Host cell invasion by the apicomplexans: the significance of microneme protein proteolysis

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Abstract

Intracellular life-style has been adopted by many pathogens as a successful immune evasion mechanism. To gain entry to a large variety of host cells and to establish an intracellular niche, Toxoplasma gondii and other apicomplexans rely on an active process distinct from phagocytosis. Calcium-regulated secretion of microneme proteins and parasite actin polymerization together with the action of at least one myosin motor act in concert to generate the gliding motility necessary to propel the parasite into host cells. During this active penetration, host cell transmembrane proteins are excluded from the forming parasitophorous vacuole hence conferring the resistance to acidification and degradative fusion. Apicomplexans possess a large repertoire of microneme proteins that contribute to invasion, but their precise role and the level of functional redundancy remain to be evaluated. Remarkably, most microneme proteins are proteolytically cleaved during biogenesis and post-exocytosis. The significance of the processing events and the identification of the proteases implicated are the object of intensive investigations. These [...]
Host cell invasion by the apicomplexans: the significance of microneme protein proteolysis

Timothy Dowse¹ and Dominique Soldati¹,²*¹

Intracellular life-style has been adopted by many pathogens as a successful immune evasion mechanism. To gain entry to a large variety of host cells and to establish an intracellular niche, Toxoplasma gondii and other apicomplexans rely on an active process distinct from phagocytosis. Calcium-regulated secretion of microneme proteins and parasite actin polymerization together with the action of at least one myosin motor act in concert to generate the gliding motility necessary to propel the parasite into host cells. During this active penetration, host cell transmembrane proteins are excluded from the forming parasitophorous vacuole hence conferring the resistance to acidification and degradative fusion. Apicomplexans possess a large repertoire of microneme proteins that contribute to invasion, but their precise role and the level of functional redundancy remain to be evaluated. Remarkably, most microneme proteins are proteolytically cleaved during biogenesis and post-exocytosis. The significance of the processing events and the identification of the proteases implicated are the object of intensive investigations. These proteases may constitute potential drug targets for intervention against malaria and other diseases caused by these parasites.

Introduction

Obligate intracellular parasites belonging to the phylum Apicomplexa are important human and veterinary pathogens, responsible for a wide variety of diseases. The most important human disease is malaria, which is caused by the Plasmodium species. Toxoplasma gondii, the etiological agent of toxoplasmosis, can be fatal in immuno-compromised patients, and can cause severe birth defects if a pregnant woman develops a primary infection. T. gondii is easily genetically tractable and has been exploited as a good model for certain aspects of apicomplexan biology, in particular for the study of motility and invasion.

Host cell invasion is a prerequisite for the establishment and maintenance of infection for all apicomplexan parasites. Although the range of host cell specificity can vary greatly between different apicomplexan species, the basic mechanisms and the machinery employed by these parasites to invade their particular host are strikingly conserved. Unlike many other intracellular pathogens, which take advantage of the phagocytic activities of the host cell, most apicomplexans invade by an active process requiring energy from the parasite and its actomyosin system [1,2,3*]. Establishment of infection is critically dependent on the sequential secretion of organelles called micronemes and rhoptries [4]. The model for host cell invasion, which has been recently reviewed [5,6,7*] suggests that, following parasite attachment to a host cell and its reorientation, a moving junction is formed upon discharge by the micronemes. This junction between the parasite and host cell moves towards the posterior pole of the parasite, pulling the host cell membrane with it. The substrate-dependent movement is driven by the translocation of microneme proteins attached to host cell receptors to the posterior pole by the actomyosin system anchored into the inner membrane complex [8]. Toxoplasma and Plasmodium aldolases have recently been identified as the first molecular link between the parasite actin cytoskeleton and the cytoplasmic tails of the transmembrane microneme proteins [9**,10] (Figure 1).

Following microneme secretion, the contents of the rhoptries are released and contribute to formation of the parasitophorous vacuole [11].

The majority of microneme proteins are subject to proteolytic cleavage, either during their transport to the micronemes, subsequent to their secretion, or both. Although it is becoming increasingly clear that proteolytic processing of microneme proteins is widespread and crucially important in the functioning of these proteins,
no gene has yet been identified that encodes for a microneme protein protease.

Here, we review the processing of *T. gondii* microneme proteins and discuss the candidate proteases responsible for these activities.

**Repertoire of microneme proteins**

The sequencing of several apicomplexan genomes has revealed the presence of multiple genomic DNA sequences showing high degrees of similarity to previously known apicomplexan microneme proteins. This considerable source of new information is currently under scrutiny for many parasites, and upon experimental confirmation the newly identified gene products will significantly augment the repertoire of microneme proteins previously described [12]. The recent survey of the *Cryptosporidium parvum* genome is an illustrative example, which led to the identification of several putative microneme proteins exhibiting diverse combinations of adhesive motifs [13].

Detailed studies on microneme content processing and release have been best conducted in *T. gondii* (Table 1). Microneme proteins are produced as complexes, which are released by the organelle upon a rise in intracellular calcium [14] and involving the action of multiple protein kinases [15,16**].

Three distinct microneme protein complexes have been identified to date, TgMIC1/MIC4/MIC6, TgMIC3/MIC8 and TgMIC2/M2AP, which are assembled in the endoplasmic reticulum, stored in the micronemes and ultimately secreted [17–19] (Figure 2). These complexes comprise a transmembrane escorter protein (TgMIC6, TgMIC8, TgMIC2) containing sorting signals in the cytoplasmic tail essential for the accurate targeting of the complex to the micronemes [20] and soluble proteins exhibiting host cell binding properties (TgMIC1, TgMIC4 and TgMIC3) [21–24,25**] reviewed in [26,27]. Assembly of microneme proteins into functional complexes is critical for invasion and appears to be a conserved feature among at least some of the apicomplexans [28**,29].

**Proteolytic processing of microneme proteins**

All microneme proteins described to date contain a signal peptide and traffic to their resident organelle via the Golgi apparatus. In addition to signal peptide cleavage occurring during translocation into the endoplasmic reticulum, most microneme proteins are also subject to further proteolytic modifications. A summary of the proteolytic cleavages occurring on the components of the three known microneme protein complexes is depicted in Figure 2. The best studied, TgMIC2, is processed post-exocytosis by two distinct protease activities called microneme protein protease 1 and 2 (MPP1 and MPP2) [30] (Figure 2). MPP1 activity results in the release of the soluble extracellular domain of TgMIC2 into the extracellular milieu, and the concomitant release of its accessory protein TgM2AP. This event is essential for host cell invasion [31**]. The MPP1 cleavage site has been mapped on TgMIC6 and TgMIC2 within their single transmembrane (TM) domain at the site IA*GG (where * represents the cleavage site) [32**,33**]. A detailed mutational analysis of TgMIC2 revealed that cleavage by MPP1 is also dependent on a lysine located 11 residues upstream of the TM domain of TgMIC2 [31**]. TgMIC12, a very
large protein incompletely characterized in *T. gondii* and homologous to EmTFP250 and EtMIC4 described in *Eimeria* species [34,35], is also cleaved by MPP1 [32**]. The repertoire of substrates for this protease may include most if not all the transmembrane microneme proteins expressed in tachyzoites and notably TgMIC8 and TgAMA1 [19,36]. In addition, several microneme proteins in *Plasmodium* and other apicomplexan species exhibit the same conserved cleavage site in their membrane-spanning domain. PbTRAP (thrombospondin-related anonymous protein), the homolog of TgMIC2 in *Plasmodium berghei* is processed when expressed in *T. gondii* and hence TRAP is a possible substrate for MPP1. It remains to be demonstrated if such an intramembrane processing occurs in the rodent malaria parasite [32**]. MPP1 cleavage of TgMIC2 results in a reduction in the host cell binding capacity of TgMIC2 [37].

In a previous study, inhibitors were used in an attempt to identify the type of protease activity of MPP1 [30]. The irreversible serine protease inhibitor, 3,4-dichloroisocoumarin (DCI) inhibited the release of TgMIC2 into the supernatant, but the concomitant inhibition of TgMIC4 release was also observed and interpreted at that time as an impairment of microneme secretion. The fact that the soluble TgMIC4 is associated with TgMIC1/TgMIC6 implies that its release is also dependent on MPP1 activity. DCI has previously been reported to inhibit host cell invasion [38] and to affect parasite growth and replication in *T. gondii* [39]. The assignment of MPP1 activity to a serine protease sensitive to DCI remains open.

MPP2 processing results in trimming of the amino-terminal domain of TgMIC2 at multiple sites up to the beginning of the A-domain, and this activity does not seem to be required for MPP1 cleavage to occur [30]. MPP2 activity is also responsible for multiple cleavages of TgM2AP near its carboxy-terminal domain. A refined and exquisite analysis has revealed that TgMIC2/M2AP is subjected to a two-step proteolysis involving MPP2 and the carboxy-terminal cleavage of TgM2AP by a newly identified MPP3 in a primary processing, followed by secondary cleavage by MPP1 to shed the complex. It is not yet clear what the functions of TgM2AP processing are, or if MPP3 activity is a prerequisite for MPP2 cleavage. MPP2 is blocked by a subset of serine and cysteine protease inhibitors and is also likely to cleave the microneme subtilisin-like protease TgSUB1 [35**] and TgMIC4 within its fifth apple domain [23]. TgMIC4, as well as being cleaved by MPP2, is also processed at the parasite surface near its amino terminus by an unknown protease [23] (Figure 2).

Several microneme proteins are produced as proproteins that undergo proteolytic processing during their traffic

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**Table 1**

*T. gondii* microneme proteins currently characterized: their properties and functions.

<table>
<thead>
<tr>
<th>Name</th>
<th>Domains/homologies</th>
<th>Complexes</th>
<th>Proteolytic processing</th>
<th>Homologues</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>TgMIC1</td>
<td>2 TSP-like</td>
<td>MIC4–MIC1–MIC6</td>
<td>N-term, C-term post-exocytosis</td>
<td>EtMIC3</td>
<td>Transport/folding of MIC4, MIC6</td>
</tr>
<tr>
<td>TgMIC2</td>
<td>1 Integrin, 5 TSRs</td>
<td>M2AP (hexameric)</td>
<td>N-term, C-term post-exocytosis</td>
<td>EtMIC1, NcMIC2</td>
<td>Transport M2AP</td>
</tr>
<tr>
<td>TgMIC3</td>
<td>1 Lectin-like, 5 EGF-like</td>
<td>MIC3–MIC8, dimerisation of MIC3</td>
<td>N-term intracellular</td>
<td>Plasmodium TRAP</td>
<td>Binding to host cells</td>
</tr>
<tr>
<td>TgMIC4</td>
<td>6 Apples</td>
<td>MIC4–MIC1–MIC6</td>
<td>N-term, C-term post-exocytosis</td>
<td>EtMIC5</td>
<td>Binding to host cells</td>
</tr>
<tr>
<td>TgMIC5</td>
<td>PPLase</td>
<td>MIC4–MIC1–MIC6</td>
<td>N-term intracellular</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>TgMIC6</td>
<td>3 EGF-like, TM, C-tail</td>
<td>MIC4–MIC1–MIC6</td>
<td>N-term intracellular</td>
<td>?</td>
<td>Escorter of MIC1 and MIC4</td>
</tr>
<tr>
<td>TgMIC7</td>
<td>5 EGF-like, TM, C-tail</td>
<td>MIC3–MIC8</td>
<td>C-term post-exocytosis</td>
<td>?</td>
<td>Escorter of MIC3</td>
</tr>
<tr>
<td>TgMIC8</td>
<td>1 Lectin-like, 10 EGF-like, TM, C-tail</td>
<td>MIC3–MIC8</td>
<td>C-term post-exocytosis</td>
<td>?</td>
<td>Escorter of MIC3</td>
</tr>
<tr>
<td>TgMIC9</td>
<td>3 EGF-like, TM, C-tail</td>
<td>?</td>
<td>C-term post-exocytosis</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>TgMIC10</td>
<td>-</td>
<td>?</td>
<td>-</td>
<td>NcMIC10, SnMIC10</td>
<td>?</td>
</tr>
<tr>
<td>TgM2AP</td>
<td>-</td>
<td>?</td>
<td>-</td>
<td>NcMIC11</td>
<td>?</td>
</tr>
<tr>
<td>TgSUB1</td>
<td>Subtilase, GPI?</td>
<td>?</td>
<td>C-term post-exocytosis</td>
<td>EmMIC4</td>
<td>?</td>
</tr>
<tr>
<td>TgAMA1</td>
<td>Cysteine rich</td>
<td>?</td>
<td>N-term intracellular and post-exocytosis</td>
<td>EtMIC2, NcM2AP</td>
<td>Transport/folding of MIC2</td>
</tr>
<tr>
<td>TgM2AP</td>
<td>-</td>
<td>?</td>
<td>N-term intracellular and post-exocytosis</td>
<td>Pf AMA1, EtAMA1</td>
<td>Host cell invasion</td>
</tr>
</tbody>
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**Current Opinion in Microbiology 2004, 7:388–396 www.sciencedirect.com**
along the secretory pathway. The cleavage of TgMIC6 occurs in the trans-Golgi network resulting in the removal of the first epidermal growth factor (EGF) domain, at the site VQLS*ETP [19]. The significance of this processing is unclear as deletion of the first EGF-like domain does not appear to affect the targeting or the ability of TgMIC6 to associate with TgMIC1, and TgMIC6 does not exhibit any detectable cell adhesive property [17]. TgMIC3 is a host cell binding protein composed of a lectin-like domain and five overlapping EGF-like domains. The protein forms dimers that are synthesized as 40 kDa precursors and proteolytically cleaved to 38 kDa products before reaching the micronemes. Removal of the propeptide of TgMIC3 is required for host cell binding activity [25]. TgMIC8 functions as an escorter for TgMIC3 and also contains a lectin-like domain, ten EGF-like domains, a transmembrane spanning domain and a short cytoplasmic tail. The protein is cleaved on secretion, most probably by MPP1, resulting in the release of a 65 kDa product [19]. However, it is not known if an additional processing similar to TgMIC3 occurs at the amino terminus (Figure 2).

TgMIC5 is processed several times, probably in the trans-Golgi network, from its 26 kDa precursor to a 22 kDa product via 23 kDa and 22.5 kDa intermediates. The processing does not appear to go to completion, and although present in small amounts, the 26 kDa product is still secreted along with the mature form of the protein. Interestingly, disruption of the TgMIC5 gene appears to enhance the MPP2-dependent processing of other microneme proteins (V Carruthers, International Toxoplasmosis meeting New York 2002). Although the mechanism responsible for this effect is
not known it is plausible that TgMIC5 regulates the activity of MPP2.

TgAMA1 is homologous to the apical merozoite antigen previously described in *Plasmodium* species. Amino-terminal processing is thought to occur during secretion, although the processing site has not been determined. Furthermore, TgAMA1 is shed from the parasite surface as a result of a proteolytic cleavage in the region of the TM domain, possibly within it [41].

In *P. falciparum*, PfAMA1 is thought to be essential for successful invasion [42]. The maturation of this protein during erythrocyte invasion has been studied in great detail. After removal of its propeptide, PfAMA1 is processed in two sites at the parasite surface to form a 44 kDa and a 48 kDa product. The 44 kDa form results from a nick in the mature PfAMA1, and the resulting fragments remain in complex with each other because of disulfide links. Shedding of the extracellular domain of PfAMA1 from the parasite surface [43] causes release of the 48 kDa form, or the 44 kDa form if the nick has previously occurred. This sheddase, sensitive to PMSF (phenylmethylsulfonyl fluoride), cleaves at the site AEVT*SNNE just upstream of the TM domain. The inhibition profile of this protease activity suggests that it is the same protease as that responsible for PfMSP1 secondary processing, a vital step in erythrocyte invasion [44,45]. Interestingly, in sporozoites, PfAMA1 is processed in a similar way but by a different sheddase, sensitive to TLCK (N-tosyl-L-lysylchloromethane) [46]. This protease also appears to cleave PfTRAP and to operate on the parasite surface even in the presence of cytochalasin D, which inhibits gliding motility and invasion as also observed for the MPP1 cleavage of TgMIC2 [30].

### Parasite proteases potentially implicated in invasion

None of the proteases implicated in the processing of microneme proteins have been formally identified. Nevertheless, a battery of available protease inhibitors that block host cell invasion suggest that several distinct classes of proteases are expected to play a critical role in this process [47]. Some of these proteases might act by cleaving microneme proteins, while others might perform yet undefined roles. Parasite genome sequence comparisons and experimental studies undertaken in *Plasmodium* and *Toxoplasma* have exposed several very interesting candidates. A recent data–mining approach of the *P. falciparum* genome has reported the identification of up to 92 putative proteases from all major classes including aspartic, cysteine, metallo, serine and threonine [48]. Among them, the proteases potentially implicated in invasion and their counterparts in *T. gondii* will be discussed here and summarized in Table 2.

### Cysteine proteases

*P. falciparum* possesses a large repertoire of cysteine proteases including the SERAs, calpain, papain and falcipains. The SERAs are encoded by a large gene family of nine members in *P. falciparum*, showing strong homology

| Table 2 |

| Properties of *Toxoplasma gondii* proteases potentially involved in host cell invasion. |
|----------|-------------------------------------------------|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Nomenclature | Catalytic class | Domains | Processing/activation | Localization | Related genes in *P. falciparum* | Putative function | References/ accession numbers |
| Cysteine | Toxopain 1 | SP | N-term | Rhoptries | - | In Pf: Hemoglobinase and invasion | [51] AAL60053 |
| Toxopain 2 | Type II TM | ? | ? | Falcipain 1,2,3 | In Pf: invasion |
| Aspartyl | Toxopain 1 | Type II TM | N+C-term | Vesicles | Plasmepsin VI | ? | AY580011 |
| Toxopain 2 | SP, GPI | ? | ? | Plasmepsin VI | ? | AY583214 |
| Toxopain 3 | SP | ? | ? | Plasmepsins IX, X | In Pf: invasion? | AY92973 |
| Serine Subtilases | TgSub-1 | SP | N-term | Micronemes | PfSub1, 2 | In Pf: MSP1/AMA1 Sheddase? | [69] AAK94670 |
| TgSub-2 | TP, TM | N-term | Rhoptries | PfSub1, 2 | In Pf: MSP1/AMA1 Sheddase? | [70] AAO83837 |
| Serine Rhomboids | TgROM 1 | 7 TMs | - | Micronemes | chr11.phat_164 | In Tg: MPP1? | AY596191 |
| TgROM 2 | 7 TMs | - | Golgi | chr11.phat_164 | ? | AY596192 |
| TgROM 3 | 7 TMs | ? | ? | chr8.glm_34 | ? | AY683120 |
| TgROM 4 | 7 TMs | - | Plasma membrane | chr5.gen_274 | In Tg: MPP1? | AY596193 |
| TgROM 5 | 7 TMs | ? | ? | chr5.gen_274 | ? | AY634626 |

to papain proteases, except for some of them where the catalytic cysteine has been replaced with a serine. One of them, SERAS is an active enzyme, sensitive to serine protease inhibitors, abundantly expressed and potentially involved in either red blood cell egress or invasion by merozoites or both [49].

Falcipain 2 and 3 localize to the food vacuole and are involved in hemoglobin degradation [50,51]. Falcipain 2 is a dual-function protease and contributes also to merozoite release by cleavage of some components of the erythrocyte membrane skeleton [52]. By contrast, falcipain 1 localizes to the apical end of the parasite and is active specifically during the invasive merozoite stage. Specific inhibitors of falcipain 1 were identified by screening of chemical libraries and shown to block parasite invasion [53*].

In T. gondii, toxopain 1, a cathepsin B-like protease is produced as proprotein and autocatalytically cleaved to generate an active enzyme. Cysteine protease inhibitors blocking toxopain-1 activity have also been shown to impair parasite invasion and to block ROP2, ROP3 and ROP4 processing [54]. A second gene coding for toxopain-2, a potential homolog of falcipain-1 is present in the T. gondii genome but its characterization has not yet been reported. Two more putative toxopain-3 and toxopain-4 genes are awaiting proper assembly.

**Aspartyl proteases**

The P. falciparum genome contains ten aspartyl proteases named Plasmepsin I, II, IV–X and a histo-aspartyl protease (HAP, sometimes called Plasmepsin III), showing homology to mammalian cathepsins D and E and pepsinogen A. [55]. Plasmepsins I, II, IV and HAP are involved in hemoglobin metabolism [55,56], however the functions of plasmepsins V to X remain to be elucidated. In a recent transcriptome analysis of P. falciparum blood stages, a role for plasmepsins IX and X in invasion has been postulated by virtue of the timing of their expression in the schizont stage, along with several other proteins involved in invasion [57**]. Plasmepsins VI, VII and VIII were not detected in the intra-erythrocytic stages.

An aspartyl protease similar to Plasmepsins VI and VIII called Eimepsin has been characterized in Eimeria tenella [55,58,59]. Eimepsin is stored as an active form in the refractile bodies, and relocates to the apical tip of the parasite shortly before invasion but its role has yet to be determined [59]. T. gondii’s genome hosts four genes coding for aspartyl proteases. Toxomepsin 1 is a type II transmembrane protein undergoing several processing events and localized to a yet undefined and possibly lysosomal compartment (Jaekle et al., unpublished). Toxomepsin II shares a high degree of identity with Eimepsin and another protease present in the Cryptosporidium parvum genome (Accession CAD98424). The analysis of their predicted amino acids sequences strongly suggests that these proteases are anchored by glycosylphosphatidylinositol possibly to the parasite cell surface. No information is available on Toxomepsin III and IV.

**Serine proteases**

The function of two serine proteases of the subtilase family, PISUB-1 and PISUB-2 has been extensively scrutinized in P. falciparum because of their potentially critical involvement in host cell invasion [60]. PISUB-1 was reported to accumulate in the dense granules, to discharge around the time of invasion and to be the primary candidate protease to cleave the major merozoite surface protein PIMSP1 [61]. PISUB-1 is autocatalytically processed [61,62] and the two processed forms can bind to the pro-domain, which has an inhibitory effect on the mature protein as previously observed with subtilases [62,63]. The determination of the cleavage specificity of PISUB-1 after an aspartate [62–64] is incompatible with the secondary processing of PIMSP-1, which occurs after a leucine residue [65]. Additionally, peptides that inhibit the activity of PISUB-1 do not appear to inhibit erythrocyte invasion by P. falciparum, hence questioning any involvement of PISUB-1 in invasion [63]. A recent comparative study of PIMSP-1 secondary processing and PFAMA-1 shedding strongly suggests that the same protease is responsible for the two activities [43**].

PISUB-2 is a large type I membrane protein maximally expressed in schizonts and localized to the dense granules [66,67]. The 160 kDa translation product of PISUB-2 is processed to a 74 kDa product, which is further trimmed to 72 kDa [67]. In P. berghei, the PbSUB-2 gene is essential for erythrocyte invasion [68]. PISUB-2 currently constitutes the primary candidate for the sheddase activity responsible for PIMSP-1 and PFAMA-1 processing, although further characterisation is required to demonstrate this conclusively.

T. gondii also possesses two subtilases and TgSUB1 is recognized by an anti-PISUB-1 antibody, suggesting that the two apicomplexan proteases share areas of conserved conformation [69]. TgSUB1 localizes to the micronemes, and is processed from an initial 120 kDa precursor into several products, potentially by autocatalysis [69]. Substrates for this enzyme have not been identified so far. TgSUB2 is a transmembrane protein localized to the rhoptries, which is autocatalytically processed at its amino terminus from a 140 kDa protein to a 90 kDa product, then to an 85 kDa product [70]. Based on subcellular distribution, complex formation with the rhoptry protein ROP1 and cleavage site specificities, TgSUB2 has been postulated to function as a rhoptry processing protease [70]. In addition to TgSUB1 and 2, the genome of T. gondii possesses several more genes coding for putative subtilases not yet characterised.
A family of polytopic membrane rhomboid-like serine proteases are present and highly conserved throughout the phylum of Apicomplexa and in *T. gondii*, five genes have been identified to date. TgROM1–5 are differentially expressed during the parasite life cycle and localize to different successive compartments along the secretory pathway. Interestingly, the *Drosophila melanogaster* Rhomboid-1 and human RHBDL2 are able to use TgMIC2, TgMIC6 and TgMIC12 transmembrane domains as substrates [71]. Taken together, these results and the known sensitivity of DmRhomboid-1 to DCI suggest that this family of seven transmembrane domain-containing proteases constitute plausible candidates for MPP1 activity. TgROM1, TgROM2 and TgROM4 localize to the micronemes, Golgi and plasma membrane, respectively (Dowse et al. unpublished). In a previous study, MPP1 activity was detected constitutively at the plasma membrane without requiring the substrate to traffic through the micronemes, hence pointing to MPP1 being a resident plasma membrane protease [32**]. Consequently, TgROM4 appears as an excellent primary candidate responsible for MPP1 activity. Alternatively TgROM1, which localizes to the micronemes, could be responsible for MPP1 activity, but would imply that the enzyme is tightly regulated and this is not a characteristic feature of the rhomboid proteases studied so far [72*]. Further work is required to demonstrate the function of this intriguing novel family of proteases in Apicomplexans.

Concluding remarks

The proteolytic processing of microneme proteins is widespread, and in some cases demonstrably essential for invasion. The significance of the cleavages occurring in the secretory pathway is still unclear but might be linked to the specificity of complex formation, biogenesis and targeting to the organelle and masking of enzymatically active sites. The sites corresponding to the intracellular cleavages are not strongly conserved and, hence, it is plausible that more than one protease is involved, although there are no candidates known so far. The post-exocytosis processing events are likely to be directly linked to the host cell attachment and invasion process. The proteolytic action of MPP1, MPP2, MPP3 and other yet uncharacterised protease activities leads to the disruption of the adhesive microneme protein complexes and their release from the plasma parasite membrane. It remains to be determined experimentally if some of these events contribute specifically to the mechanism of gliding in addition to the presumed role in the dissociation of host–parasite interaction at the end of the entry process.

At this early stage, many proteases have been identified from genome sequence analysis. Some proteases have already been implicated in host cell invasion or egress but, for the majority, the precise biological function and the identification of substrates are still lacking. While some will act on host cell proteins, a significant proportion of the others will contribute to microneme protein processing. We are now closer to assigning specific proteolytic activities to these proteases. Primary candidates are identified and, together with the potential to generate conditional knockouts for essential genes, one suspects that it is just a matter of time before the mysteries of microneme protein processing begin to be unravelled. As proteases offer excellent targets for drug design, the field is both fascinating and important.

Acknowledgements

Work in our laboratory was funded by the Deutsche Forschungsgemeinschaft and the Wellcome Trust Program Grant to DS and Studentship to TD. We thank Anthony Keeley and Thierry Soldati for their critical reading of the manuscript. DS is a Howard Hughes Medical Institutes International Scholar.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- - of outstanding interest


The establishment of an inducible system allowed for the first time the conditional disruption of a gene essential for invasion in an apicomplexan.


An excellent and concise review highlighting the current state of knowledge regarding the mechanism used by apicomplexans to invade host cells.


This study identifies the first molecular link between the parasite actomyosin system and the microneme proteins host receptor complexes.


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This study presents compelling evidence for the presence of a multi-functional serine sheddase with a broad substrate specificity on the merozoite surface and responsible for the shedding of MSP1 and AMA1.


AMA1 is not only essential for red blood cell invasion by merozoites but also contributes to hepatocyte invasion by sporozoites. AMA1 undergoes a similar processing in two distinct life stages, apparently due to the action of two distinct proteases.


This global genome sequence survey provides an updated, yet still incomplete view, of the repertoire of proteases in Plasmodium falciparum.


This study is based on a large screen of compounds selectively inhibiting the complete intraerythrocytic developmental cycle of Plasmodium falciparum. PLoS Biol 2003, 1:E5.


This unprecedented study reports the most comprehensive view of the timing of the transcription through the complete intraerythrocytic developmental cycle of P. falciparum. The highly specialized mode of transcriptional regulation adopted by the parasite allows the identification of novel genes potentially involved in erythrocyte invasion.


An excellent review recapitulating the current state of knowledge concerning the diverse functions associated with the fascinating family of rhomboid proteases.