

# Lipid Salvage and Biosynthesis Pathways in Malaria Parasite: Potential Targets for Drug Development

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**ABSTRACT:** Apicomplexan parasite *Plasmodium*, the causative agent of malaria is a wide spread pathogen causing a significant high number of casualties worldwide annually. Symptomatic infection by the parasite in humans is caused by the asexual blood stage parasite which undergoes a 48 hrs cycle of invasion, maturation, replication and egress. Lipids are omnipresent and are essential constituents of a cell. They play a crucial role in the complex life cycle of the parasite. They are key players in various biological processes and serve as signaling molecules between cells and a highly efficient source of energy storage, intercellular building blocks, haemoglobin degradation, and are also involved in post translation modification. The asexual blood stage of *Plasmodium falciparum* infection is marked with an elevated level of phospholipid, neutral lipid and lipid-associated fatty acid (FA) content in infected red blood cells, which it uses mainly for the generation of new membranes for growth and subsequent replication. Parasite has a complex mechanism for the uptake and synthesis of lipids, which changes dramatically through various stages of its life cycle. During its intraerythrocytic development, the parasite, changing the fatty acid composition, phospholipid organization, permeability and fluidity of the host membrane, extensively modifies the host cell membrane. In addition to, scavenging of lipids/phospholipids from host milieu and their catabolism, *de novo* synthesis may play important role to fulfill parasite requirement of lipids during the erythrocytic cycle. Phospholipids are also known to be recycled in the eukaryotic cell for cellular homeostasis. The significant differences between the metabolisms of the parasite with that of the human host make *Plasmodium* lipid metabolism enzymes as potential targets for novel therapeutics. Here, in this short review we have discussed various lipid biosynthesis pathways of *Plasmodium* parasite in the blood stage cycle and the recent advances in this area of research.

**KEYWORDS :** Malaria , Plasmodium, Fatty Acids, Lipids biosynthesis , Drug Targets

## I. INTRODUCTION

*Plasmodium falciparum*, the causative agent of life-threatening human malaria has a complex life cycle involving human host and mosquito vector. All the physiological symptoms of malaria in humans are caused by blood stage asexual life cycle of the parasite. Lipid metabolism, which is almost nonfunctional in the uninfected erythrocyte, is dramatically elevated in the parasite-infected erythrocytes. The blood stage cycle of the parasite is responsible for pathogenesis of the parasite and the metabolic pathways important for this cycle are for development of new targets against malaria<sup>1-3</sup>. Following invasion, *Plasmodium* parasite leads to pathological manifestation of malaria by destruction of red blood cells in the host. During this developmental cycle, the parasite undergoes rapid growth and multiplication while consuming material from host, including lipids and its derivatives. This high demand for the lipids

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by the parasite is met from exogenous sources scavenged from the RBC membrane and human serum; parasite utilizes various metabolites to synthesize the lipids<sup>4,5</sup>. Fatty acids, serine and ethanolamine are the major requirements of the parasite during this growth period, which it utilizes to synthesize structural and regulatory phospholipids. In addition to fatty acids supplied by the host, parasite also has been reported to express all the enzymes required for the fatty acids biosynthesis<sup>6-8</sup>. Therefore, the parasite makes use of both the processes, *de novo* synthesis and uptake mechanisms, to fulfill its requirement for lipids and fatty acids. Here, we have tried to discuss the synthesis and utilization of different lipid moieties by the parasite and have also summarized fatty acid synthesis pathways in the parasite and roles of lipids in asexual phase.

## II.I LIPID METABOLISM IN *PLASMODIUM*

### II.I.I PHOSPHOLIPIDS AND THEIR SYNTHESIS PATHWAYS

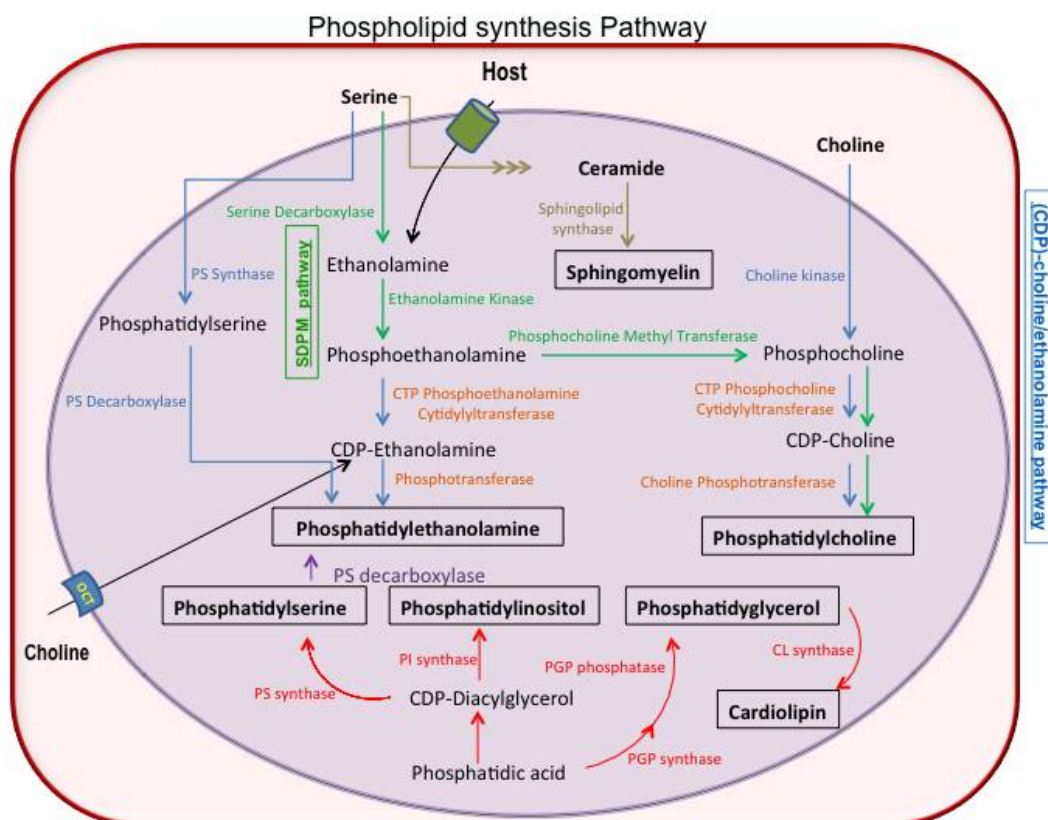
In order to multiply, *Plasmodium* requires significant amount of phospholipids during its intraerythrocytic life cycle. After invading RBC, the levels of phospholipids are shown to increase in the parasite along with the changes in the phospholipids composition of infected erythrocytes<sup>9</sup>. Of the total phospholipids in *P. falciparum*, Glycerophospholipids (GPLs), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) make up around 80% of the total phospholipids<sup>10</sup>. Other GPL species associated with parasite cells, which constitute a significant proportion of total phospholipids, are phosphatidylserine (PtdSer), phosphatidylinositol (PtdIno), and cardiolipin, though the content of these three species is sevenfold less than that of PtdCho and PtdEtn. Parasite has balanced between all these different types of GPLs with different modes of synthesis and pathways (Figure 1).

### II.I.II PHOSPHOTIDYLCHOLINE SYNTHESIS PATHWAY

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) account for a major share of phospholipids in eukaryotic membranes, playing a major functional and structural role; *Plasmodium* is not an exception to that. The synthesis of this phospholipid occurs via two routes, the CDP-choline pathway, which uses host choline as a precursor, and the plant-like serine decarboxylase-phosphoethanolamine methyltransferase (SDPM) pathway, which uses host serine-ethanolamine as a precursor.

### II.I.II.I CDP-CHOLINE (KENNEDY) PATHWAY

One of the pathways for the synthesis of Phosphotidylcholine is the PC branch of the *de novo* **Kennedy pathway**, which requires the uptake of choline from host serum into the infected erythrocyte. In order to fulfill its requirement for the uptake of choline from the serum, parasite modifies RBC and induces a new permeation pathway along with normal erythrocytic choline carrier in RBC<sup>11,12</sup>. A poly-specific organic cation transporter (OCT) functionally distinct from the known dedicated eukaryotic choline carriers, mediates choline uptake into the parasite<sup>5,13</sup>. Once transported into the parasite, choline is acted upon by a parasite specific choline kinase (PfCK) which catalyzes the ATP dependent phosphorylation of choline to produce phosphocholine. Further, by the action of CTP-phosphocholine cytidyltransferase (PfCCT) it is converted to a high-energy donor CDP-choline. In the final step of



**Figure 1: Phospholipid synthesis pathways in *Plasmodium Falciparum*.** Schematic representation showing the common lipid biosynthetic pathways in *Plasmodium*. Phospholipids are majorly synthesized by two pathways [A] Kennedy pathway [blue colour] and [B] SDPM pathway [green colour]. Parasite also uptake various phospholipids through scavenging pathway from the host [Black colour]

the reaction CDP-choline is acted upon by CDP-choline transferase in the presences of an acyl alcohol to form Phosphotidylcholine giving CMP as by-product, Phosphocholine cytidyltransferase (PfcCT) it is converted to a high-energy donor CDP-choline. In the final step of the reaction CDP-choline is acted upon by CDP-choline transferase in the presences of an acyl alcohol to form Phosphotidylcholine giving CMP as by-product. The analogous pathway of phosphoethanolamine involves CDP-ethanolamine branch of Kennedy pathway, which uses similar reactions to form Phosphatidylethanolamine (Figure.1).

### II.II.IIPHOSPHATIDYLCHOLINE BIOSYNTHESIS FROM SERINE/ETHANOLAMINE IN *P. FALCIPARUM*

Various studies<sup>5-7</sup> have pointed out that the parasite can be grown normally even in the absence of exogenous choline, which suggests that parasite must be using an alternate pathway for the synthesis of phosphotydlcholine. This alternate route is named Serine decarboxylase–phosphoethanolamine methyltransferase (SDPM) pathway. This pathway was highlighted from an earlier work, in which composition of purified parasite membrane showed (35-40%) high levels of phosphatidylethanolamine<sup>14</sup>. In this pathway, decarboxylation of parasite’s serine leads to formation of ethanolamine, which gets converted, into phosphoethanolamine. This serves as a precursor for the synthesis of PtdEtdn through the CDP-ethanolamine pathway and for the synthesis of PtdCho through the SDPM/CDP-choline pathways respectively<sup>15</sup>. Phosphocholine is synthesized from phosphoethanolamine by the action of an enzyme called Phosphoethanolamine

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Methyltransferase (PfPMT) which plays a key role in the parasite life cycle. PfPMT is expressed throughout the erythrocytic cycle with maximum expression during the trophozoite stage, when membrane biosynthesis is maximum<sup>15</sup>. Bioinformatic analysis of various *Plasmodium* species indicated the presence of orthologs of PfPMT in *P. vivax* and *P. knowlesi*, however they were absent in the rodent malaria parasites *P. berghei* and *P. yoelii*. However, no Human homologue of PfPMT was found. It also shared no homology with eukaryotic PMT proteins. Absence of phosphoethanolamine methyltransferase activity in mammalian cells has projected PfPMT as an attractive target for the development of antimalarial compounds. Microscopy based localization studies of PfPMT have shown it to be localizing to the Golgi apparatus of the parasite<sup>16</sup>. This was the first enzyme in this pathway found to be located at the same place where transmethylation reaction takes place i.e. Golgi apparatus. However, the subcellular localization of other enzymes in the phosphatidylcholine/phosphatidylethanolamine synthesis pathways is still unknown. Different reports have suggested presence of distinct pools of PtdETn from different precursors and via *de novo* pathway<sup>17</sup> which have been shown to regulate SDMP pathway<sup>18</sup>. Preliminary studies on PfPMT have shown that phosphocholine the product of the reaction is able to modulate its activity<sup>14</sup>. This study pointed out that phosphocholine analog may inhibit PfPMT activity. Hexadecylphosphocholine (miltefosine), a phosphocholine analog inhibits PfPMT activity with IC<sub>50</sub> of 50 μM and IC<sub>90</sub> of 100 μM. Further hexadecylphosphocholine is capable of inhibiting parasite growth with an IC<sub>50</sub> value of ~80 μM in parasite proliferation assays<sup>14</sup>. Although there is no direct evidence for the action of hexadecylphosphocholine on PfPMT, some studies, got PfPMT established as a reliable drug target. One study has given genetic and pharmacological evidence supporting that the pathway is essential for parasite gametocytogenesis and malaria transmission. Gene disruption studies showed that phosphoethanolamine N-methyltransferase enzyme play an important role in the asexual growth of the parasite as well as gametocyte development. Parasites lacking the *pfpm* gene were incapable of producing mature-stage gametocytes, and were therefore not transmitted to mosquitoes<sup>19</sup>. Furthermore, recent studies on PfCTP (phosphocholine cytidyltransferase) suggest that it possesses two functional catalytic domains and is inhibited by a CDP-choline analog which was selected from a virtual screening<sup>20</sup>.

### II.III TARGETING THE PHOSPHOLIPID SYNTHESIS PATHWAYS IN PLASMODIUM

Constant search for novel drug targets is one of the fundamental goals in the study of *Plasmodium*. Discovery of new metabolic pathways which play important functions in parasite development, proliferation, differentiation, and pathogenesis open up a new dimension in this direction. However, to be considered as a viable target for drug development they should be either absent in humans or different enough from their human counterparts. Since parasite essentially uptakes several lipids in order to synthesize new membranes, this was identified as possible target pathway to develop anti-malarial compounds. Several small synthetic compounds, which were structurally similar to the membrane components were tested for their antimalarial activity and many of them were found to inhibit parasite proliferation at low IC<sub>50</sub> values<sup>7,21</sup>. Biochemical analyses revealed that these analogs are transported into the parasitized erythrocyte and get incorporated into the parasite membranes which can dramatically alter the lipid composition and physico-chemical properties of the membranes leading to the parasite death<sup>21</sup>. Some groups have shown inhibition of PtdCho biosynthesis by using the structural analogue of choline. These analogs are large molecules possessing one or two quaternary ammoniums. Further works from other groups developed more potent inhibitors based on same strategy, which have shown to inhibit parasite growth with these compounds at IC<sub>50</sub> values of 0.7–10 μM<sup>22</sup>. These compounds act by blocking the entry of choline into the infected erythrocyte thus causing a decrease in the biosynthesis of PtdCho<sup>22,23</sup>. Albitiazolium specifically inhibits the Phosphocholine biosynthesis is a new class of antimalarial and is currently under phase 2 clinical trials<sup>24,25</sup>. Bis ammonium salts were also used by some groups which were found to be more potent compounds than mono ammonium salts with IC<sub>50</sub> values in the sub-nanomolar to nanomolar ranges. The proof of realistic antimalarial pharmacology of these compounds was obtained from rodent malaria and in nonhuman primate models under very severe conditions of parasitemia and short course of treatment. Biscationic choline analog have been shown to accumulate by several hundredfolds in malaria infected erythrocytes specifically and thus have made them potent and specific agents against parasite infected erythrocytes<sup>26,27</sup>. Earlier work has shed more light on the binding of these compounds with heme and their part accumulation in the parasite food vacuole. Studies have shown that heme-binding property plays an important role in drug accumulation as well as aids in the antimalarial activity of these compounds. Thus, the basic understanding about the action mechanism of these

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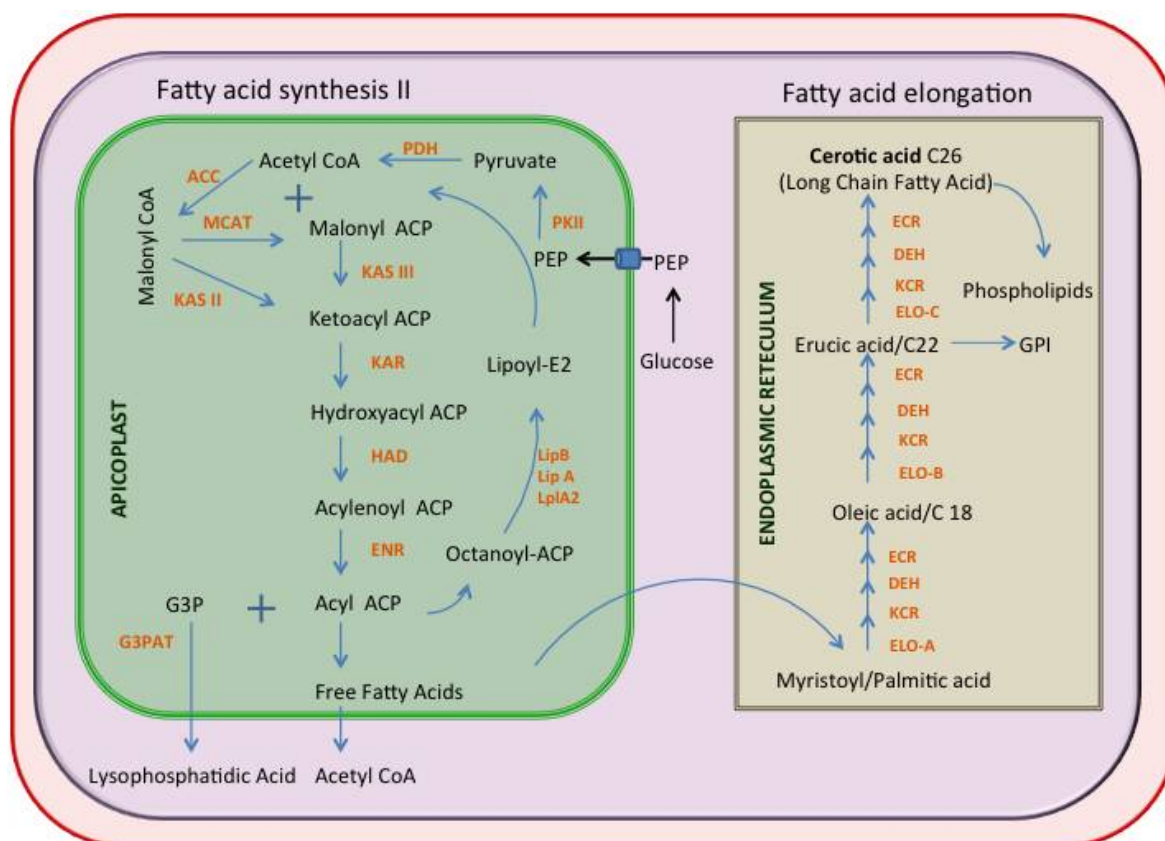
compounds on the blood stage cycle of the parasite projects PtdCho pathways as important drug targets. Transcriptome profiling of the parasite was carried out to check the global response against Bisthiazolium choline analogue, which showed cell cycle arrest, and a general induction of genes involved in gametocytogenesis; however there was no significant transcriptional change in the genes of the PtdCho biosynthetic pathways. However, proteomic and metabolomic analyses revealed a significant decrease in the level of the Cho/Eth-phosphotransferase [PfCEPT] involved in the final step of synthesis of PtdCho<sup>28</sup>. Genetic studies in the parasite have played a crucial role in validating the candidate genes as possible targets for the development of antimalarial drugs. Identification of choline analogs as primary targets is one such outcome of these studies. The choline analogs present to be promising drug candidates, as their broadspectrum inhibition capacity might be beneficial in surpassing drug resistance.

### III. FATTY ACID BIOSYNTHESIS

Fatty acids are required for membrane lipid synthesis and other essential cellular processes, and their production represents a central aspect of parasite lipid metabolism. Another very important lipid metabolism pathway in *P. falciparum* is the fatty acid (FA) biosynthesis. Previously it was thought that infected RBCs do not possess any significant activity for synthesizing fatty acids (FA) through the *de novo* pathway, and therefore, major fatty acids needed for parasite lipid metabolism have to be scavenged from the host<sup>17,21</sup>. However, with the completion of the genome sequences of *P. falciparum* and *P. yoelii* and advancement in molecular and cellular biological analyses of the parasite, it is now evident that the malaria parasites do possess the capacity to synthesize Fatty Acids, and also have the genes encoding type II FA synthase (FAS)<sup>29,30</sup>. Presence of two proteins namely, ACP (acyl carrier protein) and KASIII ( $\beta$ - ketoacyl-ACP synthase III)<sup>31</sup> have raised information about the presence of fatty acid synthesis pathway in the parasite and challenged earlier studies which had suggested that malaria parasites acquire all of the fatty acids required for blood stage growth through scavenging<sup>32</sup>. Both, ACP (acyl carrier protein) and KASIII ( $\beta$ - ketoacyl-ACP synthase III), were subsequently shown to be targeted to the apicoplast, implicating this organelle as a possible site for *de novo* fatty acid biosynthesis<sup>33</sup>. In *P. falciparum*, genes have been identified which encode for various enzymes of fatty acid biosynthesis suggesting presence of a complete machinery in parasite capable of generating fatty acids from simple precursors.

### III.1 FAS II PATHWAY

Fatty acid synthesis pathway in *Plasmodium falciparum* has been shown to have seven different proteins which comprises of dissociated type II FAS. Type II FAS pathway is commonly found in microorganisms and in the endosymbiont organelles of some eukaryotes<sup>34,35</sup>. Further, the FASII pathway can be divided into three phases namely; preparation, initiation and elongation phase. Preparation phase starts with the import of precursor for the pathway from cytoplasm. The major precursor is glycolytic intermediate phosphoenolpyruvate (PEP), which further gets converted into acetyl-CoA, ATP and reducing equivalents. The proteins involved in this import of PEP have been termed as plastidic phosphate transporters (pPTs). These proteins are antiporters which are known to move phosphorylated carbon compounds across membranes in exchange for inorganic phosphate. *Plasmodium* species possess two pPTs, which were localized in the innermost and outermost apicoplast membranes by various means<sup>36</sup>. These proteins were tested by genetic manipulations to get an insight into their roles in the survival of parasite at different stages.



**Figure 2:** Type II fatty acid synthesis and ELO pathway of *P. falciparum*. *Plasmodium* acquires fatty acids through an intricate network of synthesis and uptake. The parasite harbors two fatty acid synthesis pathways that are localized to different cellular compartments. [A] Apicoplast [green]-localized FASII pathway which produces significant amounts of free fatty acids, in addition to lipoic acid relying on cytoplasmic glycolysis for precursors. [B] *Plasmodium* also maintain an ER associated elongase system which synthesizes very long chain monounsaturated fatty acids using the activity of ELO\_A, ELO-B and ELO-C subsequently. PEP, phosphoenolpyruvate; PDH, pyruvate dehydrogenase; ACC, acetyl-CoA carboxylase; MCAT, malonyl-CoA:ACP transacylase; ER, endoplasmic reticulum; KAS, ketoacyl-ACP synthase; KAR, ketoacyl-ACP reductase; HAD, hydroxyacyl-ACP dehydratase; ENR, enoyl-ACP reductase; ELO, elongase; KCR, ketoacyl-CoA reductase; DEH, acyl-CoA dehydratase; ECR, enoyl-CoA reductase

Provided their role in different uptake mechanisms, outer and inner pPTs were shown to be important in blood stages as well as developmental stages of the parasite<sup>37</sup>. Further, in the second step of the preparation phase PEP gets converted to pyruvate by pyruvate kinase by transfer of a phosphate group from PEP to ADP, forming pyruvate and ATP. *Plasmodium* possesses two pyruvate kinases, a type I isoform (PKI) involved in glycolysis in the cytoplasm, and the type II isoform (PKII) of the apicoplast<sup>38,39</sup>. PKII is known to be a producer of pyruvate for the DOXP pathway in the parasite, and thus it is considered as essential protein for the parasite. Ensemble with the last phase of preparation step the pyruvate is converted to acetyl-CoA and carbon dioxide by the action of pyruvate dehydrogenase complex (PDH). PDH is multi-subunit enzyme complex made up of four polypeptides that assembles with thiamine pyrophosphate and NAD<sup>+</sup>. In the presence of lipoic acid as a co factor the enzyme complex catalyses the conversion of pyruvate to acetyl Co A and carbon dioxide. Knockout studies have suggested that PDH is not essential for the blood stages of the parasite, but its plays an important role in liver stages and their absence makes liver developmental stage defective. Further, ACC complex take over the acetyl CoA and starts the initiation phase of FASII pathway. Acetyl CoA carboxylase converts acetyl CoA into malonyl CoA in the presence of biotin and bi carbonate.

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Another set of enzyme termed as FabD or malonyl CoA: ACP transacylase catalyzes the transfer of a malonyl group from malonyl-CoA to Acyl Carrier Protein, producing malonyl-ACP for subsequent steps in the pathway. Apart from the structural modelling of the PfFabD, little is known about the enzyme rendering it as one of the least characterized components of FASII, and considerable scope exists for further research on the enzyme.

ACP 57 a small soluble protein plays a central role in Type II fatty acid synthesis. Nascent fatty acids get covalently linked to ACP 57 through a thioester bond and are further modified by other enzymes of the pathway. In the next step, further KASIII acts on Malonyl-ACP and catalyzes the decarboxylative condensation of the malonyl group with an acetyl group donated by acetyl-CoA. Then the combined sequential action of KAR ( $\beta$ -ketoacyl-ACP reductase), HAD ( $\beta$ -hydroxyacyl-ACP dehydratase), and ENR (enoyl-ACP reductase) converts product of KASIII to acyl-ACP. KASII ( $\beta$ -ketoacyl-ACP synthase II) carries out further elongation of the acyl chain followed by KAR, HAD, and ENR. Ketoacyl-ACP synthases substrate specificity decides the length of fatty acid produced by a type II FAS<sup>35</sup>.

Fatty acid synthesis remained a question of debate in *Plasmodium* biology for long time. Oaks *et al.*, have suggested that *P. falciparum* does not synthesize fatty acids (Oaks *et al.*, 1991), although the hypothesis have changed, with the discovery of the type II FAS in the parasite. Reports have suggested scavenging of plasma lipids<sup>7</sup>, but the uptake of key FAS products, such as lipoic acid, was not known and would have to be produced by the type II FAS pathway during the blood stages. Later work from Allary *et al* has shown that lipoic acid can be scavenged by *P. falciparum* during the blood stages<sup>40</sup>. Another important study showed essential role of enzyme ENR in *Plasmodium* life cycle using a biocide triclosan, which has highlighted importance of type FASII pathway in the parasite life cycle. Triclosan was found to be able to inhibit the ENR activity both *in vitro* growth of *P. falciparum* in culture, and *in vivo* growth in *P. berghei*. Assays showing inability of triclosan treated ENR enzyme to incorporate radiolabelled acetyl-CoA into newly synthesized fatty acids, highlighted their apparent mechanism of antiparasitic activity<sup>41</sup>. These studies taken together have provided clues to the importance of type II FAS in *Plasmodium* and also have established ENR as a drug target. Due to major difference in the type II FAS in the malaria parasite and the type I FAS present in humans, FAS had become a hot topic for people looking for drug targets against the parasite<sup>30</sup>. Work with recombinant proteins showed for ACP, MCAT, and KASIII proved that these enzymes function to initiate fatty acid biosynthesis and use acetyl-CoA and malonyl-CoA as carbon sources<sup>31</sup>. Later, HAD and KAR were also shown to be actively involved in using acetoacetyl-CoA and  $\beta$ -hydroxybutyryl-CoA<sup>42,43</sup>. In a major breakthrough crystal structures of ACP<sup>44</sup>, KAR, HAD and ENR by different workers gave a boost for the drug discovery efforts against the type II FAS pathway<sup>45,46,47,48,49</sup>. Inhibitors have been reported for several of the type II FAS enzymes from *P. falciparum* including KAR and HAD<sup>45,46</sup>. Thioloactomycin (TLM), a natural product analogue which can inhibit bacterial  $\beta$ -ketoacyl-ACP synthase enzymes (FabH, FabB, and FabF) was also reported to inhibit the growth of *P. falciparum* with an IC<sub>50</sub> value of 50  $\mu$ M<sup>50</sup>. Other workers designed various analogs of TLM and tested their potential to inhibit parasite growth *in vitro* and found that these compounds can inhibit parasite growth at low micromolar concentrations<sup>30,51,52,30,51-53</sup>.

As Triclosan was shown to be very effective against the enzyme ENR, drug discovery for ENR has mainly focused on analogs of the biocide triclosan (TRC). Genetic approaches in *P. falciparum* and two rodent malaria species<sup>54</sup> gave an unexpected result that malaria type II FAS is critical for liver stage development. *P. berghei* HAD II and KasII genetic knockout parasites were able to infect hepatocytes and develop normally but the second day of development was abnormal<sup>55</sup>. Similar findings were reported by Yu and co workers in Pb ENR knock out studies<sup>56</sup>. These studies have made it evident that the type II FAS found in malaria parasites is critical for liver stage development, and those FAS enzymes may be appropriate targets for prophylactic drugs. Apicoplast contains a prokaryote like fatty acid synthase (FASII) complex as well as key enzymes involved in phospholipid biosynthesis, indicating that *de novo* synthesized fatty acids may be incorporated into apicoplast membrane lipids<sup>55,57</sup>. Work from Cyrille *et al.*, showed that apicoplast membranes contain higher amounts of saturated fatty acids and the phospholipid PI when compared with parasite membranes. They also had a range of other lipid species like cholesterol, ceramide and sphingomyelin suggesting the scavenging of lipids from the host cell<sup>58</sup>. Together with (U-13C)-glucose labelling studies, these analysis indicate that utilization of salvage precursors plays an important role in the biogenesis of intracellular organelles. On the other hand; parasite do retains its ability to synthesize fatty acids via the apicoplast FASII complex when exogenous fatty acids get limiting and is inhibited by a CDP-choline analog which was selected from a virtual screening<sup>59</sup> (Figure 2).

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### III.II FATTY ACID ELONGASE PATHWAY (FAE):

This pathway is found in the Endoplasmic Reticulum and is similar to FAS II pathway except that the pathway starts with much longer starter molecules i.e. 16 C or longer and employs CoA as an anchor molecule rather than ACPs as used by the FAS pathway, with Myristoyl CoA and palmitoyl CoA being the preferred initiating substrates. The cycle begins with a condensation reaction catalysed by elongase; the product then gets further modified by a dehydratase and a ketoacyl reductase. Final product is elongated by two carbon unit. *Plasmodium* encodes three fatty acid elongases namely ELO-A, ELO-B and ELO-C which are found to be active during the blood stage. (Figure 2)

The products of FAE pathway are found to be essential in eukaryotes. Studies in *Toxoplasma* have shown significant proportion of fatty acids to be a product of FAE pathway<sup>60</sup>. However loss of individual elongases has no effect on parasite growth, attributed to the overlapping specificity of the elongase enzymes. However the exact role of this long chain fatty acid and functioning of this pathway in the parasite has yet to be studied.

### IV.I VARIOUS ROLES OF LIPIDS IN *PLASMODIUM* LIFE CYCLE

#### IV.I.I STRUCTURAL ROLE IN MEMBRANE MODELING

Glycosylphosphatidylinositol [GPI] is a glycolipid that can be attached to a protein during post-translational modification and is ubiquitous in eukaryotes. Several important members of *Plasmodium*'s surface proteins have been shown to play a role in membrane anchoring of proteins such as merozoite surface protein-1, which is believed to be crucial for recognition, binding and invasion of erythrocytes<sup>61,62</sup>. Basically GPIs in *P. falciparum* consist of a core Etn-phosphate-6Man<sub>1</sub>-2Man<sub>1</sub>-6Man<sub>1</sub>-4Glucosamine linked to the inositol residue of PtdIno through an  $\alpha$  1-6 bond, and an additional Man residue linked to the third Man at O-2. Although, the PtdIno moiety varies a lot with regard to fatty acyl substituents at sn-1 and sn-2 positions and at C-2 of inositol residue which impart huge structural diversity<sup>63</sup>. The structures of parasite GPIs differ significantly from those of humans, and thus the synthesis pathway for the same has been suggested as a potential chemotherapeutic target<sup>64,65</sup>.

Studies have shown that GPIs from parasite, with or without associated proteins, can induce the proinflammatory cytokine production like the tumor necrosis factor and interleukin-1 in macrophage. These secreted factors can lead to characteristic symptoms of malaria infections like pyrexia, hypoglycemia, and lethal cachexia<sup>66</sup>. Consistent with the idea that *Plasmodium* GPIs contribute to malaria pathogenesis, it has been shown that there is a good correlation between the level of resistance to clinical malaria and the level of persistent anti-GPI antibodies<sup>67</sup>. Reports suggested that the GPI-anchors are not only required for the survival of the parasite in the host, but also play an important role in intraerythrocytic parasite growth. It was found that parasites treated with mannosamine at trophozoite stage were unable to develop into schizonts and showed inhibition in GPI-anchor biosynthesis, which clearly points towards GPI biosynthetic pathways as probable drug targets<sup>67,68</sup>. However, further studies are needed to establish the mechanism of inhibition of GPI-anchor biosynthesis and to develop specific inhibitors targeting certain steps in this metabolic pathway.

#### IV.I.II ROLE OF NEUTRAL LIPIDS IN HEMOGLOBIN BREAKDOWN

Host hemoglobin is a major source of nutrients (amino acids) for the malaria parasite. During its blood stages the parasite breaks down the hemoglobin by the action of various proteases mostly inside the digestive vacuole of the parasite. Parasite converts large quantities of heme released during hemoglobin digestion, into nontoxic hemozoin crystals<sup>69</sup>. Several studies have shown hemozoin crystal to be associated with neutral lipids droplet like structures called as lipid nanospheres or with membranous structures called neutral lipid bodies<sup>70-72</sup>. Specifically, neutral lipids like triacylglycerol [TAG] and diacylglycerol [DAG], are broken down by the action of lipases and hydrolases and used as building blocks for membrane formation, as cellular messengers or for energy<sup>73,74</sup>. Of late, work by Pisciotto *et al* showed that a specific blend of mono-, di- and triacylglycerols present in the digestive vacuole play a role in hemozoin formation<sup>70</sup>. This blend when analysed by TLC and LC-MS was found to be a mixture of monoglycerides; monostearoylglycerol [MSG] and monopalmitoylglycerol [MPG] and the diglycerides; 1,3-dioleoylglycerol [DOG], 1,3-dipalmitoylglycerol [DPG] and 1,3-dilinoleoylglycerol [DLG] in a 4:2:1:1:1 ratio [21]. To gain further insight into the role of lipids in hemozoin formation Synthetic Neutral Lipid Droplets [SNLDs] were used. SNLD is a blend of mono- and diglycerides deposited onto an aqueous surface resembling physically to their *in vivo* counterpart thus



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making them useful for hemozoin degradation studies. These lipid droplets were able to mediate the production of brown pigments identical to hemozoin. Differential scanning calorimetry and kinetic studies showed that the SNLDs provide a unique environment that promotes hemozoin formation. Microscopic studies have shown formation of lipid bodies to be stage-dependent with large numbers of lipid bodies found in the late stages of the erythrocytic cycle. The overlapping production of neutral lipid and degradation of hemoglobin is unlikely to be coincidental indicating common metabolic pathways where neutral lipids play an important role in heme detoxification.

### IV.III ROLE OF *PLASMODIUM* LIPIDS IN IMMUNE MODULATION OF HOST.

GPIs of *P.falciparum* are been termed as toxins in some cases, although they are not toxic. Instead, GPIs are very much important in combating infections with *Plasmodium*, as they start proinflammatory responses in host well before the development of adaptive immunity and later help in regulating adaptive immunity against the parasite as well. Their so called toxic effects in reality are the destructive effects of unregulated proinflammatory responses. And thus, it is not correct to blame *Plasmodium* GPIs as toxic molecules. Reports have suggested that *P. falciparum* GPIs can initiate up regulation of cell adhesion molecules and hypoglycemia.<sup>75</sup> Therefore, GPIs may be called as malaria parasite-associated molecular patterns [malaria PAMPs]

GPI induces CMI based immune response mainly by TLR2<sup>76,77</sup>. It has been suggested that TLR2 further carries the signals through MAPK and NF- $\kappa$ B signaling pathways<sup>78,79</sup>. As GPIs are present on the cell surface carrying out a defined downstream signaling in the cells, it can be suggested that they are evolutionarily conserved molecular patterns of the parasite. One trivial part of malaria GPIs' recognition by TLRs is that their fatty acyl moieties remain inserted in membranes and yet are recognized by TLRs. As covalently linked fatty-acyl moieties are crucial for the activity of GPIs and in *Plasmodium* these residues are not accessible for receptor interaction, the only way through which these correct interactions could take place is the ability of GPIs to display a specific pattern for recognition by TLR2 and the co-receptors.

Apart from GPIs, Hemozoin and its complex with DNA have been suggested as specific ligands for TLR9. It is possible that hitherto unknown factors might also be involved. A deeper insight of the molecular mechanisms involved in parasite recognition by the host immune system as well as a detailed understanding of the regulation of the expression of various innate immune responses by MAPK and NF- $\kappa$ B signaling cascades will offer further avenues for the development of new drugs and therapeutics.

### V. CONCLUSION

In recent years many enzymes of the lipid metabolism have been identified some of these enzymes, involved in phospholipids and fatty acid metabolism have been studied at the functional level as well as molecular level. These enzymes have been shown to play important roles in the growth, development and differentiation of the *Plasmodium* parasite at various stages of its life cycle. Moreover these enzymes are either absent in humans or are distinctly different from their human counterparts, which makes them a potential targets for the development of novel antimalarial drugs. Recent advances in the tools available for the genetic manipulation of *Plasmodium* genes have made the study of these essential genes more feasible and informative. It is now viable to authenticate the precise steps and networks in which these enzymes are involved which can be used in designing of various therapeutic strategies. Various chemical approaches have provided us a wealth of novel inhibitors of lipid metabolism which can be used in the development of Apicomplexan specific drugs. Quaternary ammonium compounds being used as potent antimalarial are one such example.

Thus, considering the importance of lipids in the parasite life cycle, there is no question that these pathways may prove to be an exciting drug target. Using an integrated strategy involving biochemical, genetic, and structural approach will set the stage for the discovery and design of drugs that will lead the way to global eradication of malaria.

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