CHAPTER 2

Microneme Proteins in Apicomplexans

Vern B. Carruthers* and Fiona M. Tomley

Introduction

The invasive stages (zoites) of most apicomplexan parasites are polarised cells that use their actinomyosin-powered gliding motility or "glideosome" system to move over surfaces, migrate through biological barriers and invade and leave host cells. Central to these processes is the timely engagement and disengagement of specific receptors upon the regulated release of apical invasion proteins from parasite secretory organelles (micronemes, rhoptries). In this short review, we summarise recent progress on identification and functional characterisation of apical invasion proteins mobilised to the parasite surface from the microneme organelles. We have restricted our focus to *Toxoplasma*, *Eimeria*, *Cryptosporidium* and the nonerythrocytic stages of *Plasmodium* because these organisms have been the most intensively studied apicomplexans that invade nucleated cells and because invasion by erythrocytic stages of *Plasmodium* is covered in the next chapter.

Micronemes are the smallest of the apicomplexan secretory organelles that cluster at the apical end of the zoite. The number of micronemes varies enormously between different genera, species and developmental stages with those zoites displaying vigorous and extensive gliding or migration activity generally having the most. Thus, *Theileria* zoites, which are nonmotile, do not migrate and do not display active host cell invasion, have no micronemes;[1] merozoites of *Plasmodium*, which neither glide nor migrate but rapidly and actively invade erythrocytes, have few;[2] sporozoites and merozoites of *Eimeria*, which glide, migrate through intestinal contents and actively invade enterocytes have many;[3] and *Plasmodium* ookinetes, which glide and migrate through the midgut epithelium of the mosquito, but do not classically invade host cells, also have many (and by contrast, do not have rhoptries).[4] This long-standing correlation between micronemes and parasite motility, migration and invasion is well supported by a variety of biochemical and genetic studies which show: (1) that microneme secretion is rapidly up-regulated when parasites make contact with host cells;[5] (2) that some *Plasmodium* microneme proteins are targets of erythrocytic invasion-inhibitory antibodies;[6-10] (3) that parasite invasion is blocked when microneme secretion is chemically inhibited;[11,12] and (4) that genes encoding MICs either alone, or in concert with others, are essential for effective parasite motility, migration and invasion.

MICs have been identified in a variety of approaches (reviewed in ref. 13-14), most recently through the application of proteomics to gradient-purified organelles and excreted-secreted antigens.[15,67] Figure 1 summarises the current repertoires of MICs, including only those genes for which a full sequence and a verified organellar localisation is known. The majority of MICs comprise multiple copies of a limited number of adhesive domain types, which has allowed the identification of a large number of additional putative microneme

*Corresponding Author: Vern B. Carruthers—Department of Microbiology and Immunology, University of Michigan School of Medicine, Ann Arbor, Michigan, USA 48109. Email: vcarruth@umich.edu

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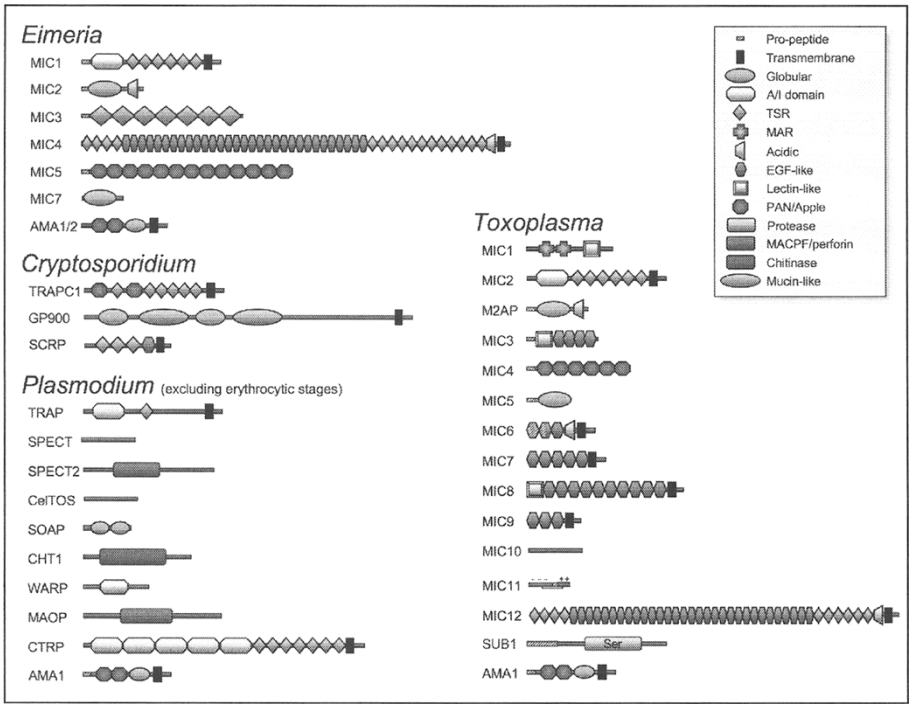

Figure1. Modular MICs. Schematic representations of known microneme proteins from four different apicomplexan genera are depicted (not to scale). Accession numbers, where available: *Eimeria tenella* MIC1, M73495; EtMIC2, Z71755; EtMIC3, AAR87667; EtMIC4, CAC34726; EtMIC5, AJ245536; *Cryptosporidium parvum* TRAP-C1, AAB92609; GP900, AAC98153; CpSCRP AF061328; *Plasmodium falciparum* TRAP, AAC1867; *Plasmodium berghei* SPECT, BAD08209; PbSPECT2, BAD83404; PbCelTOS, BAD97683; PbSOAP, AAL07530; PbCHT1, CAC40151; PbWARP, AAK83296; PbMOAP, AAV28504; PbCTRP, AAF73158; *Toxoplasma gondii* MIC1, CAA96466; TgMIC2, AAB63303; TgM2AP, AAK74070; TgMIC3, CAB56644; TgMIC4 AAD33906; TgMIC5, CAA70921; TgMIC6, AAD28185; TgMIC7, AAK35070; TgMIC8, AAK19757; TgMIC9, AAK19758; TgMIC10, AAG32024; TgMIC11, AAN16379; TgMIC12, AAK58479; TgAMA1, AF010264; TgSUB1, AAK94670. A color version of this figure is available online at www.eurekah.com.

proteins bearing these domains in the parasite databases.[16,17] Based on this it is likely that many more proteins will be shown to occupy the micronemes in future studies.

Ligand Domains and Their Cellular Receptors

Thrombospondin-1 Type 1 Domains (TSR)

Thrombospondin-1 (TSP-1) is a multifunctional, glycoprotein adhesion molecule that mediates a broad range of biological interactions via three distinct repeated domains designated types 1, 2 and 3.[18] The adhesive TSP-1 type 1 domain, TSR, is a small ~60 residue structure found in the extracellular regions of several protein families involved in immunity, cell adhesion and neuronal development, and shown to have binding activity for a number of cellular and matrix molecules (reviewed in ref. 19). The TSR is an ancient eukaryotic module that is found in many nematode and arthropod proteins as well as those from the

Apicomplexa.[20] One or more copies of the TSR are present in several apicomplexan MICs including *Plasmodium* thrombospondin-related adhesive protein (TRAP), circumsporozoite protein (CSP), and circumsporozoite-and-TRAP related protein (CTRP); *Eimeria* EtMIC1, and EtMIC4; *Cryptosporodium* TRAP-C1 and sporozoite cysteine-rich protein (SCRP) and *Toxoplasma* TgMIC2 and TgMIC12. Structures of TSR domains from TSP-1,[21] F-spondin[22] and *Plasmodium* TRAP,[23] representing two different TSR groups in respect to the organisation of their cysteine residues (Groups I and II), have been determined. Despite the different disulphide bonding patterns, these three TSRs share a highly similar elongated structure consisting of an anti-parallel, three-β-stranded fold that is additionally stabilised by stacked tryptophan and arginine residues (Fig. 2). A positively charged groove formed by the arginine stack was proposed to be the site of interaction with ligands and receptors, particularly glycosaminoglycans.[21] Several studies have shown that MICs containing TSRs bind host ligands[24-26] and recently chemical-shift mapping experiments, in which low-molecular weight heparin was titrated into ^{15}N-labelled TRAP-TSR, confirmed a site of interaction in the N-terminal half of the domain on the side of the aligned arginines.[23] Based on the structure, MICs containing TSRs would be predicted to extend out from the parasite surface after secretion from the micronemes and be thus ideally positioned to engage host surface receptors for attachment and invasion (see below).

Toxoplasma TgMIC1 and *Eimeria* EtMIC3 possess domains previously described as TSR-like, since they share some key sequence features with classical TSRs, but are now known to adopt an unrelated novel fold termed MAR (microneme adhesive repeat) (S. Matthews and D. Soldati-Favre, personal communication). The MAR domain is also able to bind host ligands[27,28] (J. Bumstead and E. Tomley, unpublished); TgMIC1 MAR binds specifically to sialic acid (S. Matthews and D. Soldati-Favre, personal communication) whereas the orthologous protein, NcMIC1, from *Neospora caninum* binds glycosaminoglycans.[29]

MIC TSRs have functions other than cell binding, for example the TSRs of TgMIC2 are implicated in its tight association with its partner protein MIC2 associated protein, TgM2AP (J Harper & V. Carruthers, unpublished) and the TSR-like domains of TgMIC1 recruit and interact with TgMIC4 in the TgMIC1-4-6 complex.[28]

Von Willebrand a Domain/ Integrin Inserted (I) Domains

The inserted (I) domain is found in the α- and β- chains of several vertebrate cell-surface integrins and is homologous to the von Willebrand A (WVA) domain, which is present in many extracellular matrix proteins. This ~200 residue A/I domain is ancient, found in proteins derived from eukaryotes, eubacteria and archaebacteria,[30] and adopts a Rossman dinucleotide binding fold consisting of five parallel and one anti-parallel β-strands that collectively are sandwiched by 7 α-helices (Fig. 2). In many A/I domains, a noncontinguous motif of amino acids is exposed on the surface of the structure to form a metal ion-dependent adhesion motif (MIDAS).[31,32] Although the MIDAS is crucial for binding in some cases, such as in type VI collagen dimerisation,[33] it appears irrelevant in others, such as in binding of the third VWA domain of von Willebrand Factor to fibrillar collagen.[34] Several TSR-containing apicomplexan MICs possess one or more A/I domain, and the MIDAS sequence is generally well conserved in these. Experimental studies have shown that the function of these apicomplexan A/I domains may be mediated by both MIDAS-dependent and independent mechanisms. Thus, mutations in the MIDAS of TRAP of *Plasmodium berghei* affect parasite invasion activity,[35] and binding of this domain to hepatocytes and to fetuin is MIDAS dependent;[36] however, binding to glycosaminoglycans is not mediated by MIDAS.[37] Similarly, the A/I domain of *Toxoplasma* TgMIC2 binds heparin in a MIDAS-independent manner.[38] Interestingly, exhaustive searching of the databases of *Cryptosporidium* has failed to identify any proteins containing A/I domains in this member of the phylum (T. Templeton, personal communication).

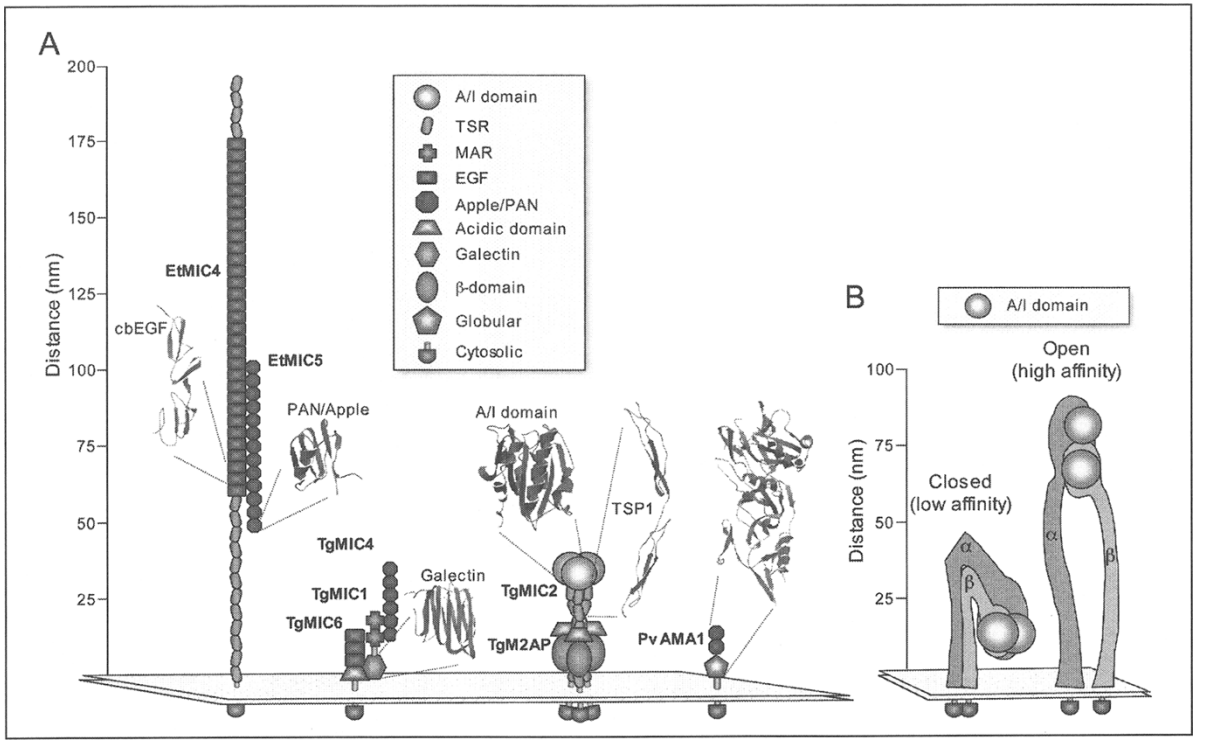

Figure 2. Structural features of micronemal ligands. (A) MICs from *Eimeria* (EtMIC4-MIC5), *Toxoplasma* (TgMIC1-4-6, TgMIC2-M2AP) and *Plasmodium* (PvAMA1) are schematically depicted with a scale (left) illustrating the approximate distance they would project from the parasite membrane, assuming a fully elongated state. Projection estimates are based on the dimensions of the domain types shown as ribbon structures, with α-helices (red), β-sheets (blue), and random coils or turns (grey). Structures are based on the following: cbEGF from fibrillin (Protein Data Bank accession number 1EMO), PAN/Apple from EtMIC5 (1HKY), A/I domain from integrin α_L (1MQA), TSR from thrombospondin (1LSL), ectodomain of PvAMA1 (1W8K). (B) Model based on of an integrin heterodimer in the closed (low affinity) and the open (high affinity) configuration.[80] A color version of this figure is available online at www.eurekah.com.

Apple/PAN Domains

Apple domains, which are a subset of the plasminogen, apple, nematode (PAN) superfamily, have been identified in plasminogen-related proteins such as coagulation factor XI, plasma prekallikrein, hepatocyte growth factor, macrophage stimulation factor and also in several nematode proteins. Apple/PAN domains have three conserved disulphide bridges that are essential for their tertiary structure, but homology in the primary amino acid sequence between domains is generally low, which may contribute to their very different and highly specific ligand binding properties. For example the four Apple/PAN domains from compliment factor XI (FXI) display very different ligand specificities: A1 binds the FXI cofactor H-kininogen and thrombin,[39,40] A2 binds the FXI substrate, FIX,[41] A3 also binds FIX and heparin[42,43] and A4 binds FXIIa.[44] Apple/PAN domains are present in several apicomplexan MIC proteins including EtMIC5, TgMIC4 and TRAP-CI and the solution structure of a single (A9) domain from *Eimeria tenella* confirmed its structural homology to the Apple/PAN superfamily[45] (Fig. 2). Very recently the crystal structures of apical membrane antigens (AMA1) of *Plasmodium* species revealed that the two most N-terminal domains of these MICs are also highly divergent members of the Apple/PAN superfamily[46,47] (Fig. 2). Most functional information on apicomplexan Apple/PAN domains has come from the study of TgMIC4, which contains 6 tandem domains and which exists as a structural heterocomplex with TgMIC1 and TgMIC6.[48,49] The first two Apple domains of TgMIC4 interact directly with the twin MAR domains of TgMIC1 and in the absence of TgMIC1 binding of TgMIC4 to host cells is almost entirely ablated;[28] however, is not known whether this is due to incorrect folding under these conditions or to the inherent lack of cell binding properties of TgMIC4. Interestingly NcMIC4, an orthologue of TgMIC4 in the closely related parasite *Neospora caninum*, is able to bind lactose, a property that is not shared by TgMIC4, which does not bind.[50] The function and binding properties of the newly defined Apple/PAN domains of AMA1 are not well defined, although recent data from *Toxoplasma* indicates that TgAMA1 cooperates with rhoptry neck proteins in the formation and maintenance of the moving junction during host cell invasion.[51,52]

EGF-Like Domains

EGF-like domains are widely distributed in membrane-bound and extracellular eukaryotic proteins and are involved in many different and diverse biological functions including blood coagulation, cell signalling, cell migration and maintenance of extracellular matrix architecture. These domains typically consist of ~50 amino acids with three conserved disulphide bridges and a subclass of EGF-like domains that bind calcium (cbEGFs) has been identified that have a conserved D/N-x-D/N-E/Q-xm-D/N*-xn-Y/F motif, (where m and n are variable and * indicates β-hydroxylation.[53] The first apicomplexan proteins containing EGF-like domains to be identified were GPI-linked proteins from *Plasmodium*[54,55] but more recently a number of apicomplexan MICs with EGF-like domains have been studied including SCRP, EtMIC4, and TgMICs 3, 6, 7, 8, 9, and 12. So far no EGF-like containing MICs have been found in *Plasmodium* species. The majority of the domains described in apicomplexan MICs are regular EGF-like, but the cbEGF motif is present in 22 of the 31 EGF-like domains of EtMIC4[56] and its homologue TgMIC12 (ToxoDB 57.m01872; F. Stavru and D. Soldati-Favre, personal communication). Study of cbEGFs in EtMIC4 has shown that in the presence of calcium these domains adopt a proteinase-resistant, extended structure that would favour the interaction of the N-terminal portion of the molecule with host cell ligands.[57] Interestingly, EtMIC4 forms a stable very high molecular mass heteromeric complex with the soluble Apple/PAN domain containing protein EtMIC5 although the precise sites of interaction between these two MICs are not yet mapped (J. Periz & F. Tomley, unpublished). In *Toxoplasma*, TgMIC3 contains both EGF-like and lectin-like domains and binds to all nucleated cells tested as well as to the tachyzoite surface.[58] The receptor-binding properties of TgMIC3 are attributed to the lectin-like domain, whereas the EGF-like domains are proposed to promote proper folding of the protein in order to expose the binding

regions. In addition, they may be involved in heteromeric polymerisation with the transmembrane MIC TgMIC8, which contains 10 EGF-like domains and which functions as an 'escorter' to ensure delivery of TgMIC3 to the micronemes. Similarly, in the TgMIC1-4-6 adhesive complex it is the transmembrane, EGF-like domain containing TgMIC6 that is responsible for targeting to the micronemes but in this case oligomerisation is promoted and stabilised by the interaction of the third EGF of TgMIC6 domain, together with its downstream acidic region, with the galectin domain of TgMIC1.[28]

Lectin Domains

Two types of domains related to lectins have been identified within apicomplexan MICs. Chitin-binding like (CBL) domains are found in a variety of plant lectins including plant defensins that have anti-fungal chitinase activity. CBLs are typically composed of ~30-43 amino acids with four conserved disulphide bridges and several conserved aromatic residues that mediate binding of the domain to N-acetyl glucosamine.[59] CBLs with lectin (or agglutinin) properties are able to bind and cross link GlcNAc-containing polymers and in *Toxoplasma*, TgMIC3 and TgMIC8 each contain a single CBL-domain at their N-termini, followed by several EGF-like domains.[58,60] Binding of the CBL-domain of TgMIC3 to host cell surfaces is dependent upon its dimerisation, which is mediated by the interaction of the C-terminal regions of each monomer,[61] and disruption of the CBL aromatic residues presumed to be important for binding results in lowered parasite virulence.[62] Fusion of the TgMIC3 dimerisation domain to the extracellular domain of TgMIC8 promotes dimerisation and binding of the chimera, indicating that the CBL of TgMIC8 also possesses binding activity when in a dimeric form.[62]

Another lectin-related domain in an apicomplexan MIC was recently identified from the three-dimensional structure of the C-terminal domain of *Toxoplasma* TgMIC1.[28] This domain has a galectin-like fold, which consists of a β-barrel formed by the association of two multi-stranded β-sheets. Galectins are soluble, calcium-independent, carbohydrate-binding animal lectins, however the critical side chains that mediate lectin activity are not conserved in the TgMIC1 galectin domain and no detectable binding to a range of carbohydrate substrates was observed in NMR chemical shift mapping experiments. Instead, the TgMIC1 galectin domain displays a large hydrophobic surface reminiscent of the protein-protein interaction domains seen in bacterial class I chaperones of the type three secretion system and both NMR and biochemical studies indicate that during the biogenesis of the TgMIC1-4-6 adhesive complex, this domain recruits and stabilises TgMIC6 providing a highly specific quality control mechanism for the exit of TgMIC6 from the ER/Golgi and for subsequent trafficking of the adhesive complex to the micronemes.

Adhesive Complexes: Assembly and Organization

Propensity to Form Oligomers

Adhesive proteins often form oligomeric complexes with themselves or other proteins that contribute to adhesion or serve a regulatory function. For example, cadherins are a family of vertebrate adhesive proteins expressed as homodimers that strengthen cell-cell junctions. Integrins are heterodimeric, transmembrane glycoproteins primarily responsible for mediating cell interactions with extracellular matrix (ECM). The propensity to form oligomeric adhesive complexes has been demonstrated in several apicomplexans, although most of the mechanistic studies have been done in *Toxoplasma*. Oligomerization bestows adhesive proteins with several important advantages.

First, oligomerization can promote the proper folding of proteins in a complex, as recently shown for the TgMIC1-4-6 complex.[28] TgMIC1 is a soluble protein that simultaneously associates with TgMIC4 through its two TSR-like MAR domains and with the transmembrane escorter protein TgMIC6 through its C-terminal (CT) galectin-like domain. As mentioned above, NMR spectroscopy revealed that the CT domain is incapable of binding sugars but

instead forms an interface with the third EGF-like domain of TgMIC6, which also contains an acidic element (TgMIC6-EGF3acid).[28] When mixed together and monitored by NMR, the TgMIC1 CT domain facilitated the folding and stabilization of the TgMIC6-EGF3acid. The TgMIC1 CT domain also rescued the secretory retention phenotype of TgMIC6 in mic1KO parasites, presumably by navigating through the quality control system that recognizes misfolded proteins. These findings reveal new molecular insights into the interdependence of adhesive proteins for correct folding and movement through the secretory pathway.

Second, assembly into protein complexes allows cooperation in trafficking to the micronemes. MIC complexes typically have one transmembrane (TM) protein. These TM MICs are also referred to as escorters since they accompany and guide the other soluble members to the micronemes based on the targeting signals in their C-terminal tails.[49,60] In TgMIC2, this signal is provided by two tyrosine-based sorting motifs capable of directing a heterologous protein to the micronemes.[63] Genetic disruption of any of the TM MICs results in retention of the other members of the complex along the secretory system or in mistargeting to the default secretory pathway, which in *T. gondii* is secretion via the dense granules. When the level of TgMIC2 expression is experimentally reduced, TgM2AP colocalizes with the dense granules and is secreted into the PV.[64] Similarly, TgMIC6 knockout parasites show a complete misrouting of TgMIC1 and TgMIC4 to the dense granules.[49] Nonetheless, escorters still depend on their cargo for proper trafficking since soluble proteins in the complexes are required for protein folding, as is the function of the galectin-like domain of TgMIC1,[28] or necessary for exiting an endosomal compartment associated with microneme biogenesis, as shown for the TgM2AP propeptide.[28a]

Third, different combinations of partners can expand the receptor repertoire and/or fine-tune the specificity of receptor binding. Humans express eighteen integrin α-subunits and eight β-subunits that form 24 heterodimers for recognition of distinct but overlapping receptors.[65,66] Although there are no firm examples of subunit mixing in the apicomplexa, these parasites often express paralogous families of adhesive proteins with the potential to participate in such a phenomenon. Four closely related putative adhesins were recently identified in a proteomic screen of *Toxoplasma* secretory proteins.[67] These proteins have four Apple/PAN domains but no predicted anchoring sequence, and, by analogy with TgMIC4 and its association with TgMIC1 and TgMIC6, they likely oligomerize with a TM protein, possibly in a manner that would expand their receptor binding capabilities. Three additional genes coding for proteins closely related to TgMIC1 are also present in the *Toxoplasma* genome (D. Soldati-Favre, personal communication).

Fourth, oligomerization allows proteins from distinct compartments to facilitate invasion collaboratively. Two studies[51,52] have recently shown that the microneme protein TgAMA1 oligomerizes with three proteins derived from the rhoptry neck: TgRON2, TgRON4, and TgRON5. Although they are discharged from different organelles during invasion, TgAMA1 and TgRON2/4/5 form an oligomeric complex on the parasite surface within the moving junction, a ring-like constriction that slides over the parasite as it penetrates the host cell. TgAMA1 is a key component of the complex since depletion of this protein causes a failure to form the moving junction and parasite invasion is arrested at the stage of apical attachment.[68] Since TgRON4 is predicted to be an integral membrane protein, this raises the hypothesis that it inserts into the host plasma membrane and acts as an autologous receptor for cell invasion.[51] In this case, oligomerization would allow the parasite to use its own receptor to support invasion of the many cell types susceptible to *Toxoplasma* invasion.

Finally, oligomerization increases valency and avidity, thereby enhancing the formation of a robust binding interface. For example, TgMIC2-M2AP is a heterohexameric complex consisting of a trimer of dimers.[38,69] The corresponding complex in *Eimeria tenella*, EtMIC1-MIC2, presumably also forms a similar hexameric assembly. Such an arrangement could promote tight binding to a complementary oligomeric receptor on the host cell surface, thereby allowing the parasite to grip sufficiently well to power its way into the target cell.

Ligand Organization in Micronemes and on Parasite Surface

It is not known precisely how adhesive ligands are organized within micronemes. However, several features suggest that ligands are packaged in an orderly fashion. First, the contents of *Cryptosporidium* micronemes are arranged in an array of 15 nm cubic crystals framing a pine-cone-like pattern.[70] Although this crystalline appearance is unique to *Cryptosporidium*, micronemes of other apicomplexa are electron dense, implying a high protein concentration. Second, since a number of micronemes are discharged in rapid succession, a strong measure of organization is presumably necessary to achieve efficient deployment. Finally, the internal dimensions of micronemes (~75 nm x 150 nm) might not accommodate some of the larger microneme proteins (e.g., EtMIC4-MIC5) in their fully extended state (see also below) and therefore these proteins are likely packaged in orderly fashion so that they are primed for secretion onto the parasite surface.

It has been proposed that some microneme proteins are involved in organizing the organellar contents. For example, TgMIC10 and TgMIC11 are small, soluble microneme proteins that display a marked charge asymmetry, which may promote electrostatic assembly into higher ordered structures.[71,72] Unlike most other microneme proteins, TgMIC10 and TgMIC11 do not associate with the parasite surface during invasion, consistent with an alternative role independent of adhesion. During transport to the micronemes, TgMIC11 is proteolytically processed to remove an internal propeptide in a manner reminiscent of insulin maturation within nascent secretory granules of pancreatic beta cells. Insulin processing is thought to promote its ordered packaging and retention in maturing secretory granules,[73,74] although this idea is somewhat controversial.[75]

During gliding and invasion the microneme contents are deployed onto the parasite's apical surface where substrate or receptor engagement occurs. Adhesive complexes are not randomly distributed. For example, TRAP is arranged in a cap or ring-like pattern on gliding *Plasmodium* sporozoites.[76] Also, the EtMIC4-MIC5 complex displays a punctate pattern on the surface of invading *Eimeria* sporozoites[77] in a manner similar to TgMIC2-M2AP during *Toxoplasma* tachyzoite invasion.[78] Invading zoites display a particularly high density of ligands at the external boundary of the moving junction. The organization of ligands in this adhesion zone may further promote multivalent, high avidity interactions with host receptors, especially if the receptors have a complementary clustering distribution. Clustering may therefore be an additional level of organization that further promotes the creation of a robust binding interface between the parasite and host cell membranes.

The Surface Ligand Landscape: Does Size (and Conformation) Matter?

Crystal structure analysis of several domain types found in apicomplexan microneme proteins is beginning to reveal both the approximate size and shape of these important ligands. For example, cbEGF domains form an elongated structure that is stabilized by interdomain Ca^{2+} binding and hydrophobic interactions between adjacent domains.[53] Since the majority of EGF domains in the extracellular portion of EtMIC4 are of the cbEGF type, EtMIC4 is predicted to adopt a highly extended conformation that could project nearly 200nm from the parasite surface. However, it is unlikely that this structure is completely rigid there is greater flexibility between noncalcium binding EGF domains.[57] This semi-rigid conformation may allow the molecule to project maximally from the membrane while still retaining some degree of flexibility to "survey" the host cell surface for receptors. The ninth Apple/PAN domain of EtMIC5 adopts a globular α/β structure with the N- and C-termini situated on the same side of the molecule.[45] Although for EtMIC5 the structure of only one domain was solved, *Plasmodium* AMA1 has two PAN/Apple domains that are stacked upon one another,[47] suggesting that EtMIC5 and other multi-PAN/Apple domain containing microneme proteins may also adopt an elongated structure that projects away from the parasite surface. Based on the crystal structures of the A/I domains from various integrins and a pair of TSR domains from thrombospondin, TRAP family members including TgMIC2 are predicted to form a

"ball-on-a-stick" type of structure that could extend up to 40 nm from the parasite surface. Six tandem TSR domains that form a highly elongated stalk provide most of the molecule's height. The trimeric arrangement likely imparts a high degree of rigidity and strength in the molecule, which may be important to form a solid connection between extracellular receptors and the parasite's intracellular motility system.

For mammalian cell adhesion, recent studies have also provided new insight into role of conformational shifting in modulating ligand affinity. Molecular electron microscopy of the integrin $\alpha_5\beta_1$ showed that it undergoes a dramatic conformational shift from a "closed" to "open" configuration upon activation by inside-out signaling and/or exposure to certain divalent cations.[79,80] As shown in Figure 2, in the closed, low affinity position the heterodimer is bent over with the paired A/I domans positioned proximal to the cell membrane. However, when Mn^{2+} binds to the MIDAS site the complex "stands up" to project the adhesive A/I domains 2-3 times further away from the cell membrane. Although no direct evidence is available, similarly dramatic conformational changes could occur in micronemal ligands. For example, if EtMIC4 is not exposed to high concentrations of Ca^{2+} during transport and packaging in the micronemes then it would be sufficiently flexible and compact to fit within the microneme lumen. However, upon secretion and exposure to millimolar concentrations of Ca^{2+} in the extracellular milieu, EtMIC4 might unfurl to attain maximum height for long-range interactions with host receptors in the initial apical docking of the parasite. Other large microneme proteins in *Cryptosporidium* (CpGP900) and *Toxoplasma* (TgMIC12) may play a similar role. In this manner the parasite could establish an initial connection between its apical pole and the host surface before using other perhaps higher affinity or more abundant micronemal ligands to strengthen the grip for active penetration.

Role of Micronemal Proteins in Migration across Biological Barriers

For *Plasmodium*, the mosquito midgut and the sinusoidal layer of the liver are two significant biological barriers against infection and cell migration activity is needed for the zoites to breach these barriers.

Ookinetes of *Plasmodium* are highly motile and they migrate through the midgut epithelium of the mosquito causing massive destruction. The microneme proteins CTRP, SOAP (soluble ookinete adhesive protein), MAOP (membrane attack ookinete protein) and CelTOS (cell-traversal protein for ookinetes and sporozoites) have been shown to play crucial roles. CTRP is essential for apical attachment to the midgut epithelial cell,[81] SOAP is involved in mosquito midgut invasion and oocyst development,[82] MAOP which has a MACPF domain is necessary for ookinetes to breach the apical plasma membrane of the epithelial cell[83] and CelTOS is needed for the ookinetes to migrate through the cell cytoplasm to reach the basal lamina where oocyst development occurs.[84]

Sporozoites of *Plasmodium* are able to glide, migrate and invade host cells. Entry of the sporozoite into the hepatocyte is controversial and has been reported to occur following direct parasite migration through cells and by 'classical' invasion, vacuole formation and egress. It has been suggested that sporozoite migration through hepatocytes has an effect on subsequent sporozoite infectivity for new hepatocytes and on permissiveness of surrounding hepatocytes (via release of hepatocyte growth factor, HGF).[85,86] However, gene-targeting experiments on sporozoite microneme proteins contradict this—SPECT (Sporozoite-protein-essential-for-cell-traversal) disrupted sporozoites are deficient in cell migration yet they show normal cell invasion and gliding motility.[87] This indicates that cell migration is not an absolute requirement for cell invasion, although it is clearly important in vivo since disruption of SPECT decreases liver infectivity ~20-fold. This decrease in infectivity was reversed by depletion of Kupfer cells that line the liver sinusoids, leading to the conclusion that the cell migration activity mediated by SPECT is required to cross the liver sinusoidal barrier.[87] Two other sporozoite MICs are implicated in liver invasion. SPECT2 contains a membrane attack complex/perforin domain and disruptants show the same phenotype as SPECT disruptants, thus SPECT2 is presumed also to

be necessary for sporozoite traversal of the liver sinusoid.[88] CelTOS is expressed in both ookinetes and sporozoites and again disruption of the gene gives essentially the same phenotype as SPECT and SPECT2 except that the disruptants maintain a low level of cell migration (cell wounding) activity.[84] It is unclear whether the proteins involved in cell migration function by binding specific receptors on the host cell surface or within the cytoplasm, or whether they function in a regulatory or sensory role (for more details see chapter IX, Frevert et al).

While *Toxoplasma* tachyzoites have not been reported to migrate through cells, recent studies suggest that they cross biological barriers by a paracellular route i.e., between host cells, using ICAM1 as a receptor.[89] TgMIC2 was shown to bind ICAM, but only upon proteolytic removal of a short N-terminal extension that preceeds the A/I domain. This proteolytic trimming phenomenon, mediated by a hypothetical surface protease called MPP2, constitutes another means of regulating adhesive activity associated with parasite migration and possibly also attachment.

Summary

Microneme secretion supports several key cellular processes including gliding motility, active cell invasion and migration through cells, biological barriers, and tissues. The modular design of microneme proteins enables these molecules to assist each other in folding and passage through the quality control system, accurately target to the micronemes, oligimerizing with other parasite proteins, and engaging a variety of host receptors for migration and cell invasion. Structural and biochemical analyses of MIC domains is providing new perspectives on how adhesion is regulated and the potentially distinct roles MICs might play in long or short range interactions during parasite attachment and entry. New access to complete genome sequences and ongoing advances in genetic manipulation should provide fertile ground for refining current models and defining exciting new roles for MICs in apicomplexan biology.

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