METABOLIC MAPS AND FUNCTIONS OF THE *PLASMODIUM FALCIPARUM* APICOPLAST

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Discovery of a relict chloroplast (the apicoplast) in malarial parasites presented new opportunities for drug development. The apicoplast — although no longer photosynthetic — is essential to parasites. Combining bioinformatics approaches with experimental validation in the laboratory, we have identified more than 500 proteins predicted to function in the apicoplast. By comparison with plant chloroplasts, we have reconstructed several anabolic pathways for the parasite plastid that are fundamentally different to the analogous pathways in the human host and are potentially good targets for drug development. Products of these pathways seem to be exported from the apicoplast and might be involved in host-cell invasion.

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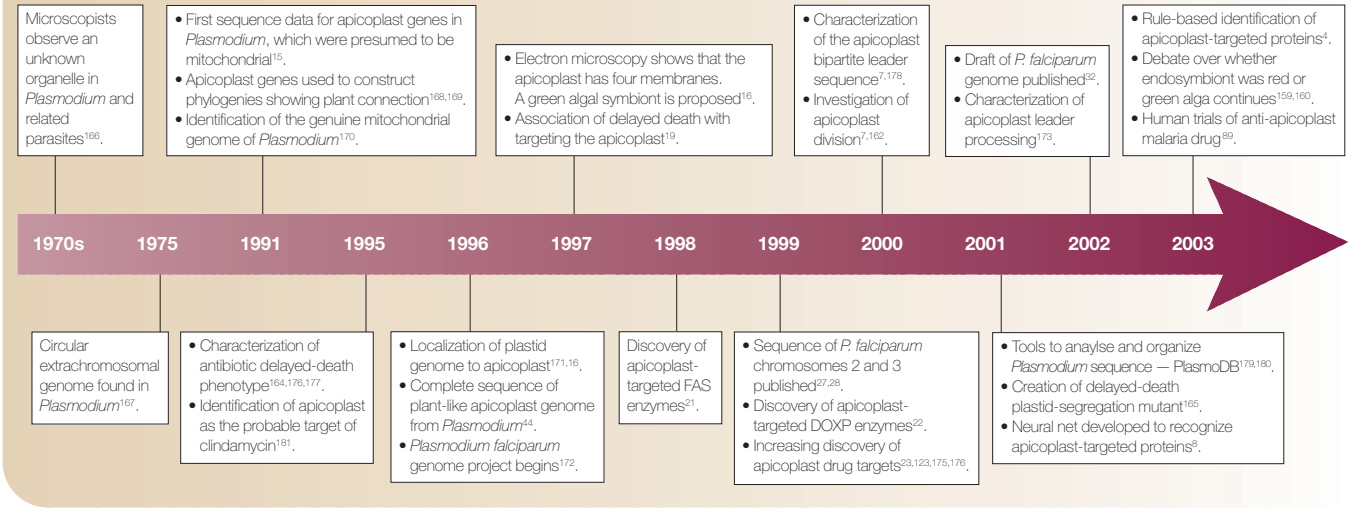
TROPICAL INFECTIOUS DISEASES

Malaria remains one of the most serious infectious diseases in the world, inflicting acute illness on more than 300 million people and leading to at least one million deaths annually[1]. In addition to the human cost, malaria imposes a massive economic burden, contributing substantially to poverty in the developing world. Malaria is estimated to reduce economic growth by approximately 1.3% each year in malaria-endemic countries, creating a vicious disease/poverty cycle that thwarts malaria control[2]. Sadly, the cheapest and most effective chemotherapies used to fight malaria are now losing efficacy due to drug resistance in the most deadly of the causative agents, *Plasmodium falciparum*. The emergence of drug-resistant parasites has led to resurgence of the disease, with malaria mortality rates redoubling in many areas[3].

Clearly, there is a need for new antimalarials — both the development of compounds against previously successful targets and the identification and exploitation of new targets are required. Many of the more exciting new targets to be revealed by the *P. falciparum* genome project are enzymes from the so-called apicoplast — a relict plastid (or chloroplast) that is a legacy of the malaria parasite's distant photosynthetic ancestry. Plastids are derived from the endosymbiosis of cyanobacteria, and the apicoplast is no exception. Importantly, the cyanobacterial heritage of the apicoplast means that many of its bacterial-like enzymes are fundamentally different from the mammalian host equivalents, making them potential drug targets. Though somewhat of a castaway from its photosynthetic origins, the apicoplast is by no means evolutionary flotsam. Indeed, the parasite is absolutely dependent on this curious organelle, which has led to speculation that the apicoplast is a potential 'Achilles' Heel' of the malaria parasite[4–6]. The *Plasmodium* genome sequencing project has unearthed several active apicoplast biosynthetic processes, and here we present a comprehensive anabolic map of this organelle. Several of the identified processes seem central to the core cellular functions of the parasite. Even though the apicoplast was only identified seven years ago (TIMELINE), thanks to the power of bioinformatics, we are now able to assemble a comprehensive picture of the organelle's metabolism.

Timeline | **Potted history of apicoplast research with major developments**



Identification of apicoplast-targeted proteins

As with all plastids, most of the proteins in the apicoplast are encoded by genes that have transferred to the nucleus of the cell. Proteins that function in the apicoplast must be targeted from the cytoplasm back to the organelle, which is accomplished by means of a BIPARTITE LEADER sequence, which is a distinctive feature attributable to the secondary endosymbiotic origins of the parasite. This leader sequence consists of a classical secretory signal peptide, which directs co-translational insertion into the ENDOMEMBRANE system. Downstream of the signal peptide there is a so-called transit peptide, which diverts the protein into the apicoplast[7]. Two independent tools have been developed for recognizing bipartite leaders in *P. falciparum* — PATS[8] and PlasmoAP[9]. PATS is an ARTIFICIAL NEURAL NETWORK that uses amino-acid sequence features to detect bipartite apicoplast-targeting sequences. PlasmoAP uses the existing SignalP software[10] to identify signal peptides, then uses a rule-based system to recognize the subsequent transit peptide. Mutational analyses of model transit peptides were conducted to test the rules underlying the PlasmoAP system. Strategic point mutations confirmed that apicoplast transit peptides conform to simple sequence requirements — first, acidic amino acids are depleted, second, basic amino acids are enriched and third, the transit peptide has a chaperone binding site[9]. An additional bioinformatic tool, PlasMit, has been trained to detect mitochondrial-targeting sequences[11], which provides extra stringency to discriminate between apicoplast and non-apicoplast-targeted proteins. Using a combination of these tools, we have extracted a list of more than 540 genes, the products of which are predicted to be targeted to the apicoplast (FIG. 1; online TABLE S1), from the sequenced *P. falciparum* genome. This list reveals a range of predicted metabolic activities as well as a high proportion of genes of unknown function in the apicoplast (FIG. 2).

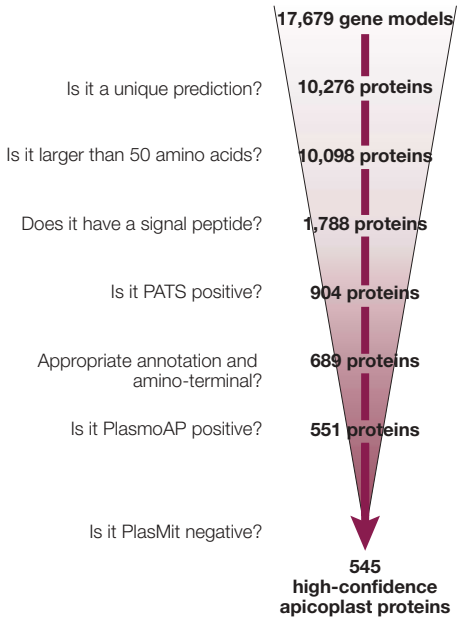


Figure 1 | **Flowchart for the identification of apicoplast-targeted proteins.** This flowchart depicts the process that is used to discriminate between apicoplast-targeted and non-apicoplast proteins. The estimate of 545 does not include the 23 proteins that are encoded by the apicoplast genome.

Apicoplast function

The function of the apicoplast has been debated since its discovery (for reviews, see REFS 5,12). Early guesses, which were based on what was known about similar relict plastids in non-photosynthetic plants[13] (reviewed recently in REF. 14), suggested that it was involved in the synthesis of haem for mitochondrial respiration[15], fatty-acid synthesis and starch storage[16], and the production of aromatic amino acids[17]. Whatever the function, it was apparent soon after its discovery that the apicoplast is indispensable to the parasite. Parasites die after treatment with drugs that interrupt apicoplast genome replication, transcription or translation[18,19,184]. Moreover, mutant parasites lacking an apicoplast are not viable[20]. Exactly what causes parasites to die when

BIPARTITE LEADER
A protein targeting segment characteristic of secondary endosymbiotic plastids, consisting of a hydrophobic signal peptide followed by a basic transit peptide.

ENDOMEMBRANE
The intracellular membrane system of a eukaryotic cell, comprising the endoplasmic reticulum, the Golgi apparatus, lysosomes and the plasma membrane. These membrane systems are interconnected by a flow of membrane from one to another using small membrane vesicles.

ARTIFICIAL NEURAL NETWORK (ANN). An information processing system that is loosely modelled on the organization of the human brain, and which possesses highly interconnected processing elements. ANNs are often useful for forming a model on the basis of a complex population of examples where no algorithm or descriptive rule exists.

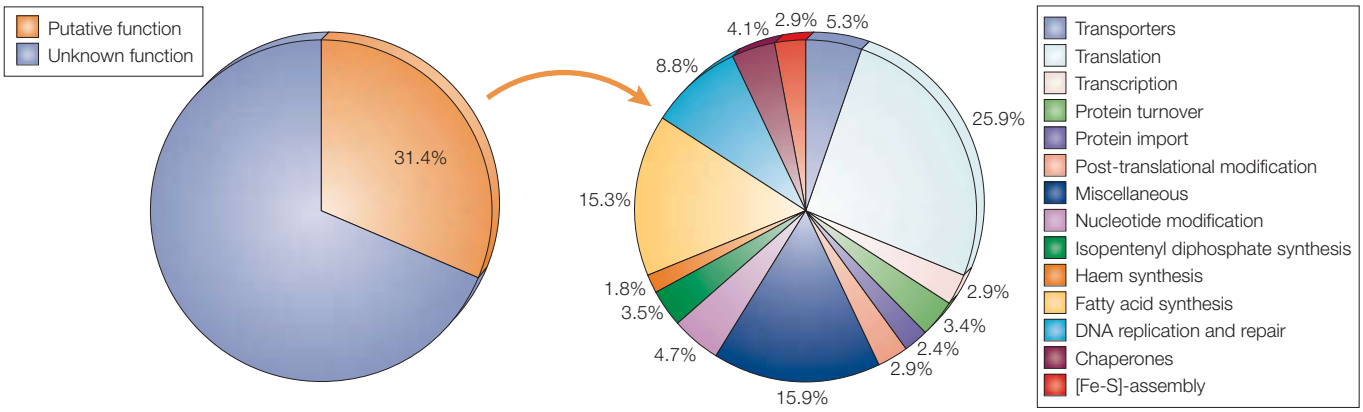


Figure 2 | **Classification of genes encoding predicted apicoplast-targeted proteins.** In addition to housekeeping activities responsible for maintenance and expression of the apicoplast genome, and a large proportion of genes with unknown functions, three anabolic pathways have been found: isoprenoid precursor synthesis, fatty-acid biosynthesis and a partial haem biosynthesis pathway.

apicoplast functions are disrupted remains a puzzle. Immediately after apicoplast perturbation (either pharmacological or genetic), parasites continue to grow normally in the host cell. However, the parasites subsequently arrest and die after infecting a new host cell. The timing of parasite death has been well characterized in *Toxoplasma gondii*[19], but less so in *Plasmodium* species. The lag in response of the parasite to the perturbation of the apicoplast is referred to as the delayed-death effect and presents a mystery, the solution to which requires that we determine the precise function of the organelle. Presumably, whatever the apicoplast provides for the parasite is crucial for a viable infection process. This could be a component of the PARASITOPHOROUS VACUOLE, which surrounds parasites in the host cell, or perhaps a resource that is usually replenished at the time of host-cell invasion. Once the preliminary *P. falciparum* genome sequence became available, a handful of apicoplast-targeted proteins that potentially fulfil these criteria were identified. These proteins have roles in fatty-acid synthesis[21] and non-mevalonate isopentenyl diphosphate synthesis[22], and they provided early clues to the pathways that we are now able to present in fine detail.

Apicoplast metabolic networks. Using the metabolic pathways of plant chloroplasts and bacteria as models, we have elucidated extensive apicoplast metabolic networks reconstructed from the list of apicoplast proteins that have been predicted using bioinformatics. These networks bring into focus a number of pathways that are not found in the vertebrate host of the parasite, and provide insights into apicoplast function. Here, we present an integrated *in silico* metabolism for apicoplast isopentenyl diphosphate, fatty-acid and haem biosynthesis, and identify putative fates for these important precursors (FIG. 3). Many housekeeping processes, such as DNA replication, transcription, translation and post-translational modification of apicoplast-encoded proteins, are also potentially excellent drug targets, but these processes have been reviewed elsewhere[4,5,18,23] and are not considered here.

PARASITOPHOROUS VACUOLE
During invasion of the host cell, the parasite initiates the formation of a membrane — the parasitophorous vacuole — which surrounds the parasite, and is substantially different from other endomembranes and the phagolysosome membrane.

REDUCING POWER
The capacity of an electron carrier to donate electrons to another compound.

Carbon and energy

The apicoplast is non-photosynthetic, so how does it obtain energy, REDUCING POWER and components, particularly carbon, for anabolic synthesis. Plant and algal plastids typically satisfy these requirements by photosynthesis. We hypothesized that the carbon and energy systems of the apicoplast would resemble that of a plant chloroplast in darkness. In plant cells that are never exposed to light, such as root cells, requirements for carbon and energy in the plastid are often fulfilled by importing hexose and triose phosphates from the cytosol. The apicoplast apparently imports phosphoenolpyruvate (PEP) by a phosphoenolpyruvate/phosphate translocator (PPT), which is otherwise unique to plants[24–26]. A *P. falciparum* gene encoding a PPT protein is predicted to be targeted to the apicoplast (online TABLE S1), which strongly indicates that the *Plasmodium* apicoplast imports PEP. In plant plastids, PEP is converted to pyruvate by a pyruvate kinase, yielding ATP[24]. The *P. falciparum* genome project has revealed a predicted apicoplast-targeted pyruvate kinase (online TABLE S1) in addition to the cytosolic isoform[27].

Another important cytosolic source of carbon for non-green plastids is dihydroxyacetone phosphate (DHAP). In plants, DHAP is imported by the triose phosphate transporter (TPT)[25], and a *P. falciparum* TPT homologue seems to be targeted to the apicoplast (online TABLE S1) — so the apicoplast most likely imports DHAP. In plant plastids, DHAP can either be converted to glyceraldehyde-3-phosphate (GA3P) — for isoprenoid synthesis for instance — by a chloroplast triose phosphate isomerase (TPI), or to glycerol-3-phosphate (G3P; a precursor for phospholipids and other molecules) by glycerol phosphate dehydrogenase (GpdA). A TPI protein from *P. falciparum* was previously annotated as being cytoplasmic[28], but more recent annotation indicates that TPI is an apicoplast-targeted protein (online TABLE S1). Similarly, an apicoplast-targeted GpdA is also present. This protein is not recognized by automated tools like other apicoplast-targeted proteins, but still has an 80 amino-acid amino-terminal extension, which contains a signal peptide followed by a region of positively

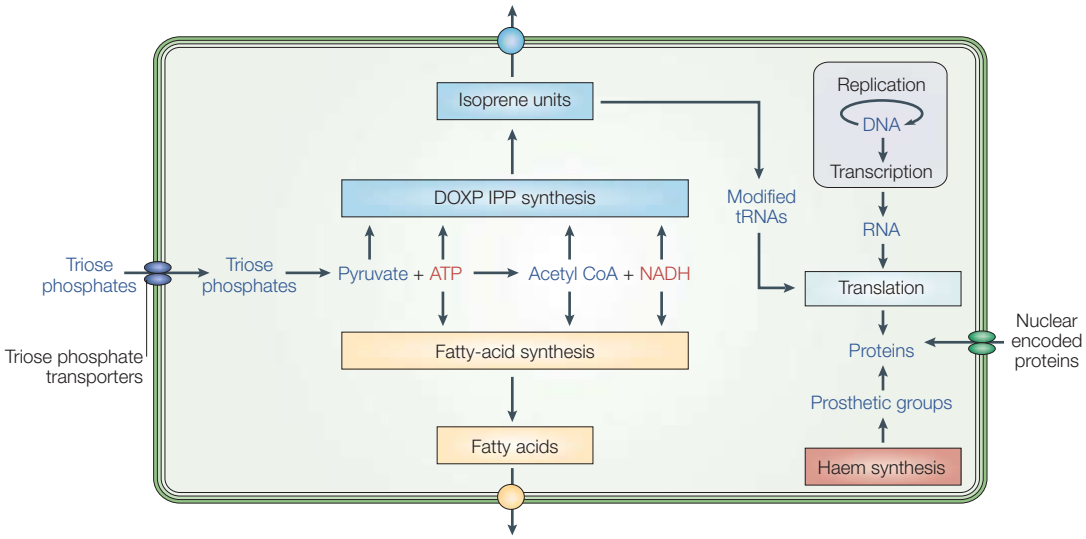


Figure 3 | **Overview of apicoplast metabolism and pathways.** The apicoplast apparently imports trioses that are converted to either fatty acids or isopentenyl diphosphate (isoprenoid precursors) by the DOXP (1-deoxy-D-xylulose-5-phosphate) IPP (isoprenoid precursor) synthesis pathway. These acyl products are likely to be exported for use elsewhere in the parasite cell, perhaps even in formation of the parasitophorous vacuole within the host. Numerous nuclear encoded proteins are imported to join the handful of endogenously produced proteins for these activities.

charged amino acids — similar to the *Plasmodium yoelii* homologue[29] — which indicates apicoplast targeting. So, the combination of the two importers PPT and TPT, with the modifying enzymes TPI, GpdA and pyruvate kinase (PYK), could provide the appropriate substrates and some of the energy and reducing power that are required to drive apicoplast-based anabolic pathways for fatty acids and isoprenoids (FIG. 4).

Dark plastids also require energy and reducing power, which they usually obtain by importing glucose for use in a plastid-localized glycolytic pathway. No evidence has been found to indicate that the apicoplast can import or process hexoses, so its main source of ATP and reducing equivalents is unclear. Similarly, no enzymes for a pentose phosphate pathway have been found in apicoplasts. Plant plastids can import ATP in exchange for ADP using an antiporter that is similar to that of the human pathogen *Rickettsia*[30], but no such transporter has been found in *Plasmodium* species. The mechanism by which the apicoplast satisfies its energy requirements remains unknown. A possible source of reducing power could be import of DHAP and conversion to GA3P by TPI, followed by conversion to 1,3-diphosphoglycerate (1,3-DPGA) by an apicoplast glyceraldehyde-3-phosphate dehydrogenase (GAPDH), with NAD(P)H produced as a by-product. The TPT could export 1,3-DPGA in exchange for import of another molecule of DHAP, thereby creating an electron shuttle (FIG. 4). An apicoplast GAPDH and a cytosolic GAPDH are both present in *Toxoplasma gondii*[31] but, curiously, only one GAPDH isoform has been found in the *P. falciparum* genome[32] and it does not seem to be targeted to the apicoplast[33].

Although no photosynthesis occurs in *P. falciparum*, some terminal components of the electron transport chain — ferredoxin and ferredoxin $NADP^+$ reductase (FNR)[34] — remain as reminders of the photosynthetic ancestry of the apicoplast[35,36]. In photosynthetic plastids, ferredoxin receives electrons from photosystem I and FNR transfers these electrons to $NADP^+$, thereby creating reduced NADPH, which can be used either to generate ATP or as a cofactor in anabolic reactions. In darkness the reverse can occur, and NADPH is reoxidised by FNR to produce reduced ferredoxin[37], which is essential for the activity of several ferredoxin-dependent enzymes. The same FNR-dependent reduction of ferredoxin has been shown in the *Toxoplasma* apicoplast[36]. Vollmer and colleagues propose that apicoplast ferredoxin might be required for apicoplast-located fatty acid desaturases[35]. A stearoyl-CoA desaturase is predicted to be an apicoplast protein (online TABLE S1), but it is unclear from the primary sequence whether or not this enzyme is ferredoxin dependent.

Another role for apicoplast ferredoxin is indicated by the presence of an iron-sulphur [Fe–S] cluster assembly pathway in the apicoplast. Biogenesis of [Fe–S] clusters was previously thought to be located exclusively in the mitochondria of eukaryotes, but this dogma has recently been overturned with the discovery of plastid-targeted[38] assembly enzymes in *Arabidopsis thaliana* and *P. falciparum*[32,38–40]. The sulphur that is required for these enzymes is probably derived from cysteine in the apicoplast, through the action of apicoplast cysteine desulphurase (SufS) (online TABLE S1). Reduction by ferredoxin has been proposed to be important in this step[41], and indeed, the mitochondrial ferredoxin Yah1p is essential for mitochondrial [Fe–S] cluster assembly[41–43]. The apicoplast ferredoxin is likely to have a similar role in apicoplast-located [Fe–S] cluster assembly, with FNR regenerating reduced ferredoxin. Curiously, ferredoxin is itself an [Fe–S]-containing protein, and SufB, which is encoded in the apicoplast genome by a gene previously

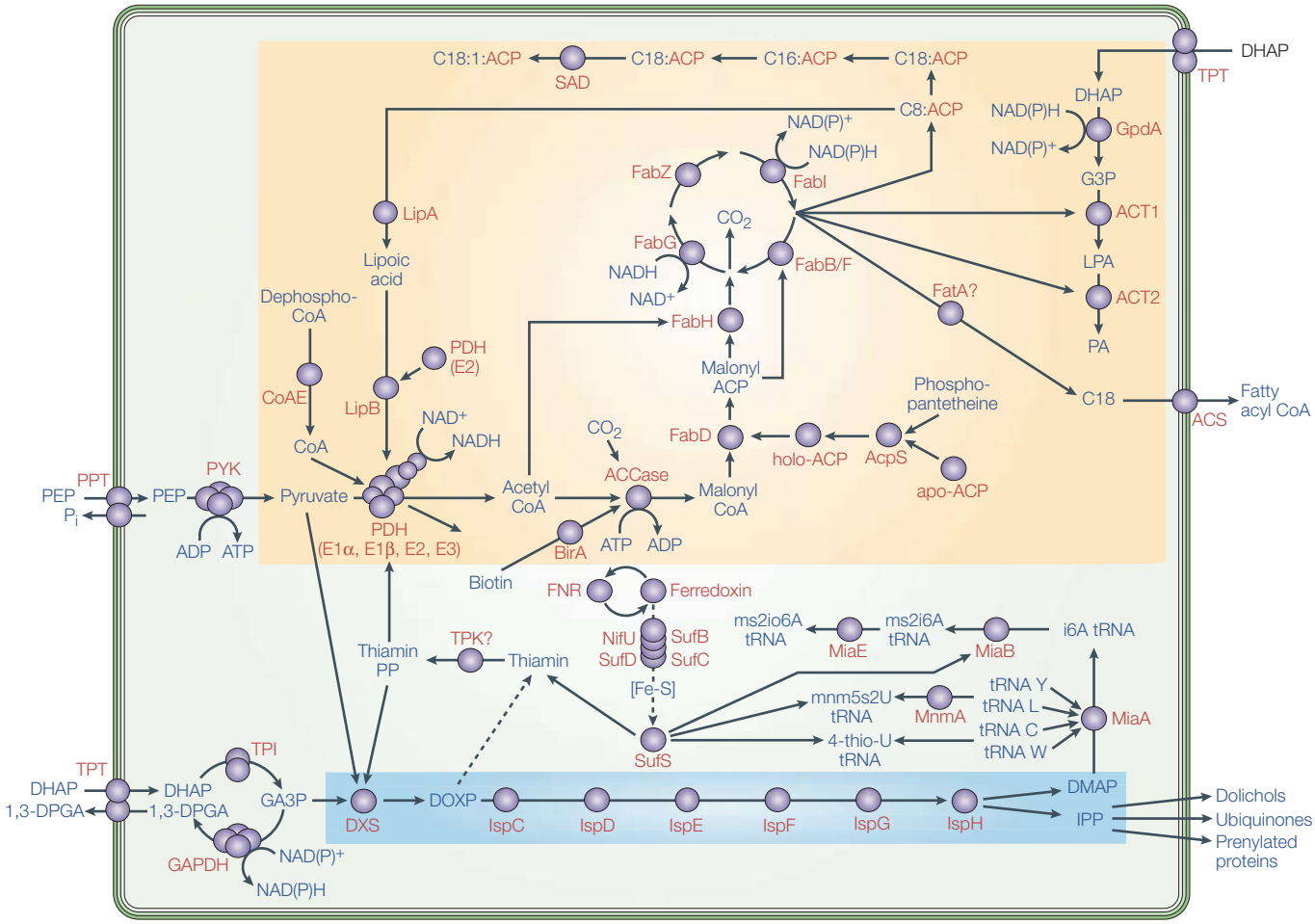


Figure 4 | **Apicoplast fatty-acid and isopentenyl diphosphate biosynthesis.** This scheme presents a model for *Plasmodium falciparum* apicoplast fatty-acid biosynthesis (shaded yellow) and isopentenyl diphosphate biosynthesis (shaded blue) on the basis of predicted apicoplast proteins. Fates for fatty acids and isopentenyl pyrophosphate (IPP) in the apicoplast and proteins with probable roles in exporting fatty acids are presented. Roles for cofactors and prosthetic groups are also shown. Enzyme names are shown in red, substrates and products are shown in blue. ACCase, acetyl-CoA carboxylase; acetyl CoA, acetyl coenzyme A; ACP, acyl carrier protein; ACS, acyl CoA synthetase; ACT1, glycerol-3-phosphate acyltransferase; ACT2, 1-acyl-glycerol-3-phosphate acyltransferase; ADP, adenosine diphosphate; ATP, adenosine triphosphate; BirA, biotin-(acetyl-CoA-carboxylase) ligase; DXS, 1-deoxy-D-xylulose-5-phosphate (DXP) synthase; FabB/F, β-ketoacyl ACP synthase I/II; FabD, malonyl-CoA transacylase; FabG, β-ketoacyl ACP reductase; FabH, β-keto-ACP synthase III; FabI, enoyl-ACP reductase; FabZ, β-hydroxyacyl-ACP dehydratase; FatA, acyl-ACP thioesterase; Ferredoxin, an electron carrier protein; FNR, ferredoxin-NADP(+)-reductase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GpdA, glycerol-3-phosphate dehydrogenase; IspC, DXP reductoisomerase ; IspD, 4-diphosphocytidyl-2*C*-methyl-D-erythritol synthetase ; IspE, 4-diphosphocytidyl-2*C*-methyl-D-erythritol kinase; IspF, 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase; IspG, (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase; IspH, 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate reductase; LipA, lipoic acid synthase; LipB, lipoate protein ligase; LPA, lysophosphatidic acid; MiaA, δ-(2)-isopentenylpyrophosphate tRNA-adenosine transferase; MiaB, tRNA methylthiotransferase; MiaE, tRNA 2-methylthio-*N*-6-isopentenyl adenosine hydroxylase; MnmA, 2-thiouridine modification of tRNA; NAD+/NADH, nicotinamide adenosine; PA, phosphatidic acid; PDH, pyruvate dehydrogenase; PDH(E2), pyruvate dehydrogenase complex E2 subunit; PEP, phosphoenolpyruvate; P_i, inorganic phosphate; PP, pyrophosphate; PPT, phosphoenolpyruvate/phosphate translocator; PYK, pyruvate kinase; SAD, stearoyl-ACP desaturase; SufBCD, SufB–SufC–SufD cysteine desulphurase complex; TPK, thiamine phosphate kinase; TPT, triose phosphate transporter.

known as *orf470* or *ycf24*[44], probably combines with SufC, SufD, SufS and NifU[38,45] (online TABLE S1) to produce holo-ferredoxin from imported apo-ferredoxin (FIG. 4). Cysteine desulphurase presumably generates sulphur for other apicoplast processes, such as the biosynthesis of thiamine and thiol-modified tRNAs, thereby implying a central and essential role for ferredoxin and FNR. The apicoplast-synthesized [Fe–S] clusters are likely to be inserted into LipA, IspG and IspH, enzymes of the fatty acid and isoprenoid pathways, and MiaB (tRNA methylthiotransferase; see below). A separate [Fe–S] cluster generation system is found in the *Plasmodium* mitochondrion.

Isopentenyl diphosphate synthesis

Isoprenoids are a diverse range of compounds, composed of repeated isopentenyl pyrophosphate (IPP) units. They form prosthetic groups on a range of enzymes, and also form the basis of ubiquinones and dolichols, which are involved in electron transport and glycoprotein formation, respectively. The existence of 1-deoxy-D-xylulose-5-phosphate (DOXP) enzymes — sometimes called non-mevalonate enzymes — for IPP biosynthesis in the apicoplast of *P. falciparum* was first reported by Jomaa and colleagues[22] and has only recently been extensively characterized. The DOXP pathway is distinct from the classical acetate/mevalonate pathway, and has

previously only been described in bacteria and chloroplasts[46–50]. We have produced a model for DOXP isoprenoid synthesis in the *P. falciparum* apicoplast, which traces the pathway from its primary precursors to the finished product (FIG. 4).

An important difference between the plastid DOXP isoprenoid pathway and the canonical mevalonate pathway is the starting compounds of each pathway — pyruvate and GA3P in plastids, compared with mevalonate in the eukaryotic cytoplasm[48]. As described above, it seems likely that triose phosphate importers and the subsequent modifying enzymes generate pyruvate and GA3P for use by the first enzyme of the DOXP pathway, DOXP synthase (DXS). DXS generates 1-deoxy-D-xylulose-5-phosphate, which is also used for the biosynthesis of thiamine pyrophosphate (TPP) and pyridoxal[51]. TPP is a necessary cofactor for the apicoplast pyruvate dehydrogenase complex (PDHC), as well as for DXS itself. The final enzyme involved in the synthesis of TPP is thiamine phosphate kinase (TPK)[52,53]. *P. falciparum* has a TPK with an amino-terminal extension resembling an apicoplast leader[32], even though this enzyme is not identified as an apicoplast protein by either of the prediction tools PATS[8] or PlasmoAP[9]. Several other candidate thiamine biosynthetic enzymes (such as ThiF and ThiD) might be apicoplast-targeted in *P. yoelii*, but the location of this pathway remains unclear.

Extending the DOXP pathway beyond the synthase and reductoisomerase (now designated IspC) described by Jomaa and colleagues[22], we searched for *Plasmodium* genes encoding downstream enzymes. In *Escherichia coli*, the product of the IspC-catalyzed reaction (2-*C*-methylerythritol 4-phosphate) is converted to 4-diphosphocytidyl-2-*C*-methylerythritol by IspD (4-diphosphocytidyl-2*C*-methyl-D-erythritol synthase)[54]. A homologue of this protein was reported in *P. falciparum* from the genome database[54], but the relevant fragment is encoded by DNA with a high G+C content (unlike *P. falciparum* DNA) and does not join to any other *P. falciparum* contig[32], so it is likely to be a contaminant. Another match to IspD is found in the *P. falciparum* genome and possesses an apparent apicoplast leader (online TABLE S1). The next step in DOXP isoprenoid synthesis is the conversion of 4-diphosphocytidyl-2-*C*-methylerythritol to 4-diphosphocytidyl-2-*C*-methylerythritol 2-phosphate by the kinase IspE[55]. This in turn is converted by IspF to 2*C*-methylerythritol 2,4-cyclodiphosphate[56], which is reduced to 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate by IspG (previously called GcpE)[57,58]. Homologues of IspF and IspG had already been identified in *P. falciparum*[59,60], and an IspE homologue was found in the *P. falciparum* genome[32]; all three enzymes possess apicoplast-targeting sequences (online TABLE S1). The subsequent and final enzyme of the DOXP pathway, IspH (previously called LytB), is a branch-point enzyme that produces the isoprenoid substrates IPP and dimethylallyl pyrophosphate (DMAPP)[61–63]. An IspH homologue is apparently apicoplast-targeted in *P. falciparum* (online TABLE S1), concluding the DOXP pathway in the apicoplast. IspG and IspH are [Fe–S] cluster proteins[64,65], further confirming the need for apicoplast [Fe–S] assembly. In some organisms, the enzyme IPP isomerase catalyses the interconversion of IPP and DMAPP, but no such enzyme is apparent in the *Plasmodium* genome[32], nor in most bacteria that use the DOXP pathway (including cyanobacteria)[66]. It is therefore highly likely that both IPP and DMAPP are produced in the apicoplast.

The mechanism by which IPP is transported out of plastids is poorly understood, but there are several roles for IPP in *Plasmodium* compartments outside the plastid. The precursors for these extraplastidic isoprenoids are unknown. Many isoprenoids are built from chains of IPP and DMAPP units, which are polymerized by prenyl diphosphate synthases. Several of these enzymes have been found in the *P. falciparum* genome, and all are apparently cytosolically located[32]. One important fate for such isoprene chains is the PRENYLATION of proteins by specific prenyl-transferases, several of which have been identified in *P. falciparum*[67,68]. Prenylation might occur in multiple compartments, but none of the *P. falciparum* prenyl transferases possess apicoplast-targeting leaders, and prenyl transferase activity has only been detected in cytosolic fractions[68]. Further extraplastidic uses for IPP and DMAPP are shown by the ability of *P. falciparum* to incorporate simple isoprenoids into dolichols[69], and the presence of prenyl-containing ubiquinones in mitochondria[70]. Dolichols are essential for the transfer of glycosylphosphatidyl inositol (GPI)-anchors onto membrane-bound proteins[71,72] — which are essential for most of the *P. falciparum* surface proteins[73–75].

Plants satisfy many of the demands for isoprenes through a cytosolic mevalonate pathway in addition to the plastidic DOXP pathway, but no mevalonate pathway is obvious in *P. falciparum*. Indeed, several lines of evidence indicate that *P. falciparum* lacks a mevalonate isoprenoid pathway. First, no mevalonate pathway genes are identifiable in the genome, despite other evolutionarily diverse homologues being highly conserved. Second, parasites show low sensitivity to the mevalonate pathway inhibitor mevastatin[69], and, finally, only very low levels of mevalonate incorporation can be measured[69]. This indicates that the cytosolic, and mitochondrial, demands for isoprene subunits are probably met by the apicoplast DOXP pathway. Such a transfer of isoprenes might explain the close link that is observed between the *P. falciparum* mitochondrion and apicoplast[7,76], and a loss of the mevalonate pathway might have made the apicoplast indispensable in apicomplexans.

Although it remains to be established whether IPP/DMAPP (or a derivative) is exported from the apicoplast, on the basis of genome analysis, utilization in the apicoplast is highly likely. DMAPP is a substrate for tRNA isopentenyltransferase (MiaA), which synthesizes isopentenylated tRNAs. Several plant chloroplast tRNAs have an isopentenyladenosine (i6A) in the anticodon loop[77,78]; the modified base is essential for binding of the charged tRNA to the ribosome–mRNA complex during translation[77]. The i6A modification is also important in the suppression of stop codons and frameshift mutations through altered codon–anticodon interactions[79–82]. The sequences of apicoplast genomes from *Plasmodium*,

PRENYLATION
The addition to a protein of a chain that has been formed by the polymerization of two or more units of isopentenyl pyrophosphate. This attachment can be covalent or non-covalent and is normally found at the carboxy terminus of the protein chain. Signals for the prenylation of a given protein are normally found in the final 3–4 amino acids of that protein.

Toxoplasma, *Eimeria* and *Neospora* have revealed predicted genes with internal stop codons and/or frameshifts[44,83,84], and i6A is probably essential for the correct translation of these sequences. A plastid-targeted version of tRNA isopentenyltransferase has been found in the genome of the plant *A. thaliana*[85], and the *P. falciparum* genome encodes a likely apicoplast-targeted homologue of tRNA isopentenyltransferase (online TABLE S1). It is not known if *P. falciparum* tRNAs are isopentenylated, but four apicoplast-encoded tRNAs ($trnY^{GUA}$, $trnL^{UAA}$, $trnC^{GCA}$ and $trnW^{CCA}$)[86] fit the parameters for modification[87]. Several downstream enzymes act to further modify i6A — MiaB adds a 2-methylthiol group to create ms2i6A, which can be hydroxylated by MiaE to form ms2io6A[88]. Apicoplast-targeted homologues of both these enzymes are found in the *P. falciparum* genome[32] (online TABLE S1). Modifications of tRNAs for the translation of apicoplast-encoded proteins are almost certainly a function of the *P. falciparum* DOXP pathway (FIG. 4).

A complete virtual pathway of plastid DOXP isoprenoid synthesis has been assembled (FIG. 4), which provides a starting point for future biochemical verification. The isoprene units that are formed by this pathway are likely to be used not only for the modification of tRNAs that are essential for apicoplast translation[78], but also for extraplastidic fates such as protein prenylation, mitochondrial ubiquinones and the formation of dolichols[69]. Drugs that target the DOXP pathway[22] might act by blocking the supply of any of these products. The recent use of the IspC inhibitor fosmidomycin in human trials[89] amply demonstrates the potential of this pathway as a drug target.

Fatty-acid synthesis

Until recently, *Plasmodium* species were believed to lack a *de novo* fatty-acid synthesis pathway[90–92]. This was supported by the poor incorporation of simple radiolabelled carbon precursors into lipids of the primate parasite *Plasmodium knowlesi*[93]. Any observed incorporation was interpreted as elongation of scavenged host fatty acids, a conclusion that is supported by the ability of the parasite to use exogenously supplied lipids[94–96]. This dogma has since been challenged by the discovery and characterization of several *P. falciparum* fatty-acid-synthesis enzymes[21,97–102], and the demonstration of acetate incorporation into *P. falciparum* fatty acids[97]. As in all plastid-bearing organisms[103–105], these fatty-acid-synthesis enzymes are targeted to the plastid[7], which strongly implicates the apicoplast as the site of fatty-acid synthesis. As with the IPP pathway, bioinformatic identification of apicoplast proteins reveals a complete biosynthetic pathway for lipids in the apicoplast (FIG. 4).

The main carbon substrate for plastid fatty-acid synthesis is acetyl-CoA, which can either be generated from acetate, by the action of acetyl-CoA synthetase, or from pyruvate by the pyruvate dehydrogenase complex (PDHC). Pyruvate is likely to be generated in the plastid from imported PEP by pyruvate kinase, and recent experiments indicate that pyruvate is the more important carbon source for plastid fatty-acid biosynthesis in plants[103,106–108]. Plastid PDHC comprises four distinct subunits (E1α, E1β, E2 and E3), each of which seems to have originated with the cyanobacterial ancestor of plastids[109]. These cyanobacterial-like subunits are also found in *P. falciparum* and *T. gondii*, and localization studies show that a PDHC is localized to the apicoplast (B.J.F., unpublished observations). *P. falciparum* seems to lack a mitochondrial pyruvate dehydrogenase, although it does possess mitochondrially targeted subunits of the related branched-chain keto-acid dehydrogenase and α-ketoglutarate dehydrogenase complexes[32,110] (B.J.F., unpublished observations).

The enzymatic activity of the PDHC involves three cofactors: lipoic acid, thiamine pyrophosphate (TPP), and coenzyme A. Lipoic acid, in conjunction with the PDHC E2 subunit, facilitates the transfer of an acetyl group to free coenzyme A[111,112]. Lipoic acid is synthesized in both mitochondria and plastids by lipoic acid synthases from an octanoyl-acyl carrier protein (ACP) precursor[113]. An apicoplast-targeted *T. gondii* lipoic acid synthase (LipA) has been characterized[114] and a *P. falciparum* homologue is also predicted to be apicoplast-targeted (online TABLE S1). Lipoic acid is attached to the E2 domain by a lipoate-protein ligase (LipB)[115] — which is found in the plastids of *A. thaliana*[116] — and *T. gondii* and *P. falciparum* LipB homologues are predicted to be apicoplast-targeted[114] (online TABLE S1). Another essential cofactor in the PDHC is TPP, which, together with the E1 subunit, transfers an acetyl group to the E2 subunit lipoic acid moiety[111]. The final enzyme in the synthesis of TPP also seems to be localized to the apicoplast. Finally, coenzyme A, to which an acetyl group is transferred by the PDHC reaction, is synthesized from dephospho-CoA[117] by the enzyme dephospho-CoA kinase. A dephospho-CoA kinase was found in the *P. falciparum* genome[32] and seems to possess an apicoplast-targeting leader (online TABLE S1). These data indicate that all substrates for PDHC, as well as the TPP and lipoic acid cofactors, are synthesized and assembled within the apicoplast (FIG. 4).

The first committed step (often considered to be the rate-limiting step) in plastidic fatty-acid synthesis is the conversion of acetyl-CoA to malonyl-CoA by the large enzyme acetyl-CoA carboxylase (ACCase)[105,118]. In bacteria and most plastids, this enzyme is a multi-subunit complex that is encoded by three or four genes and which has one subunit, AccD, that is often encoded by the plastid genome[119]. An additional single polypeptide isoform that fulfils cytosolic demands is found in most plants[120]. In grasses, a duplication of this eukaryotic (cytosolic) isoform probably replaced the multi-subunit, bacterial-type, plastidic ACCase[120]. A similar replacement has also occurred in diatoms, where the plastidic ACCase is a single, large protein[121,122]. *P. falciparum* and *T. gondii* also have eukaryotic-type ACCases[32,123], which seem to be plastid targeted (online TABLE S1). The grass plastid ACCase is susceptible to the aryloxy-phenoxypropionate class of herbicides[124], but the

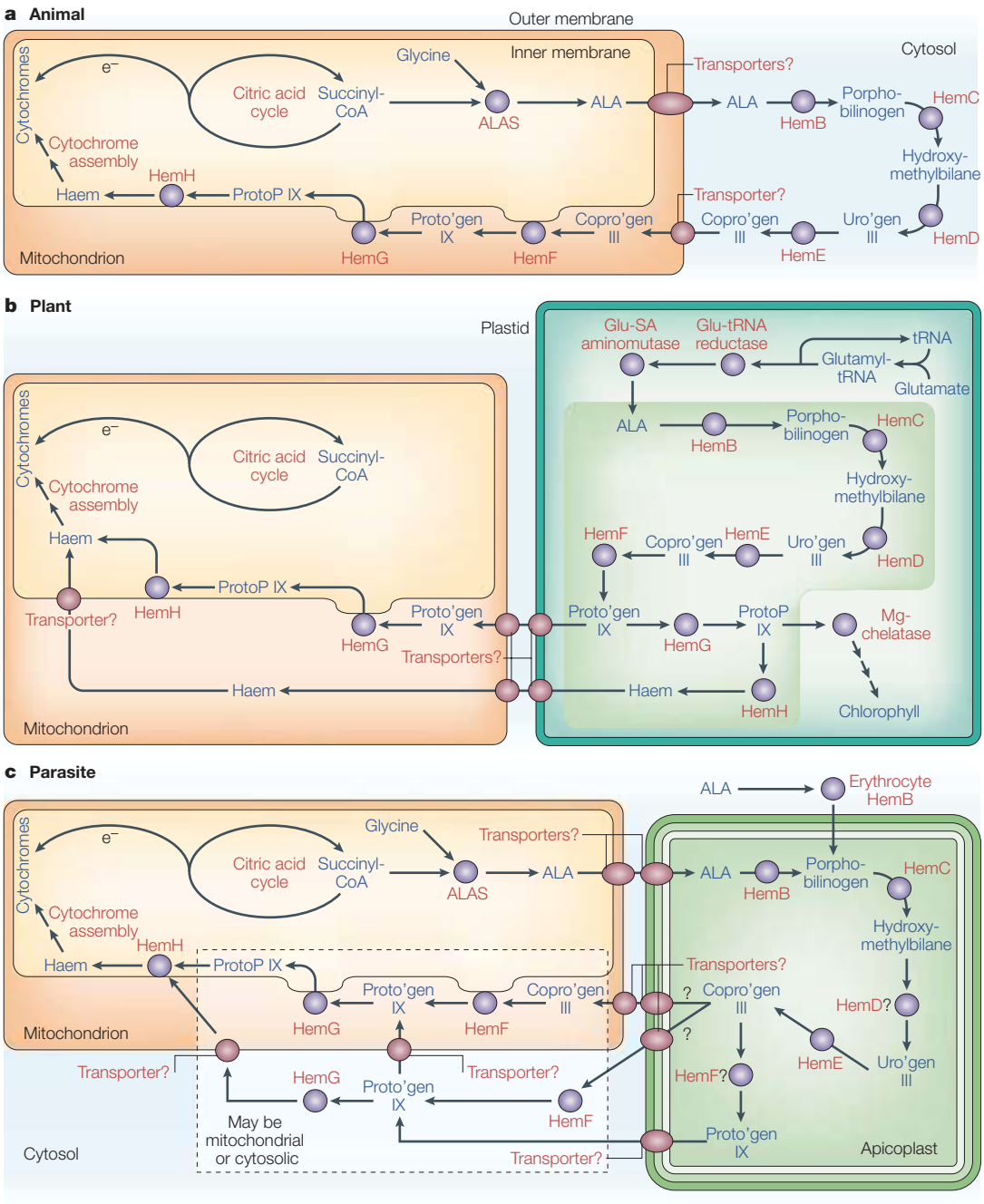


Figure 5 | **A model for *Plasmodium falciparum* haem biosynthesis. a** | Animal/fungal pathway. **b** | Plant pathway. **c** | Putative *Plasmodium* pathway. The *Plasmodium* pathway seems to be split between the apicoplast, mitochondrion and possibly the cytosol. The location of HemF, HemG and HemH is not yet clear. The contribution, if any, of imported host HemB (also known as ALAD) to the parasite haem biosynthetic pathway is also uncertain. ALA, δ-aminolevulinic acid; ALAS, ALA synthase; Copro'gen III, coproporphyrinogen III; Glu-SA, glutamate 1-semialdehyde aminomutase; Glu-tRNA reductase, glutamyl tRNA reductase; HemB, porphobilinogen synthase; HemC, porphobilinogen deaminase; HemD, uroporphyrinogen III synthase; HemE, uroporphyrinogen III decarboxylase; HemF, coproporphyrinogen oxidase; HemG, protoporphyrinogen oxidase; HemH, ferrochelatase; Proto'gen IX, protoporphyrinogen; ProtoP IX; protoporphyrinogen protein X; Uro'gen III, uroporphyrinogen III.

dicotyledonous multi-subunit form is aryloxyphenoxypropionate-resistant[125]. Aryloxyphenoxypropionates kill *T. gondii*[123] and *P. falciparum*[99], probably by targeting the plastidic ACCase[126].

ACCase is a biotin-dependent enzyme (for a review, see REF. 127), with the biotin attached by a biotin-ACCase ligase (BirA). In plants, this ligation reaction occurs in the chloroplast by a plastid-targeted enzyme isoform[128,129]. A biotin-ACCase ligase was found in the *P. falciparum* genome sequence[32] and this enzyme seems to be apicoplast-targeted. Conflicting versions of the N-terminus have been predicted for this gene , but one

convincing model has an N-terminal extension, which consists of a signal peptide and possible transit peptide, so biotin might be attached to ACCase in the apicoplast, which is consistent with avidin staining of the apicoplast[126].

Both the acetyl-CoA that is generated by the PDHC and the malonyl-CoA that is generated by ACCase are substrates for the type II fatty-acid synthase (FAS)[105]. Most apicoplast-targeted type II FAS enzymes have already been characterized in *P. falciparum*; for example ACP, malonyl-CoA transacylase (FabD), β-ketoacyl-ACP synthase III (FabH)[21,98,99,101], enoyl-ACP reductase (FabI)[97], β-ketoacyl-ACP reductase (FabG)[102] and β-hydroxyacyl-ACP dehydratase (FabZ)[100]. The only FAS subunit still to be characterized is β-ketoacyl-ACP synthase I/II (FabB/F). Gene models for *fabF* are conflicting, but several splice possibilities exist, which would create apicoplast-targeting leaders.

ACP is the core protein of type II fatty-acid biosynthesis and holds the growing acyl chain on its phosphopantotheine prosthetic group. The apicoplast ACP[21] is modified with a prosthetic group when it is expressed in *E. coli*[98]. The *acpS* gene, which encodes an ACP synthase that transfers the phosphopantotheine prosthetic group onto apo-ACP to produce the functional holo-ACP, is also encoded in the *P. falciparum* nucleus and is apparently targeted to the apicoplast (online TABLE S1). The phosphopantotheine might derive from the pantothenic acid that is imported from the host[130]. Conversion of pantothenic acid to phosphopantothenic acid could be due to the action of a cytosolic pantothenate kinase (PanK), but it is unclear how and where subsequent conversion to phosphopantothenylcysteine and then phosphopantotheine occurs. Phosphopantotheine is also required for the synthesis of coenzyme A[131,132] — so it is likely that it has several roles in the apicoplast.

The fate of the acyl chains that are synthesized in the apicoplast from imported PEP is not easy to determine from the *P. falciparum* genome. Lipoic acid is required as a prosthetic group on the E2 subunit of PDHC[113] and is probably produced in the apicoplast by octanoyl-ACP (FIG. 4). Other acyl chains are probably used in the production of phosphatidic acids (FIG. 4). G3P, which is produced by GpdA from imported DHAP (FIG. 4), could be acylated by the successive action of apicoplast-located glycerol-3-phosphate acyltransferase (ACT1) and 1-acyl-glycerol-3-phosphate acyltransferase (ACT2) to produce phosphatidic acid (FIG. 4; online TABLE 1). Additionally, the biosynthesis of free fatty acids is predicted by the existence of an apicoplast-localized stearoyl-CoA desaturase (online TABLE S1), which might produce either oleic and/or palmitoleic acids. Free fatty acids are exported from plant chloroplasts by an outer-membrane-bound acyl-CoA synthetase[133,134], although it is not known how the fatty acids (nor in fact the synthetases) arrive at the outer membrane. Acyl-CoA synthetase combines free palmitic or stearic acid with coenzyme A, then exports the acyl-CoA to the endoplasmic reticulum (ER). At least two predicted apicoplast isoforms of this enzyme are encoded in the *P. falciparum* genome (online TABLE S1), which indicates that the apicoplast exports fatty acids into the ER, perhaps from an outer-membrane-resident acetyl-CoA synthetase. Usually, a thioesterase (for example, FatA or FatB) is required to liberate palmitic or stearic acids from ACP[135,136] before conversion to acyl-CoAs and export, but no such enzymes have been found in *P. falciparum*.

The genes for several other phospholipid biosynthetic enzymes (such as those for phosphatidylcholine synthesis) are present in the *P. falciparum* genome, but none has an obvious apicoplast leader sequence[32]. Some are likely to be ER-located, whereas others are proposed to have activity in the erythrocyte cytosol[137]. Inhibitors of these enzymes are promising antimalarial compounds[138–141], reinforcing the importance of lipid biosynthesis in *Plasmodium* parasites.

Among apicomplexan parasites, *Toxoplasma* expresses enzymes associated with both type I (cytosolic) and type II (plastid) fatty-acid-synthesis pathways (M.J.C., unpublished observations). By contrast, *Plasmodium* does not seem to have a type I pathway, whereas *Cryptosporidium* does not have a type II pathway[142]. This is particularly intriguing because *Cryptosporidium parvum* might lack an apicoplast[143]. The retention of type I FAS in a plastid-lacking apicomplexan, contrasted with the presence of a type II FAS in apicoplast-harbouring apicomplexans, supports the absolute requirement for some *de novo* fatty-acid biosynthesis by these parasites, despite their ability to scavenge host lipids. The antimalarial activity of specific type II FAS and plant-like-ACCase inhibitors reinforces the reliance of blood-stage *P. falciparum* parasites on an apicoplast-based pathway, irrespective of the existence of a type I pathway. Importantly, these inhibitors present further valuable candidates for novel antimalarials[21,97,99,144].

Haem biosynthesis

In organisms such as animals and fungi, haem is an end-product of the tetrapyrrole biosynthesis pathway (FIG. 5a) and is used as a prosthetic group in proteins such as cytochromes. In plants, the tetrapyrrole biosynthesis pathway branches and produces both haem and chlorophyll (FIG. 5b). The compartmentalization of, and the initial substrate for, the haem biosynthesis pathway are substantially different in plants compared with organisms that lack a plastid (FIG. 5). How then does the malaria parasite, an organism with a plastid but no ability to synthesize chlorophyll, obtain haem? Despite ingesting vast quantities of haem-rich proteins, *P. falciparum* is capable of *de novo* haem biosynthesis, and probably produces all the haem that is required for viability. In plants, haem synthesis is initiated in the plastid using glutamate and the cofactor $tRNA^{Glu}$ in a similar manner to cyanobacteria[145] (FIG. 5b). However, in *P. falciparum*, haem synthesis is initiated in the mitochondrion — where glycine and succinyl-CoA are converted to δ-aminolevulinate (ALA) by the enzyme δ-aminolevulinate synthase (ALAS; FIG. 5c)[146,147]. Haem synthesis in *Plasmodium* is initiated by an enzyme that is likely to be of α-proteobacterial endosymbiotic origin[148]

Box 1 | Apicomplexan parasites

Apicomplexan parasites include the causative agents of malaria (*Plasmodium* spp), toxoplasmosis (*Toxoplasma gondii*), babesiosis of cattle (*Babesia* spp.), red water or East Coast cattle fever (*Theileria* spp.), coccidiosis of chickens (*Eimeria* spp.) and cryptosporidiosis (*Cryptosporidium parvum*). All apicomplexan parasites studied so far — except *Cryptosporidium* species[143] — contain an apicoplast (see figure part a for a schematic of a parasite containing an apicoplast), and the organelle is indispensable to the parasites. The apicoplast is a vestigial chloroplast (or plastid), which was named after the phylum *Apicomplexa*.

Apicoplast characteristics

The apicoplast has four membranes (shown in the figure part b; a transmission electron micrograph of a *Plasmodium* apicoplast) and has a small circular genome, which contains many genes or sequences that are clearly related to plant and algal plastid genomes[44,83]; taken together this indicates that the apicoplast arose by secondary endosymbiosis. It is still unresolved whether the secondary endosymbiont was a red or green alga[16,31,157–160]. The apicoplast is homologous to, and conceptually similar to, a plant chloroplast — a modified cyanobacterium in a eukaryotic host cell. In common with the chloroplast, the apicoplast has a separate genome, which encodes several metabolic activities. The apicoplast interacts with the environment — the cytosol of the parasite — to import and export many molecules. Malaria parasites contain one apicoplast per cell (figure part a), and replication of the organelle precedes the special form of cell division — known as schizogony; see figure part c, which shows a parasite visualized using a green fluorescent marker — that typically produces 8–24 daughter parasites in each human red blood cell host (schizont; see figure part c). Nothing is known about the activity or morphology of the apicoplast when parasites are in human liver cells or during the mosquito phase, although it must be present because plastids cannot arise *de novo*[161]. Apicomplexan cell division must ensure not only partitioning of organelles, such as mitochondria and nuclei, into each daughter cell, but also the faithful segregation of apicoplasts from generation to generation[7,162].

Apicomplexan parasite evolution

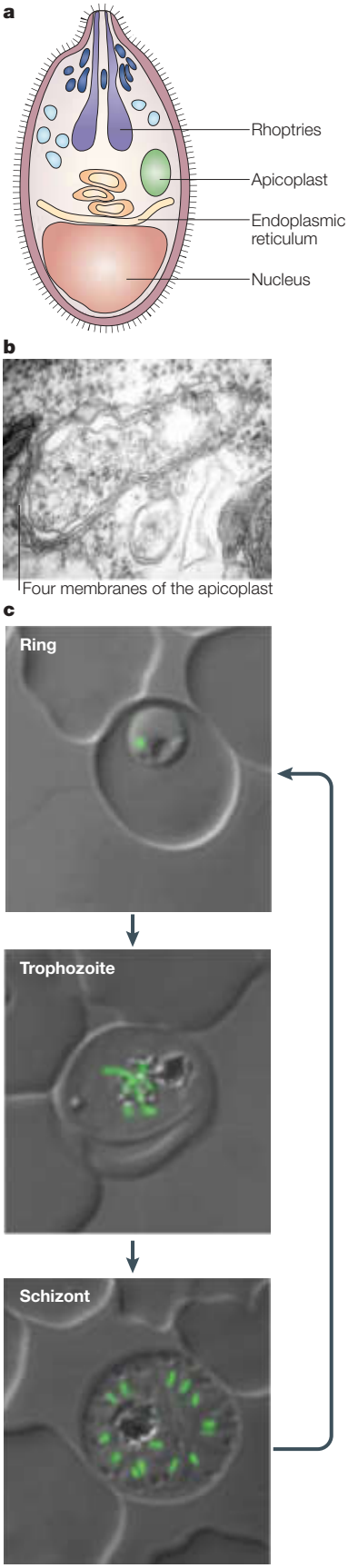
Apicomplexan parasites are both genetically and morphologically most closely related to dinoflagellate algae, and recent gene-sequence data show that both groups acquired their plastids in a single, ancestral secondary endosymbiosis[31,163]. Dinoflagellates and apicomplexans diverged at least 400 million years ago, and although many dinoflagellates have remained photosynthetic, apicomplexans have not retained this ability. Dinoflagellates are common symbionts of marine invertebrates and form mutually beneficial relationships with numerous corals (symbiotic dinoflagellates are also known as zooxanthellae). An attractive evolutionary scenario is that an ancestor of dinoflagellates and apicomplexans had the ability to live symbiotically with animals, and that one descendant lineage (apicomplexans) became parasitic, whereas a second lineage (dinoflagellates) continues to provide photosynthetically derived nutrition to the host. The retention of the apicoplast in the absence of photosynthetic activity is an important unresolved puzzle for researchers.

The apicoplast is indispensable

Two lines of evidence prove that the apicoplast is indispensable. Pharmaceutical perturbation of apicoplast metabolism results in parasite death[4,5,18,45]. Most of these studies have focused on *Toxoplasma* because the response of this parasite to drug treatments can be monitored more readily than other parasites using microscopy[19,164]. Intriguingly, parasites only die in the generation following drug intervention — which is known as 'delayed death'. Transient mutants that are unable to replicate the apicoplast are also non-viable and exhibit a similar delayed-death phenotype[19,20,164,165,178,179,184]. Indispensable apicoplast functions, which are essential to the parasite, are consistent with its retention, despite the loss of photosynthesis. Parasites can survive with no apicoplast (or a pharmacologically deactivated apicoplast) while they remain in the same host cell. However, these apicoplast-compromised parasites — despite visually appearing healthy and growing at a normal rate — are unable to establish a successful new infection. We hypothesise that the apicoplast provides reserves of a resource that is essential for establishing a new infection. One favoured hypothesis is that the apicoplast synthesizes a compound that is exported to the parasite cell for use in the infection process, either directly or indirectly. Identifying the complement of molecules that the apicoplast synthesizes is one way of determining what its vital role might be.

The apicoplast as a drug target

The sensitivity of parasites to apicoplast-perturbing compounds provides an attractive target for drug development. In common with cyanobacteria and plant chloroplasts, apicoplasts are sensitive to most antibacterials. As many antibacterials have excellent safety profiles with well-defined modes of action and mechanisms of resistance, we can rapidly determine which antibacterials could be useful for treating apicomplexan parasite infections. Selected herbicides can also specifically target plastid metabolisms. Some non-toxic herbicides might also perturb the apicoplast and have potential for drug development[22,99,123]. Understanding the delayed-death phenomenon is crucial for drug development strategies, especially in malaria parasites, where it is not well studied, since it could have profound consequences for drug therapy strategies. If the onset of parasite death is delayed, apicoplast drugs might have more value as prophylactics than as treatments for more severe malaria.



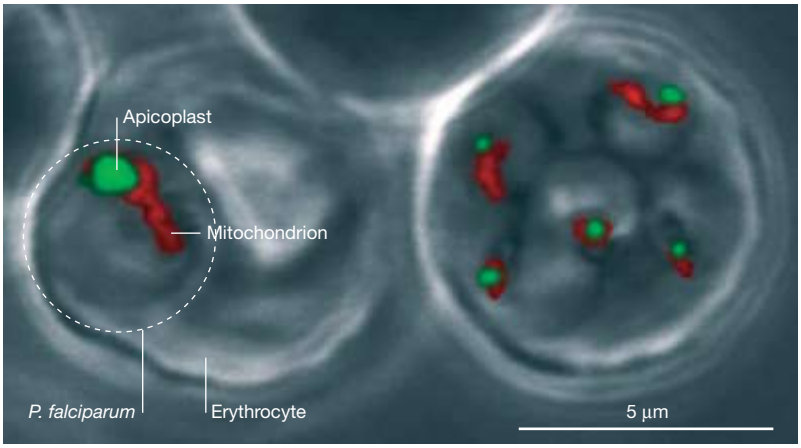


Figure 6 | **Intimate association between apicoplasts (green; GFP) and mitochondria (red; Mitotracker dye) in two infected erythrocytes.** The erythrocyte on the right harbours five parasites.

and is more similar at this stage to the canonical SHEMIN PATHWAY of animal and fungal mitochondria (FIG. 5a). This is congruent with the ability of *P. falciparum* to incorporate radiolabelled glycine into haem, and its inability to incorporate radiolabelled glutamate[149].

The next step of haem biosynthesis is the conversion of ALA to PORPHOBILINOGEN by the enzyme δ-aminolevulinate dehydratase (ALAD or HemB). In plants, this step occurs in the plastid (FIG. 5b), but in animal and fungal cells it occurs in the cytosol (FIG. 5a). The *P. falciparum* genome reveals a gene encoding an apicoplast-targeted HemB[32,150], which is enzymatically active when recombinantly expressed in *E. coli*[151]. Phylogenetic and cofactor analyses indicate that the *P. falciparum* HemB is similar to other plastid and cyanobacterial homologues[150]. Animal cells export ALA to the cytosol for this step (FIG. 5a), but it seems that in *P. falciparum*, ALA must be transferred from the mitochondrion to the apicoplast prior to HemB action (FIG. 5c). There is also evidence that erythrocyte HemB is imported into the cytosol of the parasite[152,153], which indicates that porphobilinogen might also be cytosolically produced.

Two porphobilinogen molecules are condensed into hydroxymethylbilane by porphobilinogen deaminase (HemC) — in the cytosol of animal cells and in the plastid of plants (FIG. 5a,b). A homologue of HemC is predicted to be apicoplast-targeted in *P. falciparum* (online TABLE S1), indicating a continuation of the pathway in the apicoplast, and querying the importance of cytosolic HemB scavenged from the host[153]. The next step of haem synthesis in both plants and animal cells is the flipping, and then closing, of the linear hydroxymethylbilane into a uroporphrinogen III ring by uroporphrinogen III synthase (HemD). A candidate homologue of this enzyme is not obvious in the *P. falciparum* genome[32], but the unclosed hydroxymethylbilane ring is particularly unstable and must be cyclized. HemD enzymes from other organisms have very low sequence conservation, and are not easily identifiable using bioinformatics in many organisms (including plants)[148]. A HemD orthologue is recognizable in the *T. gondii* genome, and is predicted to be targeted to the apicoplast. So, an enzyme that fulfils this function probably exists in *P. falciparum*, particularly as the next enzyme in the pathway, uroporphyrinogen decarboxylase (HemE), is present (FIG. 5c) and contains an apparent apicoplast leader (online TABLE S1).

In animals, the final three enzymes that are involved in the synthesis of protohaem — HemF, HemG and HemH — are associated with the inner mitochondrial membrane, whereas in fungi, HemF is cytosolic (FIG. 5a). In plants, it seems that HemF is localized to the plastid, whereas HemG is targeted to both the plastid and the mitochondrion. There is considerable debate surrounding the localization of HemH in plants, but several recent studies indicate that it is localized exclusively to the plastid[154]. The *P. falciparum* genome contains homologues of all three enzymes (online TABLE S1) and the HemH (ferrochelatase) homologue can complement an *E. coli hemH*$^-$ mutant, which shows that it encodes a functional protein[155]. However, the subcellular localizations of these enzymes in *Plasmodium* are unclear. HemH contains a short N-terminal extension and the *Plasmodium*-trained mitochondrial transit peptide predictor tool, PlasMit[11], indicates that this sequence could function as a mitochondrial transit peptide. The subcellular localization of HemF and HemG is more difficult to determine. HemG lacks an N-terminal extension, although mammalian HemG is targeted to the mitochondrion by unknown internal targeting motifs, which shows that the absence of an N-terminal extension does not prevent mitochondrial localization. *P. falciparum* HemF has a confusing exon/intron structure, which might indicate that the correct start codon has not yet been correctly predicted. Consequently, it is unclear whether this enzyme is localized in the mitochondrion, cytosol or apicoplast compartments. Preliminary evidence from subcellular localization experiments indicates that HemF is cytoplasmic in *T. gondii*, whereas HemG and Hem H are mitochondrial (B. Wu, unpublished observations).

In conclusion, the pathway of haem synthesis in *P. falciparum* presents a curious picture. The initial step of the pathway is clearly mitochondrial[147], but prediction tools indicate that some subsequent reactions of the pathway take place in the apicoplast (FIG. 5c). Furthermore, the steps of the pathway that follow apicoplast involvement seem to be either mitochondrial or cytosolic (or both; FIG. 5), and the pathway probably terminates in the mitochondrion, as is the case in yeast and animal cells. Haem biosynthesis in *P. falciparum* clearly has an intriguing evolutionary history. The acquisition of the secondary endosymbiont must have introduced a second haem-biosynthesis pathway of cyanobacterial origin, but perhaps the loss of photosynthetic pigments (which require porphyrin moieties) allowed part of this, now redundant, plastidic pathway to be lost (FIG. 5). It seems that the subsequent redundancy in the two pathways was resolved by the loss of several steps from each compartment, resulting in a chimeric pathway that is shared between several compartments. The haem synthesis pathway of *P. falciparum* is clearly

SHEMIN PATHWAY
The pathway by which δ-aminolevulanate (ALA) is synthesized from glycine and succinyl CoA in animals, yeast and purple photosynthetic bacteria. In plants and most bacteria ALA is made from glutamate by the C5 pathway.

PORPHOBILINOGEN
An intermediate in the biosynthesis of haem.

fertile ground for future research. Unanswered questions include the identification of a HemD, the role of erythrocyte HemB in the parasite pathway, the localization of several steps of the pathway, and how the parasite coordinates a pathway that is distributed among two organelles and perhaps the cytosol. It is noteworthy that the apicoplast and mitochondrion have an intimate physical association during specific stages of the parasite intra-erythrocytic life cycle[76] (FIG. 6), which might be conducive to substrate exchange.

Conclusions

Most of the genes encoding predicted apicoplast anabolic functions belong to the fatty-acid, haem and isopentenyl diphosphate biosynthetic pathways. Products of the fatty acid and isopentenyl diphosphate pathways have possible fates within the apicoplast, but these two pathways and the haem pathway also produce compounds that are likely to be essential for the whole parasite cell. Haem is required for mitochondrial respiration. Isoprenoids are required for mitochondrial ubiquinones, many prenylated proteins and for the synthesis of GPI and N-glycosylated proteins. Fatty acids are probably exported to the ER, where they are likely to be incorporated into phospholipids, perhaps together with the numerous fatty acids scavenged from the host. A common theme for these metabolic functions is the production and modification of lipids or lipid-bound proteins. All the pathways are likely to be crucial for the interaction between the parasite and the host, particularly in the establishment and regulation of the parasitophorous vacuole. Defects in the biogenesis or maintenance of the parasitophorous vacuole might be a key factor in the delayed-death phenotype. All three important anabolic pathways characterized — synthesis of fatty acids, IPP and haem — include steps that diverge significantly from the analogous pathways found in humans. Some of these differences have already been exploited for the identification of specific inhibitors, and one of these inhibitors — fosmidomycin — has progressed to human trials[156]. The hope is that other apicoplast drug targets will provide the basis for many more urgently required new antiparasitic drugs.

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Competing interests statement

The authors declare that they have no competing financial interests.

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