JCB Mini-Review

Secretory traffic in the eukaryotic parasite *Toxoplasma gondii*: less is more

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Name a single-celled eukaryote that boasts a small genome size, is easily cultivated in haploid form, for which a wide variety of molecular genetic tools are available, and that exhibits a simple, polarized secretory apparatus with a well-defined endoplasmic reticulum and Golgi that can serve as a model for understanding secretion. Got it? Now name a cell with all these attributes that contains at least a dozen distinct and morphologically well-defined intracellular organelles, including three distinct types of secretory vesicles and two endosymbiotic organelles. Not so sure anymore?

Toxoplasma gondii is an obligate intracellular protozoan parasite that is a leading cause of focal central nervous system infections in patients with AIDS/HIV (Luft and Remington, 1992). This parasite is a member of the phylum Apicomplexa, which includes *Plasmodium* (the cause of malaria) and ~5,000 additional species, most of which are poorly characterized (Levine, 1988). Among all of the Apicomplexa, *T. gondii* is one of the easiest to cultivate and the most amenable to genetic manipulation (Boothroyd et al., 1994; Roos et al., 1994). The nuclear genome of *T. gondii* is ~80 Mb in size; numerous ESTs are available (Ajioka et al., 1998), and a genome sequencing project is now underway. The parasite also harbors two organellar genomes associated with its mitochondrion and plastid (of which more below) (Feagin, 1994). The rapidly dividing haploid "tachyzoite" form of *T. gondii* can be propagated inside of virtually any mammalian host cell, and classical genetic crosses can be performed in cats (the parasite sexual cycle has not yet been established in vitro) (Boothroyd et al., 1994). Available tools for molecular genetic manipulation include a wide variety of selectable markers, integrating and episomal vectors, and high-efficiency transformation systems that permit gene knockouts, insertional mutagenesis, complementation cloning, antisense repression, inducible expression, etc. (Boothroyd et al., 1994; Roos et al., 1994; Black and Boothroyd, 1998; Nakaar et al., 1999; Meissner et al., 2001; Striepen et al., 2002).

A banana-shaped organism ~8-μm-long and 2 μm in diameter, *T. gondii* is substantially smaller than a typical mammalian cell (Fig. 1). The parasite's architecture can be appreciated in a few electron microscopic thin sections, displaying a single nucleus, a single mitochondrion, a single plastid, a single interconnected ER network, a single Golgi apparatus, and an apically clustered complex of secretory organelles (this apical complex gives the phylum Apicomplexa its name). Virtually all of these organelles exhibit a distinctive morphology when labeled with fluorescent protein tags (Fig. 2), permitting quantitative ultrastructural studies and time-lapse analysis in living cells. In sum, *T. gondii* can be viewed as optimally situated between the morphologically complex mammalian cell and smaller organisms with poor ultrastructural resolution, such as *Saccharomyces cerevisiae* or *Plasmodium* sp. (Hager et al., 1999). Although *T. gondii* parasites are unable to replicate outside of nucleated host cells, tachyzoites remain viable long enough in an extracellular environment to permit standard analyses of secretory processes, and a permeabilized cell secretion system has been established (Chaturvedi et al., 1998). In this mini-review, we describe insights into both unique and conserved features of the *T. gondii* secretory apparatus, providing comparisons with systems more familiar to mainstream cell biologists.

The *T. gondii* secretory pathway is highly polarized

Considered from the standpoint of an experimental system for secretion, one of the most appealing aspects of *T. gondii* is the polarized organization of its secretory organelles (Hager et al., 1999)—a consequence of the parasite's mechanism of replication, in which two daughter cells are assembled within the mother (Hu et al., 2001). The nucleus is centrally located, essentially bisecting the organism (Figs. 1 and 2). The endoplasmic reticulum, although distributed throughout the cell, is concentrated posterior to the nucleus, and is so reduced that the nuclear envelope itself provides a substantial fraction of the ER volume. Thinly coated vesicles bud from the anterior end of the nucleus/ER, destined for the closely juxtaposed Golgi stack, which consists of a limited number of cisternae (typically three to five). Reporters

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Key words: secretory pathway; vesicular trafficking; protozoan cell biology; Apicomplexan parasites; eukaryotic evolution

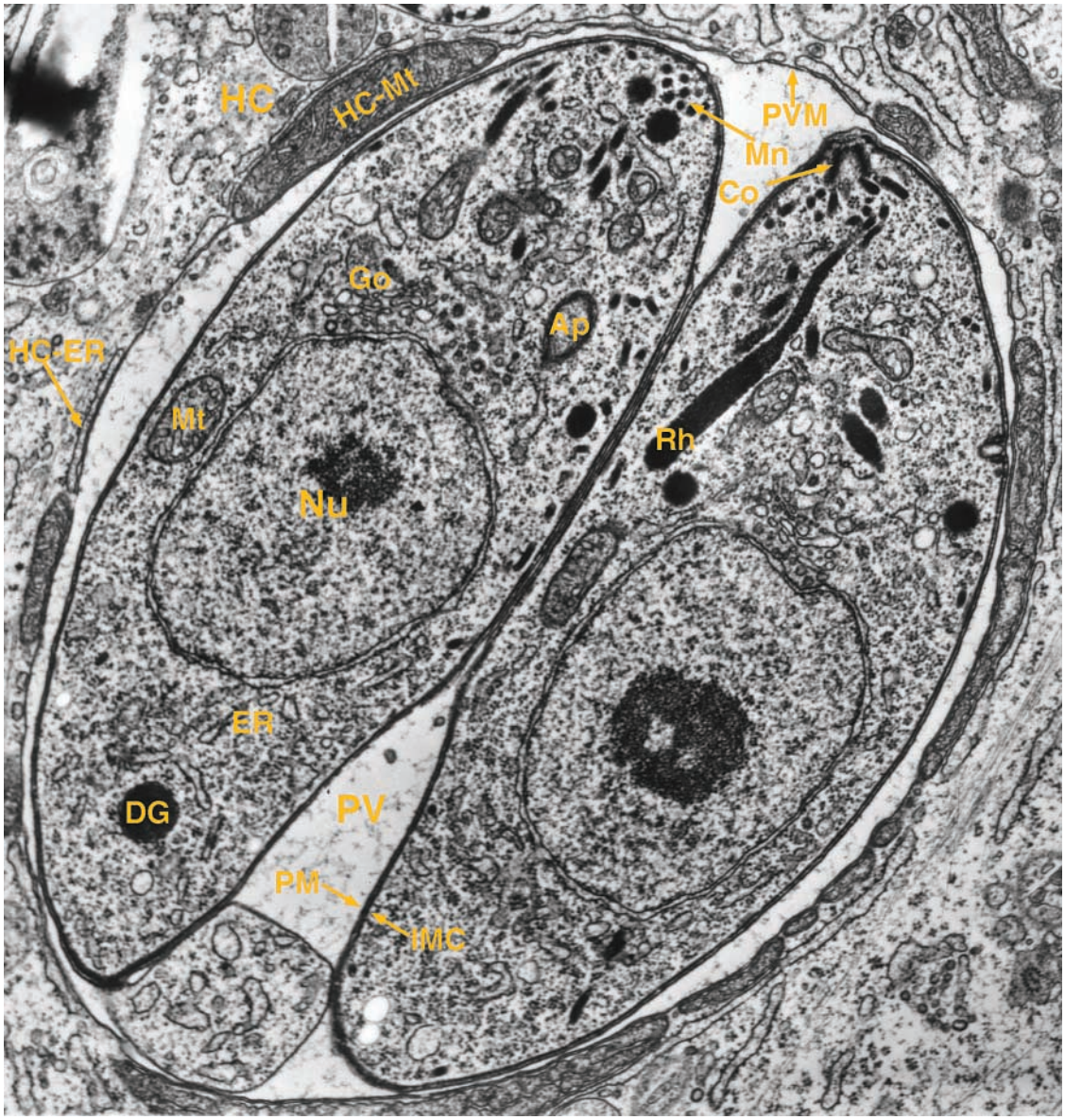

Figure 1. **Intracellular parasitophorous vacuole containing two *T. gondii* parasites within a human host cell.** The ER is distributed throughout the cell, but predominantly in the basal region. The Golgi apparatus is invariably found adjacent to the apical end of the nucleus. Rhoptries and micronemes are found at the apical end of the parasite (terminating in the conoid region), whereas dense granules are distributed throughout the cell. The inner membrane complex is comprised of a series of closed sacs of uncertain origin, underlying the plasma membrane. The micropore (not visible in this micrograph) is the only stable structure bridging the parasite plasma membrane and inner membrane complex. Clathrin-coated vesicles are often observed at the micropore (Nichols et al., 1994), suggesting that endocytosis may occur at this site. Bar, 2 μm. Ap, Apicoplast; Co, conoid; DG, dense granule; ER, endoplasmic reticulum; Go, Golgi; IMC, inner membrane complex; Mn, microneme; Mt, mitochondrion; Nu, nucleus; PM, plasma membrane; PV, parasitophorous vacuole; PVM, parasitophorous vacuole membrane; Rh, rhoptry; HC, host cell cytoplasm; HC-ER, host cell endoplasmic reticulum; HC-Mt; host cell mitochondrion.

containing the COOH-terminal ER retention signal of *T. gondii* BiP (HDEL) localize most prominently to a cup-like region anterior to the apical end of the nucleus, just below the Golgi (Figs. 1 and 2). The use of the nuclear envelope as an obligatory intermediate between the ER and Golgi is comparable to other small eukaryotic cells, such as *Pichia pastoris* (Rossanese et al., 1999) but contrasts with mammalian systems, where transitional ER elements are dispersed throughout the cell.

Forward transport from the ER to Golgi takes advantage of acidic/hydrophobic/acidic motifs in the cytoplasmic tails of secretory proteins, along with upstream tyrosines, likely by recruiting COPII coats as observed in mammalian cells and yeast (Hoppe and Joiner, 2000). Both COPII and COPI coat components (including Arf1 and Sar1) are present in *T. gondii* genome and EST databases (Ajioka et al., 1998), and COPI retrieval motifs have been shown to operate in the parasite (Liendo et al., 2001). Protein transport through the Golgi is inhibited by low temperature treatment, brefeldin A, and microtubule inhibitors (Stokkermans et al., 1996; Soldati et al., 1998). Clathrin-coated vesicles are observed at the lateral margins of the trans-most

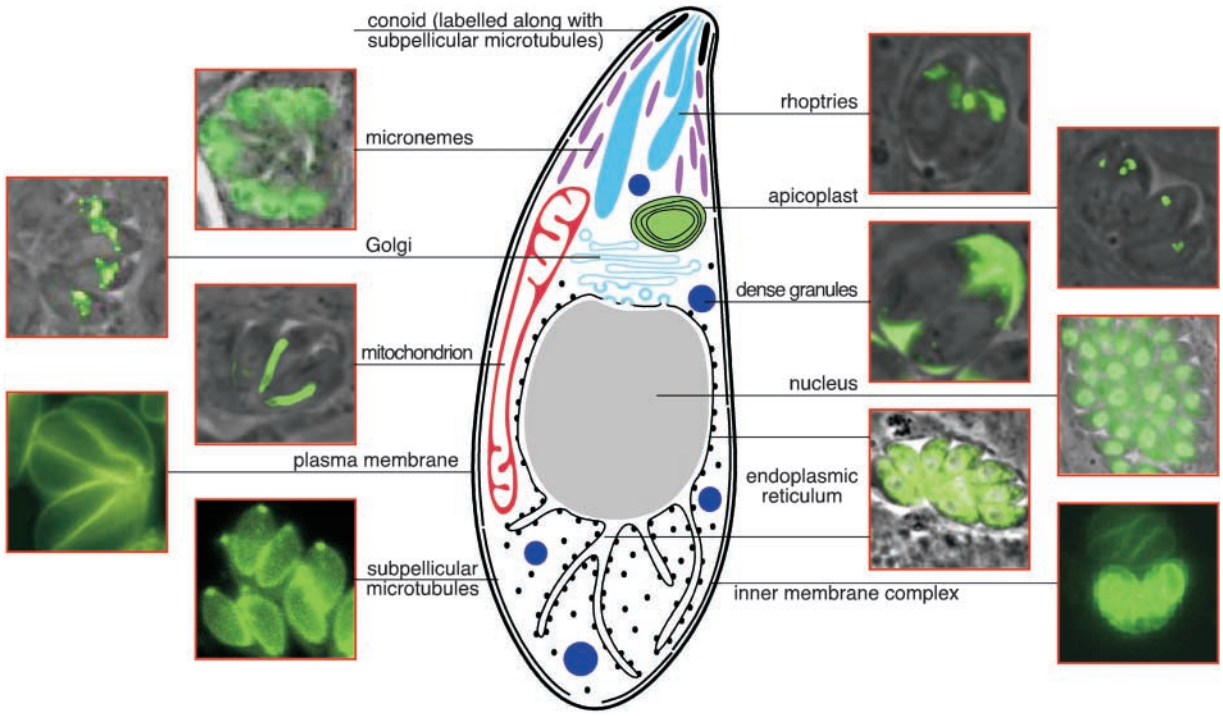

Figure 2. **Fluorescent protein labeling of subcellular organelles in *T. gondii*.** Fusions between endogenous parasite proteins and GFP, YFP, or other reporters have been expressed in transgenic *T. gondii*, and localization has been determined by fluorescence microscopy. The central cartoon, showing subcellular structures (also see Fig. 1), illustrates proper targeting of GFP chimeras. Labeling of the conoid and subpellicular microtubules was achieved using a YFP–α-tubulin construct (Striepen et al., 2000; Hu et al., 2002; Swedlow et al., 2002), micronemes using MIC3-GFP (Striepen et al., 2001), Golgi using MIC3[68–137]-GFP (Striepen et al., 2001), mitochondria using HSP60-GFP (Hu et al., 2001), plasma membrane using P30-GFP-GPI (Striepen et al., 2000); rhoptries using ROP1-GFP (Striepen et al., 1998), dense granules using P30-GFP (Striepen et al., 1998), nucleus using PCNA-GFP (Radke et al., 2001), ER using P30-GFP-HDEL (Hager et al., 1999), and inner membrane complex using IMC1-YFP (Hu et al., 2001).

Golgi stacks (Liendo et al., 2001). The target for these vesicles is likely to be one or more of the multitude of unusual secretory organelles found in *T. gondii* and other Apicomplexans, as discussed below. In contrast, proteins destined for the parasite surface are delivered via an alternative route (Karsten et al., 1998). Many *T. gondii* surface antigens are GPI anchored, and the parasite may use the GPI anchor itself as a targeting motif.

Apicomplexan parasites have three secretory organelles: micronemes, rhoptries, and dense granules

The tachyzoite form of *T. gondii* contains at least three morphologically distinct secretory organelles: micronemes, rhoptries, and dense granules (Figs. 1 and 2). The former two organelles are located in the anterior portion of the cell, whereas dense granules are more broadly distributed. Morphologically, dense granules are essentially indistinguishable from the mature secretory granules found in endocrine, neuroendocrine, or exocrine cells (Table I and Fig. 1). At the other end of the spectrum, rhoptries bear little morphological resemblance to subcellular organelles in any other cell type. These three organelles discharge sequentially: microneme exocytosis occurs upon host cell binding, rhoptry secretion coincides with invasion, and dense granule secretion is most prominent after parasite entry into the host cell (Carruthers and Sibley, 1997). Micronemes and rhoptries are thought to be critical for host cell invasion, a process that is completed within 15–20 s. Dense granule proteins are thought to be required for intracellular replication, including establishment of the parasitophorous vacuole within which parasites reside and divide until lysis of the host cell. The requirements for precise temporal regulation of differential organelle secretion are stringent, distinguishing *T. gondii* parasites from most secretory cells.

Dense granules are functionally analogous to constitutive secretory vesicles

Soluble recombinant proteins (from various sources) are delivered to dense matrix granules by the bulk flow pathway. Dense granules are quantitatively secreted in a constitutive, calcium-independent fashion (Chaturvedi et al., 1998; Karsten et al., 1998); although there is also likely to be a triggered component to the release process (Dubremetz et al., 1993; Carruthers and Sibley, 1997; Coppens et al., 1999). Even *T. gondii* proteins from which specific signals for targeting to other organelles have been deleted are routed through the dense granules as soluble proteins (Striepen et al., 1998, 2001; Reiss et al., 2001). It is therefore difficult to invoke the notion that aggregation or retention in dense granules requires specific protein sequence motifs, a low pH/high Ca^{+2} environment, lipid rafts, or other distinguishing characteristics, in contrast to observations in mammalian secretory cells (Arvan and Castle, 1998; Tooze et al., 2001) (Table I). On balance, dense granules appear to be most

Table I. **Comparison of organellar protein trafficking and secretion in *Toxoplasma* and "higher" eukaryotes**

	Secretory signal sequence	Additional targeting signals	Proteolytic processing	Secretory trigger
Toxoplasma gondii				
Dense granules	Yes	No	No	None known (constitutive)
Rhoptries	Yes	AP adaptin dependent; lysosomal	In precursor compartment	Unknown
Micronemes	Yes	AP adaptin dependent	In precursor compartment; extracellular (after secretion)	Ca^{+2}
Mitochondrion	No	Amphipathic α-helix (at NH_2 terminus)	Within the organelle	No
Apicoplast	Yes	Enriched in basic AAs (after signal sequence)	Within the organelle	???
Animals/fungi/plants				
Endo/exocrine granules	Yes	AP adaptin dependent; additional mechanisms	In precursor compartment	Ca^{+2}
Lysosome-related organelles	Yes	AP adaptin dependent; lysosomal	In precursor compartment	Ca^{+2}
Mitochondrion	No	Amphipathic α-helix (at NH_2 terminus)	Within the organelle	No
Chloroplast	No	Enriched in basic AAs (at NH_2 terminus)	Within the organelle	No

similar to the post-Golgi vesicles involved in constitutive secretion (Table I).

Rhoptries are associated with both the endocytic and secretory pathways

The name "rhoptry" is derived from the Greek word meaning "club," reflecting the bulbous shape of this organelle (Fig. 1), thought to contain vesicular/membranous material that is secreted via the long, slender neck. Labeling with DAMP (3-[2,4-dinitroanilin]-3′amino-*N*-methyldipropylamine) suggests that rhoptries are the only acidified organelles in *T. gondii*; the parasite contains no morphological equivalent of secondary lysosomes (Shaw et al., 1998). Unusual organelles designated acidocalciosomes have been reported (Moreno and Zhong, 1996), but these function in the storage of calcium and pyrophosphate (and possibly other materials as well), and do not appear to be directly related to either exocytic or endocytic trafficking. Like multivesicular bodies and late endosomes (Bishop and Woodman, 2000), rhoptries are enriched in cholesterol, but their cholesterol/phospholipid ratio of ∼1.5:1 (Foussard et al., 1991) is too high for lipid bilayer stability, suggesting that at least some of this cholesterol may be organized in a crystalline array.

Secretion from the rhoptries contributes to the formation of a distinctive parasitophorous vacuole, defining the compartment within the host cell where *T. gondii* parasites reside. The majority of lipids making up the parasitophorous vacuole at the time of invasion are of host cell (rather than rhoptry) origin (Suss-Toby et al., 1996), but rhoptry proteins are rapidly incorporated into the vacuolar membrane. The parasitophorous vacuole neither acidifies nor fuses with organelles of the host cell endomembrane system, highlighting the unusual nature of this structure (Sibley et al., 1985; Joiner et al., 1990; Mordue et al., 1999). Inhibitors of parasite actin polymerization (Sibley and Andrews, 2000) prevent host cell invasion but not rhoptry discharge, producing small vesicular structures in the host cell. These empty "e-vacuoles" (Håkansson et al., 2001) contain rhoptry markers and associate with host cell mitochondria and ER (without fusing), just as seen for parasite-containing vacuoles in the same cell (Sinai et al., 1997).

The biogenesis of rhoptries is not well understood. Rhoptries of the mother cell disappear as morphologically distinct entities during the early phases of cell division (endodyogeny). Following division of the Golgi apparatus, two distinct rhoptry antigen-positive punctae appear immediately anterior to Golgi; these structures may be regenerated de novo, as precursors to the fully formed rhoptries that will ultimately develop in the two daughter cells. Rhoptry protein processing is thought to occur in these immature rhoptries (Soldati et al., 1998), which therefore exhibit some functional similarity to immature secretory granules (Table I).

Protein targeting to the rhoptries has long been a matter of interest, since the evolutionary origin of these unique secretory structures is unknown. Soluble rhoptry proteins can harbor multiple independent targeting signals (Bradley and Boothroyd, 2001; Striepen et al., 2001). Members of the ROP2 family, which contain a putative transmembrane domain, display both YXXΦ and LL motifs within the predicted cytoplasmic tail (Hoppe et al., 2000). In higher eukaryotes, both of these motifs mediate binding to adaptor subunits and facilitate clathrin-coated vesicle formation from the trans-Golgi (Bonifacino and Dell-Angelica, 1999). Deletion or alteration of the YXXΦ motif (Hoppe et al., 2000) or LL motif (unpublished data) abolishes ROP2 delivery to mature *T. gondii* rhoptries, providing the first evidence for tyrosine-dependent sorting machinery in protozoan parasites. *T. gondii* μ1 binds to the cytoplasmic tail of ROP2 family members in a tyrosine-dependent fashion and expression of either dominant–negative *T. gondii* μ1 or antisense mRNA ablation of *T. gondii* μ1 expression impairs rhoptry targeting (unpublished data). Alteration of rhoptry targeting motifs leads to protein accumulation in a compartment located just anterior to (but distinct from) the Golgi (the precursor compartment noted in Table I). Combined with the observation that rhoptries are acidic (Shaw et al., 1998), it is tempting to consider the rhoptry a lysosome-like organelle (Dell'Angelica et al., 2000) (see Table I). A model

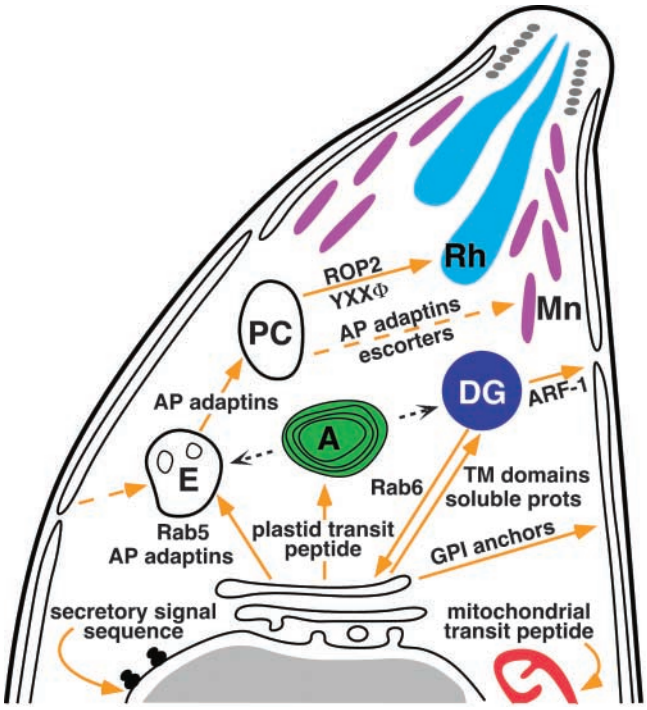

Figure 3. **Post-Golgi protein targeting in the *T. gondii* secretory pathway**. Protein traffic through the ER and Golgi likely depends on both COPI- and COPII-coated vesicles, and is regulated by forward targeting signals, ER retrieval and retention motifs, and Rab proteins. Targeting of soluble proteins from the trans-Golgi network to dense granules is signal independent, whereas targeting of membrane proteins to these organelles depends on transmembrane domain length (unpublished data). *T. gondii* rab6 mediates retrograde transport from dense granules to the parasite Golgi (unpublished data). Rhoptry proteins appear likely to be transported from the Golgi via a precursor compartment, possibly part of the endosomal pathway (Robibaro et al., 2002). Transmembrane rhoptry proteins are targeted in a tyrosine-, dileucine-, and adaptor-dependent fashion. Targeting of soluble microneme proteins proceeds by association with transmembrane escorters; transmembrane proteins are capable of using adaptor- and tyrosine-dependent signals, although typical endocytic motifs are not apparent in known microneme proteins. Results using dominant–negative adaptors suggest that microneme targeting may exploit the same precursor compartment involved in rhoptry targeting. Nuclear-encoded proteins destined for the apicoplast exhibit a bipartite NH_2-terminal domain (Roos et al., 1999; DeRocher et al., 2000; Waller et al., 2000; Yung et al., 2001), mediating transport first into the secretory pathway using a classical secretory signal sequence, and subsequently into the apicoplast using a plastid–transit peptide akin to that found in plants. Whether all secreted proteins transit this organelle after exit from the Golgi remains to be determined, as does the ultimate destination of products produced in the apicoplast (dashed black arrows). A, apicoplast; DG, dense granule; E, endosome; Mn, micronemes; PC, precursor compartment; Rh, rhoptries.

for rhoptry biogenesis consistent with the existing data is provided in Fig. 3.

Microneme targeting involves membrane escorts for soluble proteins

Microneme proteins typically exhibit one or more of a variety of adhesive domains, and are thought to be involved in host cell adhesion (Carruthers et al., 2000; Garcia-Reguet et al., 2000; Brecht et al., 2001). Chimeras containing the cytoplasmic tail of a mammalian lysosomal membrane protein are targeted to micronemes in a tyrosine-dependent fashion (Hoppe et al., 2000). No endogenous *T. gondii* microneme proteins bearing a transmembrane domain and YXXΦ or LL motifs in the cytoplasmic tail have yet been identified, however, and microneme proteins can possess multiple independent targeting domains (Striepen et al., 2001). The transmembrane protein MIC6 forms a trimeric complex with the soluble microneme proteins MIC1 and MIC4, and deletion of MIC6 prevents targeting of these molecules (Reiss et al., 2001), suggesting that MIC6 functions an escort protein. A similar escort role has been described for MIC8 in targeting MIC3 (Meissner et al., 2002), and for rhoptry proteins in *P. falciparum* (Baldi et al., 2000).

MIC2 is probably the most intensively studied microneme protein in *T. gondii. T. gondii* MIC2 is predicted to contain a transmembrane domain with cytoplasmic tail tyrosine motifs (SYHYY, EIEYE) that play a role in sorting (Di Cristina et al., 2000), and MIC2 has been shown to associate with another protein (MIC2AP) during transport to and storage in micronemes (Rabenau et al., 2001). Genetic deletion of the cytoplasmic domain of the MIC2 orthologue TRAP in *P. berghei* does not completely abolish microneme targeting (Kappe et al., 1999), however, suggesting functional redundancy in organellar targeting pathways.

The *T. gondii* plastid resides within the secretory pathway

Perhaps the most unusual subcellular organelle in *T. gondii* (and other Apicomplexan parasites) is a relict plastid, acquired by secondary endosymbiosis of a eukaryotic alga and retention of the algal plastid (Köhler et al., 1997). The Apicomplexan plastid—or "apicoplast"—is essential for parasite survival (Fichera and Roos, 1997; He et al., 2001). This organelle is known to play a role in lipid metabolism (Waller et al., 1998; Jomaa et al., 1999; Jelenska et al., 2001) and possibly other metabolic functions as well. Although the apicoplast has a its own genome (Wilson et al., 1996), this 35-kb circular element encodes only a limited protein repertoire; the majority of apicoplast proteins are synthesized on cytoplasmic ribosomes and posttranslationally imported (Waller et al., 1998; Roos et al., 1999).

As previously noted in other systems containing complex plastids, nuclear-encoded proteins destined for the apicoplast exhibit a bipartite NH_2-terminal domain. Molecular genetic manipulation in *T. gondii* and *P. falciparum* demonstrates that the extreme NH_2 terminus functions as a secretory signal sequence, whereas the subterminal domain (presumed to be exposed after cleavage of the secretory signal) functions as a plastid-targeting signal, directing the cargo protein from the secretory pathway into the apicoplast lumen (Roos et al., 1999; DeRocher et al., 2000; Waller et al., 2000; Yung et al., 2001). Remarkably, this entire process can be reconstituted from heterologous components. Thus, the combination of two normally distinct targeting processes—cotranslational translocation into the endoplasmic reticulum and posttranslational translocation into chloroplasts—combine to provide an elegant mechanism for targeting across the four membranes that surround the apicoplast. Based on the characteristics of the apicoplast targeting

signal, a large number of candidate apicoplast proteins have been identified in the *T. gondii* EST and *P. falciparum* genome databases (Ajioka et al., 1998; Bahl et al., 2002), including plastid import machinery of the tic and toc family (McFadden, G.I., personal communication).

Although the molecular details of protein targeting to the apicoplast are now clear, precisely how—in morphological terms—these proteins traffic from the secretory pathway to the apicoplast remains a mystery, as vesicles are never observed fusing with (or budding from) the organelle (compare Fig. 1). Moreover, treatment with brefeldin A or appending an ER retention signal to nuclear-encoded apicoplast proteins fails to inhibit trafficking to the organelle. These observations raise the possibility that the apicoplast lies at a proximal position within the secretory pathway—perhaps within the ER itself—and that all secreted proteins bearing an NH_2-terminal signal sequence wash over the apicoplast! As noted above, the function of the apicoplast is also uncertain, but circumstantial evidence suggests that it may play an important role in establishing the parasitophorous vacuole during host cell invasion (Fichera and Roos, 1997).

Conclusions

Protein targeting in *T. gondii* and related parasites utilizes a combination of conserved and unusual motifs and transport machinery (Ngô et al., 2000). The simplified, polarized, and morphologically distinctive organization of this cell readily permits comparison with mammalian cells and yeast, as detailed in Table I. Where mechanisms are conserved, *T. gondii* provides an excellent model for eukaryotes in general. For example, studies on the use of COPI and COPII coats in ER–Golgi transport, or the process of Golgi division, should be fruitful areas for study. Where mechanisms are different—as in the secretion of rhoptry lipids, the targeting across four membranes surrounding the apicoplast, and the trafficking of proteins destined for association with membrane compartments that lie beyond the plasma membrane—studies on these parasites are likely to reveal the diversity of eukaryotic evolution and highlight potential targets for antiparasitic drug development.

We wish to thank Lewis G. Tilney for Fig. 1, Cynthia Y. He for Fig. 2, and all members of the Joiner and Roos laboratories for helpful comments and stimulating discussions.

The authors greatfully acknowledge the National Institutes of Health and the Burroughs Wellcome Fund for support of this work.

Submitted: 28 December 2001
Revised: 15 February 2002
Accepted: 28 March 2002

References

Ajioka, J., J.C. Boothroyd, B.P. Brunk, A. Hehl, L. Hillier, I.D. Manger, M. Marra, G.C. Overton, D.S.. Roos, K.L. Wan, et al. 1998. Gene discovery by EST sequencing in *Toxoplasma gondii* reveals sequences restricted to Apicomplexa. *Genome Res.* 8:18–28.

Arvan, P., and D. Castle. 1998. Sorting and storage during secretory granule biogenesis: looking backward and looking forward. *Biochem. J.* 332:593–610.

Bahl, A., B.P. Brunk, R.L. Coppel, J. Crabtree, S.J. Diskin, M.J. Fraunholz, G.R. Grant, D. Gupta, R.L. Huestis, J.C. Kissinger, et al. 2002. PlasmoDB: the *Plasmodium* genome resource. An integrated database providing tools for accessing, analyzing and mapping expression and sequence data (both finished and unfinished). *Nucleic Acids Res.* 30:87–90.

Baldi, D.L., K.T. Andrews, R.F. Waller, D.S. Roos, R.F. Howard, B.S. Crabb, and A.F. Cowman. 2000. RAP1 controls rhoptry targeting of RAP2 in the malaria parasite *Plasmodium falciparum. EMBO J.* 19:2435–2443.

Bishop, N., and P. Woodman. 2000. ATPase-defective mammalian VPS4 localizes to aberrant endosomes and impairs cholesterol trafficking. *Mol. Biol. Cell.* 11:227–239.

Black, M., and J. Boothroyd. 1998. Development of a stable episomal shuttle vector for *Toxoplasma gondii. J. Biol. Chem.* 273:3972–3979.

Bonifacino, J.S., and E.C. Dell-Angelica. 1999. Molecular bases for the recognition of tyrosine-based sorting signals. *J. Cell Biol.* 145:923–926.

Boothroyd, J.C., K. Kim, E.R. Pfefferkorn, L.D. Sibley, and D. Soldati. 1994. Forward and reverse genetics in the study of the obligate intracellular parasite *Toxoplasma gondii. Methods Mol. Genet.* 3:1–29.

Bradley, P.J., and J.C. Boothroyd. 2001. The pro region of *Toxoplasma* ROP1 is a rhoptry-targeting signal. *Int. J. Parasitol.* 31:1177–1186.

Brecht, S., V.B. Carruthers, D.J. Ferguson, O.K. Giddings, G. Wang, U. Jakle, J.M. Harper, L.D. Sibley, and D. Soldati. 2001. The *Toxoplasma* micronemal protein MIC4 is an adhesin composed of six conserved apple domains. *J. Biol. Chem.* 276:4119–4127.

Carruthers, V.B., G.D. Sherman, and L.D. Sibley. 2000. The *Toxoplasma* adhesive protein MIC2 is proteolytically processed at multiple sites by two parasite-derived proteases. *J. Biol. Chem.* 275:14346–14353.

Carruthers, V.B., and L.D. Sibley. 1997. Sequential protein secretion from three distinct organelles of *Toxoplasma gondii* accompanies invasion of human fibroblasts. *Eur. J. Cell Biol.* 73:114–123.

Chaturvedi, S., H. Qi, D. Coleman, A. Rodriguez, P.S. Hanson, B. Striepen, D.S. Roos, and K.A. Joiner. 1998. Constitutive, calcium independent secretion of *Toxoplasma gondii* dense granules is mediated by the NSF/SNARE/SNAP/Rab machinery. *J. Biol. Chem.* 274:2424–2431.

Coppens, I., M. Andries, J. Liu, and M. Cesbron-Delauw. 1999. Intracellular trafficking of dense granule proteins in *Toxoplasma gondii* and experimental evidences for a regulated exocytosis. *Eur. J. Cell Biol.* 78:463–472.

Dell'Angelica, E.C., C. Mullins, S. Caplan, and J.S. Bonifacino. 2000. Lysosome-related organelles. *FASEB J.* 14:1265-1278.

DeRocher, A., C.B. Hagen, J.E. Froehlich, J.E. Feagin, and M. Parsons. 2000. Analysis of targeting sequences demonstrates that trafficking to the *Toxoplasma gondii* plastid branches off the secretory system. *J. Cell Sci.* 113: 3969–3977.

Di Cristina, M., R. Spaccapelo, D. Soldati, F. Bistoni, and A. Crisanti. 2000. Two conserved amino acid motifs mediate protein targeting to the micronemes of the apicomplexan parasite *Toxoplasma gondii. Mol. Cell. Biol.* 20:7332–7341.

Dubremetz, J.F., A. Achbarou, D. Bermudes, and K.A. Joiner. 1993. Kinetics of apical organelle exocytosis during *Toxoplasma gondii* host cell interaction. *Parasitol. Res.* 79:402–408.

Feagin, J.E. 1994. The extrachromosomal DNAs of apicomplexan parasites. *Annu. Rev. Microbiol.* 48:81–104.

Fichera, M.E., and D.S. Roos. 1997. A plastid organelle as a drug target in apicomplexan parasites. *Nature.* 390:407–409.

Foussard, F., M.A. Leriche, and J.F. Dubremetz. 1991. Characterization of the lipid content of *Toxoplasma gondii* rhoptries. *Parasitology.* 102:367–370.

Garcia-Reguet, N., M. Lebrun, M.N. Fourmaux, O. Mercereau-Puijalon, T. Mann, C.J. Beckers, B. Samyn, J. Van Beeumen, D. Bout, and J.F. Dubremetz. 2000. The microneme protein MIC3 of *Toxoplasma gondii* is a secretory adhesin that binds to both the surface of the host cells and the surface of the parasite. *Cell Microbiol.* 2:353–364.

Hager, K.M., B. Striepen, L.G. Tilney, and D.S. Roos. 1999. The nuclear envelope serves as an intermediary between the ER and Golgi complex in the intracellular parasite *Toxoplasma gondii. J. Cell Sci.* 112:2631–2638.

Håkansson, S., A.J. Charron, and L.D. Sibley. 2001. *Toxoplasma* evacuoles: a two-step process of secretion and fusion forms the parasitophorous vacuole. *EMBO J.* 20:3132–3144.

He, C.Y., M.K. Shaw, C.H. Pletcher, B. Striepen, L.G. Tilney, and D.S. Roos. 2001. A plastid segregation defect in the protozoan parasite *Toxoplasma gondii. EMBO J.* 20:330–339.

Hoppe, H.C., and K.A. Joiner. 2000. Cytoplasmic tail motifs mediate ER localization and export of transmembrane protein reporters in the protozoan parasite *Toxoplasma gondii. Cell. Microbiol.* 2:569–578.

Hoppe, H.C., H.M. Ngo, M. Yang, and K.A. Joiner. 2000. Targeting to rhoptry organelles of *Toxoplasma gondii* involves evolutionarily conserved mechanisms. *Nat. Cell Biol.* 2:449–456.

Hu, K., T. Mann, B. Striepen, C.J.M. Beckers, D.S. Roos, and J.M. Murray. 2001. Daughter cell assembly in the protozoan parasite *Toxoplasma gondii. Mol. Biol. Cell.* 13:593–603.

Hu, K., D.S. Roos, and M.J. Murray. 2002. A novel polymer of tubulin forms the conoid in *Taxoplasma gondii. J. Cell Biol.* 156:1039–1050.

Jelenska, J., M.J. Crawford, O. Harb, E. Zuther, R. Haselkorn, D.S. Roos, and P. Gornicki. 2001. Subcellular localization of acetyl-CoA carboxylase in the apicomplexan parasite *Toxoplasma gondii. Proc. Natl. Acad. Sci. USA.* 98: 2723–2728.

Joiner, K.A., S.A. Fuhrman, H. Mietinnen, L.L. Kasper, and I. Mellman. 1990. *Toxoplasma gondii*: Fusion competence of parasitophorous vacuoles in Fc receptor transfected fibroblasts. *Science.* 249:641–646.

Jomaa, H., J. Wiesner, S. Sanderbrand, B. Altincicek, C. Weidemeyer, M. Hintz, I. Turbachova, M. Eberl, J. Zeidler, H.K. Lichtenthaler, et al. 1999. Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs. *Science.* 285:1573–1576.

Kappe, S., T. Bruderer, S. Gantt, H. Fujioka, V. Nussenzweig, and R. Menard. 1999. Conservation of a gliding motility and cell invasion machinery in Apicomplexan parasites. *J. Cell Biol.* 147:937–944.

Karsten, V., H. Qi, C.J.M. Beckers, J.F. Dubremetz, P. Webster, and K.A. Joiner. 1998. The protozoan parasite *Toxoplasma gondii* targests proteins to dense granules and the vacuolar space using both conserved and unusual mechanisms. *J. Cell Biol.* 141:1323–1333.

Köhler, S., C. Delwiche, D. Denny, L. Tilney, P. Webster, R. Wilson, J. Palmer, and D. Roos. 1997. A plastid of probable green algal origin in Apicomplexan parasites. *Science.* 275:1485–1489.

Levine, N.D. 1988. Progess in taxonomy of the Apicomplexan protozoa. *J. Protozool.* 35:518–520.

Liendo, A., T.T. Stedman, H.M. Ngo, S. Chaturvedi, H.C. Hoppe, and K.A. Joiner. 2001. *Toxoplasma gondii* ADP-ribosylation factor 1 mediates enhanced release of constitutively secreted dense granule proteins. *J. Biol. Chem.* 276:18272–18281.

Luft, B.F., and J.S. Remington. 1992. Toxoplasmic encephalitis in AIDS. *Clin. Infect. Dis.* 15:211–222.

Meissner, M., S. Brecht, H. Bujard, and D. Soldati. 2001. Modulation of myosin A expression by a newly established tetracycline repressor-based inducible system in *Toxoplasma gondii*. *Nucleic Acids Res.* 29:E115.

Meissner, M., M. Reiss, N. Viebig, V.B. Carruthers, C. Trousel, S. Tomavo, J.W. Ajioka, and D. Soldati. 2002. A family of transmembrane microneme proteins of *Toxoplasma gondii* contain EGF-like domains and function as escorters. *J. Cell Sci.* 115:563–574.

Mordue, D.G., S. Hakansson, I. Niesman, and L.D. Sibley. 1999. *Toxoplasma gondii* resides in a vacuole that avoids fusino with host cell endocytic and exocytic vesicular trafficking pathways. *Exp. Parasitol.* 92:87–99.

Moreno, S.N., and L. Zhong. 1996. Acidocalcisomes in *Toxoplasma gondii* tachyzoites. *Biochem. J.* 313:655–659.

Nakaar, V., B.U. Samuel, E.O. Ngo, and K.A. Joiner. 1999. Targeted reduction of nucleoside triphosphate hydrolase by antisense RNA inhibits proliferation of protozoan parasite *Toxoplasma gondii. J. Biol. Chem.* 274:5083–5087.

Ngô, H., H.C. Hoppe, and K.A. Joiner. 2000. Differential sorting and post-secretory targeting of proteins in parasitic invasion. *Trends Cell Biol.* 10:67–72.

Nichols, B.A., M.L. Chiappino, and C.E.N. Pavesio. 1994. Endocytosis at the micropore of *Toxoplasma gondii. Parasitol. Res.* 80:91–98.

Rabenau, K.E., A. Sohrabi, A. Tripathy, C. Reitter, J.W. Ajioka, F.M. Tomley, and V.B. Carruthers. 2001. TgM2AP participates in *Toxoplasma gondi*i invasion of host cells and is tightly associated with the adhesive protein TgMIC2. *Mol. Microbiol.* 41:537–547.

Radke, J.R., B. Striepen, M.N. Guerini, M.E. Jerome, D.S. Roos, and M.W. White. 2001. Defining the cell cycle for the tachyzoite stage of *Toxoplasma gondii. Mol. Biochem. Parasitol.* 115:165–175.

Reiss, M., N. Viebig, S. Brecht, M.-N. Fourmaux, M. Soete, M. Di Cristina, J. Dubremetz, and D. Soldati. 2001. Identification and characterisation of an escorter for two secretory adhesins in *Toxoplasma gondii. J. Cell Biol.* 152: 563–578.

Robibaro, B., T.T. Stedman, I. Coppens, H.M. Ngô, M. Pypaert, T. Bivona, H.W. Nam, and K.A. Joiner. 2002. *Toxoplasma gondii* Rab5 enhances cholesterol acquisition from host cells. *Cell. Micro.* 4:139–152.

Roos, D.S., M.J. Crawford, R.G.K. Donald, J.C. Kissinger, L.J. Klimczak, and B. Striepen. 1999. Origins, targeting, and function of the apicomplexan plastid. *Curr. Opin. Micro.* 2:426–432.

Roos, D.S., R.G.K. Donald, N.S. Morrissette, and A.L.C. Moulton. 1994. Molecular tools for genetic dissection of the protozoan parasite *Toxoplasma gondii. Methods Cell Biol.* 45:27–63.

Rossanese, O.W., J. Soderholm, B.J. Bevis, I.B. Sears, J. O'Connor, E.K. Williamson, and B.S. Glick. 1999. Golgi structure correlates with transitional endoplasmic reticulum organization in *Pichia pastoris* and *Saccharomyces cerevisiae*. *J. Cell Biol.* 145:69–81.

Shaw, M.K., D.S. Roos, and L.G. Tilney. 1998. Acidic compartments and rhoptry formation in *Toxoplasma gondii. Parasitol.* 117:435–443.

Sibley, L.D., and N.W. Andrews. 2000. Cell invasion by un-palatable parasites. *Traffic.* 1:100–106.

Sibley, L.D., E. Weidner, and J.L. Krahenbuhl. 1985. Phagosome acidification blocked by intracellular *Toxoplasma gondii. Nature.* 315:416–419.

Sinai, A.P., P. Webster, and K.A. Joiner. 1997. Association of host cell mitochondria and endoplasmic reticulum with the *Toxoplasma gondii* parasitophorous vacuole membrane - a high affinity interaction. *J. Cell Sci.* 110:2117–2128.

Soldati, D., A. Lassen, J.F. Dubremetz, and J.C. Boothroyd. 1998. Processing of *Toxoplasma* ROP1 protein in nascent rhoptries. *Mol. Biochem. Parasitol.* 96: 37–48.

Stokkermans, T.J.W., J.D. Schwartzman, K. Keenan, N.S. Morrisette, L.G. Tilney, and D.S. Roos. 1996. Inhibition of *Toxoplasma gondii* replication by dinitroaniline herbicides. *Exp. Parasitol.* In press.

Striepen, B., M.J. Crawford, M.K. Shaw, L.G. Tilney, F. Seeber, and D.S. Roos. 2000. The plastid of *Toxoplasma gondii* is divided by association with the centrosomes. *J. Cell Biol.* 151:1423–1434.

Striepen, B., C. He, M. Matrajt, D. Soldati, and D.S. Roos. 1998. Expression, selection and organellar targeting of the green fluorescent protein in *Toxoplasma gondii. Mol. Biochem. Parasitol.* 92:325–338.

Striepen, B., D. Soldati, N. Garcia-Reguet, J.F. Dubremetz, and D.S. Roos. 2001. Targeting of soluble proteins to the rhoptries and micronemes in *Toxoplasma gondii. Mol. Biochem. Parasitol.* 113:45–53.

Striepen, B., M.W. White, C. Li, M.N. Guerini, S.-B. Malik, J.M. Logsden, Jr., C. Liu, and M.S. Abrahamsen. 2002. Genetic complementation in axiocomplexan parasites. *Proc. Natl. Acad. Sci. USA*. In press.

Suss-Toby, E., J. Zimmerberg, and G.E. Ward. 1996. *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.

Swedlow, J.R., K. Hu, P.D. Andrews, D.S. Roos, and J.M. Murray. 2002. Measurement of tubulin content in the conoid and spindle pole of the parasite *Toxoplasma gondii*: a comparison of laser scanning confocal and wide field fluorescence microscopy fro quantitative analysis in living cells. *Proc. Natl. Acad. Sci. USA.* 99:2014–2019.

Tooze, S.A., G.J. Martens, and W.B. Huttner. 2001. Secretory granule biogenesis: rafting to the SNARE. *Trends Cell Biol.* 11:116–122.

Waller, R.F., P.J. Keeling, R.G.K. Donald, B. Streipen, E. Handman, N. Lang-Unnasch, A. Cowman, G.S. Besra, D.S. Roos, and G.F. McFadden. 1998. Nuclear-encoded proteins target to the plastid in *Toxoplasma gondii* and *Plasmodium falciparum. Proc. Natl. Acad. Sci. USA.* 95:12352–12357.

Waller, R.F., M.B. Reed, A.F. Cowman, and G.I. McFadden. 2000. Protein trafficking to the plastid of *Plasmodium falciparum* is via the secretory pathway. *EMBO J.* 19:1794–1802.

Wilson, R.J., P.W. Denny, P.R. Preiser, K. Rangachari, K. Roberts, A. Roy, A. Whyte, M. Strath, D.J. Moore, P.W. Moore, and D.H. Williamson. 1996. Complete gene map of the plastid-like DNA of the malaria parasite *Plasmodium falciparum. J. Mol. Biol.* 261:155–172.

Yung, S., T.R. Unnasch, and N. Lang-Unnasch. 2001. Analysis of apicoplast targeting and transit peptide processing in *Toxoplasma gondii* by deletional and insertional mutagenesis. *Mol. Biochem. Parasitol.* 118:11–21.