Lipid transport proteins in malaria, from *Plasmodium* parasites to their hosts.

Margarida Ressurreição† and Christiaan van Ooij†

† for correspondence: Margarida.Ressurreicao@lshtm.ac.uk, Christiaan.vanOoij@lshtm.ac.uk

ORCID ID: 0000-0002-5162-2032 (MR), 0000-0002-6099-6183 (CvO)

Department of Infection Biology, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom

ABSTRACT

Eukaryotic unicellular pathogens from the genus *Plasmodium* are the etiological agents of malaria, a disease that persists over a wide range of vertebrate species, including humans. During its dynamic lifecycle, survival in the different hosts depends on the parasite's ability to establish a suitable environmental milieu. To achieve this, specific host processes are exploited to support optimal growth, including extensive modifications to the infected host cell. These modifications include the formation of novel membranous structures, which are induced by the parasite. Consequently, to maintain a finely tuned and dynamic lipid environment, the organisation and distribution of lipids to different cell sites likely requires specialised lipid transfer proteins (LTPs). Indeed, several parasite and host-derived LTPs have been identified and shown to be essential at specific stages. Here we describe the roles of LTPs in parasite development and adaptation to its host including how the latest studies are profiting from the improved genetic, lipidomic and imaging toolkits available to study *Plasmodium* parasites. Lastly, a list of predicted *Plasmodium* LTPs is provided to encourage research in this field.

INTRODUCTION

Malaria remains prevalent in tropical and subtropical regions of the world, particularly in Africa and South East Asia. Of the six species infective to humans, the most lethal is *Plasmodium falciparum* [1]. This parasite has a highly dynamic and complex lifecycle, involving a vertebrate and an insect host (**Figure 1**). Human infection occurs through the bite of a female *Anopheles* mosquito where a small number of sporozoites in the saliva, enter the circulatory system. Upon reaching the liver, through cell traversal events, the sporozoite will divide following hepatocyte invasion. The resulting progeny, referred to as merozoites, are released into the bloodstream in a merosome – a large membrane filled with thousands of merozoites [2, 3]. Rupture of the merosome frees the merozoites, allowing them to invade mature erythrocytes in the blood circulation. Following invasion, the intraerythrocytic cycle is defined by the initial ring stage, followed by the metabolically demanding trophozoite and a subsequent replicative schizont stage where new merozoites can readily invade a new erythrocyte after egress [4, 5]. It is the continuous cycle of intraerythrocytic replication and re-invasion that causes the symptoms of malaria. A proportion of the parasites from the intraerythrocytic cycle differentiate into male or female gametocytes, a prerequisite for disease transmission back to the invertebrate host. When, during a bloodmeal, male and female gametocytes are ingested into the midgut lumen of a female *Anopheles* mosquito, the abrupt environmental change triggers the differentiation of female macrogametes and flagellated male microgametes. When the male microgamete encounters the female macrogamete, the cells fuse to produce an ookinete [5]. Following one meiotic division, the ookinete migrates through the mosquito gut wall, remaining underneath the midgut basal lamina, where it transforms into an oocyst, forming a thick capsule [5]. During the lengthiest developmental multiplication stage, the oocyst undergoes several mitotic divisions, generating the sporoblast from which hundreds of sporozoites are released. These migrate through the haemolymph to reach the basal lamina of the salivary glands, where they invade acinar cells, ultimately accumulating inside the salivary duct, completing maturation, and thus becoming ready to infect hepatocytes again [5, 6].

Throughout the lifecycle, *Plasmodium* parasites remodel their host cells in numerous ways [7, 8, 9], including altering the host cell membrane and inducing the formation of various membranous compartments (**Figure 1**) [7, 8, 9]. Many of these changes are driven by the parasite, likely through the action of lipid transfer proteins (LTPs). During residence in the vertebrate host, when the parasite replicates intracellularly either in the hepatocyte or

erythrocyte, it is always entirely enveloped by the parasitophorous vacuole membrane (PVM), creating a buffer zone, the parasitophorous vacuole (PV), between the parasite plasma membrane (PPM) and the host cell cytoplasm (**Figure 1**) [7, 8]. Another prominent membranous structure formed in the infected hepatocyte and erythrocyte is the tubovesicular network (TVN), which is a highly elaborate and dynamic membranous tubular system that extends from the PVM, characterised by membrane whorls that appear to discreetly encircle host cell cytoplasm [7, 8, 9, 10, 11, 12]. During the intraerythrocytic lifecycle, thin membranous lamellae-like structures called Maurer's clefts (MCs) are formed by the parasite; these likely function to export proteins to the surface of the erythrocyte [11, 12]. Additionally, two types of mobile vesicles, the 25 nm and 80 nm vesicles, have been observed in the cytoplasm of infected erythrocytes and although uncharacterised, they are potentially involved in transport of proteins to the surface of the infected cell [7]. Another interesting group of structures located in the host cell cytosol are the cholesterol-rich J dots. J dots contain several heat-shock proteins that form a chaperone complex that interacts with multiple other proteins, including the main parasite virulence factor, the cytoadherence protein PfEMP1 [13, 14, 15, 16, 17, 18]. A likely function for J dots is to transport proteins through the aqueous environment of the erythrocyte cytosol and erythrocyte surface [15, 17, 18].

In addition to the usual organelles found in eukaryotes, the parasite contains several specialised organelles found only in apicomplexans. These include the apicoplast, an essential four-membraned vestigial plastid gained from secondary endosymbiosis of a red algae [19, 20, 21] and several apically located membranous organelles (the apical complex) necessary for invasion of host cells, comprised of micronemes, rhoptries and dense granules (**Figure 1**). Furthermore, the parasite produces the inner membrane complex, a membranous structure that provides the platform for entering the host cell. Hence, in addition to the standard phospholipid transfer requirements of a eukaryotic cell, *Plasmodium* parasites require additional mechanisms for the biogenesis of specific membranous structures and organelles as well as mechanisms involved in host cell membrane modification.

Here we review the known and studied LTPs that function during the various stages of the *Plasmodium* spp. lifecycle and describe host LTPs shown to be relevant for malaria progression. A brief overview of the lipid profiles of the parasite and the host cell is given to facilitate contextualisation of the specific lipid requirements and function that these LTPs might play during the parasite lifecycle. Lastly, we critically evaluate the current knowledge, indicating what we do not yet fully understand and provide a list of predicted *P. falciparum*

genes with putative LTP functions and their predicted essentiality (**Table 1**) to stimulate future research in the emerging field of protein-dependent lipid transport in *P. falciparum.*

PHOSPHOLIPIDS IN *PLASMODIUM*.

Glycerophospholipids constitute the major lipid class detected in parasite fractions removed from the erythrocyte, with phosphatidylcholine (PC) and phosphatidylethanolamine (PE) together accounting for ~50% of total lipid content [22, 24, 55, 89]. These structural membrane components primarily contain two saturated or monosaturated FA chains [22], consistent with parasites requiring an exogenous supply of oleic and palmitic acid to sustain growth [21, 23, 24]. Lipids enriched in uninfected erythrocytes include phosphatidylserine (PS), phosphatidic acid (PA) and ceramide. It is believed that the host cell may serve as a reservoir for parasite-mediated lipid salvage, if required in response to changes in the extracellular host environment [22, 24, 25, 26, 27, 55]. Mainly as *P. falciparum* has been shown to import several lipid species, including ceramide, complex sphingolipids and lysophosphatidylcholine (LysoPC) [28, 29, 30, 31, 32, 90].

The second major class of lipids observed in infected erythrocytes is sphingolipids, which play critical roles in both membrane structure and signalling [33, 30]. Sphingomyelin (SM) is the third most abundant lipid detected in trophozoite infected erythrocytes steadily increasing from ring stages, consistent with other studies [24, 30, 31, 33, 34, 35, 36].

Lysophospholipids are minor constituents of cell membranes, present at less than 1% of total parasite lipids. However, their levels change throughout parasite development, commensurate with a putative role in intracellular signalling to regulate varied processes, including cell signaling, protein folding and the mobilisation of intracellular Ca^{2+} stores [37, 38, 39, 40, 41, 42].

Cholesterol plays an important role in regulating the properties of phospholipid membranes, including the regulation of membrane organisation [43, 46]. Addition of cholesterol induces a condensation effect where the area per lipid decreases, leading to increased membrane stiffness [42, 43]. Cholesterol levels also affect raft structures [43, 46, 47], membrane trafficking and sorting functions that may support *P. falciparum* survival [48, 49, 50, 51]. Interestingly, a gradient of cholesterol is present in erythrocytes infected with *Plasmodium* parasites [52, 53]. Experiments using a cholesterol-sensitive fluorophore revealed that membrane cholesterol

levels in parasitised erythrocytes decrease inwardly from the erythrocyte plasma membrane (EPM), MCs/TVN, PVM and finally to the PPM [53]. Fluorescence Lifetime Imagining Microscopy (FLIM) showed little or no difference in this cholesterol gradient between parasitized HbAA erythrocytes vs HbS erythrocytes that differ in lipid content, suggesting that malaria parasites may regulate the cholesterol contents of the PVM and PM independently of levels in the host cell membrane, especially after invasion [54]. Lipid and cholesterol exchange data suggest that the cholesterol gradient involves a dilution effect from non-sterol lipids produced by the parasite and that the parasite actively maintains a level of low cholesterol [52, 53]. Furthermore, increased membrane cholesterol decreases the temperature required for the plasma membrane to maintain its liquid phase and is directly related to temperature-dependent changes to the cell membrane [43, 44, 45]. Interestingly, during gametocytogenesis, cholesterol levels in the parasite increase significantly [22, 54]. This is believed to be important for various reasons, including the production of internal reserves for further development after uptake by the mosquito, which also cannot produce cholesterol *de novo* [22, 30, 54, 112, 113, 114].

LIPID TRANSFER PROTEINS

Because the ER, mitochondrion and the apicoplast are involved in the *de novo* synthesis of lipids in *Plasmodium* parasites, a system must exist to transport these newly synthesised lipids to other organelles, membranes, membranous compartments, and the aqueous environment of the host cytoplasm [138, 139, 140]. As LTPs are candidates for the development of such processes, these proteins have become attractive in understanding the role and importance of lipid dynamics in cells infected by *Plasmodium* parasites. The parasite produces many different LTPs, some with standard functions shared with other eukaryotes and others that have been adapted for parasite-specific purposes (**Table 1**).

StAR-related lipid transfer proteins from the Bet v1-like superfamily

Plasmodium parasites encode five proteins with annotated START domains, usually ~210 amino acids long, involved in the binding and non-vesicular transport of hydrophobic molecules, including lipids and cholesterol [56]. These five proteins are PF3D7_1351000 (MAL13P1.256), PF3D7_0911100 (PFI0540w), PF3D7_0807400 (MAL8P1.300), PF3D7_01004200 (PFA0210c) and PF3D7_1463500 (PF14_0604). Of these, three have not yet been investigated: PF3D7_1351000 (MAL13P1.256), the phosphatidylinositol (PI) transfer protein orthologue; PF3D7_0911100 (PFI0540w), annotated as a conserved protein of

unknown function with a predicted C-terminal START-like domain; and PF3D7_0807400 (MAL8P1.300), a multi-domain protein with a predicted START domain and a Coenzyme Q (CoQ or ubiquinone) binding (Coq10p) domain. The PI transfer protein PF3D7_1351000 (MAL13P1.256) is likely to perform functions similarly to those in other eukaryotes, whereas PF3D7_0911100 (PFI0540w) is unique to *Plasmodium* spp. and *Hepatocystis* (a related apicomplexan transmitted by midges that skips intraerythrocytic asexual replication stages, entering directly into gametocytogenesis) [57, 58]. PF3D7_0911100 (PFI0540w) is expressed at various stages during the entire lifecycle but appears dispensable for intraerythrocytic parasite growth. Orthologues of PF3D7_0807400 (MAL8P1.300) are usually associated with mitochondria and have a role in respiratory electron transport and ATP synthesis [59, 60]. Interestingly, the Coq10 START domain protein from *Saccharomyces cerevisiae* plays a protective role against fatty acid-induced oxidative stress along with its role in ubiquinone synthesis [59, 60]. PF3D7_0104200 (PFA0210c) and PF3D7_1463500 (PF14_0604) appear to be unique to the genera *Plasmodium* and *Hepatocystis* and may perform lipid transfer functions specific to these parasites. PF3D7_0104200 (PFA0210c) is a broad-specificity phospholipid transfer protein capable of transferring PC, PE, PI, PS and SM *in vitro* [61]. Original sequence-based similarity searches indicated that it is most similar to the human protein STARD7, a PC transfer protein, while structurally it is most similar to phosphatidylcholine transfer protein STARD2. The protein has been detected in the PVM and host erythrocyte when overexpressed [61, 62, 63]. Genetic experiments have shown that PF3D7_0104200 (PFA0210c) and its orthologue in *P. knowlesi* are essential for parasite survival [63]. Intriguingly, during asexual intraerythrocytic development, PF3D7_0104200 (PFA0210c) protein synthesis initiates at entry into the trophozoite stage and protein levels drastically increase with trophozoite growth, when extension of the PPM and PVM occurs [61, 63]. This may point to a function for PF3D7_0104200 (PFA0210c) in the delivery of phospholipids to the PVM to support its expansion. One interesting aspect of PF3D7_0104200 (PFA0210c) is the unusual C-terminal extension of approximately 84 amino acids, something that is rarely detected in START domain-containing proteins [62]. Removal of the C-terminal 19 residues significantly increases lipid transfer activity *in vitro*, indicating a regulatory function of the C-terminal region. Attempts to remove the last 20 codons of the *P. knowlesi* orthologue of PF3D7_0104200 (PFA0210c) were unsuccessful, indicating that this regulatory function may be essential *in vivo* [63]. Interestingly, a recent study has revealed IgG against PF3D7_0104200 (PFA0210c) epitopes in the blood from individuals with symptomatic and even asymptomatic malaria, indicating antibodies against this protein could form a biomarker for malaria, especially since

its essentiality will likely avoid parasite adaptation, which has been observed with the non-essential, exported protein HRPII [64, 65].

PF3D7_1463900 (PF14_0607) is a member of the Fam A family of proteins and is conserved among all *Plasmodium* species. Each *Plasmodium* species encodes a single member of this family, except for the rodent malaria parasite lineage, where the Fam A family is greatly expanded. The protein is predicted to be present in the parasite cytosol and transcriptional analyses in *P. falciparum* indicate it may be produced in sporozoites [66, 67]. Modelling of the structure of PF3D7_1463900 (PF14_0604) revealed similarities with STARD3, a cholesterol transfer protein [63]. *In vitro* phospholipid transfer assays using PC revealed minimal activity, consistent with the protein acting as a cholesterol transfer protein [63]. However, no direct evidence for cholesterol transfer has been reported. Interestingly, members of the expanded family have acquired a signal sequence and are predicted to be exported from the parasite to the host cell [62, 67, 68] and in contrast to other large families in *Plasmodium spp.*, several members of the family can be expressed concomitantly in the parasite [68]. This has been shown in *P. berghei* during the hepatocyte stage and some, but not all, tested family members, transfer PC in *in vitro* assays [68]. The function of these proteins, either the ancestral gene or the members of the expanded family, remains unclear, although the altered PC metabolism in the rodent malaria parasites may require the parasite to increase the uptake and transfer of PC.

Sec14/CRAL-TRIO-like lipid transfer proteins

Sec14 proteins have a characteristic SEC14 domain, also known as CRAL-TRIO domain. They can function as PC sensors and as inducers of PI synthesis and transfer, transmitting PC metabolic information to PI synthesis via PI transfer proteins (PITPs) [69]. Other members of the Sec14 family are the alpha-tocopherol transfer proteins (alpha-TTPs) which facilitate the transfer of alpha-tocopherol (alpha-T), a form of vitamin E, to secretory lipoproteins [70], and the phosphatidylinositol transfer proteins (PITPs), key regulators of phosphoinositide signaling [71, 72]. Interestingly, soluble versions of PITPs activate inositol lipid kinases, promoting diversification and dynamics of phosphoinositide signaling [71, 72]. Therefore, these proteins can transport substrates, including alpha-tocopherol, PI or PC, between different intracellular membranes [69, 71, 72]. The *Plasmodium* spp. group of Sec14-like proteins define a novel class of multi-domain proteins with both haem-binding and PI transfer activity [72]. *Plasmodium* spp. encode four Sec14/CRAL-TRIO-like proteins: PF3D7_0626400

(PFF1280w), PF3D7_0629900 (PFF1450w) and PF3D7_1127600 (PF11_0287) and PF3D7_0920700 (PFI1015w). Although these proteins have not yet been characterised, PFI1015w and PFF1450w are predicted to be essential (**Table 1**). Interestingly, the *P. falciparum* phosphatidylinositol 3-kinase (PfPI3K), which localises to the food vacuole in trophozoites and in vesicular compartments at the PPM/PVM, may interact with PfSec14 proteins [73]. PfSec14 proteins and PfPI3K may play key roles in haemoglobin intake, as pharmacological inhibition of PfPI3K activity compromises haemoglobin endocytosis [73].

Lipid ATPases (flippases) from the P4 subfamily

Recent gene targeting approaches have uncovered an important role for the *P. falciparum* P4-ATPase subfamily, also known as lipid flippases, proteins able to actively translocate lipids from one membrane leaflet to the other, helping to generate lipid asymmetry [74, 75, 76, 77, 90, 119]. P4-ATPases maintain the asymmetric distribution of phospholipids in membranes by translocating phospholipids (most commonly PE, PS and PC) from the extracellular leaflet to the inner cytoplasmic leaflet [77]. *P. falciparum* is predicted to encode the four P4-ATPases: PfATP2, PfATP7, PfATP8 and PfATP11 [78, 79]. Whereas the first three are conserved in all *Plasmodium* species, PfATP11 is absent in *P. berghei* [80]. While the essential role of ATP7 and ATP8 remains unclear [74, 81], most ATP7-depleted ookinetes fail to internalise and translocate PC across the plasma membrane, resulting in a failure to develop [74]. This appears to result from an inability to initiate microneme secretion and a reduction of parasite survival to environmental stress, leading to elimination of ookinetes during traversal of the midgut epithelium [74, 75]. PfATP2 and its orthologue in *P. berghei* are essential during the intraerythrocytic stages [76, 81]. *P. berghei* ATP2 potentially localises to the PPM and the PVM during the erythrocytic stage [82]; higher resolution imaging or fractionation analysis will be required to fully ascertain its localisation. Interestingly, duplication of the gene encoding PfATP2 was associated with resistance to two antimalarial compounds, MMV007224 (2-N,3-N-Bis(4-bromophenyl)quinoxaline-2,3-diamine) and MMV665852 (1,3-Bis(3,4-dichlorophenyl)urea) [83], indicating that PfATP2 is either responsible for decreasing the concentration of the compounds or the direct target of the compounds in the parasite. Most P4-ATPases form heterodimeric complexes with members of the Cdc50/LEM3 protein family and this association appears to be essential for their activity [76, 85]. *P. falciparum* encodes three putative Cdc50 proteins (Cdc50A, Cdc50B and Cdc50C) (**Table 1**) which are conserved

among all *Plasmodium* species and predicted to be essential in *P. falciparum*. In fact, disruption of the genes encoding any one of the Cdc50 proteins inhibits parasite intraerythrocytic development [75]. Recently it was demonstrated, using recombinant proteins, that *P. chabaudi* ATP2 (PcATP2) forms heterodimers with PcCdc50A and PcCdc50B [76]. Moreover, the PcATP2/PcCdc50B complex displayed lipid-stimulated ATPase activity in the presence of two phospholipid substrates, POPS and POPE (1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine and 1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine). Moreover, this activity was upregulated in the presence of phosphatidylinositol-4-phosphate (PI4P). This is an essential lipid for the malaria parasite, as inhibition of PI4P synthesis blocks the development intraerythrocytic stages by disrupting membrane biogenesis around the developing merozoites [86]. Additionally, PI4P is found at the plasma membrane, like PfATP2, and the Golgi in all stages of the erythrocytic cycle [87]. It will be relevant to investigate whether the lethal effect of blocking PI4P is a direct result of the lack of PI4P or the effect it has on ATP2 function.

Interestingly, *P. falciparum* can actively import LysoPC to generate PC [88, 89]. Although the uptake mechanisms and mode of utilization of LysoPC by the parasite remain to be determined, it is possible that LysoPC is either hydrolysed or directly acylated to form PC. In yeast, LysoPC is transported by the phospholipid flippases Dnf1p and Dnf2p, which are P4-ATPases, or by Lem3p [90]. The P. *falciparum* genome encodes several orthologs of Dnf1p, Dfn2p and Lem3p (**Table 1**), although their exact functions remain to be determined. Interestingly, decreased levels of LysoPC stimulate gametocytogenesis, while the preferred environment for gametocytes, the bone marrow, also displays low levels of LysoPC, although the details of the mechanism through which the signalling occurs remains to be determined [88, 89, 91].

P. falciparum also encodes two P4-ATPase-like proteins that are fused to functional guanylyl cyclases; PF3D7_1138400 contains GCα and PF3D7_1360500 contains GCβ. Little is known about the flippase activity of these fusion proteins, although some studies have started to shed light on this matter, suggesting that the flippase component of the protein is required for survival of the parasite [93]. Whereas GCα is essential for intraerythrocytic development, GCβ plays a critical role in colonization of the mosquito midgut [96, 141]. Interestingly, the *P. falciparum* protein phosphatase 1 (PfPP1), which is essential for merozoite egress, is not only stimulated by PC but targets GCα directly, indicating a potential role for PfPP1 in the regulation of GCα-dependent lipid sensing to initiate parasite egress from the erythrocyte [95]. A recent study in *P. yoelii* has shown that Cdc50A forms a stable complex with GCβ, which is required

for the gliding motility and midgut traversal of ookinetes [96]. Meanwhile, the Cdc50A ortholog in *Toxoplasma gondii* is important for recruiting its GCβ partner to the plasma membrane prior to cellular egress [97]. The role of these unique fusion proteins and how the flippase activity may regulate the guanylyl cyclase activity will surely be further elucidated in the years to come.

ATP binding cassette (ABC) transporters (flippases)

The *P. falciparum* genome encodes multiple members of the family of ATP binding cassette (ABC) transporters. One of these, ABCG2 (PF3D7_1426500), has previously been implicated in the transport of PS and PC analogues in eukaryotic cells *in vitro*, while in *Plasmodium* parasites it is suspected to work as a lipid transporter with a specific role in lipid storage [98, 99]. In *P. berghei* ABCG2 is predominantly associated with the plasma membrane of female gametocytes and ookinetes [99]. In *P. falciparum,* this protein is produced predominantly in the gametocyte stage, where it is found in a more distinct localisation, a single dot-like lipid-rich structure within female, but not male, gametocytes [99, 100]. Interestingly, in both species ABCG2 mutant parasites produce more gametocytes of both sexes and the level of cholesteryl esters, diacylglycerols and triacylglycerols are significantly reduced in gametocytes [99, 100]. Indicating a role for ABCG2 in control of gametocyte numbers and in the accumulation of neutral lipids, potentially important for parasite development in the insect stages since neutral lipids can function as energy storage and are precursors for metabolic activity, scarcely found in the mosquito microenvironment [111, 112, 113, 114, 115]. Several reports investigating lipid species during gametocytogenesis [22, 24, 89, 90, 91, 922], found that the levels of regulators of membrane fluidity, in particular cholesterol and SM, increase significantly during gametocyte maturation [22, 24]. Neutral lipids, serving mainly as energy reserves, increase from 3% of total lipids in uninfected erythrocytes to 27% in stage V gametocyte-infected erythrocytes [24]. ABC transporters that function as flippases are poorly conserved across the eukaryotic kingdom and hence could be suitable anti-malarial drug targets [80]. Interestingly, a putative *P. falciparum* ABC phospholipid transporter ATPase, PfABCF1 (PF3D7_0813700) is predicted to be essential (**Table 1**). However, it remains to be understood how ABC transporters function throughout the parasite lifecycle as LTPs.

MULTIDOMAIN TRANSPORTERS (LIPIDS AND HAEM)

Lipocalin

Lipocalins bind to small hydrophobic molecules and can transport lipids and fatty acids and hypothesised to share an early evolutionary origin with the bacterial kingdom, followed by extreme divergence of the amino acid sequence [101]. Recently, a lipocalin PfLCN (PF3D7_0925900) with domains predicted to bind to both fatty acids and haem was identified in *P. falciparum*. It was found to localise to the PV and food vacuole (FV) of intraerythrocytic parasites and to integrate into membranes, while in free merozoites was found mainly in the cytosol [102]. Furthermore, gene inactivation revealed its importance during the intraerythrocytic stage, mutant parasites undergo abnormal schizogony with a significantly reduced number of nuclei with some disorganisation compared to wildtype parasites [102]. Furthermore, the mutant showed defects in haemozoin crystal motility, either indicating defects in the FV or a direct interaction or binding function to haemozoin. Structure predictions indicate that PfLCN is similar to the *Escherichia coli* Blc protein, which functions in the storage and transport of lipids necessary for membrane repair [103], and the human lipocalin NGAL which is a palmitate and fatty acid transport protein [104, 105].

CHOLESTEROL TRANSPORTERS

Niemann-Pick C1-related protein and cholesterol homeostasis

P. falciparum encodes a Nieman-Pick C1-related protein PfNCR1 (PF3D7_0107500), part of a family of proteins that has been shown to have a function in the transport of cholesterol in lysosomes [106]. This protein localises to the PPM and to Membrane Contact Sites (MCSs) linking the PPM with the PVM [106, 107]. Although its cholesterol transfer function remains to be experimentally confirmed, disruption of PfNCR1 made intraerythrocytic parasites susceptible to saponin, likely owing to an increased level of cholesterol in the PPM. In addition, mutant parasites showed severe alterations in the FV, indicating a role for PfNCR1 in the maintenance or biogenesis of this vacuole [107]. These findings suggest that PfNCR1 is important in the regulation and maintenance of the low cholesterol levels at the PPM and may be important for maintaining the previously observed cholesterol gradient in intraerythrocytic stages (from high levels at the erythrocyte plasma membrane to lower levels at the PVM and even lower at the PPM) [53]. A combination of cryo-EM and correlative immunofluorescence revealed that PfNCR1 is localised at MCSs, sites between the PPM and the PVM, that could potentially directly connect the PPM with the PVM [107]. Interestingly, this also revealed a

distinct spatial exclusion between PfNCR1 and the solute and protein transporter EXP2 [107], indicating that lipid and solute transfer may occur at distinct regions of the PPM and PVM. Disruption of PfNCR1 expression does not interfere with the structure of the MCSs, suggesting that another LTP may be involved in this process [107]. Previous investigations in *P. berghei* intra-hepatocytic and oocyst development have provided the first indication of the presence of MCSs between the extended ER and the PPM [108]. These transient MCSs were observed during replicative stages using live Stimulated Emission Depletion (STED) super resolution microscopy coupled with PPM and ER protein reporters fused to sfGFP and mCherry, respectively [108]. The size of MCSs (between 10-30 nm) is below the limit of fluorescence microscopy resolution, making them technically difficult to visualise. Nonetheless, MCSs might be formed throughout the various stages of the parasite lifecycle in different regions of the parasite and parasite host-interface, likely aiding the distribution of lipids. Interestingly, steroidogenic acute regulatory (StAR) domain LTPs have been implicated with the formation of MCSs in other eukaryotes [109] hence, it is possible that the same applies to parasite-encoded StAR-related lipid-transfer (START) proteins. Nonetheless, likely PfNCR1 plays an important role in the regulation of cholesterol levels in the parasite and finding that it localises at MCSs between the PPM and PVM provides for the first time an insight into how the lipids required for the expansion of the PVM and parasite growth may be transferred to and from the parasite.

Potentially another parasite-derived component of cholesterol homeostasis in the infected erythrocyte is PF3D7_0113700 (PFA0660w). This protein is a Type II Hsp40 that is present in J dots and can bind cholesterol [18]. This cholesterol binding occurs through a part of the protein separate from that that binds Hsp70x, another component of J dots. It remains unclear whether J dots function as transporters of cholesterol or whether cholesterol aids in the stabilization of the transmembrane domains of the protein cargo [15, 16, 17, 18]. The known protein components of J dots are present only in the Laveranian lineage and can be readily mutated without obvious effect on parasite growth *in vitro*, despite a significant alteration in the formation of knobs, adhesive structures at the surface of the infected erythrocyte [110]. Potentially there exists an alternative pathway for the transport of cholesterol that is conserved in all *Plasmodium* parasites.

HOST LIPID TRANSFER PROTEINS

Erythrocyte LTPs

Erythrocyte biology should be appreciated to understand the lipid transfer events occurring during the *Plasmodium* intraerythrocytic development. Mature erythrocytes are unique cells as they are anucleated, devoid of organelles and unable to synthesise phospholipids or cholesterol [116, 117, 118]. The erythrocyte maintains its membrane asymmetry using three classes of enzymes: flippases (erythrocyte P4-type ATPases) that flip mainly PS from the outer leaflet to the inner leaflet in an energy-dependent manner; floppases, which mainly externalise PC from the inner leaflet to the outer leaflet, also requiring ATP; and scramblases, which transport negatively charged phospholipids between membrane leaflets and are ATP-independent [119]. The activity of the phospholipid scramblase PLSCR1 is ATP-independent but is activated by changes in Ca^{2+} influx [120, 121]. Scramblase activity increases during invasion of the parasite owing to an influx of the Ca^{2+}, which induces the exposure of PS to the outer surface of the erythrocyte, acting *in vivo* as a signal for removal of the cell by the immune system [121]. In a recent study, a combination of lipid labelling and pharmacological interventions was used to compare membrane asymmetry between *P. falciparum*-infected and uninfected erythrocytes. Infected erythrocytes were shown to be induced by the parasite to spend energy increasing the activity of ATP-dependent flippases to counteract the increase in outer PS induced by the non-specific activity of the PLSCR1 scramblase [121]. This indicated for the first time how the parasite resolves the initial consequences of its erythrocyte invasion, which inadvertently induces host scramblase-dependent outer PS exposure owing to the increase in Ca^{2+} levels, by activating host flippases [121].

Mosquito LTPs

Little is known about LTPs in the *Anopheles* mosquito. Mosquitos lack the biochemical pathways to add a second or third double bond into fatty acids and cannot produce sterols *de novo* [111, 112, 113, 114, 115]. Female *Anopheles* mosquitoes require feeding on blood, mainly for egg production [114, 115]. For this it is dependent on the LTP lipophorin, which is used to acquire and transport lipids from blood meals. Interestingly, not only the mosquito is dependent on lipophorin but the parasite also uses this LTP for its lipid scavenging requirements; in the absence of this protein not only is the growth of the mosquito negatively impacted but the growth of the parasite in the mosquito is also significantly reduced through the obstruction of sporozoite metabolism [114, 115, 116]. Hence, this host LTP is a regulator of parasite

infectivity, and its disruption reduces virulence and potentially transmission to the vertebrate host.

Hepatocyte LTPs

During the hepatocyte stage the parasite requires the presence of several host LTP proteins, in particular those involved in cholesterol transport. The scavenger receptor binding protein 1 (SRBP1), a membrane protein important for cellular cholesterol homeostasis, is key for infection of hepatocytes by *Plasmodium* parasites *in vitro* [122]. Furthermore, the parasite scavenges PC and extracellular and intracellularly synthesised cholesterol [123, 124] and promotes lipid biosynthesis by the host cell through inhibition of the AMP-activated Protein Kinase (AMPK) pathway [125]. Furthermore, pharmacological inhibition experiments using U18666A, an inhibitor which mimics the Niemann-Pick type C 1 (NPC1) mutant phenotype by blocking its activity and hence inhibiting cholesterol trafficking, severely impairs parasite growth by mislocalising cholesterol to enlarged intracellular vacuoles. This arrest is reversed with the addition of methyl-β-cyclodextrin (MβCD), which is known to release cholesterol from membranes, re-localising it to the PVM surrounding the parasite [126]. Interestingly, during the early stages of parasite establishment in the hepatocyte, its lipid requirements are low. However, the parasite establishes itself in the apical polar region of the hepatocyte, which has a higher level of cholesterol, phospholipids and SM [127], which may provide part of the vast number of phospholipids that are required to fuel the PVM expansion and growth of the parasite, including the membranes of the newly formed merozoites [127, 128].

OUTSTANDING QUESTIONS

Although significant insight into the growth of the parasites and its modification of the host has been gained over the past fifty years, many questions remain outstanding. Little is known about the transport of lipids to and from the PVM, the origin of the lipids used by the parasite, its lipidomic profile, exchange dynamics involved in membrane biogenesis, composition, and expansion in the intraerythrocytic stage. The recent discovery of *Plasmodium* parasite LTPs such as PF3D7_01004200 (PFA0210c) and lipocalin in the PV and PfNPC1 at MCSs, with the intriguing potential to act as lipid shuttles between the closely positioned PPM and PVM, are exciting new clues to the function of LTPs in this parasite [63, 102, 106, 107]. Further investigation of the localisation and function of more LTPs throughout the *Plasmodium* parasite lifecycle will greatly enrich this rapidly expanding field. Furthermore, the role of

cholesterol remains enigmatic. There is a dramatic gradient of cholesterol in the infected erythrocyte, but how this is set up and maintained remains unclear. The parasite cannot synthesise cholesterol and hence the cholesterol gradient must be distributed from the outside inwards. Further investigation of PfNPC1 and PFA0660w, and J dots in general, are likely to shed light on this process. Another lipid that is important in the transition between different lifecycle stages is LysoPC; a decrease in LysoPC levels induces the transition from asexual to sexual stages. The uptake of LysoPC is likely to be an active process and may involve a parasite-derived transporter [89, 129]. Therefore, the biochemical and genetic characterisation of the transport and metabolism of LysoPC and other phospholipid precursors should be pursued. Many lipid transfer proteins remain unstudied, even those that have an essential function, including PF3D7_0920700, a predicted Sec14/CRAL/TRIO phospholipid transfer protein, and PF3D7_1324400, a PRELI domain-containing protein predicted to be involved in phospholipid and phosphatidic acid transport (**Table 1**). PRELI family members are known to regulate lipid accumulation in organelles by shuttling phospholipids [130]. Interestingly, while the lipid content of rhoptries in *T. gondii* has been investigated, so far, no studies have emerged with regards to the lipid contents of *Plasmodium* parasites, and only proteomic analyses have been performed [131, 132]. Such studies and proteomic analyses to identify lipids and LTPs associated with invasion organelles would be of great interest, especially to understand the specific invasion mechanisms of the parasite. Genetic and biochemical investigation of these proteins is likely to uncover fascinating new interactions between the parasite and the host and transfer pathways within the host.

Investigating these and other outstanding questions will be greatly aided by advances in research technologies to manipulate *Plasmodium* parasites [61, 102, 107, 108, 133, 134, 135, 136]. Inducible gene deletion, protein disruption and mRNA stability techniques have already proven to be indispensable to the investigation of many essential proteins and will undoubtedly be of great use in furthering our understanding of the proteins involved in the transfer of lipids in the parasite and in the host cell. Fluorescence Recovery After Photobleaching (FRAP) and fluorescent timer experiments will provide insight into the dynamics of LTPs in live cells and their directionality. In addition, cryoEM has already proven to move the boundaries of the detection of the fine structure of the parasite. As an example, the discovery of the MCSs connecting the PPM and the PVM will likely prove to be a pivotal moment in our understanding of the formation, expansion and maintenance of the PVM [107]. With these and other new

technologies, the functional role of numerous important LTPs are likely to come into better focus over the coming years.

Acknowledgments

MR and CvO are supported by MRC Career Development Award (MR/R008485/1)

CRediT author statement

Margarida Ressurreição: Conceptualization, Data curation, Writing- Original Investigation draft preparation, Writing- Reviewing and Editing. **Christiaan van Ooij***:* Supervision, Writing- Reviewing and Editing.

Keywords and abbreviations

FV Food Vacuole, **StAR** Steroidogenic acute regulatory, **START** StAR-related lipid-transfer, **MCs** Maurers's Clefts, **MCSs** Membrane Contact Sites, **PVM** Parasitophorous Vacuole Membrane, **PV Parasitophorous** Vacuole, **PPM** Parasite Plasma Membrane.

Table 1. Studied and predicted *Plasmodium falciparum* lipid transfer proteins

When proteins have been previously studied references have been indicated. If no references are indicated, then these are predicted gene products. Both Gene ID and predicted protein sequence were retrieved from plasmodb.org. Domain organisation and predicted function were obtained when available from uniprot.org and ebi.ac.uk/interpro. In certain cases, the predicted gene products were mined using specific GO searches at ebi.ac.uk, using the *Plasmodium falciparum* (isolate 3D7) Uniprot Taxon Identifier (UTI): 36329. Specific GO codes and functions searched are described at the end of this legend. Essentiality data were obtained from plasmodb.org and cross-referenced from the supplementary data provided by Zhang and colleagues [75]. Localisation was either obtained from published studies, predicted by uniprot.org or assumed due to the domain content of the respective gene product (i.e. presence or absence of transmembrane domains).

The following GO searches with included the qualifier 'enables' and excluded 'involved in'. The following GO searches were used: GO:0006869 'lipid transport', GO:0034040 'ATPase-coupled lipid transmembrane transporter activity', GO:0005319 'lipid transporter activity', GO:0120020 'cholesterol transfer activity', GO:0046624 'sphingolipid transporter activity', GO:0035627 'ceramide transport', GO:0043691 'reverse cholesterol transport', GO:0017089 'glycolipid transfer activity', GO:0035621 'ER to Golgi ceramide transport', GO:0008526 'phosphatidylinositol transfer activity', GO:0120019 'phosphatidylcholine transfer activity', GO:0120014 'phospholipid transfer activity', GO:1904121 'phosphatidylethanolamine transfer activity', GO:0005548 'phospholipid transporter activity', GO:0140337 'diacylglyceride transfer activity', GO:0140340 'cerebroside transfer activity', GO:0140339 'phosphatidylglycerol transfer activity', GO:0140338 'sphingomyelin transfer activity', GO:1990050 'phosphatidic acid transfer activity', GO:0046836 'glycolipid transport', GO:0140327 'flippase activity', GO:0030301 'cholesterol transport', GO:0015914

'phospholipid transport', GO:0090556 'phosphatidylserine floppase activity', GO:0090554 'phosphatidylcholine floppase activity'.

Figure 1. The *Plasmodium falciparum* lifecycle.
Plasmodium falciparum parasites depend on two distinct host environments, the mosquito midgut, and salivary glands, required for sexual reproduction, differentiation, replication and transmission (green background). Meanwhile the human host liver and blood cells must be invaded to sustain its differentiation and extraordinary high levels of cellular replication (beige background). A) Upon the ingestion of mature *P. falciparum* male and female gametocytes, the gametocytes are relocated to a drastically different midgut environment of the *Anopheles* mosquito (i.e. ≥5°C temperature drop, increased pH and xanthurenic acid levels). B) These changes trigger a rapid adaptational response by both gametocytes, characterised by deep nuclear reorganisation and differentiation events, resulting in eight free-swimming microgametes (male gametes) and one macrogamete (female gamete). C) The fertilisation of a macrogamete with a microgamete result in a zygote. D) The zygote becomes a motile ookinete, which traverses the mosquito midgut to encyst and become an oocyst. E) Inside the oocyst, the ookinete undergoes sporogony producing thousands of sporozoites. F) The oocyst eventually ruptures, releasing thousands of sporozoites which migrate to the mosquito salivary glands. G) Human infection occurs when sporozoite contaminated saliva is released into the blood circulation during a mosquito blood meal. H) Once in the liver, a selected hepatocyte is invaded by a single sporozoite. I) In the hepatocyte, the parasite develops inside a parasitophorous vacuole (PV) enveloped by a parasitophorous vacuole membrane (PVM). J) After several rounds of replication, through a process named schizogony, thousands of invasive merozoites are generated. K) The merozoites are released and able to invade an erythrocyte using a set of specialised invasion structures, a zoomed in depiction of a merozoite labels these apical organelles which include the micronemes (Ms), rhoptries (Rs) and dense granules (DGs). L) Merozoites initiate the intraerythrocytic asexual cycle by invading an erythrocyte through initial stochastic contact, followed by release of contents from the apical organelles, while burrowing through the erythrocyte, forming a PV and PVM. M) The parasite starts as the ring stage where for the first 24 hours it exports and imports various proteins and nutrients, moving them into and from host cytoplasm, crossing several membranous structures such as the parasite plasma membrane (PPM), the PV, PVM, tubovesicular network (TVN). N) The trophozoite the parasite growth stage, several lipid derived structures are present including the PVM, TVN, Maurer's Clefts (MCs), J dots, 25nm and 80 nm vesicles, food vacuole (FV) and membrane contact sites (MCSs). O) The schizont is the result of a highly replicative stage (erythrocytic schizogony), generating up to 32 new merozoites. P) When the schizont is mature the PVM and erythrocyte plasma membrane bursts, releasing newly invasive merozoites. Q) A subpopulation undergoes gametocytogenesis to develop into male or female gametocytes, which once fully matured can be transmitted back to the mosquito. Diagram not to scale.

References

[1] World Health Organization. World malaria report 2020: 20 years of global progress and challenges.

[2] Prudêncio M, Rodriguez A, Mota MM. The silent path to thousands of merozoites: the *Plasmodium* liver stage. *Nature Rev Microbiol*. 2006 Nov;4(11):849-56.

[3] Nyboer B, Heiss K, Mueller AK, Ingmundson A. The *Plasmodium* liver-stage parasitophorous vacuole: A front-line of communication between parasite and host. *Int J Med Microbiol*. 2018 Jan 1;308(1):107-17.

[4] Venugopal K, Hentzschel F, Valkiūnas G, Marti M. *Plasmodium* asexual growth and sexual development in the haematopoietic niche of the host. *Nature Rev Microbiol*. 2020 Mar;18(3):177-89.

[5] Cowman AF, Healer J, Marapana D, Marsh K. Malaria: biology and disease. *Cell*. 2016 Oct 20;167(3):610-24.

[6] Ghosh AK, Jacobs-Lorena M. *Plasmodium* sporozoite invasion of the mosquito salivary gland. *Current Opinion Microbiol*. 2009 Aug 1;12(4):394-400.

[7] Sherling ES, van Ooij C. Host cell remodeling by pathogens: the exomembrane system in Plasmodium-infected erythrocytes. *FEMS Microbiol Rev*. 2016 Sep 1;40(5):701-21.

[8] Matz JM, Beck JR, Blackman MJ. The parasitophorous vacuole of the blood-stage malaria parasite. *Nature Rev Microbiol*. 2020 Jul;18(7):379-91.

[9] Gilson PR, Chisholm SA, Crabb BS, de Koning-Ward TF. Host cell remodelling in malaria parasites: a new pool of potential drug targets. *Int. J Parasitol*. 2017 Feb 1;47(2-3):119-27.

[10] Wang J, Jiang N, Sang X, Yang N, Feng Y, Chen R, Wang X, Chen Q. Protein modification characteristics of the malaria parasite *Plasmodium falciparum* and the infected erythrocytes. *Mol Cell Proteomics*. 2021 Jan 1;20:100001.

[11] Grüring C, Heiber A, Kruse F, Ungefehr J, Gilberger TW, Spielmann T. Development and host cell modifications of *Plasmodium falciparum* blood stages in four dimensions. *Nature Commun*. 2011 Jan 25;2(1):1-1.

[12] Boddey JA, Cowman AF. *Plasmodium* nesting: remaking the erythrocyte from the inside out. *Annual Rev Microbiol*. 2013 Sep 8;67:243-69

[13] Petersen W, Külzer S, Engels S, Zhang Q, Ingmundson A, Rug M, Maier AG, Przyborski JM. J-dot targeting of an exported HSP40 in *Plasmodium falciparum*-infected erythrocytes. *Int J Parasitol*. 2016 Jul 1;46(8):519-25.

[14] Jha P, Laskar S, Dubey S, Bhattacharyya MK, Bhattacharyya S. *Plasmodium* Hsp40 and human Hsp70: A potential cochaperone-chaperone complex. *Mol Biochem Parasitol*. 2017 Jun 1;214:10-3

[15] Külzer S, Charnaud S, Dagan T, Riedel J, Mandal P, Pesce ER, Blatch GL, Crabb BS, Gilson PR, Przyborski JM. *Plasmodium falciparum*-encoded exported hsp70/hsp40 chaperone/co-chaperone complexes within the host erythrocyte. *Cell Microbiol*. 2012 Nov;14(11):1784-95.

[16] Przyborski JM, Diehl M, Blatch GL. Plasmodial HSP70s are functionally adapted to the malaria parasite life cycle. *Front Mol Biosci*. 2015 Jun 26;2:34.

[17] Dutta T, Singh H, Gestwicki JE, Blatch GL. Exported plasmodial J domain protein, PFE0055c, and PfHsp70-x form a specific co-chaperone-chaperone partnership. *Cell Stress Caperones*. 2021 Mar;26(2):355-66.

[18] Behl A, Kumar V, Bisht A, Panda JJ, Hora R, Mishra PC. Cholesterol bound *Plasmodium falciparum* co-chaperone 'PFA0660w'complexes with major virulence factor 'PfEMP1'via chaperone 'PfHsp70-x'. Sci Rep. 2019 Feb 25;9(1):1-7.

[19] Lim L, McFadden GI. The evolution, metabolism and functions of the apicoplast. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 2010 Mar 12;365(1541):749-63.

[20] Lemgruber L, Kudryashev M, Dekiwadia C, Riglar DT, Baum J, Stahlberg H, Ralph SA, Frischknecht F. Cryo-electron tomography reveals four-membrane architecture of the *Plasmodium* apicoplast. *Malar J*. 2013 Dec;12(1):1-9.

[21] Vaishnava S, Striepen B. The cell biology of secondary endosymbiosis–how parasites build, divide and segregate the apicoplast. *Mol Microbiol*. 2006 Sep;61(6):1380-7.

[22] Gulati S, Ekland EH, Ruggles KV, Chan RB, Jayabalasingham B, Zhou B, Mantel PY, Lee MC, Spottiswoode N, Coburn-Flynn O, Hjelmqvist D. Profiling the essential nature of lipid metabolism in asexual blood and gametocyte stages of Plasmodium falciparum. *Cell Host Microbe*. 2015 Sep 9;18(3):371-81.

[23] Hulbert AJ, Kelly MA, Abbott SK. Polyunsaturated fats, membrane lipids and animal longevity. Journal of Comparative Physiology B. 2014 Feb 1;184(2):149-66.

[24] Tran PN, Brown SH, Rug M, Ridgway MC, Mitchell TW, Maier AG. Changes in lipid composition during sexual development of the malaria parasite *Plasmodium falciparum*. *Malar J*. 2016 Dec;15(1):1-3.

[25] Vallochi AL, Teixeira L, Oliveira KD, Maya-Monteiro CM, Bozza PT. Lipid droplet, a key player in host-parasite interactions. Frontiers in immunology. 2018 May 23;9:1022.

[26] Srivastava A, Philip N, Hughes KR, Georgiou K, MacRae JI, Barrett MP, Creek DJ, McConville MJ, Waters AP. Stage-specific changes in Plasmodium metabolism required for differentiation and adaptation to different host and vector environments. PLoS pathogens. 2016 Dec 27;12(12):e1006094.

[27] Zuzarte-Luís V, Mota MM. Parasite sensing of host nutrients and environmental cues. Cell host & microbe. 2018 Jun 13;23(6):749-58.

[28] Sah RK, Pati S, Saini M, Singh S. Erythrocyte sphingosine kinase regulates intraerythrocytic development of Plasmodium falciparum. Scientific reports. 2021 Jan 13; 11(1):1-6.

[29] Sah RK, Pati S, Saini M, Boopathi PA, Kochar SK, Kochar DK, Das A, Singh S. Reduction of sphingosine kinase 1 phosphorylation and activity in plasmodium-infected erythrocytes. Frontiers in cell and developmental biology. 2020 Mar 3; 8:80.

[30] Vial HJ, Ben Mamoun C. Plasmodium lipids: metabolism and function. Molecular approaches to malaria. 2005 Sep; 1:327-52.

[31] Déchamps S, Shastri S, Wengelnik K, Vial HJ. Glycerophospholipid acquisition in Plasmodium–a puzzling assembly of biosynthetic pathways. International journal for parasitology. 2010 Oct 1; 40(12):1347-65.

[32] Asahi H, Kanazawa T, Hirayama N, Kajihara Y. Investigating serum factors promoting erythrocytic growth of Plasmodium falciparum. Experimental parasitology. 2005 Jan 1; 109(1):7-15.

[33] Gerold P, Schwarz RT. Biosynthesis of glycosphingolipids de-novo by the human malaria parasite Plasmodium falciparum. Molecular and biochemical parasitology. 2001 Jan 15; 112(1):29-37.

[34] Aerts JM, Artola M, Van Eijk M, Ferraz MJ, Boot RG. Glycosphingolipids and infection. Potential new therapeutic avenues. Frontiers in cell and developmental biology. 2019 Dec 6 ;7:324.

[35] Tewari SG, Swift RP, Reifman J, Prigge ST, Wallqvist A. Metabolic alterations in the erythrocyte during blood-stage development of the malaria parasite. Malaria journal. 2020 Dec; 19(1):1-8.

[36] MacRae JI, Lopaticki S, Maier AG, Rupasinghe T, Nahid A, Cowman AF, McConville MJ. P lasmodium falciparum is dependent on de novo myo-inositol biosynthesis for assembly of GPI glycolipids and infectivity. Molecular microbiology. 2014 Feb; 91(4):762-76.

[37] Botté CY, Yamaryo-Botté Y, Rupasinghe TW, Mullin KA, MacRae JI, Spurck TP, Kalanon M, Shears MJ, Coppel RL, Crellin PK, Maréchal E. Atypical lipid composition in the purified relict plastid (apicoplast) of malaria parasites. Proceedings of the National Academy of Sciences. 2013 Apr 30; 110(18):7506-11.

[38] Dykstra M, Cherukuri A, Sohn HW, Tzeng SJ, Pierce SK. Location is everything: lipid rafts and immune cell signaling. Annual review of immunology. 2003 Apr; 21(1):457-81.

[39] Lonez C, Vandenbranden M, Ruysschaert JM. Cationic lipids activate intracellular signaling pathways. Advanced drug delivery reviews. 2012 Dec 1; 64(15):1749-58.

[40] Lloyd-Evans E, Waller-Evans H. Lysosomal Ca2+ homeostasis and signaling in health and disease. Cold Spring Harbor perspectives in biology. 2020 Jun 1; 12(6):a035311.

[41] Okazaki Y, Saito K. Roles of lipids as signaling molecules and mitigators during stress response in plants. The Plant Journal. 2014 Aug; 79(4):584-96.

[42] Nguyen A, Rudge SA, Zhang Q, Wakelam MJ. Using lipidomics analysis to determine signalling and metabolic changes in cells. Current opinion in biotechnology. 2017 Feb 1;43: 96-103.

[43] de Meyer F, Smit B. Effect of cholesterol on the structure of a phospholipid bilayer. Proceedings of the National Academy of Sciences. 2009 Mar 10; 106(10):3654-8.

[44] Zhang Y, Li Q, Dong M, Han X. Effect of cholesterol on the fluidity of supported lipid bilayers. Colloids and Surfaces B: Biointerfaces. 2020 Dec 1; 196:111353.

[45] Somjid S, Krongsuk S, Johns JR. Cholesterol concentration effect on the bilayer properties and phase formation of niosome bilayers: a molecular dynamics simulation study. Journal of Molecular Liquids. 2018 Apr 15; 256:591-8.

[46] Clair JW, London E. Effect of sterol structure on ordered membrane domain (raft) stability in symmetric and asymmetric vesicles. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2019 Jun 1;1861(6):1112-22.

[47] Cho YY, Kwon OH, Park MK, Kim TW, Chung S. Elevated cellular cholesterol in Familial Alzheimer's presenilin 1 mutation is associated with lipid raft localization of β-amyloid precursor protein. PLoS One. 2019 Jan 25; 14(1):e0210535.

[48] Thériault C, Richard D. Characterization of a putative Plasmodium falciparum SAC1 phosphoinositide-phosphatase homologue potentially required for survival during the asexual erythrocytic stages. Scientific reports. 2017 Oct 5;7(1):1-9.

[49] Tewari SG, Rajaram K, Swift RP, Reifman J, Prigge ST, Wallqvist A. Metabolic Survival Adaptations of Plasmodium falciparum Exposed to Sublethal Doses of Fosmidomycin. Antimicrobial Agents and Chemotherapy. 2021 Mar 18;65(4).

[50] Florentin A, Cobb DW, Kudyba HM, Muralidharan V. Directing traffic: Chaperone-mediated protein transport in malaria parasites. Cellular microbiology. 2020 Jul;22(7):e13215.

[51] Counihan NA, Modak JK, Koning-Ward D, Tania F. How malaria parasites acquire nutrients from their host. Frontiers in Cell and Developmental Biology. 2021; 9:582.

[52] Hayakawa EH, Yamaguchi K, Mori M, Nardone G. Real-time cholesterol sorting in Plasmodium falciparum-erythrocytes as revealed by 3D label-free imaging. Scientific reports. 2020 Feb 17;10(1):1-2.

[53] Tokumasu F, Crivat G, Ackerman H, Hwang J, Wellems TE. Inward cholesterol gradient of the membrane system in P. falciparum-infected erythrocytes involves a dilution effect from parasite-produced lipids. Biology open. 2014 Jun 15;3(6):529-41.

[54] Fratini F, Raggi C, Sferra G, Birago C, Sansone A, Grasso F, Currà C, Olivieri A, Pace T, Mochi S, Picci L. An integrated approach to explore composition and dynamics of

cholesterol-rich membrane microdomains in sexual stages of malaria parasite. Molecular & Cellular Proteomics. 2017 Oct 1;16(10):1801-14.

[55] Couto AS, Caffaro C, Uhrig ML, Kimura E, Peres VJ, Merino EF, Katzin AM, Nishioka M, Nonami H, Erra-Balsells R. Glycosphingolipids in Plasmodium falciparum: Presence of an active glucosylceramide synthase. European journal of biochemistry. 2004 Jun;271(11):2204-14.

[56] Clark BJ. The START-domain proteins in intracellular lipid transport and beyond. Molecular and cellular endocrinology. 2020 Mar 15; 504:110704.

[57] Ejotre, I., Reeder, D.M., Matuschewski, K. and Schaer, J., 2021. Hepatocystis. Trends in Parasitology, 37(5), pp.456-457.

[58] Aunin, E., Böhme, U., Sanderson, T., Simons, N.D., Goldberg, T.L., Ting, N., Chapman, C.A., Newbold, C.I., Berriman, M. and Reid, A.J., 2020. Genomic and transcriptomic evidence for descent from Plasmodium and loss of blood schizogony in Hepatocystis parasites from naturally infected red colobus monkeys. PLoS pathogens, 16(8), p.e1008717.

[59] Allan, C.M., Hill, S., Morvaridi, S., Saiki, R., Johnson, J.S., Liau, W.S., Hirano, K., Kawashima, T., Ji, Z., Loo, J.A. and Shepherd, J.N., 2013. A conserved START domain coenzyme Q-binding polypeptide is required for efficient Q biosynthesis, respiratory electron transport, and antioxidant function in Saccharomyces cerevisiae. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1831*(4).

[60] Tsui HS, Pham NB, Amer BR, Bradley MC, Gosschalk JE, Gallagher-Jones M, Ibarra H, Clubb RT, Blaby-Haas CE, Clarke CF. Human COQ10A and COQ10B are distinct lipid-binding START domain proteins required for coenzyme Q function. Journal of lipid research. 2019 Jul 1;60(7):1293-310.

[61] van Ooij C, Withers-Martinez C, Ringel A, Cockcroft S, Haldar K, Blackman MJ. Identification of a Plasmodium falciparum phospholipid transfer protein. J Biol Chem. 2013 Nov 1;288(44):31971-83.

[62] van Ooij C, Tamez P, Bhattacharjee S, Hiller NL, Harrison T, Liolios K, Kooij T, Ramesar J, Balu B, Adams J, Waters AP, Janse CJ, Haldar K. The malaria secretome: from algorithms to essential function in blood stage infection. PLoS Pathog. 2008 Jun 13;4(6):e1000084.

[63] Hill RJ, Ringel A, Knuepfer E, Moon RW, Blackman MJ, van Ooij C. Regulation and Essentiality of the StAR-related Lipid Transfer (START) Domain-containing Phospholipid Transfer Protein PFA0210c in Malaria Parasites. J Biol Chem. 2016 Nov 11;291(46):24280-24292.

[64] Azcárate AI, Marín-García P, Abad P, Pérez-Benavente S, Paz-Artal E, Reche PA, Fobil JN, Rubio JM, Diez A, Puyet A, Bautista JM. *Plasmodium falciparum* immunodominant IgG epitopes in subclinical malaria. Sci. Rep. 2020 10, 9398

[65] Pati P, Dhangadamajhi G, Bal M, Ranjit M. High proportions of pfhrp2 gene deletion and performance of HRP2-based rapid diagnostic test in Plasmodium falciparum field isolates of Odisha. Malaria journal. 2018 Dec;17(1):1-1.

[66] Howick VM, Russell AJC, Andrews T, Heaton H, Reid AJ, Natarajan K, Butungi H, Metcalf T, Verzier LH, Rayner JC, Berriman M, Herren JK, Billker O, Hemberg M, Talman AM, Lawniczak MKN. The Malaria Cell Atlas: Single parasite transcriptomes across the complete *Plasmodium* life cycle. Science. 2019 Aug 23;365(6455):eaaw2619

[67] Frech C, Chen N. Variant surface antigens of malaria parasites: functional and evolutionary insights from comparative gene family classification and analysis. BMC Genomics. 2013 Jun 27;14:427. doi: 10.1186/1471-2164-14-427.

[68] Fougère A, Jackson AP, Bechtsi DP, Braks JA, Annoura T, Fonager J, Spaccapelo R, Ramesar J, Chevalley-Maurel S, Klop O, van der Laan AM, Tanke HJ, Kocken CH, Pasini EM, Khan SM, Böhme U, van Ooij C, Otto TD, Janse CJ, Franke-Fayard B. Variant Exported Blood-Stage Proteins Encoded by Plasmodium Multigene Families Are Expressed in Liver Stages Where They Are Exported into the Parasitophorous Vacuole. PLoS Pathog. 2016 Nov 16;12(11):e1005917. doi: 10.1371/journal.ppat.1005917. Erratum in: PLoS Pathog. 2016 Dec 14;12 (12):e1006107. Erratum in: PLoS Pathog. 2017 Jan 17;13(1):e1006128.

[69] Huang J, Ghosh R, Tripathi A, Lönnfors M, Somerharju P, Bankaitis VA. Two-ligand priming mechanism for potentiated phosphoinositide synthesis is an evolutionarily conserved feature of Sec14-like phosphatidylinositol and phosphatidylcholine exchange proteins. Molecular biology of the cell. 2016 Jul 15;27(14):2317-30.

[70] Lim Y, Traber MG. Alpha-tocopherol transfer protein (α-TTP): Insights from alpha-tocopherol transfer protein knockout mice. Nutrition research and practice. 2007 Dec 1;1(4):247-53.

[71] Lete MG, Tripathi A, Chandran V, Bankaitis VA, McDermott MI. Lipid transfer proteins and instructive regulation of lipid kinase activities: Implications for inositol lipid signaling and disease. Advances in Biological Regulation. 2020 Jul 14:100740.[71] Montag K, Hornbergs J, Ivanov R, Bauer P. Phylogenetic analysis of plant multi-domain SEC14-like phosphatidylinositol transfer proteins and structure–function properties of PATELLIN2. *Plant Mol Biol*. 2020 Dec;104(6):665-78.

[72] Khan D, Lee D, Gulten G, Aggarwal A, Wofford J, Krieger I, Tripathi A, Patrick JW, Eckert DM, Laganowsky A, Sacchettini J, Lindahl P, Bankaitis VA. A Sec14-like phosphatidylinositol transfer protein paralog defines a novel class of heme-binding proteins. *Elife*. 2020 Aug 11;9:e57081.

[73] Vaid A, Ranjan R, Smythe WA, Hoppe HC, Sharma P. PfPI3K, a phosphatidylinositol-3 kinase from *Plasmodium falciparum*, is exported to the host erythrocyte and is involved in hemoglobin trafficking. *Blood*. 2010 Mar 25;115(12):2500-7.

[74] Kenthirapalan S, Waters AP, Matuschewski K, Kooij TW. Functional profiles of orphan membrane transporters in the life cycle of the malaria parasite. *Nature Commun*. 2016 Jan 22;7(1):1-0.

[75] Zhang M, Wang C, Otto TD, Oberstaller J, Liao X, Adapa SR, Udenze K, Bronner IF, Casandra D, Mayho M, Brown J. Uncovering the essential genes of the human malaria parasite *Plasmodium falciparum* by saturation mutagenesis. *Science*. 2018 May 4;360(6388).

[76] Lamy A, Macarini-Bruzaferro E, Dieudonné T, Perálvarez-Marín A, Lenoir G, Montigny C, Le Maire M, Vázquez-Ibar JL. ATP2, the essential P4-ATPase of malaria parasites, catalyzes lipid-stimulated ATP hydrolysis in complex with a Cdc50 β-subunit. *Emerg Microbes Infect*. 2020 Dec 30:1-32.

[77] Andersen JP, Vestergaard AL, Mikkelsen SA, Mogensen LS, Chalat M, Molday RS. P4-ATPases as phospholipid flippases - structure, function, and enigmas. *Front Physiol*. 2016 Jul 8; 7:275.

[78] Krishna S, Webb R, Woodrow C. Transport proteins of Plasmodium falciparum: defining the limits of metabolism. *Int J Parasitol*. 2001 Oct;31(12):1331-42.

[79] Martin RE. The transportome of the malaria parasite. Biol Rev. 2020 Apr;95(2):305-32.

[80] Weiner J, Kooij TW. Phylogenetic profiles of all membrane transport proteins of the malaria parasite highlight new drug targets. *Microbial Cell*. 2016 Oct 3;3(10):511.

[81] Bushell E, Gomes AR, Sanderson T, Anar B, Girling G, Herd C, Metcalf T, Modrzynska K, Schwach F, Martin RE, Mather MW. Functional profiling of a *Plasmodium* genome reveals an abundance of essential genes. *Cell*. 2017 Jul 13;170(2):260-72.

[82] Kenthirapalan S, Waters AP, Matuschewski K, Kooij TW. Functional profiles of orphan membrane transporters in the life cycle of the malaria parasite. *Nature Commun*. 2016 Jan 22;7(1):1-0.

[83] Cowell AN, Istvan ES, Lukens AK, Gomez-Lorenzo MG, Vanaerschot M, Sakata-Kato T, Flannery EL, Magistrado P, Owen E, Abraham M, LaMonte G. Mapping the malaria parasite druggable genome by using in vitro evolution and chemogenomics. Science. 2018 Jan 12;359(6372):191-9.

[84] Paulusma CC, Folmer DE, Ho-Mok KS, De Waart DR, Hilarius PM, Verhoeven AJ, Oude Elferink RP. ATP8B1 requires an accessory protein for endoplasmic reticulum exit and plasma membrane lipid flippase activity. *Hepatology*. 2008 Jan;47(1):268-78.

[85] Bryde S, Hennrich H, Verhulst PM, Devaux PF, Lenoir G, Holthuis JC. CDC50 proteins are critical components of the human class-1 P4-ATPase transport machinery. *J Biol Chem*. 2010 Dec 24;285(52):40562-72.

[86] McNamara CW, Lee MC, Lim CS, Lim SH, Roland J, Nagle A, Simon O, Yeung BK, Chatterjee AK, McCormack SL, Manary MJ. Targeting Plasmodium PI (4) K to eliminate malaria. *Nature*. 2013 Dec;504(7479):248-53.

[87] Ebrahimzadeh, Zeinab, Angana Mukherjee, and Dave Richard. A Map of the Subcellular Distribution of Phosphoinositides in the Erythrocytic Cycle of the Malaria Parasite Plasmodium Falciparum. *Int J Parasitol* 2018 48 (1): 13–25.

[88] Kilian N, Choi JY, Voelker DR, Mamoun CB. Role of phospholipid synthesis in the development and differentiation of malaria parasites in the blood. *J Biol Chem*. 2018 Nov 9;293(45):17308-16.

[89] Brancucci NM, Gerdt JP, Wang C, De Niz M, Philip N, Adapa SR, Zhang M, Hitz E, Niederwieser I, Boltryk SD, Laffitte MC. Grü ring C, Ravel D, Blancke Soares A, Demas A, Bopp S, Rubio-Ruiz B, Conejo-Garcia A, Wirth DF, et al. 2017. Lysophosphatidylcholine regulates sexual stage differentiation in the human malaria parasite *Plasmodium falciparum*. *Cell*;171:1532-44.

[90] Lopez-Marques RL, Theorin L, Palmgren MG, Pomorski TG. P4-ATPases: lipid flippases in cell membranes. Pflügers Archiv-European Journal of Physiology. 2014 Jul;466(7):1227-40.

[91] Ngotho P, Soares AB, Hentzschel F, Achcar F, Bertuccini L, Marti M. Revisiting gametocyte biology in malaria parasites. *FEMS Microbiol Rev*. 2019 Jul;43(4):401-14.

[92] Venugopal K, Hentzschel F, Valkiūnas G, Marti M. Plasmodium asexual growth and sexual development in the haematopoietic niche of the host. *Nature Rev Microbiol*. 2020 Mar;18(3):177-89.

[93] Nofal SD, Patel A, Blackman MJ, Flueck C, Baker DA. Plasmodium falciparum guanylyl cyclase-alpha and the activity of its appended P4-ATPase domain are essential for cGMP synthesis and blood-stage egress. MBio. 2021 Feb 23;12(1).

[94] Moon RW, Taylor CJ, Bex C, Schepers R, Goulding D, Janse CJ, Waters AP, Baker DA, Billker O. A cyclic GMP signalling module that regulates gliding motility in a malaria parasite. *PLoS Pathog*. 2009 Sep 25;5(9):e1000599.

[95] Paul AS, Miliu A, Paulo JA, Goldberg JM, Bonilla AM, Berry L, Seveno M, Braun-Breton C, Kosber AL, Elsworth B, Arriola JS. Co-option of *Plasmodium falciparum* PP1 for egress from host erythrocytes. *Nature Commun*. 2020 Jul 15;11(1):1-3.

[96] Gao H, Yang Z, Wang X, Qian P, Hong R, Chen X, Su XZ, Cui H, Yuan J. ISP1-anchored polarization of GCβ/CDC50A complex initiates malaria ookinete gliding motility. *Curr Biol*. 2018 Sep 10;28(17):2763-76.

[97] Bisio H, Lunghi M, Brochet M, Soldati-Favre D. Phosphatidic acid governs natural egress in *Toxoplasma gondii* via a guanylate cyclase receptor platform. *Nature Microbiol*. 2019 Mar;4(3):420-8.

[98] Woehlecke H, Pohl A, Alder-Baerens N, Lage H, Herrmann A. Enhanced exposure of phosphatidylserine in human gastric carcinoma cells overexpressing the half-size ABC transporter BCRP (ABCG2). *Biochem J*. 2003 Dec 1;376(2):489-95.

[99] Kenthirapalan S, Tran PN, Kooij TW, Ridgway MC, Rauch M, Brown SH, Mitchell TW, Matuschewski K, Maier AG. Distinct adaptations of a gametocyte ABC transporter to murine and human *Plasmodium* parasites and its incompatibility in cross-species complementation. Int J Parasitol. 2020 Jun 1;50(6-7):511-22.

[100] Tran PN, Brown SH, Mitchell TW, Matuschewski K, McMillan PJ, Kirk K, Dixon MW, Maier AG. A female gametocyte-specific ABC transporter plays a role in lipid metabolism in the malaria parasite. Nature Comm. 2014 Sep 8;5(1):1-3.

[101] Ganfornina MD, Gutiérrez G, Bastiani M. A phylogenetic analysis of the lipocalin protein family. *Mol Biol Evol*. 2000 Jan 1;17(1):114-26.

[102] Burda PC, Crosskey T, Lauk K, Zurborg A, Söhnchen C, Liffner B, Wilcke L, Pietsch E, Strauss J, Jeffries CM, Svergun DI. Structure-Based Identification and Functional Characterization of a Lipocalin in the Malaria Parasite *Plasmodium falciparum*. Cell Rep. 2020 Jun 23;31(12):107817.

[103] Campanacci V, Nurizzo D, Spinelli S, Valencia C, Tegoni M, Cambillau C. The crystal structure of the Escherichia coli lipocalin Blc suggests a possible role in phospholipid binding. *FEBS Lett*. 2004 Mar 26;562(1-3):183-8.

[104] Goetz DH, Willie ST, Armen RS, Bratt T, Borregaard N, Strong RK. Ligand preference inferred from the structure of neutrophil gelatinase associated lipocalin. *Biochem*. 2000 Feb 29;39(8):1935-41.

[105] Wang Y. Small lipid-binding proteins in regulating endothelial and vascular functions: focusing on adipocyte fatty acid binding protein and lipocalin-2. *Br J Pharmacol*. 2012 Feb;165(3):603-21.

[106] Istvan ES, Das S, Bhatnagar S, Beck JR, Owen E, Llinas M, Ganesan SM, Niles JC, Winzeler E, Vaidya AB, Goldberg DE. *Plasmodium* Niemann-Pick type C1-related protein is a druggable target required for parasite membrane homeostasis. *Elife*. 2019 Mar 19;8:e40529.

[107] Garten M, Beck JR, Roth R, Tenkova-Heuser T, Heuser J, Istvan ES, Bleck CK, Goldberg DE, Zimmerberg J. Contacting domains segregate a lipid transporter from a solute transporter in the malarial host–parasite interface. *Nature Commun*. 2020 Jul 30;11(1):1-0.

[108] Burda PC, Schaffner M, Kaiser G, Roques M, Zuber B, Heussler VT. A *Plasmodium* plasma membrane reporter reveals membrane dynamics by live-cell microscopy. *Sci Rep.* 2017 Aug 29;7(1):1-4.

[109] Montoro AG, Ungermann C. StARTing to understand membrane contact sites. Trends Cell Biology. 2015 Sep 1;25(9):497-8.

[110] Diehl M, Weber S, Cyrklaff M, Sanchez CP, Beretta CA, Roling L, Simon CS, Guizetti J, Mayer MP, Przyborski JM. Co-chaperone involvement in knob biogenesis implicates host-derived chaperones in malaria virulence. BioRxiv, doi:10.1101/2021.03.11.434953

[111] Behmer ST, Nes WD. Insect sterol nutrition and physiology: a global overview. *Adv. Insect Physiol*. 2003 Jan 1;31:1-72.

[112] Sterkel M, Oliveira JH, Bottino-Rojas V, Paiva-Silva GO, Oliveira PL. The dose makes the poison: nutritional overload determines the life traits of blood-feeding arthropods. *Trends Parasitol*. 2017 Aug 1;33(8):633-44.

[113] O'Neal AJ, Butler LR, Rolandelli A, Gilk SD, Pedra JH. Lipid hijacking: a unifying theme in vector-borne diseases. *Elife*. 2020 Oct 29; 9:e61675.

[114] Costa G, Gildenhard M, Eldering M, Lindquist RL, Hauser AE, Sauerwein R, Goosmann C, Brinkmann V, Carrillo-Bustamante P, Levashina EA. Non-competitive resource exploitation within mosquito shapes within-host malaria infectivity and virulence. *Nature Commun*. 2018 Aug 27;9(1):1-1.

[115] Werling K, Shaw WR, Itoe MA, Westervelt KA, Marcenac P, Paton DG, Peng D, Singh N, Smidler AL, South A, Deik AA. Steroid hormone function controls non-competitive *Plasmodium* development in *Anopheles*. *Cell*. 2019 Apr 4;177(2):315-25.

[116] Samuel BU, Mohandas N, Harrison T, McManus H, Rosse W, Reid M, Haldar K. The role of cholesterol and glycosylphosphatidylinositol-anchored proteins of erythrocyte rafts in regulating raft protein content and malarial infection. *J Biol Chem*. 2001 Aug 1;276(31):29319-29.

[117] Harvey JW. The erythrocyte: physiology, metabolism, and biochemical disorders. In: Clinical biochemistry of domestic animals 1997 Jan 1 (pp. 157-203). Academic Press.

[118] Benz EJ. Learning about genomics and disease from the anucleate human red blood cell. *Journal Clin Inv*. 2010 Dec 1;120(12):4204-6.

[119] Montigny C, Lyons J, Champeil P, Nissen P, Lenoir G. On the molecular mechanism of flippase-and scramblase-mediated phospholipid transport. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2016 Aug 1;1861(8):767-83.

[120] Arashiki N, Takakuwa Y. Maintenance and regulation of asymmetric phospholipid distribution in human erythrocyte membranes: implications for erythrocyte functions. *Curr Opin Hematol*. 2017 May 1; 24(3):167-72.

[121] Fraser M, Matuschewski K, Maier AG. Of membranes and malaria: phospholipid asymmetry in Plasmodium falciparum-infected red blood cells. Cellular and Molecular Life Sciences. 2021 Mar 13:1-7.

[122] Yalaoui S, Huby T, Franetich JF, Gego A, Rametti A, Moreau M, Collet X, Siau A, van Gemert GJ, Sauerwein RW, Luty AJ, Vaillant JC, Hannoun L, Chapman J, Mazier D, Froissard P. Scavenger receptor BI boosts hepatocyte permissiveness to Plasmodium infection. Cell Host Microbe. 2008 Sep

[123] Labaied M, Jayabalasingham B, Bano N, Cha SJ, Sandoval J, Guan G, Coppens I. Plasmodium salvages cholesterol internalized by LDL and synthesized de novo in the liver. Cellular microbiology. 2011 Apr; 13(4):569-86.

[124] Itoe MA, Sampaio JL, Cabal GG, Real E, Zuzarte-Luis V, March S,. et al. Host cell phosphatidylcholine is a key mediator of malaria parasite survival during liver stage infection. 2014 *Cell Host & Microbe*, **16**, 778–786.

[125] Kluck GE, Wendt CH, do Imperio GE, Araujo MF, Atella TC, da Rocha I, Miranda KR, Atella GC. Plasmodium infection induces dyslipidemia and a hepatic lipogenic state in the host through the inhibition of the AMPK-ACC pathway. Scientific reports. 2019 Oct 11;9(1):1-3.

[126] Petersen W, Stenzel W, Silvie O, Blanz J, Saftig P, Matuschewski K, Ingmundson A. Sequestration of cholesterol within the host late endocytic pathway restricts liver-stage *Plasmodium* development. *Mol Biol Cell*. 2017 Mar 15; 28(6):726-35.

[127] Balasubramanian L, Zuzarte-Luís V, Syed T, Mullick D, Deb S, Ranga-Prasad H, Meissner J, Almeida A, Furstenhaupt T, Siddiqi K, Prudêncio M. Association of Plasmodium berghei With the Apical Domain of Hepatocytes Is Necessary for the Parasite's Liver Stage Development. Frontiers in cellular and infection microbiology. 2020 Jan 17; 9:451.

[128] Tarun AS, Vaughan AM, Kappe SH. Redefining the role of de novo fatty acid synthesis in *Plasmodium* parasites. *Trends Parasitol.* 2009 Dec 1; 25(12):545-50.

[129] Wein S, Ghezal S, Buré C, Maynadier M, Périgaud C, Vial HJ, Lefebvre-Tournier I, Wengelnik K, Cerdan R. Contribution of the precursors and interplay of the pathways in the phospholipid metabolism of the malaria parasite. *J Lipid Res*. 2018 Aug 1; 59(8):1461-71.

[130] Miliara X, Tatsuta T, Berry JL, Rouse SL, Solak K, Chorev DS, Wu D, Robinson CV, Matthews S, Langer T. Structural determinants of lipid specificity within Ups/PRELI lipid transfer proteins. *Nature Commun*. 2019 Mar 8 ;10(1):1-5.

[131] Besteiro S, Bertrand-Michel J, Lebrun M, Vial H, Dubremetz JF. Lipidomic analysis of Toxoplasma gondii tachyzoites rhoptries: further insights into the role of cholesterol. *Biochem J*. 2008 Oct 1;415(1):87-96.

[132] Sam-Yellowe TY, Florens L, Wang T, Raine JD, Carucci DJ, Sinden R, Yates JR. Proteome analysis of rhoptry-enriched fractions isolated from *Plasmodium* merozoites. *J Proteome Res*. 2004 Oct 11; 3(5):995-1001.

[133] De Niz M, Burda PC, Kaiser G, Del Portillo HA, Spielmann T, Frischknecht F, Heussler VT. Progress in imaging methods: insights gained into *Plasmodium* biology. *Nature Rev Microbiol*. 2017 Jan;15(1):37-54.

[134] Knuepfer E, Napiorkowska M, Van Ooij C, Holder AA. Generating conditional gene knockouts in Plasmodium–a toolkit to produce stable DiCre recombinase-expressing parasite lines using CRISPR/Cas9. *Sci Rep*. 2017 Jun 20;7(1):1-2.

[135] Kilian N, Zhang Y, LaMonica L, Hooker G, Toomre D, Mamoun CB, Ernst AM. Palmitoylated Proteins in *Plasmodium falciparum*-Infected Erythrocytes: Investigation with Click Chemistry and Metabolic Labeling. *BioEssays*. 2020 Jun;42(6):1900145.

963 [136] Cobbold SA, V Tutor M, Frasse P, McHugh E, Karnthaler M, Creek DJ, Odom John A,
964 Tilley L, Ralph SA, McConville MJ. Non-canonical metabolic pathways in the malaria
965 parasite detected by isotope-tracing metabolomics. *Mol Syst Biol*. 2021 Apr;17(4): e10023.

966 [137] Fagone P, Jackowski S. Membrane phospholipid synthesis and endoplasmic reticulum
967 function. *J Lipid Res*. 2009 Jan 1;50: S311-6.

968 [138] Horvath SE, Daum G. Lipids of mitochondria. *Prog Lipid Res*. 2013 Oct 1;52(4):590-
969 614.

970 [139] Shears MJ, Botté CY, McFadden GI. Fatty acid metabolism in the *Plasmodium*
971 apicoplast: Drugs, doubts and knockouts. *Mol Biochem Parasitology*. 2015 Jan 1;199(1-
972 2):34-50.

973 [140] Carucci DJ, Witney AA, Muhia DK, Warhurst DC, Schaap P, Meima M, Li JL, Taylor
974 MC, Kelly JM, Baker DA. Guanylyl cyclase activity associated with putative bifunctional
975 integral membrane proteins in *Plasmodium falciparum*. *J Biol Chem*. 2000 Jul
976 21;275(29):22147-56.

977 [141] Hirai M, Arai M, Kawai S, Matsuoka H. PbGCβ is essential for *Plasmodium* ookinete
978 motility to invade midgut cell and for successful completion of parasite life cycle in
979 mosquitoes. *J Biochem*. 2006 Nov 1;140(5):747-57.

980 [142] Trottein F, Triglia T, Cowman AF. Molecular cloning of a gene from *Plasmodium*
981 *falciparum* that codes for a protein sharing motifs found in adhesive molecules from
982 mammals and plasmodia. *Mol Biochem Parasitol*. 1995 Nov 1;74(2):129-41.

983 [143] Edaye S, Georges E. Characterization of native PfABCG protein in *Plasmodium*
984 *falciparum*. *Biochem Pharmacol*. 2015 Sep 15;97(2):137-46.

985 [144] Martin RE, Henry RI, Abbey JL, Clements JD, Kirk K. The 'permeome' of the malaria
986 parasite: an overview of the membrane transport proteins of *Plasmodium falciparum*.
987 *Genome Biol*. 2005 Mar;6(3):1-22.

988 [145] Matesanz F, Md T, Alcina A. The *Plasmodium falciparum* fatty acyl-CoA synthetase
989 famly (PfACS) and differential stage-specific expression in infected erythrocytes. *Mol*
990 *Biochem Parasitol*. 2003 Jan 1;126(1):109-12.

991 [146] Haase, S, Condron M, Miller C, Cherkaoui D, Jordan S, Gulbis JM, Baum J.
992 Identification and characterisation of a phospholipid scramblase in the malaria parasite
993 *Plasmodium falciparum*. *Mol. Biochem. Parasitol*. 2021 May; 243:111374

994

995

Table 1. *Plasmodium falciparum* Genes Encoding Studied and Predicted Lipid Transfer Proteins

PlasmoDB Gene ID	Annotation	Domain Organisation	Function	Essentiality	Localisation	Refs
PF3D7_0719500	LEM3/CDC50a family protein, putative	CDC50/LEM3	Flippase	Essential	ER, Golgi, PPM, integral membrane component	[84]
PF3D7_1133300	LEM3/CDC50b family protein, putative	CDC50/LEM3	Flippase	Essential	ER, Golgi, PPM, integral membrane component	[84]
PF3D7_1029400	LEM3/CDC50c family protein, putative	CDC50/LEM3	Flippase	Essential	ER, Golgi, PPM, integral membrane component	[84]
PF3D7_0104200	StAR-related lipid transfer protein	One START domain with extended 80-aa C-term	Broad specificity phospholipid transfer protein	Essential	PV, integral membrane component	[61, 63]
PF3D7_1463500	Fam-A protein, displays similarity to a cholesterol transfer protein	One START-like Domain	*Plasmodium* specific lipid transfer	Dispensable	Apicoplast	
PF3D7_1351000	Phosphatidylinositol transfer protein alpha, putative	Multiple Lipid Transfer and Binding Domains PITP, OSBP, PH and START-like Domain	Phospholipid transporter, Oxysterol-binding	Dispensable	Cytosol and membrane	
PF3D7_0911100	Conserved protein, unknown function	One C-term START-like Domain	Phospholipid transporter, putative	Dispensable	unknown	
PF3D7_0807400	Coq10p – Coenzyme Q-binding protein, START domain	Coenzyme Q Binding domain and C-term START Domain	Ubiquinone/ Coenzyme Q binding	Essential	Mitochondrion	

Table 1. *Plasmodium falciparum* Genes Encoding Studied and Predicted Lipid Transfer Proteins

PF3D7_0629900	Sec14-like cytosolic factor or phosphatidylinositol/ phosphatidylcholine transfer protein, putative (PfSec14)	Sec14p-like lipid-binding domain, overlapping CRAL-TRIO lipid binding domain	Phosphatidyl inositol/phos phatidylcholi ne transfer protein, putative	Essential	Golgi, cytosol, predicted	
PF3D7_0626400	CRAL/TRIO domain-containing protein, putative	N-terminal CRAL-TRIO domain, Sec14 domain, Phage fibre protein domain	Phospholipid transfer protein, putative	Dispensable	Golgi, cytosol, predicted	
PF3D7_0920700	CRAL/TRIO domain-containing protein, putative	N-terminal CRAL-TRIO domain, Sec14 superfamily	Phospholipid transfer protein, putative	Essential	Golgi, cytosol, predicted	
PF3D7_1127600	CRAL/TRIO domain-containing protein, putative	N-terminal CRAL-TRIO domain, Sec14 superfamily	Phospholipid transfer protein, putative	Essential	Golgi, cytosol, predicted	
PF3D7_0107500	Niemann-Pick type C1-related protein	Ptc/Disp domain, Niemann-Pick C1 domain family, sterol-sensing domain	Cholesterol transfer	Essential	PPM	[106, 107]
PF3D7_0925900	Lipocalin	FA binding domain, VDE L domain, Nitrophorin domain, THAP4-like, domain	Fatty-acid and haem binding	Essential	PV, FV, Cytosol	
PF3D7_1138400	Guanylyl cyclase alpha (GCα)	P-type (phospholipid transporting) ATPase domain, Adenyl and Guanylate cyclase domain and histidinol-phosphate phosphatase domain	Multi-domain protein catalyst of cGMP biosynthesis, signal transduction, ATP binding, phospholipid transport	Essential	PPM	[140]

Table 1. *Plasmodium falciparum* Genes Encoding Studied and Predicted Lipid Transfer Proteins

			and translocation			
PF3D7_1360500	Guanylyl cyclase beta (GCβ)	Guanylate cyclase domain, C-terminal phospholipid translocating ATPase domain	Multi-domain protein catalyst of cGMP biosynthesis and phospholipid transporter	Dispensable (*P. berghei* orthologue essential for ookinete motility)	PPM, cytosol	[140, 141]
PF3D7_0319000	P-type ATPase, putative	N-terminal phospholipid ATPase domain, Metal cation-transporting ATPase domain, C-terminal phospholipid ATPase domain	phospholipid transporting ATPase (ATP11C)	Dispensable	Membrane	[74]
PF3D7_1223400	P-type ATPase, phospholipid-transporting ATPase, putative	P-type ATPase domain and phosphorylation site, C-terminal and N-terminal P-type ATPase A/IV superfamily, HAD-like domain	Phospholipid ATPase C/N, putative (Flippase)	Essential	Membrane	
PF3D7_1219600	P-type ATPase, phospholipid-transporting ATPase 2 (PfATPase2)	P-type ATPase domain, haloacid dehydrogenase (HAD) domain	ATPase-coupled intramembrane lipid transporter activity (Flippase)	Essential	Membrane	[76, 83]
PF3D7_1468600	P-type ATPase, aminophospholipid transporter, putative	HAD-like domain, P-ATPase domain	Flippase	Dispensable	PPM	
PF3D7_1426500	ABC transporter G family member 2 (PfABCG2)	Multi-pass membrane protein, AAA+ ATPase domain, ABC-2 type transporter domain, P-loop	Flippase	Dispensable	Membrane	[98, 99, 143]

Table 1. *Plasmodium falciparum* Genes Encoding Studied and Predicted Lipid Transfer Proteins

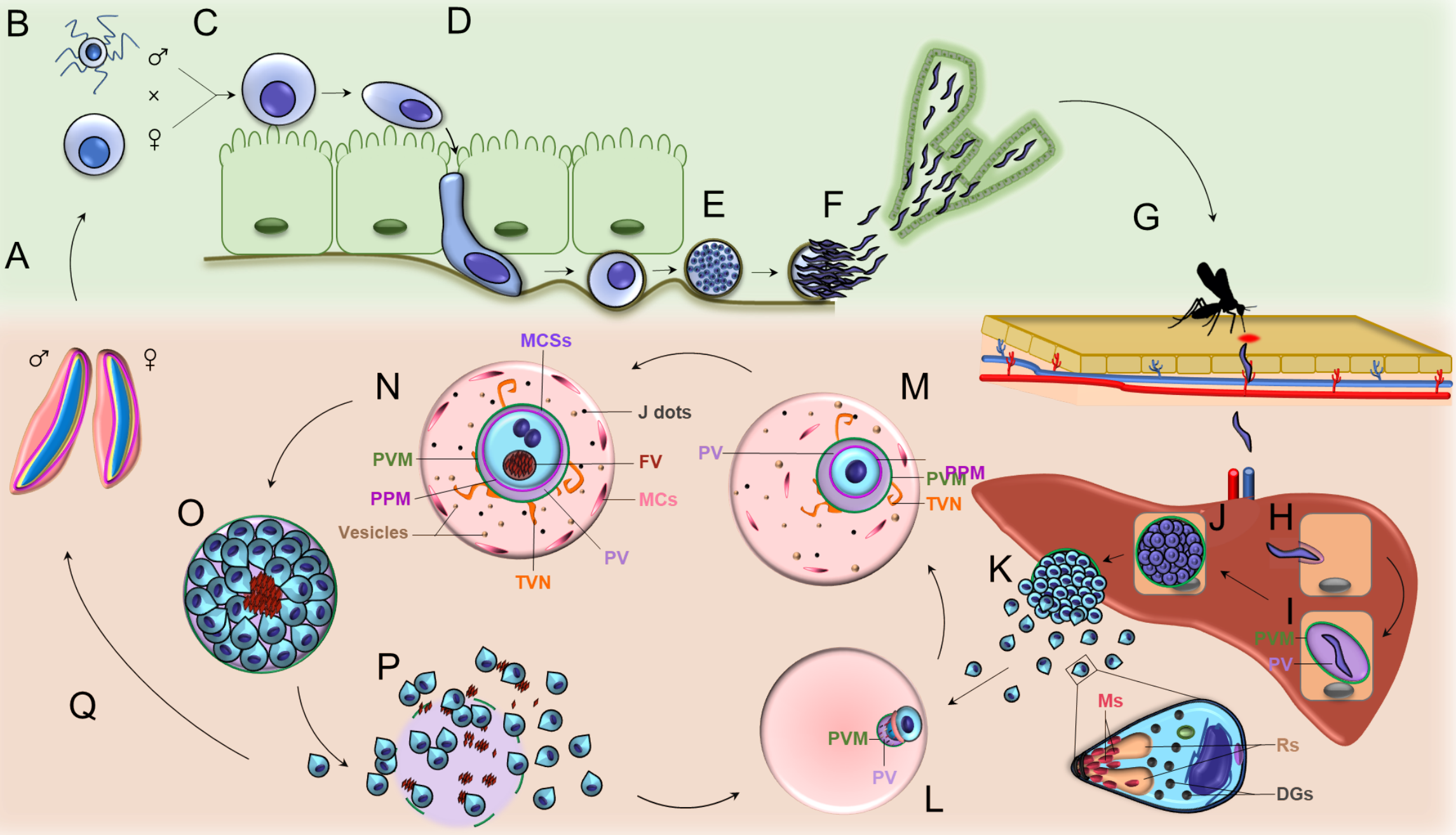
		NTPase-fold domain				
PF3D7_0319700	ABC transporter I family member 1, putative (PfABCI3)	AAA+ ATPase domain, ABC transporter-like domain, ABC transporter A domain, P-loop containing nucleoside triphosphate hydrolase domain	Lipid transporter activity (Flippase)	Dispensable	ER, putative	[74]
PF3D7_0813700	ABC transporter F family member 1 (PfABCF1)	Multi-pass membrane protein, AAA+ ATPase domain, ABC-type transporter domain, P-loop NTPase fold domain	ATPase-coupled transmembrane transporter activity (Flippase)	Essential	Apicoplast	
PF3D7_1464700	ATP synthase (C/AC39) subunit, putative	ATPase C domain, ATPase V0, V-type ATPase domain	Transmembrane transporter activity	Essential	Vacuoles, lysosomes	
PF3D7_1022700	Phospholipid scramblase, putative	Palmitoylated Ca^{2+}-activated scramblase domain with a PKC phosphorylation site and putative H3 and WW binding motifs	Phospholipid scramblase	Dispensable	PPM	[146]
PF3D7_0915800	Glycolipid transfer protein, putative	Glycolipid transfer protein domain	Ceramide transfer activity, intermembrane lipid transfer	Essential	PPM, Cytosol	
PF3D7_1131800	Oxysterol-binding protein, putative	Oxysterol-binding protein domain, PH-like domains	Sterol transport	Dispensable	Cytosol, intracellular	

Table 1. *Plasmodium falciparum* Genes Encoding Studied and Predicted Lipid Transfer Proteins

					membrane bound organelle	
PF3D7_1324400	PRELI domain-containing protein, putative	PRELI/MSF1 domain, Protein slowmo (Slmo) family.	Phospholipid and phosphatidic acid transport	Essential	Mitochondrion	
PF3D7_1011300	Protein ARV1, putative	Transmembrane domain-containing protein	Sterol transport	Essential	ER	
PF3D7_0104800	Novel putative transporter 1	MFS transporter, FMP42 protein domain	Putative glycerol-3-phosphate transporter	Essential	Membrane, putative cytosol	
PF3D7_1021700	VPS13 domain-containing protein, putative	VPS13/Chorein-N-terminal domain	Lipid transfer protein found at multiple MCSs, (presumed LTP and lipid-vesicle transfer roles)	Essential	Membrane	
PF3D7_0934700	UBX domain-containing protein, putative	Multidomain protein, UBX domain, DSBA-like thioredoxin domain, UAS domain of FAF1 proteins	Ubiquitin-regulatory protein, putative lipid droplet formation, putative sensor for long-chain unsaturated fatty acids	Dispensable	Membrane	
PF3D7_0215000	Fatty acyl-CoA synthetase	ANL, N-terminal domain, acyl-COA synthase 4 domain, AMP-dependent synthetase/ligase domain.	Long-chain fatty acid transport, putative FATP4	Dispensable	ER, membrane	

Table 1. *Plasmodium falciparum* Genes Encoding Studied and Predicted Lipid Transfer Proteins

PF3D7_0215300	Fatty acyl-CoA synthetase (PfACS)	AMP-binding domain, AMP-dependent synthetase/ligase, ANL, N-terminal domain	Long-chain fatty acid transport, long-chain fatty acid metabolic process	Dispensable	ER, membrane	[145]


A
B
C
D
E
F
G
♂
×
♀
H
I
J
K
L
M
N
O
P
Q
MCSs
J dots
PVM
FV
PPM
MCs
Vesicles
PV
TVN
PV
PPM
PVM
TVN
PVM
PV
Ms
Rs
DGs