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REVIEW

The apicoplast

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Abstract Parasites like malaria and *Toxoplasma* possess a vestigial plastid homologous to the chloroplasts of plants. The plastid (known as the apicoplast) is non-photosynthetic but retains many hallmarks of its ancestry including a circular genome that it synthesises proteins from and a suite of biosynthetic pathways of cyanobacterial origin. In this review, the discovery of the apicoplast and its integration, function and purpose are explored. New insights into the apicoplast fatty acid biosynthesis pathway and some novel roles of the apicoplast in vaccine development are reviewed.

Keywords Apicoplast · Endosymbiosis · Malaria · *Plasmodium* · Plastid · *Toxoplasma*

Abbreviations

ER	Endoplasmic reticulum
ERAD	Endoplasmic reticulum-associated protein degradation
pPTs	Plastidic phosphate translocators
SELMA	Symbiont-specific ERAD-like machinery

Introduction

The discovery of the vestigial plastid (apicoplast) in apicomplexan parasites such as malaria and *Toxoplasma gondii* radically revised our view of the evolutionary history, cell biology and metabolism of old enemies. We now know that the group originated from photosynthetic ancestors, probably similar to modern dinoflagellate zooxanthellae. We have also learned that the apicoplast contains an ensemble of bacteria-like pathways to replicate and express its genome plus an anabolic capacity generating fatty acids, haem and isoprenoid precursors. Apicoplasts are essential and perturbing them usually results in parasite death, thus making apicoplast metabolism an attractive target for drugs. In this review, I focus on the history of apicoplast discovery, the evolutionary integration of the endosymbiont from which the apicoplast is descended, and briefly recap what is known about apicoplast metabolism and potential drug targets. Finally, I examine the emerging potential of the apicoplast in whole parasite vaccine strategies.

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Discovery of the apicoplast

Araxie Kilejian found the first clues that eventually led to the identification of a plastid in malaria and related parasites. She saw a circular, extrachromosomal DNA molecule in *Plasmodium lophurae*, a malarial parasite of ducks (Kilejian 1975). Similar circles were described from *T. gondii* by Piet Borst (Borst et al. 1984). The circles assume a cross-shaped configuration best explained by the presence of inverted sequence repeats, which is a hallmark of plastid DNA (Williamson et al. 1985). However, at the time, no one suspected that this genome could be from a plastid. Indeed, since the malaria parasite was regarded as a protozoan, it was presumed that this small circular genome was the parasite's mitochondrial genome (Kilejian 1975; Williamson et al. 1985; Gardner et al. 1988). Things got complicated when other groups looked at isopycnic DNA density gradient fractions from the monkey, chicken and human malaria parasites. In addition to the circular chromosome, a linear molecule of 6 kb that encoded classical mitochondrial genes was discovered (Suplick et al. 1988;

Aldritt et al. 1989; Vaidya et al. 1989; Feagin 1992). Malaria parasites thus had two candidate mitochondrial genomes: one linear and one circular. But the first sequence data from the circular genome only served to deepen the mystery. Malcolm Gardner, then a student in Iain Wilson's group in London, examined the 35-kb circle from *Plasmodium falciparum*, which causes cerebral malaria in humans. Gardner proved that the genome did indeed have prokaryotic ancestry, but not the anticipated α-proteobacterial ancestry of the mitochondrial endosymbiont ancestor (Gardner et al. 1991a, b, 1993). Rather, Gardner's sequence data demonstrated an ancestry for the circular genome more akin to plastids of plants and algae (Gardner et al. 1991a, b, 1993). Gardner identified RNA polymerase genes then unique to plastid genomes in eukaryotes and rRNA sequences with sequence and inverted repeat arrangement similar to plastid DNA (Gardner et al. 1991a, 1991b, 1993, 1994).

At this point, the mitochondrial DNA hunters began to entertain a new hypothesis—that the circular genome was not part of the mitochondrion but derived from a new, as yet unidentified, organelle with ancestral links to plant and algal plastids (Suplick et al. 1990; Gardner et al. 1991a, b; Wilson et al. 1991; Howe 1992; Wilson 1993; Williamson et al. 1994). Wilson's group persevered with the difficult sequencing of the highly AT-rich *P. falciparum* 35-kb circular genome and produced a complete genome that was plastid-like in every detail, except that it lacked any genes involved in photosynthesis (Wilson et al. 1996). In situ hybridization studies then confirmed that the 35-kb circular genome resides in a compartment distinct from the mitochondrion, thus identifying a third DNA-containing organelle in *P. falciparum* and related apicomplexan parasites like *T. gondii* (McFadden et al. 1996; Köhler et al. 1997). This organelle is now known as the apicoplast (from apicomplexan plastid), and it occurs in all members of the phylum Apicomplexa with the definite exception of *Cryptosporidium* spp. (Zhu et al. 2000; Abrahamsen et al. 2004; Xu et al. 2004) and the possible exception of the gregarines (Toso and Omoto 2007).

Origin of the apicoplast

Three questions burned in the minds of those investigating the apicoplast: (1) how many membranes bounded it, (2) what is its evolutionary origin and (3) what is its purpose? The first two questions were initially contentious, but in this reviewer's opinion, they are now resolved. When I identified the apicoplast in 1996, I described it as having two or three membranes (McFadden et al. 1996), but others reported three (Hopkins et al. 1999), four (Köhler et al. 1997) and even five bounding membranes (Kohler 2005). With access to better electron microscope fixed material, I am now convinced that there are four membranes bounding the apicoplast (Fig. 1). The number of membranes is important to understand the evolutionary origin of the apicoplast, since more than two membranes is a hallmark of secondary endosymbiosis (Gould et al. 2008).

Multiple membranes fit with a secondary endosymbiotic origin, but much debate ensued about what kind of

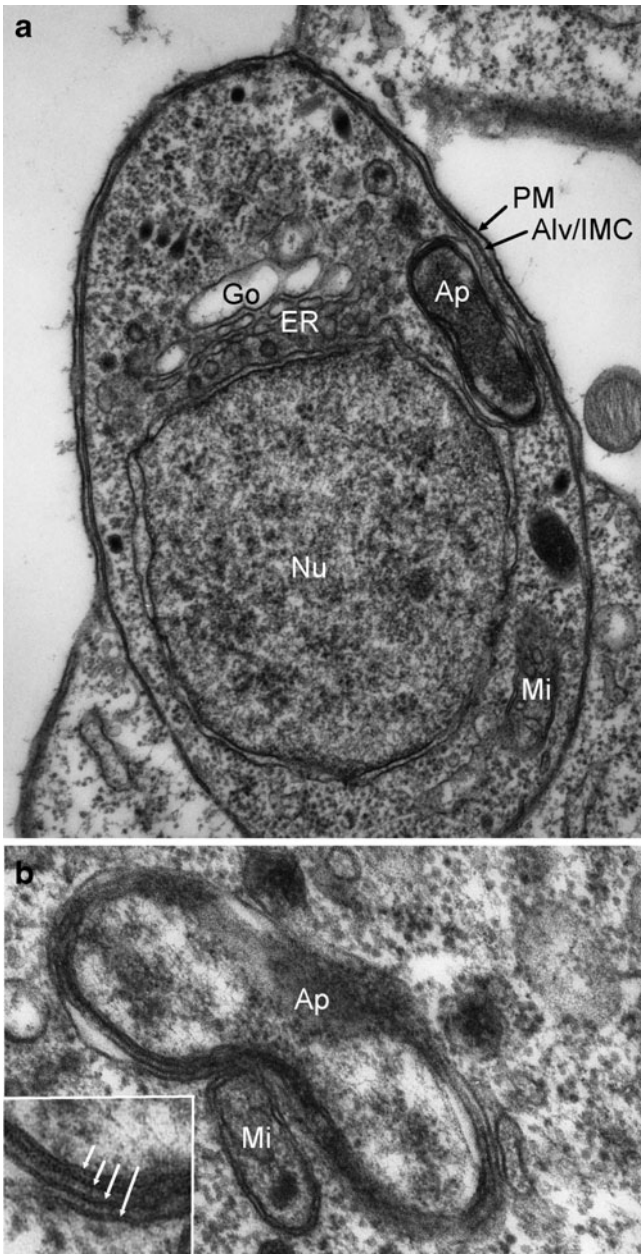

Fig. 1 **a** Transmission electron micrograph of *T. gondii* showing nucleus (*Nu*), endoplasmic reticulum (*ER*), Golgi apparatus (*Go*), apicoplast (*Ap*), mitochondrion (*Mi*), plasma membrane (*PM*) and alveoli (*Alv*). *Scale bar*=500 nm. **b** Apicoplast (*Ap*) and mitochondrion (*Mi*) showing close association and two membranes around mitochondrion. *Scale bar*=200 nm C/close-up from **b**/showing four membranes (*arrows*) bounding the apicoplast. *Scale bar*=50 nm

eukaryotic endosymbiont donated the plastid. One camp held that the endosymbiont was a green alga (Köhler et al. 1997; Funes et al. 2002, 2004; Cai et al. 2003) whereas many others favoured a red algal endosymbiont (Wilson 1993; Williamson et al. 1994; McFadden et al. 1997; Waller et al. 2003; Waller and Keeling 2006). Happily, the debate has been conclusively resolved by Dee Carter's discovery of a genuine missing link, namely *Chromera velia* (Moore et al. 2008).

When the apicoplast was first identified, we proposed that an ancestor of the obligate intracellular parasites belonging to phylum Apicomplexa was photosynthetic and similar to zooxanthellae dinoflagellates that are symbionts in corals and other invertebrates (McFadden and Waller 1997). *C. velia* is apparently the living example of such an ancestor (Okamoto and McFadden 2008). *C. velia* lives in corals, is an apicomplexan and has a photosynthetic plastid bounded by four membranes (Moore et al. 2008; Janouskovec et al. 2010; Obornik et al. 2010). Importantly for the origin debate, *Chromera* plastids share the same ancestry as apicoplasts and dinoflagellate plastids, and *Chromera* (and related algae) undoubtedly harbour a red algal endosymbiont (Janouskovec et al. 2010). The apicoplast thus derived from an engulfed red alga and was acquired before dinoflagellates and Apicomplexa diverged (Moore et al. 2008; Janouskovec et al. 2010).

Function of the apicoplast

Our third question—what does the apicoplast do?—remains mysterious, but we have a few good hypotheses. The apicoplast genome provided no clue as to the organelle's purpose (Wilson et al. 1996; Köhler et al. 1997). It was obvious that photosynthesis had been lost, but no functional pathways could be identified (Wilson et al. 1996; Köhler et al. 1997). The machinery encoded was all to do with self-perpetuation (Wilson et al. 1996; Köhler et al. 1997), prompting one to wonder if this was a remarkable case of hyperparasitism in which a selfish organelle persisted for no purpose other than to make copies of itself at the parasite's and ultimately the host's expense. However, some elegant experiments showed that the apicoplast was indispensable.

Pharmacological or genetic perturbation of the apicoplast led to parasite death (Fichera and Roos 1997; He et al. 2001), so an essential function (or even functions) for the organelle had to be found. Clues to apicoplast function emerged from the parasite genome projects. Our group identified genes for an apicoplast fatty acid biosynthesis system in *T. gondii* and *P. falciparum* (Waller et al. 1998), putting paid to the dogma that malaria parasites do not synthesise fatty acids de novo. Soon thereafter it also emerged that the apicoplast has a pathway to synthesise isopentenyl diphosphate (Jomaa et al. 1999), a precursor of isoprenoids, ubiquinone side chains, dolichols and an essential component for the modification of tRNAs (Ralph et al. 2004b). Further data mining revealed that the apicoplast of malaria parasites makes iron sulphur complexes (Seeber 2002, 2003; Ralph et al. 2004b) and also cooperates with the mitochondrion in the synthesis of haem (Ralph et al. 2004b; Sato et al. 2004; van Dooren et al. 2006; Nagaraj et al. 2008, 2009a, b, 2010a, b; Shanmugam et al. 2010). Virtual maps of these plastid-like pathways (Ralph et al. 2004b) have been validated by painstaking biochemical reconstructions and molecular genetics (reviewed in Lim and McFadden 2010). This suite of anabolic pathways is fuelled with phosphorylated three carbon sugars imported from the parasite's cytosolic glycolytic pathway using plastid-like transporters (plastidic phosphate translocators, pPTs) located in the membranes of the apicoplast (Mullin et al. 2006; Karnataki et al. 2007a; Brooks et al. 2010; Lim et al. 2010). Genetic ablation of the transporter gene in *Toxoplasma* resulted in immediate death of the parasites (Brooks et al. 2010; Lim et al. 2010). The apicoplast thus harbours a collection of plastid-like metabolic pathways that are supplied with carbon, energy and reducing power in a manner identical to a non-photosynthetic plant or algal plastid. But which of these pathways are essential and why? Presumably, the apicoplast makes something essential to the parasite, which could be any or all of the molecules mentioned above. Thus far, nothing has been demonstrated to be exported from the apicoplast to the parasite proper.

Integration of the apicoplast into the parasite

When the ancestor of Apicomplexa first acquired its red algal endosymbiont, the alga would have been a fully autonomous, free-living eukaryotic organism. Nowadays the endosymbiont is much reduced, having lost its nucleus, cytoplasm, wall and cytoskeleton. Intermediate steps in secondary endosymbiosis survive, and the nucleus and cytoplasm of the red algal endosymbiont in cryptomonads persist as vestiges allowing us to reconstruct steps in the evolutionary reduction of the symbiont (Douglas et al. 2001). In the case of the apicoplast, all that remains is the genome and stroma of the plastid plus four bounding membranes (Fig. 1). The endosymbiont plasma membrane persists (referred to as the periplastid membrane) and the two membranes bounding the original red algal plastid also survive. The fourth, outermost, membrane derives from the host's endomembrane. In the case of the apicoplast, the pigment-loaded thylakoid membranes have also disappeared, presumably when photosynthesis was relinquished.

What steps can we reconstruct in the modification from alga to apicoplast?

For a host to tame an endosymbiont, it has to achieve at least three things: (1) tap the endosymbiont's resources, (2) regulate its division so that endosymbionts can be passed on to daughter host cells and (3) stop it from escaping. Only then can the endosymbiont become a stable, integrated part of the host cell—an organelle. How the host came to tap into the photosynthate produced by the endosymbiont has been discussed elsewhere (Weber et al. 2006; Gross and Bhattacharya 2009) and was apparently achieved using the endosymbiont's own transporters (pPTs) induced to feed the host. However, in the case of the apicoplast, these transporters have to operate in reverse to feed the endosymbiont, which—being non-photosynthetic—no longer generates its own requirements (Mullin et al. 2006).

The second requirement for taming an endosymbiont—regulating endosymbiont division—is crucial. Too many endosymbiont progeny could overrun a host cell; too few could result in some daughter hosts not receiving an endosymbiont. Clearly the host had to assume some regulatory control of endosymbiont division. The division machinery of plant plastids and how the host nucleus oversees this process is quite well understood (Okazaki et al. 2010). Puzzlingly, apicoplasts appear to lack much of this machinery, despite retaining most typical plastid features (Vaishnava and Striepen 2006). Division of the apicoplast does, however, resemble plant chloroplast division in that it utilises a host-derived pinchase related to dynamin that assembles itself like a sphincter around the outermost membrane of the elongated apicoplast and pinches it in two (Striepen et al. 2000; van Dooren et al. 2009). How the inner membranes of the apicoplast divide in the absence of the canonical FtsZ, ARC and Min systems that guide and actuate plastid division in plant chloroplasts and their cyanobacterial forebears remains a mystery.

Protein translocation

The third and final trick to taming an endosymbiont is to stop it from escaping and returning to its former free-living existence. This we understand reasonably well. If the partnership between endosymbiont and host is evolutionarily more fit than the independent organisms, selection will favour any event that keeps them together. The simplest scenario is for the endosymbiont to lose autonomy and become dependent on the host. Gene transfer, where the host procures an essential endosymbiont gene and metes out the gene product to the endosymbiont, locks the partners together preventing the endosymbiont from escaping and rendering it non-autonomous. We know how and, to some extent, why DNA transfers from the endosymbiont or organelle to the host (Martin and Herrmann 1998; Howe et al. 2000; Huang et al. 2003; Martin 2003). In the case of the apicoplast proteins, the genes could either have transferred to the secondary host nucleus from the plastid genome itself or from the endosymbiont nucleus. Indeed, transfer of the latter was a prerequisite for loss of the endosymbiont nucleus and attending cytoplasm. An estimated 480 genes in the *P. falciparum* nucleus derive from the endosymbiont, and their products are believed to be targeted into the apicoplast (Gardner et al. 2002; Foth et al. 2003; Ralph et al. 2004b).

We also understand how gene products from host-acquired genes are repatriated to the organelle in plants. Translocons (Tic and Toc) residing in the inner and outer membranes of plant plastids identify appropriately tagged gene products synthesised in the host cytosol and import them in an unfolded state across the two bounding membranes (Soll and Schleiff 2004; Kessler and Schnell 2009; Bohnsack and Schleiff 2010). In recent years, good insights into how proteins are targeted across the quadruple-membrane-bound apicoplast have emerged (Bolte et al. 2009). Commencing at the innermost membrane, I outline what has been learned thus far.

Two components of the Tic translocon from plants, Tic20 and Tic22, have been identified in the apicoplast, with Tic20 clearly residing in the innermost membrane (van Dooren et al. 2008; Kalanon et al. 2009). Ablation of Tic20 in *T. gondii* stops apicoplast protein import and kills parasites (van Dooren et al. 2008). The role of Tic22 in apicoplasts is undefined but likely to be similar to plant chloroplasts where it is thought to coordinate transfer of cargo from Toc to Tic (Kalanon et al. 2009). Tic110, an essential component for plant chloroplast protein import and a possible component of the transmembrane channel (Inaba et al. 2005), has not yet been identified in the apicoplast.

The second apicoplast membrane (counting from the inside) is considered homologous to the primary plastid outer membrane that harbours the Toc translocon, which includes the cyanobacterium-derived β-barrel membrane channel Toc75 (Soll and Schleiff 2004). For a long time, the apicoplast homologue of Toc75 defied identification, but a candidate in *T. gondii* emerged via the identification of its counterpart in diatom algae (Bullmann et al. 2010). Thus far, no candidate for Toc75 in *P. falciparum* has yet been identified, but it seems likely that gene PFL1670c is Toc75 in the malaria parasite. It remains to be shown that the apicoplast Toc75 candidates are in the apicoplast and are indeed β-barrel membrane channels, but a conservation of function and process in the two inner membranes of the apicoplast with primary plastids seems likely. If porin-like proteins are shown to reside in this apicoplast membrane, its homology to the outer membrane of Gram-negative bacteria would be confirmed.

The ERAD translocon

The periplastid membrane (the third apicoplast membrane counting from the inside) was originally the plasma membrane of the red algal endosymbiont. Apicoplasts and indeed other secondary plastids derived from red algae have solved the problem of getting proteins across this membrane in a novel way. Since the space between the outermost apicoplast membrane and the periplastid membrane is topologically contiguous with the lumen of the endoplasmic reticulum (ER; see below), a protein in this zone is technically outside the host and outside the endosymbiont.

Uwe Maier's group solved this conundrum by demonstrating that secondary plastids from cryptomonads, diatoms and Apicomplexa employ a translocation motor originally derived from the endosymbiont's ER that has been relocated into the periplastid membrane (Sommer et al. 2007). This translocation system, known as the ER-associated protein degradation (ERAD) translocon, normally extracts misfolded proteins from the ER lumen for degradation in the cytoplasm (Bagola et al. 2010; Mehnert et al. 2010). In other words, the translocation system re-imports proteins back into the cytosol from the lumen of the ER, which is technically an external compartment. This is exactly the direction that is required for import across the periplastid membrane of the apicoplast, so the ERAD system was apparently ideal for the task. What is more is that it recognises unfolded proteins, which is a characteristic of plastid targeting transit peptides (Ralph et al. 2004a). Maier's team proposed that relocation of the ERAD translocon from the endosymbiont's ER to its plasma membrane, which are effectively continuous, was the critical invention for import of proteins into the secondary plastid (Sommer et al. 2007). Indeed, by simply relocating its ERAD translocon into its plasma membrane, the endosymbiont was apparently able to import proteins from the lumen of the host's ER—the space between the outermost secondary plastid membrane and the periplastid membrane.

Maier refers to the endosymbiont ERAD system as symbiont-specific ERAD-like machinery (SELMA; Hempel et al. 2009). Like canonical ERADs, SELMA comprises multiple components. Thus far, a putative channel (Der1), an ATPase motor (CDC48) and several elements of the canonical ERAD ubiquitination machinery (Ufd1, E1, E2, E3, DeUb and Ub) have been implicated in SELMA (Sommer et al. 2007; Agrawal et al. 2009; Hempel et al. 2009; Kalanon et al. 2009; Spork et al. 2009). Der1 interacts with transit peptides (Hempel et al. 2009) and is essential for apicoplast protein import in *T. gondii* (Agrawal et al. 2009). SELMA E3 (the ubiquitin ligase) and DeUb (the enzyme that removes ubiquitin from degradation [import?] tagged proteins) have been shown to be plastid-localized and active (Hempel et al. 2010). Although ubiquitination has not been demonstrated to be part of the import process, by analogy to canonical ERAD and the presence of the appropriate subunits in the appropriate places, it seems most likely that imported proteins undergo ubiquitination and de-ubiquitination during import (Hempel et al. 2010). As yet, no candidate ubiquitin substrate has been identified in the apicoplast, but a *P. falciparum* gene PF08_0067 previously identified as having a product targeted to the apicoplast is a likely ubiquitin (Ralph et al. 2004).

The outermost apicoplast membrane

The outermost apicoplast membrane is thought to derive from the phagosome membrane when the host first engulfed the red algal endosymbiont. Although there is no direct evidence for this hypothesis, the mode by which proteins begin their journey to the apicoplast is consistent with this scenario. For instance, all known apicoplast-interior-targeted proteins bear an N-terminal signal peptide, which directs the nascent protein to the Sec61 translocon in the ER. It is believed that classic co-translational insertion of the nascent protein into the ER lumen via the signal recognition system is the first step *en route* to the apicoplast (DeRocher et al. 2000; Waller et al. 2000). Exchange of signal peptides from secreted proteins still results in apicoplast targeting (so long as there is a transit peptide) so apicoplast-destined proteins enter the general secretory pathway (Tonkin et al. 2006c). Once within the lumen of the ER, it is apparent that the transit peptide (now exposed at the N terminus by signal peptidase removal of the signal peptide) is sufficient and necessary to direct proteins into the apicoplast stroma (DeRocher et al. 2000; Waller et al. 2000; Harb et al. 2004).

The mechanism by which the protein traffics from the ER to the apicoplast is not well established, but most models favour vesicular traffic that does not go via the Golgi (DeRocher et al. 2005; Tonkin et al. 2006a, c; DeRocher et al. 2008). Apicoplast protein-bearing vesicles are presumed to fuse with the outer apicoplast membrane (Tonkin et al. 2004, 2006c; DeRocher et al. 2005; DeRocher et al. 2008), thus connecting via endomembrane flow. Further support that the outermost membrane is part of the endomembrane system comes from the proteins resident in the outer membrane (Lim et al. 2009). Proteins such as the pPT of the outer apicoplast membrane (*Pf*oTPT) or *Tg*FtsH have recessed, non-cleavable signal peptides that direct them into the ER membrane and then they lodge in the outer apicoplast membrane by an unknown mechanism (Mullin et al. 2006; Karnataki et al. 2007b, 2009; Lim et al. 2009).

Thus, a bipartite leader comprising a canonical signal peptide followed by a transit peptide similar to that of

primary plant and algal plastids is sufficient to direct a protein on a convoluted journey into the ER via the Sec translocon, into the compartment between the outermost apicoplast membrane (probably via endomembrane vesicular traffic from ER to apicoplast), across a retooled ERAD translocon (SELMA) in the periplastid membrane, across a beta barrel porin (Toc75) in the second membrane from the inside (originally the outer membrane of a Gram-negative cyanobacterial ancestor of the first plastid) and, finally, across the innermost membrane courtesy of the Tic translocon. Once inside the apicoplast, the transit peptide is removed at a specific cleavage site (probably by an apicoplast homologue of the chloroplast stromal processing peptidase (SPP); van Dooren et al. 2002), but how the cleavage site is recognised is unknown.

Remarkably, the transit peptides that mediate the last four steps are a simple collection of particular amino acids with no consensus, no secondary structure and no specific length (Foth et al. 2003; Ralph et al. 2004a; Tonkin et al. 2006b, 2008). How SELMA, Toc Tic and SPP identify the hundreds of different transit peptides presented to them is mysterious. Targeting to the apicoplast is thus something of a hodgepodge of evolutionary reuse, recycling and retooling to cobble together a pathway across four membranes predominantly from pre-existing bits and pieces either acquired with the two sequential symbionts or already existing in the hosts.

Kill the apicoplast, kill the parasite?

When first discovered, the apicoplast engendered much excitement as a novel target for therapeutic drugs (Gardner et al. 1991a, b; McFadden et al. 1996; Wilson et al. 1996). The cyanobacterial pathways therein and the novel ways it was supplied with carbon, energy, reductants and proteins are all very distant from human host metabolism and cellular processes, leaving room to design or discover specific inhibitors that would perturb the apicoplast but have no side effects (Jomaa et al. 1999; McFadden and Roos 1999; Ralph et al. 2001, 2004b; Gornicki 2003; Seeber 2003; Goodman and McFadden 2007; Goodman et al. 2007).

The apicoplast is essentially a tiny bacterium inside the parasite, complete with a circular genome that has to be replicated and expressed. Numerous antibacterials target these processes, and many are parasiticidal and several (clindamycin and doxycycline) are used as an antimalarial therapy or prophylaxis. An exhaustive review of these drug leads and their current status can be found elsewhere (Wiesner et al. 2008), and this review will therefore confine itself to some recent insights.

Apicoplast type II fatty acid biosynthesis has been the focus of considerable drug attention since it was first discovered (Waller et al. 1998). Several putative inhibitors are parasiticidal and much of the biochemistry of the individual enzymes explored (reviewed in Goodman and McFadden 2007; Wiesner et al. 2008). However, gene knockout studies have recently shown that malaria parasites can do without vital enzymes of the fatty acid biosynthesis pathway during the red blood cell portion of their life cycle, implying that parasites do not need to synthesise fatty acids at this stage (Yu et al. 2008; Vaughan et al. 2009). Parasites do, however, require these enzymes in the liver stage, just after they are first introduced into the mammal by the mosquito bite (Yu et al. 2008; Vaughan et al. 2009). This insight serves as a reminder that not all pathways will be essential in all life cycle stages, and the current challenge is to establish the role of apicoplast anabolic synthesis across the life cycle. Intriguingly, apicoplast fatty acid biosynthesis is essential in *T. gondii* in mice since ablation of the acyl carrier protein in vivo clears the infection (Mazumdar et al. 2006).

Knowing that apicoplast fatty acid biosynthesis is not essential in the blood stage also forces us to re-examine the findings that anti-fatty acid synthesis compounds are parasiticidal. The best-studied example is triclosan, which was initially reported to kill parasites both in vitro and in vivo by inhibiting a fatty acid biosynthesis enzyme known as FabI or enoyl-ACP reductase (Surolia and Surolia 2001) but is now believed to be off-target, killing parasites in an unknown way (Yu et al. 2008). A range of other parasiticidal compounds with putative targets in the apicoplast fatty acid biosynthesis system have also been shown to kill blood stage parasites (Goodman and McFadden 2007; Wiesner et al. 2008), but whether or not these too are also off-target is unknown. Although fatty acid biosynthesis is not essential in blood stages, other apicoplast biosynthetic pathways such as isopentenyl diphosphate synthesis, which is targeted by fosmidomycin, are apparently essential in blood stages and are therefore good drug targets for blood stages (Jomaa et al. 1999; Wiesner and Jomaa 2007)

Kill the apicoplast, immunize the host?

At the outset, apicoplast research was focused on the organelle's potential as a drug target; no one foresaw a role for the apicoplast in vaccine strategies. Creating an efficacious vaccine against malaria has been fraught. Subunit vaccines have proven difficult, and some focus has returned to whole parasite vaccines, either low dose or attenuated parasites (McCarthy and Good 2010). Vaccinating with whole parasites carries all manner of logistical obstacles, but the fact remains that this is the only regimen thus far to imbue sterile protection, making it worthy of further exploration (McCarthy and Good 2010).

Long ago, it was learned that sterile immunity to malaria can be achieved in mice (Nussenzweig et al. 1967) and humans (Clyde et al. 1973) by injecting radiation-attenuated sporozoites (the stage normally injected by the mosquito bite). The crippled sporozoites could then establish a beachhead in the liver (as healthy parasites do) but failed to progress to the blood stage, and this exposure conferred immunity to subsequent challenges with healthy parasites (Vanderberg et al. 1968). More recently, genetic attenuation of parasites has been explored as an alternative to radiation attenuation. The idea here is to delete parasite genes that are only essential in the liver stage, allow mosquitoes to infect mice or humans with these genetically attenuated strains and monitor the progress of the liver infection and any protection against subsequence challenge. Several liver stage essential genes (p52, p36, sap1, uis3 and uis4) have proven effective in this strategy, and knockout parasites induce some immunity after failing to complete the liver phase of infection or establish blood stage patency (Vaughan et al. 2010). The longer the liver stage survives, the better the protection, and here is where the apicoplast comes in. Of all the genetically attenuated parasites trialled thus far, those with deleted genes for apicoplast fatty acid biosynthesis last longest in the liver (Yu et al. 2008; Vaughan et al. 2009, 2010). Fatty acid biosynthesis knockouts also confer the best protection, providing absolute sterile immunity in mice for 210 days thus far (Vaughan et al. 2010). It appears that the parasites only require fatty acid biosynthesis at the cytokinesis stage (schizogeny) of the liver phase as fatty acid biosynthesis lesions falter at the final stage when a large quantity of phospholipids would be required (Yu et al. 2008; Vaughan et al. 2009). Clinical trials of fatty acid biosynthesis-deficient parasites in humans are currently underway, and the outcomes are excitedly awaited.

Another way to interrupt development of liver stage parasites is by drug (chloroquine) intervention, which can also confer immunity (Roestenberg et al. 2009). Steffen Borrmann and Kai Matuschewski recently adapted this approach by specifically blocking apicoplast protein synthesis using the antibacterials clindamycin and azithromycin on liver stage parasites in mice (Friesen et al. 2010). Just as the fatty acid biosynthesis defective parasites failed to complete their liver phase, so too did the apicoplast-stressed parasites (Friesen et al. 2010). Once again, a block in parasite life cycle progression elicited robust and long-lived protection providing another avenue to engender immunity using live parasites. Importantly, there is as yet no known resistance to clindamycin and azithromycin in malaria, and the latter is safe for use in infants potentiating a strategy to administer the prophylaxis with the goal of also conferring immunity.

Conclusion

Since first being identified in 1996, the apicoplast has assumed an important role in understanding the evolutionary history, metabolic potential and unanticipated vulnerabilities in human parasitic diseases like malaria and toxoplasmosis. Early attention focused on the apicoplast as a drug target, and the discovery that one pathway—fatty acid biosynthesis—is not essential in blood stages notwithstanding, the organelle still offers tremendous potential for therapeutic intervention as it is indispensable and susceptible to a wide range of well-characterised drugs. As yet, the actual specific purpose of the apicoplast remains uncertain. Discovering why these parasites retain a plastid long after they relinquished photosynthesis is our next challenge.

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Conflict of interest The author declares that he has no conflict of interest.

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