EXPERT OPINION

Targeting apicoplasts in malaria parasites

Christopher D Goodman[†] & Geoffrey I McFadden
[†]*University of Melbourne, School of Botany, Parkville, Australia*

Introduction: The relict plastid, or apicoplast, is a characteristic feature of *Plasmodium* spp. and reflects the unusual evolutionary origins of these parasites. The essential role this organelle plays in the life of the parasite, and its unusual, non-mammalian metabolism, make the apicoplast an excellent drug target.

Areas covered: This review focuses on the biological role of the apicoplast in the erythrocytic life cycle and what that reveals about existing drug targets. We also discuss the future of the apicoplast in the development of anti-malarials, emphasizing those pathways with greatest potential as a source of novel drug targets and emphasizing the need to understand *in vitro* drug responses to optimize eventual use of these drugs to treat malaria.

Expert opinion: More than a decade of research on the apicoplast has confirmed the promise of this organelle as a source of drug targets. It is now possible to rationally assess the value of existing drugs and new drug targets, and to understand the role these drugs can play in the arsenal of anti-malarial treatments.

Keywords: antibiotics, apicoplast, delayed death, drug targets, isoprenoid, *Plasmodium*

Expert Opin. Ther. Targets [Early Online]

1. Introduction

The health impact of apicomplexan parasites [1] and the speed with which they develop resistance to existing treatments [2] make each discovery of a new source of drug targets exciting news. The discovery of the apicoplast, the reduced plastid found in most apicomplexan parasites [3-5], engendered much excitement because it provided new, prokaryotic drug targets susceptible to existing, clinically approved, drugs. The apicoplast also seemed to present many 'non-eukaryotic' processes with promise as targets for further drug development [6-9]. More than 15 years on from the identification of the apicoplast [3], and almost 10 years from the first comprehensive description of the functions of this organelle [8], it seems an opportune moment to assess the value of this organelle as a drug target and examine its future prospects.

This review will focus on the future of the apicoplast as a drug target in the most medically relevant apicomplexan, the *Plasmodium* spp. parasites that cause malaria. Many valuable reviews provide in-depth understanding of apicoplast biology in other apicomplexans and aspects of apicoplast biology not currently considered as potential drug targets. These topics will only be touched on here.

There are three prospective roles for anti-malarial compounds: prophylactic, therapeutic and transmission blocking. *Plasmodium* has two sites of infection in its human host. Following a bite from an infected mosquito, the parasite establishes itself in liver cells where it undergoes a single replicative phase, producing thousands of daughter merozoites. During this stage, the infected host is asymptomatic and the parasite does not reinvade the liver, so drugs specifically targeting this life stage can only be used prophylactically. Once the merozoites enter the bloodstream and begin replicating in the red blood cells, they produce the classic cyclical fever and other,

10.1517/14728222.2013.739158 © 2012 Informa UK, Ltd. ISSN 1472-8222, e-ISSN 1744-7631

Article highlights.

- The *Plasmodium* apicoplast is an essential organelle containing many unusual, non-mammalian metabolic pathways that are promising drug targets.
- In the erythrocytic life stage, the essential role of the apicoplast is to provide the parasite with isoprenoid precursors.
- Drugs can kill *Plasmodium* by directly targeting isoprenoid precursor synthesis or by disrupting this pathway by interfering with pathways essential for apicoplast survival.
- The isoprenoid, ferredoxin-NADP$^+$ reductase (FNR) and iron sulfur cluster biogenesis pathways are directly involved isoprenoid precursor biosynthesis and contain many promising drug targets.
- Apicoplast functions such as energy metabolism and transmembrane transport represent a pool of essential, but poorly described enzymes that are a source of future drug targets.

This box summarizes key points contained in the article.

more serious symptoms of clinical malaria. Drugs targeting this stage can be used either prophylactically or therapeutically for the treatment of clinical malaria. During clinical infection, a subset of parasites differentiate into the reproductive gametocytes, which can continue the parasite life cycle if they are taken up by a mosquito feeding on an infected individual. Eliminating gametocytes does not directly impact clinical symptoms but drugs targeting this stage are important tools for reducing malaria transmission.

1.1 Origin of the apicoplast

In assessing the potential of the apicoplast as a drug target, a vital and often overlooked question is: how did this organelle arise? The consensus phylogenetic analyses divide eukaryotes into six supergroups and place Apicomplexa in the SAR (stramenophiles, alveolates and rhizaria) supergroup [10,11], which is distinct from both its animal hosts – Opisthokonta – and the green plants – Archaeplastida (**Figure 1**). Apicomplexa started their evolutionary journey to obligate parasitism from an unlikely start point of being photosynthetic organisms; a path recently confirmed by the discovery of fully photosynthetic apicomplexan-like algae. Phylogenetic studies show that these two groups and the dinoflagellate algae arose from a common photosynthetic ancestor [12-15]. What becomes clear from the evolutionary data is that Apicomplexa are neither 'animal cells with a plastid' nor 'parasitic plants'. Rather, they have a unique evolutionary history and it therefore follows that they will have many unique biological characteristics.

The apicoplast is one of the unique biological characteristics of the apicomplexan parasites. Unlike the plant plastid, which is directly derived from a photosynthetic bacterium, the apicoplast is a secondary plastid derived from a photosynthetic eukaryote [12]. An apicomplexan ancestor engulfed a red alga and, over time, incorporated the plastid and the components necessary for its function while discarding the rest of the algal cell [15]. The most obvious remnants of this process are the third (algal outer membrane) and fourth (host cell endosome) membranes that surround the apicoplast and the transfer into the nuclear genome of most of the genes essential for apicoplast function. The presence of these extra membranes, and the need to import nuclear-encoded proteins back across them, create problems not seen in animals and most plants. In consequence, Apicomplexa possess some unusual pathways that are required to circumvent these problems [16-19]. Beyond these unusual characteristics, the apicoplast retains the basic features of a plastid: that is a set of prokaryotic/plant-like metabolic pathways that make this organelle such an exciting prospect as a drug target. However, as the biology of the apicoplast has been revealed, it is increasingly apparent that the apicoplast differs markedly from plant plastids. This divergence of the apicoplast is highlighted in a recent phylogenetic analysis of plastid origins that clearly place the apicoplast in a group that is evolutionarily distinct from plastids of the green plants and Archaeplastida algae [20]. This evolutionary divergence, and the large number of unidentified genes within this novel apicomplexan-related plastid lineage, present both obstacles and opportunities in targeting the apicoplast with anti-malarials.

2. Apicoplast as a drug target

The first complete picture of apicoplast metabolic pathways was generated from a virtual proteome assembled by combining information about the characteristic signals needed to direct nuclear-encoded proteins to the apicoplast with the function of the genes that carried those signals. The *Plasmodium* apicoplast was revealed as a stripped down version of a chloroplast, in which ~ 500 nucleus-encoded and apicoplast-targeted proteins combined with 35 proteins encoded within the apicoplast by its circular genome [4,8]. Of course, the photosynthetic pathways had been lost, but it was confirmed that the apicoplast carried four identifiable metabolic pathways: isoprenoid precursor synthesis, fatty acid synthesis, heme synthesis and iron-sulfur cluster biogenesis. Also present were the components of the 'housekeeping' functions of genome replication, transcription, translation, post-translational modification and protein turnover. In addition to these well-described pathways, other functions such as the import and export of metabolites, production of energy and reducing power and the growth and division of the organelle exist but are poorly understood. Very few molecular components for these latter processes are known, in part because some apicoplast membrane proteins lack canonical targeting motifs [21] and so are not recognized as apicoplast proteins by existing *in silico* techniques. Therefore, the utility of these processes as drug targets is uncertain. Three of the well-characterized apicoplast functions – fatty acid synthesis, isoprenoid precursor synthesis and housekeeping – were clearly bacterial in nature and, therefore, potential targets for existing antibiotic drugs. Many such antibiotics had been tested on *Plasmodium* in culture with some success [22-24]. Nothing, however, was

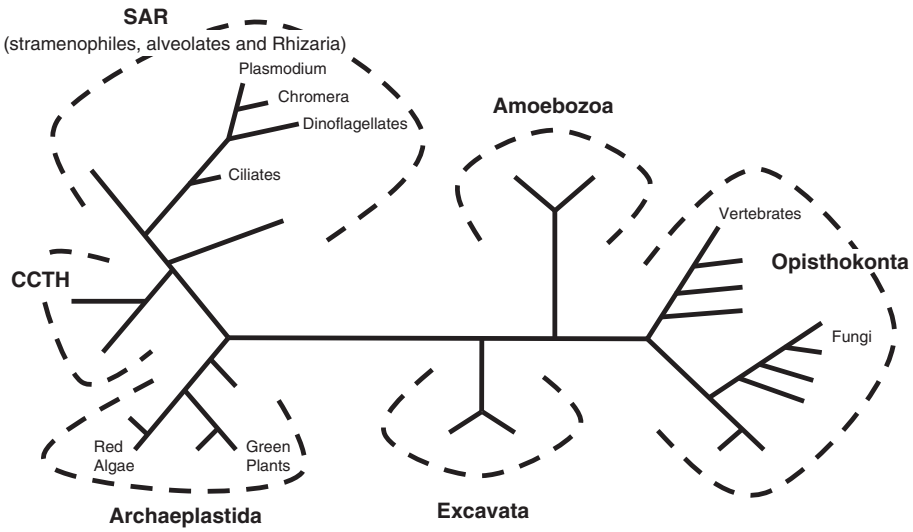

Figure 1. Cartoon of a simplified, unrooted tree of eukaryotes showing the six supergroups (dotted lines) and highlighting the distant relationships between *Plasmodium,* plants and algae, and vertebrates.

Adapted from [10].

CCTH: Cryptomonads, centrohelids, telonemids and haptophytes.

known about their mode of action prior to the characterization of the apicoplast and its metabolism. The discovery of the apicoplast essentially rationalized some hitherto enigmatic activities of antibacterials against a eukaryotic pathogen.

2.1 Apicoplast is essential for parasite survival

The complexity of *Plasmodium* has been a major impediment to quickly identifying targets for known inhibitory compounds; with a significant incidence of off-target effects confounding the interpretation of many drug screens [25,26]. Genetic experiments, both in *Plasmodium* spp. and *Toxoplasma*, proved crucial in elucidating apicoplast biology and the relevance of various drug targets. Interfering with specific apicoplast processes using reverse genetic techniques created a much clearer picture of how the apicoplast functions and its processes are integrated into the functions of the entire parasite.

The first, and most significant finding, arising from these genetic approaches was confirmation that the apicoplast is essential to the survival of the parasite. Overexpression of an unusual fusion protein in *Toxoplasma gondii* disrupted segregation of the apicoplast during cell division. Daughter cells lacking an apicoplast did not survive [27]. This study confirmed the hypothesis of apicoplast essentiality that came from numerous studies showing that inhibitors of prokaryotic genome replication and protein translation killed *Plasmodium* and *Toxoplasma* and had specific effects on the apicoplast [28-31]. Direct target validation, through identification of resistance mutations, for these compounds remains somewhat elusive, with only one such study published to date [32]. Indeed, the parasite response to some compounds originally believed to target apicoplast 'housekeeping' processes actually suggest alternative targets within the parasite [29,33,34].

2.2 'Delayed death' drug response

Studies examining the effect of drugs that target apicoplast 'housekeeping' processes revealed an unusual aspect of apicomplexan biology: the phenomenon of 'delayed death' [28-31]. When growing in human cells, *Plasmodium* spp. (and *T. gondii*) undergo a complex division process to produce multiple daughter cells within a single parasitophorous vacuole. These daughter cells are released by rupture of the vacuole and host cell and go on to invade new cells. When treated with compounds inhibiting apicoplast genome replication, transcription, protein translation, post-translation modification or protein turnover, the parasites continue to grow, divide and release daughter cells that are capable of invading new host cells [29-31,34,35]. However, once these daughter cells establish a new infection, growth fails and no further progeny are produced, hence the term delayed death. The death of the parasite following reinvasion occurs even if drug is removed at this stage [29]. The biology underpinning 'delayed death' remains poorly understood, although it is clearly mediated by the disruption in the apicoplast. Indeed, this characteristic response to compounds affecting 'housekeeping' processes has been used as a way to screen compound libraries for those targeting the apicoplast [36].

The 'delayed death' response has important ramifications for targeting apicoplast 'housekeeping' processes to treat acute infections. The long response time rules out the use of these drugs in a clinical setting, except as the longer-lasting partner in a combination therapy. Drugs targeting apicoplast translation are, however, useful for short-term prophylaxis in travelers because of their good safety profile and absence of resistance thus far. The delayed death phenotype appears to extend to the liver stages of *Plasmodium* infection, with parasites able to complete this first stage in the mammalian host

but producing daughter cells that cannot establish a viable blood cell infection [37]. Indeed, in a mouse model the treatment of artificially intense liver infections with apicoplast drugs induces sterile immunity, so these drugs and the genes they target are being investigated as a component of liver stage vaccines [37].

2.3 Isoprenoid precursor synthesis is the essential apicoplast pathway in the erythrocytic life stage

It has been clear for some time that the apicoplast is essential in red blood cell stages of *Plasmodium,* but it has been less clear why. This question was recently resolved using an innovative chemical rescue strategy. By supplementing *in vitro* cultures of *Plasmodium falciparum* with isopentenyl pyrophosphate (IPP), the product of the apicoplast localized isoprenoid precursor synthesis pathway, the parasite can be rescued from treatments blocking IPP synthesis or more generally inhibiting apicoplast housekeeping functions [38]. Indeed, long-term drug pressure under chemical rescue allows for the elimination of the apicoplast. As long as IPP supplementation continues, apicoplast-free parasites continue normal growth [38], confirming IPP synthesis as the necessary role of the apicoplast in cultured blood stage of *P. falciparum.*

Apicoplast-localized isoprenoid precursor synthesis was one the first apicoplast metabolic pathway to be described and successfully targeted with anti-bacterial drugs [6]. In bacteria and plastids, the seven enzymes of the MEP pathway convert glyceraldehyde 3-phosphate and pyruvate into the isoprenoid precursors (**Figure 2**). Mammals, and most other non-photosynthetic eukaryotes, exclusively employ the mevalonate pathway, which uses acetyl-CoA as a precursor. Plants require both pathways [39], but apicomplexan parasites have dispensed with the eukaryotic pathway and retain only the apicoplast localized MEP pathway. The anti-bacterial fosmidomycin targets the second step of MEP isoprenoid precursor synthesis, DOXP-reductase (DXR) [40,41] and kills *P. falciparum* in *in vitro* blood cell cultures at high nanomolar concentrations [6] and *Plasmodium berghei in vitro* liver stage infections at low micromolar concentrations [42]. Fosmidomycin also proved an effective anti-malarial in mice and humans, but a relatively long and complicated dosing regime and a high rate of recrudescence [43,44] raised questions about its effectiveness as a monotherapy. Subsequently, it has been included in clinical trials as a component of combination therapy (reviewed in [45]).

Despite the early success of fosmidomycin, no other MEP pathway inhibitors have progressed to clinical trials. Indeed, only a few DXR inhibitors have proven more effective than fosmidomycin in *in vitro* trials [6,46-48]. This may reflect the recently reported dual action of fosmidomycin, apparently inhibiting not only DXR but also the downstream enzyme ISPD (**Figure 2**) [49]. Attempts to improve DXR inhibition may impact adversely on ISPD inhibition, rendering fosmidomycin derivatives less effective. Among the other enzymes of the pathway, only IspE has been successfully targeted with small molecule inhibitors in *Plasmodium* [50]. Clearly, there are opportunities to employ other MEP pathway inhibitors against the parasite. The MEP pathway continues to be of interest as an antibiotic, and possibly a herbicide [51], and there are significant opportunities to use compounds identified in other systems as treatments for malaria.

Somewhat surprisingly, given the many years since inhibition of apicoplast, isoprenoid precursor synthesis proved to be a useful malaria treatment, there are little published data on the downstream effects of blocking this pathway, or on the cellular response of the parasite to treatment with fosmidomycin. Recently, it was shown that blocking isoprenoid precursor synthesis in blood stages results in an unusual growth response, with parasites surviving much of the red cell cycle, even undergoing several nuclear divisions, before stalling prior to daughter cell formation [42]. It is not clear at what point treated parasites become non-viable, but this information could prove vital to understanding treatment regimes, recrudescence and the development of resistance, as the ability to delay development to avoid drug pressure appears to be an important tool in the drug resistance arsenal of the parasite [52-54]. Fosmidomycin also blocks apicoplast development [42], but given the ability of IPP rescued parasites to survive without an apicoplast, it seems that this apicoplast-specific effect is not sufficient to kill the parasite. That leaves open the question: which of the many downstream uses of apicoplast-synthesized isoprenoid precursors are vital to parasite survival? Understanding this key metabolic question could provide many drug targets that will act synergistically with apicoplast isoprenoid precursor synthesis inhibitors.

2.4 Relevance of other apicoplast pathways as drug targets

The finding that isoprenoid precursor synthesis is the *raison d'être* of the *Plasmodium* apicoplast in the red blood cell stages greatly clarifies the study of anti-malarials targeting the apicoplast. For clinical therapeutics, it is necessary prioritize targets that directly affect isoprenoid precursor synthesis or overall apicoplast development that underpins it. Other pathways remain important for transmission blocking and prophylaxis, but are deprioritized in developing drugs to treat clinical malaria. With these criteria in mind, the known and hypothesized apicoplast pathways can be organized into three groups: those not necessary for survival in the red blood cell, those with promise as targets but no known inhibitors and those where specific target molecules need to be identified before the search for inhibitors can begin.

2.4.1 *Apicoplast fatty acid and heme synthesis are not essential for erythrocytic life stages*

Two apicoplast metabolic pathways can be eliminated from the search for blood stage targets. Genetic evidence confirmed that apicoplast fatty acid synthesis (**Figure 2**) is not essential in

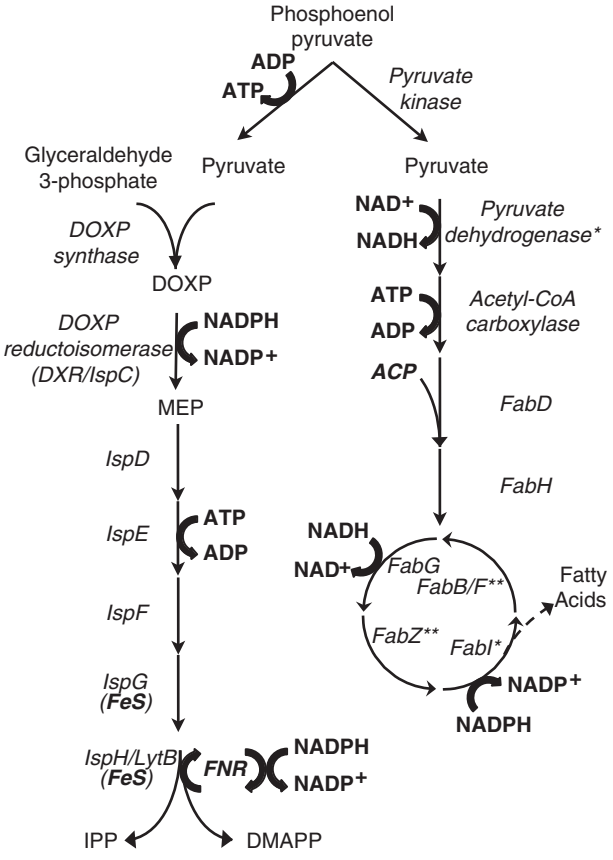

Figure 2. Synthesis pathways of apicoplast localized isoprenoid precursor and fatty acid synthesis. Highlighted are the generation and use of reducing power and ATP, enzymes containing iron-sulfur clusters (FeS), and the site of activity of ferredoxin reductase (FNR). Genes not required for erythrocytic growth in *Plasmodium berghei*/*Plasmodium falciparum** or *Plasmodium yoelii***.

Adapted from [8,44].

ACP: Acyl carrier protein, DMAPP: Dimethylallyl diphosphate, DOXP: 1-deoxy-D-xylulose-5-phosphate, IPP: Isopentenyl diphosphate, MEP: 2-*C*-methyl-D-erythritol 4-phosphate.

the red blood cell stages or gametocytes, eliminating this pathway as a clinical or transmission blocking drug target [25,26,55]. However, fatty acid biosynthesis may yet prove valuable as a prophylactic target as studies in mouse models of malaria show significant effects from knocking out this pathway on liver stages of the parasite. It has not yet been established if fatty acid biosynthesis is essential to human parasite liver stages and even the data from different species of mouse malaria suggest differences in essentiality. For instance, *Plasmodium yoelli* shows an absolute requirement for fatty acid synthesis to progress through the liver stage [26,55] but *P. berghei* fatty acid biosynthesis mutants have a less severe phenotype. Sporozoites from *P. berghei* parasites lacking a key fatty acid gene are less efficient in infecting the liver *in vitro* but show only a limited reduction in infectivity *in vivo* [25]. Sporozoites entering mice via insect bite or intravenously can successfully establish infections in the majority of cases, albeit with a 3 – 4 days delay in the appearance of parasite in the bloodstream [25]. Thus, a clearer understanding of how human malaria parasites respond to loss of fatty acid synthesis in the liver stage is needed before this pathway can rejoin the list of viable drug targets, even as a prophylactic.

The heme biosynthesis pathway is biologically intriguing because of its bizarre hybrid nature in apicomplexan parasites. During their evolution apicomplexans have combined the classic Shemin pathway, whose enzymatic reactions span the mitochondrion and cytosol with the plastid localized pathway found in plants to create a chimeric pathway in which synthesis begins in the mitochondrion and traverses both the cytosol and apicoplast before returning to the mitochondrion for the final steps [18,56-61]. The fact that chemically rescued *P. falciparum* can survive without an apicoplast [38], and hence without the heme synthesizing enzymes this organelle contains, strongly implies that parasite-derived heme synthesis is not required in red blood cell stages. The viability of apicoplast-deficient parasites in sexual or liver stages has not been assessed, so it is difficult to draw conclusions about the role of heme synthesis in these life stages. Mitochondrial activity appears to be much more important when the parasite is outside the red blood cell [62-64], so the heme synthesis may play a role in gametes and liver stage parasites. If this turns out to be the case, heme synthesis may be an important drug target for transmission blocking and prophylaxis.

2.4.2 *Apicoplast pathways with identified targets*

In the evolutionary process that integrated the apicoplast into the single-cell eukaryote that went on to become *Plasmodium* spp., most of the apicoplast genome was transferred from the endosymbiont to the host nucleus [65]. For the apicoplast to survive, the protein products of these genes need to be transported back into the organelle, a process that requires them to transit across four membranes [3,16]. From the perspective of parasite biology, this process presents good prospects as a drug target. Reducing the flow of essential proteins should impact all aspects of apicoplast activity, thereby assuring the death of the parasite. Many of the proteins involved in apicoplast protein targeting have been described and are either unusual variants of more canonical cell process, such as the ERAD complex that is a derivative of the ER protein degradation pathway [17,19,66], or plastid-specific transport proteins [67] that lack homologs in mammalian systems. Two components of this import system, the inner membrane protein Tic20 and the chaperone Tic22, are essential for apicoplast biogenesis and parasite viability in *T. gondii* [68,69]. Many components of this pathway have been characterized but thus far only one compound, 15-deoxyspergualin [70], has been proposed to directly target this pathway in *Plasmodium*. 15-Deoxyspergualin has anti-malarial activity and appears to affect the apicoplast in a manner consistent with inhibition of protein import, but definitive confirmation of the specific target of this compound remains

elusive [70-72]. Other inhibitors of the plant-like components of protein import have been studied, but the *in vitro* nature and mechanistic focus of these studies means that these compounds are not suitable for drug development.

Current evidence suggests that disrupting the apicoplast iron-sulfur biogenesis pathway will impact parasite viability. Iron-sulfur clusters are integral cofactors in many apicoplast enzymes [8], including two enzymes in the isoprenoid precursor synthesis pathway [73]. Targeting this pathway should, therefore, kill the parasite by blocking isoprenoid precursor synthesis. There is another, independent iron-sulfur cluster biogenesis pathway in the mitochondria [74,75], but iron-sulfur clusters are not readily transported between organelles in other systems [74], so mitochondrially produce iron-sulfur clusters are unlikely to rescue parasites from drugs targeting the apicoplast pathway. Unfortunately, there are no reports confirming whether iron-sulfur biogenesis is essential, and no drugs targeting iron-sulfur biogenesis have been identified in *Plasmodium* or other organisms.

Another promising drug target that is linked to the iron-sulfur biogenesis pathway is the ferredoxin-$NADP^+$ reductase (FNR) enzyme of the ferredoxin redox pathway. In energy use, apicoplasts are akin to 'dark plastids' that occur in tissues lacking light for photosynthesis. Dark plastids import sugars to provide energy and reducing power. The ferredoxin redox pathway is essential in these plastids to deliver reducing power to several pathways [76]. In the apicoplast ferredoxin is used for isoprenoid precursor synthesis and iron-sulfur cluster biogenesis [77-79] so it is likely essential for parasite viability. The apicoplast FNR is evolutionarily distinct from mammalian enzymes and should therefore be a viable drug target [78]. Several studies have addressed the structural and catalytic characteristic of FNR in *P. falciparum*, laying the foundation for inhibitor identification and development [77,79,80]. To date, however, no drug-like FNR inhibitors have been published. Somewhat surprisingly, the essential nature of FNR has not been confirmed genetically in *Plasmodium* or in *T. gondii.*

2.4.3 *Unusual but essential targets*

One of the mysteries of the apicoplast is how it obtains sufficient energy. The current understanding of the available sources of adenosine triphosphate (ATP) and reducing power suggest they are insufficient to underpin all apicoplast processes [8]. The only identified source of ATP is as a byproduct of the conversion of imported phosphoenolpyruvate to pyruvate by the apicoplast localized pyruvate kinase, but this process produces only a single ATP that is consumed in the downstream pathways that utilize pyruvate so there is no net production (**Figure 2**). Sources of reducing power, in the form of NADH and NADPH are also unclear, with the only known reaction being the conversion of pyruvate to acetyl-CoA by pyruvate dehydrogenase (PDH) [8,81]. PDH is not essential for apicoplast or parasite survival in the red blood cell [55], so there must be other, non-canonical pathways producing the energy and reducing power for the apicoplast. Such energy producing pathways could provide a rich source of drug targets because of their novel nature and the obvious need for energy to drive apicoplast metabolism.

Another promising but poorly understood aspect of apicoplast biology is the transport of metabolites across the four apicoplast membranes. To survive, the organelle must import metabolic 'raw materials', such as carbon backbones, sources of energy and essential ions and small molecules. Apicoplasts must also export the products of its anabolic pathways, primarily IPP in the red blood cell stage, and any unusable or toxic metabolic byproducts. These tasks are generally performed by membrane transporters, a class of proteins that have been intensively investigated as drug targets in other systems [82-84]. The catalog of *Plasmodium* membrane transporters is extensive [85], but identifying apicoplast-specific transport proteins is complicated by the absence of canonical apicoplast targeting information in many of these large transmembrane proteins [86]. Understanding function is also difficult because of the promiscuous nature of many of these transporters and the difficulty in defining specific substrates based on sequence similarities between species. Only two apicoplast-localized transporters have been thoroughly characterized in *Plasmodium*. These proteins are members of the plastidic triose phosphate transporter (TPT) class and are responsible for transporting phosphoenolpyruvate, a primary source of both energy and carbon backbones, into the apicoplast [21,87]. Work in *Toxoplasma* has identified an ortholog of the *Plasmodium* TPTs [88]. However, beyond the TPTs, the understanding of what transporters are present and why remains rudimentary. This is particularly true for the most important – but as yet unidentified – apicoplast transporter that is responsible for exporting IPP. Based on the known biology of the apicoplast, inhibiting IPP transport out of the apicoplast should have a significant impact on parasite viability. Unfortunately, there is little information on how IPP is moved across membranes in any system. All we know is that IPP is not a substrate of the plastidic phosphate transporters in higher plants [89].

3. Conclusion

The unusual, non-mammalian characteristics of the apicoplast continue to make it a promising target for anti-malarial drugs. In the clinically relevant red blood cell stages, the role of the apicoplast is to provide isoprenoid precursors to the rest of the parasite. The malaria parasite can be killed both by inhibitors (such as fosmidomycin) that directly target the isoprenoid precursor biosynthesis, and by many common antibiotics, which act indirectly by disrupting the viability of the apicoplast itself. *Plasmodium* spp. show specific responses to direct and indirect inhibition, and this provides valuable insight in how best to use these drugs to treat malaria. In addition to the targets of existing drugs, the apicoplast contains proteins such as FNR and the components of the protein import pathway that represent a source of well-characterized, plant-like drug targets that have yet to be exploited. Apicoplast-specific processes such as

energy metabolism and the transmembrane transport of small molecules represent poorly understood, but vital apicoplast processes that should provide a wealth of new drug targets in the future.

4. Expert opinion

Does the apicoplast have a future as a source of targets for anti-malarial drugs? The rapid increase in the knowledge of the biology of the apicoplast has closed off some promising avenues of drug discovery and, at first glance, this may seem to reduce the value of the apicoplast as a drug target. However, a deeper look into both the current knowledge and what remains unknown suggest that we are at the end of the beginning for the study of the apicoplast as a drug target, rather than the beginning of the end. The apicoplast continues to present one of the more promising targets because of the unusual, prokaryotic/plant-like characteristics of its activities. Importantly, the specificity of novel apicoplast drugs can now be assessed with simple cell-based assays and the characteristic drug responses can be used to predict the usefulness of these drugs in combination therapy. These are advantages shared by few other organelles or pathways currently being targeted by drugs in *P. falciparum.*

The evolutionary origins of apicomplexan parasites and the apicoplast point to the possibility of an unusual, non-mammalian biology in *P. falciparum* and studies into the workings of the apicoplast bear this out. The fusion of two types of heme pathways into a multi-organelle spanning synthetic pathway highlights the fact that *Plasmodium* parasites have developed many unique solutions to common biological problems. There remain many aspects of apicoplast biology that promise to yield more novel targets. Some, such as isoprenoid synthesis and transcription involve known, non-mammalian proteins that have not as yet been assessed as targets. Other processes are obviously essential for apicoplast function and parasite survival but are not driven by the genes and pathways that fulfill these functions in other organisms. This is true for two of the most promising areas of study: apicoplast energy metabolism and the import and export of metabolic intermediates. Obviously, a great deal of work is needed to understand the activity and druggability of these targets, but the basic biology of the parasite suggests that such efforts will be worthwhile.

The two clear drug response phenotypes, IPP rescue and delayed death, that definitively identify the apicoplast as the target of inhibitory compounds present an enormous advantage for identifying drugs and understanding their effects. There are many described compounds that non-specifically inhibit *Plasmodium* growth *in vitro*, and a major hurdle in anti-malarial drug development programs is procuring confirmation that the expected target is being inhibited. This problem is exacerbated by the difficulty in using molecular techniques to identify drug targets in *Plasmodium*, a problem highlighted by the ongoing debate over the target of artemisinin [90-94]. Without a confirmed target, linking *in vitro* enzyme inhibition and structure–activity relationships (SAR) to parasite effects become problematic. In some cases, these extensive and time-consuming studies actually provided the first evidence for off-target effects. With the known phenotypes in the apicoplast, this problem is largely eliminated. IPP rescue experiments can definitively show apicoplast targeting. Similarly, delayed-death assays can show which apicoplast process is inhibited. There are few other drug targets where this type of target confirmation can be gathered with simple, cell-based assays.

Understanding how the apicoplast responds to drugs is also an advantage for the development of combination therapies. The 'delayed-death' effect of many compounds targeting the apicoplast limits their effectiveness as monotherapies, but understanding the biological nature of their effects means that they are more easily combined with fast-acting drugs to yield effective combination therapies. Also, those drugs shown to target apicoplast isoprenoid precursor synthesis could be combined with drugs targeting downstream pathways that utilize isoprenoids to improve the efficacy of both drugs.

To take advantage of the apicoplast as a drug target, there are three research goals that need to be met. In the short term, the essential nature of known pathways needs to be confirmed via genetic and/or cell biological approaches. Once confirmed as essential, the process of identifying inhibitors of these pathways should begin in earnest. Over the longer term, elucidating the mysterious aspects of apicoplast biology should be pursued, as this will provide drug targets for the future. Second, efforts should be made to identify the ultimate destination of the apicoplast-synthesized isoprenoid precursors. These downstream processes represent the drug targets most likely to yield synergistic effects with drugs targeting the apicoplast. Finally, efforts should be made to link known drug responses in *in vitro* grown parasites with those responses in *in vivo* systems to better assess the value of targeting the apicoplast in the context of the combination therapies used to treat clinical malaria. It appears that most existing apicoplast targeting compounds produce only a small number of *in vitro* drug responses, so understanding how these *in vitro* responses correspond to *in vivo* outcomes will determine the most appropriate use of apicoplast targeting drugs in the fight to eliminate malaria.

Declaration of interest

The authors have received support from the National Health and Medical Research Council and the Australian Research Council for the completion of this paper. They declare no other conflicts of interest.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. WHO GMP. World malaria report: 2011. World Health Organization; Geneva Switzerland: 2011
2. Hayton K, Su X-z. Drug resistance and genetic mapping in Plasmodium falciparum. Curr Genet 2008;54(5):223-39
3. McFadden GI, Reith ME, Munholland J, Lang-Unnasch N. Plastid in human parasites. Nature 1996;381(6582):482

•• **The first description of the apicoplast in an apicomplexan parasite.**

4. Wilson RJ, Denny PW, Preiser PR, et al. Complete gene map of the plastid-like DNA of the malaria parasite Plasmodium falciparum. J Mol Biol 1996;261(2):155-72

•• **The first description of P. falciparum extrachromosomal DNA molecule bearing plastid-like sequences.**

5. Kohler S, Delwiche CF, Denny PW, et al. A plastid of probable green algal origin in Apicomplexan parasites. Science 1997;275(5305):1485-9
6. Jomaa H, Wiesner J, Sanderbrand S, et al. Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs. Science 1999;285(5433):1573-6

•• **The first description of a apicoplast targeting drug effective both in vitro and as a malaria treatment.**

7. McFadden GI, Waller RF. Plastids in parasites of humans. Bioessays 1997;19(11):1033-40
8. Ralph SA, van Dooren GG, Waller RF, et al. Tropical infectious diseases: metabolic maps and functions of the Plasmodium falciparum apicoplast. Nat Rev Microbiol 2004;2(3):203-16

•• **A comprehensive description of apicoplast metabolic pathways.**

9. Surolia N, Surolia A. Triclosan offers protection against blood stages of malaria by inhibiting enoyl-ACP reductase of Plasmodium falciparum. Nat Med 2001;7(2):167-73
10. Walker G, Dorrell RG, Schlacht A, Dacks JB. Eukaryotic systematics: a user's guide for cell biologists and parasitologists. Parasitology 2011;138(13):1638-63
11. Adl SM, Simpson AGB, Lane CE, et al. The revised classification of eukaryotes. J Eukaryot Microbiol 2012;59(5):429-514
12. Obornik M, Janouskovec J, Chrudimsky T, Lukes J. Evolution of the apicoplast and its hosts: from heterotrophy to autotrophy and back again. Int J Parasitol 2009;39(1):1-12
13. Moore RB, Obornik M, Janouskovec J, et al. A photosynthetic alveolate closely related to apicomplexan parasites. Nature 2008;451(7181):959-63

• **The first description of a photosynthetic apicomplexan-like organism.**

14. Obornik M, Modry D, Lukes M, et al. Morphology, ultrastructure and life cycle of Vitrella brassicaformis n. sp., n. gen., a novel chromerid from the Great Barrier Reef. Protist 2012;163(2):306-23
15. Janouskovec J, Horak A, Obornik M, et al. A common red algal origin of the apicomplexan, dinoflagellate, and heterokont plastids. Proc Natl Acad Sci USA 2010;107(24):10949-54
16. Foth BJ, Ralph SA, Tonkin CJ, et al. Dissecting apicoplast targeting in the malaria parasite Plasmodium falciparum. Science 2003;299(5607):705-8
17. Kalanon M, Tonkin CJ, McFadden GI. Characterization of two putative protein translocation components in the apicoplast of Plasmodium falciparum. Eukaryot Cell 2009;8(8):1146-54

• **The first description of the apicoplast-associated ERAD complex in P. falciparum (with [19]).**

18. Sato S, Clough B, Coates L, Wilson RJ. Enzymes for heme biosynthesis are found in both the mitochondrion and plastid of the malaria parasite Plasmodium falciparum. Protist 2004;155(1):117-25
19. Spork S, Hiss JA, Mandel K, et al. An unusual ERAD-like complex is targeted to the apicoplast of Plasmodium falciparum. Eukaryot Cell 2009;8(8):1134-45

• **The first description of the apicoplast-associated ERAD complex in P. falciparum (with [17]).**

20. Janouskovec J, Horak A, Barott KL, et al. Global analysis of plastid diversity reveals apicomplexan-related lineages in coral reefs. Curr Biol 2012;22(13):R518-19
21. Mullin KA, Lim L, Ralph SA, et al. Membrane transporters in the relict plastid of malaria parasites. Proc Natl Acad Sci USA 2006;103(25):9572-7

• **Characterization of the apicoplast localized plant-type TPTs in P. falciparum.**

22. Divo AA, Geary TG, Jensen JB. Oxygen- and time-dependent effects of antibiotics and selected mitochondrial inhibitors on Plasmodium falciparum in culture. Antimicrob Agents Chemother 1985;27(1):21-7

• **The earliest description of the effects of common antibiotics on P. falciparum in vitro and of the 'delayed death' response to these drugs.**

23. Yeo AE, Rieckmann KH. Increased antimalarial activity of azithromycin during prolonged exposure of Plasmodium falciparum in vitro. Int J Parasitol 1995;25(4):531-2
24. Yeo AE, Rieckmann KH. Prolonged exposure of Plasmodium falciparum to ciprofloxacin increases anti-malarial activity. J Parasitol 1994;80(1):158-60
25. Yu M, Kumar TRS, Nkrumah LJ, et al. The fatty acid biosynthesis enzyme FabI plays a key role in the development of liver-stage malarial parasites. Cell Host Microbe 2008;4(6):567-78

• **Genetic evidence that the apicoplast fatty acid synthesis pathway is not essential in the red blood cell stage in P. berghei and P. falciparum.**

26. Vaughan AM, O'neill MT, Tarun AS, et al. Type II fatty acid synthesis is essential only for malaria parasite late liver stage development. Cell Microbiol 2009;11(3):506-20

• **Genetic evidence that the apicoplact fatty acid synthesis pathway is not essential in the red blood cell stage in Plasmodium yoelii.**

27. He CY, Shaw MK, Pletcher CH, et al. A plastid segregation defect in the protozoan parasite Toxoplasma gondii. EMBO J 2001;20(3):330-9

•• **First direct evidence that the apicoplast is essential in apicomplexan parasites.**

28. Fichera ME, Roos DS. A plastid organelle as a drug target in apicomplexan parasites. Nature 1997;390(6658):407-9
29. Dahl EL, Rosenthal PJ. Multiple antibiotics exert delayed effects against

the Plasmodium falciparum apicoplast. Antimicrob Agents Chemother 2007;51(10):3485-90

30. Dahl EL, Shock JL, Shenai BR, et al. Tetracyclines specifically target the apicoplast of the malaria parasite Plasmodium falciparum. Antimicrob Agents Chemother 2006;50(9):3124-31

31. Goodman CD, Su V, McFadden GI. The effects of anti-bacterials on the malaria parasite Plasmodium falciparum. Mol Biochem Parasitol 2007;152(2):181-91

32. Sidhu AbS, Sun Q, Nkrumah LJ, et al. In vitro efficacy, resistance selection, and structural modeling studies implicate the malarial parasite apicoplast as the target of azithromycin. J Biol Chem 2007;282(4):2494-504

• **One of the few papers definitively identifying the apicoplast target of an anti-bacterial drug.**

33. Lee Y, Choi JY, Fu H, et al. Chemistry and biology of macrolide antiparasitic agents. J Med Chem 2011;54(8):2792-804

34. Aminake MN, Schoof S, Sologub L, et al. Thiostrepton and derivatives exhibit antimalarial and gametocytocidal activity by dually targeting parasite proteasome and apicoplast. Antimicrob Agents Chemother 2011;55(4):1338-48

35. Rathore S, Sinha D, Asad M, et al. A cyanobacterial serine protease of Plasmodium falciparum is targeted to the apicoplast and plays an important role in its growth and development. Mol Microbiol 2010; Epub ahead of print

36. Ekland EH, Schneider J, Fidock DA. Identifying apicoplast-targeting antimalarials using high-throughput compatible approaches. FASEB J 2011;25(10):3583-93

37. Friesen J, Silvie O, Putrianti ED, et al. Natural immunization against malaria: causal prophylaxis with antibiotics. Sci Transl Med 2010;2(40):40-9

38. Yeh E, DeRisi JL. Chemical rescue of malaria parasites lacking an apicoplast defines organelle function in blood-stage Plasmodium falciparum. PLoS Biol 2011;9(8):e100138

•• **Study showing that isoprenoid precursor biosynthesis is the only essential role the apicoplast plays during the erythrocytic life stage.**

39. Rodriguez-Concepcion M, Boronat A. Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics. Plant Physiol 2002;130(3):1079-89

40. Kuzuyama T, Shimizu T, Takahashi S, Seto H. Fosmidomycin, a specific inhibitor of 1-deoxy-d-xylulose 5-phosphate reducoisomerase in the nonmevalonate pathway for terpenoid biosynthesis. Tetrahedron Lett 1998;2239(43):7913-16

41. Zeidler J, Schwender J, Muller C, et al. Inhibition of the non-mevalonate 1-deoxy-D-xylulose-5-phosphate pathway of plant isoprenoid biosynthesis by fosmidomycin. Z Naturforsch 1998;53c:980-6

42. Nair SC, Brooks CF, Goodman CD, et al. Apicoplast isoprenoid precursor synthesis and the molecular basis of fosmidomycin resistance in Toxoplasma gondii. J Exp Med 2011;208(7):1547-59

43. Missinou MA, Borrmann S, Schindler A, et al. Fosmidomycin for malaria. Lancet 2002;360(9349):1941-2

44. Lell B, Ruangweerayut R, Wiesner J, et al. Fosmidomycin, a novel chemotherapeutic agent for malaria. Antimicrob Agents Chemother 2003;47(2):735-8

45. Wiesner J, Reichenberg A, Heinrich S, et al. The plastid-like organelle of apicomplexan parasites as drug target. Curr Pharm Des 2008;14(9):855-71

46. Devreux V, Wiesner J, Goeman JL, et al. Synthesis and biological evaluation of cyclopropyl analogues of fosmidomycin as potent Plasmodium falciparum growth inhibitors. J Med Chem 2006;49(8):2656-60

47. Haemers T, Wiesner J, Giessmann D, et al. Synthesis of beta- and gamma-oxa isosteres of fosmidomycin and FR900098 as antimalarial candidates. Bioorg Med Chem 2008;16(6):3361-71

48. Haemers T, Wiesner J, Van Poecke S, et al. Synthesis of alpha-substituted fosmidomycin analogues as highly potent Plasmodium falciparum growth inhibitors. Bioorg Med Chem Lett 2006;16(7):1888-91

49. Zhang B, Watts KM, Hodge D, et al. A second target of the antimalarial and antibacterial agent fosmidomycin revealed by cellular metabolic profiling. Biochemistry 2011;50(17):3570-7

50. Hirsch AK, Lauw S, Gersbach P, et al. Nonphosphate inhibitors of IspE protein, a kinase in the non-mevalonate pathway for isoprenoid biosynthesis and a potential target for antimalarial therapy. ChemMedChem 2007;2(6):806-10

51. Rodriguez-Concepcion M. The MEP pathway: a new target for the development of herbicides, antibiotics and antimalarial drugs. Curr Pharm Des 2004;10(19):2391-400

52. Witkowski B, Lelievre J, Barragan MJ, et al. Increased tolerance to artemisinin in Plasmodium falciparum is mediated by a quiescence mechanism. Antimicrob Agents Chemother 2010;54(5):1872-7

53. Veiga MI, Ferreira PE, Schmidt BA, et al. Antimalarial exposure delays Plasmodium falciparum intra-erythrocytic cycle and drives drug transporter genes expression. PLoS One 2010;5(8):e12408

54. Teuscher F, Gatton ML, Chen N, et al. Artemisinin-induced dormancy in Plasmodium falciparum: duration, recovery rates, and implications in treatment failure. J Infect Dis 2010;202(9):1362-8

55. Pei Y, Tarun AS, Vaughan AM, et al. Plasmodium pyruvate dehydrogenase activity is only essential for the parasite's progression from liver infection to blood infection. Mol Microbiol 2010;75(4):957-71

56. Nagaraj VA, Arumugam R, Gopalakrishnan B, et al. Unique properties of Plasmodium falciparum porphobilinogen deaminase. J Biol Chem 2008;283(1):437-44

57. Nagaraj VA, Prasad D, Rangarajan PN, Padmanaban G. Mitochondrial localization of functional ferrochelatase from Plasmodium falciparum. Mol Biochem Parasitol 2009;168(1):109-12

58. Varadharajan S, Dhanasekaran S, Bonday ZQ, et al. Involvement of delta-aminolaevulinate synthase encoded by the parasite gene in de novo haem synthesis by Plasmodium falciparum. Biochem J 2002;367(Pt 2):321-7

59. Varadharajan S, Sagar BK, Rangarajan PN, Padmanaban G. Localization of ferrochelatase in

Plasmodium falciparum. Biochem J 2004;384(Pt 2):429-36

60. van Dooren GG, Su V, D'Ombrain MC, McFadden GI. Processing of an apicoplast leader sequence in Plasmodium falciparum and the identification of a putative leader cleavage enzyme. J Biol Chem 2002;277(26):23612-19

61. Nagaraj VA, Arumugam R, Prasad D, et al. Protoporphyrinogen IX oxidase from Plasmodium falciparum is anaerobic and is localized to the mitochondrion. Mol Biochem Parasitol 2010;174(1):44-52

62. Krungkrai J, Prapunwattana P, Krungkrai SR. Ultrastructure and function of mitochondria in gametocytic stage of Plasmodium falciparum. Parasite 2000;7(1):19-26

63. Okamoto N, Spurck TP, Goodman CD, McFadden GI. Apicoplast and mitochondrion in gametocytogenesis of Plasmodium falciparum. Eukaryot Cell 2009;8(1):128-32

64. Young JA, Fivelman QL, Blair PL, et al. The Plasmodium falciparum sexual development transcriptome: a microarray analysis using ontology-based pattern identification. Mol Biochem Parasitol 2005;143(1):67-79

65. Martin W. Gene transfer from organelles to the nucleus: frequent and in big chunks. Proc Natl Acad Sci USA 2003;100(15):8612-14

66. Agrawal S, van Dooren GG, Beatty WL, Striepen B. Genetic evidence that an endosymbiont-derived endoplasmic reticulum-associated protein degradation (ERAD) system functions in import of apicoplast proteins. J Biol Chem 2009;284(48):33683-91

67. Kalanon M, McFadden GI. Malaria, Plasmodium falciparum and its apicoplast. Biochem Soc Trans 2010;38(3):775-82

68. Van Dooren GG, Tomova C, Agrawal S, et al. Toxoplasma gondii Tic20 is essential for apicoplast protein import. Proc Natl Acad Sci USA 2008;105(36):13574-9

• **Study confirming the essential nature of a plant-like protein translocon component in the apicoplast.**

69. Glaser S, Van Dooren GG, Agrawal S, et al. Tic22 is an essential chaperone required for protein import into the apicoplast. J Biol Chem 2012; Epub ahead of print

70. Ramya TNC, Karmodiya K, Surolia A, Surolia N. 15-deoxyspergualin primarily targets the trafficking of apicoplast proteins in Plasmodium falciparum. J Biol Chem 2007;282(9):6388-97

71. Banerjee T, Singh RR, Gupta S, et al. 15-Deoxyspergualin hinders physical interaction between basic residues of transit peptide in PfENR and Hsp70-1. IUBMB Life 2012;64(1):99-107

72. Ramya TN, Surolia N, Surolia A. 15-deoxyspergualin inhibits eukaryotic protein synthesis through eIF2alpha phosphorylation. Biochem J 2007;401(2):411-20

73. Rohrich RC, Englert N, Troschke K, et al. Reconstitution of an apicoplast-localised electron transfer pathway involved in the isoprenoid biosynthesis of Plasmodium falciparum. FEBS Lett 2005;579(28):6433-8

• **In vitro evidence that the FNR system is required for apicoplast isoprenoid precursor synthesis.**

74. Seeber F. Eukaryotic genomes contain a [2Fez.sbnd;2S] ferredoxin isoform with a conserved C-terminal sequence motif. Trends Biochem Sci 2002;27(11):545-7

75. Van Dooren GG, Stimmler LM, McFadden GI. Metabolic maps and functions of the Plasmodium mitochondrion. FEMS Microbiol Rev 2006;30(4):596-630

76. Vollmer M, Thomsen N, Wiek S, Seeber F. Apicomplexan parasites possess distinct nuclear-encoded, but apicoplast-localized, plant-type ferredoxin-NADP+ reductase and ferredoxin. J Biol Chem 2001;276(8):5483-90

77. Milani M, Balconi E, Aliverti A, et al. Ferredoxin-NADP+ reductase from Plasmodium falciparum undergoes NADP+-dependent dimerization and inactivation: functional and crystallographic analysis. J Mol Biol 2007;367(2):501-13

78. Seeber F, Aliverti A, Zanetti G. The plant-type ferredoxin-NADP+ reductase/ferredoxin redox system as a possible drug target against apicomplexan human parasites. Curr Pharm Des 2005;11(24):3159-72

79. Balconi E, Pennati A, Crobu D, et al. The ferredoxin-NADP+ reductase/ferredoxin electron transfer system of Plasmodium falciparum. FEBS J 2009;276(14):3825-36

80. Crobu D, Canevari G, Milani M, et al. Plasmodium falciparum ferredoxin-NADP+ reductase His286 plays a dual role in NADP(H) binding and catalysis. Biochemistry 2009;48(40):9525-33

81. Foth BJ, Stimmler LM, Handman E, et al. The malaria parasite Plasmodium falciparum has only one pyruvate dehydrogenase complex, which is located in the apicoplast. Mol Microbiol 2005;55(1):39-53

82. Kose M, Schiedel AC. Nucleoside/nucleobase transporters: drug targets of the future? Future Med Chem 2009;1(2):303-26

83. Zhou SF, Wang LL, Di YM, et al. Substrates and inhibitors of human multidrug resistance associated proteins and the implications in drug development. Curr Med Chem 2008;15(20):1981-2039

84. Wickenden A, Priest B, Erdemli G. Ion channel drug discovery: challenges and future directions. Future Med Chem 2012;4(5):661-79

85. Martin RE, Henry RI, Abbey JL, et al. The 'permeome' of the malaria parasite: an overview of the membrane transport proteins of Plasmodium falciparum. Genome Biol 2005;6(3):R26

86. DeRocher AE, Karnataki A, Vaney P, Parsons M. Apicoplast targeting of a Toxoplasma gondii transmembrane protein requires a cytosolic tyrosine-based motif. Traffic 2012;13(5):694-704

87. Lim L, Linka M, Mullin KA, et al. The carbon and energy sources of the non-photosynthetic plastid in the malaria parasite. FEBS Lett 2010;584(3):549-54

88. Karnataki A, Derocher A, Coppens I, et al. Cell cycle-regulated vesicular trafficking of Toxoplasma APT1, a protein localized to multiple apicoplast membranes. Mol Microbiol 2007;63(6):1653-68

89. Flugge UI, Gao W. Transport of isoprenoid intermediates across chloroplast envelope membranes. Plant Biol (Stuttg) 2005;7(1):91-7

90. Wang J, Huang L, Li J, et al. Artemisinin directly targets malarial mitochondria through its specific

mitochondrial activation. PLoS ONE 2010;5(3):e9582

91. Arnou B, Montigny C, Morth JP, et al. The Plasmodium falciparum Ca(2+)-ATPase PfATP6: insensitive to artemisinin, but a potential drug target. Biochem Soc Trans 2011;39(3):823-31

92. Eckstein-Ludwig U, Webb RJ, Van Goethem ID, et al. Artemisinins target the SERCA of Plasmodium falciparum. Nature 2003;424(6951):957-61

93. Haynes RK, Chan WC, Wong HN, et al. Facile oxidation of leucomethylene blue and dihydroflavins by artemisinins: relationship with flavoenzyme function and antimalarial mechanism of action. ChemMedChem 2010;5(8):1282-99

94. Kannan R, Kumar K, Sahal D, et al. Reaction of artemisinin with haemoglobin: implications for antimalarial activity. Biochem J 2005;385(Pt 2):409-18

Affiliation

Christopher D Goodman[†1] & Geoffrey I McFadden[2]
[†]Author for correspondence
[1]Research Fellow,
University of Melbourne,
School of Botany, Professor's Walk,
Parkville, Vic, 3010, Australia
Tel: +61 3 83445053;
Fax: +61 3 93475460;
E-mail: deang@unimelb.edu.au
[2]Professor,
University of Melbourne,
School of Botany, Professor's Walk,
Parkville, Vic, 3010, Australia