Chapter 2

Complex Endosymbioses I: From Primary to Complex Plastids, Multiple Independent Events

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Abstract

A substantial portion of eukaryote diversity consists of algae with complex plastids, i.e., plastids originating from eukaryote-to-eukaryote endosymbioses. These plastids are characteristic by a deviating number of envelope membranes (higher than two), and sometimes a remnant nucleus of the endosymbiont alga, termed the nucleomorph, is present. Complex plastid-bearing algae are therefore much like living matryoshka dolls, eukaryotes within eukaryotes. In comparison, primary plastids of Archaeplastida (plants, green algae, red algae, and glaucophytes) arose upon a single endosymbiosis event with a cyanobacterium and are surrounded by two membranes. Complex plastids were acquired several times by unrelated groups nested within eukaryotic heterotrophs, suggesting complex plastids are somewhat easier to obtain than primary plastids. This is consistent with the existence of higher-order and serial endosymbioses, i.e., engulfment of complex plastid-bearing algae by (tertiary) eukaryotic hosts and functional plastid replacements, respectively. Plastid endosymbiosis is typical by a massive transfer of genetic material from the endosymbiont to the host nucleus and metabolic rearrangements related to the trophic switch to phototrophy; this is necessary to establish metabolic integration of the plastid and control over its division. Although photosynthesis is the main advantage of plastid acquisition, algae that lost photosynthesis often maintain complex plastids, suggesting their roles beyond photosynthesis. This chapter summarizes basic knowledge on acquisition and functions of complex plastid.

Key words Complex endosymbiosis, Plastid replacement, Reductive evolution

1 Introduction

Phototrophic organisms are essential for life on Earth for their ability to capture the energy of sunlight. Light harvesting complexes of plastids couple the transfer of energy from photons to create NADPH and proton gradient across (thylakoid) membranes, yielding energy that is needed to incorporate CO_2 into organic compounds. The history of all plastids traces back to the initial association between a single-celled heterotrophic eukaryote, the ancestor of plants, and a phototrophic bacterium (cyanobacterium), which we refer to as prokaryote-to-eukaryote, or primary endosymbiosis (Fig. 1) [1]. Streptophytes (land plants),

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chlorophytes (green algae), rhodophytes (red algae), and glaucophytes, altogether forming the group Archaeplastida, host primary plastids believed to have evolved from the domesticated cyanobacterium. However, lineages of phototrophic eukaryotes are scattered all around the tree of life, not directly related to Archaeplastida, and represent a major fraction of eukaryotic diversity (Fig. 2). The role of these lineages in the food chain is also substantial; algae such as diatoms, dinoflagellates, or haptophytes are responsible for the majority of the primary production in the ocean. For instance, the primary production of diatoms is comparable to that of all terrestrial rain forests combined [2]. Also, they have interesting evolutionary stories to tell. Except for *Paulinella* [3], plastids in these lineages did not emerge as a result of primary endosymbiosis. Instead, they were acquired horizontally via engulfment of a photosynthetic eukaryote (an alga) (Fig. 2). Secondary endosymbiosis occurs when primary alga is taken up for endosymbiont and, by extension, higher-order endosymbioses (tertiary, quaternary, etc.) are the result of more complex interactions, leaving behind deeply composite chimeric organisms (Fig. 1).

Complex plastids distinguish from primary plastids based on their ultrastructure and phylogeny (*see* below). Their envelopes consist of multiple (3 or more) membranes, while envelopes of primary plastids constitute double membranes (Fig. 1). This is believed to be a direct consequence of the secondary or higher-order eukaryote-to-eukaryote endosymbiosis; the additional biomembranes represent derived structures that have supposedly evolved from the host endomembrane system and/or the cytoplasmic membrane of the symbiont [1]. However, some of the membranes seem to be lost following the engulfment [4], as even higher-order plastids never possess envelopes with membrane number higher than five (Fig. 1) [5]. The origins of plastids in particular lineages are rather hypothetical and still subject to passionate debates. These evolutionary events happened a long time ago, hundreds of millions of years from now. As a result, phylogenetic signals eroded to large extent, and sometimes we cannot tell apart endosymbionts that are of rhodophyte and complex (rhodophyte-derived) origin. We, therefore, prefer to use the term "complex plastid" not only when speaking of general principles of endosymbiosis but also bearing in mind the undisclosed sequence of plastid acquisitions in different rhodophyte-derived lineages (Fig. 2) [6, 7].

To understand how plastids establish as organelles is essential to recognize how phototrophic lineages repeatedly evolved to play significant roles in the planet's ecosystems. Plastids play roles in the biochemistry of cells well beyond photosynthesis. They are the keynote in balancing carbon and energy levels, nitrogen and sulfur assimilation, and biosynthesis of essential compounds such as vitamins, tetrapyrroles, fatty acids, and isoprenoids. It is then

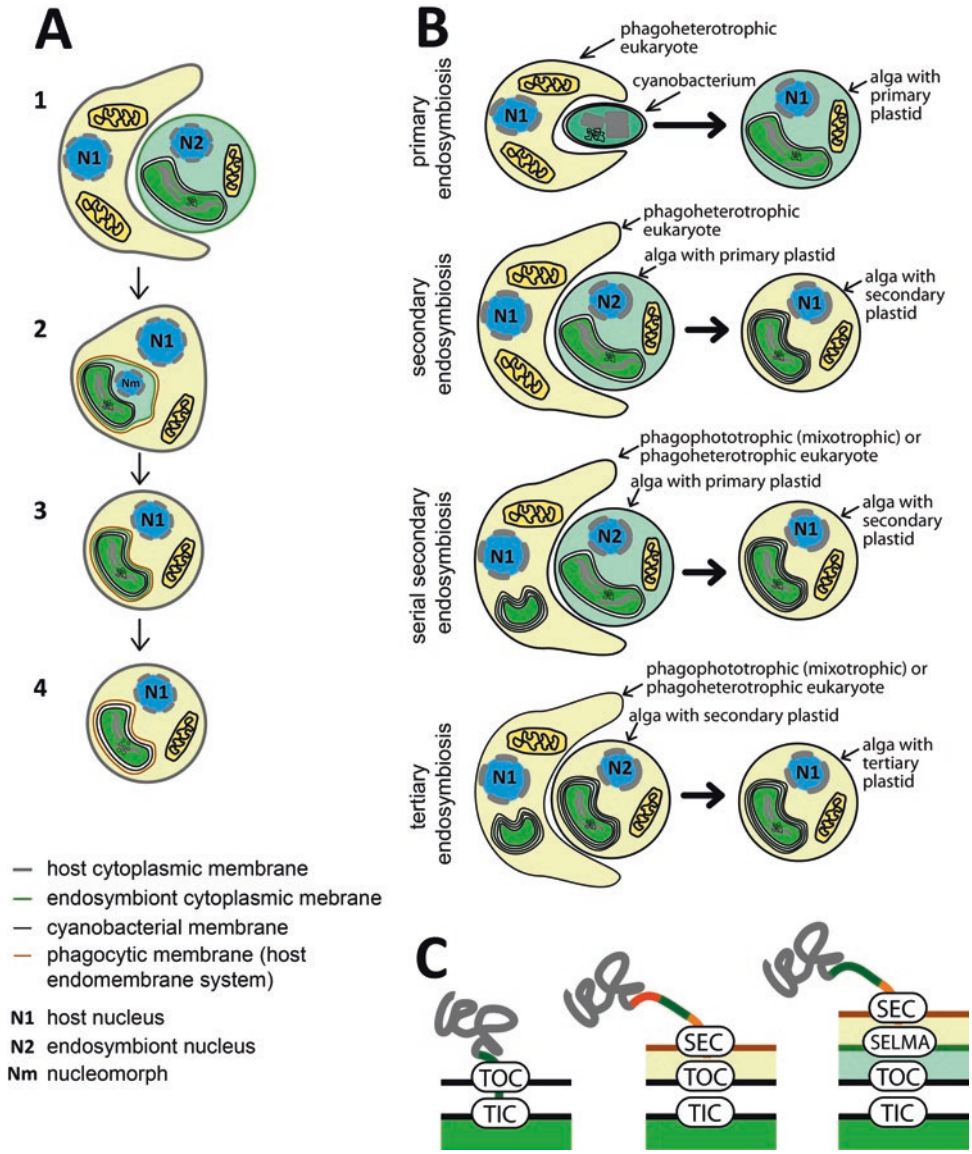

Fig. 1 Evolution of plastid envelopes. (**a**) Phases of the endosymbiont reduction over the course of time. (1) Feeding on algal prey; (2) gradual reduction of the endosymbiont structures and gene transfer to host nucleus resulting in a reduced endosymbiont nucleus, the nucleomorph; (3) progressing dependence on host factors, nucleomorph lost; (4) loss of the endosymbiont-derived membrane. (**b**) Envelope structures under various endosymbiosis scenarios. As apparent from the scheme, different-order complex endosymbioses may result in plastids with the same envelope arrangement. (**c**) More complex envelopes require an additional protein-translocating machinery, which is reflected by altered protein targeting presequences. Color code of protein domains: green, plastid transit peptide; orange, signal peptide; red, transmembrane anchor domain; gray, mature protein. TOC/TIC, translocons of the outer/inner chloroplast membrane; SEC, signal peptide translocon at the endoplasmic reticulum membrane; SELMA, symbiont-derived translocon (*see* text)

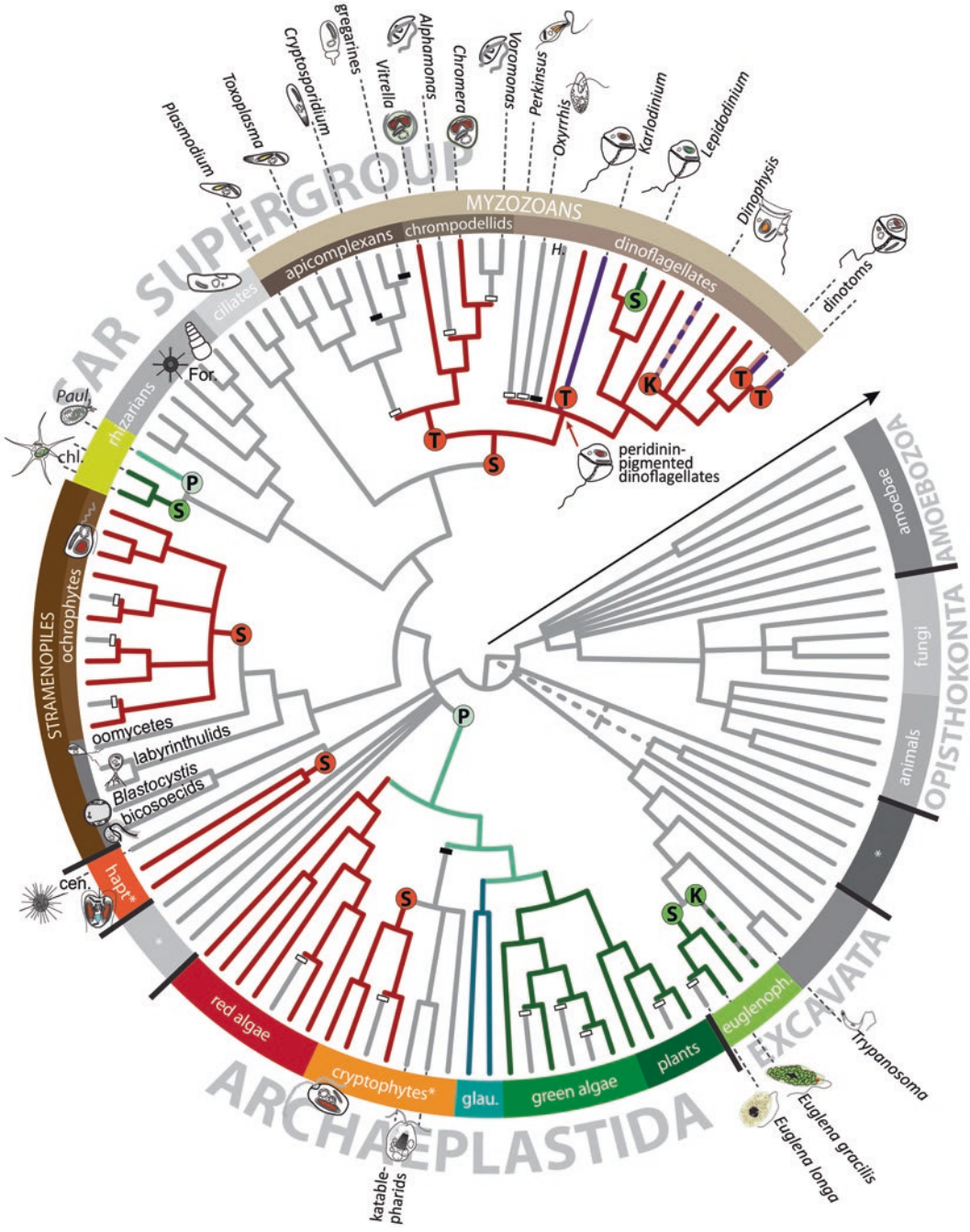

Fig. 2 Eukaryotic tree of life with an accent on the diversity of algae. Major eukaryotic groups are shown around the outermost circle, in gray. Inner circles mark lower-rank groups of organisms, nonphotosynthetic clades boxed in shades of gray, the "green lineage" boxed in shades of green, and the "red lineage" boxed in shades of red and brown. Note that cryptophytes and haptophytes do not robustly associate with currently recognized major eukaryotic groups (*). The cladogram shows schematic relationship between taxa, with the course of evolution from the center to the margin, as marked by the black arrow. Red- and green-colored nodes and branches denote the red- and green-algal plastid descendants, respectively, light-green indicates

unsurprising that many lineages maintain plastids even after they lose photosynthesis. In this chapter, we summarize the basic knowledge on the evolution of complex plastids and their role in organisms that possess them.

2 Distribution of Complex Plastids Among Eukaryotes

Currently, five major groups of eukaryotes are recognized (and a handful of *incertae sedis*, Fig. 2; [8]). Archaeplastida is the only group where plastids are omnipresent. In contrast, fungi, animals, and amoebozoans likely never bear permanent plastids, although endosymbionts of other origins occasionally occur in various lineages (e.g., [9, 10]). In other groups, plastid-bearing lineages are nested within heterotrophs, suggesting six unrelated acquisitions of complex plastids (Fig. 2): (1) myzozoans (collective designation for plastid-bearing alveolates, dinoflagellates, apicomplexans, and related lineages), (2) ochrophytes (phototrophic stramenopiles), (3) haptophytes (currently unassigned to any major eukaryotic group), (4) cryptophytes (tentatively placed within Archaeplastida [6]), (5) euglenophytes (phototrophic excavates), and (6) chlorarachniophytes (phototrophic rhizarians) [11]. Examples of complex algae are depicted in Fig. 3. *Paulinella*, yet another rhizarian genus, obtained a primary plastid during a relatively recent, independent endosymbiosis [3].

Plastid envelopes differ in the number of membranes they comprise. The envelope ultrastructure is usually conserved in major lineages, which supports the notion that endosymbiotic events define monophyletic clades. Dinoflagellates (except those that underwent serial and higher-order endosymbiosis) and euglenophytes possess three-membrane-bound plastids, while other complex algae possess four-membrane-bound plastids, compared to two-membrane-bound plastids of primary algae (Fig. 1).

Cryptophytes and chlorarachniophytes both possess in their plastids a remnant nucleus of the engulfed algal endosymbiont. Termed the nucleomorph, it is the most convincing evidence supporting the eukaryotic origin of complex plastids. This structure resides in the periplastid space between two outermost and two inner membranes of their complex plastids, topologically analogous to the cytosol of the endosymbiont, as expected (Fig. 1). The

Fig. 2 (continued) the primary endosymbiont of *Paulinella* and Archaeplastida before the divergence of red algae, (cryptophytes?), glaucophytes, and green algae. Letters mark levels of particular symbiotic events: P—primary, S—secondary, T—tertiary, K plus a dashed branch line—kleptoplasty. Narrow rectangles show losses of photosynthesis (white) or entire plastids (black). Taxa abbreviations: *chlo.*—chlorarachniophytes, *euglenoph.*—euglenophytes, *For.*—Foraminifera, *glau.*—glaucophytes, *H.—Hematodinium*, *hapt*—haptophytes, *Paul.—Paulinella*, **—incertae sedis*, uncertain evolutionary position

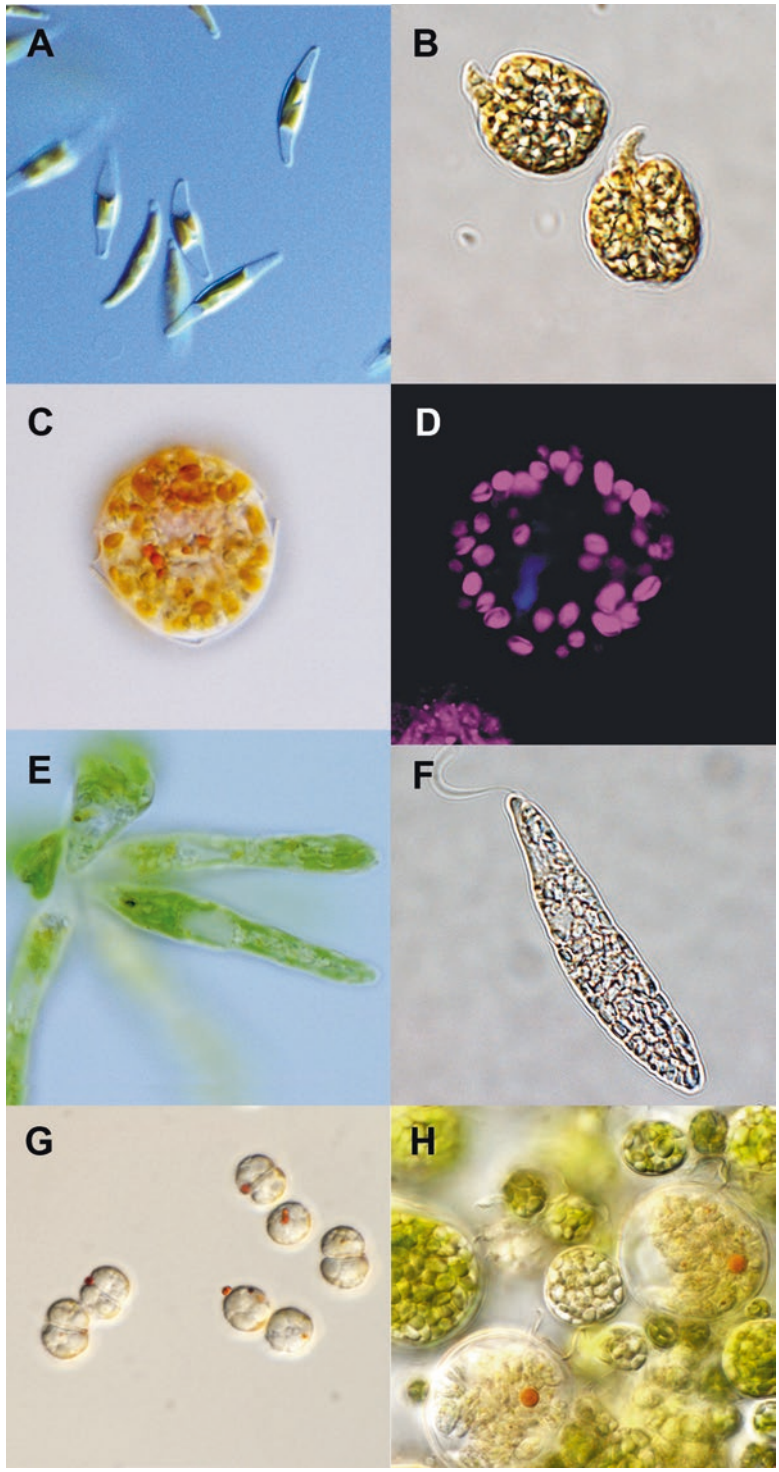

Fig. 3 Examples of algae with complex plastids. (**a**) Diatom *Phaeodactylum tricornutum*, (**b**) peridinin dinoflagellate *Amphidinium carterae*, (**c**) dinoflagellate with a diatom endosymbiont, also called a dinotom, *Glenodinium foliaceum*, (**d**) autofluorescence of the diatom plastids in the dinoflagellate *G. foliaceum* (plastid—magenta; nucleus—blue), (**e**)

nucleomorph is highly reduced and consists of just three small chromosomes that encode 548 and 288 housekeeping proteins in the cryptophyte *Guillardia theta* and the chlorarachniophyte *Bigelowiella natans*, respectively ([12], updated). Its presence also demonstrates that periplastid space is metabolically active. Indeed, analyses of the genomic sequence data of *Guillardia* and *Bigelowiella* revealed host-encoded proteins targeted to the periplastid space [12].

Myzozoans, ochrophytes, haptophytes, and cryptophytes all contain rhodophyte-derived plastids, while euglenophytes and chlorarachniophytes contain chlorophyte-derived plastids. In initial works, to raise parsimonious evolutionary scenarios, the "red lineage" and the "green lineage" were considered to be monophyletic [13]. Sometimes this is referred to as early plastid acquisition. Nevertheless, accumulating evidence consistently disproved these notions and it has become clear that these six phototrophic lineages with complex plastids most likely arose independently (late acquisition) [11, 14–16]. First of all, the plastids of euglenophytes and chlorarachniophytes show phylogenetic affinity to different taxa of chlorophytes [11]. Furthermore, phylogenetic analyses performed on host nuclear genes suggest that lineages with complex plastids branch deeply in the evolution of eukaryotes, predating the diversification of red algae and green algae (Fig. 2) [17, 18]. The apparent similarity of the plastids of the "red lineage" then most likely results from the horizontal transfer of established organelles from one lineage to another (higher-order endosymbioses), or independent secondary endosymbioses that involved closely related taxa of rhodophytes. Currently, we lack convincing evidence for either of these scenarios, though the former seems to enjoy wider acceptance (e.g., [6, 7, 15, 19]). In this scenario, a founder secondary alga developed chlorophyll *c* and engaged in endosymbiotic relationships with the ancestors of cryptophytes, haptophytes, stramenopiles, and myzozoans, in an order still unresolved. Notably, only rhodophyte-derived complex algae contain chlorophylls *a* and *c*; the green lineage (including chlorarachniophytes and euglenophytes) contains chlorophylls *a* and *b*, while rhodophytes contain chlorophylls *a* and *d*.

Another controversy emerged recently when the plastid genome of the chromerid *Vitrella brassicaformis* (Fig. 3h) showed phylogenetic affinity to plastid genomes of eustigmatophytes, which is an ochrophyte subgroup [20, 21]. Based on some data, chromerids [22, 23] and related apicomplexan parasites (*Plasmodium*, *Toxoplasma*) could have obtained their plastids via higher-order

Fig. 3 (continued) excavate alga *Euglena gracilis*, (**f**) excavate colorless (osmotrophic) alga *Euglena longa*, (**g**) alveolate alga *Chromera velia*, (**h**) alveolate alga *Vitrella brassicaformis*. Image courtesy of K. Jiroutová (**a**), Z. Füssy (**b**, **e**, **f**), J. Cihlář (**c**), J. Kručinská (**d**), and D. Modrý (**g**, **h**)

endosymbiosis with an ochrophyte [14, 21], independently on dinoflagellates. Further support comes from the absence of chlorophyll *c* from both chromerids and eustigmatophytes. Some researchers suggested that higher-order endosymbiosis with an ochrophyte alga gave rise to entire Myzozoa (including dinoflagellates) [15]. Nevertheless, differences between dinoflagellate and chromerid/apicomplexan plastids, notably the envelope ultrastructure, pigmentation, and genome organization, suggest separate origins of endosymbionts in these sister lineages [14].

Dinoflagellates are textbook examples of the evolution of endosymbiotic relationships [1, 5, 24]. Enjoying broad ecological plasticity, dinoflagellates experienced various events, ranging from loss of photosynthesis or the plastid in parasitic and predatory species, through maintaining the ancestral rhodophyte-derived plastid in peridinin-pigmented species, to kleptoplasty (plastid theft from prey) and serial endosymbiosis, i.e., plastid replacement (Fig. 2). Most outstanding examples of serial endosymbiosis are *Lepidodinium* spp. with a green algal endosymbiont, dinotoms that maintain a higher-order endosymbiont derived from ochrophytes (diatoms) (Fig. 3b), and *Karlodinium* spp. with a cryptophyte endosymbiont (again higher-order endosymbiosis) (Fig. 2) [1, 24]. Importantly, these evolutionary events in dinoflagellates occur at shorter timescales and allow a more "real-time" analysis of processes that accompany them. *Dinophysis* is the most renowned kleptoplastic lineage [25], but the recent discovery of frequent plastid promiscuity in dinotoms, some of them found frozen in the state of kleptoplasty, seems promising for tackling early plastid evolution [26].

3 Integration of Plastids

The evolution of plastids is extremely complex and involves two important processes. Enhancement of the host control over the biochemistry and division of the endosymbiont represents the first component crucial for transformation into fully integrated organelles [27, 28]. Evolutionary innovations, in the form of new genes or existing genes repurposed for roles in the evolving organelle, were necessary to underpin the molecular machinery for the emerging organelle. These innovations include protein import complexes for host nuclear-encoded organellar proteins [29, 30], plastid solute transporters [28, 31], and proteins to execute control over the organelle division [32]. Along with the establishment of metabolite exchange mechanisms, genetic material is massively transferred from the endosymbiont to the host nucleus or lost; reductive evolution is the second major process to take place during symbiogenesis [33]. As a result, primary plastids lost most of their genome complexity compared to free-living cyanobacteria. Analogously in complex plastids, the nuclear genome of the endo-

symbiont, ranging from 16 to 105 Mb in free-living red algae [34], is considerably reduced to the form of a nucleomorph or, even more frequently, completely lost.

Host nuclear-encoded proteins targeted to plastids possess a topogenic presequence recognized by the translocon machinery that guides the protein across the plastid envelopes (Fig. 1) [35]. Genes being transferred from the plastid genome to the host nucleus must acquire this topogenic presequence to regain their plastid function. Proteins with a chloroplast transit peptide presequence can reach primary plastids. Proteins directed to complex plastids need to cross over additional membranes, and therefore they are decorated with an additional domain, the signal peptide, just upstream of the transit peptide. Over the course of evolution, most plastid proteins became encoded by the host nucleus. Plastid proteomes of primary algae (and plants) consist of about 1000–1500 proteins (but up to 3000 according to [36]), with just 87 proteins encoded by the plastid genome (in *A. thaliana*) [37]. The complexity of plastid proteomes might be similar in diatoms, cryptophytes, and chlorarachniophytes [12, 38] but there are no data for other complex algae. The necessity for hundreds of proteins to obtain a plastid-targeting topogenic signal is thought to be a leading cause for primary and secondary endosymbioses to occur so rarely [39]. Also, molecular machines such as photosystems are unlikely to evolve de novo in eukaryotes. Besides being highly composite as for subunit composition, they strictly bind to specific ("genetic" or inherited) membranes that too do not appear de novo. Genetic membranes, such as the membranes of the plastid envelope, often exhibit specific lipid and protein composition and only derive semi-conservatively from preexisting biomembranes, similarly as new DNA strand derives from the template strand [39]. Development and dispersal of photosynthesis in eukaryotes were hence exclusively mediated by endosymbiosis.

Topologically, primary plastids localize to the cytosol of Archaeplastida, lacking additional membrane barriers other than those inherited from the two-membrane cyanobacterium (Fig. 1). In comparison, complex plastids are only accessible via the endomembrane system, from which the outer membrane of the envelope originates. Protein import routes in primary and complex algae indeed reflect this fundamental difference (Fig. 1) [35]. The presequences (transit peptides) of primary plastid-targeted proteins are recognized directly by the translocon complexes at the outer and inner membrane of the envelope (TOC and TIC). To traverse the additional membrane(s) of complex plastids, proteins must enter the endomembrane system via the endoplasmic reticulum (ER) and thus require the signal peptide (SP) in their presequence. Following ER entry and cleavage of the SP, the fate of proteins differs in three- and four-membrane plastids (Fig. 1). In three-membrane plastids, the cleavage of the SP exposes the transit peptide, which then guides the protein entry through the inner two membranes via the TOC/

TIC. Notably, proteins imported into three-membrane plastids tend to possess a hydrophobic domain, a membrane anchor, downstream of the transit peptide [40, 41]. Four-membrane complex plastids need an additional protein translocator system to pass the second outermost lipid layer. This translocon derives from the endosymbiont ERAD (ER-associated protein degradation) that originally exported misfolded proteins from the ER. Following redirection to the second outermost plastid membrane (thought to be homologous to the endomembrane system or the cytoplasmic membrane of the endosymbiont), this complex started to import proteins from the compartment between the first and the second outermost plastid membrane. Termed SELMA (Symbiont-specific ERAD-Like MAchinery), this translocator allowed proteins to cross this membrane and continue their way to the TOC/TIC complexes (Fig. 1) [42]. Five-membrane plastids (in dinotoms) are thought to be inaccessible to host-encoded proteins but maintain a nucleomorph (below the outermost membrane) that encodes all the necessary plastid proteins translocated as in four-membrane plastids of diatoms [43].

For metabolic integration, plastids use a set of transporters to connect with the cytosolic pool of compounds [28]. Triose phosphate/phosphate translocators were supposedly the pioneers of the connection between the host and the endosymbiont as they allow the exchange of three-carbon sugar intermediates synthesized during CO_2 fixation (Calvin-Benson cycle). Other phosphate translocators include the glucose 6-phosphate, xylulose 5-phosphate, phosphoenolpyruvate, and glutathione transporters, although their distribution among complex plastids might not be universal [44]. Members of the mitochondrial carrier family facilitate the transport of substrates like folates, S-adenosylmethionine, NAD, ADP-glucose, or adenosine nucleotides; ATP:ADP antiporters ensure the exchange of ATP and thus maintaining physiological ATP/ADP ratio in the plastid [44]. Dicarboxylate transporters play a role in nitrogen assimilation by allowing the circulation of 2-oxoglutarate and glutamate into and out of the plastid, respectively. 2-oxoglutarate is the acceptor for ammonia in the glutamate synthase reaction; glutamate then serves as the donor of nitrogen for biosynthesis of nitrogen-containing compounds. Presence and function of other transporters, such as the pyruvate carrier, are unknown in most complex algal species [44].

Once established, plastids play dominant roles in the biochemistry of algae. With four major multisubunit protein complexes and around 80 participating proteins [45], photosynthesis is the most outstanding process of plastids. These proteins are required for the assembly, function, and regulation of the light harvesting antennae, photosystems, and electron transfer factors and enable the production of ATP and reducing agent NADPH as the cosubstrates for

carbon fixation. Besides photosynthesis, plastids act like biochemical factories, synthesizing vitamins, polysaccharides, amino acids, fatty acids, isoprenoids, tetrapyrroles, and Fe–S clusters. Photosynthesis directly requires most of these compounds, but in many organisms, plastid synthesis supplies the entire cell. As discussed below, plastids tend to take over metabolic functions of other cellular compartments to achieve a streamlined and light-regulated biochemistry. Plastids also cooperate with mitochondria and cytosol in balancing metabolic and energy flows during day/night cycles and under nutrition limitation [46, 47].

Plastid proteomes are highly mosaic regarding evolutionary origin. Individual plastid proteins in primary algae descend from eukaryotes (host nuclear genes), cyanobacteria (introduced with the plastid), alpha-proteobacteria (introduced with the mitochondrion) and genes from other sources such as those obtained from non-endosymbiotic gene transfer [48, 49]. While pathways that do not have a eukaryotic counterpart originate almost entirely from the cyanobacterium (e.g., photosystems, type II fatty acid synthesis, and non-mevalonate isoprenoid synthesis), other pathways consist of a mosaic of cyanobacterial proteins and proteins retargeted from other cellular compartments. Typical examples of chimeric pathways are the tetrapyrrole biosynthesis and the Calvin-Benson cycle [50, 51]. Complex endosymbioses substantially increase the genetic complexity of organisms by adding genes of another eukaryotic symbiotic partner(s) to the pool. Secondary endosymbiosis brings together two mosaic genomes, those of the primary alga and the host (hence additional eukaryotic, proteobacterial, and horizontally acquired genes with different evolutionary histories) [33]. In extension, higher-order endosymbioses further increase the genetic complexity. These boosts of gene richness, possibly analogous to genome-wide duplications, may enhance metabolic adaptations to changing environmental conditions and drive the rapid radiation of complex algae. Unfortunately for scientists, this genetic chimerism, in addition to conflicting nuclear and plastid gene phylogenies, complicates interpretations concerning the history of complex endosymbioses [33].

4 Reductive Evolution of Plastids

The increased genetic complexity after endosymbiotic events is a temporary stage before the gradual loss of unnecessary genes. Genome complexification occurs at faster-than-exponential rate followed by an exponential decay, and this biphasic pattern seems to be recurrent in the evolution [52]. Genomic simplification is a general process for both organisms with small effective populations

(parasites and endosymbionts) and evolutionarily successful free-living organisms with larger effective populations [52].

Different genes have different probabilities to be lost after a genetic burst. Orphan or redundant proteins are almost immediately lost, while proteins working in complexes and pathways are more frequently retained [53]. Occasionally, the loss of a component may lead to a domino-effect loss of the entire module [54]. Continued reductive evolution resulted in an extremely reduced genome of dinoflagellate plastids; peridinin-pigmented plastid genomes consist of only a few minicircles, i.e. small molecules 2–3 kbp in size that generally encode one gene each [55]. Reductive evolution acts on serial complex plastids as well, although supposedly at various paces for individual acquisition events [5].

Acquired plastids represent a metabolic redundancy for the host; carbohydrates, fatty acids, isoprenoids, and tetrapyrroles are synthesized both in the host and the plastid. These compounds are essential for eukaryotic cells, and therefore we assume that there is an interim stage during endosymbiosis when host and endosymbiont pathways are concurrently used [19, 56]. For instance, chlorarachniophytes (*Bigelowiella natans*) and euglenophytes (*Euglena gracilis*, Fig. 3e) possess two redundant pathways for tetrapyrrole biosynthesis, one host-derived and one localized to the plastid [19, 56]. Similarly, dinotoms represent an intermediate (or evolutionarily frozen) lineage as both the host and the diatom endosymbiont operate independent tetrapyrrole pathways; this arrangement might result from the inability of host-encoded proteins to translocate to the endosymbiont compartment [19, 24, 43]. In the long term, however, streamlining of cellular biochemistry might prefer the retention of only one of the complementary pathways, the other doomed for disappearance [56–58]. Consistently, in most other eukaryotic algae investigated, tetrapyrroles are synthesized exclusively in the plastid.

5 Loss of Photosynthesis in Complex Plastids

Photosynthetic abilities of eukaryotic algae are not permanent; there are many examples of independent loss of this hallmark plastid feature. Loss of photosynthesis can be seen as the continuation of the reductive evolution in algae (*see* above). Nonphotosynthetic (cryptic) plastids occur in many species after close examination. Notably, about 50% of dinoflagellate species secondarily became heterotrophs or parasites [24, 59]. Nonphotosynthetic taxa also appear in other lineages, e.g., ochrophytes, chrompodellids (the group of chromerids and their nonphotosynthetic relatives, colpodellids), and apicomplexan parasites (Fig. 2) [14, 15]. Primary phototrophs, i.e., plants, chlorophytes, and rhodophytes, also

developed numerous colorless lineages [60–62], including a few green algal parasites and free-living heterotrophs such as *Helicosporidium* or *Polytomella*, respectively. Euglenophytes lost photosynthesis several times. For instance, the plastid of the alga *Euglena gracilis* (Fig. 3e) can be bleached by antibiotics or physical stress, and natural hetero-osmotrophic mutants such as *Euglena longa* are quite common and investigated for mechanisms underlying loss of photosynthesis in this lineage (Fig. 3f) [63]. This tendency for secondary heterotrophy is supposedly due to an intermediary and highly redundant state of cellular biochemistry in euglenophytes [56]. Cases of outright plastid loss seem strikingly rare, and the only accepted cases are the apicomplexans *Cryptosporidium* spp. and *Gregarina niphandrodes* [64, 65] and the parasitic dinoflagellate genus *Hematodinium* (Fig. 2) [66].

Plastids are supposedly retained after the loss of photosynthesis because they host other essential biochemical pathways [67–69]. The apicoplast of the apicomplexan parasites (e.g., *Plasmodium falciparum*), the best-studied relic plastid, illustrates this. The four-membrane-bound apicoplast holds a reduced circular genome of about 35 kb which lacks any traces of genes involved in photosynthesis (Fig. 4) [67, 70]. The organelle appears to be essential for the parasite survival, and its disruption causes the so-called "delayed death effect," when parasite progeny ceases to develop in erythrocytes. The bloodstream form of *P. falciparum* needs the apicoplast to synthesize isoprenoids, and the insect form is dependent on apicoplast fatty acid and heme synthesis [71]. These compounds are essential for the survival of most eukaryotes (but see [72]) and they must be synthesized autonomously or obtained from external sources, such as prey or host. The apicoplast imports phospho*enol*pyruvate (PEP) and dihydroxyacetone phosphate (DHAP) from the cytosol. These carbohydrate phosphates are the starting substrates for fatty acid, phospholipid, and isoprenoid biosynthesis. Also, the conversion of PEP to pyruvate and acetyl-CoA generates ATP and NADH, both required for the phosphorylation and reduction steps in the pathways above. The apicoplast also performs several intermediate steps of the heme synthesis, making it a biochemical hub of the parasite cell (Fig. 4). There is only limited knowledge of the actual metabolic functions of other cryptic plastids. Some of them host similar pathways as the apicoplasts [61, 73, 74], while others retain only carbohydrate metabolism [63].

To fight parasitic organisms, such as apicomplexans, we can exploit their reliance on plastid biosynthetic pathways. Apicomplexans are unicellular protists, obligatory parasites with typical morphological characters such as the apicoplast and the apical complex, a set of tubular and secretory organelles used for penetrating the host cell. Diseases caused by apicomplexans have a high impact on humans, e.g., hundreds of thousands of fatalities yearly

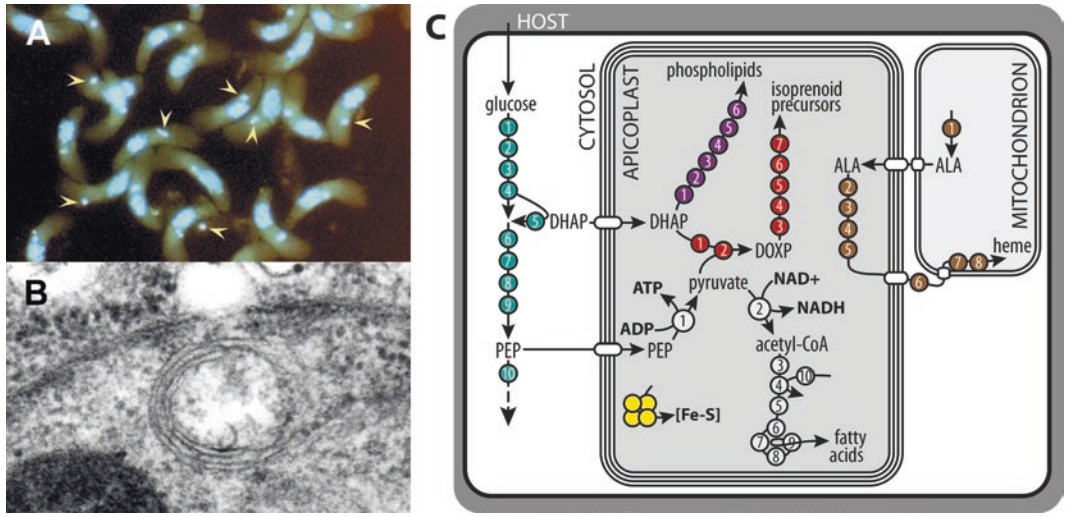

Fig. 4 Apicoplast. (**a**) Apicomplexan plastid as seen in DAPI stained trophozoites of *Sarcocystis muris*. (**b**) Electron micrograph of *Goussia janae* showing four membranes surrounding the apicoplast. Image courtesy of M. Oborník (**a**), and J. Lukeš (**b**). (**c**) Pathways of the apicoplast. Numbered circles denote enzymes, according to KEGG pathways. Turquoise, glycolysis: (1) hexokinase; (2) glucose-6-phosphate isomerase; (3) 6-phosphofructokinase; (4) aldolase; (5) triose-phosphate isomerase; (6) glyceraldehyde-phosphate dehydrogenase; (7) phosphoglycerate kinase; (8) bisphosphoglycerate mutase; (9) enolase; (10) pyruvate kinase. White, fatty acid synthesis: (1) pyruvate kinase; (2) pyruvate dehydrogenase complex; (3) acetyl-CoA carboxylase; (4) FabD; (5) FabH; (6) FabG; (7) FabZ; (8) FabI; (9) FabB/F; (10) acyl-carrier protein synthase. Red, isoprenoid precursor biosynthesis: (1) DOXP synthase; (2) DOXP reductase; (3) CDP-ME synthase; (4) CDP-ME kinase; (5) MEcPP synthase; (6) HMB-PP synthase; (7) HMB-PP reductase. Purple, phospholipid synthesis: (1) glycerol-3-phosphate dehydrogenase; (2), glycerol-3-phosphate acyltransferase; (3) acyl-glycerol-3-phosphate acyltransferase; (4) phosphatidic acid cytidyltransferase; (5) phosphatidylglycerol phosphate synthase; (6) phosphatidylglycerol phosphatase. Brown, heme synthesis: (1) ALA synthase; (2) ALA dehydratase; (3) PBG deaminase; (4) URO synthase; (5) URO decarboxylase; (6) CP oxidase; (7) PP oxidase; (8) heme ferrochelatase. Yellow, iron–sulfur cluster assembly machinery SUF, three-step synthesis, seven proteins required. Only key metabolites are shown, ALA—δ-aminolevulinic acid, DHAP—dihydroxyacetone-phosphate, DOXP—1-deoxy-D-xylulose 5-phosphate, PEP—phospho*enol*pyruvate; in bold, production of cosubstrates ATP and NADH from PEP

(malaria, toxoplasmosis) and severe economic losses (eimeriosis, toxoplasmosis, cryptosporidiosis). While several drugs have been used to treat malaria, drug-resistant strains of *Plasmodium* are quickly spreading over the affected countries in Asia and Africa. Most recently, combined treatment with antibiotics such as azithromycin is frequently employed [75]. Antibiotics target organellar replication, transcription, and translation and affect the mitochondria and the apicoplast of the parasite. Some antimalarials act on proteins unique to the apicoplast of *Plasmodium*, hence therapeutic targets not present in the mammalian host and supposedly less harmful for patients [71]. These novel targets include transporters and enzymes of the biosynthesis of amino acids (pyrimethamine, cycloguanil), fatty acids (thiolactomycin, cerulenin, triclosan), heme (succinylacetone), and isoprenoids (fosmidomycin). Unfortunately, some of these compounds might be toxic to humans at high doses

[71, 75], and clinical trials are needed to determine the efficiency of these new therapeutics. In addition, further research is expected to develop more efficient ones.

6 Conclusion

The photosynthetic organelles of eukaryotes, plastids, display extraordinary diversity and drove significant changes in the cellular biology of many lineages. The evolution into an organelle was a gradual process. The host cell had to develop mechanisms of metabolic exchange and protein import into the endosymbiont, leading to an entire dependence of the symbiont on its host. The relocation of the plastid into the endomembrane system (secondary endosymbiosis) necessitated the adoption of more complex targeting and integration machinery. Once established, secondary plastids were capable of horizontal spread across eukaryotic kingdoms, possibly taking advantage of the universal protein targeting machinery via the ER. In some lineages, most obviously dinoflagellates, complex plastids underwent further evolution, replaced by plastids from other lineages (serial or higher-order endosymbioses). Horizontal spread of plastids between eukaryotic lineages caused massive evolutionary radiations. Complex algae (mainly diatoms, haptophytes, and dinoflagellates) became key players in Earth's environments. Other, nonphotosynthetic, complex algae are parasites of animals and humans. In fact, complex plastids became nonphotosynthetic in many lineages, but most of these organelles remained present and are biochemically essential for the host cells. While complex plastids were gained to become an evolutionary advantage over aplastidic lineages, their uniqueness represents a chance to find specific drug targets to fight some of the most deadly parasitoses of humans.

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