REVIEW

Parasitology

How does *Toxoplama gondii* invade host cells?

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ABSTRACT. *Toxoplasma gondii* is a highly prevalent protozoon that can infect all warm-blooded animals, including humans. It is frequently used as an Apicomplexan parasite model in research. In this review, the invasion mechanism of *T. gondii* is described as a representative Apicomplexan parasite. The invasion machinery of *T. gondii* consists of the moving junction and the glideosome, which is a specific motor system for Apicomplexan parasites. I provide details about the moving junction, parasite-secreted proteins and host adhesion receptors, the glideosome, and calcium signaling, which generates the power for the gliding mobility of *T. gondii*. A detailed understanding of parasite invasion can be useful for the development of new effective drugs to inhibit this event and disrupt the Apicomplexan life cycle.

KEY WORDS: glideosome, host adhesion receptor, invasion, moving junction, *Toxoplasma gondii*

TOXOPLASMA GONDII

Toxoplasmosis is a common parasitic disease caused by *Toxoplasma gondii* [21]. *T. gondii* is an obligate intracellular parasite of the phylum Apicomplexa, which includes the human and animal parasites *Plasmodium*, *Eimeria*, *Neospora*, *Theileria*, *Babesia*, and *Cryptosporidium*. Approximately one-third of humans worldwide are chronically infected with *T. gondii* [20, 44]. In healthy individuals, *T. gondii* infection is usually asymptomatic; however, in pregnant women or people with suppressed immunity, it can be fatal [23]. After infecting its intermediate host, *T. gondii* differentiates into tachyzoites that rapidly infect host tissues. In principle, most active *T. gondii* infections are cleared by the immune system or appropriate drugs; however, some tachyzoites differentiates into bradyzoites, which form cysts in the infected tissues. The relatively quiescent tissue cysts may serve as a source for subsequent exacerbations, particularly in immunosuppressed individuals. Unlike most other Apicomplexa, *Toxoplasma* can invade and replicate in almost all of the nucleated cells of warm-blooded animals. The cell invasion machinery among Apicomplexa parasites is highly conserved.

T. gondii isolates from Europe and North America mainly belong to major three lines, referred to as types I, II, and III. These three genetic types differ in bioactivity. Type I is the most virulent, whereas types II and III have moderate virulence to mouse model. The life cycle of *Toxoplasma* is illustrated in Fig. 1. In both the intermediate and definitive host feline species, *T. gondii* replicates quickly and causes acute infection as tachyzoites. Once the parasite is exposed to a stressful environment, which may be the host immune system or particular host cell types, the parasite replicates slowly, evades the immune system in the cyst wall and causes persistent infection as bradyzoites. Host cell invasion by *T. gondii* involves the sequential secretion of two distinct secretory organelles, termed micronemes and rhoptries (Fig. 2), which are characteristic of the Apicomplexa phylum [9].

T. gondii is frequently used as an Apicomplexan parasite model because it can be cultured in almost all types of mammalian cells and has proven to be a valuable experimental research tool. In this review, I described the invasion mechanism of *T. gondii* as a representative Apicomplexan parasite.

THE MOVING JUNCTION

A key structure for host cell invasion is a tight junction structure known as the moving junction (Fig. 2), which is formed by intimate contact between the apical tip of the tachyzoite and the host cell membrane. As the invasion advances, the tachyzoite propels itself by means of an internal actomyosin motor into the host cell, thereby leading to the formation of a parasitophorous vacuole surrounded by the parasitophorous vacuole membrane inside the host cell [41]. In *T. gondii*, the moving junction consists of TgRON2, TgRON4, TgRON5, and TgRON8, secreted from rhoptry necks, and apical membrane antigen 1 (TgAMA1), secreted from micronemes (Fig. 3) [1, 4, 27, 39, 45]. TgRON2 is inserted as an integral transmembrane protein into the host plasma membrane, whereas TgRON4, TgRON5, and TgRON8 are exposed to the cytosolic face of the host cell membrane during
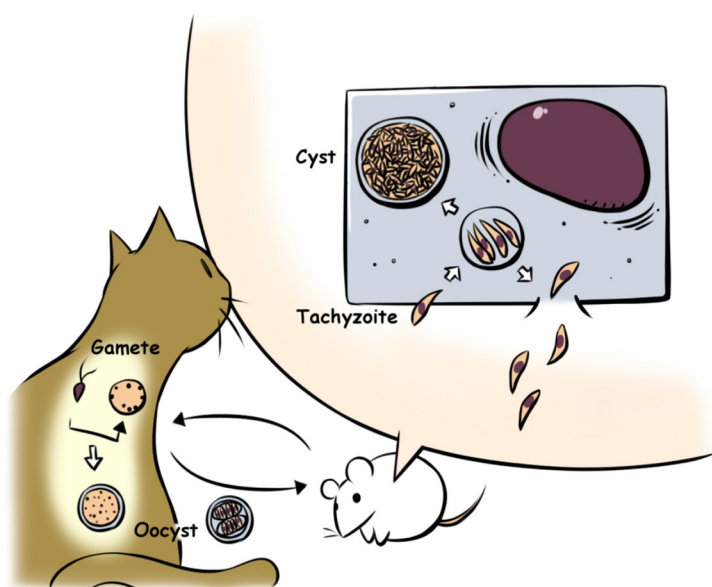
Fig. 1. Schematic image of the life cycle of *T. gondii* (partially modified from a previous review [24]). In both the intermediate and definitive host feline species, *T. gondii* replicates quickly and causes acute infection as tachyzoites. Once the parasite is exposed to a stressful environment, the parasite replicates slowly, evades the immune system in the cyst wall, and causes persistent infection as bradyzoites. The bradyzoites in the hosts can be eaten and infect the predator. In the definitive host feline species, gametocytogenesis occurs as well as tachyzoite replication. The macrogamete and microgamete are fertilized and become an oocyst. The oocysts are excreted in the stool of feline species and can be ingested by host thereby leading to infection.
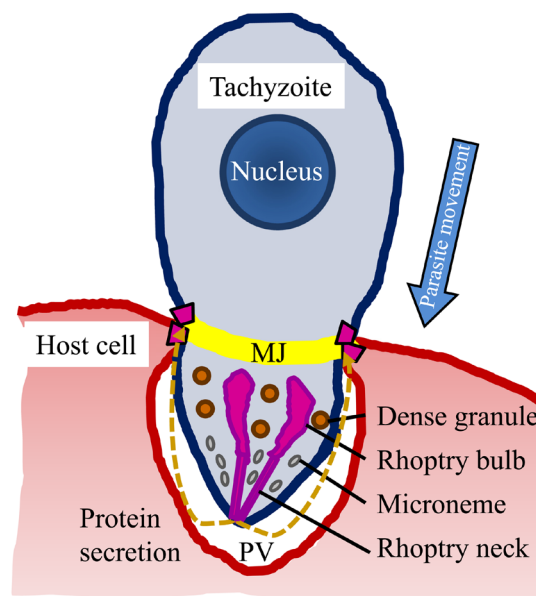

Fig. 2. The *Toxoplasma* invasion system. The parasite pushes itself through the moving junction (MJ) and invades via the parasitophorous vacuole (PV), which is formed as an invagination of the host plasma membrane. Parasite proteins are secreted from its organelles (e.g., the microneme, rhoptry, and dense granules). The large arrow indicates the direction of parasite movement.

invasion [4]. The components of this complex are conserved among Apicomplexan species [3, 26, 37], except for TgRON8, which seems to be coccidian-specific [38]. From the study of TgRON8-knockout parasites, it has been proposed that TgRON8 forms a firm intracellular grip that commits the parasite to invasion by anchoring it to the host cytoskeleton [39]. A different study with a TgRON5 conditional knockout strain demonstrated that TgRON5 is required for TgRON2 stability and the proper targeting of TgRON4 [2]. TgRON4 and TgRON5 were not detected at the moving junction during invasion of a TgRON2 conditional knockout parasite [28]. In addition to the importance of TgRON2 and TgRON5, TgRON4 is thought to be crucial for parasite growth as attempts to knock it out have been unsuccessful in *T. gondii* tachyzoites [1].

After invasion is completed, the moving junction can still be detected at the posterior pole of the parasite for a few hours [29]. In intracellular parasites, TgRON4, TgRON5, and TgRON8 are exposed on the cytosolic face of the host cell plasma membrane [1, 4, 7, 29]. TgRON4, but not TgAMA1, is associated with the moving junction during ionophore-induced egress [1]. Moreover, TgRON4 shows a typical ring-like signal in conditional TgAMA1 null mutant tachyzoites, indicating that TgAMA1 is not essential for the formation of a functional moving junction [16].

THE INTERACTIONS BETWEEN PARASITE-SECRETED PROTEINS AND HOST ADHESION RECEPTORS

T. gondii uses several adhesion receptors to build a scaffold of host cellular molecules and cellular matrix during the invasion steps (Table 1). TgRON4 is exported to the cytosolic side of the host cell where it can associate with β-tubulin, the cortical host cytoskeleton [43]. TgRON4 interacts with glypican 1, one of the components of the host cell surface, during *Toxoplasma* invasion [42]. In one study, the ability of *T. gondii* to infect Chinese hamster ovary (CHO) cells deficient in sialic acids was reduced by 26.9% compared to wild-type cells, indicating that sialic acid is critical for the attachment and invasion of *T. gondii* [35]. *T. gondii* microneme protein 1 (TgMIC1) forms a macromolecular complex with TgMIC4 and TgMIC6. Single deletion of the *TgMIC1* gene significantly

Table 1. The interactions between secreted proteins and host cell receptors during the invasion of *T. gondii*

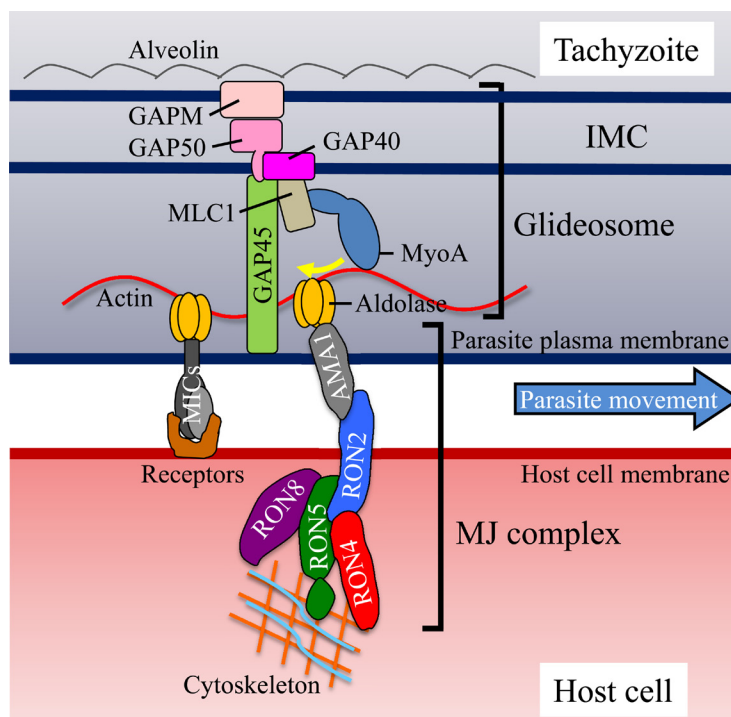| Secreted protein	Host cell receptor	References
MIC1	Sialic acid	[5]
MIC4	Galactose	[32]
MIC13	Sialic acid	[12]
RON4	β-tublin	[43]
	Heparan sulfate	[42]
P104	Chondrontin sulfate	[18]
MCP4a	HSC70	[17]

Fig. 3. Details of the MJ in Fig. 2. The glideosome-associated proteins (GAP40, GAP45, and GAP50) form a complex with GAPM in association with alveolin. MLC1 interacts with MyoA with GAP45. This motor complex is anchored to the inner membrane complex (IMC) by GAP40 and GAP50. The small arrow indicates the direction of the MyoA power stroke. The MJ complex is formed by the AMA1-RON complex (RON2, RON4, RON5, and RON8) complex and MIC protein-host cell surface receptors, connected by aldolase. The MJ complex links the host cytoskeletons.

decreases *T. gondii* invasion of host cells, suggesting an essential role for TgMIC1 in host cell attachment and invasion [10]. Structural analysis of TgMIC1 revealed a novel cell-binding motif called MARR (microneme adhesive repeat region), which provides a specialized structure for glycan discrimination [5]. Structural analysis of TgMIC4 revealed its binding specificity to a variety of galactose-containing carbohydrate ligands [32].

Carbohydrate microarray analyses have shown that TgMIC13, TgMIC1, and its homologue *Neospora caninum* MIC1, share a preference for α2-3- over α2-6-linked sialyl-N-acetyllactosamine sequences [12]. P104, a PAN/apple domain-containing protein expressed at the apical end of the extracellular parasite, functions as a ligand in the attachment of *T. gondii* to chondroitin sulfate or other receptors on the host cell, facilitating invasion by the parasite [18]. Immunoprecipitation analyses confirmed the interaction of heat shock cognate protein 70 (HSC70) with *T. gondii* MAR domain-containing protein 4a (TgMCP4a) [17].

THE GLIDEOSOME

In the *T. gondii* tachyzoite, the involvement of the myosin motor in the glideosome machinery powers motility and invasion (Fig. 3). The conserved, short single-headed myosin heavy chain A (MyoA) was identified in *T. gondii* as the motor that was able to generate the rearward traction force critical for motility and entry into host cells [34]. The glideosome is composed of TgMyoA [19], a myosin light chain (TgMLC1), and three gliding-associated proteins, TgGAP45, TgGAP50 [14], and TgGAP40 [11]. TgGAP45 spans the space between the inner membrane complex and the parasite plasma membrane. This motor complex is anchored to the inner membrane complex by TgGAP40, TgGAP50, and GAPs with multiple-membrane spans (TgGAPMs) via an association with alveolin [8]. The connection between the glideosome and adhesion is mediated by aldolase through its actin-binding activity [22]. The micronemal proteins, TgMIC2 and TgAMA1, have been shown to bind to aldolase [6].

CALCIUM SIGNALING

A spike in calcium concentration can mediate signaling by activating specific kinases and coordinating microneme secretion, and thereby has an effect on gliding motility, invasion, and egress [30]. A conditional knockout study revealed the precise function of *T. gondii* calcium-dependent protein kinase 1 (TgCDPK1) in the calcium-dependent micronemal secretion steps, which are related to egress and invasion [31]. TgCDPK3 is important for egress [13, 33], and the initiation of motility, because it phosphorylates TgMyoA. *T. gondii* calmodulin-like domain protein kinase isoform 3 (TgCDPKif3: TgCDPK1_2 in ToxoDB (http://toxodb.org/)

annotation) is expressed in tachyzoites and localizes to the apical end under extracellular conditions. An *in vitro* kinase assay demonstrated that TgCDPKif3 can phosphorylate Aldolase 1 [40], a component of the glideosome. More than 20 CDPK-related kinases are encoded in the *T. gondii* genome [36], and many of them may also contribute to invasion. For example, *T. gondii* cGMP-dependent protein kinase (TgPKG) is involved in invasion. TgGAP45, which is a member of the glideosome complex, is needed for active host cell penetration and is also phosphorylated in invasive parasites [15]. An *in vitro* kinase assay demonstrated that *T. gondii* calmodulin-dependent protein kinase-related kinase (TgCaMKrk) can phosphorylate TgGAP45 [25].

CONCLUSIONS

The *T. gondii* invasion machinery consists of the moving junction, the glideosome, and parasite-secreted proteins and their corresponding receptors, which unite the moving junction with the glideosome. Signal transduction, mainly calcium signaling, generates the power for the gliding mobility. As the basis for movement is the MyoA/actin motor for *T. gondii*, one of Apicomplexa, this machinery for movement is specific to some Apicomplexa and interesting. The motility systems including motor complex are also critical for other species to survive. Apicomplexa has a specific motor system. The analysis on Apicomplexan motility system may contribute more knowledge for the motility systems of other species. All of the components of the moving junction and the glideosome have not yet been elucidated. The discovery of more of these components and further analyses of the working system and signal transduction events involving these components are needed. The information obtained will assist in the development of new effective drugs that inhibit invasion and disrupt the Apicomplexan life cycle. Such knowledge would also contribute anti-protozoan strategies including that for malaria.

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