability to glide or to attach to host cells but fail to invade, suggesting that AMA-1 function is conserved across the phylum*.

Alternative pathways for host cell attachment and invasion

Host cell invasion is essential for the survival of obligate intracellular parasites. The development of redundant pathways would assure host cell entry in the face of specific host immune responses and receptor polymorphisms. Indeed, *P. falciparum* merozoites invade erythrocytes through multiple ligand–receptor interactions, with redundancies in each pathway. The merozoite expresses several members of the erythrocyte-binding-like (ebl) family, which includes EBA-175, EBA-140 (also known

* G. Ward *et al.* (2003) Studying the function of *Toxoplasma gondii* apical membrane antigen–1 (TgAMA-1) through generation of a parasite with an inducible TgAMA-1 deficiency. Molecular Parasitology Meeting XIV, held 14–18 September 2003 at Marine Biological Laboratory, Woods Hole, MA, USA.

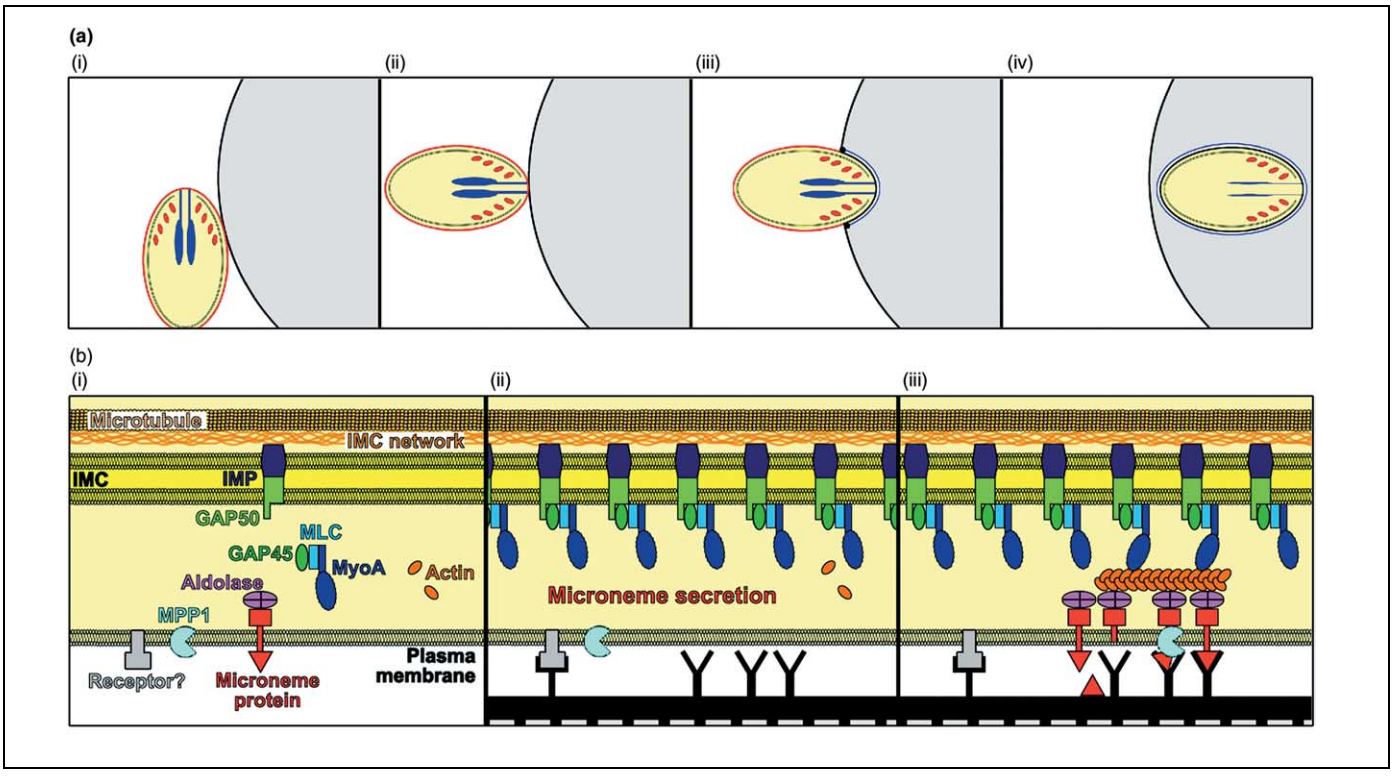

Figure 2. Model of invasion and gliding machinery in *Toxoplasma gondii*. **(a)** Model of host cell invasion by *Toxoplasma gondii*. For the animation available online, go to doi:10.1016/j.pt.2004.09.009. During invasion, microneme (red) and rhoptry (blue) contents are secreted sequentially. Micronemal proteins are discharged onto the parasite surface, redistribute towards the posterior pole and are excluded from the forming vacuole during the invasion process. By contrast, rhoptry proteins accumulate in the forming parasitophorous vacuole membrane. **(b)** Current understanding of the molecular machinery underlying gliding motility, as well as host cell invasion and egress by apicomplexan parasites. For the animation available online, go to doi:10.1016/j.pt.2004.09.009. The apicomplexan pellicle contains three membrane bilayers: the plasma membrane and the flattened sacs of the inner membrane complex (IMC). Half of the molecular motor complex [myosin A (MyoA) and its myosin light chain (MLC)] is anchored by gliding-associated protein (GAP) 45 and GAP50 to the parasite cytoskeleton comprising the inner membrane particles (IMP), the subpellicular filaments (IMC network) subtending the IMC, and the microtubules. The other half of the motor complex (F-actin) is anchored to an extracellular fixture (i.e. the host cell surface or a solid substrate) courtesy of aldolase, which links F-actin and the micronemal adhesive protein TRAP–MIC2. Because the different components of the molecular motor are attached to both the parasite and to an extracellular fixture, the motile force exerted by myosin A (MyoA) on actin results in moving the parasite relative to its extracellular anchor point. The ATP duty cycle of TgMyoA indicates that this motor detaches rapidly from F-actin, suggesting that an array of motors must bind to the same filament to generate movement. Finally, the cleavage of TgMIC2 by microneme protein protease 1 (MPP1) is necessary for successful invasion and contributes to trail formation when the parasite glides on solid substrates.

as Baebl) and EBA-181 (also known as Jesbl). These proteins bind to specific glycoproteins on the surface of the erythrocyte and play a role in the invasion process. Although the exact molecular details are unknown, EBA-175 binds to glycophorin A and this ligand–receptor interaction appears to be important in many strains of *P. falciparum*, as shown by the finding that specific antibodies can inhibit the invasion process [50]. Despite this, it is clear that EBA-175 is not absolutely essential for merozoite invasion because recombinant parasites lacking expression of this ligand can invade and grow normally [51]. Indeed, some parasites are capable of switching reliance to other ligand–receptor interactions for merozoite invasion to compensate the loss of EBA-175 function.

EBA-140 specifically binds to glycophorin C on red blood cells and this interaction contributes to invasion as antibodies to the ligand can partially inhibit the process [52,53]. Glycophorin C is responsible for the Gerbich (Ge) blood group system. Deletion of exon 3 in the glycophorin C gene has been observed in Melanesians and this alteration changes the serological phenotype, resulting in Ge negativity. EBA-140 does not bind to the altered form of glycophorin C in Ge-negative erythrocytes, nor can *P. falciparum* invade these cells using this invasion pathway. This suggests that Ge negativity has arisen in 50% of the Melanesian population through natural selection by severe malaria [52]. EBA-181 binds to a neuraminidase-sensitive and trypsin-resistant receptor [54] and might play a role in the glycophorin B-independent pathway described recently [55]. This paralog of EBA-175 also appears to be essential in some parasite lines, whereas in others its function is redundant [54]. Indeed, the ability to disrupt EBA-181, EBA-175 and EBA-140 genetically in some *P. falciparum* parasite lines but not others suggests that a minimal number of ligands is required for functional merozoite invasion, most likely to provide sufficient affinity for binding of the parasite to the host cell.

A second family of proteins in *P. falciparum* also appears to play a key role in merozoite invasion [56,57]. The PfRh (*P. falciparum* reticulocyte-binding-like proteins) family of proteins is homologous to Py235 and PvRBP (*P. vivax* reticulocyte-binding proteins) in *P. yoelii* and *P. vivax*, respectively. In *P. vivax*, two proteins have been identified (PvRBP1 and 2) that bind to reticulocytes; these proteins have been hypothesized to play a role in the preference of *P. vivax* for invasion of these specific host cells [58]. PvRBP1 and 2 are located at the apical end of

the merozoite in *P. vivax* and appear to play a key role in binding host cell receptors after reorientation. It has been hypothesized that this protein family in the different *Plasmodium* species is required for sensing of the host erythrocyte, activation of the invasion process and development of the tight junction [59]. The PfRh proteins are differentially expressed and this can, to some extent, explain the different patterns of receptor utilization that have been defined as invasion pathways. Disruption of the different *PfRh* genes has provided evidence that this protein family plays a key role in merozoite invasion ([57]; Triglia *et al.*, unpublished).

TRAP

Distinct members of the TRAP family are expressed in each invasive stage of *Plasmodium*. These type 1 transmembrane proteins are stored in the micronemes and are released onto the parasite surface during invasion, where they associate with host cell receptors to facilitate parasite motility and invasion (Figure 2, movie 2). TRAP, circumsporozoite protein (CS), and circumsporozoite- and TRAP-related protein (CTRP) have previously been shown to be key players in host cell invasion by both sporozoites (TRAP and CS) and ookinetes (CTRP). More recently, PTRAMP has been identified as a conserved *Plasmodium* thrombospondin-related apical merozoite protein [60]. In *T. gondii*, the TRAP homolog TgMIC2 forms a macromolecular hexamer complex with MIC2-associated protein (M2AP) [61]. Disruption of the gene encoding TgM2AP provided indirect evidence that TgMIC2 is crucial for host cell invasion [62]. The short cytoplasmic domains of TgMIC2 and TRAP are implicated in protein trafficking using a tyrosine-based motif [63,64]. The extreme C-terminus, which includes a conserved tryptophan residue, is involved in the interaction with aldolase, which exhibits a dual function as a glycolytic enzyme and an F-actin-binding protein [44,45].

Microneme protein proteases

The proteolytic cleavage of microneme proteins including the members of the TRAP family and AMA-1 is a key event for successful host cell invasion. In *T. gondii*, the processing of TgMIC2 in particular has been extensively characterized [65]. The microneme protein protease 1 (MPP1) cleaves TgMIC2 and other *T. gondii* MICs in the transmembrane domain [66], causing their release from the parasite surface, which is an event required for host cell invasion [67]. A recent report suggests that Rhomboid-like intramembrane proteases could be involved in this processing [68] and Rhomboid–like proteases are present in the, as-yet incomplete, repertoire of *Plasmodium* proteases revealed by database mining of the genome [69]. Five rhomboid-like proteases have been characterized in *T. gondii* as plausible candidates for MPP1 activity [70]. Currently, several parasite proteases are under scrutiny for their biological role and possible contribution in invasion [71].

Host range and cell type specificity

The molecular mechanism underlying host range and cell-type specificity is poorly understood and probably involves the intricate contribution of many different factors. The analysis of the binding affinity for the liver cell receptor of several circumsporozoite proteins originating from diverse *Plasmodium* species suggests that adhesive proteins contribute in maintaining the host specificity by the malaria parasite [72]. The *T. gondii* tachyzoite is the most ubiquitous apicomplexan parasite, and the fact that it expresses a large repertoire of microneme proteins composed of diverse combinations of adhesive modules possibly contributes to the broad range of host cell types and at the same time provides plenty of alternative pathways for invasion.

Perspective

The molecular machinery of apicomplexan parasites responsible for motility and host cell invasion comprises intracellular components as well as parts that are exposed to the extracellular milieu: the former includes the cytoskeleton, the actin–myosin complex and various interconnecting molecules, whereas a whole range of micronemal proteins and ligands with adhesive properties fall into the latter category. From the point of view of the parasite, the extracellular environment is a dangerous place. The vertebrate immune system and the innate immune defense of invertebrate hosts pose a constant threat, creating a selective pressure that has led to the expression of diverse ligands and surface molecules both in the various apicomplexan parasites and in various life stages within a species. *Plasmodium* differentially utilizes a whole array of ligands, providing the parasite with the flexibility to survive host immune responses and the polymorphic nature of the erythrocyte surface in the host populations. At the same time, the inter-species diversity of these molecules probably accounts for the vast differences in host range and host tissue specificities of apicomplexan parasites. By contrast, because of the lack of similarly strong selective pressures, purely intracellular components of the molecular invasion machinery are much more conserved throughout the Apicomplexa than are the extracellular molecules. Therefore, information garnered from more-tractable organisms such as *Toxoplasma* is providing important knowledge for many other parasites. Furthermore, one might expect these intracellular components to be effective targets for clinical intervention. The fast-evolving and diverse surface molecules of apicomplexan parasites have been optimized during evolution to be able to evade the host defense responses and have so far thwarted decades worth of efforts into vaccine development; by contrast, the much more conserved intracellular invasion machinery might prove to be a more vulnerable 'soft spot' of these parasites.

Acknowledgements

We apologize for not including many references from the work of colleagues, which was unavoidable due to the space limits placed on us. We thank R.E. Sinden and J.D. Raine for sharing with us unpublished data, and B. Shiels, J-F. Dubremetz and D. Ferguson for helpful discussions.

References

1 Su, C. *et al*. (2003) Recent expansion of *Toxoplasma* through enhanced oral transmission. *Science* 299, 414–416
2 Soldati, D. and Meissner, M. (2004) *Toxoplasma* as a novel system for motility. *Curr. Opin. Cell Biol.* 16, 32–40
3 Hu, K. *et al*. (2002) A novel polymer of tubulin forms the conoid of *Toxoplasma gondii*. *J. Cell Biol.* 156, 1039–1050
4 Shaw, M.K. (2003) Cell invasion by *Theileria* sporozoites. *Trends Parasitol.* 19, 2–6
5 Mordue, D.G. *et al*. (1999) Invasion by *Toxoplasma gondii* establishes a moving junction that selectively excludes host cell plasma membrane proteins on the basis of their membrane anchoring. *J. Exp. Med.* 190, 1783–1792
6 Sam-Yellowe, T.Y. *et al*. (2004) A *Plasmodium* gene family encoding Maurer's cleft membrane proteins: structural properties and expression profiling. *Genome Res.* 14, 1052–1059
7 Wickham, M.E. *et al*. (2001) Trafficking and assembly of the cytoadherence complex in *Plasmodium falciparum*-infected human erythrocytes. *EMBO J.* 20, 5636–5649
8 Chen, X.M. *et al*. (2004) Cdc42 and the actin-related protein/neural Wiskott-Aldrich syndrome protein network mediate cellular invasion by *Cryptosporidium parvum*. *Infect. Immun.* 72, 3011–3021
9 Chen, X.M. *et al*. (2004) Phosphatidylinositol 3-kinase and Frabin mediate *Cryptosporidium parvum* cellular invasion via activation of Cdc42. *J. Biol. Chem.* 279, 31671–31678
10 Hoff, E.F. and Carruthers, V.B. (2002) Is *Toxoplasma* egress the first step in invasion? *Trends Parasitol.* 18, 251–255
11 Wickham, M.E. *et al*. (2003) Selective inhibition of a two-step egress of malaria parasites from the host erythrocyte. *J. Biol. Chem.* 278, 37658–37663
12 Dhawan, S. *et al*. (2003) Ankyrin peptide blocks falcipain-2-mediated malaria parasite release from red blood cells. *J. Biol. Chem.* 278, 30180–30186
13 Sijwali, P.S. and Rosenthal, P.J. (2004) Gene disruption confirms a critical role for the cysteine protease falcipain-2 in hemoglobin hydrolysis by *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. U. S. A.* 101, 4384–4389
14 Moudy, R. *et al*. (2001) The loss of cytoplasmic potassium upon host cell breakdown triggers egress of *Toxoplasma gondii*. *J. Biol. Chem.* 276, 41492–41501
15 Bozdech, Z. *et al*. (2003) The transcriptome of the intraerythrocytic developmental cycle of *Plasmodium falciparum*. *PLoS Biol.* 1, E5
16 Han, Y.S. and Barillas-Mury, C. (2002) Implications of time bomb model of ookinete invasion of midgut cells. *Insect Biochem. Mol. Biol.* 32, 1311–1316
17 Dessens, J.T. *et al*. (2003) SOAP, a novel malaria ookinete protein involved in mosquito midgut invasion and oocyst development. *Mol. Microbiol.* 49, 319–329
18 Kappe, S.H. *et al*. (2003) The *Plasmodium* sporozoite journey: a rite of passage. *Trends Parasitol.* 19, 135–143
19 Mota, M.M. *et al*. (2002) Migration through host cells activates *Plasmodium* sporozoites for infection. *Nat. Med.* 8, 1318–1322
20 Carrolo, M. *et al*. (2003) Hepatocyte growth factor and its receptor are required for malaria infection. *Nat. Med.* 9, 1363–1369
21 Ishino, T. *et al*. (2004) Cell-passage activity is required for the malarial parasite to cross the liver sinusoidal cell layer. *PLoS Biol.* 2, E4
22 Hakansson, S. *et al*. (1999) Time-lapse video microscopy of gliding motility in *Toxoplasma gondii* reveals a novel, biphasic mechanism of cell locomotion. *Mol. Biol. Cell* 10, 3539–3547
23 Vlachou, D. *et al*. (2004) Real-time, *in vivo* analysis of malaria ookinete locomotion and mosquito midgut invasion. *Cell. Microbiol.* 6, 671–685
24 Frischknecht, F. *et al*. (2004) Imaging movement of malaria parasites during transmission by *Anopheles* mosquitoes. *Cell. Microbiol.* 6, 687–694
25 Barragan, A. and Sibley, L.D. (2003) Migration of *Toxoplasma gondii* across biological barriers. *Trends Microbiol.* 11, 426–430
26 Barragan, A. and Sibley, L.D. (2002) Transepithelial migration of *Toxoplasma gondii* is linked to parasite motility and virulence. *J. Exp. Med.* 195, 1625–1633
27 Carruthers, V.B. and Sibley, L.D. (1997) Sequential protein secretion from three distinct organelles of *Toxoplasma gondii* accompanies invasion of human fibroblasts. *Eur. J. Cell Biol.* 73, 114–123
28 Carey, K.L. *et al*. (2004) A small-molecule approach to studying invasive mechanisms of *Toxoplasma gondii*. *Proc. Natl. Acad. Sci. U. S. A.* 101, 7433–7438
29 Moreno, S.N. and Docampo, R. (2003) Calcium regulation in protozoan parasites. *Curr. Opin. Microbiol.* 6, 359–364
30 Moreno, S.N. and Zhong, L. (1996) Acidocalcisomes in *Toxoplasma gondii* tachyzoites. *Biochem. J.* 313, 655–659
31 Marchesini, N. *et al*. (2000) Acidocalcisomes and a vacuolar H^+-pyrophosphatase in malaria parasites. *Biochem. J.* 347, 243–253
32 Lovett, J.L. and Sibley, L.D. (2003) Intracellular calcium stores in *Toxoplasma gondii* govern invasion of host cells. *J. Cell Sci.* 116, 3009–3016
33 Lovett, J.L. *et al*. (2002) *Toxoplasma gondii* microneme secretion involves intracellular Ca(2+) release from inositol 1,4,5-triphosphate (IP_3)/ryanodine-sensitive stores. *J. Biol. Chem.* 277, 25870–25876
34 Arrizabalaga, G. and Boothroyd, J.C. (2004) Role of calcium during *Toxoplasma gondii* invasion and egress. *Int. J. Parasitol.* 34, 361–368
35 Gazarini, M.L. *et al*. (2003) Calcium signaling in a low calcium environment: how the intracellular malaria parasite solves the problem. *J. Cell Biol.* 161, 103–110
36 Kieschnick, H. *et al*. (2001) *Toxoplasma gondii* attachment to host cells is regulated by a calmodulin-like domain protein kinase. *J. Biol. Chem.* 276, 12369–12377
37 Li, J.L. *et al*. (2000) Sexual stage-specific expression of a third calcium-dependent protein kinase from *Plasmodium falciparum*. *Biochim. Biophys. Acta* 1491, 341–349
38 Fowler, R.E. *et al*. (2004) The cytoskeleton and motility in apicomplexan invasion. *Adv. Parasitol.* 56, 213–263
39 Meissner, M. *et al*. (2002) Role of *Toxoplasma gondii* myosin A in powering parasite gliding and host cell invasion. *Science* 298, 837–840
40 Herm-Gotz, A. *et al*. (2002) *Toxoplasma gondii* myosin A and its light chain: a fast, single-headed, plus-end-directed motor. *EMBO J.* 21, 2149–2158
41 Bergman, L.W. *et al*. (2003) Myosin A tail domain interacting protein (MTIP) localizes to the inner membrane complex of *Plasmodium* sporozoites. *J. Cell Sci.* 116, 39–49
42 Morrissette, N.S. and Sibley, L.D. (2002) Cytoskeleton of apicomplexan parasites. *Microbiol. Mol. Biol. Rev.* 66, 21–38
43 Gaskins, E. *et al*. (2004) Identification of the membrane receptor of a class XIV myosin in *Toxoplasma gondii*. *J. Cell Biol.* 165, 383–393
44 Jewett, T.J. and Sibley, L.D. (2003) Aldolase forms a bridge between cell surface adhesins and the actin cytoskeleton in apicomplexan parasites. *Mol. Cell* 11, 885–894
45 Buscaglia, C.A. *et al*. (2003) Sites of interaction between aldolase and thrombospondin-related anonymous protein in *Plasmodium*. *Mol. Biol. Cell* 14, 4947–4957
46 Healer, J. *et al*. (2004) Allelic polymorphisms in apical membrane antigen-1 are responsible for evasion of antibody-mediated inhibition in *Plasmodium falciparum*. *Mol. Microbiol.* 52, 159–168
47 Bannister, L.H. *et al*. (2003) *Plasmodium falciparum* apical membrane antigen 1 (PfAMA-1) is translocated within micronemes along subpellicular microtubules during merozoite development. *J. Cell Sci.* 116, 3825–3834
48 Mitchell, G.H. *et al*. (2004) Apical membrane antigen 1, a major malaria vaccine candidate, mediates the close attachment of invasive merozoites to host red blood cells. *Infect. Immun.* 72, 154–158
49 Silvie, O. *et al*. (2004) A role for apical membrane antigen 1 during invasion of hepatocytes by *Plasmodium falciparum* sporozoites. *J. Biol. Chem.* 279, 9490–9496
50 Pandey, K.C. *et al*. (2002) Bacterially expressed and refolded receptor binding domain of *Plasmodium falciparum* EBA-175 elicits invasion inhibitory antibodies. *Mol. Biochem. Parasitol.* 123, 23–33
51 Duraisingh, M.T. *et al*. (2003) Erythrocyte-binding antigen 175 mediates invasion in *Plasmodium falciparum* utilizing sialic acid-dependent and -independent pathways. *Proc. Natl. Acad. Sci. U. S. A.* 100, 4796–4801
52 Maier, A.G. *et al*. (2003) *Plasmodium falciparum* erythrocyte invasion through glycophorin C and selection for Gerbich negativity in human populations. *Nat. Med.* 9, 87–92
53 Mayer, D.C. *et al*. (2002) Polymorphism in a *Plasmodium falciparum* erythrocyte-binding ligand changes its receptor specificity. *J. Exp. Med.* 196, 1523–1528

54 Gilberger, T.W. *et al*. (2003) A novel erythrocyte binding antigen-175 paralogue from *Plasmodium falciparum* defines a new trypsin-resistant receptor on human erythrocytes. *J. Biol. Chem.* 278, 14480–14486
55 Gaur, D. *et al*. (2003) *Plasmodium falciparum* is able to invade erythrocytes through a trypsin-resistant pathway independent of glycophorin B. *Infect. Immun.* 71, 6742–6746
56 Rayner, J.C. *et al*. (2001) A *Plasmodium falciparum* homologue of *Plasmodium vivax* reticulocyte binding protein (PvRBP1) defines a trypsin-resistant erythrocyte invasion pathway. *J. Exp. Med.* 194, 1571–1581
57 Duraisingh, M.T. *et al*. (2003) Phenotypic variation of *Plasmodium falciparum* merozoite proteins directs receptor targeting for invasion of human erythrocytes. *EMBO J.* 22, 1047–1057
58 Galinski, M.R. *et al*. (1992) A reticulocyte-binding protein complex of *Plasmodium vivax* merozoites. *Cell* 69, 1213–1226
59 Duraisingh, M.T. *et al*. (2002) Negative selection of *Plasmodium falciparum* reveals targeted gene deletion by double crossover recombination. *Int. J. Parasitol.* 32, 81–89
60 Thompson, J. *et al*. (2004) PTRAMP; a conserved *Plasmodium* thrombospondin-related apical merozoite protein. *Mol. Biochem. Parasitol.* 134, 225–232
61 Jewett, T.J. and Sibley, L.D. (2004) The toxoplasma proteins MIC2 and M2AP form a hexameric complex necessary for intracellular survival. *J. Biol. Chem.* 279, 9362–9369
62 Huynh, M.H. *et al*. (2003) Rapid invasion of host cells by *Toxoplasma* requires secretion of the MIC2–M2AP adhesive protein complex. *EMBO J.* 22, 2082–2090
63 Di Cristina, M. *et al*. (2000) Two conserved amino acid motifs mediate protein targeting to the micronemes of the apicomplexan parasite *Toxoplasma gondii*. *Mol. Cell. Biol.* 20, 7332–7341
64 Bhanot, P. *et al*. (2003) Defective sorting of the thrombospondin-related anonymous protein (TRAP) inhibits *Plasmodium* infectivity. *Mol. Biochem. Parasitol.* 126, 263–273
65 Zhou, X.W. *et al*. (2004) Proteomic analysis of cleavage events reveals a dynamic two-step mechanism for proteolysis of a key parasite adhesive complex. *Mol. Cell. Proteomics* 3, 565–576
66 Opitz, C. *et al*. (2002) Intramembrane cleavage of microneme proteins at the surface of the apicomplexan parasite *Toxoplasma gondii*. *EMBO J.* 21, 1577–1585
67 Brossier, F. *et al*. (2003) C-terminal processing of the *Toxoplasma* protein MIC2 is essential for invasion into host cells. *J. Biol. Chem.* 278, 6229–6234
68 Urban, S. and Freeman, M. (2003) Substrate specificity of rhomboid intramembrane proteases is governed by helix-breaking residues in the substrate transmembrane domain. *Mol. Cell* 11, 1425–1434
69 Wu, Y. *et al*. (2003) Data-mining approaches reveal hidden families of proteases in the genome of malaria parasite. *Genome Res.* 13, 601–616
70 Dowse, T. and Soldati, D. (2004) Host cell invasion by the apicomplexans: the significance of microneme protein proteolysis. *Curr. Opin. Microbiol.* 7, 388–396
71 Kim, K. (2004) Role of proteases in host cell invasion by *Toxoplasma gondii* and other Apicomplexa. *Acta Trop.* 91, 69–81
72 Rathore, D. *et al*. (2003) Molecular mechanism of host specificity in *Plasmodium falciparum* infection: role of circumsporozoite protein. *J. Biol. Chem.* 278, 40905–40910