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Anna Stina Sandelius • Henrik Aronsson
Editors

The Chloroplast

Interactions with the Environment

Springer

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Editors

Anna Stina Sandelius pursued her PhD degree in Plant Physiology with Prof Conny Liljenberg at the University of Gothenburg. She graduated in 1983 and spent the following year and a half with Prof D. James Morré at Purdue University in West Lafayette, Indiana, USA. She returned to the University of Gothenburg in 1985, where she attained full professorship in 1999. She is presently vice dean of the science faculty. As a graduate student, she studied the role of galactolipids during chloroplast development. She then switched to the plasma membrane and its lipid metabolism and supply, initially focusing on inositolphospholipids. The interest in lipid trafficking brought back plastids as an object of study and, thanks to her group's recent discovery that the plasma membrane uses plastid-synthesized galactolipids to replace phospholipids during phosphate-limiting cultivation, the two objects of study, along with the endoplasmic reticulum, have been brought together in efforts to elucidate lipid trafficking between plastids and the plasma membrane.

Henrik Aronsson pursued his PhD degree in Plant Physiology with Dr Clas Dahlin at the University of Gothenburg. He graduated in 2001 and spent the following year and a half as a postdoctoral student in Dr Paul Jarvis' group at Leicester University. The next year he spent at Gotland University and Skövde University as senior lecturer. He then returned to the University of Gothenburg in 2004, where he attained associate professorship in 2007. As a graduate student, he studied plastid protein targeting of the light-dependent enzyme NADPH: protochlorophyllide reductase (POR) both to the envelope and the internal membrane system. He then switched to studying the chloroplast protein import machinery with a focus on the components that make up the machinery. His group has recently started studying the plastid vesicular transport system between the envelope and the internal membrane system with emphasis on putative proteins involved in the process.

Preface

A complete book on chloroplast would contain a vast number of chapters! We chose to focus on interactions between the chloroplast and its immediate as well as distant environments, with a first chapter on plastid evolution. When we received the manuscripts, also the chapters related to communication and/or physical interactions between chloroplasts and their surroundings maintained this temporal interaction as a background theme. Communication, physical interactions, evolution – but hardly anything on photosynthesis or pigments. The latter topics are probably the most clearly obvious ones for a chloroplast book, but here also lies our rationale behind the choice of chapter subjects; we want to present chloroplasts in a different perspective. The recent rapid evolvement of the presented research areas, largely made possible by the development of molecular techniques and genetic screens of an increasing number of plant model systems, makes the interactive theme timely. We are truly grateful to all the contributing authors for providing exciting chapters!

The first two chapters set the stage: in the first, the evolution of plastids is presented and the structural, functional and genomic variations among plastids of land plants and algae are described in an evolutionary context. Double membrane-bound plastids, which are believed to derive directly from a cyanobacterial endosymbiont, as well as plastids of a more complex ancestry with more than two delimiting membranes are covered. The former kind, well studied in land plants and green algae, is the main object in the following chapters. The second chapter defines the borderline, the chloroplast envelope. A current state-of-the-art list of chloroplast envelope proteins is presented, which, together with the lipid setup of this membrane system, reflects the prokaryotic origin of the chloroplast as well as its integration into the host cell.

Three chapters focus on transport across the envelope. The reduced genome of the chloroplast, compared to its ancestors, necessitates import of nuclear-encoded proteins from the cytoplasm. Chapter three presents the protein import machinery and its constituents in the two envelope membranes and how import is regulated and the chloroplast protein level maintained. Several of the imported proteins are involved in chloroplast lipid metabolism and the fourth chapter presents the interdependence of the chloroplast and the rest of the cell in providing lipid constituents to all membranes, within or outside plastids, during various environmental conditions.

This and the following chapter, on metabolite transporters, share an evolutionary feature, that plastids have acquired the role of sole provider of certain compounds once synthesized in the host cell, such as fatty acids and certain amino acids. The metabolite transport chapter uses an evolutionary perspective to present the vast array of metabolite transporters that connect chloroplast metabolism with that of the surrounding cell and also address the specific features of apicoplast transporter systems.

Transport of proteins, lipids and metabolites between plastids and the surrounding cell is regulated by feedback controls at several levels, stemming from intracellular as well as external conditions. The last four chapters cover different aspects of communication between chloroplasts and their surroundings. Plastid-nucleus signalling is the topic of the sixth chapter, with focus on the retrograde information flow, from the plastid to the nucleus, through different pathways. The next chapter presents plastid division at the molecular and cellular level with emphasis on the integration of the host and former endosymbiont and the roles of environmental and endogenous signals in controlling the process. Chapter eight also deals with communication and the chloroplast as a physical entity. The focus is on chloroplast movement and positioning in relation to light quality and quantity, where cytosolic components are involved in motility changes to optimize chloroplast function and survival. Sensing the environment and communication are also central themes of the final chapter. Here the focus switches to the involvement of chloroplasts as providers of metabolites that benefit plant individuals and communities, and the examples include defence coordination compounds and attractants for pollinators and seed dispersal.

Again, we are greatly indebted to all authors! We also extend our thanks to all colleagues in Göteborg and elsewhere who helped us with the review process, to the series editor David G. Robinson for trust and encouragement and last but not least to Anette Lindqvist, Christina Eckey and Elumalai Balamurugan of Springer-Verlag for continuous and patient support and help.

August 2008

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Diversity and Evolution of Plastids and Their Genomes

E. Kim and J. M. Archibald (✉)

Abstract Plastids, the light-harvesting organelles of plants and algae, are the descendants of cyanobacterial endosymbionts that became permanent fixtures inside nonphotosynthetic eukaryotic host cells. This chapter provides an overview of the structural, functional and molecular diversity of plastids in the context of current views on the evolutionary relationships among the eukaryotic hosts in which they reside. Green algae, land plants, red algae and glaucophyte algae harbor double-membrane-bound plastids whose ancestry is generally believed to trace directly to the original cyanobacterial endosymbiont. In contrast, the plastids of many other algae, such as dinoflagellates, diatoms and euglenids, are usually bound by more than two membranes, suggesting that these were acquired indirectly via endosymbiotic mergers between nonphotosynthetic eukaryotic hosts and eukaryotic algal endosymbionts. An increasing amount of genomic data from diverse photosynthetic taxa has made it possible to test specific hypotheses about the evolution of photosynthesis in eukaryotes and, consequently, improve our understanding of the genomic and biochemical diversity of modern-day eukaryotic phototrophs.

1 Introduction

The origin and evolution of plastids,[1] the light-gathering organelles of photosynthetic eukaryotes, is a subject that has intrigued biologists for more than a century. Since the original musings of Schimper (1885) and Mereschkowsky (1905), a wealth of structural, biochemical and, most recently, molecular sequence data

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[1]The term "chloroplast" is sometimes used to refer to all photosynthetic plastids among eukaryotic phototrophs and, occasionally, only in reference to the photosynthetic organelle of green algae and land plants.

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has accumulated and shown convincingly that these important cellular structures are of endosymbiotic origin (Gray and Spencer 1996). The exact timing of the first plastid-generating endosymbiosis, and the ecological and physiological conditions that facilitated such an event is not known, but it is commonly thought that plastids evolved from once free-living cyanobacteria that were originally ingested as food by a heterotrophic eukaryote. Under this scenario, rather than being digested, these prokaryotic cells escaped the confines of their phagocytic vacuole and gradually became fully integrated components of their eukaryotic hosts. As an obvious consequence of the cyanobacterial ancestry of their plastids, eukaryotic phototrophs perform oxygenic photosynthesis and have contributed greatly to the burial of organic carbon and oxygenation of Earth's atmosphere (Katz et al. 2004).

While the notion that plastids are derived from endosymbiotic cyanobacteria is now widely accepted, many important questions about the origin and diversification of plastids remain. The challenges associated with inferring the evolutionary history of plastids are in large part due to the exceptional structural, biochemical and molecular diversity seen in modern-day photosynthetic organisms. In this chapter, we provide an overview of the distribution of plastids across the known spectrum of eukaryotic life, summarize the diversity of photosynthetic pigments and storage carbon biochemistry seen in plants and algae, and present recent advances in our understanding of the tempo and mode of plastid diversification. Finally, we discuss the evolution of the plastid genome and proteome, providing recent insight into the significant role of lateral (or horizontal) gene transfer (LGT). As we shall see, the evolutionary history of plastids is exceedingly complex and while the use of molecular phylogenetics has improved our understanding of plastid evolution significantly, it has also raised as many questions as it has answered.

2 Distribution of Plastids

From an evolutionary perspective, the most fundamental distinction between different plastid types is between those whose ancestry can be traced directly to the original cyanobacterial endosymbiont (i.e., "primary" plastids) and those that were acquired indirectly as a result of an endosymbiosis between a plastid-bearing eukaryote and an unrelated eukaryotic host (i.e., "secondary" or "tertiary" plastids). The plastids of glaucophytes, rhodophytes (red algae) and Viridiplantae (green algae and land plants) are bound by two envelope membranes, which are thought to be derived from the inner and outer membranes of the original cyanobacterial endosymbiont (Jarvis and Soll 2001) (Fig. 1a–c). The lack of additional plastid membranes, a feature of the organelle in many other organisms, led to the notion that these plastids arose through a primary endosymbiotic event involving a cyanobacterial endosymbiont (Gibbs 1981). The evidence for and against a single origin for all primary plastids will be discussed in Sect. 4.1.

In contrast to the plastids of glaucophytes, rhodophytes and Viridiplantae, the euglenids and chlorarachniophytes are believed to have acquired their plastids from green

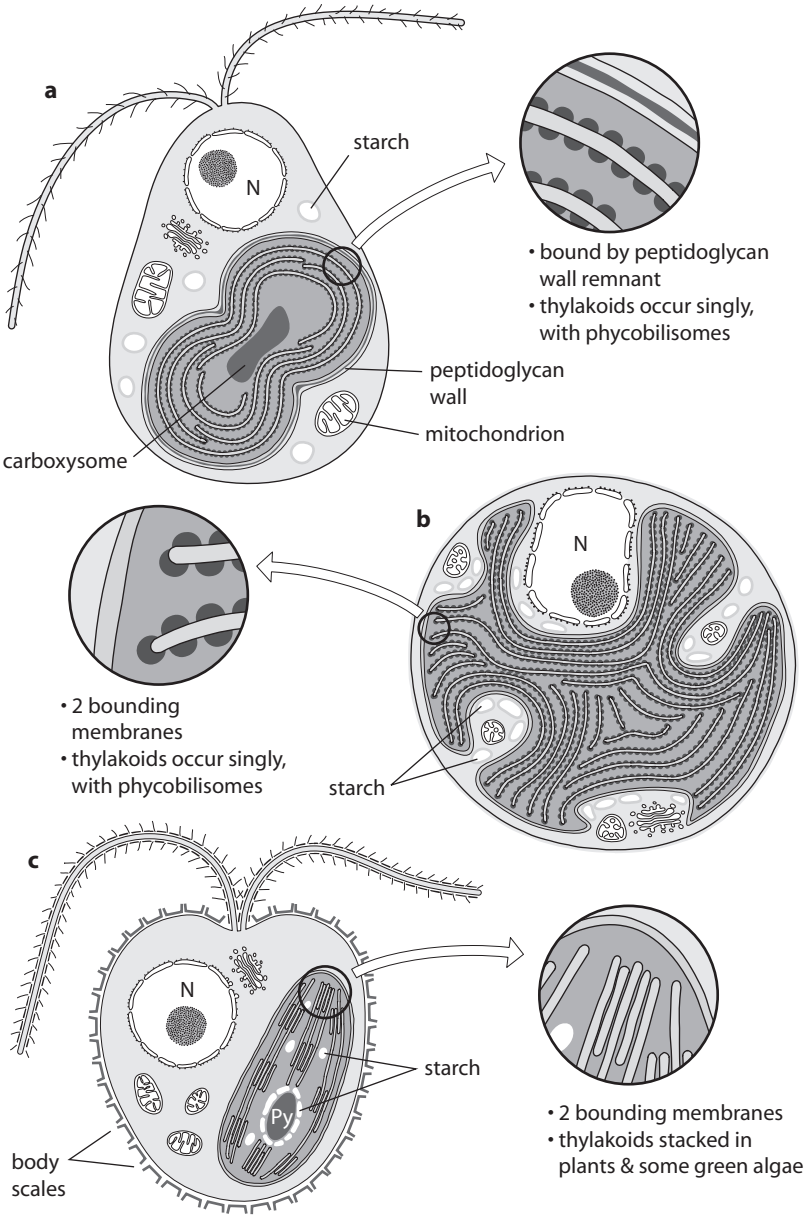


Fig. 1 Diversity of photosynthetic eukaryotes and their plastids. The plastids of glaucophytes (**a**), rhodophytes (**b**) and Viridiplantae (**c**) are bound by two membranes. Note the presence of phycobilisomes attached to the lumen side of the thylakoid membrane in **a** and **b**. The euglenids (**d**) and chlorarachniophytes (**e**) both possess plastids of green algal ancestry, which are surrounded by three and four membranes, respectively. The plastids of haptophytes (**f**), cryptophytes (**g**) and stramenopiles (**h**) are of red algal origin and are each surrounded by four membranes. Note the continuity of the nuclear envelope and the outermost plastid membrane, and the presence of ribosomes on the outer plastid membrane. Cells shown in **i** and **j** correspond to peridinin-containing dinoflagellates and apicomplexans, respectively. *N* nucleus, *Py* pyrenoid. (Art by L. Wilcox)

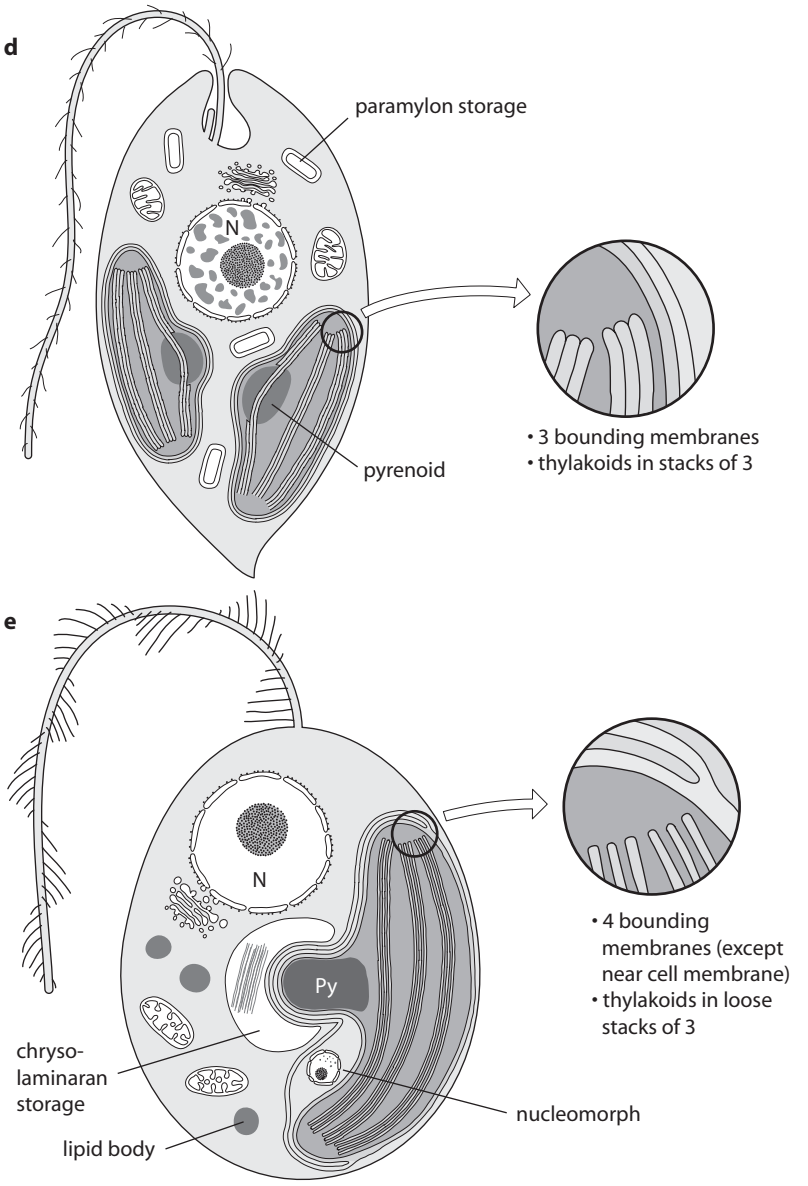
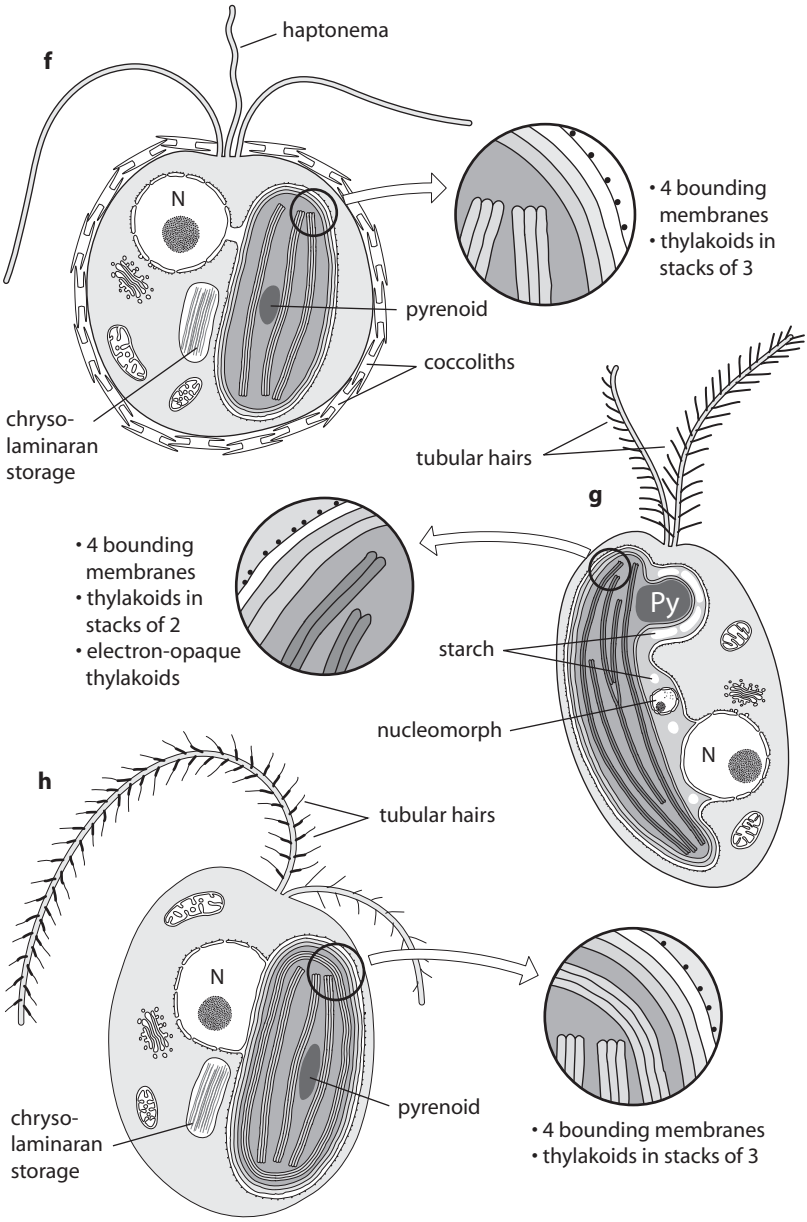


Fig. 1 (continued)

algae via secondary endosymbiosis (Table 1, Fig. 1d, e). Secondary or tertiary origins are proposed for the red-algae-derived plastids of cryptophytes, haptophytes, stramenopiles, most photosynthetic dinoflagellates, and apicomplexans (Table 1, Fig. 1f–j), although the origin of the apicomplexan plastid remains controversial (see below). With



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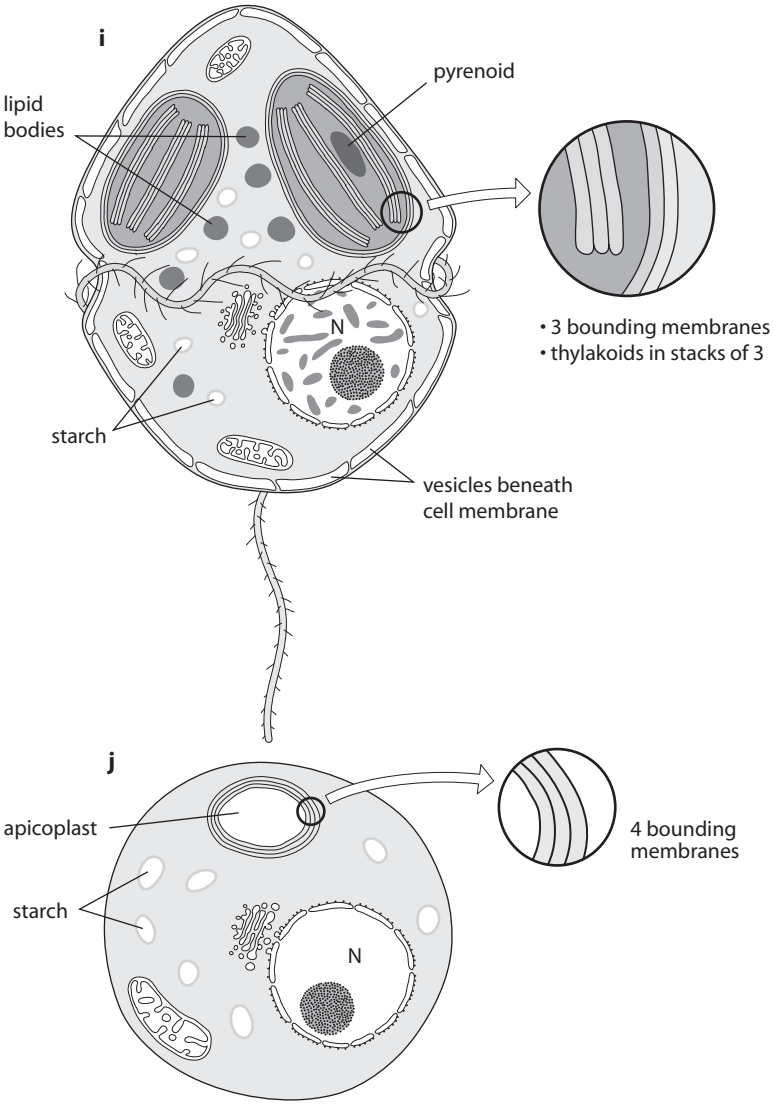
respect to the abundance of plastid-containing species in each of these groups, all known members of the glaucophytes, rhodophytes, Viridiplantae and haptophytes possess photosynthetic or nonphotosynthetic plastids, indicating that plastid acquisition



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occurred prior to the diversification within each of these lineages. While the chlorarachniophytes are an exclusively photosynthetic lineage, they belong to the eukaryotic "supergroup" Rhizaria, which is composed mostly of plastid-less taxa (Archibald and Keeling 2004; Nikolaev et al. 2004). Similarly, alveolates, cryptophytes, euglenids and stramenopiles each contain plastid-less members that are closely related to plastid-containing taxa. As will be elaborated upon below, the study of nonphotosynthetic

Table 1 General features of plastids

Taxonomic classification		Chlorophyll	Phycobilisomes	Number of plastid membranes	Putative plastid origin(s)	Nucleomorph?	Storage carbon (location)	Plastid-nuclear envelope continuity	Number of thylakoid stack
Glaucophyta		*a*	Yes	2	Primary	No	Starch (cytoplasm)	No	1
Rhodophyta		*a*	Yes	2	Primary	No	Starch (cytoplasm)	No	1
Viridiplantae		*a*, *b*	No	2	Primary	No	Starch (plastid stroma)	No	Multiple
Cryptophyta		*a*, *c*	No	4	Secondary (red)	Yes	Starch (periplastid space)	Yes	2
Haptophyta		*a*, *c*	No[a]	4	Secondary (red)	No	Chrysolaminaran (cytoplasm)	Yes	3
Stramenopiles		*a*, *c*[b]	No	4	Secondary (red)	No	Chrysolaminaran (cytoplasm)	Yes (not always)	3 (usually)
Alveolata Dinophyta	Peridinin type	*a*, *c*	No	3 (occasionally 2[c])	Secondary (red)?	No	Starch (cytoplasm)	No	3
	Green algal origin	*a*, *b*	No	4	Secondary (green)	?[d]	Starch (cytoplasm)	No	2–3
	Diatom origin	*a*, *c*	No	3	Tertiary (diatom)	?[d]	Starch (cytoplasm)	No	3
	Haptophyte origin	*a*, *c*	No	2–4[e]?	Tertiary (haptophyte)	No	Starch (cytoplasm)	No	3
Apicomplexa		None	No	2–4[f]?	Secondary (red or green?)	No	Starch (cytoplasm)	No	NA
Euglenida		*a*, *b*	No	3	Secondary (green)	No	Paramylon (cytoplasm)	No	3
Chlorarachniophyta		*a*, *b*	No	4[g]	Secondary (green)	Yes	Chrysolaminaran (cytoplasm)	No	3

NA not available

[a] Phycobiliproteins – phycoerythrin or phycocyanin – occur in the plastid lumen

[b] Members of Eustigomatophyceae lack chlorophyll *c* (Andersen 2004)

[c] Schnepf and Elbrachter (1999)

[d] Although the nuclear genomes of the algal endosymbionts seem to be retained, the extent of genome reduction, characteristic of a "genuine" nucleomorph, is not known in these plastids

[e] Bergholtz et al. (2006) and Scnhepf and Elbrachter (1999)

[f] Hopkins et al. (1999), Kohler (2005), McFadden and Roos (1999) and Tomova et al. (2006)

[g] Near the plasmalemma, the chloroplast envelope is commonly incomplete and usually composed of only two membranes (Moestrup and Sengco 2001)

relatives of plastid-containing lineages has the potential to improve our understanding of the timing of plastid acquisition and loss in eukaryotic evolution.

The Alveolata is comprised of three major subclades, the apicomplexans, dinoflagellates and ciliates, of which the first two groups include plastid-containing members. About half of known dinoflagellates harbor plastids obtained from diverse algal sources (see below for details), while the rest are plastid-less (Schnepf and Elbrachter 1999). Although the "early-diverging" dinoflagellate genera *Perkinsus* and *Oxyrrhis* were originally thought to lack plastids (Leander and Keeling 2003; Saldarriaga et al. 2003), a recent study identified a four-membrane-bound plastid-like organelle in *Perkinsus atlanticus* (Teles-Grilo et al. 2007). Apicomplexans such as *Plasmodium* and *Toxoplasma* contain two to four membrane-bound nonphotosynthetic plastids known as apicoplasts (Kohler et al. 1997; Hopkins et al. 1999; Kohler 2005; Tomova et al. 2006). On the other hand, other apicomplexan parasites, including *Cryptosporidium parvum* and the gregarines, are apparently devoid of such organelles (Toso and Omoto 2007). In addition, plastids have thus far not been identified in colpodellids, predatory, free-living heterotrophs that are closely related to Apicomplexa (Brugerolle 2002; Leander et al. 2003). Nevertheless, the recent discovery of *Chromera velia*, a photosynthetic relative of apicomplexans, suggests that these plastid-less apicomplexans and colpodellids might have lost their plastids secondarily (Moore et al. 2008).

In Cryptophyta, most genera possess four membrane-bound plastids and are noteworthy in that the relic nucleus of their red algal endosymbiont, known as the nucleomorph, still persists between the second and the third plastid membranes (Hoef-Emden et al. 2002; Archibald 2007). Molecular phylogenies have shown that the single plastid-less cryptophyte genus *Goniomonas* is sister to the plastid-containing cryptophytes and includes three species, which show substantial genetic diversity comparable to that of all other cryptophytes combined (Deane et al. 2002; Von der Heyden et al. 2004). The cryptophytes are sister to the katablepharids, an enigmatic lineage comprising plastid-less, free-living biflagellates common in aquatic environments (Lee and Kugrens 1991; Okamoto and Inouye 2005; Kim et al. 2006).

The Euglenida include plastid-containing members such as *Euglena gracilis* and paraphyletic plastid-less taxa such as the primary osmotroph *Distigma* and phagotrophs such as *Petalomonas* and *Entosiphon* (Busse et al. 2003; Breglia et al. 2007). The plastids of euglenids are surrounded by three membranes (Fig. 1d), and unlike the plastids of cryptophytes are not directly associated with the nuclear envelope (van Dooren et al. 2001). The Euglenida are closely related to Diplonemida and Kinetoplastida, which include free-living (e.g., *Bodo*) and parasitic (e.g., *Trypanosoma*) plastid-less heterotrophs (Busse and Preisfeld 2002; Leander 2004).

Stramenopiles (also known as heterokonts) include morphologically diverse forms of eukaryotes ranging from pico-sized (less than 3 μm) flagellates to giant kelps. Molecular phylogenies using the small subunit ribosomal RNA (rRNA) gene suggest that plastid-containing stramenopiles form a clade (i.e., Ochrophyta; Cavalier-Smith and Chao 1996) to the exclusion of paraphyletic plastid-less subgroups such as Bicosoecida, Developayella, Hyphochytriales, labyrinthulomycetes, Opalinata, peronospromycetes, Placididea and *Pirsonia* (Guillou et al. 1999;

Moriya et al. 2000, 2002; Karpov et al. 2001; Andersen 2004; Kuhn et al. 2004). Within Ochrophyta, loss of photosynthesis has occurred multiple times, especially in the Chrysophyceae, but plastids usually persist as in the common freshwater flagellate *Paraphysomonas* (Preisig and Hibberd 1983). Although a few ochrophyte taxa were once thought to have completely lost their plastids (Cavalier-Smith et al. 1995/1996), bona fide organelles were subsequently identified or plastid-derived *rbcL* genes were successfully PCR-amplified, supporting the presence of plastids in the genera *Pteridomonas* and *Ciliophrys* (Sekiguchi et al. 2002). Comparable investigations are needed to test for the presence or absence of plastids in *Oikomonas* and *Picophagus*, two additional nonphotosynthetic ochrophyte taxa (Cavalier-Smith et al. 1995/1996; Guillou et al. 1999).

Several additional examples of photosynthetic eukaryotes are worthy of mention, two of which are somewhat controversial. First, a putative plastid-like organelle has been identified in the "picobiliphytes," a newly discovered eukaryotic lineage that has yet to be cultured in the laboratory (Not et al. 2007). These cells appear to harbor a photosynthetic body that emits orange autofluorescence under blue light, suggesting the presence of phycobiliproteins (Not et al. 2007). A small DNA-containing region was identified in close association with the picobiliphyte "plastid" and proposed to be a nucleomorph, though this has not been proven. Second, the thecate amoeba *Paulinella chromatophora* (Rhizaria) possesses two to four elongate blue-green photosynthetic bodies that recent molecular investigations have shown to be very closely related to cyanobacteria of the *Prochlorococcus*/*Synechococcus* clade (Lukavsky and Cepak 1992; Marin et al. 2005, 2007; Yoon et al. 2006; Nowack et al. 2008). Finally, the diatom *Rhopalodia gibba* (Stramenopiles) harbors *Cyanothece*-like, cyanobacterium-derived spheroid bodies that fix nitrogen using ATP and/or photosynthate derived from the plastids of its host (Prechtl et al. 2004). While the cyanobacterial-derived entities of both *P. chromatophora* and *R. gibba* appear to be "permanent" cellular inclusions that cannot be cultured in isolation, there is considerable debate as to whether the term "endosymbiont" or "organelle" is most appropriate (see Archibald (2006), Bhattacharya and Archibald (2006), Theissen and Martin (2006) and Bodyl et al. (2007) for recent discussion).

3 Biochemical Diversity of Plastids

3.1 Photosynthetic Pigments

In photosynthetic plants and algae, the harvesting of light energy involves three major types of pigments: chlorophylls (Chl), carotenoids and phycobilins (Graham and Wilcox 2000). Chl *a* is an essential component of the core complexes of photosystems I and II and is universally distributed in photosynthetic plastids and cyanobacteria, whereas Chl *b*, *c* and *d* are regarded as accessory pigments, which absorb and transfer excitation energy to Chl *a* (Falkowski and Raven 2007). Chl *b* occurs in the plastids of Viridiplantae and their secondary derivatives (chlorarachniophytes, euglenids and

the dinoflagellate *Lepidodinium*) and in three cyanobacterial genera, *Prochlorococcus*, *Prochloron* and *Prochlorothrix*, which are collectively referred to as prochlorophytes even though they do not form a monophyletic assemblage (Green and Durnford 1996; Griffiths 2006). Biosynthesis of Chl *b* requires the enzyme Chl *a* oxygenase (CAO), which converts Chl *a* into Chl *b* (Tomitani et al. 1999). Molecular sequence analyses suggest that the CAO gene sequences of Viridiplantae and two prochlorophytes, *Prochloron* and *Prochlorothrix*, share a common evolutionary origin (Tomitani et al. 1999), while that of *Prochlorococcus* may be of a separate origin (Hess et al. 2001). On the other hand, Chl *a*/*b*-binding proteins of green plastids belong to the eukaryotic light-harvesting complex (LHC) family, and are not related to the prochlorophyte functional equivalent, prochlorophyte-like Chl binding protein (Pcb) (Green and Durnford 1996; La Roche et al. 1996). The eukaryotic LHC family is thought to be derived from the high-light-inducible protein (HLIP) through successive gene duplication events, whereas prochlorophyte Pcb is related to the iron stress-induced protein (IsiA) or the photosystem II protein PsbC (Chen et al. 2005; Green 2005).

Chl *d* occurs in the cyanobacterium *Acaryochloris marina* as a major pigment (Miyashita et al. 2003). Although several red algae were thought to produce a small amount of Chl *d*, its detection seems to be due to the presence of epiphytic *Acaryochloris* on them (Murakami et al. 2004). While the molecular structure suggests that Chl *d* is likely synthesized directly from Chl *a*, its biosynthetic pathway is poorly understood (Beale 1999). Chl *c* occurs in red algal-derived plastids (although not in red plastids themselves) as a major pigment fraction, as well as in some phycobilisome-lacking cyanobacteria and prasinophycean green algae (Wilhelm 1987; Larkum et al. 1994; Green and Durnford 1996; Miyashita et al. 2003; Six et al. 2005). Unlike Chl *b* and *d*, which are structurally chlorin-based like Chl *a*, Chl *c* (c_1, c_2, c_3) is structurally more similar to a Chl *a* precursor, protochlorophyllide (porphyrin), and generally does not possess a hydrophobic phytol tail (Zapata and Garrido 1997). The existence of Chl *c* in red algal-derived plastids has been argued as evidence for their common origin (Cavalier-Smith 1999; see below). However, the biosynthetic pathway underlying the synthesis of Chl *c* is essentially unknown (Beale 1999), and, hence, its utility as a phylogenetic marker is unclear.

Carotenoids, which are structurally related to tetraterpenoids, exist as two major types, the hydrocarbon carotenes and their oxygenated derivatives, xanthophylls such as alloxanthin, peridinin and fucoxanthin (Cunningham and Gantt 1998). They are a vital component of the thylakoid membrane and play an important role in energy transfer as well as photoprotection by dissipating excess energy (Cunningham and Gantt 1998). With respect to carotenoid distribution, β-carotene occurs universally in chloroplasts, whereas other carotenoids show a more restricted distribution and are recognized as potentially valuable phylogenetic markers. Alloxanthin, for example, is uniquely found among cryptophyte algae (Reid et al. 1990). Peridinin is another unique pigment, which is found in the majority of plastid-containing dinoflagellates and occurs within the lumen as water-soluble peridinin–Chl *a*–protein complexes (Green and Durnford 1996).

Phycobilin pigments – phycourobilin, phycoerythrobilin and phycocyanobilin – are linear tetrapyrroles that bind to proteins to form phycobiliproteins, which can be further assembled into a large hemispherical structure, about 40 nm in diameter, known as a phycobilisome (Falkowski and Raven 2007). Anchored into the stromal side of the thylakoid membrane, phycobilisomes are visible under the electron microscope and appear to be responsible for the spatial separation of thylakoid membranes. Prochlorophytes and plastids that are devoid of phycobilisomes apparently have paired or stacked thylakoid membranes (Walsby 1986) (Fig. 1c–i). While cryptophytes and the cyanobacterium *Prochlorococcus* do not possess typical phycobilisomes, they nevertheless possess phycoerythrin and/or phycocyanin in the lumen part of the thylakoid (Hess et al. 1999; Griffiths 2006; Dammeyer et al. 2007).

It has been suggested that the ancestral cyanobacterium that gave rise to the plastid possessed both Chl *b* and phycobilisomes and during plastid evolution Chl *b* was lost in glaucophytes and rhodophytes, whereas phycobilisomes were lost in the lineage leading to green algae and land plants (Tomitani et al. 1999). This hypothesis, however, is not supported by pigment composition patterns seen in extant cyanobacteria. To date, no cyanobacterium possessing both Chl *b* and phycobilisomes is known. Even in the case where Chl *b* and phycobiliproteins co-occur as in the prochlorophyte *Prochlorococcus*, these phycobiliproteins do not form highly organized phycobilisomes. This suggests that the Chl *b* biosynthetic capacity of green plastids was likely acquired after the divergence of green plastids from red plastids through LGT of the CAO gene and potentially other gene compliments from *Prochlorothrix* or *Prochloron*-like cyanobacteria or other vectors such as cyanophage.

3.2 Storage Carbon Biochemistry

The end products of photosynthesis in algae and plants are stored as polysaccharides comprising d-glucose monomers, linked via either α-1,4-glycosidic bonds with α-1,6-branches (starch), or β-1,3-glycosidic bonds with occasional β-1,6-branches (chrysolaminaran and paramylon). The storage polysaccharides of most algal groups are found in the cytoplasm, with cryptophytes and members of the green algae and land plants being interesting exceptions. In cryptophytes, starch accumulates in the space between the second and third plastid envelopes (i.e., the periplastidal compartment; Fig. 1g), which corresponds to the cytoplasm of the red algal endosymbiont (McFadden et al. 1994). In green algae and land plants, starch accumulates within the plastid stroma (Ball and Morell 2003).

Starch is found in photosynthetic members of the cryptophytes, dinoflagellates, glaucophytes, rhodophytes, Viridiplantae and their nonphotosynthetic derivatives (Raven 2005). Apicomplexans such as *Toxoplasma gondii* and even *C. parvum*, which lacks a plastid, also produce starch granules in the cytoplasm (Harris et al. 2004). Unlike the glycogen seen in animals, fungi and prokaryotes, which is a

water-soluble and highly branched (10–12%) polymer of less than 50 nm in diameter, starch is a large (0.1 to over 50 μm) and complex semicrystalline polymer generally made of amylopectin and amylose (Ball and Morell 2003). Amylose is a linear chain of α-1,4-linked glucose containing less than 1% α-1,6-branches, whereas amylopectin is a much larger molecule with frequent α-1,6-branches (5–6%) (Buleon et al. 1998). In land plants, where starch has been extensively studied, the starch granule is organized as concentric rings of alternating semicrystalline and amorphous layering patterns resulting from regularly branching amylopectin molecules (Buleon et al. 1998; Buleon et al. 2007). Like green algae and land plants, the starch granules of cryptophytes and dinoflagellates are composed of amylose (up to 40% for cryptophytes) and amylopectin and stain blue-black with iodine solution (Vogel and Meeuse 1968; Antia et al. 1979; McFadden et al. 1994; Coppin et al. 2005). In contrast, the storage carbohydrate of apicomplexans appears to lack amylose and consist only of amylopectin (Coppin et al. 2005). Red algae show more variations in storage polysaccharides, containing either glycogen (*Cyanidium caldarium*) or amylopectin (florideophycean red algae) alone, or a mixture of amylose and semi-amylopectin (*Porphyridium purpureum*) (Yu et al. 2002; Shimonaga et al. 2007). Interestingly, some cyanobacteria produce semi-amylopectin instead of glycogen, which shows a chain length distribution similar to that of the red alga *P. purpureum* (Nakamura et al. 2005; Shimonaga et al. 2007); however, unlike *P. purpureum*, these cyanobacteria do not synthesize amylose and it is not clear whether these organisms share a similar biochemical machinery to produce semi-amylopectin (Nakamura et al. 2005).

Paramylon is the β-1,3-glucose linked storage carbohydrate of euglenids, and does not stain with iodine solution. A paramylon granule, bound by a single membrane, is composed of triangular and rectangular segments, each segment made of several layers (Kiss et al. 1987). Similar to cellulose, paramylon is organized into microfibrils (of 4.0 nm in diameter) composed of triple helices of β-1,3-glucose chains, which are further bundled into thicker fibers (Marchessault and Deslandes 1979; Kiss et al. 1987). The higher-order assembly of microfibrils and their interactions with water molecules contribute to the highly crystalline nature of paramylon (Marchessault and Deslandes 1979; Kiss et al. 1988). The paramylon granules occur in the cytoplasm and generally near the pyrenoid region of photosynthetically active plastids (Kiss et al. 1986). However, heterotrophically grown *Euglena* cells and even some plastid-less "primitive" euglenids such as certain *Petalomonas* species also contain abundant paramylon granules (Kiss et al. 1986; Lee et al. 2000). This suggests that when the colorless euglenid ancestor acquired its plastid through the secondary endosymbiotic engulfment of a green algal cell, the carbon storage system of the host was utilized, as opposed to that of the algal endosymbiont.

Chlorarachniophytes, haptophytes and stramenopiles also store photosynthetic end products as β-1,3-polyglucans and are referred to as chrysolaminaran (also known as leucosin or laminaran) (McFadden et al. 1997; Granum and Myklestad 2001; Chiovitti et al. 2006; Hirokawa et al. 2007). However, unlike paramylon, which is insoluble, chrysolaminaran is water-soluble and generally consists of only 20–60 glucose units (Janse et al. 1996). In chlorarachniophytes, the cap-shaped chrysolaminaran vesicle is

tightly associated with the pyrenoid, albeit separated from it by the four membranes surrounding the plastid (Fig. 1e) (McFadden et al. 1997; Moestrup and Sengco 2001). Some brown seaweeds (Phaeophyceae) have manitol groups attached to the ends of the β-1,3-polyglucan chain (Chizhov et al. 1998). The haptophyte *Pavlova* is unusual in that it produces water-insoluble, crystalline β-1,3-polyglucans like the paramylon of euglenoids, although the two crystalline granules have different structures and likely arose independently (Kiss and Triemer 1988).

Storage polyglucans are synthesized from either ADP glucose- or UDP glucose-based pathways (Ball and Morell 2003). Green algae and plants are unique among eukaryotic algae in that their starch synthesis pathway utilizes ADP glucose as a donor, as in bacteria, where the pathway is likely to have originated (Ball and Morell 2003). In contrast, other eukaryotic algae utilize UDP glucose as precursors to synthesize glucose polymers, similar to glycogen synthesis in animals and fungi (Viola et al. 2001; Ball and Morell 2003; Barbier et al. 2005; Deschamps et al. 2006). During the acquisition of plastids, the majority of algal groups other than Viridiplantae and cryptophytes have transferred the location of their storage carbohydrate to the host cytoplasm, which suggests that the endosymbiont's photosynthetic carbon metabolism has been amalgamated into the host carbohydrate biochemistry. Indeed, analyses of starch synthesis pathway genes have revealed that red algae and even land plants integrated the endosymbiont and host carbohydrate pathways (Patron and Keeling 2005).

4 Origin of Plastids

4.1 Origin of Primary Plastids

The question of single or multiple origins for the primary plastids of glaucophytes, rhodophytes and Viridiplantae has been extensively debated (Nozaki et al. 2003; Palmer 2003; Stiller et al. 2003; Larkum et al. 2007; Stiller 2007). Several plastid-related characters support the hypothesis that plastids evolved from a single type of cyanobacterial ancestor. First, the plastids of Rhodophyta and Viridiplantae (and their derivatives) share eukaryote-specific LHCs that are not present in cyanobacteria (Durnford et al. 1999). LHC homologs, however, have not been identified in the plastids of Glaucophyta (Rissler and Durnford 2005). Second, Tic110, an important component of the protein import apparatus, is present in all three primary plastids and their descendants, but is absent in cyanobacteria (McFadden and van Dooren 2004). This suggests that the nucleus-encoded Tic110 may represent a postendosymbiotic innovation (McFadden and van Dooren 2004). Third, the organization of the *atpA* gene cluster is another line of evidence in support of the common origin of the three types of primary plastids (Stoebe and Kowallik 1999). Plastid-encoded gene phylogenies also support the notion that the three primary plastids are closely related to each other, suggesting their common origin (Rodriguez-Ezpeleta et al. 2005), although the acquisition of similar cyanobacterial endosymbionts on multiple occasions could also produce such a

topology. Currently available data thus favor a single origin for all primary plastids. Nevertheless, it should be emphasized that plastid-related characters do not directly address whether or not the three primary plastids share a single endosymbiotic origin. This is because the number of plastid membranes, upon which the concept of "primary" plastids depends, does not necessarily reflect the primary, secondary or tertiary origin of the organelle because of possible losses of membranes during or after endosymbiosis (Stiller and Hall 1997). For example, the plastids of some peridinin-containing dinoflagellates have lost one membrane and are now bound by only two plastid membranes, although these are not likely to be of primary origin (Schnepf and Elbrachter 1999). The relationships among the nucleocytoplasmic component of Glaucophyta, Rhodophyta and Viridiplantae are also unsettled. While the monophyly of the three groups was initially strongly supported in a combined nuclear-encoded gene phylogeny by Rodriguez-Ezpeleta et al. (2005), addition of sequences from cryptophytes and haptophytes resulted in a reduction in statistical support or the monophyly of the three groups was no longer inferred (Burki et al. 2007; Patron et al. 2007).

4.2 Evolution of Green Algal-Derived Secondary Plastids

A wealth of biochemical, ultrastructural and molecular data has shown that euglenids and chlorarachniophytes (Fig. 1 d, e) acquired photosynthesis secondarily through the uptake of green algal endosymbionts (McFadden 2001; Archibald and Keeling 2002). Cavalier-Smith (1999) hypothesized that the plastids in these two groups are specifically related to one another, i.e., that the secondary endosymbiosis occurred in their common ancestor. This is parsimonious in the sense that it requires only a single endosymbiotic event, but is problematic when one considers that their respective host cells are not obviously related to one another: euglenids belong to the Euglenozoa, while the chlorarachniophytes reside within an entirely different eukaryotic supergroup, the Rhizaria (Adl et al. 2005; Keeling et al. 2005). Indeed, recent phylogenies of the plastid-targeted psbO protein and concatenated plastid-encoded proteins support the hypothesis that the euglenid and chlorarachniophyte plastids are of independent origin (Rogers et al. 2007; Takahashi et al. 2007). Finally, it is worth noting that some dinoflagellates have plastids of green algal origin (Hansen et al. 2007), having replaced their red algal secondary plastids (or reacquired a plastid after having lost it) (see below).

4.3 Evolution of Red Algal-Derived Secondary Plastids

A number of eukaryotes acquired their plastids through secondary or tertiary endosymbioses involving red algal endosymbionts. These include the cryptophytes, haptophytes and stramenopiles, whose plastids are generally bound by four membranes (Fig. 1f–h, Table 1). An interesting feature of these plastids, albeit with

some exceptions in stramenopiles (Andersen 2004), is the confluence of the outer plastid membrane with the nuclear envelope either directly, or via endoplasmic reticulum (Fig. 1f–h; Cavalier-Smith 1999). Consequently, the outer membrane of these plastids is typically studded with cytoplasmic ribosomes. Apicoplasts and the peridinin-containing plastids of dinoflagellates may also have originated from red algal endosymbionts (see below), although their outer membranes are not continuous with the host cell endomembrane system (Schnepf and Elbrachter 1999). The origin and evolution of red algal secondary plastids is hotly debated, and there is currently no generally agreed upon consensus on the pattern of plastid gain and/or loss that best describes the current distribution of these organelles (Falkowski et al. 2004; Grzebyk et al. 2004; Keeling et al. 2004).

Cavalier-Smith (1999) hypothesized that the red algal-derived plastids of cryptophytes, haptophytes, stramenopiles (i.e., "chromists") and plastid-bearing alveolates (apicomplexans and dinoflagellates; see below) are the product of an ancient secondary endosymbiosis in a common ancestor each of these lineages shared to the exclusion of all other eukaryotic groups. As was the case for the origin of green algal secondary plastids, the so-called chromalveolate hypothesis was postulated in order to minimize the number of secondary endosymbioses needed to explain the observed distribution of red secondary plastids. A wide range of data has been brought to bear on the question of chromalveolate monophyly, most notably, phylogenies of plastid and host nuclear gene sequences. Host gene phylogenies and the presence of a number of plastid-less sister taxa, for example, conflict with the chromalveolate hypothesis, or require additional underlying assumptions (e.g., extensive plastid loss; Kim et al. 2006; Hackett et al. 2007; Patron et al. 2007). Analyses of plastid-encoded genes also do not consistently support the chromalveolate hypothesis; the relationships among red algal-derived plastids vary depending on taxonomic sampling, analytical methods, and types and the number of genes included (Martin et al. 2002; Yoon et al. 2002b; Ohta et al. 2003; Sanchez-Puerta et al. 2007). Furthermore, the monophyly of the plastid component of cryptophytes, haptophytes, stramenopiles and alveolates can also be explained by the "serial hypothesis," which proposes serial transfers of red algal-derived plastids among different host lineages (Bachvaroff et al. 2005; Bodyl 2005; Sanchez-Puerta et al. 2007). The acquisition of similar red algal endosymbionts by different host eukaryotes is also predicted to produce a gene topology where their plastid genes are clustered together (Grzebyk et al. 2004).

The most widely cited molecular marker in support of the chromalveolate hypothesis is glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Photosynthetic eukaryotes possess cytosolic and plastid isoforms of this protein and unlike Viridiplantae and rhodophytes, in which the plastid-targeted GAPDH likely derived from a cyanobacterial donor, red plastid-containing eukaryotes – apicomplexans, cryptophytes, dinoflagellates, haptophytes and stramenopiles – possess a plastid-targeted GAPDH that arose through duplication of the eukaryotic cytosolic isoform (Fast et al. 2001; Harper and Keeling 2003); notable exceptions exist in some dinoflagellates (Fagan and Hastings 2002; Takishita et al. 2003). Although a close relationship among these plastid-targeted GAPDH copies was interpreted as evidence

for a single origin for red algal-derived plastids (Harper and Keeling 2003), these results have been called into question (Bodyl 2005; Bodyl and Moszczynski 2006). One prominent reason is the apparent discrepancy between the branching pattern of the cytosolic and the plastid-targeted GAPDH subtrees. Although the plastid-targeted GAPDH sequences of "chromalveolate" taxa cluster together with strong bootstrap support (more than 95%), their cytosolic GAPDH sequences do not form a clade (Fast et al. 2001; Harper and Keeling 2003). The observed disparities between the two homologs would seem to refute the underlying assumption of Fast et al. (2001) that the two homologs have coevolved since the endosymbiotic common origin. Secondly, plastid-targeted GAPDH protein phylogenies are not always consistent with accepted organismal relationships. For example, in the plastid-targeted GAPDH subtree the apicomplexan *T. gondii* is strongly associated with haptophytes to the exclusion of peridinin-containing dinoflagellates (Harper and Keeling 2003; Takishita et al. 2004). This suggests that *T. gondii* and peridinin-containing dinoflagellates obtained the genes for their plastid-targeted GAPDH proteins independently.

The phylogeny of several other nucleus-encoded plastid-targeted proteins, such as sedoheptulose bisphosphatase, fructose bisphosphatase, phosphoribulokinase and fructose bisphosphate aldolase, has also been explored in an attempt to elucidate the origin of red algal-derived plastids. Unfortunately, these analyses are largely inconclusive because of the complex evolutionary history of such genes (Kroth et al. 2005; Petersen et al. 2006; Teich et al. 2007). With the growing recognition of the impact of LGT in eukaryotic evolution, especially in phagotrophs (Andersson 2005), the complex evolutionary patterns of plastid-targeted proteins may have arisen during the early stages of endosymbiosis when the host–endosymbiont relationship had not been permanently established. If ancient host eukaryotes were exposed to, and "experimented" with, diverse kinds of algae in the context of transient endosymbioses, analogous to modern sea-slug-plastid symbioses (Rumpho et al. 2000), nuclear genes for plastid-targeted proteins could have originated from multiple sources, and thus display a mosaic evolutionary pattern. Additionally, the mixotrophic life style of many plastid-containing eukaryotes could provide a continuous source of "foreign" genes to the host. Seen in this light, the analysis and interpretation of plastid-targeted proteins requires caution.

4.4 Alveolate Plastids

The red or green algal secondary endosymbiotic origin of the apicoplast has been intensely debated (Funes et al. 2002, 2003; Waller et al. 2003). While sequence analyses of *tufA*, *rpoB*, *rpoC1* and *rpoC2* genes suggest that apicoplasts are related to green plastids (Kohler et al. 1997; Cai et al. 2003), rRNA, transfer RNA (tRNA) and ribosomal protein gene trees tend to support (when long-branching sequences of euglenids are excluded) the alternative hypothesis that apicoplasts are of red algal origin (Blanchard and Hicks 1999). Consistent with the results of

phylogenetic analyses, the ribosomal protein gene cluster of apicoplasts shares structural similarity with that of red algal plastids (Blanchard and Hicks 1999). As noted above, a photosynthetic relative of apicomplexans, *C. velia*, has been discovered recently (Moore et al. 2008), the plastid of which appears to be related to apicoplasts on the basis of plastid rRNA gene phylogeny and to be of red algal origin. This result suggests that the hypothesis of green algal ancestry of the apicoplast is unlikely, although it is still possible that the apicoplast is of chimeric origin, i.e., derived from both red and green plastids through multiple endosymbiotic events, as has been suggested (Funes et al. 2004). Comprehensive phylogenetic analysis of plastid-encoded genes in *C. velia* will be necessary to test whether the apicoplast is of red algal origin or of chimeric origin.

Dinoflagellates are remarkable in that they have acquired plastids from diverse algal sources (Schnepf and Elbrachter 1999). Approximately half of known dinoflagellate species possess "genuine" plastids, while other photosynthetic dinoflagellates harbor transient cyanobacterial endosymbionts or plastids (i.e., kleptoplastids) borrowed from haptophytes (e.g., *Dinophysis mitra*), cryptophytes (e.g., *Dinophysis acuminata*, *Gymnodinium acidoum*) or green algae (e.g., *Noctiluca scintillans*) (Wilcox and Wedemayer 1984; Schnepf and Elbrachter 1999; Takishita et al. 2002; Koike et al. 2005; Minnhagen and Janson 2006).

Among the plastid-containing dinoflagellates, most are characterized by the presence of the pigment peridinin as a major carotenoid fraction, as well as a nuclear-encoded form II ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which otherwise only occurs in some proteobacteria (Morse et al. 1995). In contrast, the plastids of the dinoflagellate genera *Karenia*, *Karlonidium* and *Takayama* contain fucoxanthin and its derivatives (19′-hexanoyloxyfucoxanthin and 19′-butanoyloxyfucoxanthin) instead of peridinin, reminiscent of certain haptophytes (Takishita et al. 2004). Yet other dinoflagellates harbor plastids taken from prasinophyte green algae (*Lepidodinium viride*, *L. chlorophorum*) or diatoms (*Kryptoperidinium foliaceum*, *Durinskia baltica*, *Galeidinium rugatum* and *Peridinium quinoquecorne*), some of which seem to retain the relic nucleus and, in some cases, even the mitochondria of their algal endosymbionts (Schnepf and Elbrachter 1999; Horiguchi and Takano 2006; Hansen et al. 2007; Imanian and Keeling 2007). The thecate dinoflagellate *Podolampas bipes* harbors plastids that originate from a dictyophyte (Stramenopiles) (Schnepf and Elbrachter 1999; Schweiker and Elbrachter 2004), but whether these plastids are permanent or transient remains to be demonstrated.

The origins of peridinin-containing and fucoxanthin-containing (19′-hexanoyloxyfucoxanthin and 19′-butanoyloxyfucoxanthin) plastids of dinoflagellates have been difficult to discern (Ishida and Green 2002; Yoon et al. 2002a; Bodyl and Moszczynski 2006). Growing evidence suggests that the two types of plastids arose separately and that the fucoxanthin-containing plastids originated from a haptophyte ancestor (Ishida and Green 2002; Yoon et al. 2002a; Bodyl and Moszczynski 2006). This hypothesis is supported by the phylogenies of two nuclear-encoded and plastid-targeted proteins, psbO and GAPDH, and the plastid-encoded psbC (Ishida and Green 2002; Takishita et al. 2004, 2005). Although combined DNA sequence

analysis of plastid-encoded *psaA* and *psbA* genes suggested a strong affiliation between the two types of plastids and consequently a common haptophyte origin (Yoon et al. 2002a), this relationship has been suggested to be a phylogenetic artifact caused by codon usage heterogeneity (Inagaki et al. 2004). The true origin of peridinin-containing plastids is thus presently unclear (Cavalier-Smith 1999; Bodyl and Moszczynski 2006). Unfortunately, the plastid genome of peridinin-containing dinoflagellates is fragmented into multiple minicircles (see Sect. 1.3) (Zhang et al. 2002) and as a result, information on gene order cannot be used as a phylogenetic character. In addition, only a handful of plastid-encoded genes remain in the peridinin plastid and those that do are highly derived in sequence (Sanchez-Puerta et al. 2007) and thus susceptible to phylogenetic artifacts.

5 Plastid Genome Evolution

5.1 Plastid Genome Structure

As of January 2008, approximately 200 plastid genomes have been completely sequenced. The vast majority of these are from land plants and green algae, with only a single plastid genome sequence currently available from members of the Chlorarachniophyta, Glaucophyta and Haptophyta. Consequently, our views on the "typical" features of plastid genomes are significantly biased. Nevertheless, it is possible to identify a number of near-universal features of plastid genomes and in some cases these features are shared with the genomes of modern-day cyanobacteria.

One of the most widely distributed features of plastid genomes is the presence of ribosomal DNA (rDNA) containing repeats that form a quadripartite structure consisting of two inverted repeats and small and large single-copy regions (Stirewalt et al. 1995; Oudot-Le Secq et al. 2007). The rDNA repeat unit typically contains three rRNA genes (*rns*, *rnl*, *rrn5*) and two tRNAs (*trnA*, *trnI*), but can harbor as few as four genes or up to 161 genes through contraction or expansion of this region (Chumley et al. 2006) (Table 2). Although the repeats are rarely 100% identical to one another, they are always highly similar, and apparently evolve by concerted evolution. The presence of rDNA-containing repeats in all three plastid types and in some cyanobacteria such as *Synechococcus* sp. WH8102 suggests that the repeats likely predate the origin of plastids (Glockner et al. 2000). Some plastids, however, have apparently lost rDNA-containing repeats, or have rearranged the repeats in tandem (Ohta et al. 2003; Turmel et al. 2005; De Koning and Keeling 2006). In the case of the red alga *Porphyra*, the repeats are directly oriented (Reith and Munholland 1993).

Far and away the most unusual plastid genomes belong to the peridinin-containing dinoflagellates, which are composed of minicircles 2–10 kbp in size. Individual minicircles usually carry one to three genes, although "empty" minicircles have also been detected (Barbrook et al. 2006). The noncoding region of the

Table 2 Plastid genome features

Taxonomic classification	Genome size (nt)	No. of ORFs/structural RNAs[a]	Size of rDNA repeat unit (nt)	Genes in rDNA-containing repeats[b]	LSC/SSC ratio	Intron[a]
Glaucophyta						
Cyanophora paradoxa	135,599	145/37	11,285	(SSC)-*rns-trnI(gau)-trnA(ugc)-rnl-rrn5-dnaK-trnC(gca)-groEL-groES-clpP*-(LSC)	5.3	1 (group I)
Rhodophyta and red-plastid-containing eukaryotes						
Cyanidium caldarium (Rhodophyta)	164,921	197/33	Not present	NA	NA	0
Cyanidioschyzon merolae (Rhodophyta)	149,987	207/35	Not present	NA	NA	0
Gracilaria tenuistipitata (Rhodophyta)	183,883	203(1)/33	Not present	NA	NA	0
Porphyra yezoensis (Rhodophyta)	191,952	209/50	4,829	DR: *rns-trnI(gau)-trnA(ugc)-rnl-rrn5*	4.4	0
Porphyra purpurea (Rhodophyta)	191,028	209/38	4,826	DR: *rns-trnI(gau)-trnA(ugc)-rnl-rrn5*	4.4	0
Emiliania huxleyi (Haptophyta)	105,309	119/31	4,841	(LSC)-*rns-trnI(gau)-trnA(ugc)-rnl-rrn5*-(SSC)	7.6	0
Guillardia theta (Cryptophyta)	121,524	147/31	4,967	(LSC)-*rns-trnI(gau)-trnA(ugc)-rnl-rrn5*-(SSC)	6.2	0
Rhodomonas salina (Cryptophyta)	135,854	146(3)/32	4,927	(LSC)-*rns-trnI(gau)-trnA(ugc)-rnl-rrn5*-(SSC)	6.6	2 (group II)
Odontella sinensis (diatom, Stramenopiles)	119,704	137/29	7,725	(LSC)-*trnP(ugg)-orf355-rns-trnI(gau)-trnA(ugc)-rnl-rrn5-ycf32-rpl32*-(SSC)	1.7	0
Phaeodactylum tricornutum (diatom, Stramenopiles)	117,369	130/30	6,912	(LSC)-*trnP(ugg)-ycf89-rns-trnI(gau)-trnA(ugc)-rnl-rrn5-psbY*-(SSC)	1.6	0
Thalassiosira pseudonana (diatom, Stramenopiles)	128,814	127/30	18,337	(LSC)-*trnP(ugg)-ycf89-rns-trnI(gau)-trnA(ugc)-rnl-rrn5-psbY-rpl32-trnL(uag)-rbcR-rpl21-rpl27-secA-rpl34-ycf46-ccs1-psbA-ycf35-psaC-ccsA*-(SSC)	2.4	0

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