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Current Opinion in Microbiology

Recent insights into the export of PEXEL/HTS-motif containing proteins in *Plasmodium* parasites

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Protein export in intra-erythrocytic *Plasmodium* parasites is of considerable interest in the malaria field because the process is inextricably linked to virulence and survival mechanisms in the human host. Despite many and varied functions, a common link between many exported proteins is their actual mode of export. Most exported proteins must traverse two membranes to their destination in the infected erythrocyte cytosol, the parasite plasma membrane and surrounding parasitophorous vacuole membrane (PVM). In recent years, several studies have shone light on the common molecular mechanism by which the major class of exported proteins, the so-called PEXEL/HTS motif-containing proteins, are translocated across these membranes. Roles for parasite-specific molecular processes in two distinct sites, the endoplasmic reticulum and the PVM have been revealed.

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Current Opinion in Microbiology 2012, **15**:699–704

This review comes from a themed issue on **Growth and development: eukaryotes**

Edited by **Nicholas J Talbot**

For a complete overview see the Issue and the Editorial

Available online 22th October 2012

1369-5274/$ – see front matter,

http://dx.doi.org/10.1016/j.mib.2012.09.008

Introduction

Apicomplexan parasites such as *Plasmodium* spp. cause a multitude of illnesses through infection of both human and livestock hosts. Of the *Plasmodium* species that infect humans, *P. falciparum* is associated with a large proportion of symptomatic malarial disease, resulting in approximately 800 000 deaths and a further 250 million clinical cases annually (WHO World Malaria Report, 2010). Although there are many facets that contribute to the global health catastrophe caused by malaria, including social, geographical, economic and political; the biology of the parasite itself is a major contributing factor. *P. falciparum* has a complex lifecycle involving stages in both its mosquito vector and human host, within which it carries out its development in distinct phases in both the liver and blood stream. Symptomatic malaria is associated with blood-stage infection and the virulence of *Plasmodium* parasites can largely be attributed to their ability to radically modify and remodel the vertebrate host cell inside of which these obligate intra-cellular parasites reside. Host cell remodelling is achieved by the export of effector proteins across two encasing membranes, the parasite plasma membrane and the adjacent parasitophorous vacuole membrane (PVM), and into the host cell cytosol. This process is particularly important during erythrocytic infection in mammals. Mammalian erythrocytes are enucleate, terminally differentiated cells that possess no secretory system to assist in delivering exported parasite proteins to their final destinations. Because of this, parasites export their own trafficking system, which has contributed to the expansion of parasite exportomes (reviewed in [1]).

The best-studied and probably largest exportome belongs to the most virulent and pathogenic human malaria parasite, *P. falciparum*, which exports approximately 5% of its encoded genome across the parasitophorous vacuole and into the erythrocyte cytosol [2–5]. Exported proteins play roles in modifying the host cell in one of three main ways: (A) by altering the cytoskeleton to provide a robust cell inside of which the parasite can safely grow and replicate [6–12], (B) by increasing the membrane conductance of the erythrocyte to facilitate nutrient and solute acquisition [13,14,15•,16–18], or (C) by presenting parasite-derived adhesion proteins, most notably PfEMP1, on the surface of the infected cell to assist in preventing splenic clearance [7,19–22]. The nature of this exportome has recently been extensively reviewed elsewhere [7,19–22].

The key common link between many exported proteins is thought to be the unique means by which they are trafficked to their destination. Understanding the molecular details of this nexus is particularly relevant to drug development as the common pathway(s) offer a means by which many different key protein functions can be targeted simultaneously. This review focuses on the recent major advances in our understanding of the trafficking pathways of the major class of exported proteins.

Export begins in the ER and is predicted to follow a targeted vesicular route to the PVM

To gain entry into the ER, the majority of exported *P. falciparum* proteins utilise a recessed N-terminal leader

sequence, 25–30 amino acids downstream of a motif known as a PEXEL or HTS motif with the consensus sequence RxLxE/Q/D [2,3,23,24]. Because of the presence of this motif, PEXEL/HTS containing proteins have been the most extensively studied exported proteins to date and thus form the major focus of this review. It should be noted however that there also exists a group of exported proteins lacking any discernable export motif and these proteins are termed PEXEL-negative exported proteins (PNEPs).

Despite suggestions that the PEXEL motif may act as a 'barcode' for a downstream protein translocation machinery involved in protein export across the PVM, it was discovered that upon import into the ER the PEXEL motif is cleaved immediately downstream of the conserved leucine residue [25,26••,27••]. Mutation of any of the key conserved residues in the PEXEL motif to alanine prevents the export of reporter proteins. Whilst mutation of the R and L residues prevents processing and leads to ER retention, mutation of the downstream E/Q/D residue has no effect on processing but leads to accumulation of the reporter protein in the parasitophorous vacuole (PV). Hence, processing is not sufficient for export but is necessary for proteins destined for export to leave the ER.

Since we last reviewed this general topic [28], significant advances have been made in our understanding of this cleavage event. In 2010 it was discovered by two separate groups that PEXEL/HTS motif cleavage is carried out by an ER-resident aspartic protease termed Plasmepsin-V, which cleaves the PEXEL/HTS motif downstream of its central L residue leaving an N-terminal xE/Q/D motif on mature proteins destined for export [26••,27••,29]. Plasmepsin V is present in all *Plasmodium* species, yet appears to be functionally divergent from other members of the *Plasmodium* plasmepsin family, all of which are involved in haemoglobin degradation [26••,27••,30]. A structural modelling study has suggested that three specific residues in this enzyme (Tyr13, Glu77 and Ala117) are important in recognising the arginine residue in the PEXEL motif to promote specific cleavage [29].

Importantly, Boddey et al. [26••] showed that cleavage in the ER seems to specifically require Plasmepsin V, rather than another enzyme that could artificially produce an N-terminal xE/Q/D motif in an ER-resident protein. This was achieved by engineering cleavage of a reporter gene by signal peptidase to reveal an identical sequence to a reporter protein that is known to be exported if cleaved by Plasmepsin V. This signal peptidase-cleaved reporter trafficked effectively to the PV but did not go beyond into the erythrocyte cytosol. The authors conclude that Plasmepsin V is likely to be a part of a more elaborate molecular process that directs exported proteins along a specific vesicular pathway, presumably to a specific region of the PV where they will encounter a protein translocon.

Recent data indicate that whilst in the ER, the full-length PEXEL/HTS motif associates with the phospholipid PI(3)P in distinct regions of enrichment within the ER membrane [31•]. The reason for this binding is yet to be fully elucidated however it remains possible that this association may enable PEXEL proteins to be held in specific 'export zones' of the ER before their cleavage by Plasmepsin-V and transit to specific 'translocation competent zones' of the PVM as suggested by Boddey et al. [26••] (Figure 1).

As mentioned earlier, there also exists a group of proteins termed PNEPs that whilst exported, lack any discernable export motif. Numerous PNEPs have been characterised, including the Maurer's cleft-resident proteins REX2 and SBP1. In place of a definable export motif, these proteins have been found to contain either a single transmembrane domain or a hydrophobic stretch of amino acids likely to be involved in trafficking to the erythrocyte cytosol [32,33]. Additional PNEPs include members of the small exported protein (SEP) family, which are conserved across the *Plasmodium* genus and characterised by a predicted signal peptide, a short lysine-rich stretch, an internal transmembrane domain and a highly charged C-terminal region [34]. These proteins are mentioned here as they have been shown to form complexes within distinct micro-domains of the PVM in both *Plasmodium berghei* and *P. falciparum* suggesting that they may be involved in defining specialised zones wherein exported proteins are organised and/or delivered [34], thus fitting with a 'regional' model of protein export [28]. As there is no obvious PNEP export motif, the number of proteins contained within this group remains undefined, as are the mechanism(s) by which these proteins are trafficked into the host cell, however it remains possible that all exported proteins utilize a common export pathway [35].

Translocation across the PVM: the Plasmodium Translocon of EXported proteins (PTEX)

Consistent with a regional model of protein export as described above, cleaved PEXEL proteins are deposited into the PV in specific regions thought to contain a proteinaceous translocon [36]. It is known that for translocation across the PVM to occur, exported proteins need to be unfolded indicating the likely involvement of a protein unfolding channel or translocon containing a protein unfolding chaperone ATPase [36]. A large macromolecular protein complex with characteristics of a PEXEL protein translocon has been identified and termed the *Plasmodium* Translocon of EXported proteins (PTEX) [37••,38]. Whilst direct functional proof of this putative translocon is currently lacking, our recent