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Protein–export pathway illuminated

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Abstract

Two studies provide evidence that the protein complex PTEX is needed for export of malaria-parasite proteins into the cytoplasm of infected cells, and that such export is essential for parasite survival.

Malaria parasites export hundreds of proteins into the red blood cells that they infect. These proteins increase nutrient uptake from blood plasma, facilitate adhesion of the infected cell to endothelial cells in blood vessels and markedly remodel the red blood cell for the parasite's benefit. A parasite protein complex called *Plasmodium* translocon of exported proteins (PTEX) has been proposed to traffic these proteins across the membrane of the cellular vacuole that separates the parasite from the cytoplasm of the infected cell¹. In two papers in this issue, Beck *et al.*² (page 592) and Elsworth *et al.*³ (page 587) definitively show protein transport through PTEX.

Protein interaction studies¹ have suggested that the PTEX translocon consists of five proteins and that one of these, EXP2, forms a pore through which proteins are threaded after they have been unfolded, in an energy-dependent process, by the chaperone protein HSP101. The new studies demonstrate the function of this protein complex in the infected cell by ablating the activity of HSP101 and PTEX150, a PTEX component with unknown function. Suppression of either component, which was achieved by transcriptional repression or protein destabilization, was found to prevent export of two broad categories of parasite protein.

In *Plasmodium falciparum*, one of the main human-infecting malaria parasites, most exported proteins contain a sequence of five amino-acid residues, called the PEXEL motif, near their amino terminus^{4,5}. Cleavage within this motif by the enzyme plasmepsin V commits the mature protein for export to the host cell, but the precise nature of the commitment step remains debated⁶. The second category of exported proteins lacks this motif. Such PEXEL-negative exported proteins, of which there is an expanding list, lack unifying features and thus have defied attempts to predict how they are recognized and exported⁷. The authors' observations of inhibited export of proteins from both categories — which cover the full spectrum of export timings, protein biophysical properties and destinations in the host cell — implicates PTEX as a crucial bottleneck in parasite-induced remodelling of the red blood cell.

These findings raise new questions about the translocation process. How does PTEX recognize and transport such diverse proteins, but allow other proteins to remain in the parasitophorous vacuole (the parasite-containing compartment in the host cell that is formed during invasion and further modified during parasite growth)? Are retained proteases, chaperones and other enzymes not recognized by PTEX or do they carry specific signals that prevent export? To be threaded through PTEX, exported proteins must first be unfolded, as has been shown with a reporter protein that was prohibited from unfolding by a tightly bound substrate⁸. Whether HSP101 or other chaperones actually catalyse the unfolding of each exported protein is unknown. Also still unclear are if EXP2 indeed defines the pore and what roles the other PTEX components might serve. Could membrane proteins passing through PTEX undergo lateral transfer into the parasitophorous vacuolar membrane to allow migration along membranous extensions (Fig. 1), as established for translocons in other organisms⁹? Finally, proteins that pass into the host-cell cytoplasm will require refolding, presumably by parasite chaperones that are also exported and must somehow be refolded themselves¹⁰.

Another fundamental finding of these studies is that suppression of protein export interferes with intracellular parasite growth, indicating that exported proteins have essential roles in parasite survival. The authors observed adverse effects on parasite development *in vitro* and *in vivo*, with immature ring-stage parasites unable to mature to the trophozoite stage. By contrast, inhibiting PTEX after maturation to the trophozoite stage was well tolerated, with no effect on parasite egress from the cell or invasion of new red blood cells, suggesting that these latter processes do not depend on proteins exported late in the cycle. But development of early-stage gametocytes, the sexual stage of the parasite life cycle required for malaria transmission by mosquitoes, was also severely compromised.

Which activities of the numerous exported proteins account for the parasite growth inhibition seen in these studies? Although binding of infected cells to endothelial receptors is required for parasite survival *in vivo*, it is dispensable for *in vitro* culture. A leading candidate is the uptake of nutrients by the plasmodial surface anion channel (PSAC), an essential activity associated with the parasite protein cytoadherence-linked antigen 3 (CLAG3)^{11,12}. Beck and colleagues found that CLAG3 still enters the host-cell cytoplasm when PTEX is suppressed, implying that it is exported by a distinct mechanism, perhaps during invasion. At the same time, solute transport by PSAC was curtailed, suggesting that other exported proteins are required for nutrient-channel formation.

In human malaria, binding of infected cells to the endothelium averts their destruction by the spleen and is primarily mediated by members of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) protein family. Each member has multiple binding domains at its extracellular face and a single transmembrane domain to anchor the protein over parasite-induced knobs on the infected cell¹³. How does PfEMP1 move from the parasite to the red-blood-cell membrane? Although the protein is not cleaved by plasmepsin V, its atypical PEXEL motif and transmembrane domain both seem to contribute to its export⁴. Subsequent refolding of the endothelium-binding domains in the host cytoplasm presumably requires disulphide-isomerase enzymes to bring numerous cysteine amino-acid residues together correctly and may involve a battery of chaperones^{14,15}. Specialized sorting organelles

known as Maurer's clefts and proteins at the surface knobs also seem to be required for the ultimate insertion of PfEMP1 in the host membrane¹⁶. In light of the complex folded structure of PfEMP1 and the possible involvement of many chaperones, the compromised export of this protein observed by both Beck *et al.* and Elsworth *et al.* could reflect an indirect effect of PTEX inhibition. Further study will be required to determine the precise mechanisms for trafficking and presentation of this key virulence factor.

The two new articles reveal a remarkably broad range of substrates for the translocon and provide compelling evidence that protein export is essential for the parasite and therefore represents a potential therapeutic target. We foresee that combinations of drugs that target both PTEX and exported parasite activities, such as PSAC-mediated nutrient uptake, may be highly synergistic antimalarial therapies.

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Figure 1. Transport of parasite proteins into the host red blood cell

The protein complex PTEX mediates export of malaria-parasite proteins across the parasitophorous vacuolar membrane (PVM), which separates the cytoplasm of an infected cell from the vacuole in which the parasite resides. Parasite proteins are first secreted into the vacuolar space, in which they are unfolded before transport through PTEX; they are then refolded in the host-cell cytoplasm. Some membrane proteins might undergo lateral transfer (dashed arrow) from PTEX into the PVM, a process that would allow movement within membranes to reach vesicles known as Maurer's clefts. Exported proteins then localize to specific sites in the cell or on the red-cell membrane, at which they serve functions that are crucial to intracellular parasite growth. Examples include nutrient uptake through PSAC and PfEMP1-mediated binding of infected cells to endothelial cells that line blood vessels.