Kiss and spit: the dual roles of *Toxoplasma* rhoptries

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Abstract | *Toxoplasma gondii* is a single-celled, eukaryotic parasite that can only reproduce inside a host cell. Upon entry, this Apicomplexan parasite co-opts host functions for its own purposes. An unusual set of apical organelles, named rhoptries, contain some of the machinery that is used by *T. gondii* both for invasion and to commandeer host functions. Of particular interest are a group of injected protein kinases that are among the most variable of all the *T. gondii* proteins. At least one of these kinases has a major effect on host-gene expression, including the modulation of key regulators of the immune response. Here, we discuss these recent findings and use them to propose a model in which an expansion of host range is a major force that drives rhoptry-protein evolution.

Apicomplexa
A phylum of unicellular eukaryotes that are obligate parasites and defined by a collection of apical organelles that are involved in invasion of a host cell.

Microneme
A small, cylindrical organelle that is found at the periphery of the anterior end of Apicomplexan parasites that secretes its contents onto the surface of a gliding or invading parasite.

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The phylum Apicomplexa includes a large number of obligate intracellular parasites. Among these are some notorious human and animal pathogens from genera such as *Plasmodium* (the causative agent of malaria), *Toxoplasma* (an important cause of congenital disease and infection in immuno-compromised patients[1]; see BOX 1 for a brief description of the *Toxoplasma* life cycle and the clinical spectrum of human toxoplasmosis), *Cryptosporidium* (a cause of serious gastrointestinal disease) and *Eimeria* (a major problem in the poultry industry and cause of chicken coccidiosis). The phylum is defined by the presence of an apical complex that comprises a microtubule anchoring ring through which dedicated secretory organelles release their contents[2]. There are two types of apical secretory organelle — the small, rod-shaped micronemes and the much larger, bulb-shaped rhoptries (Greek for club). FIG. 1a provides an electron micrograph of one of the asexual forms of *Toxoplasma gondii*, which clearly shows an apical cytoskeleton and a substantial complement of micronemes and rhoptries.

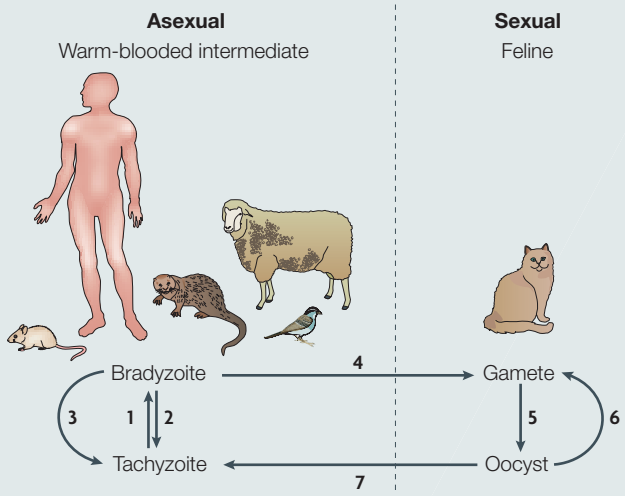
Given their conservation across much of the phylum, it has long been suspected that rhoptries have a key role in the intracellular lifestyle of these pathogens. Recently, exciting results have shed light on at least two functions of the rhoptries. In this Review, we will discuss the ultrastructure and content of rhoptries, their role in invasion and function in subverting host-cell processes, as well as the evolutionary pressures that might underlie the extreme variability of rhoptry proteins.

Rhoptry ultrastructure and content

The size, electron density and number of rhoptries varies among Apicomplexan species and between the different developmental stages of a single species. In this Review, we will mainly focus on the rapidly dividing, asexual form of *T. gondii* that is known as the tachyzoite[3]. Tachyzoites predominate during the acute stages of infection in an intermediate host, which can be virtually any warm-blooded animal (BOX 1). This breadth of host range also extends to the cellular level, as tachyzoites can invade and replicate in almost any host cell that they encounter, at least *in vitro*. *In vivo*, the cellular tropism of tachyzoites has not been well characterized as, until recently[4,5], methods to survey parasite growth in the entire animal have been lacking. Most *in vitro* studies use fibroblasts as the host cell because of their availability, the fact that they are a primary cell line (making studies on host-gene expression more relevant than in a transformed cell line) and the ease with which they can be examined by light microscopy (as they have full contact inhibition and grow as a uniform, flat monolayer).

Tachyzoites typically have ~12 rhoptries, each of which is ~2 to 3 micrometres in length[6] (FIG. 1). Using light microscopy, the rhoptries can be visualized as an elongated cluster that is present at the anterior end of the parasite and is frequently located on one side. They are visible using differential interference contrast microscopy, but are more easily observed by immunofluorescence using a range of antibodies that are specific for their protein contents. Interestingly, most of these antibodies

Box 1 | ***Toxoplasma gondii* biology**



T. gondii is an extremely common protozoan parasite of warm-blooded animals that has a host range that extends from birds to humans. Its life cycle comprises two, potentially independent cycles, one asexual and one sexual, although movement between the two is almost certainly key to efficient transmission (see the figure).

The asexual cycle (see the figure, left-hand panel) can occur in virtually any warm-blooded animal and ~100 mammalian and avian species have been documented as being infected with *T. gondii* in nature. In these 'intermediate' hosts, the parasite population initially expands by rapid proliferation of the tachyzoite form ('tachy' means fast in Greek and in this context refers to speedy replication). Once an immune response is elicited, the parasite differentiates to the more slowly growing bradyzoite form ('brady' means slow in Greek) (arrow 1). The bradyzoite form can persist for the life of the host in cyst-like structures that are present deep in the brain and other tissues. During immunosuppression (for example, in patients with AIDS) the parasite can resume rapid replication in the form of tachyzoites (arrow 2). Transmission between intermediate hosts is by the ingestion of raw or under-cooked meat and other organs that contain the infectious, encysted bradyzoites (for example, a mouse eaten by a hawk or an under-cooked lamb chop eaten by a human) (arrow 3). The sexual cycle (see the figure, right-hand panel), which begins when bradyzoite-bearing tissue from an intermediate host is ingested by a feline (arrow 4), involves gametogenesis and fertilization (arrow 5) in the gut epithelium of felines. The cat family is the only group of animals that is known to serve as a definitive host for this parasite; that is, it is the only host in which sexual reproduction occurs, although felines can also support asexual reproduction. The sexual cycle culminates in the shedding of up to 100,000,000 highly stable oocysts in the faeces of an infected cat (these oocysts are initially shed in an immature state and require approximately 2 days to mature in the environment and gain full infectivity). Mature oocysts are highly infectious and can either infect another cat (arrow 6) or, more probably, a grazing intermediate host (arrow 7) that is foraging in a farm.

T. gondii causes serious disease in humans, but this disease is primarily confined to the developing foetus of a woman who acquires her first infection during pregnancy and individuals who are immunocompromised as a result of HIV-1 infection, lymphoma or other immune-suppressive syndromes. Disease pathologies range from asymptomatic to severe and, sometimes, fatal[55]. Occasionally, otherwise healthy adults can also experience acute symptoms, especially in the eye[54]. These different disease outcomes may be related to which of the three most common strains of *T. gondii* (Type I, II or III) is responsible for the infection[46,47,55].

Rhoptry
A club-shaped secretory organelle that is found at the anterior end of Apicomplexan parasites that releases its contents during invasion; subdivided into a bulbous base and tapering-neck.

stain either the bulbous base[7,8] or the tapering neck of the rhoptry[9], but not both. It seems, therefore, that the rhoptry contents are not a random mixture but are sorted into discrete subcompartments. No internal membranes seem to separate these domains and the mechanism by which proteins are sorted to a particular region is not yet known. After translation, at least initially, rhoptry proteins move through a conventional eukaryotic secretory pathway that involves rough endoplasmic reticulum and the Golgi apparatus, but the process of rhoptry formation and the signals that ultimately target proteins to this organelle have yet to be precisely identified[10–14]. There is some evidence that rhoptries are related to exosomes[15], which are membrane-limited bodies that are extruded by some cells. This has interesting implications for the final topology of a rhoptry protein during release, as exosome formation might involve an invagination of the rhoptry membrane and, therefore, place an integral membrane protein in an inverted orientation relative to their position by alternative models[16].

There are 29 proven rhoptry proteins, of which 24 are present in the rhoptry bulb (most of these are therefore termed ROP proteins) and 5 are present in the rhoptry neck (termed RON proteins) (TABLE 1). Many of these were first discovered by proteomic analyses of purified rhoptries[9], which identified an additional 28 proteins that have yet to be verified as being truly rhoptry in origin. Given the high percentage of proteins that were verified as being rhoptry proteins from the first group that was analysed, most of these 28 proteins are probably also from this compartment. Further evidence of a rhoptry origin comes from the fact that many of these 28 proteins are paralogues of known rhoptry proteins, as are the predicted products of several additional genes that have yet to be analysed in detail (TABLE 2).

The largest rhoptry gene family shows clear homology to protein kinases[17]. The canonical member of this family is ROP2 and, in at least one instance, a member of this family (ROP18) has been confirmed to have kinase activity[17,18], although the vast majority seem to have lost the key catalytic residues and, hence, the ability to phosphorylate proteins[19] (TABLE 1). Apart from their kinase domains, these proteins seem to be unique to the *Toxoplasma* genus and its close relatives (for example, species of *Neospora*); no *bona fide* homologues have been described in the distantly related *Plasmodium* spp. This could reflect an incomplete knowledge of the rhoptry proteome in species of *Plasmodium* and/or a difference in the intracellular niches that they occupy. Other ROPs are homologous to phosphatases[20] and proteases[21,22], but several ROPs, including ROP1, ROP6 and ROP9, seem to be unique to the *Toxoplasma* genus and are of unknown function[23,24].

In contrast to the extensive ROP2 family, each of the RON proteins is encoded either by a unique gene that has no similarity to any other gene in the *T. gondii* genome (for example, RON1 and RON5) or by a gene that has one or two paralogues elsewhere in the *T. gondii* genome (for example, RON2, RON3 and RON4) (TABLE 2). The initial discovery of the sequence of the RON proteins gave no clue as to their biological function[9]. Several *T. gondii* RONs have clear orthologues in related genera, including *Plasmodium*, which suggested their involvement in processes that are common to the Apicomplexa phylum. Although their exact function has not been determined, the unusual secretion of RON4 and its subsequent migration down the length of the parasite during invasion has led to clear models about the overall role of RON4 and other RONs, as discussed below.

Finally, in addition to their protein complement, rhoptries also contain lipids[25] and this lipid complement has a high ratio of cholesterol to phospholipids. Interestingly, these lipids are sometimes present as membrane whorls inside the rhoptry organelle, which can be visualized using electron microscopy. This might explain the unusual topology of ROP proteins that are released into the host cell during invasion; as discussed below, some ROPs seem to enter the host-cell cytosol in a freely soluble form whereas other ROPs are associated with unusual vesicle-like bodies that seem to fuse with the nascent parasitophorous vacuole.

Role of rhoptries in cell invasion

The only circumstance in which rhoptries are known to secrete their contents is during the process of invasion into a host cell (FIGS 2,3; see Supplementary information S1 (movie)). The trigger for release is unknown, but it evidently depends on a direct recognition between the apical surface of the parasite and the receptor molecule (or molecules) on the host cell. The identities of the molecules on either side of this interaction are also unknown and no stimulus has yet been identified that will induce rhoptry secretion in the absence of host-cell contact. The mechanics of the fusion event that allows rhoptry protein release are a mystery. It could be as simple as fusion of the rhoptry to the parasite's plasma membrane or, perhaps, an intermediate, anterior compartment. Whatever the process, a distinct opening at the anterior-most tip of the tachyzoite is clearly observed during host-cell invasion[26] and this is presumed to be the opening through which the rhoptry contents ultimately flow.

Once released, rhoptry proteins have various destinations (FIG. 3). The RON proteins, RON2, 4 and 5, form a complex with the micronemal protein AMA1 and this multimeric complex colocalizes with the so-called moving junction (MJ)[27,28]. The MJ is a ring-like structure that represents the circular point of contact between the parasite surface and host plasma membrane[29]. During invasion, the MJ migrates down the length of the parasite (FIGS 2,3; see Supplementary information S1 (movie)). AMA1 seems to be necessary for the MJ to form, because parasites in which AMA1 expression has been reduced to ~1% of the wild type (using parasites that harbour a tetracycline-regulated copy of the *AMA1* gene) release the RONs, but the MJ fails to form and the parasites do not invade host cells[28].

The MJ might represent the mechanism by which the parasite makes contact with the host cytoskeleton. The most widely accepted model is one in which the parasite anchors itself on integral proteins of the host plasma membrane and drags them backward (relative to the parasite surface), effectively converting the host plasma membrane into parasitophorous vacuole membrane that surrounds the intracellular portion of the parasite (FIG. 2). This wrapping of the parasite in parasitophorous vacuole membrane is simultaneous with a clear forward motion of the parasite into the host cell, relative to the host-cell's normal perimeter (Supplementary information S1 (movie)), which strongly argues that the parasite must have a firm grip on something that is connected to fixed anchors within the host cell; that is, the host plasma membrane integral proteins must be connected to the host-cell cytoskeleton. Finally, the model predicts that the

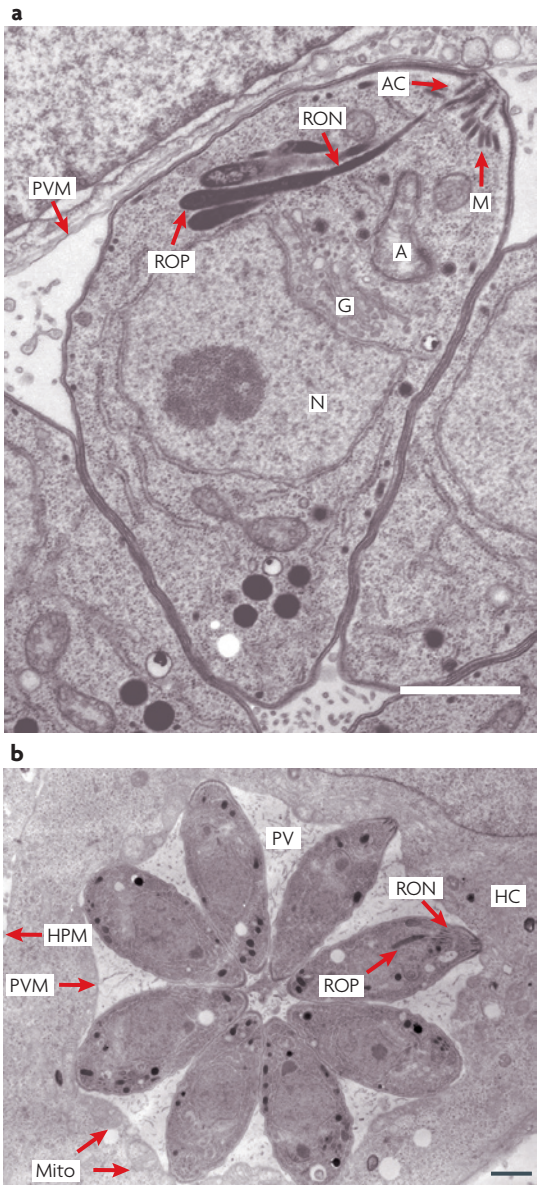


Figure 1 | ***Toxoplasma gondii* ultrastructure. a** | An intracellular *T. gondii* tachyzoite inside a parasitophorous vacuole membrane (PVM), showing the apical cytoskeleton (AC) and neighbouring micronemes (M), rhoptry bulbs (ROP) and rhoptry necks (RON). Other components of the parasite, such as the nucleus (N), the Golgi apparatus (G) and the plastid that is specific to the Apicomplexa phylum, the 'Apicoplast' (A), are shown. The scale bar represents 0.5 µm. **b** | A lower magnification of a rosette of *T. gondii* in the parasitophorous vacuole (PV) inside an infected cell (HC). The host plasma membrane (HPM), as well as host mitochondria (Mito) in close apposition to the PVM, can also be seen. The scale bar represents 1 µm. Image in part **a** reproduced, with permission, from REF. 56 © Elsevier Science. Image in part **b** reproduced, with permission, from REF. 57 © Elsevier Science.

Paralogue
A gene that shares a common evolutionary origin and has evolved in parallel with another gene that is located in the same genome or organism, typically to serve different but related functions.

Orthologue
A gene in one species that shares a common evolutionary origin with a related gene in a different species and that serves essentially the same function.

Parasitophorous vacuole
The vacuole that harbours the parasite.

Moving junction
(MJ). A migrating ring of contact between a host-cell plasma membrane and the surface of an invading parasite.

Table 1 | **Known rhoptry proteins**

Protein name	Gene identification number*; GenBank accession number	Final destination‡	Predicted coding function	Biological function	Comments	*Plasmodium falciparum*§	Refs‖
ROP1	583.m00003; M71274	PV	Unknown	Unknown	Knock-out in a type I strain is still virulent	No	23
ROP2A¶	33.m01398; Z36906	PVM	Protein kinase	Mitochondria recruitment	More adjacent genes probably exist but are missing from ToxoDB4.2	No	37
ROP2B¶	63.m00146	PVM	Protein kinase	Unknown	Should be a tandem, identical copy of ROP2A but appears as a fragment in the ME49 sequence in ToxoDB4.2	No	37,58
ROP4¶	83.m02145; Z71787 or AY662677	PVM	Non-catalytic kinase	Unknown	Phosphorylated; mis-annotated in ToxoDB4.2 as one gene (fusion with ROP7)	No	19,37
ROP5¶	551.m00238; EF466101 or DQ116423	PVM	Non-catalytic kinase	Unknown	Approximately 3 or 4 more tandem copies exist that are not present in the ME49 sequence in ToxoDB4.2	No	9,35,59
ROP7¶	83.m02145; AM056071	PVM	Non-catalytic kinase	Unknown	Mis-annotated in ToxoDB4.2 as one gene (fusion with ROP4)	No	36
ROP8¶	33.m00005; AF011377	PVM	Non-catalytic kinase	Unknown	None	No	58
ROP9	49.m00048; AJ401616	Unknown	Unknown	Unknown	Distinct from another protein named ROP9 (REF. 60), for which the sequence and gene are unidentified	No	24
ROP10	583.m05686; DQ124368	Unknown	Unknown	Unknown	None	No	9
ROP11¶	42.m03584; AAZ29607 or DQ077905	Unknown	Protein kinase	Unknown	None	No	9
ROP12	20.m08222; DQ096559	Unknown	Unknown	Unknown	None	No	9
ROP13	583.m09115; DQ096560	Unknown	Unknown	Unknown	Mis-annotated in ToxoDB4.2; encoded on the opposite strand	No	9
ROP14	583.m00692; DQ096565	Unknown	Transmembrane	Unknown	None	Yes	9
ROP15	27.m00091; DQ096561	Unknown	Unknown	Unknown	None	No	9
ROP16¶	55.m08219; DQ116422	Host nucleus	Protein kinase	Host subversion and virulence	Extremely different in *T. gondii* type I, II and III strains	No	9
ROP17¶	55.m08191; AM075203	Unknown	Protein kinase	Unknown	None	No	17
ROP18¶	20.m03896; AM075204 or EF092842	PVM	Protein kinase	Virulence	Extremely different in *T. gondii* type I, II and III strains	No	17
TgSUB2	583.m00011; AF420596	Unknown	Serine protease	ROP protein processing	None	Yes	22
Toxopain1	50.m00008; AY071839	Unknown	Cathepsin B protease	ROP protein processing	None	No	21
TgPP2C-hn	74.m00766 + 74m.00767; EF450457	Host nucleus	Protein phosphatase 2C	Unknown	Injected into host cell where it ends up in the nucleus; knock-out has a slight growth defect *in vitro*	No	20
TgNHE2	129.m00252; AY735393	Unknown	Na^+ and H^+ exchanger	Ionic homeostasis	Knock-out in type I strain is still virulent	Yes	61
Toxofilin	33.m02185; AJ132777	Unknown	Actin-binding	Unknown	Possible interference with host actin	No	62
BRP1	583.m09133	Unknown	Unknown	Bradyzoite- and merozoite-specific	Knock-out in type II strain is still virulent	No	63
TgRAB11	80.m00009	Unknown	Small GTPase	Protein trafficking	None	Yes	9
RON1	583.m11443 + 583.m00597; DQ096562	Unknown	Possibly GPI-anchored	Unknown	None	Yes	9
RON2	145.m00331; DQ096563	MJ	Transmembrane	Invasion	Covalently attached to RON4	Yes	9
RON3	42.m00026; DQ096564	Unknown	Transmembrane	Unknown	None	Yes	9
RON4	44.m06355; DQ096566	MJ	Unknown	Invasion	Covalently attached to RON2	Yes	9
RON5#	583.m09191 + 583.m09192 + 583.m00636	Possibly MJ	Unknown	Invasion	Cleaved into three major fragments	Yes	None

*The ToxoDB4.2 (*Toxoplasma gondii* Genome resource; see Further information[64]) gene identifier is provided for the *T. gondii* ME49 strain, along with the GenBank entry for (typically, but not always) the *T. gondii* RH strain, if such an entry exists. The GenBank entry should be a verified coding sequence and, therefore, encode the definitive amino-acid sequence of the primary translation product. The gene identifiers in ToxoDB4.2 are tentative predictions that in most cases have yet to be experimentally confirmed. Consequently, although they are approximately correct, the transcription start sites, splice junctions and predicted protein sequences might be incorrect. This is why some genes have multiple identifiers — for example, the large RON1 gene is currently incorrectly annotated as two different genes (indicated by the + symbol, which separates the gene identifiers listed). ‡Final location of the protein after invasion. §Significant orthologue, with homology that extends beyond the presence of a conserved enzymatic domain (for example, more than just a kinase or protease active site is conserved throughout evolution), exists in *P. falciparum*. ‖The reference that reports the definitive sequence and/or identification of this protein as a rhoptry protein. ¶Homologue of ROP2. No gene has yet been linked to the ROP2 family member that has been dubbed ROP3. ROP3 could simply be a post-translational modification of one of the known ROP2 family members or encoded by one of the ROP2-like genes for which the protein product has yet to be detected. #P. Bradley and M. Lebrun, unpublished observations.
GPI, glycophosphatidylinositol; MJ, moving junction; PV, parasitophorous vacuole; PVM, parasitophorous vacuole membrane.

Table 2 | **Suspected rhoptry proteins**

Protein name	Gene identification number*; GenBank accession number	Final destination‡	Predicted coding function	Comments	*Plasmodium falciparum*§	Refs‖
ROP6	55.m00092; AY792971	Possibly HPM	Possible protease and GPI-anchored	None	No	665
ROP2L3¶	55.m08224	Unknown	Protein kinase	Mass spectrometry data for expression**; detected in rhoptry proteome‡‡	No	9,17
ROP2L4¶	57.m01774	Unknown	Protein kinase	None	No	17
ROP2L5¶	49.m03275	Unknown	Protein kinase	None	No	17
ROP2L6¶	80.m02343	Unknown	Non-catalytic kinase	Mass spectrometry data for expression**; detected in rhoptry proteome‡‡	No	9,17
ROP2L7¶	49.m03159	Unknown	Protein kinase	None	No	None
ROP2L8¶	52.m01543	Unknown	Protein kinase	Mass spectrometry data for expression**; detected in rhoptry proteome‡‡	No	9
ROP2L9¶	55.m04788	Unknown	Protein kinase	Mass spectrometry data for expression**	No	None
ROP2L10¶	59.m06126	Unknown	Protein kinase	None	No	None
ROP2L11¶	86.m00398	Unknown	Unknown	Truncated pseudogene	No	None
ROP2L12¶	86.m00844	Unknown	Unknown	Truncated pseudogene	No	None
ROP2L13¶	25.m01746	Unknown	Non-catalytic kinase	None	No	None
RON2L1#	83.m01266	Unknown	Unknown	None	Yes	28
RON2L2#	57.m01722	Unknown	Unknown	None	Yes	28
RON3L1#	20.m03905	Unknown	Unknown	Mass spectrometry data for expression**	Yes	None
RON4L1#	52.m01582	Unknown	Unknown	Mass spectrometry data for expression**	Yes	28

*The ToxoDB4.2 (*Toxoplasma gondii* Genome resource; see Further information[64]) gene identifier is provided for the *T. gondii* ME49 strain, along with the GenBank entry for (typically, but not always) the *T. gondii* RH strain, if such an entry exists. The GenBank entry should be a verified coding sequence and, therefore, encode the definitive amino-acid sequence of the primary translation product. The gene identifiers in ToxoDB4.2 are tentative predictions that in most cases have yet to be experimentally confirmed. Consequently, although they are approximately correct, the transcription start sites, splice junctions and predicted protein sequences might be incorrect in their detail. ‡Final location of the protein after invasion. §Significant orthologue, with homology that extends beyond the presence of a conserved enzymatic domain (for example, more than just a kinase or protease active site is conserved throughout evolution), exists in *P. falciparum*. ‖Reference that originally reported this gene or protein. ¶ROP2-like (ROP2L) protein predicted in ToxoDB4.2 but not yet confirmed to be rhoptry localized and, except as noted, not even confirmed to be expressed. #RON2-, RON3- or RON4-like protein (RON2L, RON3L and RON4L, respectively) predicted in ToxoDB4.2 but not yet confirmed to be rhoptry localized and, except as noted, not even confirmed to be expressed. **Mass spectrometry has revealed that one or more peptides are present in tachyzoites based on data presented in ToxoDB4.2. ‡‡Detected by mass spectrometry in rhoptry-enriched fraction but not yet confirmed to be rhoptry-localized. The biological function of these proteins is unknown. GPI, glycophosphatidylinositol; HPM, host plasma membrane.

region of contact between the parasite and the host cell needs to be a circular band, as otherwise a gliding parasite would not be able to take a 'dive' into the host cell — it would simply keep moving along the surface. AMA1, which was first described in *Plasmodium falciparum*, might organize the MJ into a ring[28]. The association of AMA1 with RON4 is also observed in *P. falciparum*, which indicates that the overall collaboration of rhoptry RONs with micronemal AMA1 is a conserved feature of most, if not all, species of the Apicomplexa phylum[30].

How the RON proteins (RON2, 4 and 5) that form the MJ complex associate with each other is not known, although RON2 and RON4 seem to be linked by disulphide bonds[28]. How these proteins further associate with a micronemal protein such as AMA1 is also unclear; it is presumed, however, that they come into contact with one another at, or just below, the apical surface of the parasite. Likewise, the topology of the various components of the MJ complex within the membrane (or membranes) has not yet been determined. RON2 has at least 2, and possibly 3, predicted transmembrane domains, but it is unclear which membrane (host or parasite) they are embedded within. One exciting possibility is that RON2, or a different MJ protein, is inserted into the host plasma membrane during the first steps of invasion and that it then contacts the host cytoskeleton to provide the anchoring that is described above. This is analogous to a phenomenon that has been reported for enteropathogenic *Escherichia coli*, in which the bacterium inserts a protein (Tir) into the host cell that then plays a part in attachment and subsequent invasion[31]. An appealing feature of such a model is that it would explain the ability of *T. gondii* to invade almost any cell from a wide range of warm-blooded animals; if they provide their own anchor, they could make a home in almost any port, as long as they are able to make contact with a highly conserved cytoskeletal protein. Alternatively, of course, the MJ proteins could remain anchored in the parasite's plasma membrane and associate with host-plasma-membrane structures that are, in turn, anchored to the host-cell cytoskeleton. If so, a portion, or domain, of that host-plasma-membrane molecule would need to be structurally conserved among many species and cell types to explain the extraordinary range of hosts and cells that *T. gondii* can productively infect.

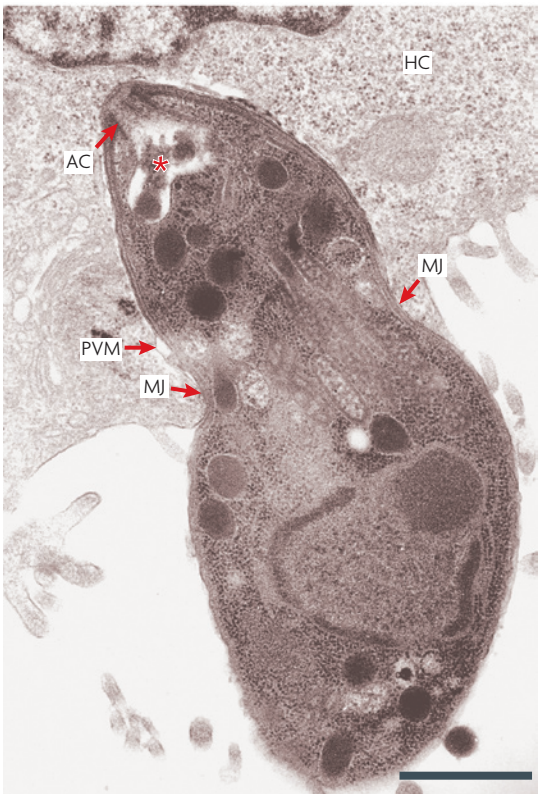


Figure 2 | ***Toxoplasma gondii* invasion.** A *T. gondii* tachyzoite invading an HeLa cell (HC). An irregularly shaped organelle that is derived from rhoptry exocytosis (asterisk) is found near the apical cytoskeleton (AC). The parasitophorous vacuole membrane (PVM) that is derived from the host cell membrane is found around the portion of the parasite that has invaded the host cell. The invasion is thought to be driven by parasite motors acting at the moving junction (MJ). The scale bar represents 0.5 µm. Image reproduced, with permission, from REF. 56 © Elsevier Science.

ROP proteins are injected into host cells

ROPs seem to have completely different functions from RONs. Like the neck proteins, ROPs are released during invasion but they do not form organized structures and are not found at the MJ. Instead, following release, they migrate to one of three general locations: the lumen of the nascent parasitophorous vacuole; the parasitophorous vacuole membrane; or the interior of the host cell (FIG. 3).

ROP1 is an example of migration to the first location: it is released during invasion and accumulates within the lumen of the nascent parasitophorous vacuole[23]. Remarkably, based on studies that used a combination of ROP1 knock-out parasites and parasite lines that express epitope-tagged versions of ROP1, it seems that ROP1 can be synthesized in one parasite but end up in the parasitophorous vacuole of another[32]. This suggests that ROP1 is not simply 'dumped' into the nascent parasitophorous vacuole during invasion, but instead is released into the host cell where it then migrates to the nearest parasitophorous vacuole. Because in nature most cells will be infected by only one parasite, the nearest parasitophorous vacuole is usually that of the same parasite that released the ROP1 during invasion. Small vesicle-like structures referred to as evacuoles have been observed that contain ROP1 but are devoid of parasites (hence they have also been named 'empty'-vacuoles or 'e'-vacuoles[32]). Evacuoles are observed in a small, but significant, percentage of invasion events in tissue culture and their frequency can be increased if invasion is blocked by drug treatment. For example, treatment with cytochalasin D prevents the actin and myosin motors of the parasite from exerting their propulsive force, although attachment is not affected (the host cell's actin and myosin motors appear to be irrelevant to parasite invasion[17,33]). The result is a 'frustrated' parasite that is stuck to the outside of a host cell that contains many evacuoles but lacks a developing parasitophorous vacuole membrane for the ROP1-containing evacuoles to fuse to.

The ROP2 family of proteins generally migrates to the second location for rhoptry proteins, the parasitophorous vacuole membrane[19,34–37]. It seems that several members of this protein family are intimately associated with the parasitophorous vacuole membrane, and are possibly even integral membrane proteins. Early suggestions that a hydrophobic alpha helix functions as a transmembrane domain[34] have been called into question now that we know that this helix is a conserved feature of most protein kinases and its hydrophobicity is a necessary feature of its being buried within the interior of the protein. Although no crystal structure of a ROP2 family member has been reported, it seems unlikely that a helix would be used to span a membrane, especially given the clear conservation of sequence (and presumably structure) on either side of the hydrophobic portion. ROP2 has also been implicated in the recruitment of host-cell mitochondria[38,39]. This has been postulated to be through recognition of the processed amino terminus of ROP2, which resembles a mitochondrial-import signal (that is, an amphipathic helix). The proposal is that host mitochondria mistakenly attempt to import ROP2, which is somehow firmly tethered to the parasitophorous vacuole membrane. The result is that as the mitochondria attempt to 'reel in' the ROP2 protein, they ratchet down onto the parasitophorous vacuole membrane, which is where they are routinely observed by electron microscopy.

Recent data on ROP18, a member of the ROP2 family, might indicate an interesting aspect of its association with the parasitophorous vacuole membrane; when expressed inside an infected host cell, by direct transfection of the *ROP18* gene (minus the portion encoding a signal peptide), the protein is eventually found concentrated on the parasitophorous vacuole membrane, presumably on the face that is exposed to the host cytosol[18]. This is also the location of a putative parasite-derived kinase that phosphorylates host iκB[40]. Whether ROP18 or another member of the ROP2 family is responsible for iκB phosphorylation remains to be directly investigated and the mechanism by which these proteins associate with the parasitophorous vacuole membrane is likewise unknown.

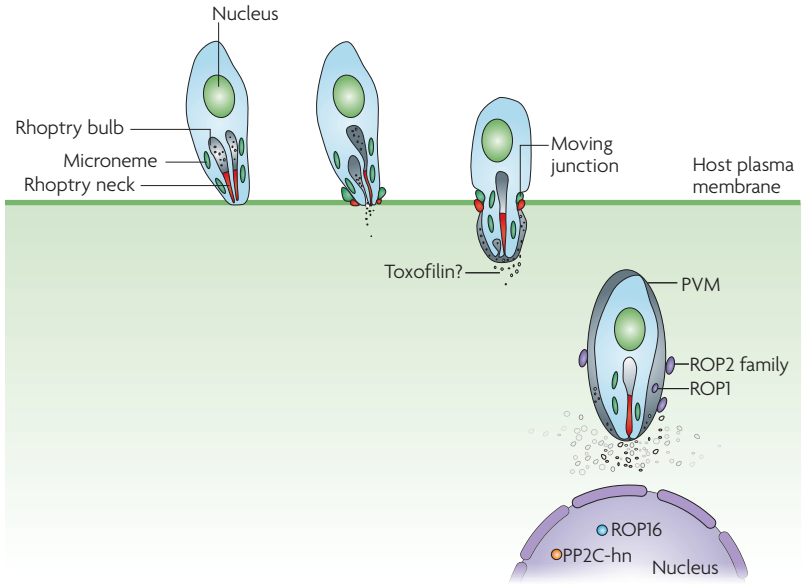


Figure 3 | **Schematic model for rhoptry contribution to invasion.** Rhoptry bulbs (grey) and rhoptry necks (red) release their contents during the invasion process in concert with simultaneous release by micronemes (green). RON2, RON4 and RON5 collaborate with micronemal AMA1 to create the moving junction, which migrates down the surface of the parasite, forming a ring of contact with the host plasma membrane. This effectively excludes many host plasma membrane integral proteins and results in the generation of a parasitophorous vacuole membrane (PVM), which envelopes the parasite. ROP2 family members are injected during invasion, perhaps in association with small vesicles, and ultimately end up on the host cytosolic side of the PVM. ROP1 is also observed in association with the injected vesicles, but most of this protein ends up inside the parasitophorous vacuole lumen. ROP16 and PP2C-hn (hn is the abbreviation for host nucleus) are not observed in the vesicles, but accumulate inside the host nucleus. Other soluble rhoptry proteins (for example, toxofilin) are also presumed to be injected, but in the absence of a concentrating mechanism they will be present at too low a concentration to be detectable (only a few rhoptries secrete their contents during invasion and any proteins that they release will be diluted by up to a million-fold or more in the host cytosol). Figure adapted from REF. 28.

The third known destination for ROPs is the interior of the host cell or, more specifically, the host-cell nucleus. So far, two rhoptry proteins have been observed in the nucleus, a protein phosphatase of the 2C class (PP2C-hn; hn is the abbreviation for host nucleus)[20] and a putative protein kinase that is a highly divergent member of the ROP2 family (ROP16 (REF. 41)). As for the other ROPs, how these proteins enter the host cell is a mystery. The only clues come from patch-clamp experiments, which show that there is a break in the continuity of the host plasma membrane at the earliest times of invasion[42], perhaps reflecting the moment when injection occurs (FIGS 2,3). The process is clearly an early one, as both ROP16 and PP2C-hn are detectable in the host nucleus within 15 minutes of allowing infection to commence, which is close to the time that such proteins would need to reach the host nucleus if they were introduced at time zero. It should be noted that once ROP16 and PP2C-hn enter the host cytosol, conventional nuclear localization signals (NLSs) are used to traffic them to the nucleus. This has been demonstrated by showing that ablation of a classic NLS signature, which both proteins have, stops these proteins from concentrating in the nucleus[41,43]. There is no evidence for the existence in *T. gondii* of genes or proteins that are related to those used by bacteria for introducing proteins into a host cell (for example, type III or type IV secretion systems[44]), although there are many parallels between these processes.

The function of the rhoptry PP2C-hn is unknown, as a knock-out strain showed no changes in host-gene expression (based on microarray analysis of infected cells) or virulence (based on infection studies in mice), although the knock-out strain was partially compromised in its ability to grow in fibroblasts *in vitro*[43]. Any conclusion concerning the lack of a virulence phenotype must be qualified, however, because the knock-out of PP2C-hn was in an RH strain in which virulence is so high (LD_{100} of 1 parasite) that anything short of a dramatic reduction in virulence might still yield a parasite that is capable of killing a mouse.

ROP16 and ROP18 — roles in virulence

It has long been known that different strains of *T. gondii* produce radically different pathologies in mice and maybe even in humans[45]. Using F1 progeny from crosses between two strains that differ in their virulence, two research groups have mapped the parasite loci that are responsible for the different pathogenicities[46,47]. The results showed that virulence in a given host (in these cases, mice) is influenced by which allele is present at each of at least five loci. Two of the most important loci are *ROP16* and *ROP18*; for example, depending on which allele of *ROP18* a strain carries, its LD_{50} in mice can vary by over 4 logs. The impact of the *ROP16* locus is less dramatic but still significant. The identities of the other three virulence loci have yet to be determined.

For *ROP18*, there is still much to be learned about the exact mechanism by which the different alleles effect such dramatic differences in disease outcome. It is known that the allele that is associated with low virulence yields a tiny fraction (~0.1%) of *ROP18* mRNA compared with the amount that is produced by the alleles found in more virulent strains[46,47]. This seems to be due to the presence of a large insertion and a small deletion within the presumptive promoter region of the low virulence allele, which seems to render the promoter inactive. The precise function of the ROP18 protein, however, is not yet known, although it clearly does have potent kinase activity, and a parasite substrate has been observed but not yet identified[18].

ROP16 subverts host gene expression

Microarray experiments using infected host cells *in vitro* have shown that the infected host cell responds differently depending on which strain of *T. gondii* is used and that these differences also segregate as distinct phenotypes among the F1 progeny[41]. Importantly, *ROP16* is among the main parasite loci that have been shown to be responsible for these differences. Testing of specifically engineered strains showed that this putative protein kinase somehow intersects the host signal transducer and activator of transcription (STAT) pathways[41]. These pathways are central to the regulation of many host genes,

including several cytokines and other immune-response mediators. This suggested the obvious possibility that differences in how a host cell responds to *T. gondii* might result in differences in the disease pathology. One of the main immune mediators that ROP16 affects by the perturbation of STAT pathways seems to be interleukin (IL)-12, which is central to the host response to *T. gondii* infection[48,49]. Too much, or too little, IL-12 can have serious and negative consequences for the host by causing too much or too little of the necessary immune responses, and so its variable expression, which depends on the allele of *ROP16* that is carried by the invading parasite, is an attractive explanation for the differences in the disease that are caused by the various strains. This possibility has been explored *in vivo* and ROP16 does indeed seem to have a role in determining serum IL-12 levels and, therefore, the overall course of infection[41].

Using the terminology that is favoured by those studying bacterial pathogens, ROPs are crucial 'effector proteins'. They might equally be called 'negotiator proteins', owing to their role in managing the interaction between host and parasite, as the vast majority of *T. gondii* infections result in a persistent infection with little, if any, disease.

Rhoptries and *T. gondii* evolution

ROP16 and *ROP18* are members of a large gene family (the ROP2 family) and are two of the most variable loci in the entire *T. gondii* genome. The ratio of synonymous to nonsynonymous substitutions is also extremely high, thus strengthening the argument that these two genes are subject to a strong positive selection for change[41,46,47]. What is the pressure that drives this process? One possibility is simply that it is the immune pressure of the sort that drives variation in many pathogen proteins. ROP16 and ROP18 may particularly be subject to such selection, relative to other *T. gondii* antigens, because they seem to be accessible to the host cytosol and are, therefore, freely available to the host cell for class I major histocompatibility complex presentation. As yet, however, there are no data to indicate that these proteins are efficiently presented or are crucial as targets of the immune response in the conventional sense. This is in contrast to the well-characterized family of surface antigens that is known as the SAG1 related sequences or SRS family, which are immunodominant antigens (at least for the humoral response) that show a more modest level of variability between strains[50].

An alternative hypothesis for the diversification of ROP16 and ROP18 is selection for an expanded host range. This model posits that if a strain found itself in a new ecological niche, and therefore in a new host, as long as it could infect to some degree (and be transmitted), there would be a strong pressure to optimize the 'fit' between a negotiator protein and the new host. This could lead to a powerful and rapid selection for new gene variants or, as is the case for *ROP18*, selection for dramatic events that downregulate or upregulate its expression (as discussed above, the *ROP18* locus in type III strains of *T. gondii* has a large disruption in the promoter region that is thought to be responsible for the massive decrease in its expression relative to type I and II strains). The hypothesis is that, in some hosts, high levels of expression of ROP18 are so problematic to the host or parasite that either the host or parasite is killed prematurely, with the result that there is little, if any, transmission. For example, the highly expressed type II allele of ROP18, if present in a type III background, generally causes that strain to be fatal to mice[46]. Hence, the promoter disruption of ROP18 might have been necessary for *T. gondii* to productively infect mice, whereas full-on expression might be needed for the infection of some other intermediate hosts. The differences in the coding region (as opposed to the promoter) might be evidence for more subtle changes that are needed for optimization of the interaction of ROP18 with its respective target in related host species. Ultimately, the situation might be somewhat analogous to that of the influenza virus, in which both immune pressure and host range seem to play important parts in the evolution of several of its genes. For example, the haemagglutinin gene of H3N2 viruses is constantly evolving under selective pressure from the immune system, but for an H5N1 virus to become transmissible between humans other mutations may need to occur that allow binding to the particular receptors that are present in the relevant human tissue[51].

When did these differences arise? To answer this question, additional sequences of *ROP16* and *ROP18* genes, from many more strains that infect diverse hosts and, therefore, have diverse ecological niches, are clearly needed. It is possible that the extremely large number of coding-sequence differences are a vestige of a long evolutionary time period, during which time the sequences became more divergent as the genes were optimized to different niches. The disruption of the promoter might be a more recent event that in one stroke expanded the parasite's host range to accommodate the emergence of a new niche, which, perhaps, is related to human migrations. One scenario might be that an ancestral North American strain that experienced the promoter disruption suddenly became a productive infector of Norwegian roof rats or European house sparrows, both of which were introduced to North America only in the past few centuries. Genotyping of some of the currently most common strains has shown that one or two matings can have a dramatic impact on the population biology of *T. gondii*[52] and that recent mixing of gene pools from distant geographic locations has probably occurred; South America appears to be the source of some of the greatest diversity[53]. Such matings may have given rise to recombinant strains that have just the right mix of crucial rhoptry proteins, which has enabled the productive infection of hosts that were previously not susceptible to this parasite.

Clinical implications of rhoptry biology

A detailed understanding of rhoptry-protein functions could have profound clinical implications that are well

beyond the usual opportunities for the development of new vaccines or drugs to block parasite metabolism. A compound that interferes with assembly or migration of the MJ, for example, would clearly be lethal to these obligate intracellular parasites. Much of the MJ machinery is conserved in the malaria parasite[9,30], so the benefit of such a drug could also extend to treating this worldwide scourge. ROP16 and ROP18 are kinases that are injected into the host cell, where their different allelic forms substantially alter the host–pathogen interaction[18,41,46,47]. This finding might be key to appropriate decision making if treating severe ocular toxoplasmosis with steroids that suppress inflammation[54]. On the one hand, if infection is with a strain in which the ROP16 allele causes an excessive IL-12 response, dampening of inflammation might be clinically appropriate to prevent damage to the eye itself. On the other hand, suppressing the immune response to a strain that expresses a ROP16 allele, which is associated with a weak IL-12 response (and therefore is naturally producing little inflammation), might result in an inadequate immune defence, leading to uncontrolled parasite growth. Knowing which strain is infecting a patient, therefore, might allow future clinicians who are armed with a refrigerator full of immune-modulators (for example, recombinant cytokines), to tweak the immune response in the right direction and to the right degree.

Conclusions

Rhoptries contain many of the key molecules that are used for parasite entry into host cells and subversion of host functions. The MJ, which contains four known proteins, AMA1, RON2, RON4 and RON5, and probably several other proteins that have yet to be identified, plays a central part during invasion. Determining how these various molecules interact to create the MJ is, therefore, of great importance to understanding how the parasitophorous vacuole forms. Such knowledge is also likely to yield some novel findings about interactions between membranes, as migration of a circular ring of contact between two membranes without fusion is an unusual process in biology. The ROP2 family (which includes ROP16 and ROP18) is extensive and we have almost no understanding of how this large family of proteins interacts with itself, let alone other host or parasite proteins. Clearly, based on their high sequence divergence and role in virulence they are crucial to the host–pathogen interaction, but the molecular details remain to be discovered. Even those members of the ROP2 family that seem to have lost kinase activity might be important and could mediate an effect by regulating the activity of those members that retain the ability to phosphorylate other proteins. The prediction that rhoptries contain many of the molecules that are key to an intracellular lifestyle is proving correct and recent results are a tantalizing glimpse of an exciting future.

1. Luft, B. J. & Remington, J. S. Toxoplasmic encephalitis in AIDS. *Clin. Infect. Dis.* **15**, 211–222 (1992).
2. Dubremetz, J. F., Garcia-Reguet, N., Conseil, V. & Fourmaux, M. N. Apical organelles and host-cell invasion by Apicomplexa. *Int. J. Parasitol.* **28**, 1007–1013 (1998).
3. Dubey, J. P. in *Parasitic Protozoa* (ed. Kreier, J. P.) 1–158 (Academic, California, 1993).
4. Pepper, M., Dzierszinski, F., Crawford, A., Hunter, C. A. & Roos, D. Development of a system to study $CD4^+$-T-cell responses to transgenic ovalbumin-expressing *Toxoplasma gondii* during toxoplasmosis. *Infect. Immun.* **72**, 7240–7246 (2004).
5. Gubbels, M. J., Striepen, B., Shastri, N., Turkoz, M. & Robey, E. A. Class I major histocompatibility complex presentation of antigens that escape from the parasitophorous vacuole of *Toxoplasma gondii*. *Infect. Immun.* **73**, 703–711 (2005).
6. Dubey, J. P., Lindsay, D. S. & Speer, C. A. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. *Clin. Microbiol. Rev.* **11**, 267–299 (1998).
7. Sadak, A., Taghy, Z., Fortier, B. & Dubremetz, J. F. Characterization of a family of rhoptry proteins of *Toxoplasma gondii*. *Mol. Biochem. Parasitol.* **29**, 203–211 (1988).
8. Saffer, L. D., Mercereau-Puijalon, O., Dubremetz, J. F. & Schwartzman, J. D. Localization of a *Toxoplasma gondii* rhoptry protein by immunoelectron microscopy during and after host cell penetration. *J. Protozool.* **39**, 526–530 (1992).
9. Bradley, P. J. *et al.* Proteomic analysis of rhoptry organelles reveals many novel constituents for host–parasite interactions in *Toxoplasma gondii*. *J. Biol. Chem.* **280**, 34245–34258 (2005).
10. Hoppe, H. C., Ngo, H. M., Yang, M. & Joiner, K. A. Targeting to rhoptry organelles of *Toxoplasma gondii* involves evolutionarily conserved mechanisms. *Nature Cell Biol.* **2**, 449–456 (2000).
11. Bradley, P. J. & Boothroyd, J. C. The pro region of *Toxoplasma* ROP1 is a rhoptry-targeting signal. *Int. J. Parasitol.* **31**, 1177–1186 (2001).
12. Striepen, B., Soldati, D., Garcia-Reguet, N., Dubremetz, J. F. & Roos, D. S. Targeting of soluble proteins to the rhoptries and micronemes in *Toxoplasma gondii*. *Mol. Biochem. Parasitol.* **113**, 45–53 (2001).
13. Ngo, H. M. *et al.* AP-1 in *Toxoplasma gondii* mediates biogenesis of the rhoptry secretory organelle from a post-Golgi compartment. *J. Biol. Chem.* **278**, 5343–5352 (2003).
14. Bradley, P. J., Li, N. & Boothroyd, J. C. A GFP-based motif-trap reveals a novel mechanism of targeting for the *Toxoplasma* ROP4 protein. *Mol. Biochem. Parasitol.* **137**, 111–120 (2004).
15. Ngo, H. M., Yang, M. & Joiner, K. A. Are rhoptries in Apicomplexan parasites secretory granules or secretory lysosomal granules? *Mol. Microbiol.* **52**, 1531–1541 (2004).
16. Fevrier, B. & Raposo, G. Exosomes: endosomal-derived vesicles shipping extracellular messages. *Curr. Opin. Cell Biol.* **16**, 415–421 (2004).
17. El Hajj, H. *et al.* The ROP2 family of *Toxoplasma gondii* rhoptry proteins: proteomic and genomic characterization and molecular modeling. *Proteomics* **6**, 5773–5784 (2006).
18. El Hajj, H. *et al.* ROP18 is a rhoptry kinase controlling the intracellular proliferation of *Toxoplasma gondii*. *PLoS Pathog.* **3**, e14 (2007).
The first direct demonstration of the kinase activity of the rhoptry protein ROP18 and its role in *T. gondii* proliferation *in vitro*.
19. Carey, K. L., Jongco, A. M., Kim, K. & Ward, G. E. The *Toxoplasma gondii* rhoptry protein ROP4 is secreted into the parasitophorous vacuole and becomes phosphorylated in infected cells. *Eukaryot. Cell* **3**, 1320–1330 (2004).
20. Gilbert, L. A., Ravindran, S., Turetzky, J. M., Boothroyd, J. C. & Bradley, P. J. *Toxoplasma gondii* targets a protein phosphatase 2C to the nuclei of infected host cells. *Eukaryot. Cell* **6**, 73–83 (2007).
The first study to reveal the targeting of a rhoptry protein to the host-cell nucleus after invasion.
21. Que, X. *et al.* The cathepsin B of *Toxoplasma gondii*, toxopain-1, is critical for parasite invasion and rhoptry protein processing. *J. Biol. Chem.* 277, 25791–25797 (2002).
22. Miller, S. A., Thathy, V., Ajioka, J. W., Blackman, M. J. & Kim, K. TgSUB2 is a *Toxoplasma gondii* rhoptry organelle processing proteinase. *Mol. Microbiol.* **49**, 883–894 (2003).
23. Ossorio, P. N., Schwartzman, J. D. & Boothroyd, J. C. A *Toxoplasma gondii* rhoptry protein associated with host cell penetration has unusual charge asymmetry. *Mol. Biochem. Parasitol.* **50**, 1–15 (1992).
24. Reichmann, G., Dlugonska, H. & Fischer, H. G. Characterization of TgROP9 (p36), a novel rhoptry protein of *Toxoplasma gondii* tachyzoites identified by T cell clone. *Mol. Biochem. Parasitol.* **119**, 43–54 (2002).
25. Foussard, F., Leriche, M. A. & Dubremetz, J. F. Characterization of the lipid content of *Toxoplasma gondii* rhoptries. *Parasitology* **102** Pt 3, 367–370 (1991).
26. Nichols, B. A., Chiappino, M. L. & O'Connor, G. R. Secretion from the rhoptries of *Toxoplasma gondii* during host-cell invasion. *J. Ultrastruct. Res.* **83**, 85–98 (1983).
First visualization of apparent rhoptry secretion into an infected host cell.
27. Lebrun, M. *et al.* The rhoptry neck protein RON4 re-localizes at the moving junction during *Toxoplasma gondii* invasion. *Cell. Microbiol.* **7**, 1823–1833 (2005).
28. Alexander, D. L., Mital, J., Ward, G. E., Bradley, P. & Boothroyd, J. C. Identification of the moving junction complex of *Toxoplasma gondii*: a collaboration between distinct secretory organelles. *PLoS Pathog.* **1**, e17 (2005).
This paper was the first to identify a cooperation between rhoptry and microneme proteins in invasion. Together with reference 27 it was also the first to show the involvement of RON proteins in the MJ.
29. Aikawa, M., Miller, L. H., Johnson, J. & Rabbege, J. Erythrocyte entry by malarial parasites: a moving junction between erythrocyte and parasite. *J. Cell Biol.* **77**, 72–82 (1978).
30. Alexander, D. L., Arastu-Kapur, S., Dubremetz, J. F. & Boothroyd, J. C. *Plasmodium falciparum* AMA1 binds a rhoptry neck protein homologous to TgRON4, a component of the moving junction in *Toxoplasma gondii*. *Eukaryot. Cell* **5**, 1169–1173 (2006).
31. Kenny, B. *et al.* Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. *Cell* **91**, 511–520 (1997).

32. Hakansson, S., Charron, A. J. & Sibley, L. D. *Toxoplasma* evacuoles: a two-step process of secretion and fusion forms the parasitophorous vacuole. *EMBO J.* **20**, 3132–3144 (2001).
First direct visualization of rhoptry-protein trafficking within an infected cell.
33. Dobrowolski, J. M. & Sibley, L. D. *Toxoplasma* invasion of mammalian cells is powered by the actin cytoskeleton of the parasite. *Cell* **84**, 933–939 (1996).
34. Beckers, C. J. M., Dubremetz, J. F., Mercereau-Puijalon, O. & Joiner, K. A. The *Toxoplasma gondii* rhoptry protein ROP2 is inserted into the parasitophorous vauole membrane, surrounding the intracellular parasite, and is exposed to the host cell cytoplasm. *J. Cell Biol.* **127**, 947–961 (1994).
First report that rhoptry proteins can be directly exposed to the infected host-cell cytosol.
35. El Hajj, H., Lebrun, M., Fourmaux, M. N., Vial, H. & Dubremetz, J. F. Inverted topology of the *Toxoplasma gondii* ROP5 rhoptry protein provides new insights into the association of the ROP2 protein family with the parasitophorous vacuole membrane. *Cell. Microbiol.* **9**, 54–64 (2007).
36. El Hajj, H., Lebrun, M., Fourmaux, M. N., Vial, H. & Dubremetz, J. F. Characterization, biosynthesis and fate of ROP7, a ROP2 related rhoptry protein of *Toxoplasma gondii*. *Mol. Biochem. Parasitol.* **146**, 98–100 (2006).
37. Herion, P., Hernandez-Pando, R., Dubremetz, J. F. & Saavedra, R. Subcellular localization of the 54-kDa antigen of *Toxoplasma gondii*. *J. Parasitol.* **79**, 216–222 (1993).
38. Sinai, A., Webster, P. & Joiner, K. Association of host cell endoplasmic reticulum and mitochondria with the *Toxoplasma gondii* parasitophorous vacuole membrane: a high affinity interaction. *J. Cell. Sci.* **110**, 2117–2128 (1997).
39. Sinai, A. P. & Joiner, K. A. The *Toxoplasma gondii* protein ROP2 mediates host organelle association with the parasitophorous vacuole membrane. *J. Cell. Biol.* **154**, 95–108 (2001).
40. Molestina, R. E., Payne, T. M., Coppens, I. & Sinai, A. P. Activation of NF-κB by *Toxoplasma gondii* correlates with increased expression of antiapoptotic genes and localization of phosphorylated IκB to the parasitophorous vacuole membrane. *J. Cell Sci.* **116**, 4359–4371 (2003).
41. Saeij, J. P. *et al. Toxoplasma* co-opts host gene expression by injection of a polymorphic kinase homologue. *Nature* **445**, 324–327 (2007).
42. Suss-Toby, E., Zimmerberg, J. & Ward, G. E. *Toxoplasma* invasion: the parasitophorous vacuole is formed from host cell plasma membrane and pinches off via a fission pore. *Proc. Natl Acad. Sci. USA* **93**, 8413–8418 (1996).
43. Gilbert, R. E. *et al.* Screening for congenital toxoplasmosis: accuracy of immunoglobulin M and immunoglobulin A tests after birth. *J. Med. Screen.* **14**, 8–13 (2007).
44. Ghosh, P. Process of protein transport by the type III secretion system. *Microbiol. Mol. Biol. Rev.* **68**, 771–795 (2004).
45. Saeij, J. P., Boyle, J. P. & Boothroyd, J. C. Differences among the three major strains of *Toxoplasma gondii* and their specific interactions with the infected host. *Trends Parasitol.* **21**, 476–481 (2005).
46. Saeij, J. P. *et al.* Polymorphic secreted kinases are key virulence factors in toxoplasmosis. *Science* **314**, 1780–1783 (2006).
Demonstrated the major role of two rhoptry proteins (ROP16 and ROP18) in *T. gondii* virulence in mice.
47. Taylor, S. *et al.* A secreted serine-threonine kinase determines virulence in the eukaryotic pathogen *Toxoplasma gondii*. *Science* **314**, 1776–1780 (2006).
Identified the major role that ROP18 has in *T. gondii* proliferation *in vitro* and virulence in mice.
48. Hunter, C. A. & Remington, J. S. The role of IL12 in toxoplasmosis. *Res. Immunol.* **146**, 546–552 (1995).
49. Scharton-Kersten, T., Denkers, E. Y., Gazzinelli, R. & Sher, A. Role of IL12 in induction of cell-mediated immunity to *Toxoplasma gondii*. *Res. Immunol.* **146**, 539–545 (1995).
50. Jung, C., Lee, C. Y. & Grigg, M. E. The SRS superfamily of *Toxoplasma* surface proteins. *Int. J. Parasitol.* **34**, 285–296 (2004).
51. Parrish, C. R. & Kawaoka, Y. The origins of new pandemic viruses: the acquisition of new host ranges by canine parvovirus and influenza A viruses. *Annu. Rev. Microbiol.* **59**, 553–586 (2005).
52. Boyle, J. P. *et al.* Just one cross appears capable of dramatically altering the population biology of a eukaryotic pathogen like *Toxoplasma gondii*. *Proc. Natl Acad. Sci. USA* **103**, 10514–10519 (2006).
53. Lehmann, T., Marcet, P. L., Graham, D. H., Dahl, E. R. & Dubey, J. P. Globalization and the population structure of *Toxoplasma gondii*. *Proc. Natl Acad. Sci. USA* **103**, 11423–11428 (2006).
54. Holland, G. N. & Lewis, K. G. An update on current practices in the management of ocular toxoplasmosis. *Am. J. Ophthalmol.* **134**, 102–114 (2002).
55. Montoya, J. G. & Liesenfeld, O. Toxoplasmosis. *Lancet* **363**, 1965–1976 (2004).
56. Dubremetz, J. F. Host cell invasion by *Toxoplasma gondii*. *Trends Microbiol.* **6**, 27–30 (1998).
57. Ferguson, D. J. P. & Dubremetz, J. F. in *Toxoplasma gondii: the Model Apicomplexan. Perspectives and Methods* (eds. Weiss, L. M. & Kim, K.) 19–48 (Elsevier, London, 2007).
58. Beckers, C. J., Wakefield, T. & Joiner, K. A. The expression of *Toxoplasma* proteins in *Neospora caninum* and the identification of a gene encoding a novel rhoptry protein. *Mol. Biochem. Parasitol.* **89**, 209–223 (1997).
59. Leriche, M. A. & Dubremetz, J. F. Characterization of the protein contents of rhoptries and dense granules of *Toxoplasma gondii* tachyzoites by subcellular fractionation and monoclonal antibodies. *Mol. Biochem. Parasitol.* **45**, 249–259 (1991).
60. Park, Y. K. & Nam, H. W. Early recognized antigen (p34) of *Toxoplasma gondii* after peroral ingestion of tissue cyst forming strain (Me49 strain) in mice. *Korean J. Parasitol.* **37**, 157–162 (1999).
61. Karasov, A. O., Boothroyd, J. C. & Arrizabalaga, G. Identification and disruption of a rhoptry-localized homologue of sodium hydrogen exchangers in *Toxoplasma gondii*. *Int. J. Parasitol.* **35**, 285–291 (2005).
62. Poupel, O., Boleti, H., Axisa, S., Couture-Tosi, E. & Tardieux, I. Toxofilin, a novel actin-binding protein from *Toxoplasma gondii*, sequesters actin monomers and caps actin filaments. *Mol. Biol. Cell* **11**, 355–368 (2000).
63. Schwarz, J. A., Fouts, A. E., Cummings, C. A., Ferguson, D. J. & Boothroyd, J. C. A novel rhoptry protein in *Toxoplasma gondii* bradyzoites and merozoites. *Mol. Biochem. Parasitol.* **144**, 159–166 (2005).
64. Kissinger, J. C., Gajria, B., Li, L., Paulsen, I. T. & Roos, D. S. ToxoDB: accessing the *Toxoplasma gondii* genome. *Nucleic Acids Res.* **31**, 234–236 (2003).
65. Ahn, H. J., Kim, S. & Nam, H. W. Molecular cloning of a rhoptry protein (ROP6) secreted from *Toxoplasma gondii*. *Korean J. Parasitol.* **44**, 251–254 (2006).

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DATABASES
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=geneomeprj
Escherichia coli | *Plasmodium falciparum* | *Toxoplasma gondii*
GenBank: http://www.ncbi.nlm.nih.gov/Genbank/index.html
RON1 | RON2 | RON3 | RON4 | ROP1 | ROP2A | ROP4 | ROP5 | ROP6 | ROP7 | ROP8 | ROP9 | ROP10 | ROP11 | ROP12 | ROP13 | ROP14 | ROP15 | ROP16 | ROP17 | ROP18 | TgNHE2 | TgPP2C-hn | TgSUB2 | Toxofilin | Toxopain1

FURTHER INFORMATION
John C. Boothroyd's homepage: http://med.stanford.edu/profiles/John_Boothroyd/
ToxoDB4.2: http://www.toxodb.org/toxo/home.jsp

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