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How Apicomplexa Parasites Secrete and Build Their Invasion Machinery

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Abstract

Apicomplexa are obligatory intracellular parasites that sense and actively invade host cells. Invasion is a conserved process that relies on the timely and spatially controlled exocytosis of unique specialized secretory organelles termed micronemes and rhoptries. Microneme exocytosis starts first and likely controls the intricate mechanism of rhoptry secretion. To assemble the invasion machinery, micronemal proteins—associated with the surface of the parasite—interact and form complexes with rhoptry proteins, which in turn are targeted into the host cell. This review covers the molecular advances regarding microneme and rhoptry exocytosis and focuses on how the proteins discharged from these two compartments work in synergy to drive a successful invasion event. Particular emphasis is given to the structure and molecular components of the rhoptry secretion apparatus, and to the current conceptual framework of rhoptry exocytosis that may constitute an unconventional eukaryotic secretory machinery closely related to the one described in ciliates.

Contents

INTRODUCTION

Apicomplexa parasites such as *Plasmodium*, *Toxoplasma*, and *Cryptosporidium* prevail as pathogens with major health and socioeconomic importance worldwide, especially in developing countries. Every year, *Plasmodium* parasites account for more than 400,000 deaths due to malaria (161). *Toxoplasma gondii* is one of the most widespread human pathogens, infecting up to one-third of the population. Although toxoplasmosis is a not a life-threatening disease in healthy adults, it is clinically relevant in pregnant women and immunocompromised patients (117) and induces serious ocular consequences even in healthy individuals. Cryptosporidiosis, caused by *Cryptosporidium*, is one of the major causes of devastating diarrhea and malnutrition in children (137).

The apical complex is the essential armament of Apicomplexa enabling their obligate intracellular lifestyle. It comprises a unique set of secretory organelles—micronemes, rhoptries, and dense granules—involved in host cell attachment and invasion and formation of an intracellular secluded environment, namely the parasitophorous vacuole (PV), which separates the parasite from the host cell cytoplasm to facilitate its intracellular development (80). Invasion is an essential and unique mechanism shared by many apicomplexans and occurs in less than 30 s (52, 93). A hallmark of the Apicomplexa invasion process is an electron-dense structure between the parasite

and host cell membranes that is referred to as the moving junction (MJ) (1). The MJ serves as a firm anchor to the host cell, allowing it to endure shear forces during invasion, and also provides a support for the gliding machinery, an actomyosin-dependent process that propels the parasite inside the host (48). Its formation requires coordinated assembly of an intricate protein complex arising from the discharge of both microneme and rhoptry proteins (3, 17, 81, 136).

TWO TIMELY REGULATED SECRETION EVENTS TO BUILD UP THE INVASION MACHINERY

During invasion, the parasite exquisitely orchestrates the sequential exocytosis of micronemes, followed by that of rhoptries (27, 113). Not only is the timing of secretion tightly regulated, but the location where it takes place is also restricted to the apical tip of the parasite. Microneme secretion initiates prior to the exit (egress) of the parasite from the host cell and persists during gliding and attachment of the parasite to a new host cell (20, 42). Rhoptry discharge is likely triggered upon contact of the apex of the parasite with the host cell just before the beginning of invasion (43), and it is completed by the time the nascent PV is formed (113). Upon release, micronemal (MIC) proteins relocate either in the PV or to the surface of the parasite, where they facilitate disruption of the cell membrane for egress and cell traversal during tissue passage (122) and allow motility (48) and recognition and attachment to the host cell (28, 38, 100). In contrast, rhoptry proteins are injected into the host cell, where they reach different locations: the host plasma membrane (HPM), the cytosol, the nucleus, the PV membrane (PVM), or the PV lumen. Rhoptry proteins are involved in various cellular processes during the lytic cycle of Apicomplexa, notably invasion, PV formation, nutrient import, and control of cell-autonomous defenses (15, 51, 59, 72, 80, 94). When targeted to the HPM, at the attachment site of the parasite, rhoptry proteins associate with MICs to form cooperative complexes that contribute to the elaboration of the MJ [apical membrane antigen 1 (AMA1)–rhoptry neck protein (RON) complex] and possibly to the delivery of rhoptry proteins within the host (Rh5-Ripr-CyRPA complex), at least in *Plasmodium*, as detailed below (see the section titled Crossing the Host Cell Membrane Barrier). The assembly of these essential complexes at the host-parasite interface in such a short time frame (a few seconds) must be finely controlled in time and space to result in productive invasion. One ingenious yet simple way to coordinate these two sequential exocytic processes is to ensure that rhoptry discharge is triggered only following the interaction between surface-translocated MICs and host cell receptors. For instance, the binding of *Plasmodium falciparum* merozoite micronemal protein EBA175 to glycophorin A on the red blood cell (RBC) correlates with rhoptry secretion (132). Similarly, *Plasmodium berghei* and *Plasmodium yoelii* p36 and p52, two micronemal proteins of the 6-*cys* protein family (7, 89), and *Toxoplasma* MIC8 (73) are also implicated in triggering rhoptry secretion.

The sensing of external cues, i.e., a drop in pH or a rise in phosphatidic acid (PA), by intracellular parasites sets the start of microneme secretion. A complex signaling pathway is then initiated in the parasite cytoplasm, primarily relayed through the modulation of cyclic GMP (cGMP) and intracellular Ca^{2+} concentrations. These second messengers in turn will eventually lead to the release of micronemes (for a compelling review see Reference 20). Then, the parasite stops microneme exocytosis to prevent further motility and escape from the newly formed vacuole. This switch-off mechanism is controlled by cyclic AMP (cAMP) signaling and its downstream effector protein kinase A (PKA), which suppress cytosolic Ca^{2+} level elevation (69, 151). This feedback mechanism is likely activated before the parasite begins its internalization, as, with the exception of AMA1 (65), MICs are not found in the lumen of the vacuole nor associated with the surface of the intracellular part of the parasite. In contrast to micronemes, the signaling pathways governing rhoptry secretion and the interconnection with the binding of MICs to host cell receptors are

currently unknown. However, in recent years, the characterization of some key molecular actors has shed light on fusion events of the parasite plasma membrane (PPM) with both micronemes and rhoptries—a process referred to as exocytosis.

MICRONEME AND RHOPTRY EXOCYTOSIS: A SNARE-INDEPENDENT MECHANISM?

Exocytosis is classically divided into three steps: docking of vesicles at specialized sites, priming that renders them competent for exocytosis, and fusion with the plasma membrane. Some components involved in these steps are highly conserved in eukaryotes and are the core machinery for fusion. These include *N*-ethyl maleimide sensitive factor (NSF), soluble NSF adaptor proteins (SNAPs), SNAP receptors (SNAREs), the Sec1/Munc18 (SM) protein Munc18–1, and Munc13s (116). Apicomplexa genomes encode some of these factors, but recent studies showed that they are involved in organelle trafficking rather than exocytosis (8–10, 18, 26). Consequently, regulated exocytosis of apicomplexan secretory organelles might rely on different molecular effectors. Besides the aforementioned core machinery, specialized factors like synaptotagmins bring additional regulation to the exocytic process, usually via an ancestral calcium-binding domain known as the C2 domain, which also confers lipid- and protein-binding properties (116). Interestingly, some C2-containing proteins are conserved in Apicomplexa and have been shown to control secretory organelle exocytosis. For instance, the double C2 (DOC2) protein homolog in Apicomplexa, while very divergent from its mammalian counterpart, fulfills similar calcium-dependent exocytosis activation. *Plasmodium* DOC2 binds to Ca^{2+} when associated with membranes (68), and both *Toxoplasma* and *Plasmodium* DOC2 mutants are affected in microneme secretion (46). Mammalian DOC2 is a vesicular protein that interacts with many exocytosis-related proteins—including Munc13 and SNAREs—and regulates neurotransmitter release (105). In *Toxoplasma*, DOC2 may act as a broad calcium-dependent exocytosis activator, as depletion of *Tg*DOC2 also affects secretion by rhoptries and dense granules (143). Two additional C2-containing proteins, FER1 and FER2, are also involved in microneme exocytosis and rhoptry exocytosis, respectively (35, 142). They are members of the conserved family of ferlins, which typically contain five to seven C2 domains, but are part of a unique clade that originated independently from mammalian ferlins (143). Although ferlins are absent from the conventional model of calcium-dependent exocytosis established in neurons, they can play a direct role in exocytosis in other cell types, as demonstrated for otoferlin. Otoferlin is present at a specialized synaptic zone of the inner ear cells and controls the fusion of vesicles upon mechanical stimulation by sound (120). Surprisingly, otoferlin-mediated exocytosis does not require typical SNARE proteins (96), thereby supporting an alternative model of exocytosis independent of SNAREs. At present, it is still unknown whether microneme and rhoptry release may also follow this unconventional route, but the description of the rhoptry secretion machinery below fits well with this model.

MICRONEME EXOCYTOSIS

Micronemes are small, rod-like organelles clustered at the apex of apicomplexans. Distinct populations have been proposed in both *Toxoplasma* and *Plasmodium* that may correlate with different biological functions. These populations, their abundance, and their morphology have been recently covered by comprehensive reviews (6, 42). In *Toxoplasma*, micronemes accumulate beneath the conoid—an apical truncated-cone-shaped structure (99)—or at the lateral periphery. A few micronemes are also visible engaged inside the conoid (42, 57, 88, 125) and one microneme vesicle has been recently shown by cryo-electron tomography (cryo-ET) (57) to be docked at the exocytic site, close to but distinct from the rhoptry exocytic site described below (see the section titled

Structural Elements of the Rhoptry Secretion Apparatus Pinpointed by Cryo-ET). Microneme exocytosis is likely driven by a fusion process during which the vesicles completely merge with the PPM. Few molecular players of microneme exocytosis have been identified. *Toxoplasma* FER1 controls both the traffic and exocytosis of micronemes (142). Concordant with this dual function, it localizes to several compartments of the parasite's secretory pathway as well as to an apical spot of the conoid that may correspond to the microneme exocytosis site. Interestingly, the ortholog of *Tg*FER1 in the rodent malaria model, *P. berghei* ferlin-like protein (*Pb*FLP), is crucial for gamete egress (98), a process relying on the exocytosis of microneme-like vesicles called osmiophilic bodies and egress vesicles. Thus, the phenotype of a *Pb*FLP mutant supports a broad and conserved function of FER1 in microneme and microneme-like organelle exocytosis. Transporters have also been reported as required for microneme secretion, although their precise function during this process remains elusive (60, 71). Two pleckstrin-homology (PH) domain–containing proteins conserved in Apicomplexa are crucial for microneme exocytosis, namely APH and PH2. *Tg*APH decorates the surface of micronemes, where it is ideally positioned to sense and bind PA produced at the inner leaflet of the PPM (23). By doing so, APH clusters PA molecules at the lipid bilayer, facilitating the close apposition of micronemes with the PPM and ultimately providing a stable molecular scaffold for the subsequent recruitment of priming and fusion molecular players (41), such as FER1 and DOC2 (46, 142). *Pf*PH2 is an apically localized protein of the merozoite with a phosphoinositide-binding property, which controls exocytosis of only a subset of micronemes containing *Pf*EBA proteins (45). Interestingly, in the rodent malaria parasite *P. yoelii*, APH controls the secretion not of EBA proteins but rather other MIC proteins, including AMA1 and MTRAP (29). This suggests that in *Plasmodium*, APH and PH2 might have a mutually exclusive role to regulate exocytosis of different sets of microneme vesicles. Apart from PA, other lipids are important for microneme secretion. In *T. gondii*, maintenance of the phosphatidylserine (PS) asymmetry by the flippase ATP2A (a P type-IV ATPase also named P4-ATPase1 in Reference 31) is crucial for efficient microneme exocytosis (19). The protein candidates binding PS enriched at the inner leaflet of the plasma membrane remain to be identified. To conclude, while the core components of exocytosis described in mammals are not shared within Apicomplexa, the molecular players regulating microneme exocytosis identified so far support a similar lipid- and calcium-dependent process.

A UNIQUE ALVEOLATA SECRETION MACHINERY PROMOTES INJECTION OF RHOPTRY PROTEINS INTO THE HOST CELL

Apicomplexa Rhoptries: A Unique Shape

The number of rhoptries varies among apicomplexan parasites and even between different developmental stages within a given species. *T. gondii* tachyzoites possess 8 to 12 clustered together at the apex (43), *P. falciparum* merozoites have 2, and sporozoites have 2 to 4 (77, 124), while ookinetes, a noninvasive stage, are devoid of them. *Cryptosporidium parvum* sporozoites have a single rhoptry (88). However, rhoptries are morphologically similar, with a unique club shape comprising a narrow tubular duct or neck that opens at the anterior pole of the parasite and a posterior sac-like body or bulb (43, 61). These delimited rhoptry regions define two classes of luminal proteins, the rhoptry neck (RON) proteins and the rhoptry bulb (ROP) proteins (22, 80, 86, 119). These proteins are released sequentially upon exocytosis to fulfill distinct functions. RONs are secreted first and mainly used for invasion, whereas ROPs contribute to postinvasion processes (37, 38, 43, 51, 59, 80, 94). This subcompartmentation of RONs/ROPs is clearly visible in *Toxoplasma* tachyzoites and *Plasmodium* merozoites but is less obvious for *Plasmodium* sporozoites, which may reflect distinct protein secretion mechanisms (145). How rhoptries maintain their shape and dimorphism

is currently unknown, but mutants that lose this neck/bulb organization (30, 60, 129) fail to invade host cells, supporting a crucial link between rhoptry structure and exocytosis. Despite the lack of physical separation between the neck and the bulb, EM reveals a region of intermediate density that connects the electron-dense neck to the less electron-dense bulb (82). Freeze-fracture studies of *Toxoplasma* rhoptries also revealed pore-like structures along the bulb and particles arranged in a parallel row along the entire surface of the organelle (82). Recently, cryo-ET of both *Toxoplasma* and *Cryptosporidium* rhoptries uncovered another layer of structural complexity specifically in the neck region. It revealed a short anterior tapering tip followed by two types of membrane-associated helical filaments that delineate anterior and posterior neck regions (88) (**Figure 1**), as also shown by expansion microscopy (83). These filamentous arrangements may contribute to the proper allocation of the rhoptry proteins to the different subcompartments of the organelle.

Structural Elements of the Rhoptry Secretion Apparatus Pinpointed by Cryo-ET

Unlike micronemes, rhoptries are predocked at the PPM (88). In *Toxoplasma*, *Cryptosporidium*, and *Plasmodium*, cryo-ET showed that rhoptries are not in direct apposition with the PPM but in contact with an intermediate vesicle called the apical vesicle (AV) (5, 88, 90), previously identified in *Sarcocystis* (107), *Eimeria* (44), and *Toxoplasma* (108). A pair of rhoptries in *T. gondii* and *P. falciparum*, and the single rhoptry in *C. parvum*, are linked to the AV. Via this AV, rhoptries are connected to a rosette of intramembranous particles embedded in the PPM that represent the docking site for rhoptry exocytosis (5, 88, 90) (**Figure 1**). This unique arrangement requires two fusion events: the rhoptries with the AV, and the AV with the PPM, as discussed below. The AV has a characteristic shape and size that differ in *Plasmodium*, *Cryptosporidium*, and *T. gondii*, where it is circular, teardrop-shaped, and nearly ellipsoid, respectively (88, 90). The docking of the AV to the PPM is sustained by an elaborate multicomponent structure named the rhoptry secretory apparatus (RSA), conserved in Apicomplexa (88, 90). The particular differences observed between the RSA structures of *Toxoplasma*, *Plasmodium*, and *Cryptosporidium* (90) may reflect adaptations in the molecular mechanisms for their specific parasitic activities. **Figure 1*b*,*c*** details the RSA and the possible functions of its different elements. The RSA is a prime candidate to exert the mechanical force needed to bring opposing membranes (the AV membrane and PPM) in proximity, allowing their fusion and thus rhoptry exocytosis. It defines a new class of secretion machinery in pathogenic eukaryotes that is likely conserved within Alveolata, a large group of unicellular eukaryotes including Apicomplexa, Ciliata, Dinoflagellata, Chromerida, and Perkinsozoa.

Rhoptry Exocytosis Relies on an Alveolata Conserved Machinery

The rosette was first described by freeze-fracture in the late 1970s in both Ciliata (*Paramecium* and *Tetrahymena*) and Apicomplexa (*Toxoplasma*, *Eimeria*, *Plasmodium*, and *Sarcocystis*) (reviewed in 134). This structure is essential for exocytosis of secretory organelles in Ciliata (trichocysts in *Paramecium* and mucocysts in *Tetrahymena*). The genes required for rosette assembly or for exocytosis per se were named *nd*, for "nondischarged," in *Paramecium*. Interestingly, comparative genomic analysis evidenced the conservation of some *nd* genes within Alveolata, particularly *nd6* and *nd9*, and reverse-genetics studies of these *nd* genes in *Toxoplasma* and *P. falciparum* demonstrated that the rosette is the site for rhoptry exocytosis in Apicomplexa (5). Nd9 depletion led to a strong decrease in the number of rosettes visible at the apex of *T. gondii* tachyzoites (5) and to drastic structural defects of both RSA and AV anchoring (88). This finding establishes the link between the formation of the rosette and the exocytosis of the rhoptries, highlighting the importance of the RSA and AV docking to the PPM in this process. In *Toxoplasma*, Nd6 and Nd9 are part of

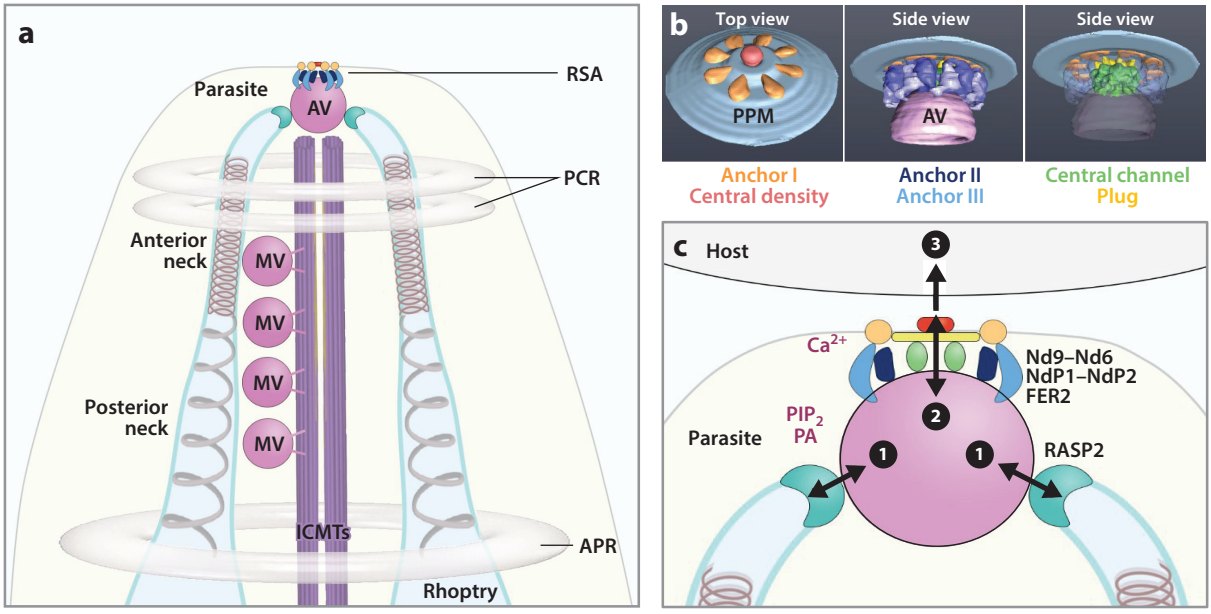

Figure 1

A multifaceted secretion machinery supports rhoptry protein exocytosis. (*a*) Scheme of *Toxoplasma gondii* apex with a focus on the rhoptry neck and the rhoptry secretion machinery structures. The anterior and posterior regions of two rhoptry necks are represented with their respective membrane-associated filaments. The anterior tapering tips of the two rhoptries are in close contact with an AV via rhoptry tip densities (*teal*). The AV is anchored to the PPM via the RSA. Three to five vesicles, similar to AVs, are associated with a pair of ICMTs. These MVs could serve as new AVs by moving along the ICMTs, allowing the reloading of a new RSA for subsequent rounds of rhoptry secretion. (*b*) 3D display of the PPM, RSA, and AV in *T. gondii*. The RSA is a complex ultrastructure that comprises a central density, three anchors (anchors I, II, and III), and an anterior central channel between the AV and the PPM. The central density is exposed on the extracellular side, where it sits on top of a cytoplasmic structure with radiating spokes (*yellow plug*), and on an anterior channel (*green*) located between the AV and the plug. The central density and anchor I correspond to the apical rosette previously spotted by freeze-fracture. The central density may be involved in the establishment of a transient pore within the PPM. Anchor I might have a structural role, as it physically connects the PPM to the AV via anchors II and III. The peripheral contact of anchors II and III with the AV may have a double function: rigid anchoring of the AV and prevention of spontaneous membrane fusion. Upon signaling, the radial dispersion or torque motion of anchor I in the RSA could exaggerate the twist existing in anchors II and III, thereby pulling the AV closer to the PPM for fusion. (*c*) Close-up view of the molecular actors involved in the mechanism of rhoptry secretion. RASP2, associated with the tip of the neck, binds to PA and PIP_2. These signaling lipids might trigger fusion between the rhoptry necks and the AV (❶). An Alveolata-specific complex (Nd9, Nd6, NdP1, NdP2, FER2) is involved in assembly of the RSA and fusion of the AV with the PPM (❷). Given the presence of calcium lipid-binding domains on NdP2 and Fer2, calcium may act as a trigger for the fusion step. The molecular players responsible for the export of the rhoptry content into the host are unknown (❸). Abbreviations: APR, apical polar ring; AV, apical vesicle; ICMT, intraconoidal microtubule; MV, microtubule-associated vesicle; Nd6, nondischarged 6; NdP1, Nd partner 1; PA, phosphatidic acid; PCR, preconoidal ring; PIP_2, phosphatidylinositol 4,5-bisphosphate; PPM, parasite plasma membrane; RASP2, rhoptry apical secretion protein 2; RSA, rhoptry secretion apparatus. Panel *b* adapted with permission from Reference 88.

a molecular complex comprising other factors: NdP1 (Nd partner 1), NdP2 (Nd partner 2), and FER2. NdP1 and Ndp2 are conserved and unique to Alveolata. They are equally important for exocytosis of mucocysts in *Tetrahymena* and rhoptries in *Toxoplasma* (5), thus supporting the existence of a conserved Alveolata secretory machinery.

The bewildering complexity of the RSA poses new challenges in terms of understanding its assembly, composition, and how it assists the membrane fusion event between the AV and PPM. Nd6 and NdP2 are the only proteins detected at the site of exocytosis, raising the question as to whether the others might be only transiently associated or present in such a low amount that

they cannot be observed by microscopy. Nd6 may have a direct role in exocytosis, as a *Toxoplasma* Nd6 mutant is compromised in rhoptry secretion while the rosette appears normal (5). However, a subtle defect in the RSA structure is not excluded since Nd6 lacks transmembrane domains and is thus likely associated with the cytosolic part of the RSA. Nd6 may regulate fusion events through its predicted RCC1-like domain, which could act as a guanine exchange factor (GEF) toward small GTPases (58). Intriguingly, Nd9, NdP1, and NdP2 also lack transmembrane domains, making them unlikely to be structural components of the rosette, yet the rosette is disassembled in the corresponding mutants (5). Consequently, they might assist RSA assembly or stabilization. The presence in Nd9 and NdP1 of armadillo-like and leucine-rich repeats, two domains involved in protein-protein interactions, would support this hypothesis. NdP2 (5) and FER2 (35) may act as Ca^{2+} sensors through their C2 domains. In contrast to the other members of the Nd complex, FER2 contains a transmembrane domain. However, it does not accumulate at the site of exocytosis in *Toxoplasma* but is detected along the cytosolic face of the inner-membrane complex in intracellular tachyzoites and relocates to the cytosolic face of the rhoptry neck in extracellular parasites (35). The functional significance of these observations and the role of FER2 (if any) in rosette and RSA assembly require further investigation.

The conservation of the secretion process between Apicomplexa and Alveolata is not only restricted to the membrane fusion event; it also extends to the signaling events leading to exocytosis. Recent studies in *T. gondii* described membrane-bound proteins, named CRMPs (cysteine repeat modular proteins), as part of a large complex essential for rhoptry secretion and invasion (109, 131, 133). CRMPs are conserved in *Tetrahymena* and required for mucocyst release (133). Unlike Nd proteins, *T. gondii* CRMPs are not required for assembly of the RSA and are only transiently associated with the exocytic site prior to invasion (133). CRMPs and their partners contain putative host cell–binding domains, suggesting that they may act as molecular host sensors to ensure timely coordination of host cell contact and rhoptry exocytosis.

Rhoptry Exocytosis: More Than One Fusion Event

The bulk of the rhoptry organelle membrane does not become integrated into the PM upon exocytosis, as electron-lucent rhoptries, or empty rhoptries, are still visible upon invasion and connected by their apical end to the PPM (2, 24, 61, 95, 101) (**Figure 2*a***). The mechanism for their post-discharge disassembly or recycling is unknown. In *Plasmodium*, there are two rhoptries that dock and likely fuse to the AV (2, 61, 90). This event might also occur in *Toxoplasma* (95), as suggested by the merge between two rhoptries during invasion and by the docking of two rhoptries with the AV (88).

In *Cryptosporidium*, the single rhoptry can be imaged either in a prefusion state with the AV in the absence of any exocytosis trigger or not fused at all (88). In *Toxoplasma*, the AV shape changes upon calcium ionophore treatment, which might suggest a prefusion state (125). In *Plasmodium* merozoites, two additional states have been observed by cryo-ET (90), both with the AV absent. In the first one, the two rhoptries are distinct, with their necks often twisted around each other, and only one of the two rhoptry tips is docked to the RSA. In the second state, the two rhoptries are fused together and their common tip is directly docked to the RSA. In these different organizations, the RSA adopts different conformations, including the opening of a gate-like density in the rhoptry-fused state (90). These observations support the existence of a dynamic process of rhoptry docking/fusion to the AV preceding exocytosis at the PPM. Due to its location and function in *Toxoplasma*, RASP2 (rhoptry apical secretion protein 2) is a prime candidate to regulate the fusion event of the rhoptry to the AV (138) (**Figure 1*c***). This protein, unique and conserved among Apicomplexa, caps the extremity of the rhoptry neck on its cytosolic face, where it interacts with RASP1 and RASP3. Together they might correspond to the distinct density detected

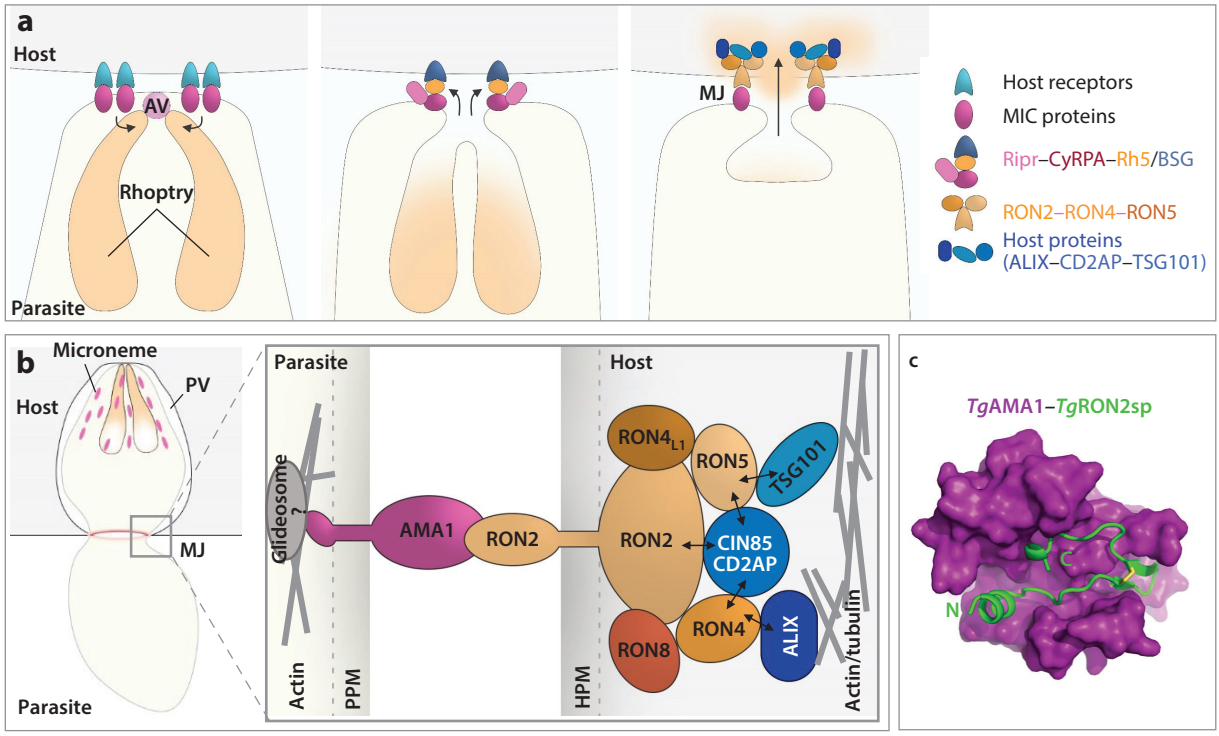

Figure 2

Cooperative roles of microneme and rhoptry proteins for invasion. (*a*) Rhoptry secretion relies on host cell attachment mediated by recognition of host cell receptors by MICs (*left*). The two rhoptry necks fuse with the AV and with the PPM. Rhoptry neck proteins are then released and establish complexes with MICs to initiate the formation of a fusion pore in the HPM (*middle*) and then to build the MJ (*right*). In *Plasmodium falciparum*, pore formation may be dependent on formation of a stable complex between two microneme proteins, Ripr and CyRPA, and the rhoptry neck protein Rh5, which assemble at the host-parasite interface to interact with the host receptor basigin. This in turn allows delivery of the rhoptry neck proteins RON2, RON4, and RON5 into the host cell and intimate interaction of the extracellular part of the transmembrane protein RON2 with the micronemal protein AMA1. In *Toxoplasma*, the RON proteins recruit cytosolic host proteins such as ALIX, TSG101, CD2AP, and CIN85. (*b*) Close-up of the MJ complex during invasion. The MJ connects the PPM and HPM via the interaction between the ectodomain of AMA1 and the C-terminal region of RON2. On the parasite side, the cytoplasmic tail of AMA1 might be connected to the glideosome machinery. On the host side, actin and tubulin accumulate at the MJ and RONs subvert host protein networks. RON4 possesses two YP(x)nL motifs that specifically recruit two ALIX proteins. RON5 has one PPPY and two P(T/S)AP motifs that account for accumulation of TSG101 at the MJ. Moreover, RON2, RON4, and RON5 contain multiple Px(P/A)xPR motifs that bind to CIN85 and CD2AP and massively recruit CIN85/CD2AP to the MJ. (*c*) Top view of the cocrystal structures of *Toxoplasma gondii* AMA1 (*purple*) with its RON2-interacting peptide (RON2sp) (*green*). *Tg*RON2sp is anchored into the apical groove of *Tg*AMA1 in a disulfide-anchored U-shaped conformation. Abbreviations: ALIX, apoptosis-linked gene 2–interacting protein X; AMA1, apical membrane antigen 1; AV, apical vesicle; BSG, basigin; CD2AP, CD2-associated protein; CIN85, Cbl-interacting protein of 85 kDa; CyRPA, cysteine-rich protective antigen; HPM, host plasma membrane; MIC, micronemal; MJ, moving junction; PPM, parasite plasma membrane; PV, parasitophorous vacuole; Rh5, reticulocyte-binding protein homolog 5; Ripr, Rh5-interacting protein; RON2, rhoptry neck protein 2; TSG101, tumor susceptibility gene 101. Figure adapted with permission from Reference 147.

at the tip of the rhoptry by cryo-ET (88). RASP2 depletion compromises rhoptry secretion, further leading to invasion defects in both *Plasmodium* and *Toxoplasma* (138). Through its C2-like (Ca^{2+}-dependent lipid-binding) and PH-like domains, *Tg*RASP2 binds to PA and phosphatidylinositol 4,5-bisphosphate (PIP_2)—two lipids known to participate in membrane fusion events—thereby supporting a function in rhoptry docking/fusion. However, the interaction of RASP2 with

lipids is calcium independent, as its C2 domain is divergent and has lost its ability to bind calcium (138). Further studies are required to generalize the function of RASP2, as a recent study in *Plasmodium* showed that *Pf*RASP2 [named cytosolically exposed rhoptry-leaflet-interacting protein 1 (CERLI1)] localizes exclusively to the rhoptry bulb, a location that does not fit well with a function in rhoptry exocytosis (87).

The last identified factor for rhoptry secretion is *Tg*PL3, a patatin-like phospholipase present at the apical cap but also associated with the tip of the parasite in extracellular tachyzoites (159). It is mechanistically unclear how *Tg*PL3 is involved in rhoptry secretion, but its function is dependent on its phospholipase A2 (PLA2) domain. The use of a PLA2 inhibitor also impairs rhoptry discharge, further supporting a role of phospholipase in rhoptry exocytosis, although the target of this compound needs to be verified (103).

A Reload Machinery for Successive Rhoptry Discharge?

During invasion, *Toxoplasma* discharges only a subset of its rhoptries (108). Concordantly, only two are docked at the AV, while several proximal free rhoptries appear to wait for successive rounds of secretion (88). Once invasion is completed, nondischarged rhoptries are still visible at the parasite apex (95, 108). In contrast, in *Plasmodium* merozoites, no intracellular rhoptry labeling is detected by immunofluorescence following RBC invasion (113), and the rhoptries are not detected by cryo-ET (21), advocating for the simultaneous discharge of the two rhoptries.

Why would some parasite species exhibit more than two rhoptries? They might permit successive attempts of invasion in case of a failed attempt and/or may be used to inject rhoptry proteins into uninfected cells, as shown for *Toxoplasma* (75), to restrict the magnitude of parasite-specific T cell responses (32). In the case of *Plasmodium* sporozoites, the parasites undergo two successive invasion steps: The midgut-derived sporozoites first enter the mosquito salivary glands, and then upon transmission to the host, they invade hepatocytes. Both invasion processes are dependent on rhoptry secretion (47, 66, 115). Interestingly, midgut-derived sporozoites have four rhoptries (124), whereas only two are reported in sporozoites in salivary glands (77) and none are reported in hepatocytes after infection (115), suggesting that sporozoites successively discharge two rhoptries at a time.

In *T. gondii*, three to six vesicles accumulate within the conoid in close association with a pair of intraconoidal microtubules (ICMTs) (57, 88, 101, 125) (**Figure 2*a***). Referred to as microtubule-associated vesicles (MVs), they are similar in shape and size to the AV and are coated with a discontinuous layer of protein density, similar to the one observed on the AV (88). An emerging model proposes that MVs function as new AVs in subsequent rounds of rhoptry docking and exocytosis. They would move along the microtubules to reach the apical tip of the parasite, where their docking to the PPM would induce assembly of a new RSA, a process already described in *Paramecium* when secretory organelles dock to the plasma membrane (see review in 134). The involvement of microtubules in transporting MVs needs to be experimentally demonstrated, yet the requirement for the microtubule dynein motor DLC8a (dynein light chain 8a) in the proper apical docking of the rhoptries supports this model (84). This reloading process might exist in other coccidian parasites, e.g., *Eimeria* sporozoites that display several rhoptries, similar vesicles, and ICMTs (24).

Crossing the Host Cell Membrane Barrier

It is widely reported in the literature that upon exocytosis, the rhoptry content accesses the host cytosol via the formation of a pore in the HPM, but the data supporting this assumption are very scarce. A few images have captured an opening between the apical rhoptry and the HPM (24, 61, 95, 140). An ~40-nm pore has been observed by freeze-fracture in the PVM (initially HPM)

over the apical tip of the parasite during *T. gondii* invasion (43). Finally, a transient spike of HPM conductance was measured by electrophysiology when the apical end of the parasite attached to the cell, before penetration (140). This result has been interpreted as the formation of a transient opening in the HPM to allow injection of the rhoptry material inside the host.

In *P. falciparum*, a tripartite complex is speculated to be important for the formation of this pore (**Figure 2*a***). This complex results from assembly of the rhoptry neck protein Rh5 (reticulocyte-binding protein homolog 5) (63, 118) with two MICs, namely Ripr (Rh5-interacting protein) (33) and CyRPA (cysteine-rich protective antigen) (112). *Pf*Rh5, *Pf*CyRPA, and *Pf*Ripr colocalize at the site of interaction between the merozoite and the RBC. *Pf*Rh5 binds to an RBC via its direct interaction with basigin (BSG) (40). This ligand-receptor interface is essential for invasion, and Rh5 is one of the most promising target for a next-generation blood-stage vaccine against malaria (111). The resolution of an Rh5 crystal structure alone or in complex with BSG revealed a novel protein fold resembling a flat, kite-shaped architecture that exposes binding sites for BSG (34, 162). Independent functional analysis of Rh5, Ripr, and CyRPA revealed that the proteins are required for a calcium flux within the erythrocyte. This calcium signal is itself correlated with RBC echinocytosis upon invasion, a dramatic dehydration-type morphology of the RBC presumably linked to rhoptry secretion (4, 152, 157). A recent study regarding the structure and biophysical properties of the Rh5-CyRPA-Ripr complex supports their insertion into membranes (160). This study places CyRPA as a critical mediator of complex assembly and proposes that following binding to BSG, the dissociation of Rh5 and Ripr from CyRPA allows membrane insertion of the Rh5-Ripr complex and formation of a pore in the HPM (160). In addition, the Rh5 coiled-coil domain has some structural homology with the coiled-coil domain of SipB, a bacterial protein that forms part of the *Salmonella* type III secretion system, and is thus responsible for transport of bacterial effectors across the HPM (64). These observations led to the proposal that Rh5-CyRPA-Ripr might have a role in the establishment of a pore between the merozoite and the HPM, through which rhoptry proteins could be transferred. However, there are conflicting data regarding the capacity of a recombinant Rh5 protein alone or in complex to trigger calcium release within the erythrocyte (4, 160), and the biochemical properties of *Pf*Rh5 do not fit with membrane insertion and pore formation (34, 162). If the Rh5-CyRPA-Ripr complex is responsible for the formation of a pore to inject the rhoptry content in *P. falciparum*, its function cannot be extended to Apicomplexa in general nor to all *Plasmodium* species, as genes encoding Rh5 protein are restricted to the *Laverania* subgenus (49).

Alternatively, the RSA might have a direct role in pore formation, with the anterior central channel (**Figure 1*b*,*c***) being inserted into the HPM to provide a transmembrane route for rhoptry delivery (88). In line with this idea, the AV, which organizes this putative pore, is absent from *Paramecium* and *Tetrahymena*, which discharge the content of their secretory organelles into the extracellular milieu, and present in Apicomplexa that contain rhoptries that inject proteins into host cells.

BUILDING THE MOVING JUNCTION: AN APICOMPLEXA-SPECIFIC DEVICE

Moving Junction: Self-Assembly of Parasite Proteins at the Parasite-Host Interface

The MJ corresponds to an intimate attachment between the host and parasite PMs. It is restricted to the tip of the parasite at the beginning of invasion and evolves into a ring shape encircling the parasite as it progresses into the host cell (1). Once the parasite is fully internalized, the MJ eventually disassembles at the posterior end of the parasite when the PV pinches off from the HPM.

First described at the molecular level in *Toxoplasma*, this unique invasion structure is shaped by the micronemal protein AMA1 and several rhoptry neck proteins (RON2, RON4, and RON5) (3, 81, 136) (**Figure 2**). Remarkably, apicomplexan parasites that use an MJ-dependent invasion rely on this same protein complex, which is highly conserved within Apicomplexa (for reviews, see 16, 128). Additional RON components of the MJ are restricted to Coccidia, e.g., $RON4_{L1}$ and RON8 (56, 136). Upon microneme secretion, the transmembrane protein AMA1 is translocated at the parasite surface, while the subsequent discharge of rhoptries targets the RON2/RON4/RON5 proteins to the HPM (17) (**Figure 2*b***). RON2 is the only transmembrane protein of the RON complex. Its extracellular C terminus directly binds AMA1 (17, 78, 135, 150), while its large N-terminal portion localizes to the cytosolic face of the HPM, where it interacts with the other RONs (16, 56, 136).

Importantly, disrupting the AMA1-RON2 interaction using antibodies (or peptides), or by reverse genetics, stressed the crucial role of this complex to the invasion process (for reviews, see 16, 128). *Toxoplasma* AMA1 or RON2 mutants are motile and successfully attach to the host cell—likely via MIC-receptor interactions—but eventually detach from the host cell owing to a strong defect in firm MJ-dependent attachment to the host cell (79, 91). Strategic point mutations within the *Tg*RON2 binding site for *Tg*AMA1 mimic the phenotype of a RON2 knockdown, supporting the view that the AMA1-RON2 interface accounts for the structural integrity of the MJ (79). Despite being initially controversial in *Plasmodium* (review in 13, 62, 128), the role of AMA1 in establishment of the merozoite MJ is now well accepted. The loss of *Pf*AMA1 leads to a complete block in RBC invasion without preventing the merozoites from extensively interacting with the RBC membrane (36). Its functions in sporozoites' invasion of the salivary glands and hepatocytes have been recently clarified (47). AMA1 interacts with RON2 and RON4, and these proteins are required for sporozoite invasion in both the mosquito and mammalian hosts (47, 53, 66, 130, 163), supporting the view that AMA1 and RONs facilitate host cell invasion across *Plasmodium* invasive stages.

Toxoplasma gondii tachyzoites use AMA1/RON2, but interestingly, this parasite can deploy three additional AMA-RON complexes: AMA2/RON2 (79), $AMA3/RON2_{L2}$ (110), and $AMA4/RON2_{L1}$ (79, 103). The latter two are highly expressed and functional at the sporozoite stage (103, 110). Remarkably, the loss of AMA1 in tachyzoites leads to parasite adaptation, with upregulation and use of the AMA2/RON2 and AMA4 complexes (79, 103). It is still unclear why the parasite would require distinct MJ complexes for different stages and even several pairs for a given stage, but importantly, these results illustrate the diversity and flexibility of the MJ components to fulfill the invasion process. Altogether, the organization of the MJ clearly highlights a self-reliant mechanism in which the parasite provides both the ligand (AMA) and the receptor (RON) to build up a key structural feature of invasion.

Structural Details of the AMA1-RON2 Interaction Reveal an Exceptionally Stable Structure and the Separate Coevolution of the AMA1-RON2 Pairs in Apicomplexa

The co-crystal X-ray structures of AMA1 in complex with its interacting RON2 peptide (RON2sp) revealed a conserved binding paradigm in *Toxoplasma* and *Plasmodium*. In its apo form, AMA1 displays a flexible loop (DII loop) intimately associated with the base of a large hydrophobic groove (39, 106). This loop can be readily displaced upon RON2 binding, exposing the full functional binding surface of the AMA1 cleft, a prerequisite for the proper insertion of RON2 in a U-shaped conformation (147, 154). The RON2 N-terminal helix is anchored on one side of the groove and extends to a disulfide-closed β-hairpin loop (cystine loop) at the opposite side

(**Figure 2*c***), resulting in a very high-affinity interaction in the nanomolar range and representing one of the widest surfaces of interface reported so far for a binary ligand-receptor interaction bridging a pathogen to a host cell (147, 154). Globally, the co-crystal structures of other AMA-RON pairs follow the same archetype, with AMA4-RON2$_{L1}$ being the most divergent (103, 110).

The AMA1-binding site of RON2 corresponds to the most conserved region between apicomplexan RON2 orthologs. Despite this apparent conservation, there is no cross-reactivity between AMA1 and RON2 from different Apicomplexa (78, 110, 146) or with only low affinity, as observed between *P. falciparum* and *Plasmodium vivax* (153). Functional mutagenesis studies coupled to the comparison of several AMA1-RON2 binding interfaces identified the RON2 cystine loop as a major driver for the separate coevolution of AMA1-RON2 pairs within Apicomplexa (11, 147, 154). Moreover, the DII loop of AMA1 acts as a structural gatekeeper to govern RON2 selectivity to the AMA1 apical groove in *T. gondii* (102). Overall, the structural insights into AMA1-RON2 interaction revealed an exceptionally stable structure that could offset the mechanical stresses encountered by the parasite to pass through the constricted MJ ring.

AMA1-RON2 Complex: Connecting Host and Parasite Cytosolic Components

Apicomplexa gliding motility provides the mechanical force required to actively penetrate host cells (48). In this mechanism, the MJ not only needs to be firmly attached to the host cell to withstand the sheer mechanical forces associated with the parasite movement but also serves as an anchoring point to engage the parasite motility machinery. The transmembrane AMA1 and RON2 proteins expose domains to the cytosolic face of the parasite and host cell, respectively, and are therefore ideally positioned to mediate such functions. Supporting this model, accumulation of F-actin is detected on the parasite (114, 148) and host cell (54) sides of the MJ.

It has been demonstrated that the cytoplasmic tail of AMA1 is essential for invasion, although its precise function remains enigmatic (126, 149). Despite being quite divergent among Apicomplexa, AMA1 tails are functionally interchangeable between parasite species (e.g., *P. falciparum* with *P. berghei* or *P. vivax*) (149). Two conserved aromatic residues close to the C terminus of the protein (*Pf*AMA1-FW$^{602–603}$ and *Tg*AMA1-FW$^{519–520}$) are necessary for AMA1 function, as mutation of both into alanine abrogates invasion (126, 149) without affecting attachment or MJ formation in *Toxoplasma* (79). These results suggest that AMA1 also contributes to the invasion process through interactions with parasite cytosolic proteins. One such appealing partner would be the glideosome-associated connector (GAC), a central player in the gliding machinery that connects transmembrane MICs to actin filaments (67). Interestingly, the AMA1 cytoplasmic tail is also subject to posttranslational modifications. In *Plasmodium*, phosphorylation of Ser-610 by PKA induces structural changes of the tail (104) and is required to promote efficient invasion (85, 149). Correspondingly, disrupting PKA activity abrogates merozoite invasion and proteolytic shedding of AMA1 (see below) (104, 158), which may be at least partly explained by the lack of AMA1 phosphorylation. Although the significance of this phosphorylation is not yet clear, it may have a role in signaling and/or in recruiting the gliding machinery. In *Toxoplasma*, dephosphorylation of Ser-527 is induced upon binding of RON2 to AMA1, a prerequisite to achieve fully efficient invasion (76). Altogether, these results highlight the fact that AMA1 is not a simple tether but likely also exerts important functions related to invasion, such as linking the MJ to the gliding machinery or relaying crucial downstream parasite signaling. Paradoxically, while serving as a firm anchor to the host cell, transmembrane MIC proteins, including AMA1, are constitutively shed by parasite surface proteases belonging to the rhomboid family (ROM4 and ROM5) (12, 65, 121, 127) or the subtilisin family (SUB2) (36), thereby releasing their ectodomains into the extracellular milieu. One way to reconcile these two processes is based on the observation that

the binding of *Tg*RON2 to *Tg*AMA1 reduces *Tg*AMA1 rhomboid–mediated cleavage (76). The parasite–host cell connection would be specifically maintained at the MJ where it could engage with the parasite traction force to drive internalization.

On the host cell side, the RON components of the MJ are exposed to the cytosolic face of the HPM. Most of them are essential for invasion, but understanding of their respective contribution has been hampered by their intermingling as a protein complex (14, 55, 56, 78, 97, 155). Their function might depend on their phosphorylation status, as in *Toxoplasma*, RON13 phosphorylates RON4, among other rhoptry substrates, and is required for MJ assembly (83). Studies in *T. gondii* revealed direct interaction of RON4 with host cellular β-tubulin (144) and also evidenced a direct role of RONs in recruiting host proteins, namely ALIX (apoptosis-linked gene 2–interacting protein X), CIN85 (Cbl-interacting protein of 85 kDa), CD2AP (CD2-associated protein), and TSG101 (tumor susceptibility gene 101) (55) (**Figure 2**). Their accumulation at the MJ is strictly dependent on their capacity to interact with specific motifs displayed on RON proteins. Importantly, parasite mutants unable to recruit ALIX, TSG101, and CIN85/CD2AP exhibit a decrease in their invasion efficiency (55). These host factors are known to interact with each other and to form cargo-specific intermediates involved in various biological processes. Of relevance are their interaction with actin and tubulin—both reported to accumulate at the MJ (54, 141)—and their role in connecting membrane proteins to the underlying cytoskeleton to establish and maintain intercellular junctions (25, 70, 123). Therefore, they might physically anchor the RON complex to the host cortical actin cytoskeleton, and by doing so provide the necessary tensile strength required to withstand forces exerted by the parasite during host cell penetration. Furthermore, the proteins of the complex exposed to the host cell cytosol (RON4, $RON4_{L1}$, RON5, and RON8) might gate the access of host PM molecules to the PVM, working as a selective and protective barrier against fusion of the PV with the endo-lysosomal system (92).

MOVING JUNCTION FORMATION AND RHOPTRY SECRETION: ONE CANNOT BE EFFECTIVE WITHOUT THE OTHER

MJ formation requires prior exocytosis of the rhoptries to first inject the RON complex in the host cell to properly bridge the parasite to the host cell. Intriguingly, there is some evidence indicating that the reverse might also be true. In *Plasmodium*, antibodies (or peptides) targeting the AMA1-RON2 interface or the disruption of AMA1 do not prevent RBC echinocytosis, suggesting that rhoptry secretion is likely taking place (50, 129). However, during these defective invasions, ROP proteins remain associated with the surface of the RBC (50, 74, 113). Similarly, in *Toxoplasma*, conditional AMA1 and RON5 mutants show improper targeting of the rhoptry content inside the host (3, 83, 91). By maintaining a tight apposition between the PPM and HPM, the MJ may secure the continuum between the rhoptry lumen and the host cytoplasm, ensuring injection of the rhoptry content at the desired target site—the host cell. One notable observation is that upon contact with its host, *P. falciparum* AMA1 knockout merozoites trigger RBC lysis (36). This suggests a role for the MJ in the safeguarding the pore formed in the HPM and highlights the intricate dependence between the rhoptry secretion machinery and the MJ structure.

FUTURE DIRECTIONS

Recent technical breakthroughs have shed light on the structure of the RSA (88) but also more broadly on the organization of the apex of the parasites (21, 24, 57, 125, 139, 156). Some molecular players of the rhoptry exocytosis process have been identified, but further mapping of these factors onto the in situ structure of the RSA will be necessary to provide an insightful understanding of this unique eukaryotic secretion machinery. Despite these recent advances, many important

questions remain. How does the tiny space of the parasite apex accommodate both microneme and rhoptry fusion events? Following invasion, how are the empty rhoptry saccules disassembled and/or recycled? How do successive rounds of rhoptry exocytosis take place in coccidian parasites? What are the signaling events that coordinate microneme and rhoptry release? Why does the rhoptry secretion machinery display an additional layer of complexity with an AV? What is the molecular composition of the AV, and does it play a role in breaching the HPM? To what extent is the rhoptry secretion machinery conserved in Ciliata?

Beyond these burning questions, another challenge will be to directly visualize the injection apparatus using in situ cryo-EM on invading parasites. Data will not only elucidate the fold of the RSA upon exocytosis and possibly evidence of the formation of the pore within the host but also contribute to our understanding of how the MJ is organized and integrated with these structures.

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LITERATURE CITED

1. Aikawa M, Miller LH, Johnson J, Rabbege J. 1978. Erythrocyte entry by malarial parasites: a moving junction between erythrocyte and parasite. *J. Cell Biol.* 77(1):72–82
2. Aikawa M, Miller LH, Rabbege JR, Epstein N. 1981. Freeze-fracture study on the erythrocyte membrane during malarial parasite invasion. *J. Cell Biol.* 91(1):55–62
3. Alexander DL, Mital J, Ward GE, Bradley P, Boothroyd JC. 2005. Identification of the moving junction complex of *Toxoplasma gondii*: a collaboration between distinct secretory organelles. *PLOS Pathog.* 1(2):e17
4. Aniweh Y, Gao X, Hao P, Meng W, Lai SK, et al. 2017. *P. falciparum* RH5-Basigin interaction induces changes in the cytoskeleton of the host RBC. *Cell Microbiol.* 19(9):e12747
5. Aquilini E, Cova MM, Mageswaran SK, Dos Santos Pacheco N, Sparvoli D, et al. 2021. An Alveolata secretory machinery adapted to parasite host cell invasion. *Nat. Microbiol.* 6(4):425–34
6. Arredondo SA, Schepis A, Reynolds L, Kappe SHI. 2021. Secretory organelle function in the *Plasmodium* sporozoite. *Trends Parasitol.* 37(7):651–63
7. Arredondo SA, Swearingen KE, Martinson T, Steel R, Dankwa DA, et al. 2018. The micronemal *Plasmodium* proteins P36 and P52 act in concert to establish the replication-permissive compartment within infected hepatocytes. *Front. Cell. Infect. Microbiol.* 8:413
8. Ayong L, DaSilva T, Mauser J, Allen CM, Chakrabarti D. 2011. Evidence for prenylation-dependent targeting of a Ykt6 SNARE in *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* 175(2):162–68
9. Ayong L, Pagnotti G, Tobon AB, Chakrabarti D. 2007. Identification of *Plasmodium falciparum* family of SNAREs. *Mol. Biochem. Parasitol.* 152(2):113–22

10. Ayong L, Raghavan A, Schneider TG, Taraschi TF, Fidock DA, Chakrabarti D. 2009. The longin domain regulates the steady-state dynamics of Sec22 in *Plasmodium falciparum*. *Eukaryot. Cell* 8(9):1330–40
11. Bai T, Becker M, Gupta A, Strike P, Murphy VJ, et al. 2005. Structure of AMA1 from *Plasmodium falciparum* reveals a clustering of polymorphisms that surround a conserved hydrophobic pocket. *PNAS* 102(36):12736–41
12. Baker RP, Wijetilaka R, Urban S. 2006. Two *Plasmodium* rhomboid proteases preferentially cleave different adhesins implicated in all invasive stages of malaria. *PLOS Pathog*. 2(10):e113
13. Bargieri D, Lagal V, Tardieux I, Menard R. 2012. Host cell invasion by apicomplexans: What do we know? *Trends Parasitol*. 28(4):131–35
14. Beck JR, Chen AL, Kim EW, Bradley PJ. 2014. RON5 is critical for organization and function of the *Toxoplasma* moving junction complex. *PLOS Pathog*. 10(3):e1004025
15. Ben Chaabene R, Lentini G, Soldati-Favre D. 2021. Biogenesis and discharge of the rhoptries: key organelles for entry and hijack of host cells by the Apicomplexa. *Mol. Microbiol.* 115(3):453–65
16. Besteiro S, Dubremetz J-F, Lebrun M. 2011. The moving junction of apicomplexan parasites: a key structure for invasion. *Cell Microbiol*. 13(6):797–805
17. Besteiro S, Michelin A, Poncet J, Dubremetz J-F, Lebrun M. 2009. Export of a *Toxoplasma gondii* rhoptry neck protein complex at the host cell membrane to form the moving junction during invasion. *PLOS Pathog*. 5(2):e1000309
18. Bisio H, Chaabene RB, Sabitzki R, Maco B, Marq JB, et al. 2020. The ZIP code of vesicle trafficking in Apicomplexa: SEC1/Munc18 and SNARE proteins. *mBio* 11(5):e02092-20
19. Bisio H, Krishnan A, Mark JB, Soldati-Favre D. 2022. *PLOS Pathog*. 18(3):e1010438
20. Bisio H, Soldati-Favre D. 2019. Signaling cascades governing entry into and exit from host cells by *Toxoplasma gondii*. *Annu. Rev. Microbiol.* 73:579–99
21. Bisson C, Hecksel CW, Gilchrist JB, Fleck RA. 2021. Preparing lamellae from vitreous biological samples using a dual-beam scanning electron microscope for cryo-electron tomography. *J. Vis. Exp.* 174:e62350
22. Bradley PJ, Ward C, Cheng SJ, Alexander DL, Coller S, et al. 2005. Proteomic analysis of rhoptry organelles reveals many novel constituents for host-parasite interactions in *Toxoplasma gondii*. *J. Biol. Chem.* 280(40):34245–58
23. Bullen HE, Jia Y, Yamaryo-Botté Y, Bisio H, Zhang O, et al. 2016. Phosphatidic acid-mediated signaling regulates microneme secretion in *Toxoplasma*. *Cell Host Microbe* 19(3):349–60
24. Burrell A, Marugan-Hernandez V, Moreira-Leite F, Ferguson DJP, Tomley FM, Vaughan S. 2021. Cellular electron tomography of the apical complex in the apicomplexan parasite *Eimeria tenella* shows a highly organised gateway for regulated secretion. bioRxiv 2021.06.17.448283, June 17
25. Campos Y, Qiu X, Gomero E, Wakefield R, Horner L, et al. 2016. Alix-mediated assembly of the actomyosin-tight junction polarity complex preserves epithelial polarity and epithelial barrier. *Nat. Commun.* 7:11876
26. Cao S, Yang J, Fu J, Chen H, Jia H. 2021. The dissection of SNAREs reveals key factors for vesicular trafficking to the endosome-like compartment and apicoplast via the secretory system in *Toxoplasma gondii*. *mBio* 12(4):e0138021
27. Carruthers VB, Sibley LD. 1997. Sequential protein secretion from three distinct organelles of *Toxoplasma gondii* accompanies invasion of human fibroblasts. *Eur. J. Cell Biol.* 73(2):114–23
28. Carruthers VB, Tomley FM. 2008. Microneme proteins in apicomplexans. *Subcell. Biochem.* 47:33–45
29. Chaiyawong N, Ishizaki T, Hakimi H, Asada M, Kazuhide Y, Kaneko O. 2022. Distinct effects on the secretion of MTRAP and AMA1 in *Plasmodium yoelii* following deletion of acylated pleckstrin homology domain-containing protein. *Parasitol. Int.* 86:102479
30. Chasen NM, Asady B, Lemgruber L, Vommaro RC, Kissinger JC, et al. 2017. A glycosylphosphatidylinositol-anchored carbonic anhydrase-related protein of *Toxoplasma gondii* is important for rhoptry biogenesis and virulence. *mSphere* 2(3):e00027-17
31. Chen K, Günay-Esiyok Ö, Klingeberg M, Marquardt S, Pomorski TG, Gupta N. 2021. Aminoglycerophospholipid flipping and P4-ATPases in *Toxoplasma gondii*. *J. Biol. Chem.* 296:100315
32. Chen L, Christian DA, Kochanowsky JA, Phan AT, Clark JT, et al. 2020. The *Toxoplasma gondii* virulence factor ROP16 acts in cis and trans, and suppresses T cell responses. *J. Exp. Med.* 217(3):e20181757

33. Chen L, Lopaticki S, Riglar DT, Dekiwadia C, Uboldi AD, et al. 2011. An EGF-like protein forms a complex with PfRh5 and is required for invasion of human erythrocytes by *Plasmodium falciparum*. *PLOS Pathog*. 7(9):e1002199
34. Chen L, Xu Y, Healer J, Thompson JK, Smith BJ, et al. 2014. Crystal structure of PfRh5, an essential *P. falciparum* ligand for invasion of human erythrocytes. *eLife* 3:e04187
35. Coleman BI, Saha S, Sato S, Engelberg K, Ferguson DJP, et al. 2021. A member of the ferlin calcium sensor family is essential for *Toxoplasma gondii* rhoptry secretion. *mBio* 9(5):e01510-18
36. Collins CR, Hackett F, Howell SA, Snijders AP, Russell MR, et al. 2020. The malaria parasite sheddase SUB2 governs host red blood cell membrane sealing at invasion. *eLife* 9:e61121
37. Counihan NA, Kalanon M, Coppel RL, de Koning-Ward TF. 2013. *Plasmodium* rhoptry proteins: why order is important. *Trends Parasitol*. 29(5):228–36
38. Cowman AF, Tonkin CJ, Tham WH, Duraisingh MT. 2017. The molecular basis of erythrocyte invasion by malaria parasites. *Cell Host Microbe* 22(2):232–45
39. Crawford J, Tonkin ML, Grujic O, Boulanger MJ. 2010. Structural characterization of apical membrane antigen 1 (AMA1) from *Toxoplasma gondii*. *J. Biol. Chem.* 285(20):15644–52
40. Crosnier C, Bustamante LY, Bartholdson SJ, Bei AK, Theron M, et al. 2011. BASIGIN is a receptor essential for erythrocyte invasion by *Plasmodium falciparum*. *Nature* 480(7378):534–37
41. Darvill N, Dubois DJ, Rouse SL, Hammoudi PM, Blake T, et al. 2018. Structural basis of phosphatidic acid sensing by APH in apicomplexan parasites. *Structure* 26(8):1059–1071.e6
42. Dubois DJ, Soldati-Favre D. 2019. Biogenesis and secretion of micronemes in *Toxoplasma gondii*. *Cell. Microbiol.* 21(5):e13018
43. Dubremetz JF. 2007. Rhoptries are major players in *Toxoplasma gondii* invasion and host cell interaction. *Cell Microbiol*. 9(4):841–48
44. Dubremetz JF, Torpier G. 1978. Freeze fracture study of the pellicle of an eimerian sporozoite (Protozoa, Coccidia). *J. Ultrastruct. Res.* 62(2):94–109
45. Ebrahimzadeh Z, Mukherjee A, Crochetière M-È, Sergerie A, Amiar S, et al. 2019. A pan-apicomplexan phosphoinositide-binding protein acts in malarial microneme exocytosis. *EMBO Rep*. 20(6):e47102
46. Farrell A, Thirugnanam S, Lorestani A, Dvorin JD, Eidell KP, et al. 2012. A DOC2 protein identified by mutational profiling is essential for apicomplexan parasite exocytosis. *Science* 335(6065):218–21
47. Fernandes P, Loubens M, Le Borgne R, Marinach C, Ardin B, et al. 2022. The AMA1-RON complex drives *Plasmodium* sporozoite invasion in the mosquito and mammalian hosts. bioRxiv 2022.01.04.474787, Jan. 04
48. Frénal K, Dubremetz JF, Lebrun M, Soldati-Favre D. 2017. Gliding motility powers invasion and egress in Apicomplexa. *Nat. Rev. Microbiol.* 15(11):645–60
49. Galaway F, Yu R, Constantinou A, Prugnolle F, Wright GJ. 2019. Resurrection of the ancestral RH5 invasion ligand provides a molecular explanation for the origin of *P. falciparum* malaria in humans. *PLOS Biol.* 17(10):e3000490
50. Geoghegan ND, Evelyn C, Whitehead LW, Pasternak M, McDonald P, et al. 2021. 4D analysis of malaria parasite invasion offers insights into erythrocyte membrane remodeling and parasitophorous vacuole formation. *Nat. Commun.* 12(1):3620
51. Ghosh S, Kennedy K, Sanders P, Matthews K, Ralph SA, et al. 2017. The *Plasmodium* rhoptry associated protein complex is important for parasitophorous vacuole membrane structure and intraerythrocytic parasite growth. *Cell. Microbiol.* 19(8):e12733
52. Gilson PR, Crabb BS. 2009. Morphology and kinetics of the three distinct phases of red blood cell invasion by *Plasmodium falciparum* merozoites. *Int. J. Parasitol.* 39(1):91–96
53. Giovannini D, Spath S, Lacroix C, Perazzi A, Bargieri D, et al. 2011. Independent roles of apical membrane antigen 1 and rhoptry neck proteins during host cell invasion by Apicomplexa. *Cell Host Microbe* 10(6):591–602
54. Gonzalez V, Combe A, David V, Malmquist NA, Delorme V, et al. 2009. Host cell entry by Apicomplexa parasites requires actin polymerization in the host cell. *Cell Host Microbe* 5(3):259–72
55. Guérin A, Corrales RM, Parker ML, Lamarque MH, Jacot D, et al. 2017. Efficient invasion by *Toxoplasma* depends on the subversion of host protein networks. *Nat. Microbiol.* 2(10):1358–66

56. Guérin A, El Hajj H, Penarete-Vargas D, Besteiro S, Lebrun M. 2017. RON4L1 is a new member of the moving junction complex in *Toxoplasma gondii*. *Sci. Rep.* 7(1):17907
57. Gui L, O'Shaughnessy WJ, Cai K, Reetz E, Reese M, Nicastro D. 2022. Cryo-electron tomography of the apicomplexan invasion machinery in its native state reveals rigid body motion of the conoid and docked secretory machinery. bioRxiv 2022.04.23.489287, Apr. 24
58. Hadjebi O, Casas-Terradellas E, Garcia-Gonzalo FR, Rosa JL. 2008. The RCC1 superfamily: from genes, to function, to disease. *Biochim. Biophys. Acta Mol. Cell Res.* 1783(8):1467–79
59. Hakimi M-A, Olias P, Sibley LD. 2017. *Toxoplasma* effectors targeting host signaling and transcription. *Clin. Microbiol. Rev.* 30(3):615–45
60. Hammoudi P-M, Maco B, Dogga SK, Frénal K, Soldati-Favre D. 2018. *Toxoplasma gondii* TFP1 is an essential transporter family protein critical for microneme maturation and exocytosis. *Mol. Microbiol.* 109:225–44
61. Hanssen E, Dekiwadia C, Riglar DT, Rug M, Lemgruber L, et al. 2013. Electron tomography of *Plasmodium falciparum* merozoites reveals core cellular events that underpin erythrocyte invasion. *Cell Microbiol.* 15(9):1457–72
62. Harvey KL, Yap A, Gilson PR, Cowman AF, Crabb BS. 2014. Insights and controversies into the role of the key apicomplexan invasion ligand, Apical Membrane Antigen 1. *Int. J. Parasitol.* 44(12):853–57
63. Hayton K, Gaur D, Liu A, Takahashi J, Henschen B, et al. 2008. Erythrocyte binding protein PfRH5 polymorphisms determine species-specific pathways of *Plasmodium falciparum* invasion. *Cell Host Microbe* 4(1):40–51
64. Hayward RD, McGhie EJ, Koronakis V. 2000. Membrane fusion activity of purified SipB, a *Salmonella* surface protein essential for mammalian cell invasion. *Mol. Microbiol.* 37(4):727–39
65. Howell SA, Hackett F, Jongco AM, Withers-Martinez C, Kim K, et al. 2005. Distinct mechanisms govern proteolytic shedding of a key invasion protein in apicomplexan pathogens. *Mol. Microbiol.* 57(5):1342–56
66. Ishino T, Murata E, Tokunaga N, Baba M, Tachibana M, et al. 2019. Rhoptry neck protein 2 expressed in *Plasmodium* sporozoites plays a crucial role during invasion of mosquito salivary glands. *Cell Microbiol.* 21(1):e12964
67. Jacot D, Tosetti N, Pires I, Stock J, Graindorge A, et al. 2016. An apicomplexan actin-binding protein serves as a connector and lipid sensor to coordinate motility and invasion. *Cell Host Microbe* 20(6):731–43
68. Jean S, Zapata-Jenks MA, Farley JM, Tracy E, Mayer DCG. 2014. *Plasmodium falciparum* double C2 domain protein, PfDOC2, binds to calcium when associated with membranes. *Exp. Parasitol.* 144:91–95
69. Jia Y, Marq J, Bisio H, Jacot D, Mueller C, et al. 2017. Crosstalk between PKA and PKG controls pH-dependent host cell egress of *Toxoplasma gondii*. *EMBO J.* 36(21):3250–67
70. Johnson RI, Seppa MJ, Cagan RL. 2008. The *Drosophila* CD2AP/CIN85 orthologue Cindr regulates junctions and cytoskeleton dynamics during tissue patterning. *J. Cell Biol.* 180(6):1191–204
71. Kehrer J, Singer M, Lemgruber L, Silva PAGC, Frischknecht F, Mair GR. 2016. A putative small solute transporter is responsible for the secretion of G377 and TRAP-containing secretory vesicles during *Plasmodium* gamete egress and sporozoite motility. *PLOS Pathog.* 12(7):e1005734
72. Kemp LE, Yamamoto M, Soldati-Favre D. 2013. Subversion of host cellular functions by the apicomplexan parasites. *FEMS Microbiol. Rev.* 37(4):607–31
73. Kessler H, Herm-Götz A, Hegge S, Rauch M, Soldati-Favre D, et al. 2008. Microneme protein 8—a new essential invasion factor in *Toxoplasma gondii*. *J. Cell Sci.* 121(Part 7):947–56
74. Koch M, Baum J. 2016. The mechanics of malaria parasite invasion of the human erythrocyte—towards a reassessment of the host cell contribution. *Cell Microbiol.* 18(3):319–29
75. Koshy AA, Dietrich HK, Christian DA, Melehani JH, Shastri AJ, et al. 2012. *Toxoplasma* co-opts host cells it does not invade. *PLOS Pathog.* 8(7):e1002825
76. Krishnamurthy S, Deng B, del Rio R, Buchholz KR, Treeck M, et al. 2016. Not a simple tether: Binding of *Toxoplasma gondii* AMA1 to RON2 during invasion protects AMA1 from rhomboid-mediated cleavage and leads to dephosphorylation of its cytosolic tail. *mBio* 7(5):e00754-16
77. Kudryashev M, Lepper S, Stanway R, Bohn S, Baumeister W, et al. 2010. Positioning of large organelles by a membrane-associated cytoskeleton in *Plasmodium* sporozoites. *Cell Microbiol.* 12(3):362–71

78. Lamarque M, Besteiro S, Papoin J, Roques M, Normand BV-L, et al. 2011. The RON2-AMA1 interaction is a critical step in moving junction-dependent invasion by apicomplexan parasites. *PLOS Pathog.* 7(2):e1001276
79. Lamarque MH, Roques M, Kong-Hap M, Tonkin ML, Rugarabamu G, et al. 2014. Plasticity and redundancy among AMA-RON pairs ensure host cell entry of *Toxoplasma* parasites. *Nat. Commun.* 5(1):4098
80. Lebrun M, Carrthers VB, Cesbron-Delauw M-F. 2020. *Toxoplasma* secretory proteins and their roles in parasite cell cycle and infection. In Toxoplasma gondii: *The Model Apicomplexan; Perspectives and Methods*, ed. LM Weiss, K Kim, pp. 607–704. Amsterdam: Elsevier
81. Lebrun M, Michelin A, El Hajj H, Poncet J, Bradley PJ, et al. 2005. The rhoptry neck protein RON4 re-localizes at the moving junction during *Toxoplasma gondii* invasion. *Cell Microbiol.* 7(12):1823–33
82. Lemgruber L, Lupetti P, Souza WD, Vommaro RC. 2011. New details on the fine structure of the rhoptry of *Toxoplasma gondii*. *Microsc. Res. Tech.* 74(9):812–18
83. Lentini G, Ben Chaabene R, Vadas O, Ramakrishnan C, Mukherjee B, et al. 2021. Structural insights into an atypical secretory pathway kinase crucial for *Toxoplasma gondii* invasion. *Nat. Commun.* 12(1):3788
84. Lentini G, Dubois DJ, Maco B, Soldati-Favre D, Frénal K. 2019. The roles of Centrin 2 and Dynein Light Chain 8a in apical secretory organelles discharge of *Toxoplasma gondii*. *Traffic* 20(8):583–600
85. Leykauf K, Treeck M, Gilson PR, Nebl T, Braulke T, et al. 2010. Protein kinase A dependent phosphorylation of apical membrane antigen 1 plays an important role in erythrocyte invasion by the malaria parasite. *PLOS Pathog.* 6(6):e1000941
86. Liffner B, Balbin JM, Wichers JS, Gilberger T-W, Wilson DW. 2021. The ins and outs of *Plasmodium* rhoptries, focusing on the cytosolic side. *Trends Parasitol.* 37(7):638–50
87. Liffner B, Frölich S, Heinemann GK, Liu B, Ralph SA, et al. 2020. PfCERLI1 is a conserved rhoptry associated protein essential for *Plasmodium falciparum* merozoite invasion of erythrocytes. *Nat. Commun.* 11(1):1411
88. Mageswaran SK, Guérin A, Theveny LM, Chen WD, Martinez M, et al. 2021. In situ ultrastructures of two evolutionarily distant apicomplexan rhoptry secretion systems. *Nat. Commun.* 12(1):4983. Erratum. 2021. *Nat. Commun.* 12(1):6203
89. Manzoni G, Marinach C, Topçu S, Briquet S, Grand M, et al. 2017. *Plasmodium* P36 determines host cell receptor usage during sporozoite invasion. *eLife* 6:e25903
90. Martinez M, Chen WD, Cova MM, Molnár P, Mageswaran SK, et al. 2022. In situ structure and priming mechanism of the rhoptry secretion system in *Plasmodium* revealed by cryo-electron tomography. bioRxiv 2022.01.11.475861, Jan. 11
91. Mital J, Meissner M, Soldati D, Ward GE. 2005. Conditional expression of *Toxoplasma gondii* apical membrane antigen-1 (TgAMA1) demonstrates that TgAMA1 plays a critical role in host cell invasion. *Mol. Biol. Cell* 16(9):4341–49
92. Mordue DG, Håkansson S, Niesman I, Sibley LD. 1999. *Toxoplasma gondii* resides in a vacuole that avoids fusion with host cell endocytic and exocytic vesicular trafficking pathways. *Exp. Parasitol.* 92(2):87–99
93. Morisaki JH, Heuser JE, Sibley LD. 1995. Invasion of *Toxoplasma gondii* occurs by active penetration of the host cell. *J. Cell Sci.* 108(Part 6):2457–64
94. Nguitragool W, Bokhari AA, Pillai AD, Rayavara K, Sharma P, et al. 2011. Malaria parasite *clag3* genes determine channel-mediated nutrient uptake by infected red blood cells. *Cell* 145(5):665–77
95. Nichols BA, Chiappino ML, O'Connor GR. 1983. Secretion from the rhoptries of *Toxoplasma gondii* during host-cell invasion. *J. Ultrastruct. Res.* 83(1):85–98
96. Nouvian R, Neef J, Bulankina AV, Reisinger E, Pangršič T, et al. 2011. Exocytosis at the hair cell ribbon synapse apparently operates without neuronal SNARE proteins. *Nat. Neurosci.* 14(4):411–13
97. Nozaki M, Baba M, Tachibana M, Tokunaga N, Torii M, Ishino T. 2020. Detection of the rhoptry neck protein complex in *Plasmodium* sporozoites and its contribution to sporozoite invasion of salivary glands. *mSphere* 5(4):e00325-20
98. Obrova K, Cyrklaff M, Frank R, Mair GR, Mueller A-K. 2019. Transmission of the malaria parasite requires ferlin for gamete egress from the red blood cell. *Cell Microbiol.* 21(5):e12999
99. Pacheco NDS, Tosetti N, Koreny L, Waller RF, Soldati-Favre D. 2020. Evolution, composition, assembly, and function of the conoid in Apicomplexa. *Trends Parasitol.* 36(8):688–704

100. Paing MM, Tolia NH. 2014. Multimeric assembly of host-pathogen adhesion complexes involved in apicomplexan invasion. *PLOS Pathog*. 10(6):e1004120
101. Paredes-Santos TC, de Souza W, Attias M. 2012. Dynamics and 3D organization of secretory organelles of *Toxoplasma gondii*. *J. Struct. Biol.* 177(2):420–30
102. Parker ML, Boulanger MJ. 2015. An extended surface loop on *Toxoplasma gondii* apical membrane antigen 1 (AMA1) governs ligand binding selectivity. *PLOS ONE* 10(5):e0126206
103. Parker ML, Penarete-Vargas DM, Hamilton PT, Guérin A, Dubey JP, et al. 2016. Dissecting the interface between apicomplexan parasite and host cell: insights from a divergent AMA-RON2 pair. *PNAS* 113(2):398–403
104. Patel A, Perrin AJ, Flynn HR, Bisson C, Withers-Martinez C, et al. 2019. Cyclic AMP signalling controls key components of malaria parasite host cell invasion machinery. *PLOS Biol*. 17(5):e3000264
105. Pinheiro PS, Houy S, Sørensen JB. 2016. C2-domain containing calcium sensors in neuroendocrine secretion. *J. Neurochem.* 139(6):943–58
106. Pizarro JC, Vulliez-Le Normand B, Chesne-Seck M-L, Collins CR, Withers-Martinez C, et al. 2005. Crystal structure of the malaria vaccine candidate apical membrane antigen 1. *Science* 308(5720):408–11
107. Porchet E, Torpier G. 1977. Etude du germe infectieux de *Sarcocystis tenella* et *Toxoplasma gondii* par la technique du cryodécapage [Freeze fracture study of *Toxoplasma* and *Sarcocystis* infective stages]. *Z. Parasitenkd* 54(2):101–24
108. Porchet-Hennere E, Nicolas G. 1983. Are rhoptries of *Coccidia* really extrusomes? *J. Ultrastruct. Res.* 84(2):194–203
109. Possenti A, Cristina MD, Nicastro C, Lunghi M, Messina V, et al. 2022. Functional characterization of the thrombospondin-related paralogous proteins rhoptry discharge factor 1 and 2 unveils phenotypic plasticity in *Toxoplasma gondii* rhoptry exocytosis. bioRxiv 2022.03.02.482699, Mar. 2
110. Poukchanski A, Fritz HM, Tonkin ML, Treeck M, Boulanger MJ, Boothroyd JC. 2013. *Toxoplasma gondii* sporozoites invade host cells using two novel paralogues of RON2 and AMA1. *PLOS ONE* 8(8):e70637
111. Ragotte RJ, Higgins MK, Draper SJ. 2020. The RH5-CyRPA-Ripr complex as a malaria vaccine target. *Trends Parasitol*. 36(6):545–59
112. Reddy KS, Amlabu E, Pandey AK, Mitra P, Chauhan VS, Gaur D. 2015. Multiprotein complex between the GPI-anchored CyRPA with PfRH5 and PfRipr is crucial for *Plasmodium falciparum* erythrocyte invasion. *PNAS* 112(4):1179–84
113. Riglar DT, Richard D, Wilson DW, Boyle MJ, Dekiwadia C, et al. 2011. Super-resolution dissection of coordinated events during malaria parasite invasion of the human erythrocyte. *Cell Host Microbe* 9(1):9–20
114. Riglar DT, Whitehead L, Cowman AF, Rogers KL, Baum J. 2016. Localisation-based imaging of malarial antigens during erythrocyte entry reaffirms a role for AMA1 but not MTRAP in invasion. *J. Cell Sci.* 129(1):228–42
115. Risco-Castillo V, Topcu S, Son O, Briquet S, Manzoni G, Silvie O. 2014. CD81 is required for rhoptry discharge during host cell invasion by *Plasmodium yoelii* sporozoites. *Cell. Microbiol.* 16(10):1533–48
116. Rizo J, Xu J. 2015. The synaptic vesicle release machinery. *Annu. Rev. Biophys.* 44:339–67
117. Robert-Gangneux F, Dardé M-L. 2012. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin. Microbiol Rev*. 25(2):264–96
118. Rodriguez M, Lustigman S, Montero E, Oksov Y, Lobo CA. 2008. PfRH5: a novel reticulocyte-binding family homolog of *Plasmodium falciparum* that binds to the erythrocyte, and an investigation of its receptor. *PLOS ONE* 3(10):e3300
119. Roger N, Dubremetz JF, Delplace P, Fortier B, Tronchin G, Vernes A. 1988. Characterization of a 225 kilodalton rhoptry protein of *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* 27(2–3):135–41
120. Roux I, Safieddine S, Nouvian R, Grati M, Simmler M-C, et al. 2006. Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse. *Cell* 127(2):277–89
121. Rugarabamu G, Marq J-B, Guérin A, Lebrun M, Soldati-Favre D. 2015. Distinct contribution of *Toxoplasma gondii* rhomboid proteases 4 and 5 to micronemal protein protease 1 activity during invasion. *Mol. Microbiol.* 97(2):244–62
122. Sassmannshausen J, Pradel G, Bennink S. 2020. Perforin-like proteins of apicomplexan parasites. *Front. Cell Infect. Microbiol.* 10:578883

123. Schmidt MHH, Chen B, Randazzo LM, Bogler O. 2003. SETA/CIN85/Ruk and its binding partner AIP1 associate with diverse cytoskeletal elements, including FAKs, and modulate cell adhesion. *J. Cell Sci.* 116(Part 14):2845–55
124. Schrevel J, Asfaux-Foucher G, Hopkins JM, Robert V, Bourgouin C, et al. 2008. Vesicle trafficking during sporozoite development in *Plasmodium berghei:* ultrastructural evidence for a novel trafficking mechanism. *Parasitology* 135(Part 1):1–12
125. Segev-Zarko L, Dahlberg PD, Sun S, Pelt DM, Sethian JA, et al. 2022. Ionophore-stimulation promotes re-organization of the invasion machinery of *Toxoplasma gondii*. bioRxiv 2022.01.12.476068, Jan. 12
126. Sheiner L, Santos JM, Klages N, Parussini F, Jemmely N, et al. 2010. *Toxoplasma gondii* transmembrane microneme proteins and their modular design. *Mol. Microbiol.* 77(4):912–29
127. Shen B, Buguliskis JS, Lee TD, Sibley LD. 2014. Functional analysis of rhomboid proteases during *Toxoplasma* invasion. *mBio* 5(5):e01795-14
128. Shen B, Sibley LD. 2012. The moving junction, a key portal to host cell invasion by apicomplexan parasites. *Curr. Opin. Microbiol.* 15(4):449–55
129. Sherling ES, Perrin AJ, Knuepfer E, Russell MRG, Collinson LM, et al. 2019. The *Plasmodium falciparum* rhoptry bulb protein RAMA plays an essential role in rhoptry neck morphogenesis and host red blood cell invasion. *PLOS Pathog.* 15(9):e1008049
130. Silvie O, Franetich JF, Charrin S, Mueller MS, Siau A, et al. 2004. A role for apical membrane antigen 1 during invasion of hepatocytes by *Plasmodium falciparum* sporozoites. *J. Biol. Chem.* 279(10):9490–96
131. Singer M, Simon K, Forné I, Meissner M. 2022. A central protein complex essential for invasion in *Toxoplasma gondii*. bioRxiv 2022.02.24.481622, Feb. 24
132. Singh S, Alam MM, Pal-Bhowmick I, Brzostowski JA, Chitnis CE. 2010. Distinct external signals trigger sequential release of apical organelles during erythrocyte invasion by malaria parasites. *PLOS Pathog.* 6(2):e1000746
133. Sparvoli D, Delabre J, Penarete-Vargas DM, Mageswaran SK, Tsypin LM, et al. 2022. An apical membrane complex controls rhoptry exocytosis and invasion in *Toxoplasma*. bioRxiv 2022.02.25.481937, Feb. 25
134. Sparvoli D, Lebrun M. 2021. Unraveling the elusive rhoptry exocytic mechanism of Apicomplexa. *Trends Parasitol.* 37(7):622–37
135. Srinivasan P, Beatty WL, Diouf A, Herrera R, Ambroggio X, et al. 2011. Binding of *Plasmodium* merozoite proteins RON2 and AMA1 triggers commitment to invasion. *PNAS* 108(32):13275–80
136. Straub KW, Cheng SJ, Sohn CS, Bradley PJ. 2009. Novel components of the Apicomplexan moving junction reveal conserved and coccidia-restricted elements. *Cell Microbiol.* 11(4):590–603
137. Striepen B. 2013. Parasitic infections: time to tackle cryptosporidiosis. *Nature* 503(7475):189–91
138. Suarez C, Lentini G, Ramaswamy R, Maynadier M, Aquilini E, et al. 2019. A lipid-binding protein mediates rhoptry discharge and invasion in *Plasmodium falciparum* and *Toxoplasma gondii* parasites. *Nat. Commun.* 10(1):4041
139. Sun SY, Segev-Zarko L, Chen M, Pintilie GD, Schmid MF, et al. 2022. Cryo-ET of *Toxoplasma* gives subnanometer insight into tubulin-based structures. *PNAS* 119(6):e2111661119
140. Suss-Toby E, Zimmerberg J, Ward GE. 1996. *Toxoplasma* invasion: The parasitophorous vacuole is formed from host cell plasma membrane and pinches off via a fission pore. *PNAS* 93(16):8413–18
141. Sweeney KR, Morrissette NS, LaChapelle S, Blader IJ. 2010. Host cell invasion by *Toxoplasma gondii* is temporally regulated by the host microtubule cytoskeleton. *Eukaryot. Cell* 9(11):1680–89
142. Tagoe DNA, Drozda AA, Coppens I, Coleman BI, Gubbels M-J. 2020. *Toxoplasma* ferlin1 is a versatile and dynamic mediator of microneme trafficking and secretion. bioRxiv 2020.04.27.063628, Apr. 28
143. Tagoe DNA, Drozda AA, Falco JA, Bechtel TJ, Weerapana E, Gubbels M-J. 2021. Ferlins and TgDOC2 in *Toxoplasma* microneme, rhoptry and dense granule secretion. *Life* 11(3):217
144. Takemae H, Sugi T, Kobayashi K, Gong H, Ishiwa A, et al. 2013. Characterization of the interaction between *Toxoplasma gondii* rhoptry neck protein 4 and host cellular beta-tubulin. *Sci. Rep.* 3:3199
145. Tokunaga N, Nozaki M, Tachibana M, Baba M, Matsuoka K, et al. 2019. Expression and localization profiles of rhoptry proteins in *Plasmodium berghei* sporozoites. *Front. Cell. Infect. Microbiol.* 9:316

146. Tonkin ML, Crawford J, Lebrun ML, Boulanger MJ. 2013. *Babesia divergens* and *Neospora caninum* apical membrane antigen 1 structures reveal selectivity and plasticity in apicomplexan parasite host cell invasion. *Protein Sci*. 22(1):114–27
147. Tonkin ML, Roques M, Lamarque MH, Pugnière M, Douguet D, et al. 2011. Host cell invasion by apicomplexan parasites: insights from the co-structure of AMA1 with a RON2 peptide. *Science* 333(6041):463–67
148. Tosetti N, Dos Santos Pacheco N, Soldati-Favre D, Jacot D. 2019. Three F-actin assembly centers regulate organelle inheritance, cell-cell communication and motility in *Toxoplasma gondii*. *eLife* 8:e42669
149. Treeck M, Zacherl S, Herrmann S, Cabrera A, Kono M, et al. 2009. Functional analysis of the leading malaria vaccine candidate AMA-1 reveals an essential role for the cytoplasmic domain in the invasion process. *PLOS Pathog*. 5(3):e1000322
150. Tyler JS, Boothroyd JC. 2011. The C-terminus of *Toxoplasma* RON2 provides the crucial link between AMA1 and the host-associated invasion complex. *PLOS Pathog*. 7(2):e1001282
151. Uboldi AD, Wilde M-L, McRae EA, Stewart RJ, Dagley LF, et al. 2018. Protein kinase A negatively regulates Ca^{2+} signalling in *Toxoplasma gondii*. *PLOS Biol*. 16(9):e2005642
152. Volz JC, Yap A, Sisquella X, Thompson JK, Lim NTY, et al. 2016. Essential role of the PfRh5/PfRipr/CyRPA complex during *Plasmodium falciparum* invasion of erythrocytes. *Cell Host Microbe* 20(1):60–71
153. Vulliez-Le Normand B, Saul FA, Hoos S, Faber BW, Bentley GA. 2017. Cross-reactivity between apical membrane antgen 1 and rhoptry neck protein 2 in *P. vivax* and *P. falciparum*: a structural and binding study. *PLOS ONE* 12(8):e0183198
154. Vulliez-Le Normand B, Tonkin ML, Lamarque MH, Langer S, Hoos S, et al. 2012. Structural and functional insights into the malaria parasite moving junction complex. *PLOS Pathog*. 8(6):e1002755
155. Wang M, Cao S, Du N, Fu J, Li Z, et al. 2017. The moving junction protein RON4, although not critical, facilitates host cell invasion and stabilizes MJ members. *Parasitology* 144(11):1490–97
156. Wang X, Fu Y, Beatty WL, Ma M, Brown A, et al. 2021. Cryo-EM structure of cortical microtubules from human parasite *Toxoplasma gondii* identifies their microtubule inner proteins. *Nat. Commun.* 12(1):3065
157. Weiss GE, Gilson PR, Taechalertpaisarn T, Tham W-H, de Jong NWM, et al. 2015. Revealing the sequence and resulting cellular morphology of receptor-ligand interactions during *Plasmodium falciparum* invasion of erythrocytes. *PLOS Pathog*. 11(2):e1004670
158. Wilde M-L, Triglia T, Marapana D, Thompson JK, Kouzmitchev AA, et al. 2021. Protein kinase A is essential for invasion of *Plasmodium falciparum* into human erythrocytes. *mBio* 10(5):e01972-19
159. Wilson SK, Heckendorn J, Martorelli Di Genova B, Koch LL, Rooney PJ, et al. 2020. A *Toxoplasma gondii* patatin-like phospholipase contributes to host cell invasion. *PLOS Pathog*. 16(7):e1008650
160. Wong W, Huang R, Menant S, Hong C, Sandow JJ, et al. 2019. Structure of *Plasmodium falciparum* Rh5-CyRPA-Ripr invasion complex. *Nature* 565(7737):118–21
161. World Health Organ. 2020. *World Malaria Report 2020: 20 Years of Global Progress and Challenges*. Geneva: World Health Organ.
162. Wright KE, Hjerrild KA, Bartlett J, Douglas AD, Jin J, et al. 2014. Structure of malaria invasion protein RH5 with erythrocyte basigin and blocking antibodies. *Nature* 515(7527):427–30
163. Yang ASP, Lopaticki S, O'Neill MT, Erickson SM, Douglas DN, et al. 2017. AMA1 and MAEBL are important for *Plasmodium falciparum* sporozoite infection of the liver. *Cell. Microbiol.* 19(9):e12745