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Review

Fatty acid metabolism in the *Plasmodium* apicoplast: Drugs, doubts and knockouts

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ABSTRACT

The malaria parasite *Plasmodium* possesses a relict, non-photosynthetic plastid known as the apicoplast. The apicoplast is essential for parasite survival, and harbors several plant-like metabolic pathways including a type II fatty acid synthesis (FASII) pathway. The FASII pathway was discovered in 1998, and much of the early research in the field pursued it as a therapeutic drug target. These studies identified a range of compounds with activity against bloodstage parasites and led to the localization and characterization of most enzymes in the pathway. However, when genetic studies revealed FASII was dispensable in bloodstage parasites, it effectively discounted the pathway as a therapeutic drug target, and suggested these compounds instead interfered with other processes. Interest in FASII then shifted toward its disruption for malaria prophylaxis and vaccine development, with experiments in rodent malaria models identifying a crucial role for the pathway in the parasite's transition from the liver to the blood. Unexpectedly however, the human malaria parasite *P. falciparum* was recently found to differ from rodent models and require FASII for mosquito stage development. This requirement blocked the production of the FASII-deficient forms that might be used as a genetically attenuated parasite vaccine, suggesting the pathway was also unsuitable as a vaccine target. This review discusses how perception of FASII has changed over time, and presents key findings about each enzyme in the pathway to identify remaining questions and opportunities for malaria control.

Contents

Abbreviations: ACC, acetyl-coenzyme A carboxylase; ACP, acyl carrier protein; ACPS, acyl carrier protein synthase; ACS, acyl-coenzyme A synthetase; CoA, coenzyme A; DHAP, dihydroxyacetone phosphate; dim, cyclohexanedione; DOXP pathway, 1-deoxy-D-xylulose-5-phosphate/non-mevalonate isoprenoid precursor synthesis pathway; ER, endoplasmic reticulum; G3PAT, glycerol-3-phosphate acyltransferase; G3PDH, glycerol-3-phosphate dehydrogenase; GAP, genetically attenuated parasite; GFP, green fluorescent protein; FASI, type I fatty acid synthesis; FASII, type II fatty acid synthesis; fop, aryloxphenoxypropionate; PDH, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; pPT, plastidic phosphate transporter.

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1. Introduction

Malaria is the most significant parasitic disease of humans, with over a third of the world's population considered at risk, and approximately 200 million cases reported each year [1]. Malaria is caused by *Plasmodium*, a single-celled protist in the phylum Apicomplexa. The *Plasmodium* life cycle is fascinating and complex, and several reviews provide detailed descriptions of its stages and their potential to be targeted for malaria control [2–5]. Briefly, infection begins when a small number of *Plasmodium* sporozoites are injected into the skin by the bite of a female *Anopheles* mosquito. The sporozoites travel to the liver and invade hepatocytes, where they proliferate asymptomatically to produce tens of thousands of liver stage merozoites. The merozoites are then released into the bloodstream, where they infect red blood cells to begin the replication cycle responsible for the symptoms of malaria. As parasite numbers increase, sexual forms of the parasite called gametocytes also start to appear in the blood. If ingested by a mosquito, these differentiate into gametes and fuse to produce zygotes in the lumen of the insect midgut. Zygotes then develop into motile ookinetes, which traverse the midgut epithelium and transform into oocysts on the outside of the gut wall. Finally, these oocysts divide to produce thousands of sporozoites, which migrate to the salivary glands ready for transmission to the next human host.

Malaria researchers utilize a range of tools to study the biology of *Plasmodium* across its life cycle. The bloodstages of the human malaria parasite *P. falciparum* can be maintained indefinitely *in vitro* [6], and gametocytes can be readily generated through manipulation of the culture conditions [7,8]. Gametocyte cultures can also be used to infect mosquitoes, and the resulting sporozoites can be isolated for infection of human hepatocytes *in vitro* [9]. More commonly, however, studies of the *Plasmodium* mosquito and liver stages rely on rodent malaria models such as *P. berghei* and *P. yoelii*. These models enable the entire *Plasmodium* life cycle to be safely perpetuated *in vivo*, and also allow for blood and liver stage parasites to be analyzed *in vitro* [10]. Complementing these experimental systems are technologies for the genetic modification of each species [11–15] and resources such as the *Plasmodium* genome database PlasmoDB [16]. Malaria research has also benefited enormously from studies in the related apicomplexan parasite *Toxoplasma gondii*. The *T. gondii* life cycle has multiple developmental stages, but the majority of research is focused on the tachyzoite stage, which is easily maintained in nucleated human cells *in vitro*. *T. gondii* is far more genetically tractable than *Plasmodium* and offers a wider range of tools for its modification [17,18]. These features have made the parasites invaluable as a surrogate for *Plasmodium*, and numerous discoveries about shared aspects of biology have emerged from studies in *T. gondii*.

Both *Plasmodium* and *T. gondii* possess an apicoplast, a relict non-photosynthetic plastid homologous to the chloroplasts of plants and algae [19]. The apicoplast was acquired by secondary endosymbiosis, a process by which the ancestor of the parasite engulfed and retained a eukaryotic alga and its plastid [19–22]. This intriguing evolutionary history has resulted in the apicoplast being surrounded by four membranes, and has shaped numerous other aspects of the organelle's biology. Like other plastids, the apicoplast has its own genome and machinery for transcription and translation [23]. However, the vast majority of apicoplast proteins are encoded by the parasite nucleus and must be imported into the organelle, in most cases courtesy of a distinctive two-part targeting sequence at their N-terminus [24,25]. There are approximately 500 proteins putatively targeted to the apicoplast in *P. falciparum*, and a detailed map of the organelle's metabolism has been assembled [26]. The apicoplast harbors pathways with similarities to those in plants plastids and cyanobacteria, including a FASII pathway, a non-mevalonate (DOXP) isoprenoid precursor synthesis pathway, an iron–sulfur cluster assembly pathway, and part of a heme synthesis pathway. These pathways are fed by precursors imported from the cytoplasm and mitochondrion, and produce metabolites and cofactors required for a range of cellular processes [19,20]. These metabolic activities undoubtedly account for why the apicoplast is essential in both *Plasmodium* and *T. gondii* [27–30], although not every pathway appears to be required at each stage of the life cycle.

Since its identification in 1998, the FASII pathway of the apicoplast has received considerable attention as a potential drug target [24,31–33] (Fig. 1). Fatty acids are required for membrane lipid synthesis and other essential cellular processes, and their production represents a central aspect of parasite lipid metabolism. As *Plasmodium* was previously assumed to rely entirely on the host for fatty acids [34,35], the discovery of FASII offered an exciting new opportunity for drug design. Indeed, the pathway appeared to be the ideal target, with no homologue in humans, and a range of existing compounds already established as FASII inhibitors in other organisms [36–38]. When these compounds showed activity against bloodstage parasites [24,39,40], it indicated FASII was essential, and seemingly validated the pathway as a therapeutic drug target. In efforts to develop more potent inhibitors, a myriad of studies were then undertaken in both *Plasmodium* and *T. gondii*. Numerous compounds were tested for inhibition of parasite growth [41–61], and many FASII enzymes were characterized [49,52,55,62–79], allowing further optimization of lead compounds against their putative targets. These studies identified several promising anti-malarial drug candidates, some of which displayed activity against bloodstage parasites at nanomolar concentrations [54,59].

Alongside these drug studies, researchers also began to investigate the fate of fatty acids produced by FASII. Two pathways for