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

2

3   Margarida Ressurreição† and Christiaan van Ooij†

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

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

6   Department of Infection Biology, London School of Hygiene & Tropical Medicine, Keppel

7   Street, London WC1E 7HT, United Kingdom

8

9   ABSTRACT

10   Eukaryotic unicellular pathogens from the genus *Plasmodium* are the etiological agents of

11   malaria, a disease that persists over a wide range of vertebrate species, including humans.

12   During its dynamic lifecycle, survival in the different hosts depends on the parasite's ability to

13   establish a suitable environmental milieu. To achieve this, specific host processes are exploited

14   to support optimal growth, including extensive modifications to the infected host cell. These

15   modifications include the formation of novel membranous structures, which are induced by the

16   parasite. Consequently, to maintain a finely tuned and dynamic lipid environment, the

17   organisation and distribution of lipids to different cell sites likely requires specialised lipid

18   transfer proteins (LTPs). Indeed, several parasite and host-derived LTPs have been identified

19   and shown to be essential at specific stages. Here we describe the roles of LTPs in parasite

20   development and adaptation to its host including how the latest studies are profiting from the

21   improved genetic, lipidomic and imaging toolkits available to study *Plasmodium* parasites.

22   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*

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

91

92 PHOSPHOLIPIDS IN *PLASMODIUM.*

93 Glycerophospholipids constitute the major lipid class detected in parasite fractions removed
94 from the erythrocyte, with phosphatidylcholine (PC) and phosphatidylethanolamine (PE)
95 together accounting for ~50% of total lipid content [22, 24, 55, 89]. These structural membrane
96 components primarily contain two saturated or monosaturated FA chains [22], consistent with
97 parasites requiring an exogenous supply of oleic and palmitic acid to sustain growth [21, 23,
98 24]. Lipids enriched in uninfected erythrocytes include phosphatidylserine (PS), phosphatidic
99 acid (PA) and ceramide. It is believed that the host cell may serve as a reservoir for parasite-
100 mediated lipid salvage, if required in response to changes in the extracellular host environment
101 [22, 24, 25, 26, 27, 55]. Mainly as *P. falciparum* has been shown to import several lipid species,
102 including ceramide, complex sphingolipids and lysophosphatidylcholine (LysoPC) [28, 29, 30,
103 31, 32, 90].

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

108 Lysophospholipids are minor constituents of cell membranes, present at less than 1% of total
109 parasite lipids. However, their levels change throughout parasite development, commensurate
110 with a putative role in intracellular signalling to regulate varied processes, including cell
111 signaling, protein folding and the mobilisation of intracellular Ca^{2+} stores [37, 38, 39, 40, 41,
112 42].

113 Cholesterol plays an important role in regulating the properties of phospholipid membranes,
114 including the regulation of membrane organisation [43, 46]. Addition of cholesterol induces a
115 condensation effect where the area per lipid decreases, leading to increased membrane stiffness
116 [42, 43]. Cholesterol levels also affect raft structures [43, 46, 47], membrane trafficking and
117 sorting functions that may support *P. falciparum* survival [48, 49, 50, 51]. Interestingly, a
118 gradient of cholesterol is present in erythrocytes infected with *Plasmodium* parasites [52, 53].
119 Experiments using a cholesterol-sensitive fluorophore revealed that membrane cholesterol

120   levels in parasitised erythrocytes decrease inwardly from the erythrocyte plasma membrane

121   (EPM), MCs/TVN, PVM and finally to the PPM [53]. Fluorescence Lifetime Imagining

122   Microscopy (FLIM) showed little or no difference in this cholesterol gradient between

123   parasitized HbAA erythrocytes vs HbS erythrocytes that differ in lipid content, suggesting that

124   malaria parasites may regulate the cholesterol contents of the PVM and PM independently of

125   levels in the host cell membrane, especially after invasion [54]. Lipid and cholesterol exchange

126   data suggest that the cholesterol gradient involves a dilution effect from non-sterol lipids

127   produced by the parasite and that the parasite actively maintains a level of low cholesterol [52,

128   53]. Furthermore, increased membrane cholesterol decreases the temperature required for the

129   plasma membrane to maintain its liquid phase and is directly related to temperature-dependent

130   changes to the cell membrane [43, 44, 45]. Interestingly, during gametocytogenesis, cholesterol

131   levels in the parasite increase significantly [22, 54]. This is believed to be important for various

132   reasons, including the production of internal reserves for further development after uptake by

133   the mosquito, which also cannot produce cholesterol *de novo* [22, 30, 54, 112, 113, 114].

134

135   LIPID TRANSFER PROTEINS

136   Because the ER, mitochondrion and the apicoplast are involved in the *de novo* synthesis of

137   lipids in *Plasmodium* parasites, a system must exist to transport these newly synthesised lipids

138   to other organelles, membranes, membranous compartments, and the aqueous environment of

139   the host cytoplasm [138, 139, 140]. As LTPs are candidates for the development of such

140   processes, these proteins have become attractive in understanding the role and importance of

141   lipid dynamics in cells infected by *Plasmodium* parasites. The parasite produces many different

142   LTPs, some with standard functions shared with other eukaryotes and others that have been

143   adapted for parasite-specific purposes (**Table 1**).

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

145   *Plasmodium* parasites encode five proteins with annotated START domains, usually ~210

146   amino acids long, involved in the binding and non-vesicular transport of hydrophobic

147   molecules, including lipids and cholesterol [56]. These five proteins are PF3D7_1351000

148   (MAL13P1.256),   PF3D7_0911100   (PFI0540w),   PF3D7_0807400   (MAL8P1.300),

149   PF3D7_01004200 (PFA0210c) and PF3D7_1463500 (PF14_0604). Of these, three have not

150   yet been investigated: PF3D7_1351000 (MAL13P1.256), the phosphatidylinositol (PI) transfer

151   protein orthologue; PF3D7_0911100 (PFI0540w), annotated as a conserved protein of

152 unknown function with a predicted C-terminal START-like domain; and PF3D7_0807400

153 (MAL8P1.300), a multi-domain protein with a predicted START domain and a Coenzyme Q

154 (CoQ or ubiquinone) binding (Coq10p) domain. The PI transfer protein PF3D7_1351000

155 (MAL13P1.256) is likely to perform functions similarly to those in other eukaryotes, whereas

156 PF3D7_0911100 (PFI0540w) is unique to *Plasmodium* spp. and *Hepatocystis* (a related

157 apicomplexan transmitted by midges that skips intraerythrocytic asexual replication stages,

158 entering directly into gametocytogenesis) [57, 58]. PF3D7_0911100 (PFI0540w) is expressed

159 at various stages during the entire lifecycle but appears dispensable for intraerythrocytic

160 parasite growth. Orthologues of PF3D7_0807400 (MAL8P1.300) are usually associated with

161 mitochondria and have a role in respiratory electron transport and ATP synthesis [59, 60].

162 Interestingly, the Coq10 START domain protein from *Saccharomyces cerevisiae* plays a

163 protective role against fatty acid-induced oxidative stress along with its role in ubiquinone

164 synthesis [59, 60]. PF3D7_0104200 (PFA0210c) and PF3D7_1463500 (PF14_0604) appear to

165 be unique to the genera *Plasmodium* and *Hepatocystis* and may perform lipid transfer functions

166 specific to these parasites. PF3D7_0104200 (PFA0210c) is a broad-specificity phospholipid

167 transfer protein capable of transferring PC, PE, PI, PS and SM *in vitro* [61]. Original sequence-

168 based similarity searches indicated that it is most similar to the human protein STARD7, a PC

169 transfer protein, while structurally it is most similar to phosphatidylcholine transfer protein

170 STARD2. The protein has been detected in the PVM and host erythrocyte when overexpressed

171 [61, 62, 63]. Genetic experiments have shown that PF3D7_0104200 (PFA0210c) and its

172 orthologue in *P. knowlesi* are essential for parasite survival [63]. Intriguingly, during asexual

173 intraerythrocytic development, PF3D7_0104200 (PFA0210c) protein synthesis initiates at

174 entry into the trophozoite stage and protein levels drastically increase with trophozoite growth,

175 when extension of the PPM and PVM occurs [61, 63]. This may point to a function for

176 PF3D7_0104200 (PFA0210c) in the delivery of phospholipids to the PVM to support its

177 expansion. One interesting aspect of PF3D7_0104200 (PFA0210c) is the unusual C-terminal

178 extension of approximately 84 amino acids, something that is rarely detected in START

179 domain-containing proteins [62]. Removal of the C-terminal 19 residues significantly increases

180 lipid transfer activity *in vitro*, indicating a regulatory function of the C-terminal region.

181 Attempts to remove the last 20 codons of the *P. knowlesi* orthologue of PF3D7_0104200

182 (PFA0210c) were unsuccessful, indicating that this regulatory function may be essential *in vivo*

183 [63]. Interestingly, a recent study has revealed IgG against PF3D7_0104200 (PFA0210c)

184 epitopes in the blood from individuals with symptomatic and even asymptomatic malaria,

185 indicating antibodies against this protein could form a biomarker for malaria, especially since

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

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

204

205   <u>Sec14/CRAL-TRIO-like lipid transfer proteins</u>

206   Sec14 proteins have a characteristic SEC14 domain, also known as CRAL-TRIO domain. They
207   can function as PC sensors and as inducers of PI synthesis and transfer, transmitting PC
208   metabolic information to PI synthesis via PI transfer proteins (PITPs) [69]. Other members of
209   the Sec14 family are the alpha-tocopherol transfer proteins (alpha-TTPs) which facilitate the
210   transfer of alpha-tocopherol (alpha-T), a form of vitamin E, to secretory lipoproteins [70], and
211   the phosphatidylinositol transfer proteins (PITPs), key regulators of phosphoinositide signaling
212   [71, 72]. Interestingly, soluble versions of PITPs activate inositol lipid kinases, promoting
213   diversification and dynamics of phosphoinositide signaling [71, 72]. Therefore, these proteins
214   can transport substrates, including alpha-tocopherol, PI or PC, between different intracellular
215   membranes [69, 71, 72]. The *Plasmodium* spp. group of Sec14-like proteins define a novel
216   class of multi-domain proteins with both haem-binding and PI transfer activity [72].
217   *Plasmodium* spp. encode four Sec14/CRAL-TRIO-like proteins: PF3D7_0626400

218 (PFF1280w), PF3D7_0629900 (PFF1450w) and PF3D7_1127600 (PF11_0287) and

219 PF3D7_0920700 (PFI1015w). Although these proteins have not yet been characterised,

220 PFI1015w and PFF1450w are predicted to be essential (**Table 1**). Interestingly, the *P.*

221 *falciparum* phosphatidylinositol 3-kinase (PfPI3K), which localises to the food vacuole in

222 trophozoites and in vesicular compartments at the PPM/PVM, may interact with PfSec14

223 proteins [73]. PfSec14 proteins and PfPI3K may play key roles in haemoglobin intake, as

224 pharmacological inhibition of PfPI3K activity compromises haemoglobin endocytosis [73].

225

226 Lipid ATPases (flippases) from the P4 subfamily

227 Recent gene targeting approaches have uncovered an important role for the *P. falciparum*

228 P4-ATPase subfamily, also known as lipid flippases, proteins able to actively translocate lipids

229 from one membrane leaflet to the other, helping to generate lipid asymmetry [74, 75, 76, 77,

230 90, 119]. P4-ATPases maintain the asymmetric distribution of phospholipids in membranes by

231 translocating phospholipids (most commonly PE, PS and PC) from the extracellular leaflet to

232 the inner cytoplasmic leaflet [77]. *P. falciparum* is predicted to encode the four P4-ATPases:

233 PfATP2, PfATP7, PfATP8 and PfATP11 [78, 79]. Whereas the first three are conserved in all

234 *Plasmodium* species, PfATP11 is absent in *P. berghei* [80]. While the essential role of ATP7

235 and ATP8 remains unclear [74, 81], most ATP7-depleted ookinetes fail to internalise and

236 translocate PC across the plasma membrane, resulting in a failure to develop [74]. This appears

237 to result from an inability to initiate microneme secretion and a reduction of parasite survival

238 to environmental stress, leading to elimination of ookinetes during traversal of the midgut

239 epithelium [74, 75]. PfATP2 and its orthologue in *P. berghei* are essential during the

240 intraerythrocytic stages [76, 81]. *P. berghei* ATP2 potentially localises to the PPM and the

241 PVM during the erythrocytic stage [82]; higher resolution imaging or fractionation analysis

242 will be required to fully ascertain its localisation. Interestingly, duplication of the gene

243 encoding PfATP2 was associated with resistance to two antimalarial compounds,

244 MMV007224 (2-N,3-N-Bis(4-bromophenyl)quinoxaline-2,3-diamine) and MMV665852 (1,3-

245 Bis(3,4-dichlorophenyl)urea) [83], indicating that PfATP2 is either responsible for decreasing

246 the concentration of the compounds or the direct target of the compounds in the parasite. Most

247 P4-ATPases form heterodimeric complexes with members of the Cdc50/LEM3 protein family

248 and this association appears to be essential for their activity [76, 85]. *P. falciparum* encodes

249 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

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

288

289 ATP binding cassette (ABC) transporters (flippases)

290 The *P. falciparum* genome encodes multiple members of the family of ATP binding cassette
291 (ABC) transporters. One of these, ABCG2 (PF3D7_1426500), has previously been implicated
292 in the transport of PS and PC analogues in eukaryotic cells *in vitro*, while in *Plasmodium*
293 parasites it is suspected to work as a lipid transporter with a specific role in lipid storage [98,
294 99]. In *P. berghei* ABCG2 is predominantly associated with the plasma membrane of female
295 gametocytes and ookinetes [99]. In *P. falciparum,* this protein is produced predominantly in
296 the gametocyte stage, where it is found in a more distinct localisation, a single dot-like lipid-
297 rich structure within female, but not male, gametocytes [99, 100]. Interestingly, in both species
298 ABCG2 mutant parasites produce more gametocytes of both sexes and the level of cholesteryl
299 esters, diacylglycerols and triacylglycerols are significantly reduced in gametocytes [99, 100].
300 Indicating a role for ABCG2 in control of gametocyte numbers and in the accumulation of
301 neutral lipids, potentially important for parasite development in the insect stages since neutral
302 lipids can function as energy storage and are precursors for metabolic activity, scarcely found
303 in the mosquito microenvironment [111, 112, 113, 114, 115]. Several reports investigating lipid
304 species during gametocytogenesis [22, 24, 89, 90, 91, 922], found that the levels of regulators
305 of membrane fluidity, in particular cholesterol and SM, increase significantly during
306 gametocyte maturation [22, 24]. Neutral lipids, serving mainly as energy reserves, increase
307 from 3% of total lipids in uninfected erythrocytes to 27% in stage V gametocyte-infected
308 erythrocytes [24]. ABC transporters that function as flippases are poorly conserved across the
309 eukaryotic kingdom and hence could be suitable anti-malarial drug targets [80]. Interestingly,
310 a putative *P. falciparum* ABC phospholipid transporter ATPase, PfABCF1 (PF3D7_0813700)
311 is predicted to be essential (**Table 1**). However, it remains to be understood how ABC
312 transporters function throughout the parasite lifecycle as LTPs.

313

314 MULTIDOMAIN TRANSPORTERS (LIPIDS AND HAEM)

<u>Lipocalin</u>

316 Lipocalins bind to small hydrophobic molecules and can transport lipids and fatty acids and

317 hypothesised to share an early evolutionary origin with the bacterial kingdom, followed by

318 extreme divergence of the amino acid sequence [101]. Recently, a lipocalin PfLCN

319 (PF3D7_0925900) with domains predicted to bind to both fatty acids and haem was identified

320 in *P. falciparum*. It was found to localise to the PV and food vacuole (FV) of intraerythrocytic

321 parasites and to integrate into membranes, while in free merozoites was found mainly in the

322 cytosol [102]. Furthermore, gene inactivation revealed its importance during the

323 intraerythrocytic stage, mutant parasites undergo abnormal schizogony with a significantly

324 reduced number of nuclei with some disorganisation compared to wildtype parasites [102].

325 Furthermore, the mutant showed defects in haemozoin crystal motility, either indicating defects

326 in the FV or a direct interaction or binding function to haemozoin. Structure predictions

327 indicate that PfLCN is similar to the *Escherichia coli* Blc protein, which functions in the

328 storage and transport of lipids necessary for membrane repair [103], and the human lipocalin

329 NGAL which is a palmitate and fatty acid transport protein [104, 105].

330

331 <u>CHOLESTEROL TRANSPORTERS</u>

332 <u>Niemann-Pick C1-related protein and cholesterol homeostasis</u>

333 *P. falciparum* encodes a Nieman-Pick C1-related protein PfNCR1 (PF3D7_0107500), part of

334 a family of proteins that has been shown to have a function in the transport of cholesterol in

335 lysosomes [106]. This protein localises to the PPM and to Membrane Contact Sites (MCSs)

336 linking the PPM with the PVM [106, 107]. Although its cholesterol transfer function remains

337 to be experimentally confirmed, disruption of PfNCR1 made intraerythrocytic parasites

338 susceptible to saponin, likely owing to an increased level of cholesterol in the PPM. In addition,

339 mutant parasites showed severe alterations in the FV, indicating a role for PfNCR1 in the

340 maintenance or biogenesis of this vacuole [107]. These findings suggest that PfNCR1 is

341 important in the regulation and maintenance of the low cholesterol levels at the PPM and may

342 be important for maintaining the previously observed cholesterol gradient in intraerythrocytic

343 stages (from high levels at the erythrocyte plasma membrane to lower levels at the PVM and

344 even lower at the PPM) [53]. A combination of cryo-EM and correlative immunofluorescence

345 revealed that PfNCR1 is localised at MCSs, sites between the PPM and the PVM, that could

346 potentially directly connect the PPM with the PVM [107]. Interestingly, this also revealed a

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

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

377

378 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

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

412 Hepatocyte LTPs

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

430

431 OUTSTANDING QUESTIONS

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

442 cholesterol remains enigmatic. There is a dramatic gradient of cholesterol in the infected

443 erythrocyte, but how this is set up and maintained remains unclear. The parasite cannot

444 synthesise cholesterol and hence the cholesterol gradient must be distributed from the outside

445 inwards. Further investigation of PfNPC1 and PFA0660w, and J dots in general, are likely to

446 shed light on this process. Another lipid that is important in the transition between different

447 lifecycle stages is LysoPC; a decrease in LysoPC levels induces the transition from asexual to

448 sexual stages. The uptake of LysoPC is likely to be an active process and may involve a

449 parasite-derived transporter [89, 129]. Therefore, the biochemical and genetic characterisation

450 of the transport and metabolism of LysoPC and other phospholipid precursors should be

451 pursued. Many lipid transfer proteins remain unstudied, even those that have an essential

452 function, including PF3D7_0920700, a predicted Sec14/CRAL/TRIO phospholipid transfer

453 protein, and PF3D7_1324400, a PRELI domain-containing protein predicted to be involved in

454 phospholipid and phosphatidic acid transport (**Table 1**). PRELI family members are known to

455 regulate lipid accumulation in organelles by shuttling phospholipids [130]. Interestingly, while

456 the lipid content of rhoptries in *T. gondii* has been investigated, so far, no studies have emerged

457 with regards to the lipid contents of *Plasmodium* parasites, and only proteomic analyses have

458 been performed [131, 132]. Such studies and proteomic analyses to identify lipids and LTPs

459 associated with invasion organelles would be of great interest, especially to understand the

460 specific invasion mechanisms of the parasite. Genetic and biochemical investigation of these

461 proteins is likely to uncover fascinating new interactions between the parasite and the host and

462 transfer pathways within the host.

463   Investigating these and other outstanding questions will be greatly aided by advances

464 in research technologies to manipulate *Plasmodium* parasites [61, 102, 107, 108, 133, 134, 135,

465 136]. Inducible gene deletion, protein disruption and mRNA stability techniques have already

466 proven to be indispensable to the investigation of many essential proteins and will undoubtedly

467 be of great use in furthering our understanding of the proteins involved in the transfer of lipids

468 in the parasite and in the host cell. Fluorescence Recovery After Photobleaching (FRAP) and

469 fluorescent timer experiments will provide insight into the dynamics of LTPs in live cells and

470 their directionality. In addition, cryoEM has already proven to move the boundaries of the

471 detection of the fine structure of the parasite. As an example, the discovery of the MCSs

472 connecting the PPM and the PVM will likely prove to be a pivotal moment in our understanding

473 of the formation, expansion and maintenance of the PVM [107]. With these and other new

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

476

479

480 **CRediT author statement**

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

484

485 **Keywords and abbreviations**

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

489

490 **Table 1. Studied and predicted *Plasmodium falciparum* lipid transfer proteins**
491 When proteins have been previously studied references have been indicated. If no references
492 are indicated, then these are predicted gene products. Both Gene ID and predicted protein
493 sequence were retrieved from plasmodb.org. Domain organisation and predicted function were
494 obtained when available from uniprot.org and ebi.ac.uk/interpro. In certain cases, the predicted
495 gene products were mined using specific GO searches at ebi.ac.uk, using the *Plasmodium*
496 *falciparum* (isolate 3D7) Uniprot Taxon Identifier (UTI): 36329. Specific GO codes and
497 functions searched are described at the end of this legend. Essentiality data were obtained from
498 plasmodb.org and cross-referenced from the supplementary data provided by Zhang and
499 colleagues [75]. Localisation was either obtained from published studies, predicted by
500 uniprot.org or assumed due to the domain content of the respective gene product (i.e. presence
501 or absence of transmembrane domains).
502 The following GO searches with included the qualifier 'enables' and excluded 'involved in'.
503 The following GO searches were used: GO:0006869 'lipid transport', GO:0034040 'ATPase-
504 coupled lipid transmembrane transporter activity', GO:0005319 'lipid transporter activity',
505 GO:0120020 'cholesterol transfer activity', GO:0046624 'sphingolipid transporter activity',
506 GO:0035627 'ceramide transport', GO:0043691 'reverse cholesterol transport', GO:0017089
507 'glycolipid transfer activity', GO:0035621 'ER to Golgi ceramide transport', GO:0008526
508 'phosphatidylinositol transfer activity', GO:0120019 'phosphatidylcholine transfer activity',
509 GO:0120014 'phospholipid transfer activity', GO:1904121 'phosphatidylethanolamine
510 transfer activity', GO:0005548 'phospholipid transporter activity', GO:0140337
511 'diacylglyceride transfer activity', GO:0140340 'cerebroside transfer activity', GO:0140339
512 'phosphatidylglycerol transfer activity', GO:0140338 'sphingomyelin transfer activity',
513 GO:1990050 'phosphatidic acid transfer activity', GO:0046836 'glycolipid transport',
514 GO:0140327 'flippase activity', GO:0030301 'cholesterol transport', GO:0015914

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

517

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

555

556

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.

629 [28] Sah RK, Pati S, Saini M, Singh S. Erythrocyte sphingosine kinase regulates
630 intraerythrocytic development of Plasmodium falciparum. Scientific reports. 2021 Jan 13;
631 11(1):1-6.

632 [29] Sah RK, Pati S, Saini M, Boopathi PA, Kochar SK, Kochar DK, Das A, Singh S.
633 Reduction of sphingosine kinase 1 phosphorylation and activity in plasmodium-infected
634 erythrocytes. Frontiers in cell and developmental biology. 2020 Mar 3; 8:80.

635 [30] Vial HJ, Ben Mamoun C. Plasmodium lipids: metabolism and function. Molecular
636 approaches to malaria. 2005 Sep; 1:327-52.

637 [31] Déchamps S, Shastri S, Wengelnik K, Vial HJ. Glycerophospholipid acquisition in
638 Plasmodium–a puzzling assembly of biosynthetic pathways. International journal for
639 parasitology. 2010 Oct 1; 40(12):1347-65.

640 [32] Asahi H, Kanazawa T, Hirayama N, Kajihara Y. Investigating serum factors promoting
641 erythrocytic growth of Plasmodium falciparum. Experimental parasitology. 2005 Jan 1;
642 109(1):7-15.

643 [33] Gerold P, Schwarz RT. Biosynthesis of glycosphingolipids de-novo by the human
644 malaria parasite Plasmodium falciparum. Molecular and biochemical parasitology. 2001 Jan
645 15; 112(1):29-37.

646 [34] Aerts JM, Artola M, Van Eijk M, Ferraz MJ, Boot RG. Glycosphingolipids and
647 infection. Potential new therapeutic avenues. Frontiers in cell and developmental biology.
648 2019 Dec 6 ;7:324.

649 [35] Tewari SG, Swift RP, Reifman J, Prigge ST, Wallqvist A. Metabolic alterations in the
650 erythrocyte during blood-stage development of the malaria parasite. Malaria journal. 2020
651 Dec; 19(1):1-8.

652 [36] MacRae JI, Lopaticki S, Maier AG, Rupasinghe T, Nahid A, Cowman AF, McConville
653 MJ. P lasmodium falciparum is dependent on de novo myo-inositol biosynthesis for assembly
654 of GPI glycolipids and infectivity. Molecular microbiology. 2014 Feb; 91(4):762-76.

655 [37] Botté CY, Yamaryo-Botté Y, Rupasinghe TW, Mullin KA, MacRae JI, Spurck TP,
656 Kalanon M, Shears MJ, Coppel RL, Crellin PK, Maréchal E. Atypical lipid composition in
657 the purified relict plastid (apicoplast) of malaria parasites. Proceedings of the National
658 Academy of Sciences. 2013 Apr 30; 110(18):7506-11.

659 [38] Dykstra M, Cherukuri A, Sohn HW, Tzeng SJ, Pierce SK. Location is everything: lipid
660 rafts and immune cell signaling. Annual review of immunology. 2003 Apr; 21(1):457-81.

661 [39] Lonez C, Vandenbranden M, Ruysschaert JM. Cationic lipids activate intracellular
662 signaling pathways. Advanced drug delivery reviews. 2012 Dec 1; 64(15):1749-58.

663 [40] Lloyd-Evans E, Waller-Evans H. Lysosomal Ca2+ homeostasis and signaling in health
664 and disease. Cold Spring Harbor perspectives in biology. 2020 Jun 1; 12(6):a035311.

665

666 [41] Okazaki Y, Saito K. Roles of lipids as signaling molecules and mitigators during stress
667 response in plants. The Plant Journal. 2014 Aug; 79(4):584-96.

668 [42] Nguyen A, Rudge SA, Zhang Q, Wakelam MJ. Using lipidomics analysis to determine
669 signalling and metabolic changes in cells. Current opinion in biotechnology. 2017 Feb 1;43:
670 96-103.

671 [43] de Meyer F, Smit B. Effect of cholesterol on the structure of a phospholipid bilayer.
672 Proceedings of the National Academy of Sciences. 2009 Mar 10; 106(10):3654-8.

673 [44] Zhang Y, Li Q, Dong M, Han X. Effect of cholesterol on the fluidity of supported lipid
674 bilayers. Colloids and Surfaces B: Biointerfaces. 2020 Dec 1; 196:111353.

675 [45] Somjid S, Krongsuk S, Johns JR. Cholesterol concentration effect on the bilayer
676 properties and phase formation of niosome bilayers: a molecular dynamics simulation study.
677 Journal of Molecular Liquids. 2018 Apr 15; 256:591-8.

678 [46] Clair JW, London E. Effect of sterol structure on ordered membrane domain (raft)
679 stability in symmetric and asymmetric vesicles. Biochimica et Biophysica Acta (BBA)-
680 Biomembranes. 2019 Jun 1;1861(6):1112-22.

681 [47] Cho YY, Kwon OH, Park MK, Kim TW, Chung S. Elevated cellular cholesterol in
682 Familial Alzheimer's presenilin 1 mutation is associated with lipid raft localization of β-
683 amyloid precursor protein. PLoS One. 2019 Jan 25; 14(1):e0210535.

684 [48] Thériault C, Richard D. Characterization of a putative Plasmodium falciparum SAC1
685 phosphoinositide-phosphatase homologue potentially required for survival during the asexual
686 erythrocytic stages. Scientific reports. 2017 Oct 5;7(1):1-9.

687 [49] Tewari SG, Rajaram K, Swift RP, Reifman J, Prigge ST, Wallqvist A. Metabolic
688 Survival Adaptations of Plasmodium falciparum Exposed to Sublethal Doses of
689 Fosmidomycin. Antimicrobial Agents and Chemotherapy. 2021 Mar 18;65(4).

690 [50] Florentin A, Cobb DW, Kudyba HM, Muralidharan V. Directing traffic: Chaperone-
691 mediated protein transport in malaria parasites. Cellular microbiology. 2020
692 Jul;22(7):e13215.

693 [51] Counihan NA, Modak JK, Koning-Ward D, Tania F. How malaria parasites acquire
694 nutrients from their host. Frontiers in Cell and Developmental Biology. 2021; 9:582.

695 [52] Hayakawa EH, Yamaguchi K, Mori M, Nardone G. Real-time cholesterol sorting in
696 Plasmodium falciparum-erythrocytes as revealed by 3D label-free imaging. Scientific reports.
697 2020 Feb 17;10(1):1-2.

698 [53] Tokumasu F, Crivat G, Ackerman H, Hwang J, Wellems TE. Inward cholesterol gradient
699 of the membrane system in P. falciparum-infected erythrocytes involves a dilution effect
700 from parasite-produced lipids. Biology open. 2014 Jun 15;3(6):529-41.

701 [54] Fratini F, Raggi C, Sferra G, Birago C, Sansone A, Grasso F, Currà C, Olivieri A, Pace
702 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

740 [65] Pati P, Dhangadamajhi G, Bal M, Ranjit M. High proportions of pfhrp2 gene deletion
741 and performance of HRP2-based rapid diagnostic test in Plasmodium falciparum field
742 isolates of Odisha. Malaria journal. 2018 Dec;17(1):1-1.

743 [66] Howick VM, Russell AJC, Andrews T, Heaton H, Reid AJ, Natarajan K, Butungi H,
744 Metcalf T, Verzier LH, Rayner JC, Berriman M, Herren JK, Billker O, Hemberg M, Talman
745 AM, Lawniczak MKN. The Malaria Cell Atlas: Single parasite transcriptomes across the
746 complete *Plasmodium* life cycle. Science. 2019 Aug 23;365(6455):eaaw2619

747 [67] Frech C, Chen N. Variant surface antigens of malaria parasites: functional and
748 evolutionary insights from comparative gene family classification and analysis. BMC
749 Genomics. 2013 Jun 27;14:427. doi: 10.1186/1471-2164-14-427.

750 [68] Fougère A, Jackson AP, Bechtsi DP, Braks JA, Annoura T, Fonager J, Spaccapelo R,
751 Ramesar J, Chevalley-Maurel S, Klop O, van der Laan AM, Tanke HJ, Kocken CH, Pasini
752 EM, Khan SM, Böhme U, van Ooij C, Otto TD, Janse CJ, Franke-Fayard B. Variant
753 Exported Blood-Stage Proteins Encoded by Plasmodium Multigene Families Are Expressed
754 in Liver Stages Where They Are Exported into the Parasitophorous Vacuole. PLoS Pathog.
755 2016 Nov 16;12(11):e1005917. doi: 10.1371/journal.ppat.1005917. Erratum in: PLoS
756 Pathog. 2016 Dec 14;12 (12):e1006107. Erratum in: PLoS Pathog. 2017 Jan
757 17;13(1):e1006128.

758 [69] Huang J, Ghosh R, Tripathi A, Lönnfors M, Somerharju P, Bankaitis VA. Two-ligand
759 priming mechanism for potentiated phosphoinositide synthesis is an evolutionarily conserved
760 feature of Sec14-like phosphatidylinositol and phosphatidylcholine exchange proteins.
761 Molecular biology of the cell. 2016 Jul 15;27(14):2317-30.

762 [70] Lim Y, Traber MG. Alpha-tocopherol transfer protein (α-TTP): Insights from alpha-
763 tocopherol transfer protein knockout mice. Nutrition research and practice. 2007 Dec
764 1;1(4):247-53.

765 [71] Lete MG, Tripathi A, Chandran V, Bankaitis VA, McDermott MI. Lipid transfer
766 proteins and instructive regulation of lipid kinase activities: Implications for inositol lipid
767 signaling and disease. Advances in Biological Regulation. 2020 Jul 14:100740.[71] Montag
768 K, Hornbergs J, Ivanov R, Bauer P. Phylogenetic analysis of plant multi-domain SEC14-like
769 phosphatidylinositol transfer proteins and structure–function properties of PATELLIN2.
770 *Plant Mol Biol*. 2020 Dec;104(6):665-78.

771 [72] Khan D, Lee D, Gulten G, Aggarwal A, Wofford J, Krieger I, Tripathi A, Patrick JW,
772 Eckert DM, Laganowsky A, Sacchettini J, Lindahl P, Bankaitis VA. A Sec14-like
773 phosphatidylinositol transfer protein paralog defines a novel class of heme-binding proteins.
774 *Elife*. 2020 Aug 11;9:e57081.

775 [73] Vaid A, Ranjan R, Smythe WA, Hoppe HC, Sharma P. PfPI3K, a phosphatidylinositol-3
776 kinase from *Plasmodium falciparum*, is exported to the host erythrocyte and is involved in
777 hemoglobin trafficking. *Blood*. 2010 Mar 25;115(12):2500-7.

778   [74] Kenthirapalan S, Waters AP, Matuschewski K, Kooij TW. Functional profiles of orphan
779   membrane transporters in the life cycle of the malaria parasite. *Nature Commun*. 2016 Jan
780   22;7(1):1-0.

781   [75] Zhang M, Wang C, Otto TD, Oberstaller J, Liao X, Adapa SR, Udenze K, Bronner IF,
782   Casandra D, Mayho M, Brown J. Uncovering the essential genes of the human malaria
783   parasite *Plasmodium falciparum* by saturation mutagenesis. *Science*. 2018 May 4;360(6388).

784   [76] Lamy A, Macarini-Bruzaferro E, Dieudonné T, Perálvarez-Marín A, Lenoir G,
785   Montigny C, Le Maire M, Vázquez-Ibar JL. ATP2, the essential P4-ATPase of malaria
786   parasites, catalyzes lipid-stimulated ATP hydrolysis in complex with a Cdc50 β-subunit.
787   *Emerg Microbes Infect*. 2020 Dec 30:1-32.

788   [77] Andersen JP, Vestergaard AL, Mikkelsen SA, Mogensen LS, Chalat M, Molday RS. P4-
789   ATPases as phospholipid flippases - structure, function, and enigmas. *Front Physiol*. 2016 Jul
790   8; 7:275.

791   [78] Krishna S, Webb R, Woodrow C. Transport proteins of Plasmodium falciparum:
792   defining the limits of metabolism. *Int J Parasitol*. 2001 Oct;31(12):1331-42.

793   [79] Martin RE. The transportome of the malaria parasite. Biol Rev. 2020 Apr;95(2):305-32.

794   [80] Weiner J, Kooij TW. Phylogenetic profiles of all membrane transport proteins of the
795   malaria parasite highlight new drug targets. *Microbial Cell*. 2016 Oct 3;3(10):511.

796   [81] Bushell E, Gomes AR, Sanderson T, Anar B, Girling G, Herd C, Metcalf T, Modrzynska
797   K, Schwach F, Martin RE, Mather MW. Functional profiling of a *Plasmodium* genome
798   reveals an abundance of essential genes. *Cell*. 2017 Jul 13;170(2):260-72.

799   [82] Kenthirapalan S, Waters AP, Matuschewski K, Kooij TW. Functional profiles of orphan
800   membrane transporters in the life cycle of the malaria parasite. *Nature Commun*. 2016 Jan
801   22;7(1):1-0.

802   [83] Cowell AN, Istvan ES, Lukens AK, Gomez-Lorenzo MG, Vanaerschot M, Sakata-Kato
803   T, Flannery EL, Magistrado P, Owen E, Abraham M, LaMonte G. Mapping the malaria
804   parasite druggable genome by using in vitro evolution and chemogenomics. Science. 2018
805   Jan 12;359(6372):191-9.

806   [84] Paulusma CC, Folmer DE, Ho-Mok KS, De Waart DR, Hilarius PM, Verhoeven AJ,
807   Oude Elferink RP. ATP8B1 requires an accessory protein for endoplasmic reticulum exit and
808   plasma membrane lipid flippase activity. *Hepatology*. 2008 Jan;47(1):268-78.

809   [85] Bryde S, Hennrich H, Verhulst PM, Devaux PF, Lenoir G, Holthuis JC. CDC50 proteins
810   are critical components of the human class-1 P4-ATPase transport machinery. *J Biol Chem*.
811   2010 Dec 24;285(52):40562-72.

812   [86] McNamara CW, Lee MC, Lim CS, Lim SH, Roland J, Nagle A, Simon O, Yeung BK,
813   Chatterjee AK, McCormack SL, Manary MJ. Targeting Plasmodium PI (4) K to eliminate
814   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.

890 [112] Sterkel M, Oliveira JH, Bottino-Rojas V, Paiva-Silva GO, Oliveira PL. The dose makes
891 the poison: nutritional overload determines the life traits of blood-feeding arthropods. *Trends*
892 *Parasitol*. 2017 Aug 1;33(8):633-44.

893 [113] O'Neal AJ, Butler LR, Rolandelli A, Gilk SD, Pedra JH. Lipid hijacking: a unifying
894 theme in vector-borne diseases. *Elife*. 2020 Oct 29; 9:e61675.

895 [114] Costa G, Gildenhard M, Eldering M, Lindquist RL, Hauser AE, Sauerwein R,
896 Goosmann C, Brinkmann V, Carrillo-Bustamante P, Levashina EA. Non-competitive
897 resource exploitation within mosquito shapes within-host malaria infectivity and virulence.
898 *Nature Commun*. 2018 Aug 27;9(1):1-1.

899 [115] Werling K, Shaw WR, Itoe MA, Westervelt KA, Marcenac P, Paton DG, Peng D,
900 Singh N, Smidler AL, South A, Deik AA. Steroid hormone function controls non-competitive
901 *Plasmodium* development in *Anopheles*. *Cell*. 2019 Apr 4;177(2):315-25.

902 [116] Samuel BU, Mohandas N, Harrison T, McManus H, Rosse W, Reid M, Haldar K. The
903 role of cholesterol and glycosylphosphatidylinositol-anchored proteins of erythrocyte rafts in
904 regulating raft protein content and malarial infection. *J Biol Chem*. 2001 Aug
905 1;276(31):29319-29.

906 [117] Harvey JW. The erythrocyte: physiology, metabolism, and biochemical disorders. In:
907 Clinical biochemistry of domestic animals 1997 Jan 1 (pp. 157-203). Academic Press.

908 [118] Benz EJ. Learning about genomics and disease from the anucleate human red blood
909 cell. *Journal Clin Inv*. 2010 Dec 1;120(12):4204-6.

910 [119] Montigny C, Lyons J, Champeil P, Nissen P, Lenoir G. On the molecular mechanism of
911 flippase-and scramblase-mediated phospholipid transport. Biochimica et Biophysica Acta
912 (BBA)-Molecular and Cell Biology of Lipids. 2016 Aug 1;1861(8):767-83.

913 [120] Arashiki N, Takakuwa Y. Maintenance and regulation of asymmetric phospholipid
914 distribution in human erythrocyte membranes: implications for erythrocyte functions. *Curr*
915 *Opin Hematol*. 2017 May 1; 24(3):167-72.

916 [121] Fraser M, Matuschewski K, Maier AG. Of membranes and malaria: phospholipid
917 asymmetry in Plasmodium falciparum-infected red blood cells. Cellular and Molecular Life
918 Sciences. 2021 Mar 13:1-7.

919 [122] Yalaoui S, Huby T, Franetich JF, Gego A, Rametti A, Moreau M, Collet X, Siau A, van
920 Gemert GJ, Sauerwein RW, Luty AJ, Vaillant JC, Hannoun L, Chapman J, Mazier D,
921 Froissard P. Scavenger receptor BI boosts hepatocyte permissiveness to Plasmodium
922 infection. Cell Host Microbe. 2008 Sep

923 [123] Labaied M, Jayabalasingham B, Bano N, Cha SJ, Sandoval J, Guan G, Coppens I.
924 Plasmodium salvages cholesterol internalized by LDL and synthesized de novo in the liver.
925 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	Phosphatidylinositol/phosphatidylcholine 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]