

EXPORT AND TRAFFICKING OF *Plasmodium* PROTEINS WITHIN THE HOST ERYTHROCYTE

Tráfico y exportación de proteínas de *Plasmodium* en el eritrocito hospedero

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SUMMARY

The export and trafficking of parasite proteins within the infected erythrocyte is a complex process and not well understood. Export of proteins from the parasite was previously speculated to involve a specialized compartment originally designated as the secondary endoplasmic reticulum of the Apicomplexa, or sERA. The properties of this *Plasmodium* export compartment are reviewed in regards to more recent observations about the trafficking of *Plasmodium* proteins within the host erythrocyte. In addition, a calcium ATPase unique to the Apicomplexa and with homology to the sarcoplasmic-endoplasmic reticulum calcium ATPase is discussed in the context of this unique export compartment. It is also speculated that the *Plasmodium* export element, PEXEL, may play a role in targeting proteins to this parasite export compartment. Exported proteins are then proposed to move into the parasitophorous vacuole and those destined for the host erythrocyte are transported to the erythrocyte cytoplasm as soluble proteins. Chaperones probably play a role in escorting parasite proteins to their final destinations and assembly on the erythrocyte membrane.

Key words: secretory pathway, SERCA, protein trafficking, malaria, *Plasmodium*.

RESUMEN

El tráfico y exportación de proteínas del parásito dentro del citoplasma del eritrocito es un proceso complejo, que no es bien comprendido. Inicialmente se especuló sobre la manera como el parásito exporta éstas proteínas y se involucró un compartimiento que se denominó retículo endoplasmático secundario de los Apicomplexa (sERA). En este trabajo se revisan las propiedades de este compartimiento exportador de acuerdo con las observaciones más recientes del tráfico de proteínas de *Plasmodium* al salir del parásito. La discusión se enmarca dentro del contexto de este único compartimiento exportador relacionado con una ATPasa de calcio única de los Apicomplexa que presenta homología con la ATPasa de calcio del retículo endoplasmático sarcoplásmico

(SERCA). Además, se especula que *Plasmodium* exporta elementos PEXEL, que podrían estar asociados con el direccionamiento de proteínas de *Plasmodium* hacia el compartimiento exportador y se propone que éstas proteínas van posteriormente a la vacuola parasitófora, y aquellas cuyo destino es la célula hospedera se transportan al citoplasma del eritrocito como proteínas solubles. Las chaperonas posiblemente escoltan las proteínas del parásito hasta su destino final y se ensamblan en la membrana del eritrocito.

Palabras clave: vía secretoria, SERCA, tráfico de proteínas, malaria, *Plasmodium*.

INTRODUCTION

The malaria parasite exhibits a complex life cycle involving a mosquito vector and a vertebrate host. During one stage of its life cycle the parasite infects erythrocytes. The blood stage is primarily responsible for the disease manifestations and generally establishes a chronic infection within the host. Like other Apicomplexa, the malaria parasite has specialized invasive forms (eg., merozoites) which are capable of entering the host cell. Invasion of host cells is accomplished through specialized secretory organelles called rhoptries, micronemes and dense granules (Cowman and Crabb, 2006). During parasite entry the merozoite forms a parasitophorous vacuole which is derived in part from the host erythrocyte membrane and the contents of the rhoptries (Soldati *et al.*, 2004). Thus, the parasite does not lie within the cytoplasm of the erythrocyte, but is surrounded by a parasitophorous vacuolar membrane (PVM). In addition to these organelles that are specialized in the invasion process, there are other unique organelles within the parasite such as the food vacuole and apicoplast (Tonkin *et al.*, 2006).

The malaria parasite also extensively modifies the host erythrocyte during the intraerythrocytic stage (Cooke *et al.*, 2004b). For example, following infection the host erythrocyte becomes more permeable to low molecular metabolites and nutrients and new permeability pathways are induced in the host erythrocyte membrane (Kirk, 2004). This reflects the huge demand the actively growing and replicating parasite has for metabolites in the relatively impermeable erythrocyte. These new permeability pathways are probably the result of parasite proteins transported to the erythrocyte membrane (Baumeister *et al.*, 2006).

Ultrastructural alterations are also observed in the host erythrocyte following infection. Well known ultrastructural alterations include the electron-dense knobs on *P. falciparum*-infected erythrocytes and caveola-vesicle complexes on *P. vivax*-infected erythrocytes. The knobs on *P. falciparum*-infected erythrocytes play a role in the cytoadherence of infected erythrocytes to endothelial cells. This cytoadherence leads to a sequestration of the infected erythrocytes in the tissues and contributes to parasite survival by avoidance of the spleen and providing an environment of low oxygen tension. This sequestration of infected erythrocytes also plays a role in the pathogenesis of severe malaria.

Membranous structures are found throughout the host cell cytoplasm of infected erythrocytes. For example, Maurer's clefts are flattened lamellae of a single unit mem-

brane usually located close to the erythrocyte membrane (Lanzer *et al.*, 2006). Other membranous structures which appear to be extensions of the PVM are also found in the cytoplasm of the infected erythrocyte (Atkinson and Aikawa, 1990; Elford and Ferguson, 1993). These extensions of the PVM have also been referred to as the tubovesicular membrane (TVM) network (Elmendorf and Haldar, 1993a). Serial sections of infected erythrocytes suggest that the Maurer's clefts form a continuous membrane network which originates from the PVM at multiple sites and extends across the host erythrocyte (Wickert *et al.*, 2004). It has also been suggested that the Maurer's clefts bud off from this membranous network and are distinct entities (Spycher *et al.*, 2006).

In summary, the malaria parasite exhibits a unique cell biology with many specialized compartments that will require specific protein targeting and trafficking within the parasite (Tonkin *et al.*, 2006). In addition, the parasite induces new compartments in the host erythrocyte and is able to specifically target proteins to distinct extracellular locations: the parasitophorous vacuole, the PVM (including membranous extensions continuous with the PVM), Maurer's clefts, and the modified host erythrocyte membrane. How the parasite induces these new compartments in the host erythrocyte and is able to specifically target proteins to extracellular locations is a central question in *Plasmodium* cell biology. This review will discuss a novel secretory compartment in the malaria parasite and its possible role in modifying the host erythrocyte.

A NOVEL SECRETORY COMPARTMENT IN *PLASMODIUM*

The malaria parasite is a eukaryotic organism and presumably exhibits a secretory pathway similar to other eukaryotic organisms. In this regard, components of the secretory pathway have been identified in the genome of *Plasmodium* (Carlton *et al.*, 2002; Gardner *et al.*, 2002). The first step in the export of proteins is generally the endoplasmic reticulum. However, many *Plasmodium* proteins destined for export into the host erythrocyte appear to accumulate in a compartment distinct from the ER following treatment with brefeldin A (BFA). BFA is a fungal metabolite that blocks secretion by leading to disassembly of the Golgi apparatus and an accumulation of exported proteins in the ER (Chardin and McCormick, 1999). Treatment of infected erythrocytes with BFA results in the accumulation of exported *Plasmodium* proteins in a single compartment located near the parasite periphery (Wiser *et al.*, 1997; Wiser *et al.*, 1999b). This compartment appears as either a flattened disk or has a tubular shape when examined by immunofluorescence. Ultrastructural studies and analysis by density gradients indicate that this novel compartment is membrane bound (Wiser *et al.*, 1999a). Several *Plasmodium* proteins, which are exported into the host erythrocyte, accumulate in this novel compartment located at the parasite periphery following BFA treatment (Table 1). Furthermore, proteins destined for different locations within the infected erythrocyte co-localize to the same compartment following BFA treatment (Wiser *et al.*, 1997).

In contrast, merozoite surface protein-1, a protein on the plasma membrane of the parasite, exhibits a diffuse mottled pattern throughout the cytoplasm of the parasite following BFA treatment, which is similar to the pattern exhibited by ER protein BiP (Wiser *et al.*, 1997). This same reticular pattern has been previously reported for BiP

(Kumar *et al.*, 1991; Elmendorf and Haldar, 1993b) as well as the *P. falciparum* reticulocalbin homologue, a Ca²⁺-binding protein localized to the ER (La Greca *et al.*, 1997). This reticulated pattern and the ultrastructural localization of BiP and reticulocalbin to endomembranes throughout the parasite cytoplasm are consistent with the parasite ER being a loose network of vesicles as previously described (Langreth *et al.*, 1978). Thus it appears that there are two BFA-sensitive export pathways in the malaria parasite: one characterized by a diffuse reticular distribution and representing the classical ER, and the other characterized by a single compartment located at the periphery of the parasite.

Location	Protein	Reference
Erythrocyte membrane	Pc(em)93	(Wisner <i>et al.</i> , 1997)
	Pb(em)65	(Wisner <i>et al.</i> , 1997)
	Ag-Pf332	(Hinterberg <i>et al.</i> , 1994)
	MESA	(Mattei <i>et al.</i> , 1999)
	KAHRP	(Wickham <i>et al.</i> , 2001)
	PfEMP1	(Wickham <i>et al.</i> , 2001)
Membranes in erythrocyte cytoplasm	Pb(ec)31	(Wisner <i>et al.</i> , 1997)
	Pb(ec)13	(Wisner <i>et al.</i> , 1997)
	Pc(ec)19	(Wisner <i>et al.</i> , 1997)
	GBP	(Benting <i>et al.</i> , 1994)
	HRP-II	(Haldar <i>et al.</i> , 2002)
PVM	Exp-1	(Mattei <i>et al.</i> , 1999)
	Ag-3008	(Wisner <i>et al.</i> , 1997)

Table 1. Exported *Plasmodium* proteins accumulating in novel compartment following BFA treatment.

This BFA-induced compartment at the parasite periphery is proposed to be involved in the export of *Plasmodium* proteins into the host erythrocyte. Proteins destined to be exported to the host erythrocyte traverse this *Plasmodium* export compartment instead of the ER. This novel export compartment may be part of an alternate secretory pathway distinct from the classical ER and Golgi. Although many exported *Plasmodium* proteins utilize this novel compartment, other mechanisms of protein export probably co-exist since some protein export occurs via a BFA-insensitive pathway (Elmendorf *et al.*, 1992; Moura and Pudles, 1999).

Several *Plasmodium* proteins are associated with a compartment with a similar morphology as the proposed *Plasmodium* export compartment in the absence of BFA treatment (Table 2). For example, several monoclonal antibodies raised against a membranous fraction isolated from *P. falciparum*-infected erythrocytes exhibited an immunofluorescence pattern similar to the novel BFA-induced compartment (Cortes *et al.*, 2003). Co-localization studies confirm that the proteins recognized by these monoclonal antibodies are localized to the proposed *Plasmodium* export compartment. These co-localization studies indicate that this compartment is not simply an artifact of BFA treatment in that this compartment exists in the absence of BFA. Three distinct proteins of molecular masses 68, 45 and 22 kDa are localized to the *Plasmodium* export

compartment and these three proteins are conserved between *P. falciparum* and the rodent malaria parasites. The identities of these proteins are not known. Knowing the identities of these proteins will provide some insight into the nature of this *Plasmodium* export compartment.

Protein	Comments	Reference
68 kDa	Recognized by mAb-7 and mAb-IG2	(Cortes <i>et al.</i> , 2003)
45 kDa	Recognized by mAb-4F8	(Cortes <i>et al.</i> , 2003)
22 kDa	Recognized by mAb-134	(Cortes <i>et al.</i> , 2003)
<i>PfSar1</i>	COP II component	(Albano <i>et al.</i> , 1999)
<i>PfSec31</i>	COP II component	(Adisa <i>et al.</i> , 2001)
<i>PfATPase4</i>	SERCA-like ATPase unique to Apicomplexa	(Dyer <i>et al.</i> , 1996)

Table 2. Proteins localized to subcellular compartment at the parasite periphery in the absence of BFA.

These monoclonal antibodies have been used to characterize the export compartment during erythrocytic stage schizogony. The structure recognized by these monoclonal antibodies is observed in the early ring stage and is continuously present throughout the entire blood stage (Cortes *et al.*, 2003). The structure increases in size during the trophozoite stage as the parasite grows and may reflect an increase in protein trafficking as the parasite matures. During schizogony the compartment recognized by the monoclonal antibodies divides into smaller structures that are associated with the individual budding merozoites.

Two other proteins possibly localized to the *Plasmodium* export compartment are *PfSar1* (Albano *et al.*, 1999) and *PfSec31* (Adisa *et al.*, 2001). Both of these proteins are homologues of COPII components of which form coats around vesicles involved in ER-Golgi transport (Wieland and Harter, 1999). Co-localization studies using antibodies against the COPII components and monoclonal antibodies discussed above reveal that there is substantial overlap between the compartments recognized by the anti-COPII antibodies and these monoclonal antibodies. However, the compartments recognized by anti-*PfSar1*p and anti-*PfSec31*p do not coincide exactly with the monoclonal antibodies (Cortes *et al.*, 2003). The COPII components and the 68, 45 and 22 kDa proteins co-localize to the compartment along the parasite periphery. But the COPII components exhibit a more extensive distribution within the parasite in that there is a diffuse mottled labeling over the entire parasite. This diffuse mottled pattern is quite reminiscent of the ER and these COPII components partially co-localize with the ER markers BiP and *PfERC* (Albano *et al.*, 1999; Adisa *et al.*, 2001). These results suggest that COPII components are localized to both the ER and to the *Plasmodium* export compartment.

The accumulation of exported proteins following BFA treatment and the co-localization of COPII components to the *Plasmodium* export compartment suggests that it has similar properties as the ER. In addition, an ER-like calcium ATPase is speculated to be localized to this novel compartment (see below). These observations suggest that the *Plasmodium*

export compartment may be a specialized domain of the ER or a separate ER-like organelle. The more extensive distribution of the COPII components in the parasite as compared to the 68, 45 and 22 kDa proteins is consistent with functionally distinct regions of ER. Similarities between this novel compartment and the ER have been previously noted (Wiser *et al.*, 1997; Wiser *et al.*, 1999b) and this compartment was called the secondary ER of Apicomplexa (sERA). These ER-like properties are also consistent with the proposal that this novel export compartment is the first step in an alternate secretory pathway specialized in the export of proteins into the host erythrocyte. Proteins destined for export into the host erythrocyte would be targeted to this special ER domain or ER-like organelle whereas proteins destined for compartments within the parasite would be targeted to other domains of the ER.

ER-LIKE CALCIUM ATPASES OF APICOMPLEXA

Antibodies raised against a P-type ATPase from *P. falciparum*, called ATPase4, label a flattened disk-shaped structure at the parasite periphery (Dyer *et al.*, 1996). Unfortunately these antibodies were no longer available and it could not be determined if this compartment is the same as the novel *Plasmodium* export compartment (Wiser *et al.*, 1997). ATPase4 and another *P. falciparum* ATPase, called ATPase6, both exhibit homology to the sarcoplasmic-endoplasmic reticulum calcium ATPase (SERCA). The observation that *Plasmodium* has two distinct SERCA-like genes and that both PfATPase4 (Dyer *et al.*, 1996) and PfATPase6 (Kimura *et al.*, 1993) are expressed during the blood stage is consistent with the hypothesis that *Plasmodium* has functionally distinct ER-like compartments. No localization data are available for ATPase6.

Searching all of the available sequence databases indicate that orthologs of both ATPase4 and ATPase6 are found in other *Plasmodium* species as well as other Apicomplexa. Orthologs for both ATPase4 and ATPase6 were found in all Apicomplexa genomes which are completely sequenced. Phylogenetic analysis indicates that the ATPase4 orthologs form a clade distinct from the ATPase6 orthologs (Figure 1). Previous studies also indicate that ATPase6 is more homologous to SERCA and that ATPase4 may define a group of Ca²⁺-ATPases which is specific to Apicomplexa (Krishna *et al.*, 2001; Nagamune and Sibley, 2006).

Interestingly, antibodies raised against the *Cryptosporidium* ATPase4 ortholog recognize two distinct proteins of 160 kDa and 146 kDa on immunoblots and localization studies reveal two distinct patterns (Zhu and Keithly, 1997). One pattern is a diffuse perinuclear location, presumably the ER, and the other pattern is discrete vesicles near the parasite periphery at its apical end. These results suggest that the antibodies are recognizing two distinct proteins in two separate compartments. Furthermore, the sizes of the proteins recognized by the antibody are consistent with the predicted sizes of the ATPase4 and ATPase6 orthologs from *Cryptosporidium*. *Cryptosporidium* is more closely related to the gregarines and probably emerged early in Apicomplexan evolution (Zhu *et al.*, 2000) suggesting that the duality of ER-like calcium ATPases may be a feature common among the Apicomplexa. Clearly more work on the locations and functions of the SERCA-like ATPases from the Apicomplexa is needed.

Other interesting observations in regards to Ca^{2+} -ATPases are the labeling of clefts within *P. vivax*-infected erythrocytes with monoclonal antibodies which recognizes SERCA from a wide variety of organisms (Bracho *et al.*, 2002) and the histochemical localization of Ca^{2+} -ATPase activity to Mauers' clefts in *P. falciparum* (Caldas and Wasserman, 2001).

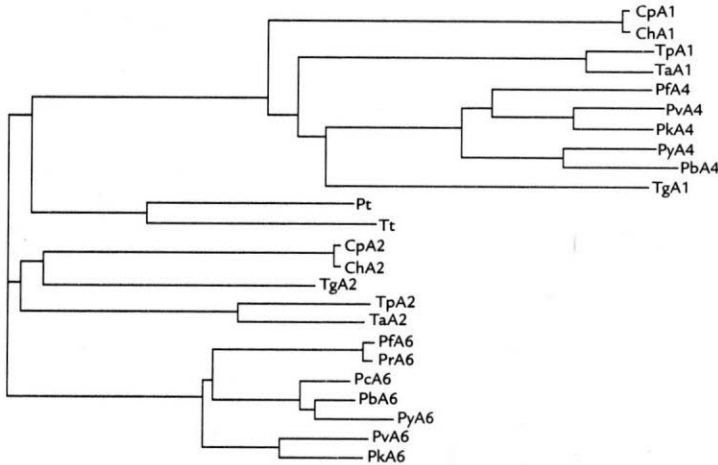


Figure 1. Phylogram of ER-like calcium ATPases from Apicomplexa. Complete sequences were aligned using ClustalW and the phylogram tree was generated with software available at <http://www.ebi.ac.uk/clustalw/>. Two major groups of ER-like calcium ATPases are observed. One appears to be unique to the Apicomplexa (APICA) and the other is homologous to the sarco-endoplasmic reticulum calcium ATPase (SERCA). The protozoa are *Cryptosporidium parvum* (Cp), *C. hominis* (Ch), *Theileria parva* (Tp), *T. annulata* (Ta), *Plasmodium falciparum* (Pf), *P. vivax* (Pv), *P. knowlesi* (Pk), *P. chabaudi* (Pc), *P. berghei* (Pb), *P. yoelii* (Py), *P. reichenowi* (Pr), *Toxoplasma gondii* (Tg), *Paramecium tetraurelia* (Pt), and *Tetrahymena thermophila* (Tt). Accession numbers of sequence derived from Genbank are: EAK87832 (CpA1), XP_667379 (ChA1), EAK90400 (CpA2), EAL38338 (ChA2), CAA76764 (Pt), EAR82769 (Tt), EAN33958 (TpA1), EAN32807 (TaA2), CAI73381 (TaA1), CAI74461 (TaA2), AAF17245 (PfA4), EAA15769 (PyA4), CAH97611 (PbA4), CAD49022 (PfA6), BAD73969 (PrA6), ABA56283 (PcA6), CAH94552 (PbA6), EAA17851 (PyA6) and AAU93917 (TgA2). Identifiers of sequences obtained from <http://www.sanger.ac.uk> are: Pv084625 (PvA4), PK12_1050c (PkA4), Pv081455 (PvA6) and PK1_0430c (PkA6). The TgA1 sequence was obtained from <http://www.toxodb.org> and has the identifier 65.m01184.

The SERCA ATPase from ciliates exhibits approximately equal homology to both ATPase4 and ATPase6 (Figure 1). The ciliates are a sister group to the apicomplexa and dinoflagellates within the Alveolata (Leander and Keeling, 2004). The ciliates probably only have one SERCA since the genome of *Tetrahymena* is completely sequenced (Eisen *et al.*, 2006). Therefore the duplication of SERCA in the apicomplexa likely occurred after the divergence of the apicomplexa and ciliates. SERCA from the ciliates is localized to the alveolar sacs (Hauser *et al.*, 1998), which are flatten membrane-bound compartments found just below the plasma membrane. In Apicomplexa the structures homologous to aveolar sacs are found in the invasive stages and are often called the inner membrane complex. Similarities between alveolar sacs and the ER have been previously noted (Länge *et al.*, 1995; de Melo and de Souza, 1997).

EXTRAPARASITE TRAFFICKING

The observation that many parasite proteins destined for different locations within the host erythrocyte appear to utilize this novel *Plasmodium* export compartment raises some questions about the sorting process within the infected erythrocyte. Presumably proteins will move from this novel compartment into the parasitophorous vacuole. Some exported proteins are known to pass through the parasitophorous vacuole en route to their final destinations within the infected erythrocyte (Ansorge *et al.*, 1996; Wickham *et al.*, 2001). The juxtaposition of the novel export compartment with the parasite plasma membrane implies that proteins may move directly into the parasitophorous vacuole. However, there is no evidence of any transient direct connections between the export compartment and the plasma membrane.

Once within the parasitophorous vacuole the exported proteins need to be sorted and trafficked to their final destinations. The movement of proteins from compartments within the parasite to compartments in the host erythrocyte presents some topological problems and models to account for these problems have been proposed (Przyborski and Lanzer, 2005; Lingelbach and Przyborski, 2006). Many of these models involve a vesicle-mediated transport within the infected erythrocyte. However, many exported *Plasmodium* proteins appear to be exported into the host erythrocyte as soluble proteins. For example, studies on the trafficking of *P. falciparum* erythrocyte membrane protein-1 (PfEMP1) suggest that the protein is trafficked as a complex rather than in vesicles (Knuepfer *et al.*, 2005a; Papakrivov *et al.*, 2005). In addition, the C-terminal region of PfEMP1 binds to knob-associated histidine rich protein (KAHRP; Rug, 2006). These observations imply that PfEMP1 is exported into the host erythrocyte as a soluble protein and is directed to its final destination via interactions with other proteins. PfEMP3 is also trafficked as a complex with other proteins in the cytoplasm of the host erythrocyte (Knuepfer *et al.*, 2005b).

In addition, several *Plasmodium* proteins that are localized to the host erythrocyte membrane, including KAHRP, bind to the submembrane cytoskeleton on the cytoplasmic face of the erythrocyte membrane (Lustigman *et al.*, 1990; Wiser *et al.*, 1990; Foley *et al.*, 1991; Kilejian *et al.*, 1991). These observations imply that a potential mechanism of trafficking within the infected erythrocyte involves the secretion of soluble proteins into the erythrocyte cytoplasm and targeting involves binding to other proteins. Interestingly, many of the proposed exported proteins are chaperones (Hiller *et al.*, 2004; Sargeant *et al.*, 2006) and several chaperones are found in parasitophorous vacuole (Nyalwidhe and Lingelbach, 2006). The role of these exported chaperones may be to escort secreted proteins to their final destinations and assist in the formation of supramolecular structures such as knobs. Host chaperones have also been shown to be associated with knob-enriched erythrocyte membrane fractions (Banumathy *et al.*, 2001).

Such a model for trafficking within the erythrocyte raises questions about how proteins are released from the parasitophorous vacuole. One possibility is that transporters are found on the PVM which transfer proteins from the parasitophorous vacuole to the erythrocyte cytoplasm. However, no such transporters have been identified on the PVM.

Pores on alveolar membranes of the malaria parasite have been described and these pores are speculated to be involved in protein transport (Raibaud *et al.*, 2001). In addition, diffuse membranous structures found at the PVM have been proposed to be involved in the release of material from the parasitophorous vacuole into the erythrocyte cytoplasm (Lanners *et al.*, 1999). Once released into the erythrocyte cytoplasm the exported proteins could then bind to their final destination or associate with chaperones which will assist in targeting the proteins to their correct destinations.

Several exported proteins destined for the erythrocyte membrane are transiently found on Maurer's clefts before arriving at their final erythrocyte membrane destination (Hinterberg *et al.*, 1994; Wickham *et al.*, 2001; Kriek *et al.*, 2003). This suggests that the Maurer's clefts function as an intermediate step in the trafficking of proteins within the host erythrocyte. In particular, the KAHRP (Wickham *et al.*, 2001) and PfEMP1 (Kriek *et al.*, 2003) are known to be associated with Maurer's clefts before the final assembly of the knobs on the erythrocyte membrane. For example, PfEMP1 is synthesized and trafficked as a peripheral membrane protein to the Maurer's clefts and then subsequently inserted through erythrocyte membrane from the Maurer's clefts (Papakrivov *et al.*, 2005). This trafficking to the Maurer's clefts and insertion of PfEMP1 into the erythrocyte membrane requires a Maurer's cleft protein called skeleton binding protein-1 (SBP1) (Cooke *et al.*, 2006; Maier *et al.*, 2007). However, other erythrocyte membrane associated proteins do not require SBP1 for their trafficking to erythrocyte membrane. Therefore, SBP1 may play a specific role in the trafficking of PfEMP1 and its insertion into the host membrane. In addition, the cholesterol rich domains on the erythrocyte membrane are important for the insertion of PfEMP1 into the erythrocyte membrane (Frankland *et al.*, 2006).

Another possibility is that proteins can move along the extensions of the PVM. Such a mechanism may be used by proteins which are resident within these PVM extensions and ultimately the Maurer's clefts. For example, GRA3, a *Toxoplasma* protein, integrates into the PVM following secretion of a soluble form into the parasitophorous vacuole (Ossorio *et al.*, 1994), and a similar mechanism for the incorporation of *P. falciparum* Exp-2 in the PVM has been proposed (Fischer *et al.*, 1998).

TARGETING SEQUENCES

Another major question in regards to the trafficking of proteins to the host erythrocyte concerns targeting sequences. Some exported *Plasmodium* proteins contain canonical N-terminal signal sequences, whereas other exported proteins contain a hydrophobic region resembling a signal sequence that is recessed by up to 80 amino acids (Cooke *et al.*, 2004a). This hydrophobic signal sequence is sufficient for targeting proteins to the parasitophorous vacuole but additional signals are needed for trafficking into the host erythrocyte. A short sequence motif necessary for transport beyond the PVM has been identified (Hiller *et al.*, 2004; Marti *et al.*, 2004). This *Plasmodium* export element (PEXEL) is located 15-20 amino acids downstream of the hydrophobic signal sequence and has a consensus sequence of RxLxE/Q. Many of the genes contain an intron between the signal sequence and PEXEL with the PEXEL motif close to the beginning of the second

exon. Not all exported proteins have this targeting motif though. SBP1 has neither a signal sequence nor the PEXEL, PfEMP1 has the PEXEL but no signal sequence, and EXP-1 has a signal sequence, but no PEXEL. Such proteins may need to be escorted to their final destinations by other proteins with the proper signals.

Proteins destined for different locations within the infected erythrocyte contain the PEXEL. In addition, both soluble and membrane exported proteins have the PEXEL. This implies that the PEXEL functions at a step before the proteins have separated into distinct trafficking pathways. In other words, PEXEL probably functions in the secretory pathway before the parasitophorous vacuole. For example, PEXEL in combination with the signal sequence may target proteins to *Plasmodium* export compartment. As discussed above, the exported proteins are then transferred to the parasitophorous vacuole for subsequent sorting and trafficking. Thus, PEXEL may function twice in the secretory process. The first function is to target the protein to the *Plasmodium* export compartment and then later as a signal to exit the parasitophorous vacuole.

The PEXEL motif has not been identified in other apicomplexa and may be unique to *Plasmodium* (Sargeant *et al.*, 2006). If PEXEL is involved in targeting exported proteins to the *Plasmodium* export compartment, then this compartment may also be unique to *Plasmodium*. Thus, the earlier designation of this compartment as the secondary ER of the apicomplexa (sERA) may not be accurate. However, all Apicomplexa do appear to have the unique SERCA-like calcium ATPase. Clearly more work is needed on resolving if PEXEL plays a role in targeting proteins to the *Plasmodium* export compartment and the function and location of the Apicomplexan calcium ATPase.

SUMMARY AND MODEL

The malaria parasite exports many proteins to distinct locations within the host erythrocyte. Many of these exported proteins appear to traverse a specialized compartment within the parasite that functions in the export of proteins into the host erythrocyte. This *Plasmodium* export compartment exhibits many ER-like properties and may be a specialized domain of the ER or a distinct ER-like organelle. The observation that *Plasmodium* and other Apicomplexa have two SERCA-like ATPases is consistent with two ER-like organelles. It is proposed that proteins destined for compartments within the parasite utilize the classical ER, whereas proteins destined for export into the host erythrocyte are targeted for the *Plasmodium* export compartment (Figure 2). Proteins then move from the *Plasmodium* export compartment into the parasitophorous vacuole by an unknown mechanism. Some sorting may occur within the parasitophorous vacuole. For example, proteins that are resident to the PVM or the extensions of the PVM and possibly the Maurer's clefts may move along these extensions. Proteins destined for the erythrocyte are likely exported into the cytoplasm of the host erythrocyte as soluble proteins. Some of these proteins may bind directly to their final destination such as the erythrocyte membrane. Others may associate transiently with Maurer's clefts or other membranes within the infected erythrocyte. Chaperones may also be involved in the trafficking of proteins to their final destinations and the assembly of supramolecular complexes such as knobs.

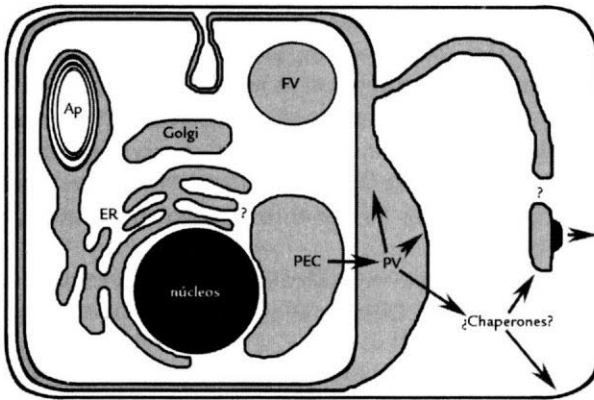


Figure 2. Schematic representation of proposed trafficking of *Plasmodium* proteins to the host erythrocyte. Proteins destined for locations within the parasite are trafficked through the endoplasmic reticulum (ER) and sorted to the various compartments such as the apicoplast (Ap), food vacuole (FV), and parasite plasma membrane. Many exported proteins are targeted to the *Plasmodium* export compartment (PEC) instead of the ER in route to the parasitophorous vacuole (PV). The PEC could also be a specialized domain of the ER. Some proteins will be retained in the PV or associate with the PVM or the extensions of the PVM, whereas others will be transported into the host erythrocyte cytoplasm as soluble proteins. These proteins will then associate with membranous compartments within the erythrocyte or the erythrocyte membrane. Chaperones may play a role in the trafficking of exported proteins or their association with supramolecular structures. Many proteins, especially those associated with knobs, assemble on the Maurer's clefts or other membranes within the cytoplasm of the host erythrocyte before their final transfer to the erythrocyte membrane.

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