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Invited Review

Host cell remodelling in malaria parasites: a new pool of potential drug targets

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ABSTRACT

When in their human hosts, malaria parasites spend most of their time housed within vacuoles inside erythrocytes and hepatocytes. The parasites extensively modify their host cells to obtain nutrients, prevent host cell breakdown and avoid the immune system. To perform these modifications, malaria parasites export hundreds of effector proteins into their host cells and this process is best understood in the most lethal species to infect humans, *Plasmodium falciparum*. The effector proteins are synthesized within the parasite and following a proteolytic cleavage event in the endoplasmic reticulum and sorting of mature proteins into the correct vesicular trafficking pathway, they are transported to the parasite surface and released into the vacuole. The effector proteins are then unfolded before extrusion across the vacuole membrane by a unique translocon complex called *Plasmodium* translocon of exported proteins. After gaining access to the erythrocyte cytoplasm many effector proteins continue their journey to the erythrocyte surface by utilising various membranous structures established by the parasite. This complex trafficking pathway and a large number of the effector proteins are unique to *Plasmodium* parasites. This pathway could, therefore, be developed as new drug targets given that protein export and the functional role of these proteins are essential for parasite survival. This review explores known and potential drug targetable steps in the protein export pathway and strategies for discovering novel drug targets.

1. Introduction

Lacking a nucleus and most organelles, erythrocytes are uncomplicated cells packed with haemoglobin so that they can transport oxygen and carbon dioxide around the circulatory system. They also contain a membrane-bound skeleton comprised primarily of spectrin that makes them extremely deformable to pass through capillaries. However when *Plasmodium* parasites, the causative agents of the devastating disease malaria, take up residence in erythrocytes, they impart major morphological changes that alter the erythrocyte's physical characteristics (Cooke et al., 2004; Boddey and Cowman, 2013). The most notable of these are raised knob-like protrusions on the surface of the infected erythrocyte that provide a scaffold for the correct presentation of the parasite's major virulence protein, erythrocyte membrane protein 1 (PfEMP1) (Baruch et al., 1995; Su et al., 1995; Maier et al., 2009). PfEMP1 facilitates binding of the infected erythrocyte to particular surface ligands lining the microvasculature of the human host (Fig. 1a). Modulation of the erythrocyte cytoskeleton also increases the rigidity of the infected cell, further improving its capacity to bind to host endothelial receptors (Nash et al., 1989). The ability to cytoadhere and sequester within the vasculature is important for parasite survival because it prevents the infected erythrocytes from passaging through the spleen and being destroyed by resident macrophages. Unfortunately, the infected erythrocytes can also congest the vasculature, leading to tissue damage, and this is a major contributor to malaria pathogenesis. The parasite also establishes new permeability pathways in the erythrocyte membrane to facilitate the uptake of nutrients from host plasma, which fuels rapid growth and the production of more offspring (Kirk et al., 1994) (Fig. 1b).

All of these modifications to the erythrocyte are induced by several hundred parasite proteins, most of which initially traffic from the parasite via the secretory pathway to the parasitophorous vacuole (PV) that surrounds the growing parasite (Fig. 1). A relative few proteins such as the CLAG3.1 (see Section 7) are injected by

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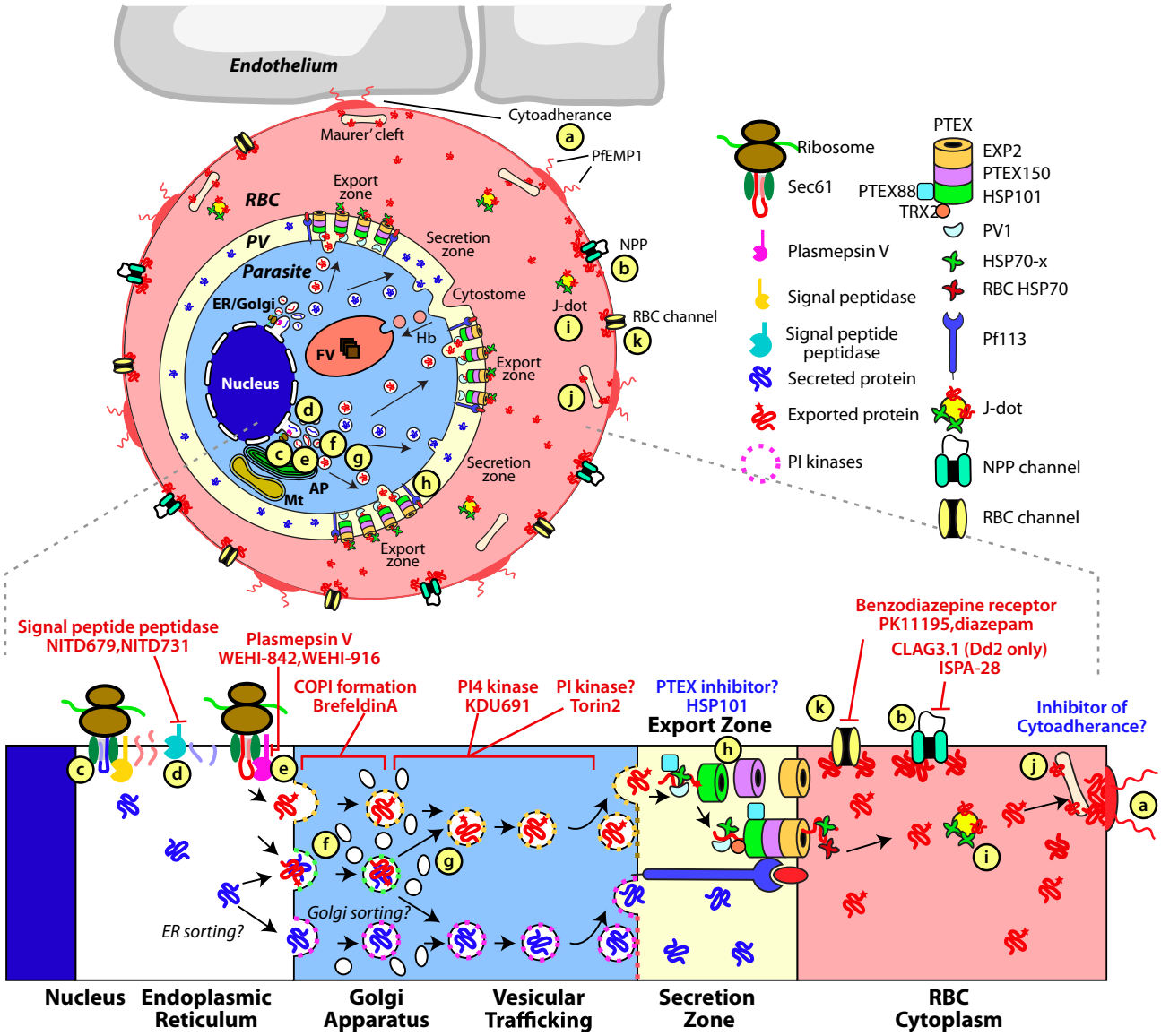

Fig. 1. Diagram of the protein export pathway in *Plasmodium falciparum* as well as a selection of protein trafficking steps and exported protein functions that can be chemically inhibited. *Plasmodium falciparum* extensively modifies the red blood cell it infects by exporting hundreds of proteins into them that help the parasites survive in their human host. One of the most important modifications is the deployment of *P. falciparum* erythrocyte membrane protein 1 onto the erythrocyte surface that enables cytoadherence to endothelial cells, removal from circulation and consequent splenic clearance (a). Another important function carried out by exported proteins is the creation of new permeability pathways that allow access to plasma nutrients to promote rapid parasite growth (b). Proteins exported into the erythrocyte compartment begin their journey with synthesis at the endoplasmic reticulum where most are cleaved by Plasmepsin V (e). This licenses them for transport to the parasite surface after various vesicular sorting steps in the endoplasmic reticulum/Golgi apparatus (f, g). Once deposited in the parasitophorous vacuole at possible export zones, the *Plasmodium* translocon of exported protein transfers them into the erythrocyte cytoplasm (h). Membranous chaperone rich structures such as J-dots (i) and Maurer's clefts (j) possibly help refold and assemble exported proteins such as *P. falciparum* erythrocyte membrane protein 1 into functional complexes. Validated chemical inhibitors of each trafficking step and their targets are indicated in red text and desirable targets are in blue text. AP, apicoplast; COPI, coat protein I; EXP, exported protein; FV, food vacuole; Hb, haemoglobin; HSP, heat shock protein; Mt, mitochondrion; PI, phosphatidyl inositol; TRX, thioredoxin.

invading merozoites and appear to gain access to the erythrocyte compartment during or shortly after invasion. The majority of exported proteins, however, must traverse the bounding PV membrane to gain access to the host cell, from where they traffic to various destinations in the host cell compartment to exert their functions. To do this, the exported proteins utilise an elaborate exomembrane system established by the parasite because erythrocytes lack a trafficking machinery that can be exploited. The importance of protein export to the parasite can be highlighted by a large scale knockout screen of 51 known or predicted exported proteins, which showed that 23% of these proteins could not be deleted and were therefore considered likely to be essential (Maier et al., 2008). Thus at least a subset of exported proteins are critical for parasite growth and survival. Hence in recent years there has been a concerted effort to attempt to understand how proteins are exported with the long-term aim of developing new targets for malaria drug development. Such research has demonstrated that the trafficking pathway is indeed quite complex. Excitingly, however, this research has also revealed that the many hundreds of unique proteins rely on a common trafficking pathway for export and accordingly, they represent potential drug targets. This review focuses on some of the key events in the protein export and host cell remodelling pathway that are already being exploited for drug development. It also highlights some of our research presented at Molecular Approaches to Malaria conference, 21–25 February 2016 (MAM2016), Lorne Australia, in which reverse genetics has been used to functionally analyse some of the components that engage in particular steps of the protein export pathway. This

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