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Review article

Understanding the biology of the *Plasmodium falciparum* apicoplast; an excellent target for antimalarial drug development

Arnish Chakraborty

Malaria Biology Group, International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110067, India

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ABSTRACT

Malaria is a life-threatening tropical disease, caused by the intracellular parasite *Plasmodium falciparum*. The World Health Organization counts malaria as one of the top ten causes of worldwide death. The unavailability of a successful malaria vaccine and the ever-increasing instances of drug resistance in the malaria parasite demand the discovery of new targets within *P. falciparum* for the development of next generation antimalarials. Fortunately, all apicomplexan parasites, including *P. falciparum* harbor a relict, non-photosynthetic plastid known as the apicoplast. The apicoplast is a semi-autonomous organelle within *P. falciparum* containing a 35 kb circular genome. Despite a genome of its own, majority of the apicoplast proteins are encoded by the parasite nucleus and imported into the apicoplast. The organelle has been shown to be essential to *P. falciparum* survival and the loss the apicoplast manifests as a 'delayed death' response in the parasite. The apicoplast has evolved out of cyanobacteria in a complex, two step endosymbiotic event. As a result the architecture and the gene expression machinery of the apicoplast is quite bacteria-like and is susceptible to a wide range of antibiotics such as fosmidomycin, tetracycline, azithromycin, clindamycin and triclosan. The biosynthetic pathways for isoprenoids, fatty acids and heme operate within the malaria apicoplast, making the organelle an excellent target for drug development. The review focuses on the evolution, biology and the essentiality of the apicoplast within the malaria parasite and discusses some of the recent achievements towards the design and discovery of apicoplast targeted antimalarial compounds.

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1. Introduction

Malaria is an infectious parasitic disease affecting large populations dwelling in the tropical and sub-tropical regions of the world. Tropical climate (which favors mosquito breeding) coupled with the poor hygiene, sanitation and public health infrastructure of the under-developed nations have been instrumental in elevating this disease to life-threatening proportions in Sub-Saharan Africa and South-East Asia. The disease is responsible for more than 6,000,000 deaths annually and is a major burden on the public health and economy of the tropical nations [1]. The causative agents are intracellular apicomplexan parasites of the *Plasmodium* species, with *Plasmodium falciparum* being responsible for the highest recorded mortality. The intra-cellular, parasitic life-cycle of *P. falciparum* enables it to efficiently evade host immune responses, as a result of which vaccine development against

E-mail addresses: arnish@icgeb.res.in, arnishchakraborty@gmail.com.

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malaria has been an uphill task with little success [2]. For generations, the major artillery against malaria has been antimalarial medications. However, the widespread use of antimalarial medications in the endemic regions for a prolonged time unintentionally subjects *P. falciparum* to selective drug-pressure. This has resulted in the evolution of drug resistant parasites [3]. Drug resistance in malaria is currently a global health concern in several parts of the world, cutting down the effective life-span of antimalarial drugs and making treatment difficult overtime [4]. Several parasite strains, resistant to front-line antimalarials such as chloroquine, sulfadoxine-pyrimethamine and artemisinin have been identified and reported from different malaria endemic regions of the world [5]. The current scenario is alarming in several regions of Myanmar, Thailand, and Cambodia, where multi-drug resistant malaria outbreaks have compelled the medical authority to shift to the last line of defense; the synthetic derivatives of artemisinin [5]. On the face of ever-increasing reports of drug-resistance, new targets in *P. falciparum* has to be identified and characterized structurally and biochemically to facilitate the development of next-generation antimalarials. Intracellular parasites such as *Plasmodium* and *Toxoplasma* are members of the phylum Apicomplexa. The members of this large phyla of protists are characterized by the presence of a unique plastid-like organelle called the apicoplast [6]. This relict, non-photosynthetic plastid within the malaria parasite is similar to those of plants, houses prokaryote-like metabolic pathways and is believed to have evolved out of an endosymbiotic relationship with cyanobacteria [7]. The apicoplast is indispensable for the survival of *P. falciparum*. The presence of a prokaryotic type gene expression machinery within the apicoplast makes it susceptible to several antibiotics and offers promises of exploiting this organelle for parasite-specific drug development [8,9].

The review presents a focused discussion on the biology of the malaria apicoplast with reference to its origin and essentiality in *P. falciparum*. Mechanism of action of several apicoplast targeting drugs have been discussed which demonstrates the feasibility of exploiting this unusual organelle for novel drug development. The review concludes with the highlights of some of the recently developed inhibitors against *P. falciparum* apicoplast enzymes for parasite-specific malaria chemotherapy.

2. The apicoplast is an unusual organelle

The presence of a unique membrane bound organelle apart from the mitochondrion was observed as early as in the 1960s in *P. falciparum* and other related intra-cellular parasites, despite any information about its function [10]. The apicoplast of *P. falciparum* appears to be a four-membraned structure under electron microscope with distinct granulations [11]. When this organelle was studied in detail, it was found to contain a 35 kb circular genome of its own. The sequence elucidation of this 35 kb circular DNA revealed that its architecture, gene content as well as its arrangement was highly similar to the genomes of plant plastids [12,13]. The discovery that this circular DNA was maternally inherited in the parasite further confirmed its existence to be separate from that of the parasite's nucleus [14]. The difference in the size of this circular DNA and the mitochondrial genome (6 kb) and their apparent lack of co-purification during sub-cellular fractionation, strongly suggested that this organelle was different from the mitochondrion [15]. Striking sequence similarity between the plastid genomes of apicomplexan parasites *P. falciparum* and *Toxoplasma gondii* strongly suggests that the apicoplast biology is strongly conserved across all species of intra-cellular parasites and is inherited from a common ancestor [16]. Using GFP tagged apicoplast markers, Waller et al. traced the dynamics of the apicoplast in live parasitized red blood cells throughout the asexual blood stages of malaria. It was found that each parasite contains only a single copy of the apicoplast and that the apicoplast extensively interacts with the mitochondrion during different life-cycle stages [17]. The fact that the apicoplast and mitochondrion are always observed together during the asexual (human) as well as sexual (mosquito) stages of the parasite suggests strong metabolic dependence between the two organelles [18–20]. The plastids within plants and intracellular parasites such as *Plasmodium* and *Toxoplasma* are believed to have evolved from a symbiotic event where an eukaryotic cell engulfed and established an endosymbiotic relationship was a cyanobacteria-like organism [21]. However symbiotic relationship between *P. falciparum* and its internalized apicoplast is a manifestation of two independent symbiotic events. Primary endosymbiosis is defined as the symbiotic relationship between a prokaryotic and a eukaryotic cell (Fig. 1). The engulfed prokaryote in turn evolves into a plastid and is characterized by galactolipid rich membranes. Primary plastids are commonly observed in plant cells, green and red algae [21]. Secondary endosymbiosis is defined as the symbiotic relationship between two eukaryotic cells in which the eukaryotic cell containing a primary plastid is phagocytized by a second eukaryotic cell, as in the case of the evolution of *P. falciparum* apicoplast (Fig. 1). Along the evolutionary timescale, the engulfed eukaryote loses its eukaryotic features with concomitant transfer of most of its genetic material to the host nucleus. This happens until the internalized eukaryote is stripped down to only its plastid [22]. Secondary plastids thus contain either one or two extra phospholipid rich membranes which are remnants of the former eukaryotic host cell's plasma membrane [10]. A key feature of a typical endosymbiotic organelle such as a plastid is its ability to transfer some of its genes to the nuclear genome during the gradual process of evolution and the *Plasmodium* apicoplast is no exception [23]. Typically, the 35 kb apicoplast genome codes for less than 50 proteins while the vast majority of the enzymes, essential for apicoplast functioning are coded by the nuclear genome of the parasite and are targeted to the apicoplast [24,25]. This protein targeting process is similar to that of the mitochondrion. Nuclear encoded proteins which are destined to be targeted into the apicoplast are tagged with a bipartite transit peptide at the protein's N-terminal end. This transit peptide interacts with the lippo-protein complexes Tic and Toc (translocon at the inner/outer membrane of chloroplast) on the apicoplast surface resulting in subsequent import into the organelle [26,27]. Presence of polycistronic mRNA transcripts and 70S ribosomes within the apicoplast provide direct evidence that the gene expression machinery within the apicoplast is prokaryote-like [28,29]. The apicoplast genome houses a complete set of rRNA and tRNA genes, several essential ribosomal proteins, transcription and translation factors [12]. Several apicoplast specific mRNA and rRNA bound polysomes have been purified from the erythrocytic stages of *P .falciparum* indicating a partial autonomy of this unusual organelle in gene expression and regulation within the malaria parasite [30].

3. The apicoplast is essential to *P. falciparum* survival

The presence of a plastid-like organelle in the malaria parasite had baffled scientists and raised obvious curiosity regarding its role in a parasitic organism. Investigations into the role of this relict plastid in apicomplexan parasites using reverse genetics showed that the organelle was essential for survival. It was demonstrated first in *T. gondii* and later in *P. falciparum*, that interference in the process of apicoplast segregation during parasite division, produced daughter cells which despite being viable failed to divide in the subsequent growth cycles [31,32]. This effect was described as the 'delayed death' phenomenon. Due to the prokaryote-like gene expression in the apicoplast, several antibiotics which target DNA replication, transcription and translation in bacteria also kill the parasite demonstrating a delayed death response [33]. In the erythrocytic cycle of malaria, the parasite undergoes nuclear division followed by cytokinesis to produce daughter merozoites. Parasites treated with apicoplast targeting drugs, divide normally to produce viable merozoites capable of infecting new erythrocytes. However, upon establishment on new infection, growth ceases due to the absence of apicoplasts in these daughter cells and hence no further progeny is produced (Fig. 2) [9,32–34]. Due to this delayed death phenotype, antibiotics such as tetracycline and clindamycin, despite slow acting have