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Review

Apicoplast: keep it or leave it

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Abstract

Most Apicomplexans possess a relic plastid named apicoplast, originating from secondary endosymbiosis of a red algae. This non-photosynthetic organelle fulfils important metabolic functions and confers sensitivity to antibiotics. The tasks of this organelle is compared across the phylum of Apicomplexa, highlighting its role in metabolic adaptation to different intracellular niches

Keywords: Apicomplexa; Apicoplast; Metabolism; Isoprenoid; Fatty acids; Iron–sulfur cluster; Haem; Lipoic acid

1. Introduction

The phylum Apicomplexa comprises many medically and economically important, obligate intracellular parasites. *Plasmodium falciparum,* the causative agent of the most deadly form of malaria is responsible for up to two million deaths per year, primarily in sub-Saharan Africa [1]. Infection with *Toxoplasma gondii*, the most widespread parasite of this phylum, leads to significant morbidity in immunocompromised patients and can cause severe congenital defects to the unborn child when primary infection occurs during pregnancy [2]. *Cryptosporidium* sp. is an opportunistic parasite responsible for mammalian diarrhoeal disease [3]. The phylum also includes livestock parasites such as *Eimeria* sp. – that causes coccidiosis in a variety of wild and domesticated animals, including chickens. *Neospora caninum* is responsible for neosporosis, a major cause of abortion in cattle. Two additional Apoicomplexan are responsible for tick borne severe diseases of cattle, *Babesia bovis* and *Theileria parva* the etiologic agent of East Coast fever [4].

In accordance to their diverse niches and lifestyles the apicomplexans exhibit different metabolic needs that reflect in the composition of their genome by the maintenance or loss of metabolic pathways [5]. The intraerythrocytic stages of *Plasmodium* are surrounded by a parasitophorous vacuole (PV), express, amongst others, a hexose phosphate transporter PfHT [6] and remodel the permeability of the infected red blood cells to small solutes increasing access to nutrients [7]. In contrast, *T. gondii* can replicate inside a non-fusogenic PV in most types of nucleated cells, entering in direct competition with the host cell for nutrients. *Toxoplasma* genome encodes for a broad range of metabolic pathways, some of which are not found in other apicomplexans [5]. *Cryptosporidium* infects mainly enterocytes of vertebrates and lacks many metabolic pathways and hence exhibits numerous auxotrophies. Remarkably this parasite possesses a considerably reduced mitochondrion, called mitome, which lacks a genome and the ability to produce ATP. *Theileria* and *Babesia* are directly accessing host cell metabolites as these parasites rapidly escape their vacuole and replicate freely in the cytosol of lymphocytes and erythrocytes, respectively. Their adaptation to these niches is reflected by the loss of numerous metabolic pathways and the abundance of transporters as compared to *Plasmodium* or *Toxoplasma* [8].

Abbreviations: FASII, Fatty-acid type II; IPP, Isopentenyl-pyrophosphate; [FE–S], Iron–sulfur; PDH, Pyruvate–dehydrogenase complex; DOXP, 1-Deoxy-D-xylulose 5-phosphate; MEP, 2-C-methyl-erythritol 4-phosphate; GA3P, Glyceraldehyde-3-phosphate; FA, Fatty acid; PEP, Phosphoenol-pyruvate; TPI, Triosephosphate-isomerase; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; PGK, Phosphoglycerate kinase.

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The apicomplexans possess a plastid-like organelle termed apicoplast [9,10] that is found in nearly all members of this phylum, with the exception of *Cryptosporidium* sp., which appears to have lost it [11]. This organelle is derived from a secondary endosymbiosis, when the ancestor of the chromalveolates engulfed a red algae, resulting in a plastid surrounded by four membranes [12]. The outermost and periplastid membranes are presumed to originate from the endomembrane system and the algal plasma membrane, respectively. The two innermost membranes are believed to correspond to the original chloroplast membranes [13].

By adopting an intracellular life style, the apicomplexans have lost the genes that conferred the ability to harness light as an energy source. Interestingly, *Chromera velia* was recently identified as the closest relative of this phylum that is still capable of photosynthesis [14].

The apicoplast genome is the smallest described so far for plastids, with a size of 35 kb encoding only for 30–50 housekeeping genes, mainly involved in transcription and translation and a few additional unknown ORFs [15]. Throughout evolution, most of the genes coding for resident proteins of the apicoplast have been transferred to the nuclear genome and their products are targeted to the organelle. Soluble proteins are addressed to the apicoplast via a bipartite N-terminal extension, 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 towards the organelle and mediates its import [13,16]. Until now, no common motif for the transit peptide could be identified although the presence of positively charged amino acids appears to be essential for successful trafficking [17].

Genome database mining for proteins harbouring a bipartite signal led to the identification of around 500 genes that represent 5–10% of the apicomplexan genomes [18]. More recently several proteins were localized to the apicoplast, despite the absence of a bipartite targeting signal. All these proteins seem to be located in – or adjacent to – the membranes of this organelle. It is assumed that trafficking of these proteins is mediated by an internal hydrophobic sequence that probably acts as an internal signal sequence [19]. In consequence, the number of proteins targeted to the apicoplast might be considerably underestimated.

The apicoplast is essential for parasite survival, and drugs targeting this organelle are mainly causing the so-called delayed death phenomenon but our understanding of the vital aspects of the organelle is fractal. By comparing several complete apicomplexan genomes it became clear, that the apicoplast is heterogeneous and fulfils different purposes depending on the niche and life style occupied by each apicomplexan. Strikingly, the apicoplast of *T. gondii* and *Plasmodium* spp. harbours a number of common pathways; namely a fatty-acid type II (FASII), haem-, isoprenoid- (IPP), lipoic acid and iron–sulfur [Fe–S] cluster biosynthesis [20]. In contrast in *Theileria* and *Babesia* species, the organelles only hosts the isoprenoid and Fe–S cluster biosynthesis pathways (Fig. 1A, B; Table 1). The server addresses to access the detailed pathways hosted by the apicoplast are listed in Table 2.

2. One for all – the isoprenoid biosynthesis

Isoprenoids are an important class of lipid compounds that play key roles in a variety of cellular processes. They are used for prenylation of proteins, which takes place in the cytosol and mitochondria, as a precursor for abscisic acid and in vitamin B6 biosynthesis [20–23]. Thiamine pyrophosphate synthesis is also dependent on a functional isopentenyl-pyrophosphate (IPP) biosynthesis, as it uses an intermediate product of this pathway. Thiamine serves as cofactor for the E1-subunit of the PDH-complex and the DOXP-synthase in the apicoplast of *P. falciparum* and *T. gondii*. The tRNAs are among the other IPP consumers that are usually isoprenylated. These modified tRNAs are needed for the correct translation of the organellar genome.

In mammals, archebacteria, higher plants and yeast, IPP is synthesized via the mevalonate pathway, which starts with the generation of the precursor, mevalonate from acetyl-CoA. In 1993, an alternative pathway leading to IPP production was discovered in bacteria and plants [24] and subsequently shown to be hosted by the apicoplast [25]. This pathway utilises pyruvate and glyceraldehyde 3-phosphate (GA3P) as substrates to produce 1-deoxy-D-xylulose 5-phosphate (DOXP) that is then used for IPP synthesis. In a second step DOXP is converted into 2-C-methyl-erythritol 4-phosphate (MEP) and the process is therefore called DOXP/MEP or non-mevalonate pathway. With the exception of *Cryptosporidium*, all apicomplexans are predicted to import the seven enzymes of the DOXP/MEP biosynthesis pathway into apicoplast and some of them have been experimentally confirmed [20,25] (Table 1; Fig. 1A, B). In *Plasmodium* this pathway has been intensively studied and is considered as a promising target for therapy [26]. Fosmidomycin, a specific inhibitor of the DOXP reductoisomerase, is an effective against *Plasmodium* parasites *in vitro* and *in vivo*, leading to immediate death of the parasite [25]. Despite the fact that all genes of the DOXP/MEP are present and expressed in several developmental stages of *T. gondii* and *Eimeria tenella* [27,28], fosmidomycin is poorly effective against these parasites. Moreover, a recent study showed that *Theileria* species are not affected by this drug [29]. This differential sensitivity to the drug might be explained by a poor bioavailability due to the absence of specific transporters. Indeed, the plasma membrane of *P. falciparum* infected red blood cells is dramatically modified by the introduction of the so-called new permeation pathways, which gives access to numerous molecules [30]. Such a perturbation of the permeability of the host cell plasma membrane does not appear to occur in the nucleated cells infected by *T. gondii* and *Theileria* sp. [29]. Although the site of synthesis of isoprenoids is exclusively limited to the apicoplast, these molecules are incorporated in different cellular compartments and it is unclear how IPP crosses the four membranes of the apicoplast.