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Do apicomplexan parasite-encoded proteins act as both ligands and receptors during host cell invasion?

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Abstract

Apicomplexan parasites are responsible for a wide range of diseases in animals, including humans, in whom *Plasmodium* species cause the devastating disease malaria. Several recent discoveries now indicate that these intracellular parasites may use a conserved mechanism to infect their host cells by using parasite-encoded proteins as both parasite ligands and receptors anchored to the host cells.

Introduction and context

Apicomplexa are a phylum of unicellular eukaryotic organisms that often parasitise multiple animal hosts during their life cycles. This group of obligate intracellular parasites are so called because the invasive zoite stages possess a characteristic cell apex containing several secretory organelles and associated cytoskeletal machinery specialised for penetrating host cells and tissues. The diseases associated with apicomplexan infections are of great health, social, and economic importance. Leading them is malaria caused by *Plasmodium* species, the most deadly form of which is *P. falciparum* [1]. Other apicomplexans that infect humans include *Toxoplasma gondii* and *Cryptosporidium*, but they usually cause less severe disease – except in the developing foetus and immunocompromised individuals. Understanding how parasites enter their hosts' cells is of great interest because this offers an attractive target for the development of novel therapeutics.

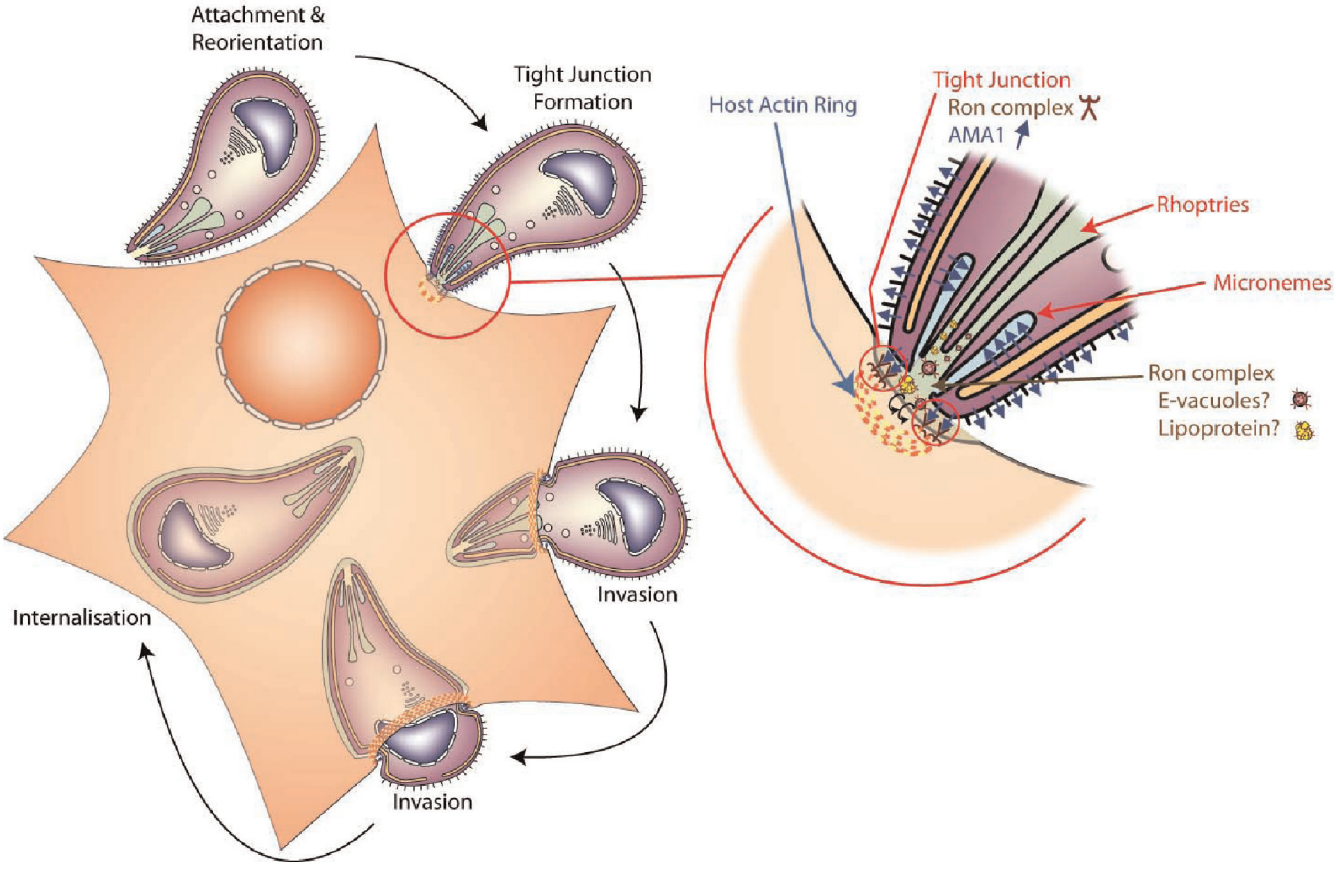
Some apicomplexans such as *T. gondii* are able to invade a wide variety of host cells despite the enormous variety of host surface proteins between tissues and species. Other apicomplexans such as *Plasmodium* species are highly selective for their target species and can even bias their infectivity toward cells of a particular level of maturation. In addition, some plasmodia, in particular the red blood cell invasive stage of *P. falciparum*, are able to switch invasion pathways to use different parasite ligands [members of the erythrocyte-binding antigen (EBA) family and reticulocyte-binding protein homologue (Rh) family] to mediate interaction with different host cell receptors [2-6]. For example, an individual parasite clone can adapt from using host-cell glycophorin A as a key receptor to relying on a completely different host cell receptor for entry.

Major recent advances

How are these parasites able to have so much adaptability in the receptors and ligands they use to determine host specificity yet apparently maintain similar invasion efficiency? Recently, Besteiro *et al.* [7] provided evidence for how efficiency might remain constant; not only do apicomplexans carry their own invasion ligands but they may provide their own invasion receptors, which they 'plug' into their hosts' cells and which serve as anchoring points for host cell penetration.

In Figure 1, we have illustrated the basic steps of invasion, using *Toxoplasma* tachyzoites as an example. After initial attachment and recognition, presumably involving the parasite genus-specific ligands and host cell-encoded receptors referred to above, the apical end of the parasite becomes juxtaposed to the host cell surface and a strong ring of attachment, or tight junction, is formed (Figure 1). Parasite actin/myosin motor

Figure 1. During the invasion of their host cells apicomplexan zoites may transfer ligands into their hosts which they then bind to during invasion



Apicomplexan parasites may insert their own ligands into their host cells to provide an adhesive point for invasion, as illustrated by a *Toxoplasma* tachyzoite invading an animal cell. After host cell contact the parasite apically reorientates. Apical membrane antigen 1 (AMA1) is secreted from micronemes onto the tachyzoite surface, where a small amount interacts with the rhoptry neck proteins (RONs) to form the tight junction. How this transfer is mediated is unknown but could involve the release of RON-containing evacuoles or lipoprotein complexes. Concurrent with the formation of the AMA1-RON tight junction is the creation of an F-actin ring below the host cell surface which possibly helps anchor the tight junction. Internalisation of the parasites ensues after activation of the parasite's actin/myosin motor. The parasite migrates into a vacuole formed from host and secreted parasite lipids and proteins.

proteins are engaged and the parasite pulls itself through the tight junction and enters the host cell [8-13]. The parasite concomitantly secretes proteins and lipids from its apical secretory organelles that help form a parasitophorous vacuole into which the parasite enters (Figure 1). The parasite ligand relevant to the work of Besteiro *et al.* [7] is known as apical membrane antigen 1 (AMA1), a type 1 integral membrane protein with a large ectodomain and small cytoplasmic region [14,15]. AMA1 is stored in secretory microneme organelles and released onto the plasma membrane prior to host cell attachment (Figure 1) [16,17]. Prior to internalisation, a subset of AMA1 molecules become restricted to the tight junction and appear to interact with a complex of three or four rhoptry neck proteins (RONs) [18-20]. These interactions have been known for some time, but Besteiro and colleagues suggest that, rather than residing on the parasite side of the junction, the RON complex might reside on the host side of the junction, with one or more RON components penetrating the host plasma membrane making contact with the host cytoskeleton (Figure 1). Many apicomplexans possess homologues of AMA1 and the RONs, raising the possibility that this invasion mechanism is commonly used within the phylum.

Although the interaction between AMA1 and the RONs has been studied by other groups, similar conclusions about the orientation and host cell localisation of the RON complex have not been reached previously [18-20]. If this observation is substantiated by follow-up studies, it will be of tremendous significance for the field. Some support for the findings of Besteiro *et al.* [7] has been provided by another study that indicates that a ring of

host cell F-actin rapidly forms beneath the tight junction in *Toxoplasma* tachyzoites and *P. berghei* sporozoites invading tissue-cultured cells [21]. F-actin ring formation greatly assists parasite invasion by presumably acting as a solid anchoring point, and inhibition of its formation with actin-destabilising drugs significantly reduces invasion efficiency. Actin polymerisation appears to be stimulated by recruitment of F-actin nucleating factors such as the Arp2/3 complex and cortactin. The proteins that ultimately recruit the F-actin nucleators are unknown, but the possibility of parasite effector proteins being involved has been raised [21]. These may well be members of the RON complex, and examples of direct parasite protein-mediated stimulation of host cell F-actin formation are known from bacterial pathogens [22].

If a common mechanism of tight junction formation and internalisation is used for apicomplexan invasion, then the preceding molecular interactions presumably provide the necessary specificity between the parasite and host. Initial contact between parasite and host is seemingly mediated by low-affinity long-range interactions between parasite surface coat proteins and the host surface. These may trigger the release of high-specificity EBA and Rh family proteins from apical secretory microneme organelles in *Plasmodium* parasites that bind blood cell surface proteins such as the glycophorins. It will be interesting to discover whether the apical concentration of these parasite receptors aids reorientating of the parasite and/or triggers a signal cascade that results in the release of the RON complex and formation of the tight junction. In *Toxoplasma*, the microneme (MIC) proteins perform this role, with MIC8 acting upstream of AMA1 and regulating the release of the RONs [23].

Future directions

Although early contact events between host cells and apicomplexan parasites require specific parasite-host cell recognition events, the process of tight junction formation and host cell penetration may involve a core of conserved parasite-encoded proteins acting as both ligands and receptors. AMA1 is already a leading vaccine candidate, and understanding its conserved interaction with the RON complex offers the promise of novel intervention approaches with a possible broad effect for multiple parasite species. Furthermore, the involvement of intracellular host cell proteins during invasion presents new targets for intervention that centre on the functions of host cell components.

Abbreviations

AMA1, apical membrane antigen 1; EBA, erythrocyte-binding antigen; MIC, microneme protein; Rh, reticulocyte-binding protein homologue; RON, rhoptry neck protein.

Competing interests

The authors declare that they have no competing interests.

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