PARASITES AND PLASTIDS: BRIEF ROADMAP OF A COMPLEX RELATIONSHIP

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Received: 20.11.2018
Accepted:28.11.2018

Abstract.

Phylum apicomplexa form an important group of pathogens that include many parasitic organisms of medical and veterinary importance. Major members of that phylum that are responsible for debilitating diseases in humans and animals are Toxoplasma, Plasmodium, Babesia, Theileria, Eimeria and Cryptosporidium. Lack of effective vaccines and emerging resistance against available drugs demands improved and effective new strategies for prevention and treatment of the diseases caused by these pathogens. One curious and important organelle shared by these pathogens is named as apicoplast because of its similarity to plant chloroplasts. Apicoplast is indispensable for parasites belonging to phylum apicomplexa as it provides the parasites with important metabolic intermediates. The metabolic pathways hosted by the apicoplast that provides these essential metabolites are different from the corresponding pathways in the host organisms as the apicoplast has prokaryotic origins. This makes apicoplast and its metabolic pathways important and divergent drug targets that could be utilized to develop improved chemotherapeutic agents against apicomplexan parasites. The current review focuses on the origins of the apicoplast, the assimilation of the apicoplast after its acquirement, the chief reasons for its retention and the available drug targets against the essential cellular and metabolic pathways in the apicoplast.

Eukaryotic life is divided into four main kingdoms, animals, plants, fungi and protists. Protists are eukaryotic organisms with a very diverse life styles and includes organisms that are unicellular or unicellular colonies without any complex organizations. Apicomplexans form an important phylum under the kingdom Protista that includes a wide range of parasitic organisms. Most of the members of the phylum are obligate intracellular parasites, meaning they require life inside a host cell for the completion of its life cycle. Members of phylum Apicomplexa cause major parasitic diseases in humans and animals. Important pathogenic members are the

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Plasmodium , Toxoplasma, Babesia, Eimeria, Theileria and Cryptosporidium. Plasmodium is responsible for malaria, a lifethreatening disease responsible for half a million deaths a year. Toxoplasma, on the other hand is the most ubiquitous pathogen in the planet with about 75% of world's population infected without any apparent clinical symptoms, although the disease can be deadly in immunocompromised conditions. Other members of the phylum like Eimeria, Babesia and Theileria are responsible for important diseases in the animals, while Cryptosporidium is responsible for fatal diarrheal disease in infants.

Apicomplexans are named so because of the presence of the "apical complex" that is usually used for the purpose of cellular invasion and establishment of infection inside the cell (Morrissette *et al.,* 2002). Another important feature of the members of this phylum is the presence of a chloroplast like organelle, aptly named as the apicoplast *ie.* **apico**mplexan chloro**plast** (Lim *et al*., 2009). This short reviews aims to shed some light on the origins of this important organelleand the complex relationship that led to its retention through evolution. Finally , the efficacy of this organelle as an important drug target and a possible "achilles heel" for this diverse group of pathogens is also discussed briefly.

Discovery and origins of apicoplast

Apicoplast, the apicomplexan chloroplast, traces its origin to a photosynthetic chloroplast in another eukaryote , most probably a red algae (Janouškovec *et al*., 2010). Most chloroplast that we see in plants today are originated through endosymbiotic events through evolution, like mitochondria. Endosymbiosis is defined as a process through which one organism engulfs another for evolutionary benefits. The relict genome associated with this newly acquired endosymbiont is the primary evidence towards the independent existence of the endosymbiont before the acquirement process. For example, mitochondria in all eukaryotic cells has a very reduced genome for itself indicative of its free existence in time. The discovery of apicoplast in apicomplexan parasites was also based on the discovery of this extra chromosomal genome that pelleted differently in a density gradient centrifugation of parasite extract (Gardner *et al.,* 1991). The presence of this non nuclear 35Kb genome paved way to speculations of another endosymbiotic organelle, other than mitochondria that had a 6Kb genome size. Sequencing of the genome and further analysis speculated that this newly discovered organellar genome was similar to chloroplast genome in plants. The physical presence of apicoplast was first visualized through insitu hybridization using probes targeted against the apicoplast genome in *Toxoplasma gondii* (McFadden *et al.,* 1996, McFadden and Waller, 1997). This exciting discovery was an opening door towards further research directed at understanding the origins as well as the roles of this endosymbiotic organelle in parasitic protists. Apicoplast in these parasitic organisms can be easily visualized using advanced microscopic techniques and immune staining (Fig 1 A and B)

Control and assimilation of the endosymbiont

"Power and control means everything", as cliched as it sounds, it holds true even in case of smallest forms of eukaryotic life. Endosymbiosis is a messy process, engulfment of another organism requires stabilization and control over this newly acquired prey. This control is usually established by extensive transfer of genetic material from the acquired organism to the host organisms. Apicomplexan parasites also exerted control over its prey by extensive genetic transfer from apicoplast to its nuclear genome. Hence apicoplast was left out with a small genome of 35Kb in size, that primarily encodes for proteins required for the duplication of the genome (Wilson *et al*., 1996). All the other major proteins that are a part of metabolic pathways in the apicoplast are nuclear encoded and transported to the apicoplast using vesicular transport machinery. The vesicular transport of those proteins to the apicoplast is mediated by signal sequences at the beginning of the protein(Fig 2).

Figure 2

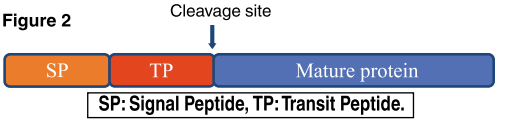


Figure 1A

Apicoplast in *Toxoplasma gondii*: This image on the top is human fibroblast cellular monolayer infected with the apicomplexan parasite *Toxoplasma gondii*. These parasites are expressing a green fluorescent protein in the apicoplast lumen. The presence of this protein in the apicoplast lumen helps easy visualization of this distinct compartment inside the parasite as a green dot.

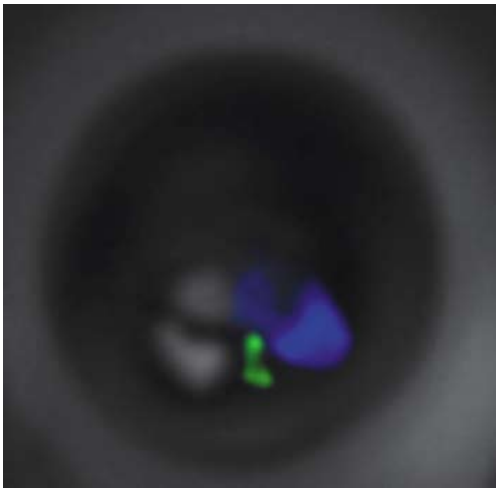


Figure 1B

Apicoplast in human malaria parasite *Plasmodium falciparum*: The image on the top is human red blood cell infected with the malaria parasite *Plasmodium falciparum*. For easy visualization of the parasite, its nucleus is stained with a blue satin called DAPI. The apicoplast of the parasite is visualized using a green fluorescent protein that was targeted to the apicoplast from the nucleus of the parasite(see below for targeting information).

All the proteins that is required for the apicoplast is encoded by the nuclear genome, made in the cytoplasm and then targeted to the apicoplast. The targeting is mediated by two signal sequences at the beginning of the protein; one signal peptide(SP) that takes it to the transport machinery in the endoplasmic reticulum and a transit peptide(TP) that directs it to the apicoplast. The mature protein is released into the apicoplast lumen after cleaving off the SP and TP in the apicoplast lumen by a transpeptidase enzyme.

Loss of photosynthesis and harnessing the metabolic benefits

Although the plastid was acquired by the ancestors of apicomplexan parasites for photosynthetic process, they soon had lineages that no longer used light as a source of energy. Chloroplasts in these lineages of eukaryotic life completely lost their photosynthetic abilities through time.(Fig 3)

The chief reason for the retention of chloroplasts in these organisms through evolution was the metabolic benefits associated with it. Ralph *et al.* (2004) reported that the major metabolic pathways hosted by the apicoplast are the fatty acid biosynthetic pathway, isoprenoid biosynthetic pathway and a shared heme biosynthetic pathway (between apicoplast and mitochondria(Fig4). The enzymes needed

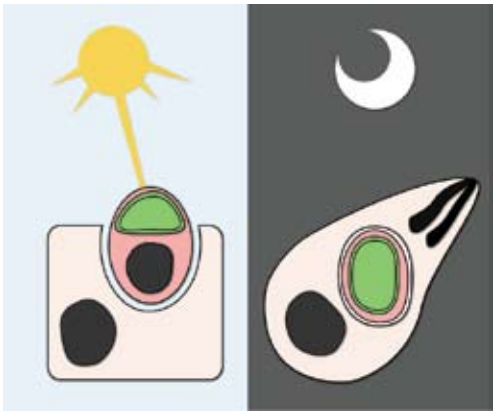


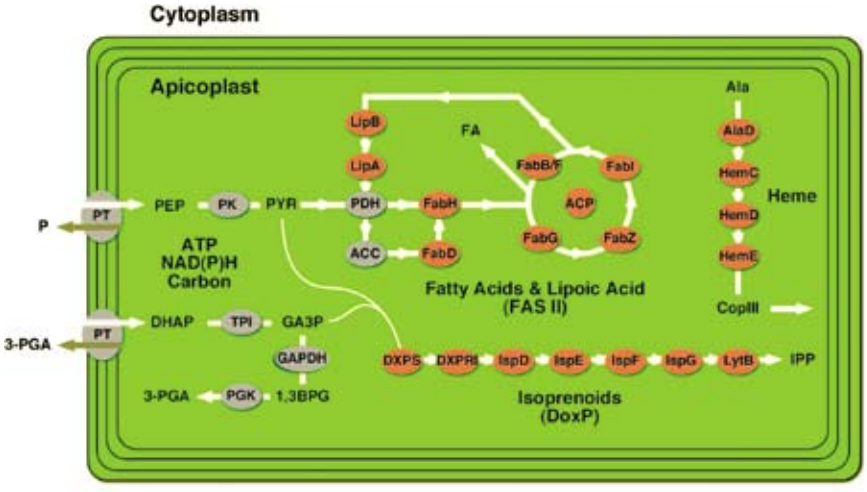
Figure 3

Cartoon depicting the probable course of events that led to the evolution of the non photosynthetic chloroplast, apicoplast: The apicoplast resulted from the endosymbiosis of a eukaryote that carried a photosynthetic chloroplast. The ancestral organism that carried this photosynthetic chloroplast had lineages that lived in the dark that could not use the organelle for photosynthetic benefits. Hence, the chloroplast lost its photosynthetic abilities through time and was retained primarily because of its metabolic benefits

for these biosynthetic pathways are nuclear encoded and then transported to the apicoplast (Foth *et al.,* 2003). The import of the metabolites that is required for the initiation of metabolic pathways is achieved using transporters on the membranes surrounding the apicoplast. These transporters called as triose phosphate transporters has been shown to be essential in Toxoplasma and Plasmodium (Brooks *et al.,* 2010). The requirement of these metabolic pathways has been studied in apicomplexan parasites through genetic as well as biochemical approaches. For example,in *Toxoplasma gondii*, genetic studies has shown that fatty acid and isoprenoid pathway are essential in the tachyzoite stages of the parasites(Nair *et al.,* 2011, Mazumdar *et al.,* 2006). In malaria parasite on the other hand, the fatty acid biosynthesis is completely dispensable during the blood stages of the parasite, but is only required during the mosquito and liver stages of the parasite(Van Schaijk *et al*., 2014). The only metabolic pathway that is required during the blood stages of malaria parasites is the isoprenoid biosynthetic pathway as the blood stages are susceptible to fosmidomycin, a drug targeting the isoprenoid pathway(Wiesner *et al.,* 2003). This led to further speculations that one of the chief forces that was behind the retention of the apicoplast might be its need for the isoprenoid subunits from the apicoplast. This is further supported by the fact that most of the apicomplexan parasites processes redundant fattyacid biosynthetic pathways in the cytosol (Ramakrishnan *et al.,* 2013), but the only pathway that can make isoprenoid subunits comes from the apicoplast. Bioinformatic analysis across the apicomplexan parasites also clearly showed that the apicoplast localized isoprenoid biosynthetic pathway is one of the most conserved pathways across phylum. This argument was supported by recent experiments where the supplementation of the isoprenoid subunits in the culture medium used for invitro growth of *Plasmodium falciparum* resulted in the generation of apicoplast less parasites or parasites that could live without apicoplast in the presence of drug pressure (Yeh, E. and DeRisi, 2011). These apicoplast negative parasites is an excellent testimony to the hypothesis that isoprenoid biosynthesis could be the chief raison d'etre for the apicoplast (Nair and Striepin, 2011).

"Achilles heel" of apicomplexans.

Another exciting prospect of having a prokaryotic organelle in a eukaryotic organism is the possibility of using it as an excellent target for chemotherapeutic agents.



Metabolic pathways in the apicoplast: Apicoplast harbors three important metabolic pathways , Isoprenoid biosynthesis(DoxP), fatty Acids and Lipoic acid biosynthesis (FAS II) and Heme biosynthesis shared with mitochondria. The enzymes involved in the pathway are in pink circles. Other abbreviations, PT: Transporter for importing 3-Carbon compounds from cytoplasm like pyruvate and Phosphoenol pyruvate, PEP: Phosphoenol pyruvate, PK: Pyruvate kinase, PYR: Pyruvate, PDH: Pyruvate dehydrogenase, ACC: acetyl-coA carboxylase, DHA: Dihydroxyacetone phosphate, TPI: Triose phosphate isomerase, GA3P: Glyceraldehyde 3-phosphate, 1,3BPG: I,3 Bisphosphoglycerate, PGK: Phosphoglycerate kinase, 3-PGA: 3 Phosphoglycerate.

Table. 1

No	Name of drug	Mode of action
1	Ciprofloxacin	DNA replication, Plastid DNA topoisomerase inhibition
2	Rifampacin	Plastid RNA polymerase inhibition
3	Doxycycline	Inhibition of plastid protein translation, 16SrRNA inhibition
4	Chloramphenicol	Inhibition of plastid protein translation ,23SrRNA inhibition
5	Erythromycin	Inhibition of plastid protein translation ,23SrRNA inhibition
6	Fosmidomyicn	Inhibition of isoprenoid biosynthesis, DOXPRI inhibition
7	Triclosan	Inhibition of fatty acid biosynthesis, Fab H inhibition
8	Thialactomycin	Inhibition of fatty acid biosynthesis, Fab I inhibition
9	Amythiamycin	Inhibition of plastid protein translation , elongation factor protein inhibition

Apicoplast , being a member of all apicomplexan parasites, serves as an excellent "achilles heel" for this important group of pathogens. Apicoplast is host to important biochemical pathways that can serve as good targets for chemotherapeutic agents (Chakraborty, 2016). Isoprenoid biosynthetic pathway, the most conserved of all, is the target of the drug fosmidomycin that has been used clinically to treat malaria. Unfortunately, fosmidomycin shows little efficacy in Toxoplasma, possible due to inability of the drug to reach apicoplast (Ralph *et al.,* 2011). Fatty acid pathway is also an excellent target for drugs, commonly used against Toxoplasma and Plasmodium. Another way to target the organelle is to use drugs that can inhibit apicoplast DNA replication and translation. These are excellent broad spectrum antibiotics commonly used against bacterial infections, but also shows efficacy against apicomplexan parasites because of the presence of apicoplast. A comprehensive list of drugs that can target apicoplast and their possible mode of action is listed in Table 1.

Conclusions

Apicomplexan parasites are an important threat to both human and animal life. The emerging resistance against the available drugs and the lack of effective vaccines against members of this phylum is a real threat. Most members of this phylum has an important organelle closely resembling plant chloroplast famously named as apicoplast, short for apicomplexan chloroplast. Hypothesized to be acquired during eukaryotic evolution for photosynthetic purpose by apicomplexan ancestors, the organelle lost its photosynthetic ability as many other members of the phylum started living in the dark. But the organelle was retained because of its metabolic benefits to the host organism. These metabolic benefits from the apicoplast is also the biggest weakness of the parasite as the pathways encoded by the apicoplast are essentially prokaryotic and hence divergent from its eukaryotic host. Researchers have indeed used this inherent weakness of this parasite by developing effective chemotherapeutic agents against metabolic and housekeeping functions of the apicoplast. This notoriety associated with the apicoplast has earned it the name "the achilles heel of apicomplexans"

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