Review

Plasmodium rhoptry proteins: why order is important

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Apicomplexan parasites, including the *Plasmodium* species that cause malaria, contain three unusual apical secretory organelles (micronemes, rhoptries, and dense granules) that are required for the infection of new host cells. Because of their specialized nature, the majority of proteins secreted from these organelles are unique to Apicomplexans and are consequently poorly characterized. Although rhoptry proteins of *Plasmodium* have been implicated in events central to invasion, there is growing evidence to suggest that proteins originating from this organelle play key roles downstream of parasite entry into the host cell. Here we discuss recent work that has advanced our knowledge of rhoptry protein trafficking and function, and highlight areas of research that require further investigation.

Plasmodium rhoptry proteins are important for multiple steps of host-cell invasion

The Apicomplexan phylum comprises intracellular parasites including *Plasmodium*, *Toxoplasma*, and *Cryptosporidium*, all of which are important human pathogens. Despite great variation in the target host cell of each parasite and subsequent disease pathology, all members of this phylum share a common feature: the presence of three specialized secretory organelles. The micronemes, rhoptries, and dense granules are apically positioned structures that secrete their contents into the target host cell in a rapid and coordinated sequence of events to facilitate parasite invasion.

The invasion process of *Plasmodium* begins after initial contact between the merozoite form of the parasite and the host red blood cell (RBC); this adherence is of low affinity and reversible (reviewed in [1]). The parasite then orientates so that its apical end is juxtaposed to the RBC membrane to facilitate closer interactions, with invasion proceeding via the formation of a tight junction (TJ; see Glossary). The parasite enters the cell by pulling itself through this junction using an actin–myosin motor [2], simultaneously creating a vacuole (parasitophorous vacuole; PV) that separates it from the host-cell cytoplasm. The parasitophorous vacuole membrane (PVM) fuses to surround the invaded parasite, thus providing an environment hospitable for parasite replication [3]. Throughout these stages of invasion, the parasite sequentially discharges mediators from its three secretory organelles to facilitate entry into the host cell. Microneme proteins are the first to be released, rhoptry discharge follows, and dense granule contents are secreted once the PV has been established. Although the micronemes and dense granules are involved in establishing the early stages of invasion (including reorientation of the parasite and establishing the TJ [4]) and in modifying the host cell, respectively, rhoptry proteins have been implicated in a diverse array of functions at various stages throughout invasion; these range from merozoite adhesion, TJ formation, and establishment of the PVM as well as modification of the host cell, all of which will be explored further in this review.

The rhoptries and its constituents

The rhoptries are the most prominent of the secretory organelles in *Plasmodium* and are synthesized *de novo* during parasite development. Their evolutionary origin remains unknown, being unique to Apicomplexans and bearing little morphological resemblance to other

Glossary

Adhesins: merozoite surface proteins involved in host cell interactions and induction of invasion. Adhesins include the micronemal erythrocyte binding antigens (EBA) family, EBA175, EBA140, EBA181, and the rhoptry-localized reticulocyte-binding homologs (Rh) family, Rh1, Rh2a, Rh2b, Rh4, and Rh5.
Basigin (Ok blood group antigen, CD147, EMMPRIN, M6): an erythrocyte receptor that interacts with the rhoptry adhesin Rh5.
Invasins: a new term describing parasite ligands involved in establishing invasion mediated by tight junctions.
Parasitophorous vacuole (PV): vacuole found in infected host cells where most Apicomplexan parasites reside and develop.
RhopH complex: a high molecular weight protein complex comprising RhopH1, RhopH2, and RhopH3.
Rhoptry-associated membrane antigen (RAMA): an essential and abundant glycophosphatidyl inositol (GPI)-anchored protein that accumulates in the ER and Golgi compartments in lipid rafts prior to rhoptry development, and which is implicated in rhoptry biogenesis.
Rhoptry-associated protein (RAP) complex: a low molecular weight protein complex localized to the rhoptry bulb, and composed of heterodimers of RAP1 and RAP2, or RAP1 and RAP3.
R1 inhibitory peptide: a 20-residue peptide that is able to inhibit invasion of *P. falciparum* merozoites by binding to AMA1 and preventing TJ formation.
Sushi domains: cysteine-rich domains that typically regulate complement ligands, also known as short complement repeats (SCRs). These domains are found in the rhoptry neck protein ASP, as well as in CR1, the erythrocyte receptor for Rh4.
Tight junction (TJ): a dynamic junction formed between the apical end of the invading merozoite and the host erythrocyte and hence is often also referred to as the moving junction. The *Plasmodium* merozoite TJ is predicted to be composed of the RON complex (RON2, RON4, RON5) and the micronemal protein AMA1.

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Keywords: rhoptry; *Plasmodium*; neck; bulb; trafficking; function; biogenesis; tight junction.

1471-4922/$ – see front matter
 http://dx.doi.org/10.1016/j.pt.2013.03.003

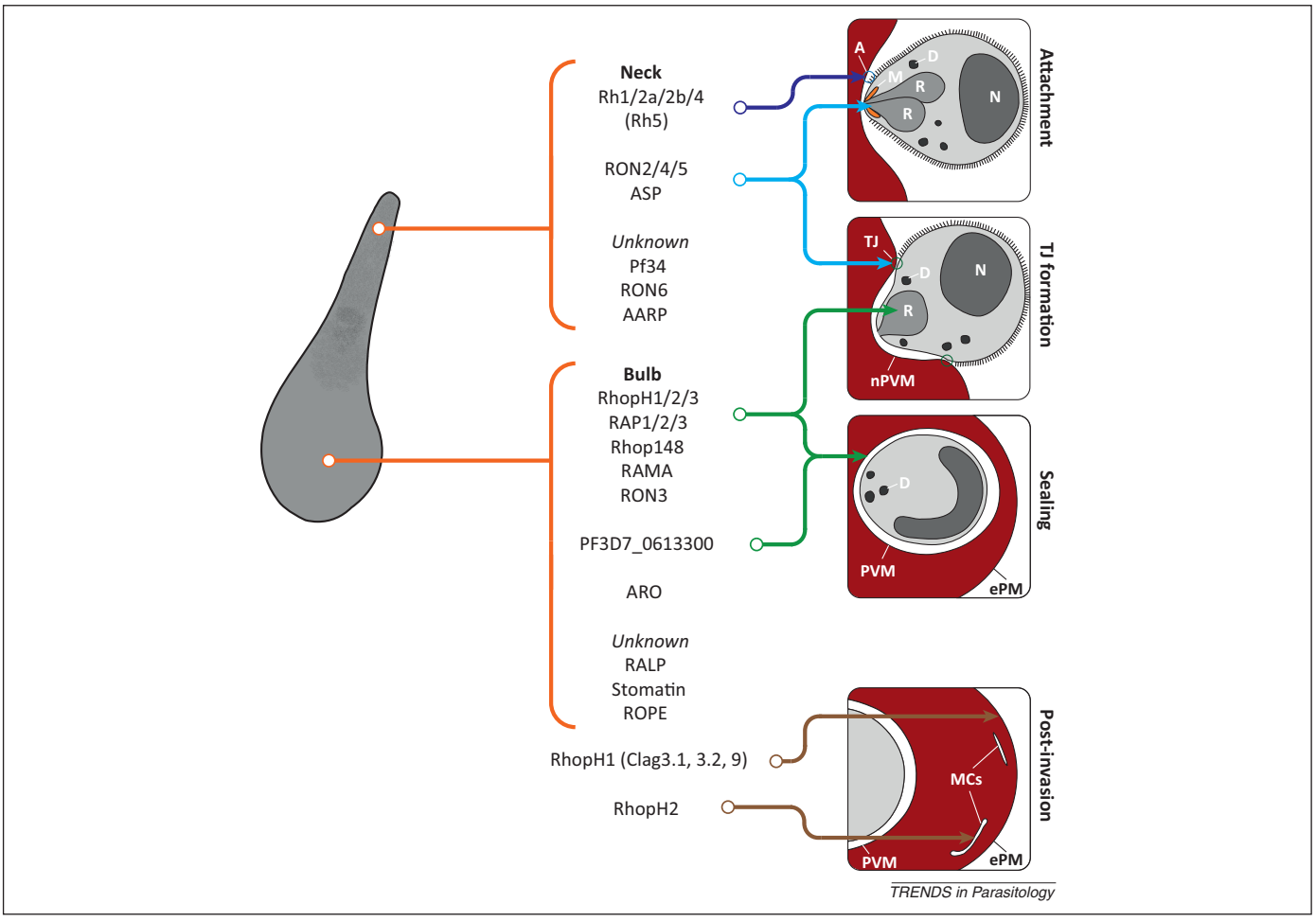

Figure 1. Rhoptry neck and bulb proteins (RONs and ROPs, respectively) during and after invasion. RONs predominantly localize to the merozoite surface adhesion site or tight junction during attachment and invasion, although the function and final localization of several RONs remain unknown. ROPs are secreted after the parasite has fully entered the erythrocyte, and may be involved in forming the parasitophorous vacuole membrane (PVM). Some ROPs have been detected at the Maurer's clefts and even at the ePM after invasion. Again, the final localization of some ROPs remains unknown.

eukaryotic organelles, although studies in *Toxoplasma gondii* reveal rhoptries possess some characteristics typical of secretory lysosomes [5]. Rhoptries consist of two distinct regions: an apical duct known as the rhoptry neck, and a larger lipid-rich region termed the rhoptry bulb, with each compartment containing distinct protein constituents (Figure 1). Some rhoptry neck proteins (RONs) are conserved between *Toxoplasma*, *Eimeria*, and *Plasmodium* [6–8], whereas rhoptry bulb proteins (ROPs) are generally distinct in each species (reviewed in [7]), presumably a consequence of the different host cells (nucleated vs non-nucleated) that each species invades. To date, more than 30 proteins have been classified as rhoptry proteins in the human malaria parasite *Plasmodium falciparum* (Figure 1) [9–11]. A further 27 potential rhoptry proteins with unknown functions have been identified through proteome analysis of purified rhoptries from the rodent species *Plasmodium yoelii* [12]. However, it is difficult to isolate rhoptries pure of other organelles, and parameters such as expression profile and sequence motifs do not easily discriminate rhoptry proteins from other apical organelle proteins. As a result, further experimental validation is required to put a more accurate figure on the number of rhoptry proteins.

Biogenesis of rhoptries and trafficking of its cargo

Surprisingly, there is still only limited understanding of how rhoptries are formed during each cell cycle, how proteins traffic to these structures, and the nature of the signals that determine their segregation within the rhoptry to ensure correct secretion during invasion. Transmission electron microscopy (TEM) studies performed on *P. falciparum* suggest rhoptries form from the progressive fusion of Golgi-derived coated vesicles, although how this is initiated is unknown [13]. Certainly, rhoptry proteins pass through the conventional eukaryotic secretory pathway, courtesy of a classical hydrophobic signal sequence [14], consistent with rhoptries forming from Golgi-derived vesicles. As the rhoptry matures, two distinctive regions separate within it, ultimately to become the rhoptry neck and bulb [13]. Although *P. falciparum* possesses a stripped-down version of the secretory pathway that exists in higher eukaryotes [15], it is unclear how the parasite delivers proteins specifically to the rhoptries (and indeed to either the neck/bulb region) given that this is not the only secretory organelle within the parasite. Most likely, the sorting of cargo also occurs within the Golgi, with subsequent packaging into trafficking vesicles specifically destined for the rhoptries (Figure 2). In *T. gondii*, the generation