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The apicoplast: a red alga in human parasites

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Abstract

Surprisingly, some of the world's most dangerous parasites appear to have had a benign photosynthetic past in the ocean. The phylum Apicomplexa includes the causative agents of malaria and a number of additional human and animal diseases. These diseases threaten the life and health of hundreds of millions each year and pose a tremendous challenge to public health. Recent findings suggest that Apicomplexa share their ancestry with diatoms and kelps, and that a key event in their evolution was the acquisition of a red algal endosymbiont. A remnant of this endosymbiont is still present today, albeit reduced to a small chloroplast-like organelle, the apicoplast. In the present chapter, I introduce the remarkably complex biology of the organelle. The apicoplast is bounded by four membranes, and these membranes trace their ancestry to three different organisms. Intriguingly, this divergent ancestry is still reflected in their molecular makeup and function. We also pursue the *raison d'être* of the apicoplast. Why did Apicomplexa retain a chloroplast when they abandoned photosynthesis for a life as obligate parasites? The answer to this question appears to lie in the profound metabolic dependence of the parasite on its endosymbiont. This dependence may prove to be a liability to the parasite. As humans lack chloroplasts, the apicoplast has become one of the prime targets for the development of parasite-specific drugs.

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Apicomplexa are important pathogens

Apicomplexa are small single-celled eukaryotes. Traditional taxonomy counted them among the protozoa, a hodge-podge of unicellular organisms at the base of the eukaryotic tree of life. However, Apicomplexa are not the friendly protozoans whizzing about in the microscopy class that introduced you to the wondrous menagerie of pond water. Apicomplexa are dangerous parasites and live at the expense of their host animals. Many of them have the potential to cause significant harm or even kill their hosts. Several species of Apicomplexa are important human pathogens, collectively causing more than a million deaths per year and hundreds of millions of cases of illness. These include several species of *Plasmodium* causing malaria, an infection of the blood that is characterized by episodic high fever and several life-threatening complications including severe anaemia, cerebral malaria and renal syndromes. Other Apicomplexa infecting humans are *Toxoplasma,* which if unchecked by the immune system will attack and quickly destroy the central nervous system in unborn children and AIDS patients, and *Cryptosporidium* and *Cyclospora*, which cause severe diarrhoeal diseases. There is an even larger variety of important veterinary pathogens among the Apicomplexa.

Apicomplexa, particularly the malaria parasite *Plasmodium*, remain a tremendous and largely unmet challenge to public health and medicine. The development of vaccines to prevent malaria has proven very difficult. The parasite has evolved to frequently change the proteins that the human immune system recognizes most prominently. This process of antigenic variation ensures that the parasite remains a step ahead of the immune system and it takes years of exposure and infection for people to develop robust immune control of the disease. The parasite's ability to change and adapt rapidly is also a significant problem for drug treatment. The emergence of chloroquine-resistant malaria parasites has been a public health disaster. Drug resistance of apicomplexan parasites also plagues veterinary medicine as in the case of *Eimeria* infection of chickens. A steady stream of new drugs acting upon different targets and through different mechanisms is required to keep up with an ever-evolving parasite population. Currently, one of the most hotly pursued targets is the apicoplast, a unique parasite organelle that is related to the chloroplasts of plants and algae.

A small step for an alga – a giant leap for the tree of life

The realization that Apicomplexa have an organelle related to chloroplasts was a big surprise. Apicomplexa (previously referred to as Sporozoa) were long the domain of zoology, not botany. The discovery of the apicoplast came in two major breakthroughs. The first one was the cloning and sequencing of the 'third' genome: a 35000 bp DNA circle [1]. This genome had been known about for some time and had initially been thought to be the mitochondrial genome. Studying the genes that are encoded on the 35 kb

genome, their particular organization on the circle, and their phylogenetic relationship to genes from other organisms suggested that it most closely resembles the genome of an algal chloroplast. The second breakthrough was to demonstrate that the genome has a particular home in the parasite cell, an organelle that is surrounded by four membranes [2,3]. How did Apicomplexa obtain a chloroplast, and why would this chloroplast have four membranes? An answer to this question is provided by the concept of endosymbiosis, which is outlined schematically in Figure 1(A). One cell, the endosymbiont, is engulfed by another cell, the host, and then settles into an intracellular life style that results in its transformation from a free-living organism to a dependent organelle. This theory was first developed to explain the origin of the mitochondrion, noting the many similarities between this organelle and α-proteobacteria. It was not long before biologists realized that this model can also be applied to the chloroplast, which is now viewed as a domesticated cyanobacterium. Amazingly, this was not the end of the journey. In multiple subsequent instances, previously non-photosynthetic organisms acquired and domesticated single-celled eukaryotic algae [4]. This process is referred to as secondary endosymbiosis, and the resulting organelles as secondary or complex plastids (Figure 1A). Note that most of these complex plastids are surrounded by four membranes, a tell-tale sign of their origin. The two innermost membranes are thought to be derived from the membranes of the original chloroplast (themselves derived from the inner and outer membrane of the cyanobacterium), the third one represents the plasma membrane of the endosymbiont alga, and the outermost membrane is derived from the host endosomal compartment. Secondary endosymbiosis has been described for both green and red algae. As shown in Figure 1(B), a single acquisition of a red algal endosymbiont is believed to have given rise to a major branch of the eukaryotic tree of life: the chromalveolates. The chromalveolates have conquered a tremendous breadth of ecological niches. Many of these organisms are still photosynthetic, such as kelps and diatoms, whereas others have lost photosynthesis and are now predators (such as the ciliates) or parasites (such as apicomplexans or oomycetes). Recently, a photosynthetic apicomplexan, *Chromera*, was discovered that itself lives as a symbiont of corals [5]. Overall, this suggests that Apicomplexa had a photosynthetic past in the ocean and subsequently adapted to living within animals as symbionts or parasites. This 'algal past' is an important denominator of the apicomplexan present and holds fascinating clues to the evolution of eukaryotic cells as well as real-world leads for urgently needed drug treatments.

Why does a parasite need a chloroplast?

What does the apicoplast do? It is reasonable to assume that the acquisition of photosynthesis was the main initial benefit of the endosymbiont to its host. As pointed out, many of the descendents are still photosynthetic today. However, most Apicomplexa are believed to have been parasites for hundreds of millions of

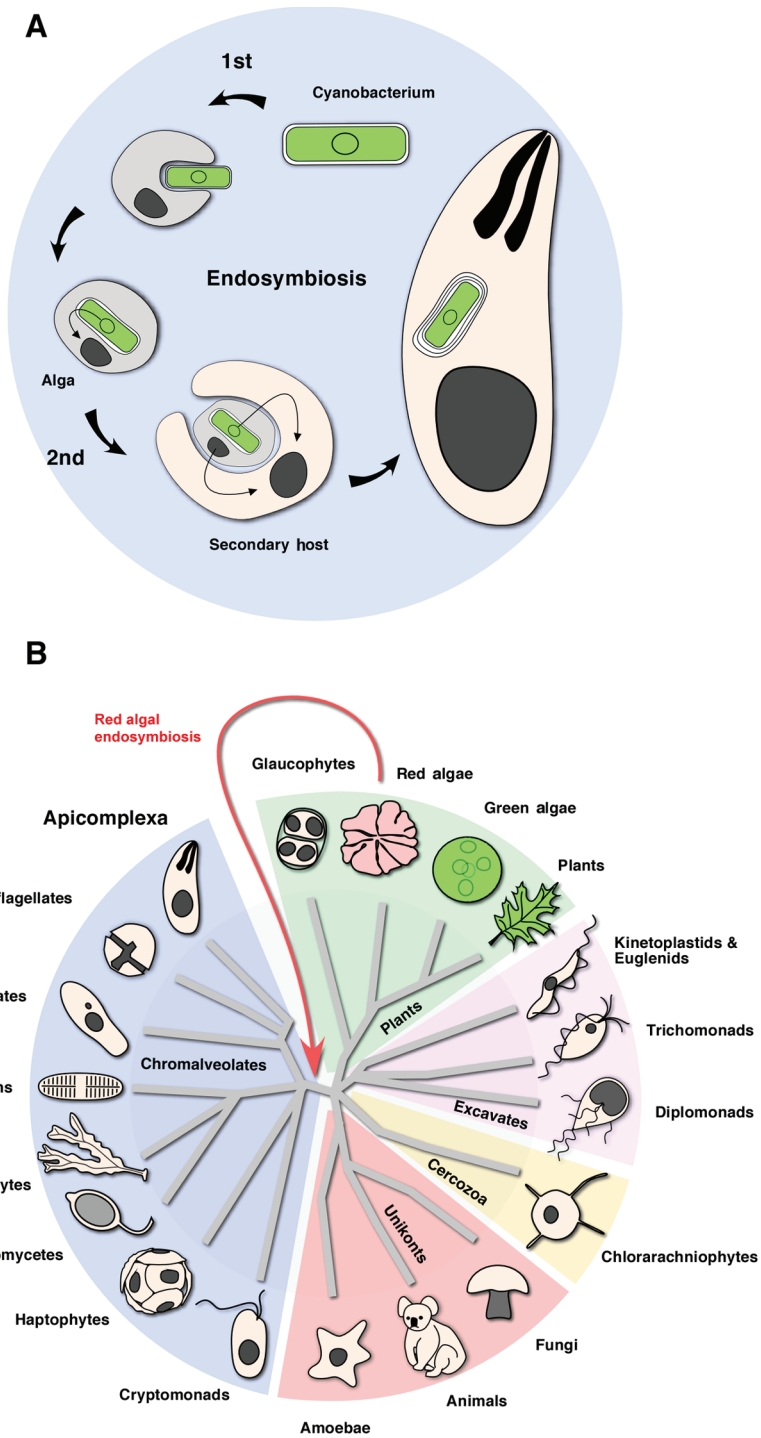

Figure 1. Secondary endosymbiosis and the origin of the phylum Apicomplexa

(A) Diagrammatic representation of the complex evolutionary process that gave rise to present day primary and secondary plastids. A cyanobacterium was engulfed by a heterotrophic eukaryote giving rise to the plastids of red and green algae (including land plants) and glaucophytes.

years with little or no access to light. In addition, none of the key genes involved in photosynthesis (e.g. those encoding the elements of the photosystems or the enzymes for the synthesis of chlorophyll) appear to be present in their genomes at this point. Nonetheless, the apicoplast is critical for the growth and development of the parasites. This was demonstrated initially in *Toxoplasma* through pharmacological and genetic experiments. The treatment of *Toxoplasma* cultures with ciprofloxacin, an antibiotic that leads to DNA breaks in bacteria and chloroplasts, produced specific loss of the apicoplast genome and subsequent death of the parasite [6]. Similarly, parasite mutants that have inducible defects in the apicoplast replication and division process die upon loss of the organelle [7,8]. It may be helpful here to consider that chloroplasts have functions in plants that go well beyond photosynthesis. Chloroplasts are major biosynthetic hubs and produce numerous important classes of compounds. In fact, all plant cells, even those not engaged in photosynthesis such as those in the roots, maintain plastids to provide these essential building blocks. This line of thought led to the proposal that the apicoplast may function like a chloroplast in the dark, providing metabolites rather than energy [9]. Endosymbiosis generated metabolic redundancy and gave the host organism the choice of using its own established biochemical capacities or to rely more heavily on those of its new acquisition. It appears that the host chose to maintain the chloroplast functions in pathways that produce highly reduced molecules (such as lipids). The probable reason is that photosynthesis generates reduction equivalents and energy in abundance by harvesting the energy of light and fixing carbon dioxide into simple carbohydrates. Tapping into these sources right where they emerge may have been the most efficient solution. In the light, these compounds came at no or little cost to the host. This redundancy might subsequently have resulted in the loss of host pathways. The catch of this streamlining was that it rendered the symbiont essential. Once addicted to the 'free' metabolites provided by the symbiont, the host had to maintain it even after abandoning photosynthesis.

Discovering the metabolic functions of the apicoplast

So what are the specific metabolic functions of the apicoplast? The traditional approach to answer this question would be to purify the apicoplast and to assay its function using biochemistry, or in a modern variation to identify all

Figure 1 (continued). In a second endosymbiotic event, an alga was taken up by and another eukaryote (note that this occurred multiple times independently). The resulting complex plastids are surrounded by four (and sometimes three) membranes. Narrow arrows indicate gene transfer from the symbiont to the host genome **(B)** Schematic tree of eukaryotic life. The relationships show here are based on phylogenetic analyses summarized by Keeling et al. [34]. The ancestor of present day chromalveolates acquired its plastid through endosymbiotic uptake of a red alga. Diversification and adaptation to different ecological niches led to subsequent loss of photosynthesis (as in Apicomplexa) or loss of entire plastids (as in ciliates or oomycetes). Reprinted from *Protist*, vol. 161, Agrawal, S. and Striepen, B., More membranes, more proteins: complex protein import mechanisms into secondary plastids, pp. 672–687, © 2010, with permission from Elsevier. <AQ1>

of the proteins present in the organelle by MS and to deduce function from that. However, purification of the apicoplast has proven quite technically challenging. In the absence of a direct route, the parasite genomes have proven a marvelous resource to indirectly piece together a metabolic map for the apicoplast. The genome of the malaria parasite *Plasmodium falciparum* was the first fully sequenced apicomplexan genome [10], followed by *Toxoplasma*, *Cryptosporidium* and many other species that now provide researchers with a remarkably complete catalogue of genes and proteins. This catalogue has been mined for potential apicoplast enzymes using several bioinformatic strategies. One straightforward and highly successful approach has been to work out a blueprint derived from the metabolism of the plant chloroplast and to directly search for homologues of known plant chloroplast enzymes [11,12]. One can also look for proteins that more generally appear 'plant- or alga-like', specifically proteins that show the highest degree of sequence similarity to proteins from plants and algae. Simple similarity, however, is not always a guarantee for shared evolutionary origin. Numerous sophisticated approaches have been developed to track more reliably the evolutionary origin of proteins by constructing, statistically evaluating and analysing phylogenetic trees in a more and more automated fashion. A variation of this model uses considerable computing power to brute-force compare complete genomes to identify genes that are shared among organisms that have plastids and are missing in organisms that lack plastids. Finally, instead of being on the lookout for proteins that share the same evolutionary origin, one can also hone in on proteins that may be in the right place. As will be detailed below, most apicoplast proteins carry a characteristic N-terminal targeting motif. Developing algorithms that scan predicted proteins for elements of this motif has also been highly informative. Putting all these tools together, the apicoplast proteome has been predicted to be composed of about 500 proteins (representing roughly 10% of the total proteome of the apicomplexan cell)[9,13,14]. Three major anabolic pathways typically found in chloroplasts are now established in the apicoplast of *Plasmodium* and *Toxoplasma*: fatty acid synthesis, isoprenoid synthesis and segments of the haem synthesis pathway. Comparing the genomes of various apicomplexan species shows interesting differences in apicoplast pathway representation summarized in Figure 2. These differences show that metabolic reliance on the apicoplast is not set in stone. There are genera, such as *Theileria*, that appear to have reduced the main function of the apicoplast to the production of isoprenoids, and others, such as *Cryptosporidium*, that have lost the endosymbiont altogether. Overall, this may indicate that certain metabolites are easier or harder to attain by different parasites, depending on the specific cell or tissue niche they occupy. Specialization on to a particularly 'rich' niche such as the intestinal epithelium or the cytoplasm of cells may allow some species to curtail or sever their dependence on the metabolites provided by the endosymbiont.

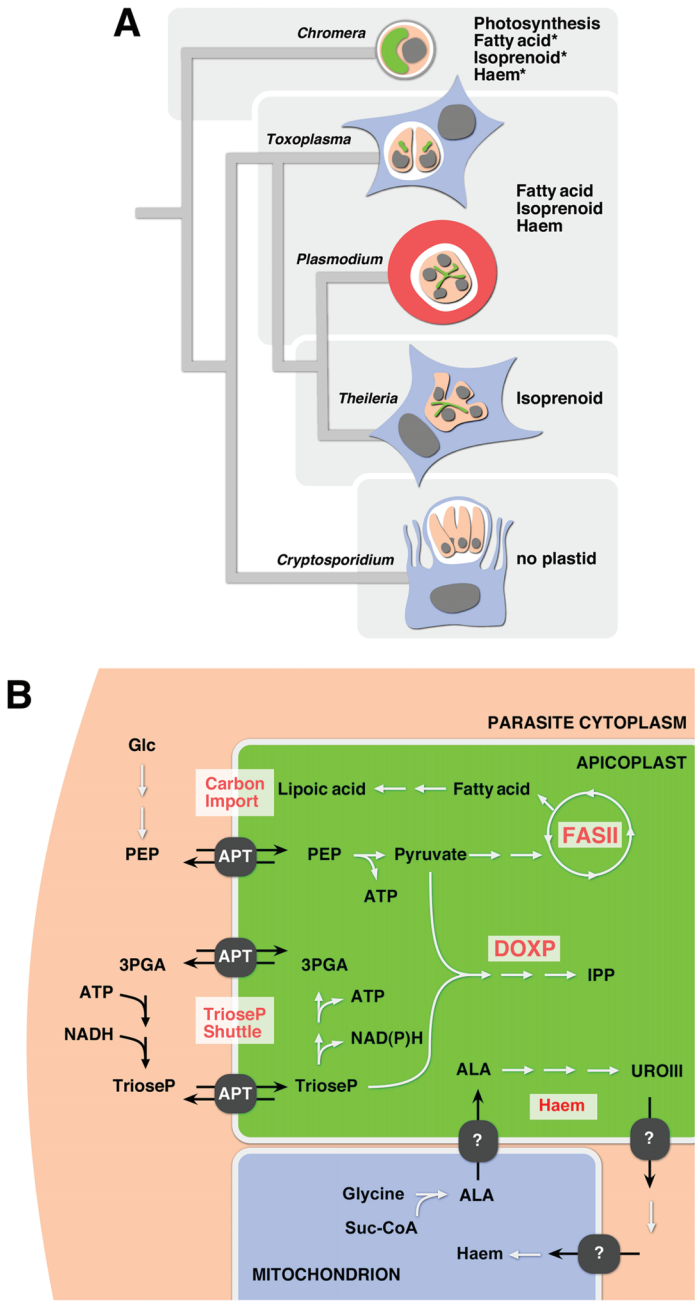

Figure 2. The metabolic functions of the apicoplast

(**A**) Schematic tree representing the evolutionary relationships among different members of the Apicomplexa. Metabolic functions are indicated and based on comparative genomic analyses. *Note that the functions indicated for *Chromera* at this time are speculative. (**B**) Highly schematic overview of the apicoplast metabolism of *Toxoplasma*. Three major pathways (FASII, DOXP and haem) have been identified and two of them depend on the activity of the APT. Note that many steps were omitted for simplicity here. Please refer to [9,14] for in-depth description of these pathways. ALA, aminolevulinic acid; Glc, glucose; PEP, phosphoenolpyruvate; suc-CoA, succinyl-CoA; UROIII, uroporphyrinogen-III.

The apicoplast is an anabolic hub

The pathways that have their home in the apicoplast actually trace their origin back to the cyanobacterium that gave rise to the chloroplast in the primary endosymbiosis event. Importantly, these pathways are not only of divergent evolutionary origin from those used by human cells, but they also show significant differences in their biochemical mechanisms resulting in differential sensitivity to inhibitors. In other words, dependence on pathways of bacterial origin is an Achilles heel of the parasite that we could exploit to develop drugs that specifically poison the parasite while leaving the human host unharmed. The pathway that initially drew the most attention is the apicoplast fatty acid synthesis pathway. Fatty acids are critical components of the lipids that form cellular membranes and they are also used as stores of energy. They are synthesized by the sequential extension of a growing hydrocarbon chain that is held by ACP (acyl-carrier protein). In each round, a two-carbon unit is added using malonyl-CoA as a donor, followed by the complete reduction of the penultimate carbon in three additional enzymatic steps. Like bacteria and chloroplasts, the apicoplast uses a FASII (fatty acid synthesis type II) system. Here, ACP and the various enzymes are encoded as individual polypeptides. In contrast, humans rely on a FASI (fatty acid synthesis type I) system in which all activities are encoded into a single giant protein. FASI and FASII show varying degrees of sensitivity to the inhibitors triclosan, thiolactamycin and cerulenin. Initial pharmacological and biochemical studies appeared to suggest that the FASII system may be a highly promising drug target. In these studies, triclosan, an inhibitor of FASII and a compound found in many antibacterial soaps, was able to inhibit the growth of *Plasmodium* and *Toxoplasma*. Subsequent genetic studies have produced knockout mutations in key components of FASII and have painted a more complex and differentiated picture [15–17]. The pathway is indeed essential for *Toxoplasma* as well as for the initial development of malaria parasites in liver cells. Surprisingly, it is dispensable for the blood stages of malaria; note that the blood phase is the clinically most important stage of malaria. These studies also demonstrate that a mutant malaria parasite lacking the gene for the FASII enzyme enoyl-reductase FabI, the presumptive target of triclosan, is just as sensitive to triclosan as is the wild-type. This suggests that the activity of triclosan against malaria parasites is due to an off-target effect, and that identifying FASII inhibitors may not be the best route to new antimalarials.

The second pathway that has been studied intensely produces isoprenoids. Isoprenoids are another important class of lipids that are based on a five-carbon building block characterized by a branched chain and two double bonds. Using the activated building block IPP (isopentenyl pyrophosphate), cells can construct a vast array of compounds that include cholesterol (a component of membranes), dolichol and farnesol (required for the synthesis of various protein modifications), and ubiquinone (an essential electron carrier for oxidative phosphorylation in the mitochondrion) to name a few examples.

As was the case for fatty acid synthesis, the apicoplast isoprenoid pathway differs from that used by humans. Humans and other animals use the mevalonate pathway that uses three molecules of acetyl-CoA to build a six-carbon precursor that is subsequently reduced and decarboxylated. This pathway is the target of mevastatin, the active component of the commercially highly successful cholesterol-lowering drug Lipitor. In contrast, the apicoplast relies on the DOXP or MEP pathway (named after its two key intermediates: deoxy-xylulose phosphate and methyl-erythritol phosphate) found in bacteria and plant chloroplasts. This pathway uses a completely different set of enzymes and starts with the two three-carbon precursors pyruvate and glyceraldehyde 3-phosphate. These are used to synthesize the five-carbon DOXP, which is subsequently reduced and remodelled to form IPP. These enzymes are essential for parasite growth, and genetic ablation of the pathway in *Toxoplasma* is lethal [17a]. Furthermore, in malaria parasites the DOXP pathway can be specifically inhibited using the antibiotic fosmidomycin. Encouragingly, fosmidomycin inhibits the growth of malaria parasites in the blood stage and has shown some efficacy in the treatment of clinical malaria in humans when used in combination with synergistic drugs [12,18]. The apicoplast also takes part in the synthesis of haem. Haem, best known for its role in co-ordinating molecular oxygen in haemoglobin, is also an important cofactor for several cellular enzymes. The haem pathway initiates in the mitochondrion and then moves to the apicoplast for three steps, followed by two cytoplasmic reactions and a final step that occurs back in the mitochondrion. This pathway not only has a complex subcellular localization, but also appears to be an evolutionary mosaic assembled from different sources. Although an inhibitor of one of the apicoplast-localized enzymes was shown to kill *Toxoplasma* at very high concentrations, further work is needed to establish the therapeutic potential of this pathway [19].

The apicoplast is fed from the cytoplasm

As we discussed above, photosynthesis provides for the vigorous anabolic synthesis activity of chloroplasts. Upon loss of photosynthesis, Apicomplexa had to feed their previously self-sufficient endosymbiont with carbon sources, energy and reduction equivalents. They found an ingenious solution to this problem by reversing the flow of the transport system that previously allowed them to reap some of the benefits of photosynthesis. The apicoplast membranes are crossed by phosphate translocators [20]. These are multispan membrane proteins of chloroplast origin that act as antiport systems and exchange inorganic phosphate for phosphorylated carbohydrates of varied structure. The chloroplast employs a selection of translocators to exchange C_3, C_5 and C_6 phosphates with the cytoplasm. The APT (apicoplast phosphate translocator) has a unique specificity and transports phosphoenol pyruvate, triose phosphate and 3PGA (3-phosphoglyceraldehyde) [21,22]. As schematically shown in Figure 2(B), this provides the building blocks for fatty acid and isoprenoid

synthesis, and in addition establishes a shuttle that generates ATP and NADPH in the lumen of the apicoplast. Genetic ablation of this transporter in *Toxoplasma* results in swift parasite death, underscoring the promise of apicoplast metabolic activity as a potential drug target. Analysis of the mutant phenotype further supports the notion that isoprenoid synthesis may be the most critical apicoplast function [21] and therefore the most promising target for drug development. With an eye on drug development, it is important to note that potential targets are not limited to anabolic pathways. Targeting the replication, transcription and translation of the apicoplast genome represents major opportunities that already have delivered drugs for clinical use (e.g. azithromycin for the treatment of toxoplasmosis or tetracycline for the treatment of malaria) [23]. Again, the divergent prokaryotic origin of the machinery can be exploited to derive drug specificity. In addition, targeting bacteria-type translation mechanisms can piggy-back on decades of work on antibiotics.

Apicoplast proteins are largely not encoded on the apicoplast genome

The apicoplast maintains a genome, but this genome is quite small and encodes only a moderate number of proteins that are restricted to housekeeping functions such as transcription and translation. Where are all the algal genes that encode apicoplast proteins active in the metabolic processes we discussed? The answer to this question lies in gene transfer. Gene transfer from the genome of the endosymbiont to the nuclear genome of the host is a hallmark of endosymbiosis. This is not limited to chloroplasts, however, and as an example, the vast majority of our own mitochondrial proteins are encoded in the nucleus and not the mitochondrion. Gene transfer provides the host with control over its endosymbiont, but the proteins are now translated on cytoplasmic ribosomes and have to be rerouted into the organelle post-translationally. This is no small feat, as the proteins have to overcome the four membranes that surround the apicoplast. The molecular mechanism of this process has only recently begun to emerge. The present chapter focuses on Apicomplexa, but please note that the view of apicoplast protein import has been heavily influenced by work in related algal systems [24,25]. For the majority of apicoplast proteins, the process is guided by a targeting sequence found at the N-terminus of the protein [11,13]. The targeting sequence is bipartite: the first component is a classical signal peptide that will result in co-translational insertion into the ER (endoplasmic reticulum) through the Sec61 translocon (see Figure 3A for a schematic representation of this and the following steps). The second component is referred to as a transit peptide and is required for the remainder of the journey. Once in the ER, proteins are believed to traffic to the apicoplast in a vesicle-mediated fashion that side-steps the Golgi apparatus [26]. This step remains poorly understood. In several related chromalveolates, the outer plastid membrane is continuous with the ER, providing a direct bridge [4]. However, in Apicomplexa no such connection is obvious. Vesicle fusion would deliver

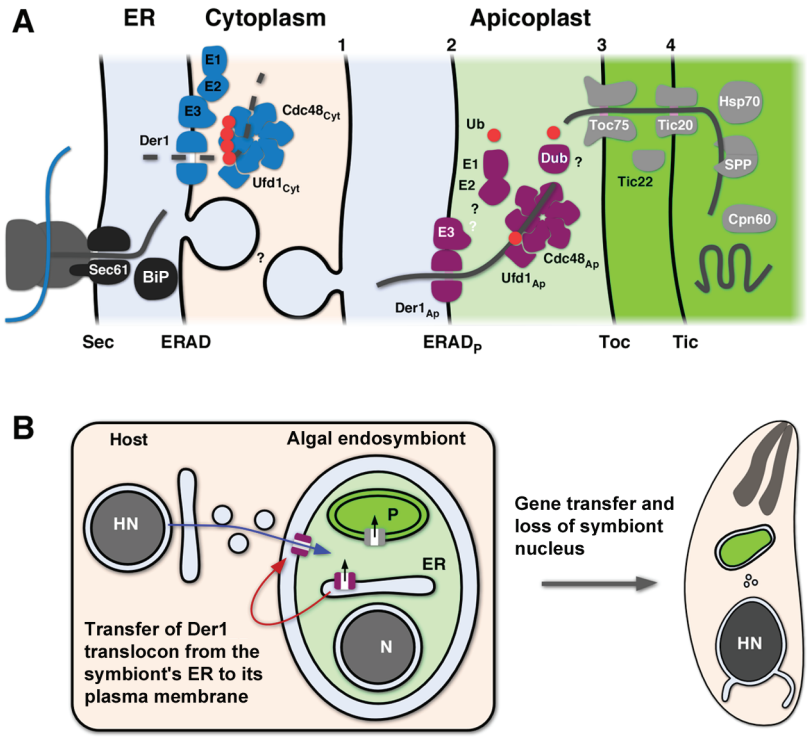

Figure 3. The mechanism and evolution of apicoplast protein import

(**A**) Schematic outline of a molecular model of the plastid protein import machinery based on results from apicomplexans and diatoms. Reprinted from *Protist*, vol. 161, Agrawal, S. and Striepen, B., More membranes, more proteins: complex protein import mechanisms into secondary plastids, pp. 672–687, © 2010, with permission from Elsevier. Note that not all elements have been experimentally validated (several such points are highlighted by a question mark). The pathway from the ER to the apicoplast remains highly speculative. Cargo proteins are shown as grey lines, proteins destined for degradation as broken lines. Sec61is an ER translocon; Der1, Cdc48 and Ufd1 are ERAD components; E1–E3 are ubiquitinylation factors; BiP (immunoglobulin heavy-chain-binding protein), Hsp70 (heat-shock protein 70) and Cpn60 (chaperonin 60) are chaperones. Ap, apicoplastic; Cyt, cytoplasmic; Dub, deubiquitination factor; $ERAD_P$, ????; SPP, signal peptide peptidase; Ub, ubiquitin. (**B**) Hypothetical model of apicoplast translocon evolution. The endosymbiont duplicated and relocated the Der1 translocon from the ER to its plasma membrane (red arrow and purple translocon). The host already had an established route to target proteins to its endosomal compartment. Combining both elements (blue arrow) permitted targeting to the symbiont's cytoplasm and was followed by import into the chloroplast using Toc and Tic (grey translocon). HN, host nucleus; N, nucleus; P, plastid. <AQ2>

proteins to the outermost compartment of the apicoplast. How the next membrane, which is thought to be the remnant of the former cell membrane of the alga, is crossed was long enigmatic. A breakthrough came from the sequencing of a truly tiny genome, the three small chromosomes of the nucleomorph of the haptophyte *Guillardia*. The nucleomorph is the remnant of the nucleus of the red algal endosymbiont. Sommer et al. [24] noted the presence of genes for multiple elements of an ERAD (ER-associated degradation) system, which

generally extracts misfolded proteins from the ER back into the cytoplasm. This was surprising as the symbiont no longer has an ER, and the authors hypothesized that this machine had been retooled to an import mechanism. Over the last three years this hypothesis has accumulated substantial experimental support. ERAD proteins are found in the periphery of complex plastids in numerous organisms including Apicomplexa, and mutation of Der1, the presumptive pore component of the translocon, ablates protein import [27–29]. The current working model is depicted in Fig 3A: cargo proteins are thought to be threaded through a pore formed by Der1, driven by the ATPase activity of Cdc48, which is aided by its cofactor Ufd1. The classical ERAD process is tightly associated with ubiquitinylation and this appears conserved in the apicoplast and other complex plastids [29,30]. This may suggest that the role of ubiquitin in ERAD is not limited to tagging proteins for proteasomal destruction later on, but that it plays a more immediate mechanistic role in the translocation process.

The two innermost membranes are believed to represent the envelope of the former chloroplast of the algal endosymbiont. It may be reasonable to hypothesize that transport phenomena across these membranes show similarities to those observed in the ancestral chloroplast. Transport across the chloroplast envelope from the cytoplasm into the stroma has been studied in great detail in plants [31]. In the plant chloroplast, proteins are imported by two multi-protein complexes, the Toc (translocon of the outer chloroplast membrane) and the Tic (translocon of the inner chloroplast membrane). Evidence for a Toc translocon in the apicoplast remains indirect. A potential homologue of Toc75, the pore component, has recently been characterized in diatoms, and the authors of that study also describe proteins with sequence similarity in Apicomplexa [32]. The Tic translocon is now well established. A divergent homologue of the pore candidate Tic20 is encoded in the genomes of all Apicomplexa that carry a plastid, yet is missing in the plastid-less *Cryptosporidium*. The protein is an integral component of the innermost apicoplast membrane, and loss of the protein (by gene targeting) results in loss of apicoplast protein import and the death of the parasites [33]. Overall, it appears that the evolutionary past of each membrane is deeply imprinted on the molecular mechanisms that are used to cross it. It is also interesting to note that the key elements for the protein import mechanism appear to be contributed by the symbiont and not the host. As an example, the apicoplast ERAD system is phylogenetically most closely related to that from organisms of the red algal lineage and is distinct from the ERAD system active in the ER of Apicomplexa (see Figure 3B and [27]). Endosymbiosis is often described in terms of enslavement or subjugation of the smaller organism. The fact that the machinery that permits protein exchange and communication between the two cells comes from the symbiont may temper these views and instead argue for an invasion or at least a cohabitation model that in no small part is driven by, and to the benefit of, the smaller partner.

Conclusion

The apicoplast is a fascinatingly complex product of evolution, combining the history and molecular features of three organisms. Much remains to be discovered about the mechanisms that evolved along the way to make this such a successful union. Unravelling these principles pertains to our general understanding of the origin and evolution of the eukaryotic cell and in addition may help to combat important diseases. It is now clear that the organelle is essential for parasite growth and development, home to several metabolic pathways that are highly divergent from their human counterparts, and an excellent drug target. Although the metabolic capabilities of the organelle have emerged in broad strokes, we still do not understand what the key metabolites are that make the apicoplast so important. Combining mutant analysis with novel approaches to measure hundreds of cellular metabolites in parallel could further advance this understanding. Overcoming the technical hurdles in purification of the apicoplast would be another route to reach this goal. Developing apicoplast pathways and enzymes into robust targets for chemotherapy will depend on a sustained medicinal chemistry effort. Exciting candidate compounds have been identified that inhibit various apicoplast functions, but significant improvements of potency, host toxicity and pharmacokinetics are required to turn these into clinical successes.

Summary

- *Apicomplexa have a photosynthetic past and maintain a unique chloroplast-like organelle, the apicoplast.*
- *The apicoplast is a red algal endosymbiont and represents an ancient acquisition prior to the radiation of chromalveolates.*
- *The apicoplast is essential and is home to synthesis pathways for fatty acids, isoprenoids and haem; these pathways are distinct from their human counterparts and the target of drug development.*
- *The apicoplast is no longer photosynthetic and is now supplied with building blocks, energy and reduction power from the cytoplasm through a phosphate translocator.*
- *Import of proteins across the four apicoplast membranes employs distinct translocon complexes, and the evolutionary origin of each translocon mirrors that of the membrane that it crosses.*

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<AQ1>In the legend to Figure 1, a word appears to be missing in the phrase 'an alga was taken up by and another eukaryote'. Please revise.
<AQ2>In Figure 3(A), please define $ERAD_P$.