Traffic Jams:
Protein Transport in *Plasmodium falciparum*

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Protein targeting in malaria parasites is a complex process, involving several cellular compartments that distinguish these cells from more familiar systems, such as yeast or mammals. At least a dozen distinct protein destinations are known. The best studied of these is the vestigial chloroplast (the apicoplast), but new tools promise rapid progress in understanding how Plasmodium falciparum *and related apicomplexan parasites traffic proteins to their invasion-related organelles, and how they modify the host by trafficking proteins into its cytoplasm and plasma membrane. Here, Giel van Dooren and colleagues discuss recent insights into protein targeting via the secretory pathway in this fascinating and important system. This topic emerged as a major theme at the Molecular Approaches to Malaria conference, Lorne, Australia, 2–5 February 2000.*

A key difference between eukaryotes and prokaryotes is the presence of a nucleus and other membrane-bound organelles in eukaryotes. Organellar sub-compartments enable eukaryotes to segregate biochemical processes. However, this compartmentalization creates a cellular dilemma: as most organellar proteins are encoded by the nuclear genome and translation is initiated within the cytosol, how are the proteins to traverse the very boundaries that keep the organelles separate? Because different organelles require different proteins, these proteins must somehow be specifically targeted from the cytosol into the organelle in which they are to function.

This review focuses on recent insights into protein targeting via the secretory pathway of *Plasmodium falciparum*, also considering relevant work on the related *Toxoplasma gondii*. Both genera are intracellular parasites, which introduces an intriguing extra level of complexity to targeting. Indeed, among all the eukaryotes, *Plasmodium* performs some of the most extraordinary protein-targeting gymnastics known. In addition to targeting proteins to multiple sites within the parasite, *Plasmodium* is also able to target proteins to various parts of the host cell, including the host cell plasma membrane (PM).

Trafficking jams in *Plasmodium falciparum*

The asexual life cycle of *P. falciparum* occurs in the blood system of humans, and has been the focus of most protein-trafficking studies in this parasite. When a merozoite comes into contact with an erythrocyte, it commences invasion, initiating the intracellular cycle. Although *T. gondii* exhibits a much broader tissue specificity (and host range), the tachyzoite form of this parasite is, in many respects, comparable to the *P. falciparum* merozoite, and it is reasonable to assume that the mechanism of cell invasion is generally conserved between the two[1]. Invasion involves the discharge of contents from three secretory organelles located at the apical end of the parasite: the micronemes, rhoptries and dense granules[1]. During initial contact, adhesive proteins from the micronemes are released from the parasite cell[1]. These proteins adhere to the host cell, and in *Toxoplasma* appear to be involved in the gliding motility that occurs during invasion[1]. As the parasite enters the erythrocyte, rhoptries discharge their contents, which coincides with parasitophorous vacuole membrane (PVM) formation. The PVM encloses a novel compartment (the 'parasitophorous vacuole'), which serves as an important biochemical barrier between host and parasite[2]. After invasion is complete, dense granules release their contents into the parasitophorous vacuole. As the parasite develops, it consumes the internal contents of the host cell, eventually dividing and breaking out of the erythrocyte to become merozoites again in the bloodstream (see also L. Bannister, this issue).

With at least three unique secretory organelles associated with invasion, it can already be seen that protein trafficking in *P. falciparum* will differ from well-studied systems such as those of yeast and mammals. These differences become even more striking when one considers the substantial modifications made to the host cell by the invading parasite[3]. Such modifications include parasite-derived proteins that are targeted to the erythrocyte cell surface, where they might be associated with blood vessel adhesion (eg. Ref. 4); proteins targeted to the erythrocyte where they might stabilize the cytoskeleton in the wake of invasion (eg. Ref. 5); and biosynthetic enzymes that are directed into the erythrocyte cytosol (eg. Ref. 6). Add to this the recent findings that proteins are directed to the chloroplast homologue (the 'apicoplast') via the endomembrane system[7–9], and you have a considerable protein 'trafficking jam' in the parasite secretory pathway. The secretory system of the parasite must differentially target nuclear-encoded proteins to the endoplasmic reticulum (ER), Golgi domains, the food vacuole, micronemes, rhoptries, dense granules, the apicoplast, the parasite PM (PPM), the parasitophorous vacuole, the PVM, the erythrocyte cytosol and the erythrocyte PM (EPM) (Fig. 1).

The unusual secretory pathway

Three lines of evidence suggest the presence of a classical secretory pathway in *P. falciparum* (ie. one similar to that found in better-studied eukaryotes). First, several homologues of proteins found in the classical secretory system occur in *P. falciparum*, including the ER proteins reticulocalbin[10] and glucose-regulated protein[11]. Both localize to an ER-like compartment in *P. falciparum* and contain a classical XDEL amino acid retention motif for ER localization. A range of Golgi-protein homologues have also been found. In addition,

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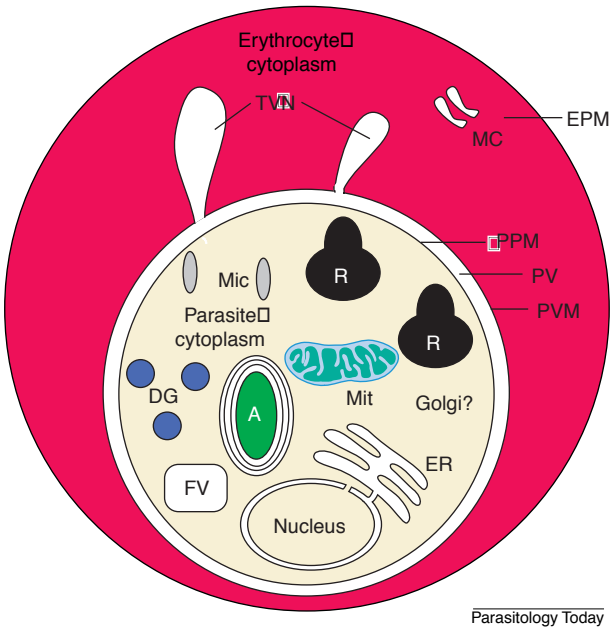

Fig. 1. Organelles and compartments in an erythrocyte infected by *Plasmodium falciparum*. The parasite targets proteins to all of the labelled structures. Except for the mitochondrion and the nucleus (which have distinct targeting mechanisms), targeting involves the endomembrane system. Abbreviations: A, apicoplast; DG, dense granules; EPM, erythrocyte plasma membrane; ER, endoplasmic reticulum; FV, food vacuole; MC, Maurer's clefts; Mic, micronemes; Mit, mitochondrion; PPM, parasite plasma membrane; PV, parasitophorous vacuole; PVM, parasitophorous vacuolar membrane; R, rhoptries; TVN, tubulovesicular network.

many secretory pathway proteins have been identified in *Toxoplasma*[12–14]. Second, many *P. falciparum* and *T. gondii* proteins contain a hydrophobic region at their N-terminus, similar to the signal peptide that targets proteins to the secretory pathway in other organisms[15]. These include proteins targeted to the rhoptries[16], micronemes[17], parasitophorous vacuole[18], PVM[19] and apicoplast[7,8]. Third, the fungal metabolite brefeldin A (BFA) – thought to inhibit the formation of vesicles involved in trafficking between the ER and Golgi complex[20] – blocks protein trafficking to the rhoptries[16], erythrocyte cytosol[21] and EPM[22,23] in *P. falciparum*. BFA also blocks trafficking in *T. gondi*[12,13,24–26].

However, although a classical secretory pathway appears to be present in *P. falciparum*, protein trafficking in this parasite is unusual in several respects. First, electron micrographs reveal no obvious Golgi complex, suggesting that this organelle is either absent or highly modified. Second, evidence suggests that some exported proteins are not trafficked via a classical pathway. Several immunolabelling experiments indicate that the export of some proteins is not inhibited by BFA[6,27]. Furthermore, not all exported proteins contain a typical signal peptide. Some proteins contain an internal hydrophobic sequence beginning 50–82 residues from the N-terminus (reviewed in Ref. 18), and others [eg. erythrocyte membrane protein 1 (EMP1)] lack a hydrophobic sequence altogether[3]. Third, secretory pathway protein trafficking in *P. falciparum* must differentially target proteins to a range of destinations not found in other eukaryotes, including the three secretory organelles (micronemes, rhoptries and dense granules), the PVM and various locations within the erythrocyte.

The unstacked functional Golgi

Electron microscope examination indicates that *P. falciparum* lacks the stacked cisternae that make up a typical Golgi. Some studies have found membrane-enclosed structures resembling a single Golgi cisterna (eg. Refs 28,29), but Golgi-protein homologues have not yet been localized to these compartments. Other Apicomplexa, such as *Toxoplasma*[13], clearly have a Golgi, suggesting that this organelle was originally present in the phylum, and has been reduced or lost in the *Plasmodium* lineage. Several lines of evidence suggest that *P. falciparum* retains a functional Golgi (ie. regions of the cell that perform Golgi functions, even though they might not be Golgi-like in appearance). Golgi-protein homologues identified to date include ERD2 (a *cis*-Golgi protein that binds to the XDEL retention sequence of ER proteins[30]), and Rab6 (a protein found in the *medial*- and *trans*-Golgi of other eukaryotes[31,32]). Interestingly, localization experiments indicate that ERD2 and Rab6 are found in different regions of the parasite cell, with ERD2 localizing to a single region near the nucleus, and Rab6 localizing to several sites distinct from ERD2 (Ref. 32). Assuming that ERD2 and Rab6 serve as *cis*- and *trans*-Golgi markers, respectively, van Wye *et al.*[32] suggest that the Golgi of *P. falciparum* is present in a highly modified 'unstacked' form, with the Golgi compartments not located adjacent to one another. It remains to be determined whether and how protein trafficking occurs between these compartments, or between the ER and these compartments.

Trafficking beyond the PPM

In other eukaryotic systems, secretory protein trafficking depends generally on forward transport signals and machinery[33]. In the absence of alternative sorting signals, the default pathway transports proteins to the PM or out of the cell. In *P. falciparum*, however, proteins must also be targeted beyond the PM to the PVM, the erythrocyte cytosol and the EPM. Two models have been proposed to account for translocating proteins across the PVM[34]. The one-step model proposes that fusion of secretory vesicles at regions where the PPM appears to coalesce with the PVM, would release proteins across the PVM, into the erythrocyte cytosol[35]. The two-step model suggests that proteins secreted into the parasitophorous vacuole are subsequently translocated across the PVM, either by protein translocation complexes or by vesicle budding.

To test these models, Ansorge *et al.*[36] permeabilized either the EPM alone (with streptolysin O), or both the EPM and PVM (with saponin). By analysing the contents of the soluble vs. pellet fractions, they could compare the proteins located in the erythrocyte cytosol, in the parasitophorous vacuole and in the parasite itself. The one-step model predicts that proteins targeted into the erythrocyte would be absent from the parasitophorous vacuole fraction, while the two-step model predicts that exported proteins would be located in both the parasitophorous vacuole and erythrocyte fractions. Ansorge *et al.* found that one erythrocyte surface protein [glycophorin-binding protein (GBP)] occurred in both the erythrocyte and

parasitophorous vacuole fractions, supporting the two-step model. Recently, the same group attached a signal peptide to an enzymatic reporter protein, and demonstrated that secretion into the erythrocyte occurs via the parasitophorous vacuole (K. Lingelbach, pers. commun.). This finding supports the two-step model, and suggests that trafficking to the erythrocyte cytosol might be the default pathway in *P. falciparum*. The mechanism of protein transport across the PVM remains to be determined.

Having crossed the PVM, proteins must be transported to various regions within the erythrocyte, including the EPM. Parasite infection causes extensive internal membranes to develop within the erythrocyte[37]. These include extensions of the PVM, called the tubulovesicular network (TVN) and Maurer's clefts (it is not known whether these two extensions are distinct from one another), both of which might be involved in protein transport[37]. Several studies have shown that proteins exported into the erythrocyte localize to Maurer's clefts[23,38], suggesting their involvement in the trafficking of these proteins. One controversial theory suggests that the TVN is connected to the EPM, resulting in a so-called 'parasitophorous duct' connecting the parasitophorous vacuole to the extracellular medium (reviewed in Ref. 37). Experiments whereby the parasitophorous vacuole was visualized using green fluorescent protein (GFP) showed clear images of the TVN[7], but no connections to the host cell membrane were observed. The inability to visualize a duct by this technique could be due to dispersion of the GFP as it is secreted into the extracellular medium. However, little new evidence has emerged to support the existence of a duct[39].

Several studies suggest that transport through the erythrocyte cytosol occurs in vesicles that bud from the PVM[29,40]. Trelka *et al*.[29] identified 60–100 nm vesicles surrounded by an electron-dense coat in the infected erythrocyte cytoplasm, aggregated on the host cell side of the vacuolar membrane, and fusing with the EPM. Incubation of infected erythrocytes in aluminium fluoride (which, in other eukaryotes, activates GTP-binding proteins and, consequently, inhibits vesicle fusion with membranes) caused the vesicles to form in strings in the erythrocyte cytosol. Immunoelectron microscopy indicates that several parasite proteins destined for the erythrocyte surface localize to these vesicles, suggesting that trafficking of these proteins to the EPM is vesicle mediated. Interestingly, Albano *et al*.[21] reported finding a *P. falciparum* homologue of Sar1p that localizes to the erythrocyte cytosol. In other eukaryotes, Sar1p is involved in the trafficking of coatomer-coated (COP11) vesicles between the ER and Golgi. These results suggest that *P. falciparum* might use existing transport machinery for trafficking through the erythrocyte cytosol, although contributions of the parasite vs erythrocyte cytosol for the multiple components needed for vesicle budding, docking and fusion remain unclear. Gormley *et al*.[41] found that some erythrocyte surface proteins are transported in vesicles, while others are transported as protein aggregates, suggesting there might be several pathways for protein trafficking beyond the PVM.

BFA: evidence for a second secretory pathway?

The lack of an obvious signal peptide in many secreted *P. falciparum* proteins[3] and the failure of BFA to inhibit the export of some proteins[6] suggests the existence of an ER–Golgi-independent secretory pathway in the parasite[42]. The presence of multiple secretory pathways would not be unprecedented. In bacteria, for example, two separate secretory pathways coexist – one mediated by signal peptide targeting and the other by a twin-Arg motif[43]. A survey of *P. falciparum* proteins showed that those targeted within the parasite and to the parasitophorous vacuole all contained classical N-terminal signal peptides, while most of those targeted to the erythrocyte contained either a putative internal signal peptide or no signal peptide at all[3]. This provides a potential mechanism for the sorting of proteins targeted within the parasite vs. those destined for the erythrocyte. There is no evidence for protein targeting from the parasite to the host cell during *T. gondii* infection, so experimental elucidation of this fascinating phenomenon will have to come from studies on *Plasmodium* spp[2,44].

Wiser *et al*.[45] have proposed a second pathway model for *Plasmodium*, based on the observation that BFA treatment causes the formation of a large ER-like compartment that is distinct from the classical ER[46]. Several proteins normally targeted to the PVM and erythrocyte were localized in this secondary ER, which could provide a pathway for protein trafficking to the PVM and beyond. It remains to be shown whether targeting to the proposed secondary ER compartment involves motifs distinct from classical ER signal sequences. Of course, the lack of a classical signal peptide does not necessarily exclude proteins being transported via the ER, with signal recognition particle (SRP)-independent translocation into the ER occurring in yeast[47].

Several authors have noted that the effects of BFA are still not well characterized in *Plasmodium* cells[21,42], and it is therefore necessary to view conclusions drawn exclusively from studies using BFA with caution. BFA is generally considered to inhibit vesicle trafficking in the pre-Golgi secretory system. However, evidence from other systems suggests BFA might interfere more strongly with trafficking between the Golgi and *trans*-Golgi network[48]. Given the dispersed nature of the Golgi of *Plasmodium*, the two observed secretory pathways in the parasite might simply represent a branch point in the secretory system after the Golgi, BFA inhibiting trafficking from only one of the branch points. Additional experiments might include the use of other agents that block membrane traffic, such as agents that disrupt the cytoskeleton, or inhibitors of vesicle acidification, phosphoinositide metabolism, or protein folding in the ER[13].

Targeting signals in *Plasmodium falciparum*

With a multitude of destinations for secretory pathway proteins both inside and outside the parasite (Fig. 1), the protein transport machinery must be able to distinguish between them. In other eukaryotes, this process can occur in several ways. The best-characterized lysosomal targeting pathway is mediated by oligosaccharide signals that are attached to proteins in the Golgi[49]. Glycosylation appears to be rare in *P. falciparum*, although recent evidence suggests that it does occur[50]. Oligosaccharides can exert dramatic effects on protein trafficking in other Protozoa[51], but their function in *P. falciparum* has not been explored. One intriguing possibility is that post-translational addition of a GPI anchor, which connects many erythrocyte surface proteins to the PM, might serve as a

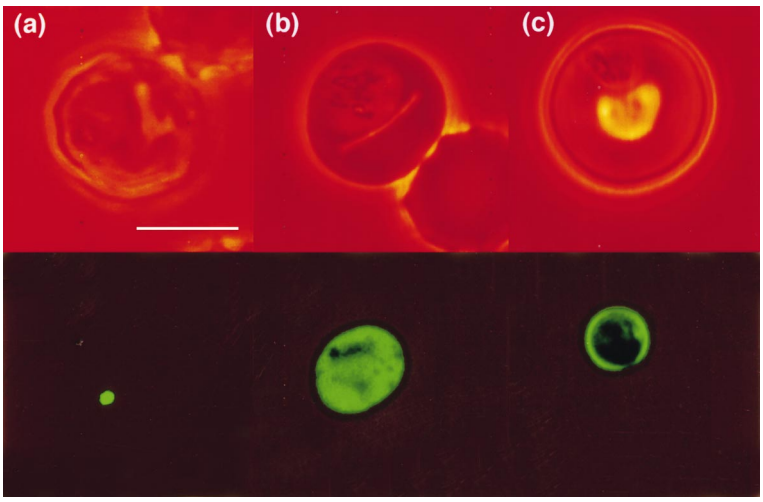

Fig. 2. Fluorescent protein studies on apicoplast targeting in *Plasmodium falciparum*. Bright field (upper panel) and GFP fluorescence (lower panel) of erythrocytes harbouring *Plasmodium* transfectants expressing green fluorescent protein (GFP) with the bipartite chloroplast leader (a), the transit peptide without the signal peptide (b) and the signal peptide without the transit peptide (c). In (a), the fluorescence is in the relict chloroplast. Without the signal peptide, GFP accumulates throughout the parasite cytoplasm (b). Without the transit peptide, GFP is secreted into the parasitophorous vacuole surrounding the parasite (c). Scale bar = 0.5μm.

targeting signal to the erythrocyte membrane[50]. In *T. gondii*, GPI anchors act as sorting signals by directing parasite surface proteins to a distinct set of vesicles[12], but no studies have yet examined possible GPI-targeting to the host cell surface in *P. falciparum*.

Sorting by timing provides another means of differentially targeting proteins to various destinations[52]. If rhoptries and micronemes are required only during invasion[1], trafficking to these organelles need only occur late in the erythrocytic life cycle, during the schizont or merozoite stages. Likewise, modification of the erythrocyte need only occur early in the erythrocytic cycle, during the ring and trophozoite stages. Apical membrane antigen 1 (AMA-1), a rhoptry protein of *Plasmodium*, was mistargeted to regions including the parasite cell surface when under control of the promoter of a protein expressed at several stages of the life cycle[53]. This suggests that the same targeting signal could conceivably target proteins to different locations at different stages of development, and that the timing of expression can be critical for proper subcellular localization in *Plasmodium*.

Another common means of targeting proteins to specific destinations is via motifs embedded in the amino acid sequence of the protein. *Toxoplasma gondii* has a well-developed system for organellar sorting of transmembrane proteins based on Tyr motifs in the cytoplasmic tail, including proteins destined for the rhoptries, micronemes and the *trans*-Golgi network[14]. Such Tyr-based motifs are also present in many *P. falciparum* microneme proteins, but their role in sorting has not yet been established.

Protein targeting to the apicoplast

The best-studied examples of amino acid motif-based targeting in *P. falciparum* are apicoplast proteins. The apicomplexan plastid organelle is nonphotosynthetic, but pharmacological analysis indicates that it is essential for cell survival[54]. Cell biological studies have yielded plastid segregation mutants, and parasites that lose their apicoplast are unable to establish a productive infection in new host cells (C.Y. He and D.S. Roos, unpublished). Recent work suggests that this organelle functions in fatty acid and isoprenoid biosynthesis[8,55], processes that also occur in plant chloroplasts. Like the chloroplasts of plants, most proteins of apicoplasts are encoded in the nuclear genome and imported post-translationally.

In plant chloroplasts, targeting of most nuclear-encoded proteins is mediated by an N-terminal sequence known as the transit peptide, which is rich in Ser and Thr residues[56]. This sequence mediates interaction with the so-called 'translocon outer chloroplast complex' (Toc) and the 'translocon inner chloroplast complex' (Tic), present on the outer and inner membranes of the chloroplast, respectively[57]. Toc–Tic complexes bind to the transit peptide and then translocate proteins across the two membranes into the chloroplast interior.

Apicoplasts differ from most plant chloroplasts in one crucial respect: they are surrounded by four membranes rather than by two[54] (but see Ref. 58). These extra membranes can be viewed conceptually as resulting from the secondary endosymbiotic origin of the apicoplast, in which a cell containing a chloroplast bound by two membranes was engulfed by a heterotrophic cell: one extra membrane being derived from the PM of the engulfed cell, and the other from the phagosome of the heterotroph[9,59]. How then are proteins transported across these extra membranes? Because such secondary endosymbiotic organelles are topologically outside the host cell (ie. within the lumen of the endomembrane system), it is not surprising that apicoplast-targeted proteins contain a signal peptide at their N-terminus[8,9].

Fusion of GFP downstream of putative leader sequences from proteins targeted to the apicoplast proves that the leader is sufficient for apicoplast targeting (Fig. 2a). Apicoplast leaders are bipartite[8] and dissection of the leader reveals that both components are required for targeting[7,9]. When the hypothetical transit peptide alone (the region directly following the signal peptide) was fused to the reporter protein, GFP accumulated in the cytoplasm (Fig. 2b). Using just the signal peptide of an apicoplast-targeted gene causes GFP to be secreted from the parasite (Fig. 2c), indicating that targeting occurs via the secretory pathway. Clearly, a further signal is required for transport to the apicoplast. It appears that the N-terminal signal peptide targets apicoplast proteins into the secretory system, and the sub-terminal targeting signal directs the protein to the apicoplast[7,9,59,60]. Experiments have shown that *Toxoplasma* apicoplast leaders function in *Plasmodium* and vice versa[7,55]. Furthermore, a completely artificial apicoplast protein can be assembled from 'spare parts'. By fusing a standard signal peptide from a *Toxoplasma* surface protein (P30)[61] with a transit peptide from a chloroplast-targeted protein of a plant (the FtsZ leader of *Arabidopsis thaliana*), it was possible to target the GFP reporter to the apicoplast (M.J. Crawford and D.S. Roos, unpublished). This demonstrates that targeting

motifs are readily interchangeable, supporting the hypothesis that apicomplexan parasite plastids use the same transit peptide system found in plants[59,60]. Indeed, several homologues of proteins from the Toc–Tic complexes have been found in *P. falciparum* (C.J. Tonkin and G.I. McFadden, unpublished). Apicoplast targeting thus brings together two separate systems of protein trafficking, namely the secretory- and the chloroplast-targeting pathways.

Remaining questions

Two key questions remain: (1) where does the apicoplast lie within the secretory system? and (2) how do proteins cross the second membrane of the apicoplast (counting from the outside)? The outer membrane of the apicoplast is clearly part of the endomembrane system, suggesting vesicular transfer to the apicoplast compartment. It is also possible that the apicoplast lies very proximal within the endomembrane system, and that all secreted proteins have to pass this organelle, with those bearing a transit peptide being removed from the default pathway by the Toc–Tic apparatus. An intriguing possibility (suggested initially by Cavalier-Smith[62]) is that the second membrane contains extra Toc proteins that recognize transit peptides within the endomembrane lumen and transport them to the space between the second and third membranes. In this zone, the transit peptide would be recognized by a second Toc apparatus (in the third membrane, homologous to the outer membrane of plant chloroplasts) that would further translocate apicoplast proteins to the Tic apparatus for passage across the fourth, and final membrane (Fig. 3). This model is consistent with the GFP studies described earlier, and explains how a transit peptide component can route proteins to the apicoplast once inside the endomembrane system. One weakness of the model is that it does not explain why the second membrane persists when its function seems redundant. Furthermore, recognition of transit peptides by the Toc apparatus is believed to be dependent on transit peptide contact with galactolipids in the chloroplast membrane[60]. If the model proposed here (Fig. 3) is correct, galactolipids would be expected to occur in both the second and third membranes. A further requirement for this model is that genes encoding the Toc apparatus would be among the first to relocate to the host nucleus as the Toc proteins presumably need to access Membrane 2 via the endomembrane system.

Although signals involved in trafficking to the apicoplast are better understood than those for other compartments within *P. falciparum*, much remains unknown, including the functional features of the transit peptide, which, even in plants, are poorly characterized[63]. Mutagenesis experiments might indicate which residues of the transit peptide are important for trafficking. One experiment deleted all Ser and Thr residues from an apicoplast transit peptide, but found that targeting was not impeded[7]. With no obvious amino acid motifs identified in apicoplast transit peptides (or chloroplast transit peptides), the important characteristics of these might lie in their secondary and tertiary structures[63].

Future directions

The presence of signal peptides and secretory pathway protein homologues indicates that a 'classical' secretory pathway exists in *Plasmodium falciparum*. This pathway

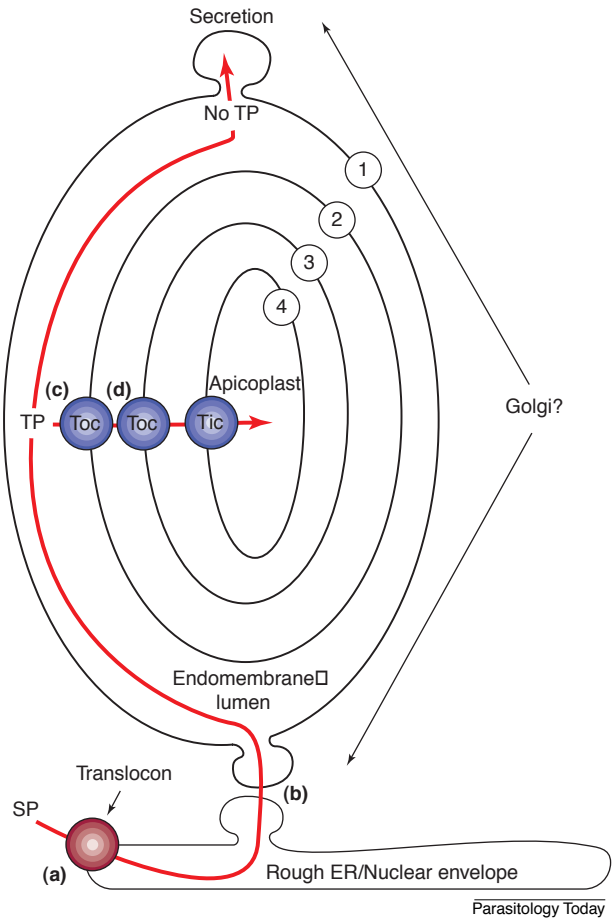

Fig. 3. Model for targeting proteins across the four apicoplast membranes (1–4). The signal peptide component (SP) of this bipartite leader engages with the signal recognition particle and ER-translocating complex (a), effecting cotranslational insertion into the lumen of the endomembrane system (b). The apicoplast lies within the endomembrane system, perhaps even upstream of the Golgi. A Toc apparatus hypothesized to be located in Membrane 2 is presumed to recognize the transit peptide component (TP) of apicoplast-targeted proteins, mediating translocation out of the endomembrane pathway (c). Another Toc apparatus (this time acting in conjunction with Tic proteins) might mediate transfer across the inner two membranes (3 and 4) (d).

clearly targets proteins via the ER – and perhaps a Golgi apparatus – to the apicoplast, the parasitophorous vacuole, the secretory organelles and possibly the erythrocyte. However, the trafficking of exported proteins lacking an obvious signal peptide remains to be explored. Also awaiting explanation is how proteins are translocated into the erythrocyte cytosol, how they are then transported to the erythrocyte membrane, and how protein targeting to the micronemes, rhoptries, dense granules and the apicoplast is specified.

Addressing these questions will require a range of experimental techniques. The value of *Toxoplasma* as a proxy system, more amenable to genetic manipulation than is *Plasmodium*, has already provided important clues about protein targeting in *Plasmodium*[12–14,64]. Transfecting both *Toxoplasma* and *Plasmodium* cells with reporter proteins that contain attached targeting sequences should provide insights into the motifs important for mediating intracellular protein transport. Developing informatics-based approaches from these data will enable recognition of targeting regions from

the genome sequence data, crucial for identifying proteins targeted to the plastid and other organelles. Determining the compartments to which proteins are targeted will help us unravel their metabolic pathways and roles in the invasion process. Used in conjunction with streptolysin O and saponin treatments, reporter proteins can also be used for determining the locations of exported proteins, and for elucidating the pathway(s) and motifs required for protein trafficking to the erythrocyte. Using aluminium fluoride to prevent vesicle fusion should prove useful in determining the importance of vesicle transport through the erythrocyte[29]. Finally, subcellular fractionation of organelles and vesicles has been valuable in determining the proteins present in the compartments of other eukaryotes (reviewed in Ref. 65). Developing such techniques in *P. falciparum* will provide important clues concerning: (1) the function of Golgi-like compartments; (2) the possible presence of a secondary ER; and (3) the proteins and mechanisms involved in protein trafficking across the membranes of the apicoplast and the ER. With these tools, we should be able to understand how *Plasmodium* proteins avoid getting 'stuck in traffic'.

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A Brief Illustrated Guide to the Ultrastructure of *Plasmodium falciparum* Asexual Blood Stages

L.H. Bannister, J.M. Hopkins, R.E. Fowler, S. Krishna and G.H. Mitchell

Interpretation of the new information arising from the Plasmodium falciparum *Genome Project requires a good working knowledge of the ultrastructure of the parasite; however many aspects of the morphology of this species remain obscure. Lawrence Bannister, John Hopkins and colleagues here give an illustrated overview of the three-dimensional (3-D) organization of the merozoite, ring, trophozoite and schizont stages of the parasite, based on available data that include 3-D reconstruction from serial electron microscope sections. The review describes the chief organelles present in these stages, emphasizing the continuity of structure in addition to specialized, stage-specific features developed during the asexual erythrocytic cycle.*

As the sequencing of the *Plasmodium falciparum* genome nears completion, two major challenges are becoming urgent: to interpret the plethora of data in terms of parasite biology, and to use this information effectively in the control of malaria. There have been many studies of malaria parasites of nonhuman host species[1–3], but relatively few papers on *P. falciparum* morphology since the first definitive ultrastructural description of the parasite in culture by Langreth *et al.* in 1978 (Ref. 4). We are in the curious position of knowing much about the molecular biology of *P. falciparum* while its structure remains only sketchily understood, making accurate immunolocalization of antigens increasingly problematical.

Three-dimensional (3-D) reconstruction from serial electron microscopic sections allows detailed analysis of cell structure, and has been used to study the mitochondrion[5], chromosome number[6] and feeding apparatus[7] of *P. falciparum*. This approach provides high spatial resolution not available by other means. Efforts have been made to image whole parasitized red blood cells (RBC) by X-ray microscopy[8], but the resolving power of this method is as yet considerably inferior to that of electron microscopy (EM). Using serial sectioning, we have also reconstructed the plastid (apicoplast)[9] and the merozoite rhoptry–Golgi complex in this species[10]. The illustrations in this *Review* outline the major features of each stage; it should be borne in mind that development is a continuous dynamic process taking 48 h to complete, with many intermediate stages and complexities that are not shown here.

The merozoite stage

The invasive merozoite stage of the parasite is important immunologically because it is briefly extracellular and thus exposed to host antibodies between leaving one RBC and entering the next. *Plasmodium falciparum*

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