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Malaria, *Plasmodium falciparum* and its apicoplast

Ming Kalanon and Geoffrey I. McFadden[1]
School of Botany, University of Melbourne, Parkville, Victoria 3010, Australia

Abstract

Malaria, which is caused by species of the parasite genus *Plasmodium*, remains a major global health problem. A vestigial plastid homologous with the chloroplasts of plants and algae was discovered in malaria and related parasites from the phylum Apicomplexa and has radically changed our view of the evolutionary origins of these disease-causing protists. We now recognize that this large group of parasites had a photosynthetic ancestry and were converted into parasitism early in the evolution of animals. Apicomplexans have probably been parasitizing the animal kingdom for more than 500 million years. The relic plastid persists in most apicomplexans and is an essential component. Perturbation of apicoplast function or inheritance results in parasite death, making the organelle a promising target for chemotherapy. Plastids, including those of malaria parasites, are essentially reduced endosymbiotic bacteria living inside a eukaryotic host. This means that plastids have bacterial-type metabolic pathways and housekeeping processes, all of which are vulnerable to antibacterial compounds. Indeed, many antibacterials kill malaria parasites by blocking essential processes in the plastid. Furthermore, a range of herbicides that target plastid metabolism of undesired plants are also parasiticidal, making them potential new leads for antimalarial drugs. In the present review, we examine the evolutionary origins of the malaria parasite's plastid by endosymbiosis and outline the recent findings on how the organelle imports nuclear-encoded proteins through a set of translocation machineries in the membranes that bound the organelle.

Plasmodium falciparum causes malaria

P. falciparum is the intracellular obligate parasite that causes the most virulent and prevalent form of human malaria, a major global disease. According to the World Health Organization (WHO) report on malaria [1], there were approx. 250 million cases of malaria globally in 2006, causing between 610000 and 1.2 million deaths, predominantly in children under 5 years of age. Malaria is endemic in 109 countries, particularly in equatorial Africa, where approx. 600 million people are at high risk of contracting the disease. In contrast, only 92 countries were malaria-free, and only one country (the United Arab Emirates) has succeeded in eradicating malaria since the 1980s [1].

Five species of *Plasmodium* are capable of causing malaria in humans; however, *P. falciparum* is by far the major cause of disease burden, accounting for 91% of cases globally [1]. Furthermore, *P. falciparum* is unique among the human *Plasmodium* parasites because it is a descendent of the chimpanzee parasite *Plasmodium reichenowi*, whereas the other four human *Plasmodium* spp., including *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi* are all derived from monkey parasites [2].

The complex life cycle of *P. falciparum* is intrinsically linked to the symptoms of malaria. Upon transmission from the mosquito, the parasite initially undergoes one massive cycle of replication in a hepatocyte, producing tens of thousands of invasive merozoites, which then proceed to infect erythrocytes. The parasite then undergoes synchronous cycles of asexual replication, causing erythrocytes to burst approximately every 48 h as new merozoites are formed and released, resulting in the symptomatic peaks of fever and anaemia associated with malaria [3]. Furthermore, serious infection with *P. falciparum* can be associated with cerebral and placental malaria, where erythrocytes infected with mature parasites attach to each other and the endothelial blood vessel walls via parasite-induced knobs, obstructing blood flow and tissue perfusion [3].

Recent large-scale malaria control programmes, such as Roll Back Malaria, have had a positive impact on the prevention and treatment of malaria in some countries by improving access to preventative and treatment measures, such as insecticide-treated bed nets and artemisinin-based combination drug therapy [1]. Despite these achievements, however, current understanding of *P. falciparum* biology remains far from complete, and access to malaria-control measures remains well below the coverage required for adequate disease control. Furthermore, the emergence of artemisinin-resistant parasites [4] has also highlighted the need for novel therapeutics to provide an alternative treatment for this first-line treatment for malaria. Significant

Key words: apicoplast, endosymbiosis, malaria, *Plasmodium falciparum*, plastid.
Abbreviations used: BiP, immunoglobulin heavy-chain-binding protein; ER, endoplasmic reticulum; ERAD, ER-associated degradation; Hsp70, heat-shock protein 70.
[1]To whom correspondence should be addressed (email gim@unimelb.edu.au).

progress in both the implementation of practical control measures, as well as increasing our understanding of the biology of the parasite, is required if the target of malaria eradication is to be achieved.

The essential and dynamic apicoplast of *P. falciparum*

Plasmodium parasites belong to a larger group of intracellular parasites, the Apicomplexa [5]. Other members of this phylum include parasites of humans, such as *Toxoplasma gondii* and *Cryptosporidium* spp., which can cause diseases in immunocompromised individuals, in addition to parasites of livestock and domesticated animals, such as *Theileria* spp. and *Babesia* spp., which have a severe impact on the agricultural industry. Among several structural features such as invasion-related organelles at the apical end of the merozoite stage, one of the defining characteristics of apicomplexan parasites is the presence of a four membrane-bounded plastid called the apicoplast [6]. *Cryptosporidium* parasites are an exception to this rule, owing to a secondary loss of the organelle [7]. Although the apicoplast of most apicomplexan parasites is no longer photosynthetic, the recent discovery of a photosynthetic apicomplexan, the coral symbiont *Chromera velia*, strongly supports the hypothesis that the apicoplast of the cenancestor of apicomplexans was also photosynthetic [8].

The apicoplast of *P. falciparum* is essential for parasite survival in both its erythrocytic [9] and liver stages [10,11]. Despite its lack of photosynthesis, the apicoplast houses several plastid-derived biochemical pathways including the biosynthesis of fatty acids, isoprenoids, haem and iron–sulfur clusters [12]. The detection of plant-like biosynthetic pathways for abscisic acid in *T. gondii* [13] and β-carotenoids in *P. falciparum* [14] corroborate the algal origin of the organelle.

Each *P. falciparum* parasite contains one apicoplast that develops from a small punctate organelle in the merozoite and ring stages into a complex reticulated structure in the multinucleated schizont stage during the erythrocytic stage of the parasite life cycle [15]. A similar process of development occurs in the hepatocyte stages of the parasite life cycle, albeit at a significantly larger scale [16]. The apicoplast also forms close associations with both the mitochondria and the ER (endoplasmic reticulum) during its development [17], suggesting that the dynamic apicoplast morphology is tightly co-ordinated and regulated together with the development of other parasite organelles. In gametocytes, the apicoplast and mitochondria are closely associated, although the mitochondria undergoes significant morphological changes whereas the apicoplast remains relatively quiescent [18]. In the related apicomplexan *T. gondii*, a tight association between the apicoplast and ER is also observed [19], whereas apicoplast elongation and division appears to be tightly associated with the daughter cell formation [20,21].

The endosymbiotic origin of the apicoplast

Plastids, like mitochondria, are derived from endosymbiosis, the process of a phagotrophic host cell engulfing, retaining and ultimately enslaving another cell, its endosymbiont [22]. A single endosymbiotic event involving a photosynthetic cyanobacteria and a biciliate eukaryotic phagotroph gave rise to the plastid-bearing common ancestor of the supergroup Archaeplastida (or Plantae), which consists of glaucocystophytes, red algae, green algae and land plants (Figure 1). The plastids in these lineages retain the double-membrane structure of their cyanobacterial antecedents. Significantly, the plastids resulting from this first endosymbiosis, called primary plastids or chloroplasts, also retain the cyanobacterial ability to photosynthesize, conferring autotrophic capacity on eukaryotic cells for the first time.

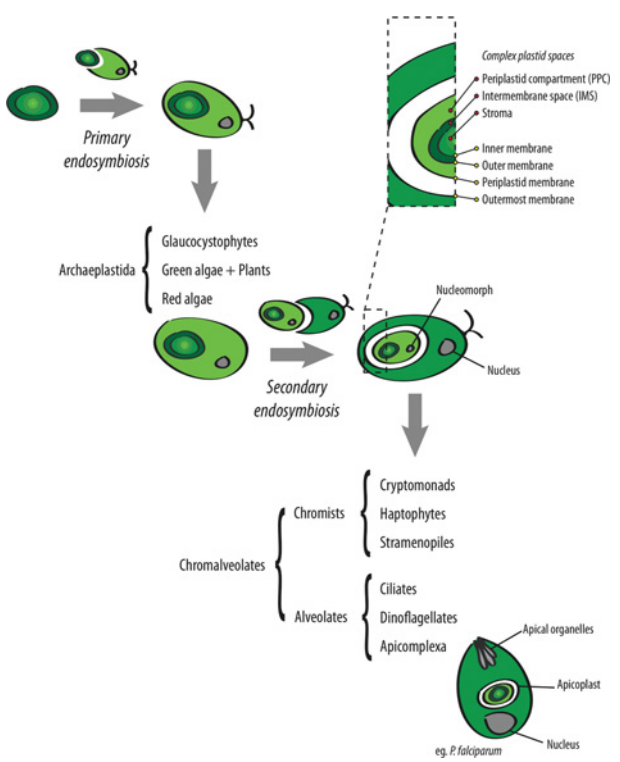
Several fundamental characteristics distinguish an enslaved organelle (the plastid) from its origin as an autonomous endosymbiont (the engulfed cyanobacterium) [23]. First, plastid membranes contain solute transporters, mostly derived from the host genome, allowing the host to harness nutrients and metabolites generated by its new organelle [24]. Secondly, plastid genomes are significantly reduced and truncated, and are completely incapable of encoding the genes for its own protein complement, leading to the hypothesis that plastid proteins were predominantly nuclear-encoded [25]. Indeed, evidence of gene transfer from the endosymbiont to the host is evident [26], and quantitative experiments have proved that transfer of genetic material, most likely in the form of double-stranded DNA, from the plastid to the nucleus is an ongoing and relatively frequent event [27]. Although nuclear-localized endosymbiont-derived genes must acquire eukaryotic characteristics such as promoters, terminators and introns in order to be transcribed and translated, endosymbiotic-gene transfer is calculated to have contributed as much as 16–18 % of expressed genes in the nuclear genomes of red algae, green algae and land plant lineages of the Archaeplastida [28].

However, gene transfer could not have led to a concomitant plastid genome reduction without the mechanism for protein translocation across the plastid membranes, coupled with the use of N-terminal transit peptides to traffic plastid proteins back to their original subcellular location [29]. These latter mechanisms of trafficking and translocation allowed the nuclear-localized gene copies to complement their plastid-localized counterparts, thereby facilitating the eventual degradation and loss of the plastid genes over evolutionary time. However, the development of novel import mechanisms across previously impervious membranes would have been a complex and evolutionarily costly process [22]. As such, the advent of protein import complexes in the plastid is considered to be one of the rate-limiting steps in the process of endosymbiosis [23].

Whereas the plastids of the Archaeplastida lineages are derived from one endosymbiosis and are surrounded by the two membranes of the engulfed cyanobacterium, three or four membranes surround the plastids of other lineages, such as the apicoplast of *P. falciparum* (Figure 1). These latter 'complex' or secondary plastids are derived from at least two serial endosymbiotic events involving the engulfment

Figure 1 | Endosymbiotic origins of the apicoplast of *P. falciparum*

The apicoplast of *P. falciparum* is a non-photosynthetic secondary plastid surrounded by four membranes. It is derived from two serial endosymbiotic events. The first, called primary endosymbiosis, occurred between a nucleated biciliate phagotroph and a photosynthetic cyanobacterium bounded by two membranes. This process gave rise to the three lineages of Archaeplastida: glaucocystophytes, red algae and Viridiplantae (including green algae and land plants). The second endosymbiosis involved the engulfment and retention of a red alga by a second phagotroph, which gave rise to the Chromalveolates, including *P. falciparum*, a parasite in the apicomplexan lineage. The outermost membrane of the secondary plastid is derived from the phagotrophic digestive vacuole that engulfed the red alga. The third membrane from the inside is called the periplastid membrane and is derived from the plasma membrane of the red alga. The first and second membranes, from the inside, are derived from the inner and outer membranes of the primary plastid respectively. As in primary plastids, the central space within the innermost membrane is called the stroma, whereas the space between the innermost and outer membrane is called the intermembrane space. The periplastid compartment, between the outer membrane and periplastid membrane, is derived from the endosymbiont cytosol and contains the nucleomorph in cryptomonads. Finally, the space between the periplastid membrane and outermost membrane is most likely to be continuous with the ER lumen. Conversion of the endosymbiont into a secondary plastid requires gene transfer from both the original plastid and the endosymbiont nucleus, now called the nucleomorph. Of the Chromalveolates, only the cryptomonads retain a nucleomorph within its secondary plastid.



and retention of a photosynthetic eukaryotic alga by another eukaryotic phagotroph [22]. In the case of the four-membrane-bounded plastids, the inner two membranes are analogous to the outer and inner membranes of the original plastid, whereas the third membrane, known as the periplastid membrane, is probably the former plasma membrane of the engulfed alga, and the outermost membrane is putatively an extension of the host's phagotrophic endomembrane system (Figure 1). The independent loss of one of these two outer membranes, probably the periplastid membrane, resulted in the formation of three-membrane bounded plastids of *Euglena* and dinoflagellates [30].

Similar to the enslavement of the cyanobacterium in primary endosymbiosis, the conversion of the secondary endosymbiont into a secondary plastid involved gene transfer to the host nucleus, this time from both the endosymbiont nucleus and plastid [31]. Unlike primary plastids, secondary plastids now have extra outer membranes that require novel mechanisms for protein targeting and translocation in order to return plastid proteins to their point of origin. Establishment of these mechanisms for protein import facilitated the complete loss of the endosymbiont nucleus (for example in the apicomplexan plastids), or its dramatic reduction, as in the cases of the nucleomorphs of the cryptomonads [32] and chlorarachniophytes [33]. Once again, the development of protein translocation is considered one of the rate-limiting processes in secondary endosymbiosis [34].

On the basis of the hypothesis that successful endosymbiotic events are rare and unusual events, dependent on the development of multiple complex adaptations between the host and the endosymbiont such as protein translocation, Cavalier-Smith [34] proposed the Chromalveolate theory which theorizes that the diverse plastids of chromists and alveolata (including apicomplexa) are all derived from just one single endosymbiotic event. In essence, this theory argues that one secondary endosymbiosis and subsequent divergence and adaptation is more parsimonious than the alternative hypothesis of multiple independent endosymbiotic events in each of these secondary plastid-bearing lineages. Subsequent studies of unusual nuclear-localized plastid-targeted genes, such as *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) [35] and *FBA* (fructose-6-phosphate aldolase) [36], as well as a genome-wide analysis of gene gains and losses across different Chromalveolate phyla [37], strongly support the hypothesis that the Chromalveolate lineages are monophyletic [38].

A corollary of the Chromalveolate hypothesis is that chromist or alveolate lineages that lack plastids (for example ciliates), actually inherited a secondary plastid from the proposed endosymbiotic event, but discarded it during their evolution. The genetic footprints of these now-absent plastids have been found in ciliates, oomycetes and *Cryptosporidium* [38].

Although initial analyses suggested that the apicomplexan secondary plastid was derived from a green alga [39], the monophyly of alveolates with chromists, which clearly have a red-algal-derived plastid [40], along with the plastid genome organization itself [41], conclusively support a red algal origin for the apicomplexan plastid [42]. However, a recent phylogenomic study of Chromalveolate genomes argues for

a more complex scenario in which these two apparently opposing arguments are not mutually exclusive. Moustafa et al. [43] found that more than 70% of the plastid-related genes in the two diatom genomes, accounting for 16% of the entire genomes, are related to green algal genes. These authors argue that the Chromalveolate cenancestor contained a prasinophyte green algal endosymbiont that was replaced by a red algal endosymbiont before the divergence of alveolates and chromists [34].

This proposed model also reflects a recent hypothesis that the primary endosymbiotic event that gave rise to the Archaeplastida was itself preceded by, or co-occurred with, an independent intracellular symbiosis with a *Chlamydia*-like prokaryote [44]. The identification of several *Chlamydia* genes in red algae and the Viridiplantae lineages, particularly among solute transporters to the plastid [24], supports this model. The authors propose that the presence of the non-photosynthetic symbiont assisted in the establishment of the endosymbiosis that produced all primary plastids [44]. Overall, these emerging hypotheses for primary and secondary endosymbioses highlight the complexity of converting an endosymbiont into an organelle, of which protein translocation is a central facet.

Protein targeting to the *P. falciparum* apicoplast

As a result of gene transfer and loss during endosymbiosis, the apicoplast of *P. falciparum* retains only a 35 kb remnant of the original endosymbiont genome [41], and the organelle itself does not contain a nucleomorph. Apicoplast biogenesis and function therefore critically relies on targeting nuclear-encoded proteins back to the organelle. Furthermore, since the apicoplast of *P. falciparum* is only one of numerous intracellular organelles in the parasite, distinctive apicoplast-targeting motifs are essential in order to traffic proteins specifically to the organelle.

In the case of the better-characterized chloroplast of land plants, the most predominant pathway for protein trafficking is with an N-terminal chloroplast transit peptide (reviewed in [45]). Here, chloroplast proteins are translated in the cytosol, and the transit peptide facilitates post-translational targeting and translocation across two membranes into the chloroplast stroma. Whereas there is no apparent consensus sequence or even prescribed length for these trafficking signals, there is an overall bias towards hydroxylated and basic amino acids. Interactions with cytosolic chaperones such as Hsp70 (heat-shock protein 70) and 14-3-3 proteins have also been shown for transit peptides of some chloroplast proteins. In solution, the chloroplast transit peptides probably lack any secondary or tertiary structure; however, transit peptides are known to interact directly with lipid bilayers and may possibly form amphipathic helices at membrane surfaces.

One of the initial observations of nuclear-encoded apicoplast-localized proteins of *P. falciparum* was that they also contain trafficking signals at their N-termini. However, unlike plant chloroplast proteins, apicoplast proteins have a chloroplast-like transit peptide that is preceded by a hydrophobic signal peptide [46]. Keystone truncation and mutagenesis experiments using the N-terminal bipartite leader of PfACP (*P. falciparum* acyl-carrier protein) fused to GFP (green fluorescent protein) subsequently showed that the signal peptide is required for targeting the apicoplast protein to the parasite endomembrane system, whereas the transit peptide is required to traffic the protein to the apicoplast [15]. Absence of the former caused cytoplasmic localization, whereas loss of the latter resulted in secretion of the protein from the parasite into the parasitophorous vacuole. This bipartite nature of the N-terminal leader of apicoplast proteins was shown to be both essential and sufficient for apicoplast localization. Mutagenesis of the transit peptide showed that basic amino acids, together with putative Hsp70-binding sites, were found to be important for transit peptide function [47]. The role of hydroxylated residues appears to be less significant in *P. falciparum* than in plants and algae, since neither serine nor threonine residues are enriched [48].

The unique combination of signal peptide followed by transit peptide, along with the distinctive characteristics of the transit peptide itself, enabled the construction of PlasmoAP, a rules-based bioinformatic approach for predicting apicoplast proteins [47]. Using PlasmoAP in a genome-wide bioinformatic search robustly predicted a pool of 466 proteins putatively targeted to the apicoplast, representing approx. 9% of the total *P. falciparum* genome [47]. These predicted apicoplast proteins were used subsequently to putatively reconstruct, at least partially, several biosynthetic pathways in the apicoplast, including fatty acid, isoprenoid, haem and iron–sulfur cluster biosynthesis [12].

Finally, a bioinformatic screen of the *P. falciparum* genome using the PlasmoAP tool found that 18.3% of exons could potentially function as transit peptides [49]. In addition, randomly generated transit peptides with positive PlasmoAP scores could also target to the apicoplast, confirming that transit peptide function is predicated solely on its amino acid composition, and not a cryptic conserved motif [49]. Overall, these observations indicate that the apicoplast-targeting transit peptide is a relatively simple and flexible trafficking signal that could have evolved by exon shuffling.

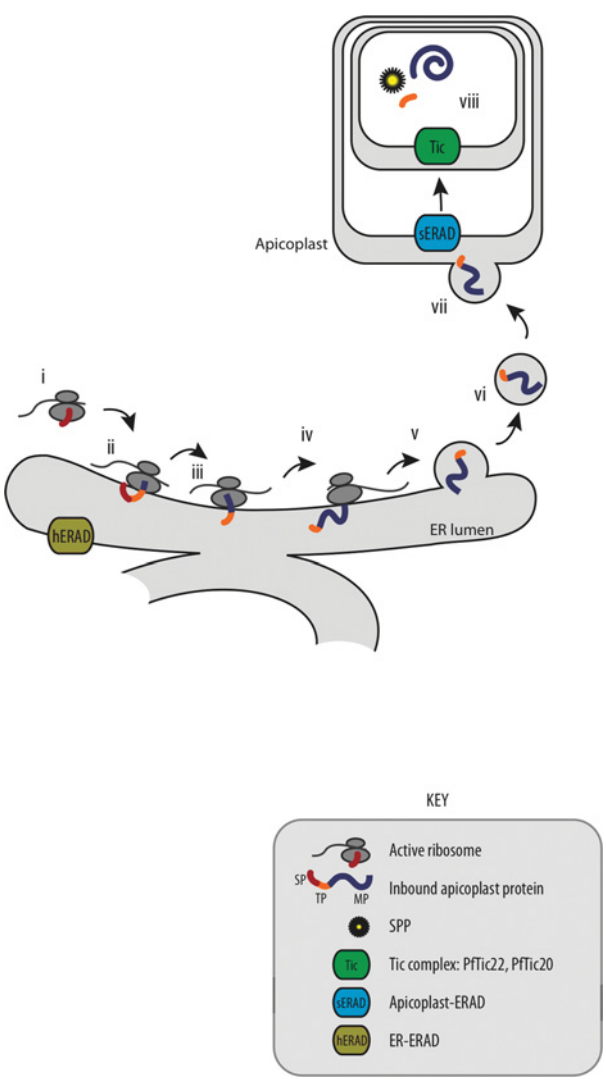
Translocation across four membranes: a model

Whereas the bipartite leader signal required for apicoplast targeting has been investigated extensively, the molecular mechanism for how these two trafficking signals facilitate protein translocation across four apicoplast membranes remains poorly understood. In the present review, we discuss an emerging model for this process (Figure 2).

Initially, apicoplast proteins enter the ER lumen by co-translation because the signal peptide of the bipartite leader of apicoplast proteins functions as a canonical signal peptide [46]. Since the outermost membrane of the apicoplast is connected, either transiently or continuously, to the ER

Figure 2 | Model for protein import into the apicoplast of *P. falciparum*

Most apicoplast proteins traffic to the organelle with N-terminal bipartite leaders, consisting of a signal peptide and a transit peptide. The signal peptide targets the nascent apicoplast protein to the ER membrane (i), where co-translational insertion into the ER occurs via the Sec translocon (ii). The signal peptide is removed during co-translation (iii), leaving the apicoplast protein in the ER lumen with only an N-terminal transit peptide (iv). How transport from the ER lumen to the apicoplast is achieved is unclear, but may involve an apicoplast-specific ER domain (v) and vesicular transport (vi–vii). Whereas vesicle-like structures similar to (vi) have been observed for some *T. gondii* outermost membrane proteins, none has been documented in *P. falciparum*. Whether by vesicles, or another unknown mechanism, the connection between the outermost membrane and the endomembrane system means that the space between the outermost membrane and the periplastid membrane is contiguous with the ER lumen. Consequently, once apicoplast proteins, now bearing only the transit peptide, have three remaining membranes to cross. Currently, protein translocation across the periplastid membrane is postulated to occur at the recently identified symbiont-derived ERAD complex (sERAD), which is distinct from the ER-localized, host-derived ERAD (hERAD). Components of the Toc and Tic complexes are predicted to facilitate translocation across the outer and innermost membrane respectively. However, functional evidence for these translocation processes has not yet been provided in *P. falciparum*. Once in the stroma, the transit peptide is removed by SPP (signal peptide peptidase), allowing the mature protein to be folded by stromal chaperones (viii).



membrane [30], the space between the outermost and periplastid membranes is therefore contiguous with the ER lumen (Figure 2). Thus co-translation at the ER membrane is effectively also translocation across the outermost apicoplast membrane [31]. As the signal peptide is removed by a signal peptidase during co-translation, apicoplast proteins retain only the transit peptide for traversing the three remaining membranes.

A new model invoking an adaptation of an apicoplast-localized ERAD (ER-associated degradation) translocation system has been proposed to explain translocation across the periplastid membrane [50].

Classically, ERAD involves the recognition, retrotranslocation and degradation of misfolded ER proteins by cytosolic proteasomes (reviewed in [51]). Briefly, misfolded proteins are detected by luminal chaperones, such as the Hsp70 homologue BiP (immunoglobulin heavy-chain-binding protein) and PDI (protein disulfide-isomerase), and subsequently targeted to a retrotranslocation site at the ER membrane where the ERAD substrate is returned to the cytosol via a proteinaceous channel. The identity of the translocation channel is disputed; however, putative channel components include the Sec61 import channel, the multiple membrane-spanning ubiquitin ligase Doa10 and the smaller membrane protein Der1. On the *trans* side of the membrane, an ATPase motor protein, Cdc48, is targeted to the ERAD translocase by its cofactors, Npl4 and Ufd1, and provides the energy that drives the translocation process. Ubiquitylation of the substrate protein by the ubiquitin-pathway enzymes E1, E2 and E3 could also occur before, or during, retrotranslocation. It is becoming evident that distinct ERAD pathways exist for different types of ERAD substrates. For instance, in the yeast *Saccharomyces cerevisiae*, three distinct pathways, ERAD-L, ERAD-M and ERAD-C facilitate translocation of luminal, membrane and cytosolic misfolds respectively. Consequently, many different proteins fulfil redundant roles in ERAD, expanding the complexity and flexibility of the retrotranslocation system.

Sommer et al. [50] identified genes encoding canonical ERAD components in the nucleomorph of the secondary plastid of the cryptomonad *Guillardia theta*. Searches in other Chromalveolate lineages quickly identified two suites of ERAD components, one putatively localized to the ER, and the other to the apicoplast by plastid-targeting bipartite leaders [50]. In *P. falciparum*, the plastid-targeted ERAD translocon consisted of an almost complete set of ERAD components, including proteins for substrate

recognition (PfBiP), two homologues of the channel-forming protein (PfsDer1-1 and PfsDer1-2, where 's' denotes symbiont), ubiquitylation components (PfUb, PfsUba1, an E1 component, and PfsUbc2, an E2 component) and translocation motor (PfsCdc48 and its cofactor PfsUfd1). Given their classical role in protein translocation, Sommer et al. [50] proposed that the plastid-localized ERAD complexes have an equivalent function to translocate proteins across the periplastid membrane [50]. This hypothesis has subsequently been supported by studies in diatoms [52], *T. gondii* [53] and *P. falciparum* [54,55].

After traversing the periplastid membrane, apicoplast proteins still retaining their transit peptides enter the periplastid compartment and face the remaining two innermost membranes (Figure 2). Both of these membranes are derived from the cyanobacterium engulfed during the first endosymbiosis, and are topologically equivalent to the outer and inner membranes of primary plastids (discussed above). As such, protein translocation across these final two membranes is also predicted to be analogous to the translocation process in primary plastids [56].

Protein import into primary plastids has been well studied in the chloroplast of higher plants over two decades, and is known to require two translocons called Toc and Tic, located on the outer and inner chloroplast membranes respectively (reviewed in [29,45,57]). Initially, the transit peptides of inbound chloroplast proteins dock with the outer membrane at receptor proteins, such as the GTP-binding heterodimers Toc34 and Toc159, or the TPR (tetratricopeptide repeat)-bearing Toc64, although the significance of this latter pathway are disputed [58,59]. Subsequently, inbound proteins are translocated across the outer membrane by the integral membrane β-barrel channel protein Toc75. The Toc complex is physically associated with components of the Tic complex forming a 0.8–1 MDa complex [60,61], allowing incoming proteins immediate entry into the stroma from the cytosol. Tic22 is an intermembrane space component that may function as a structural scaffold holding the two translocons together, or possibly as a protein chaperone ferrying the inbound chloroplast protein between the two membranes. The composition of the transmembrane channel in the Tic complex is not clear, although various components have been proposed as the translocation pore, including Tic20, Tic21 and Tic110 [57]. On the stromal side of the inner membrane, the AAA (ATPase associated with various cellular activities) chaperone ClpC docks at the inner membrane by interacting with Tic40 and Tic110, and probably functions as the motor that pulls incoming chloroplast proteins across both membranes [62]. Other Tic components probably function to modulate the translocation process, for example by sensing the redox state of the chloroplast (Tic55, Tic32 and Tic62) or by responding to calcium signals (Tic32) [45].

Although this process of protein translocation is well established in higher plants, it is less well characterized in the other primary plastids of green and red algae [56,57], let alone secondary plastids derived from two endosymbiotic events. Although it remains to be confirmed whether any Toc or Tic component is involved in apicoplast protein import in *P. falciparum*, bioinformatic searches have identified homologues of Tic22 and Tic20, with the *P. falciparum* Tic22 localizing to the apicoplast [54]. Furthermore, van Dooren et al. [63] localized a *T. gondii* Tic20 homologue to the innermost membrane of the apicoplast, although knockout data indicates that it may not be involved in protein import. Thus whether the Toc and Tic translocons are present, as well as functional as import complexes, in apicomplexan parasites remains a hypothesis to be confirmed.

Concluding remarks

The plastid of malaria and related parasites was acquired via two rounds of endosymbiosis. The primary endosymbiosis that created all plastids was followed by a secondary endosymbiosis that created a large group of photosynthetic protists known as the Chromalveolates [34], of which apicomplexan parasites are merely one lineage. The recent identification of a photosynthetic apicomplexan (*Chromera*) living symbiotically in corals [8] corroborates the hypothesis that apicomplexan parasites arose from symbiotic protists able to live mutualistically in animal tissues [64]. The capacity to translocate proteins across the four membranes of the *P. falciparum* apicoplast was critical for the evolution of the organelle from its endosymbiotic origins, and remains an essential process for organellar biogenesis and function [31]. An overview of the machinery involved in translocating proteins across the four membranes surrounding the apicoplast is emerging. Some components were inherited from the cyanobacterial endosymbionts, whereas others involved in translocation across the inner two membranes were supplied by the host [57]. The secondary endosymbiotic acquisition of a plastid such as the apicoplast necessitated adaptation of endomembrane machinery to cross the outer two membranes [50,54,55]. Thus a relatively complicated apparatus has evolved stepwise as the endosymbiotic integration into the two successive hosts unfolded. The challenges now are to dissect the workings of these machines and hopefully find a way to perturb their function as a means to combating one of humankind's deadliest foes.

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