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Relict plastidic metabolic process as a potential therapeutic target

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The alignment of the evolutionary history of parasites with that of plants provides a different panorama in the drug development process. The housing of different metabolic processes, essential for parasite survival, adds to the indispensability of the apicoplast. The different pathways responsible for fueling the apicoplast and parasite offer a myriad of proteins responsible for the apicoplast function. The studies emphasizing the target-based approaches might help in the discovery of antimalarials. The different putative drug targets and their roles are highlighted. In addition, the origin of the apicoplast and metabolic processes are reviewed and the different drugs acting upon the enzymes of the apicoplast are discussed.

Introduction

Despite encouraging advances over the past decade, malaria caused by *Plasmodium* continues to pose an enormous disease burden and is one of the most considerable global health problems. The extreme challenge is the resistance of parasites to conventional monochemotherapies like chloroquine, sulfadoxine-pyrimethamine, piperaquine and artemisinin [1]. No vaccine is yet in sight, and the efficacious drugs are also losing ground against the disease owing to the resistance of parasites. New antimalarials with novel mechanisms-of-action are needed to circumvent existing or emerging drug resistance. The rising severity and resistance against the disease toward the usual therapeutic regimen has put forth exigency for a novel drug target to restrain this disease. A new inclination for development of novel drugs becomes visible when it was discovered [2] that the malaria parasites have unrecognized evolutionary history aligned to plants. The parasites contain a subcellular compartment – the apicoplast – that is homologous to the chloroplast of plants and algae [3]. It is a vestigial plastid found in most parasites of the phylum *Apicomplexa*. The origin has been shaped by intimate interaction between different organisms through the process of symbiosis and parasitism [4]. The organelle is derived from secondary endosymbiosis in which the ancestor of the chromalveolates engulfed red algae [5], resulting in a plastid surrounded by four membranes. Cryoelectron tomography clearly described its four-membrane architecture in which a wider gap between the second and third apicoplast membrane is observed [6]. Out of four membranes, the outermost and periplastid membranes are speculated to originate from the endomembrane system and the algal plasma membrane, respectively. The two innermost membranes are believed to belong to the original chloroplast membranes. The apicoplast is found in close proximity to the nucleus. It is considered as a minibacterium living inside the parasite. Inside this 'cell-within-a-cell' occurs all the housekeeping processes such as DNA replication, transcription, translation and post-translational modifications [7–10]. Despite its minimalization, the apicoplast continues to serve imperative metabolic functions; namely fatty-acid type II (FASII), heme, isoprenoid (IPP), lipoic acid and iron sulfur (FeS) cluster biosynthesis [11–16] (Fig. 1). Morphological, biochemical and bioinformatics studies further reinforced its 'plant-like' characteristics and several features of this organelle contribute to its essentiality in the growth of the parasite [17–21]. This unusual, nonmammalian metabolism of the apicoplast opened a new insight for drug discovery and development. The indispensability of the plastid is supported by studies [22,23] in which parasites cured of their apicoplasts do not die immediately but they fail to invade new

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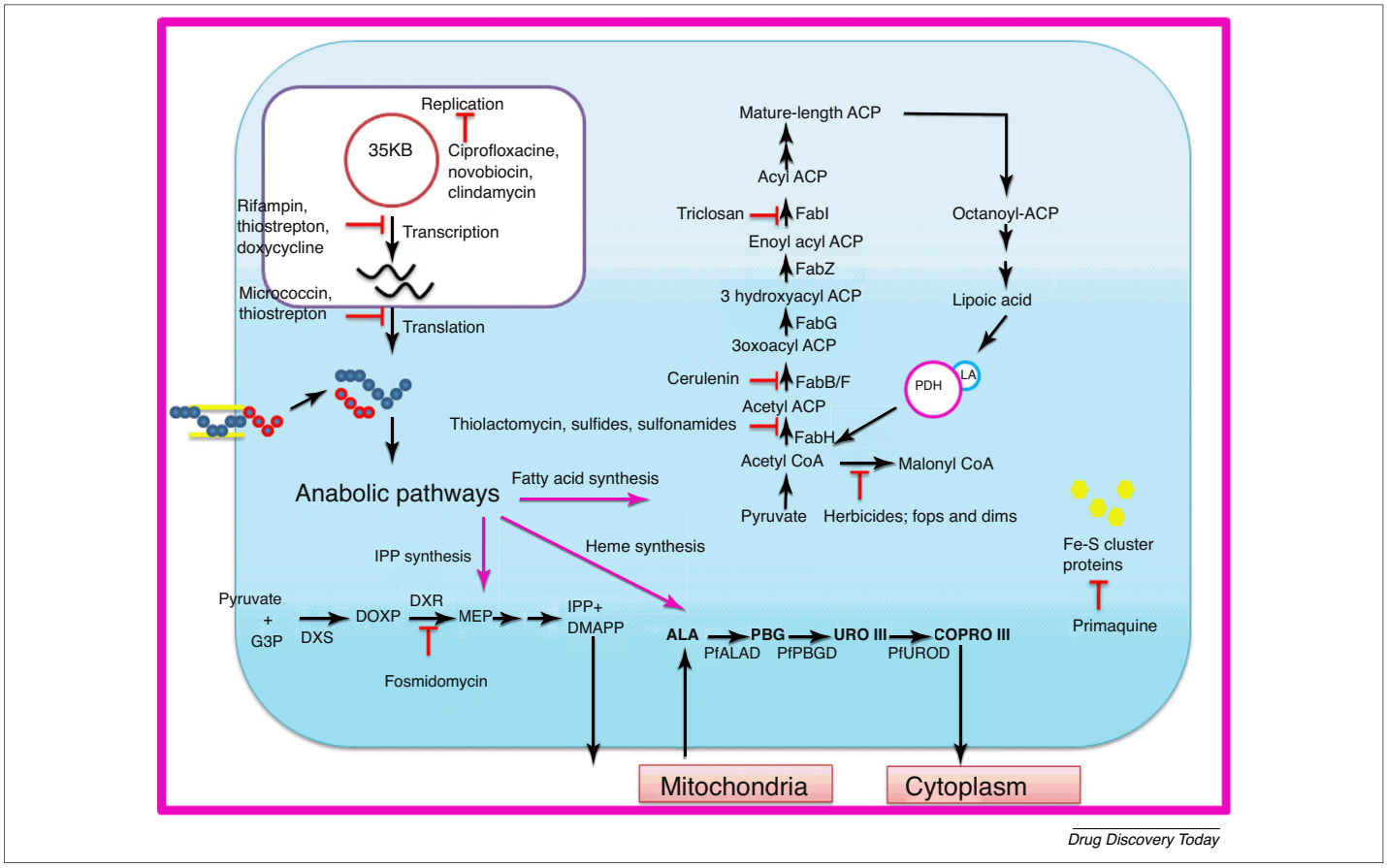

FIGURE 1

Summary diagram of apicoplast-resident pathways. The model is highlighting the apicoplast metabolism along with the drugs and their targets. The arresting of apicoplast metabolism at different stages of the lifecycle will affect the growth and development of the parasite.

host cells successfully owing to their inability to establish a functional parasitophorous vacuole. The results were consistent with the delayed-death phenotype observed for parasites treated with antibiotics targeting housekeeping functions [24].

Morphological changes during lifecycle

During the *Plasmodium* lifecycle and division processes, the single apicoplast is engaged in drastic morphological changes. The single round structure in early intraerythrocytic rings is transformed to highly branched in the trophozoite mid-liver stage and the oocyst in the mosquito [25]. The rod-shaped apicoplast tends to round up into a spherical structure during the early ring stage. The spherical conformation is maintained as the trophozoite grows with a gradual increase in size. At the late trophozoite stage, the morphology of the apicoplast exhibits momentous changes [26]. The changes encompass elongation and development into a multibranched reticulum. The branched form is maintained through successive rounds of nuclear division and a late schizont stage. The merozoite, released upon rupture of the schizont, inherits one apicoplast (per merozoite). The intriguing morphology and timing of apicoplast division is found to co-evolve with the pattern of schizogony.

Apicoplast protein trafficking and gene flow

Throughout evolution, most of the genes coding for apicoplast resident proteins are transferred to the nuclear genome [27]. The sequencing of the full *Plasmodium falciparum* genome and identification of an apicoplast-targeting sequence allowed recognition of several hundred nuclear-encoded proteins that are probably targeted to this organelle. The protein import machinery faces some obstacles during trafficking owing to the presence of extra membranes of the secondary plastid. A specialized transport system delivers these proteins to the organelle across four membranes [27]. Green fluorescent protein (GFP) reporter studies have corroborated that the N-terminal bipartite pre-sequences of apicoplast-targeted proteins are necessary and sufficient for apicoplast import in *P. falciparum*. Soluble proteins are addressed to the apicoplast via bipartite N-terminal extension [28], which is composed of a classical signal peptide followed by a plant-like transit peptide. The signal peptide facilitates the entry of the polypeptide into the secretory pathway, where the signal is cleaved thereby giving access to the following transit peptide. This transit peptide routes the protein toward the organelle and mediates its import [29]. Until now, no common motif for the transit peptide could be identified although the presence of positively charged amino acids appears to be paramount. The transit peptide is known to contain the Hsp70-chaperone-binding domain which is imperative for transit peptide function. The physical interaction between transit peptide and Hsp70 was demonstrated. The model of transit peptide recognition and apicoplast protein import suggested that, after entry into the ER, the transit peptide is bound by BiP (resident ER-Hsp70) and remains

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