

Chapter 2

Plastid Origins

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2.1 Modern-Day Plastids and Their Genomes

The evolution of oxygenic photosynthesis in the ancestors of present-day cyanobacteria transformed the biosphere of our planet (Blankenship 1994; Reyes-Prieto et al. 2007). This landmark event was also an essential prerequisite for the evolution of photosynthetic eukaryotes. Plastids, or chloroplasts, are the light-gathering organelles of algae and plants whose origin can be traced back to cyanobacteria. Mereschkowsky (1905) is usually credited as being the first biologist to speculate on the possible evolutionary significance of similarities between cyanobacteria and plastids, and in the era of molecular biology and genomics, the evidence that plastids are derived from once free-living prokaryotes is now beyond refute. Modern-day plastids and the eukaryotes that harbor them are remarkably diverse in their morphology and biochemistry, but are nevertheless sufficiently similar to one another in their core features to be able to infer common ancestry. These include similarities in their plastid light-harvesting apparatus, the existence of protein import machinery with many cyanobacterial features, and an organellar genome of demonstrable cyanobacterial ancestry (Kim and Archibald 2009).

This chapter provides an overview of the origin and diversification of plastids across the eukaryotic tree of life, an area of basic research that has benefited tremendously from advances in genomics and molecular biology. Genome sequences from an evolutionarily diverse array of eukaryotic phototrophs are now available and have made it possible to sketch a general picture of how plastids evolved. Yet, while the evidence in support of a cyanobacterial origin for plastids is stronger than ever, other questions pertaining to the biology and evolution of

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plastid-bearing organisms have become less clear in the light of more data. Most prominent among them is the issue of how – and how often – plastids have spread horizontally across the tree by endosymbioses involving two eukaryotes and the extent to which genes of algal/cyanobacterial ancestry in the genomes of plastid-lacking eukaryotes can be taken as evidence for plastid loss.

One of the major challenges associated with inferring the history of plastids is the vast amount of time that has transpired since they first evolved and the limited coding capacity of their genomes relative to those of cyanobacteria. Molecular clock-based analyses (e.g., Yoon et al. 2004) have suggested that plastids evolved >1 billion years ago, and the transition from cyanobacterial endosymbiont to fully integrated organelle is known to have involved the loss of many nonessential genes and the transfer of essential genes from the endosymbiont to the nuclear genome of its eukaryotic host. This process, referred to as endosymbiotic gene transfer (EGT), is of profound significance to the study of organelle evolution and is reviewed in detail by Timmis and colleagues in Chap. 7. Even the most gene-rich plastid genomes possess only ~200 protein genes; most possess far fewer than this (Martin et al. 1998; Kim and Archibald 2009). The nuclear genomes of algae and plants encode many hundreds of proteins of cyanobacterial/plastid ancestry, many (but not all) of which are translated on cytoplasmic ribosomes and targeted to the plastid post-translationally (Jarvis and Soll 2001; Gould et al. 2008). Interestingly, whole genome-scale analyses have revealed that genes of noncyanobacterial ancestry also contribute to the proteomes of modern-day plastids (e.g., Moustafa et al. 2008; Suzuki and Miyagishima 2010). Conversely, many of the genes donated to the nuclear genome by the cyanobacterial progenitor of the plastid subsequently acquired functions in the host eukaryote unrelated to the plastid and to photosynthesis (Martin et al. 2002; Archibald 2006; Reyes-Prieto et al. 2006). Photosynthetic eukaryotes are thus increasingly recognized as complex evolutionary “mosaics,” with genes having been acquired via EGT as well as horizontal (or lateral) gene transfer (HGT) (Lane and Archibald 2008; Elias and Archibald 2009). Establishing the significance and relative contributions of these two sources of gene flow into algal nuclear genomes remains a major hurdle to overcome as the field moves toward a comprehensive understanding of the evolutionary history of plastid-bearing eukaryotes.

2.2 Primary Plastids

Plastids are typically classified as belonging to one of two types. “Primary” plastids are those considered to stem directly from the primordial endosymbiosis between a nonphotosynthetic eukaryote and the cyanobacterial plastid progenitor, while plastids that have spread indirectly from one eukaryote to another are designated “secondary” or “tertiary” organelles (Reyes-Prieto et al. 2007; Gould et al. 2008; Archibald 2009). Primary plastids are united in their shared possession of a two-membrane envelope, the leaflets of which are thought to correspond to the inner and outer membranes of the engulfed cyanobacterium (Reyes-Prieto et al. 2007; Gould

Table 2.1 Diversity and basic characteristics of plastids^a

Lineage	Putative origin	Membranes	Pigmentation
Glaucophytes	1 ^o	2 ^b	Chl <i>a</i> + phycobiliproteins
Red algae	1 ^o	2	Chl <i>a</i> + phycobiliproteins
Green algae + land plants	1 ^o	2	Chl <i>a</i> + <i>b</i>
Cryptophytes ^c	2 ^o (Red)	4	Chl <i>a</i> + <i>c</i> + phycobiliproteins
Haptophytes	2 ^o (Red)	4	Chl <i>a</i> + <i>c</i> + fucoxanthin
Stramenopiles (Heterokonts)	2 ^o (Red)	4	Chl <i>a</i> + <i>c</i> + fucoxanthin
Dinoflagellates ^d	2 ^o (Red)	3	Chl <i>a</i> + <i>c</i> + peridinin
Perkinsids	2 ^o (Red)	4	None (non-photosynthetic)
Apicomplexans	2 ^o (Red)	4	None (non-photosynthetic)
<i>Chromera</i>	2 ^o (Red)	4	Chl <i>a</i>
Euglenophytes	2 ^o (Green)	3	Chl <i>a</i> + <i>b</i>
Chlorarachniophytes ^c	2 ^o (Green)	4	Chl <i>a</i> + <i>b</i>

^aData taken primarily from Graham and Wilcox (2000) and Larkum et al. (2007). Numerous exceptions and additional complexities exist beyond the data presented in this table. Interested readers are referred to Kim and Archibald (2009) and references therein

^bGlaucophyte plastids possess a layer of peptidoglycan between the inner and outer membranes, as in cyanobacteria

^cThe nucleus of the algal endosymbionts that gave rise to the cryptophyte and chlorarachniophyte plastids persists in a highly degenerate form called a nucleomorph. The nucleomorph is located in the periplastidial compartment, i.e., the space between the inner and outer pairs of plastid membranes

^dApproximately 50% of known dinoflagellate species are photosynthetic. Plastid-bearing species usually possess a peridinin-pigmented plastid, although some dinoflagellates have also replaced this organelle with plastids acquired from haptophytes and diatoms (tertiary endosymbiosis) or green algae (serial secondary endosymbiosis). Plastid membrane number varies depending on plastid type. Refer to Hackett et al. (2004) for review

et al. 2008). In contrast, secondary and tertiary organelles possess additional membranes, with the precise number varying from lineage to lineage (Table 2.1).

The number of secondary and tertiary endosymbiotic events that have occurred during eukaryotic evolution is still very much an open question, but there is general agreement with regard to the origin of primary plastids and the lineages that harbor them: these are the red algae, glaucophyte (or glaucocystophyte) algae, and the green algae, the latter being the group from which land plants ultimately evolved (Delwiche et al. 2004; Reyes-Prieto et al. 2007). A single endosymbiotic capture of an ancestor of modern-day cyanobacteria by a full-blown eukaryotic host cell is believed by many researchers to have occurred in a common ancestor shared by the three lineages, followed by strict vertical inheritance thereafter (Palmer 2003; Reyes-Prieto et al. 2007) (Fig. 2.1).

A broad array of biochemical, molecular, and phylogenetic data has been brought to bear on the issue of whether primary plastids evolved once or more than once. For example, the plastids of red and green algae have been shown to possess light-harvesting complex (LHC) proteins that are not related to their functional equivalents in present-day cyanobacteria (Green and Durnford 1996; Durnford et al. 1999), the implication being that they represent singular eukaryote-

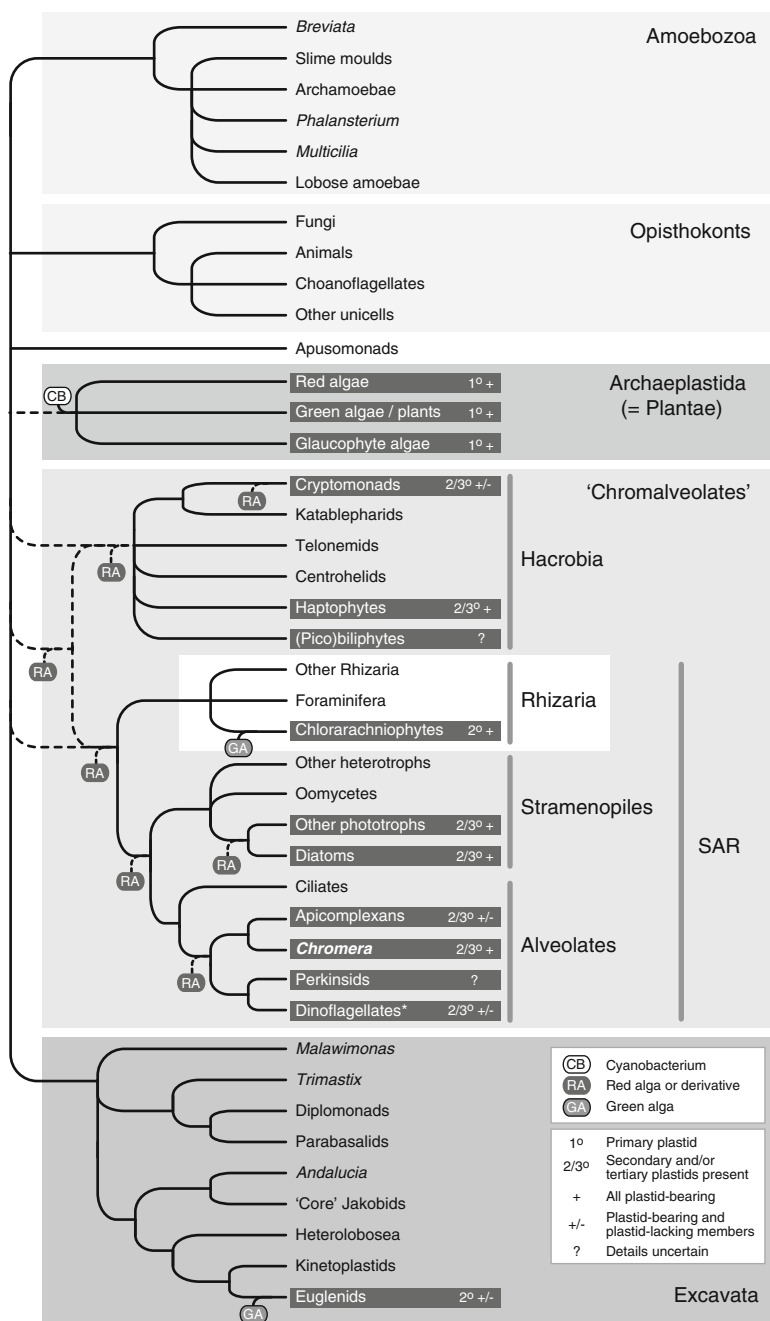


Fig. 2.1 A schematic representation of eukaryotic relationships with an emphasis on lineages containing one or more photosynthetic groups. The topology is a consensus of various nuclear multigene phylogenies (e.g., Burki et al. 2007, 2008; Hackett et al. 2007; Hampl et al. 2009; Parfrey et al. 2010). Dashed lines represent alternative topologies and/or areas of particular uncertainty. Primary and secondary/tertiary plastid-bearing lineages are indicated as described in the key. Asterisks: The dinoflagellates are known to harbor a wide diversity of plastids beyond those depicted in this figure (see main text)

specific evolutionary innovations occurring in a common ancestor of the two groups. A similar explanation has been proposed to account for the distribution of Tic110, a nucleus-encoded core subunit of the plastid protein import apparatus: Tic110 is found in red, green, and glaucophyte algae but is absent in all known cyanobacteria (McFadden and van Dooren 2004). The structure and coding capacity of primary plastid genomes is also suggestive of common ancestry. For example, the structure of the plastid *atpA* gene cluster is widely conserved among red, green, and glaucophyte algae (Stoebe and Kowallik 1999), as is the presence of ribosomal DNA (rDNA)-containing inverted repeats in the vast majority of primary plastid genomes (and those of their secondary derivatives). Inverted rDNA repeats are, however, also found in the genomes of some cyanobacteria, suggesting that this feature may have existed prior to the evolution of plastids by endosymbiosis (Glockner et al. 2000). The possibility of convergent evolution of basic plastid genome architecture is usually ignored but cannot be discounted (Palmer 2003; Stiller et al. 2003). Chapter 4 presents a detailed overview of plastid genomes and their structure.

Molecular phylogenetic analyses often, though by no means always, support the notion that red, green, and glaucophyte algae are each other's closest relatives. Such a relationship would be expected if primary plastids evolved only once in a common ancestor shared by the three lineages and if primary plastids have not been lost secondarily in plastid-lacking eukaryotic lineages. Early plastid 16S rDNA and elongation factor Tu (EF-Tu) sequence analyses supported the hypothesis of primary plastid monophyly (Delwiche et al. 1995; Helmchen et al. 1995), as have more recent multigene analyses in which dozens of plastid protein sequences are analyzed together as a single concatenate (e.g., Rodriguez-Ezpeleta et al. 2005). In contrast, phylogenies inferred from nuclear loci, such as the largest subunit of RNA polymerase II (RPB1) are sometimes much less clear (Stiller and Hall 1997; Longet et al. 2003). Tree topologies resulting from the latest "phylogenomic" analyses, including analyses of large 100+ protein data sets, have proven unexpectedly sensitive to taxon sampling and gene selection but often resolve the monophyly of "Archaeplastida" with high statistical support (e.g., Rodriguez-Ezpeleta et al. 2005, 2007a, b; Burki et al. 2007, 2008, 2009; Hackett et al. 2007). There is however no general consensus as to the branching order of red, green, and glaucophyte algae relative to one another. In cases where monophyly of these three main lines is not recovered, secondary plastid-bearing groups such as cryptophyte and haptophyte algae (see below) are often the intervening lineages (e.g., Hampl et al. 2009; Parfrey et al. 2010). Recent analysis of specific subsets of nuclear genes, in particular those deemed to be the most slowly evolving, appear to support the nonmonophyly of the primary plastid-bearing lineages and have been used as the basis for alternative scenarios in which primary plastids evolved in a common ancestor shared by red, green, and glaucophyte algae and other eukaryotic groups that currently lack a plastid (e.g., Nozaki et al. 2007). In sum, while it is widely held that primary plastids evolved only once, exactly when this occurred during eukaryotic evolution is unclear, and it is possible that primary plastid loss has occurred and/or that the double membrane-bound organelles in the three groups

have not been inherited in a strictly vertical fashion (see Stiller and Hall 1997; Andersson and Roger 2002; Nozaki et al. 2003; Larkum et al. 2007; Stiller 2007; Kim and Graham 2008 and references therein for alternative scenarios). As will be underscored in the sections that follow, assessing the extent to which nuclear and organellar gene phylogenies can be judged to be congruent or incongruent with one another has become one of the most important issues in plastid evolution.

One final note on the evolution of primary plastids relates to the “chromatophores” of the testate amoeba *Paulinella chromatophora*. First discovered over 100 years ago (Lauterborn 1895; Melkonian and Mollenhauer 2005), *P. chromatophora*, a member of the Rhizaria (Fig. 2.1), harbors within its cytoplasm cyanobacteria belonging to the genus *Synechococcus* (Marin et al. 2005; Yoon et al. 2006). The chromatophore genome is somewhat reduced relative to its closest free-living relatives, although nowhere near that of a canonical plastid (Nowack et al. 2008; Yoon et al. 2009). Much has been written about whether these “recently” acquired photosynthetic intracellular inclusions should be considered endosymbionts or true cellular organelles (Theissen and Martin 2006; Bhattacharya and Archibald 2007; Bodyl et al. 2007). Chromatophore-to-host-nucleus gene transfer has been documented (Nakayama and Ishida 2009; Nowack et al. 2011) and a mechanism for protein import into the chromatophore has even been proposed (Bodyl et al. 2010; Mackiewicz and Bodyl 2010). It will thus be interesting to see how much of what we learn from studying *P. chromatophora* and its enigmatic “organelles” will shed light on the primary endosymbiotic origin of canonical plastids, which appears to have been a singular, truly ancient event in eukaryotic evolution.

2.3 Secondary and Tertiary Plastids: Origins and Evolution

As fundamental as they are, the uncertainties surrounding the evolution of plastids within red, green, and glaucophyte algae pale in comparison to those of phototrophic eukaryotes as a whole. Researchers have long recognized the extraordinary structural and biochemical diversity of plastids, particularly in the realm of microscopic algae, and in the 1970s, the possibility that plastids had moved from one eukaryotic lineage to another began to be taken seriously (Taylor 1974; Gibbs 1978). Definitive evidence of what is referred to as secondary endosymbiosis eventually came from the study of algae that possess two bona fide nuclei, a host nucleus and a plastid-associated, eukaryotic endosymbiont-derived nucleus, the latter being referred to as a “nucleomorph” (Greenwood 1974; Greenwood et al. 1977; McFadden et al. 1994). Nucleomorphs have been shown to harbor the smallest nuclear genomes known to science and represent interesting systems with which to study the processes of genome reduction and compaction (Archibald and Lane 2009; Moore and Archibald 2009).

Two distinct nucleomorph-bearing lineages are currently recognized. The cryptophytes and chlorarachniophytes are significant not only in their shared

possession of the “smoking gun” of secondary endosymbiosis but also because they acquired photosynthesis independently. Cryptophyte nucleomorphs and plastids are derived from a red algal endosymbiont (Douglas et al. 1991, 2001; Douglas and Penny 1999; Graham and Wilcox 2000; Lane et al. 2007; Kim and Archibald 2009), while in chlorarachniophytes these organelles evolved from an endosymbiotic green alga (Gilson and McFadden 1996; Ishida et al. 1997, 1999; Gilson et al. 2006; Rogers et al. 2007). The closest relatives of their respective secondary endosymbionts within modern-day red and green algae are still uncertain, particularly in the case of cryptophytes, where comparative sequence data from diverse red algae are lacking. Nevertheless, karyotype and molecular sequence data have revealed that the cryptophyte and chlorarachniophyte nucleomorph genomes constitute a remarkable example of convergent evolution. Both harbor highly reduced, A+T-rich genomes less than 1 megabase-pair (Mbp) in size and partitioned into three chromosomes, each capped with subtelomeric ribosomal DNA (rDNA) loci (Moore and Archibald 2009). The evolutionary pressures responsible for and the biological significance of these similarities are for the most part not known.

The euglenophytes constitute a second lineage harboring green algal-derived secondary plastids. However, unlike chlorarachniophytes, whose plastid is surrounded by four membranes, the euglenophyte plastid has three membranes and lacks a plastid-associated nucleomorph (Table 2.1). Euglenophytes are classified as euglenids (or “euglenoids”), which in addition to phototrophs such as *Euglena gracilis*, include plastid-lacking heterotrophs capable of ingesting bacteria (bacteriovores) and eukaryotes (eukaryovores) (Leander et al. 2001). Together with the exclusively plastid-lacking kinetoplastids (e.g., parasites such as *Trypanosoma* and *Leishmania*), the euglenophytes reside within the “supergroup” Excavata (Hampel et al. 2009) (Fig. 2.1). In contrast, the chlorarachniophytes constitute the sole plastid-bearing lineage within the supergroup Rhizaria, a diverse collection of predominantly amoeboid, unicellular eukaryotes that include the foraminiferans and radiolarians (Nikolaev et al. 2004). The existence of green algal secondary plastids in both chlorarachniophytes and euglenophytes thus represents a case of discordant host–endosymbiont evolutionary histories and, at face value, is most consistent with the notion of independent secondary acquisitions of green algal plastids. Indeed, not only do the host components of chlorarachniophytes and euglenophytes belong to completely different supergroups, the latest plastid genome sequence comparisons suggest that their plastids evolved from distinct lines of green algae (e.g., Rogers et al. 2007; Turmel et al. 2009). Current data do not support the hypothesis of a single ancient green algal secondary endosymbiosis in a common ancestor shared by chlorarachniophytes and euglenophytes (Cavalier-Smith 1999).

An even broader array of eukaryotes harbors red algal-derived secondary plastids. In addition to the nucleomorph-bearing cryptophytes, these include the stramenopiles (e.g., diatoms and giant kelp), haptophytes (e.g., *Emiliania huxleyi*), some dinoflagellates, some apicomplexans such as the malaria parasite *Plasmodium falciparum*, as well as the newly discovered chromerids (Keeling 2009). Dinoflagellates are particularly impressive in their diversity of plastids, although

it should be noted that only ~50% of known species actually possess a photosynthetic organelle (Taylor 1980). Of those that do, most harbor a peridinin-pigmented, red algal-derived secondary (or tertiary) plastid, while others have tertiary plastids specifically derived from haptophytes (Tengs et al. 2000), cryptophytes (Schnepf and Elbrächter 1988; Hackett et al. 2003), and diatoms (Dodge 1969; Inagaki et al. 2000) (see Hackett et al. 2004 and Archibald 2005 and references therein for detailed review). The dinoflagellate *Lepidodinium* possesses a recently acquired, green algal-derived plastid of serial secondary origin (Watanabe et al. 1990), and members of the genus *Kryptoperidinium* have an “unreduced” diatom plastid with a nucleus and mitochondria still associated with it (Chesnick et al. 1997; McEwan and Keeling 2004; Imanian et al. 2007; Imanian and Keeling 2007). In addition, some dinoflagellates possess transient plastids and carry out “acquired phototrophy.” For instance, the heterotrophic dinoflagellate *Dinophysis acuminata* harbors a cryptophyte plastid that it obtains indirectly by regularly feeding on the ciliate *Myrionecta rubra*, which itself ingests cryptophytes of the *Teleaulax/Geminigera* clade (Park et al. 2008). Wisecaver and Hackett have recently shown that the nuclear genome of *D. acuminata* does not appear to be stocked with genes for plastid-targeted proteins, as is invariably the case for photosynthetic eukaryotes, and the few that have been found come primarily from algae other than cryptophytes (Wisecaver and Hackett 2010). The implication is that the *D. acuminata* plastid is truly temporary and incapable of being perpetuated to any great extent within the dinoflagellate cell.

Recent plastid acquisitions in dinoflagellates aside, how do the various red algal secondary plastids relate to one another? As for euglenids and chlorarachniophytes, demonstrating incongruent host and plastid phylogenies would support the notion of independent secondary endosymbioses involving distinct red algae and/or secondary hosts, but for various reasons, this has proven difficult to determine. For example, the highly derived nature of the plastid genomes of apicomplexans and dinoflagellates do not lend themselves to accurate phylogenetic reconstruction. The apicomplexans are nonphotosynthetic and appear to retain their plastids solely to carry out core metabolic processes, such as the synthesis of isoprenoids and fatty acids (Ralph et al. 2004). Consequently, the coding capacity of the “apicoplast” is quite limited and the genes that remain are typically highly divergent. It was in fact initially unclear whether the four-membrane-bound plastids of apicomplexans were of green or red algal ancestry. Data have been presented in support of both hypotheses (e.g., Köhler et al. 1997; Blanchard and Hicks 1999; Funes et al. 2002; Waller et al. 2003), but on balance, the evidence rests decidedly in favor of a red algal origin (Waller and McFadden 2005; Janouskovec et al. 2010; Lim and McFadden 2010). Dinoflagellates are even more problematic, as their peridinin plastid genomes are made up of single-gene minicircles encoding extraordinarily rapidly evolving genes (Zhang et al. 1999, 2000; Sanchez-Puerta et al. 2007a; Howe et al. 2008). The exciting discovery of *Chromera velia* (Moore et al. 2008), an alga with a relatively gene-rich plastid genome (Janouskovec et al. 2010), has made it possible to link both host- and plastid-associated features of dinoflagellates with those of apicomplexans (Keeling 2008; Janouskovec et al. 2010). Combined with the discovery of cryptic plastids in dinoflagellates that were previously assumed to

be lacking plastids (Sanchez-Puerta et al. 2007b) and their close relatives, such as perkinsids (Stelter et al. 2007; Teles-Grilo et al. 2007) and *Oxyrrhis* (Slamovits and Keeling 2008), it now seems likely that the common ancestor of dinoflagellates and apicomplexans possessed a red algal-derived secondary plastid.

What about the other red secondary plastid-containing lineages? A long-standing and controversial idea in the field is that the plastids of apicomplexans and dinoflagellates are truly ancient, sharing a common endosymbiotic origin with *all* other known red secondary plastids (Table 2.1, Fig. 2.1). Cavalier-Smith's "chromalveolate" hypothesis unites the "chromists" (plastid-bearing cryptomonads (i.e., cryptophytes), stramenopiles, and haptophytes) with the alveolates (dinoflagellates, apicomplexans, and ciliates) and rests on the principle that secondary endosymbiosis is a complex process and should be invoked sparingly (Cavalier-Smith 1999). With each such event, hundreds of nuclear genes for plastid-targeted proteins must be transferred from the red or green algal nucleus to the secondary host nucleus, and a mechanism for importing such proteins must evolve "from scratch" (Cavalier-Smith 1999; McFadden 1999; Cavalier-Smith 2000; Gould et al. 2008). Critics of the chromalveolate hypothesis acknowledge these difficulties but point to the existence of many plastid-lacking "chromalveolate" taxa: *Goniomonas* (a basal cryptomonad), Hacrobia such as katablepharids and telonemids, heterotrophic stramenopiles such as oomycetes, and ciliates, a huge, diverse, and entirely plastid-lacking alveolate lineage (Fig. 2.1). If the chromalveolate hypothesis were true, then plastids would have had to be lost secondarily in each of these lineages. An alternative hypothesis is that red algal-derived secondary plastids have spread by one or more cryptic tertiary endosymbioses, as is known to have occurred in the case of dinoflagellates (Hackett et al. 2004). The chromalveolate hypothesis has proven to be something of a moving target: new eukaryotic lineages continue to be discovered and evolutionary relationships must continuously be retested as genomic sequence data accumulate.

Early single-locus analyses of plastid genes, such as for 16S rDNA and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), were inconsistent with the chromalveolate hypothesis, seeming to favor the notion that the different chromist lineages, i.e., cryptophytes, stramenopiles, and haptophytes, had acquired their plastids from different red algae (Daugbjerg and Andersen 1997; Oliveira and Bhattacharya 2000; Müller et al. 2001). With time and more sequence data, however, the balance tipped in favor of chromist plastid monophyly, albeit with varying levels of confidence (e.g., Yoon et al. 2002; Khan et al. 2007). Rare genomic characters, such as endosymbiotic gene replacements involving the plastid-associated genes for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Fast et al. 2001) and fructose-1,6-bisphosphate (FBA) (Patron et al. 2004), were also presented as evidence to support plastid monophyly of subsets of chromalveolate taxa, and a rare lateral gene transfer involving the ribosomal protein gene *rpL36* suggested a specific relationship between haptophyte and cryptophyte plastids (Rice and Palmer 2006). However, a common origin of chromalveolate plastids is, as argued by Bodyl and others (Bodyl 2005, 2006; Sanchez-Puerta and Delwiche 2008; Bodyl et al. 2009), also consistent with evolutionary scenarios involving

multiple tertiary endosymbioses. The “final frontier” has thus been examination of the phylogenetic signal contained in as many different nuclear loci as possible in an effort to confirm or refute the notion that the histories of the chromalveolate hosts and endosymbionts are congruent with one another.

The answer seems to be that they are not. Phylogenomic analyses of dozens to hundreds of nucleus-encoded proteins concatenated together have revealed that the chromalveolates, as originally defined (Cavalier-Smith 1999), do not form a monophyletic group to the exclusion of other eukaryotes, even though subsets of chromalveolate lineages are clearly related to one another, such as cryptophytes and haptophytes (Hackett et al. 2007; Patron et al. 2007) and stramenopiles and alveolates (Burki et al. 2007; Burki et al. 2008; Hampl et al. 2009; Parfrey et al. 2010). One recent twist has been the realization that the supergroup Rhizaria, to which the green algal secondary plastid-containing chlorarachniophytes belong, is robustly allied with stramenopiles and alveolates. This tripartite grouping has been dubbed SAR (Burki et al. 2007, 2008). The cryptophyte–haptophyte pair does *not* branch with the other chromalveolate groups and has been expanded to include the plastid-lacking telonemids, centrohelids, and katablepharids under the term Hacrobia (Burki et al. 2009; Okamoto et al. 2009). A recent analysis using smaller data sets than the above-mentioned studies but with expanded taxonomic sampling supported some but not all of these relationships, and found no evidence for chromalveolate monophyly (Parfrey et al. 2010). Finally, a rigorous test of the phylogenetic signal contained in the nuclear, mitochondrial, and plastid genomes of the CASH group (cryptophytes, alveolates, stramenopiles, and haptophytes) led Baurain et al. to “...*reject the chromalveolate hypothesis as falsified in favor of more complex evolutionary scenarios involving multiple higher order eukaryote-eukaryote endosymbioses*” (Baurain et al. 2010). The basis for their falsification is that the CASH lineage plastid genomes appear to have diverged from one another much more recently than their respective mitochondrial and nuclear genomes. Various alternative scenarios involving “higher-order” endosymbioses have recently been explored in the literature (e.g., Bodyl 2005, 2006; Sanchez-Puerta and Delwiche 2008; Archibald 2009; Bodyl et al. 2009), but unfortunately there is as yet little information with which to distinguish between them. The original recipient lineage(s) of the red algal secondary plastid is not obvious, nor is the number (and directionality) of subsequent tertiary endosymbioses needed to account for the apparent incongruence between chromalveolate hosts and plastids.

2.4 Genome Mosaicism: Evidence for Past Endosymbioses or “You Are What You Eat”?

One of the most unexpected developments in recent years has been the extent to which the nuclear genomes of secondary plastid-containing algae, and indeed all eukaryotes, harbor genes of mixed ancestry. HGT is now a well-established factor in the evolution of eukaryotic genomes (Keeling and Palmer 2008), but in the case

of phototrophs it is often difficult to tell whether any given “foreign” gene is the product of an endosymbiotic gene transfer or was acquired in a endosymbiosis-independent fashion before, during, or after plastid acquisition. This is not a trivial distinction. Numerous studies invoking secondary plastid loss in eukaryotic groups are based entirely on the presence of algal/cyanobacterial genes in the genome (e.g., Huang et al. 2004; Tyler et al. 2006; Reyes-Prieto et al. 2008). Assessing the significance of genome mosaicism in algae thus has important implications for modeling the pattern and process of plastid evolution.

A 2003 study of the chlorarachniophyte *Bigeloviella natans*, which has a green algal secondary plastid (Table 2.1 and Fig. 2.1), provided some of the first comprehensive evidence for genome mosaicism in eukaryotes. As expected, most of the examined nucleus-encoded, plastid-targeted proteins in this organism were found to be of green algal ancestry, but red algal-derived genes, and even those from bacteria were also found (Archibald et al. 2003). The chlorarachniophytes are known to be capable of ingesting other algae and bacteria (Hibberd and Norris 1984), and the mosaic *B. natans* plastid proteome was deemed to be the product of both endosymbiotic and horizontal gene transfers, the latter related to its phagotrophic lifestyle. A more recent study of the dinoflagellate *Lepidodinium chlorophorum* revealed a similar pattern. This organism currently has a green algal plastid of serial secondary endosymbiotic origin and, not surprisingly, green algal-derived, plastid-associated genes reside in its nucleus (Minge et al. 2010). However, *L. chlorophorum* also harbors genes of red algal secondary endosymbiotic origin and, in this case, it seems reasonable to conclude that at least some of the red algal-type genes are “holdovers” from the ancestral peridinin-type plastid this dinoflagellate is believed to have harbored (Minge et al. 2010). Even though the patterns of plastid-associated gene mosaicism in *B. natans* and *L. chlorophorum* are similar, our interpretation of the underlying causes is different, invoking predominantly HGT in the former and EGT in the latter. Or is it different?

Considering that the supergroup Rhizaria, to which the chlorarachniophytes belong, now appears to be nested within traditional chromalveolate taxa (Burki et al. 2008; Parfrey et al. 2010) (Fig. 2.1), one could argue that at least some of the red algal genes in the *B. natans* genome (Archibald et al. 2003) are derived from ancient endosymbiotic gene transfer rather than HGT. The picture becomes even more complex when one considers a provocative hypothesis put forth by Moustafa et al. (2009). These authors showed that in chromalveolate taxa such as diatoms and haptophytes, genes of apparent green algal ancestry outnumber red algal genes by more than 3-to-1. Preliminary evidence for a green algal “footprint” in chromalveolates possessing a red algal-derived secondary plastid had in fact been observed previously (Frommolt et al. 2008), and Moustafa et al. interpret it as evidence for a cryptic green algal endosymbiont present in an ancient chromalveolate ancestor prior to the hypothesized red algal endosymbiotic event (Moustafa et al. 2009). Under such a model, the composition of the chlorarachniophyte nuclear genome would have conceivably been impacted by no fewer than three secondary endosymbionts at different times (green, red, then green again; Elias and Archibald 2009). Against an increasingly supported backdrop of HGT in

chlorarachniophytes and other eukaryotes (Keeling and Palmer 2008; Takishita et al. 2009), it is not clear how such a hypothesis can be rigorously tested.

A similar challenge exists when probing the nuclear genomes of plastid-lacking eukaryotes for the “footprint” of past endosymbioses. Consistent with the chromalveolate hypothesis, it was proposed that the genome of the stramenopile *Phytophthora* contains hundreds of genes of algal/cyanobacterial ancestry, evidence for a plastid-bearing phase in its history (Tyler et al. 2006). However, reanalysis of the data by Stiller et al. (2009) indicates that the number of algal-like genes in *Phytophthora* does not in fact rise above “background,” i.e., the number of algal genes found in the genomes of amoebozoans (Fig. 2.1), which would *not* be expected to possess an endosymbiotic footprint (Elias and Archibald 2009; Stiller et al. 2009). Similar concerns exist for the putative algal/cyanobacterial footprints in the genomes of ciliates (Archibald 2008; Reyes-Prieto et al. 2008), the apicomplexan *Cryptosporidium* (Huang et al. 2004), and other plastid-lacking eukaryotes (e.g., Maruyama et al. 2008, 2009).

2.5 Future Directions

The amount of genomic data with which to test hypotheses about the origin and evolution of plastids has increased tremendously. However, if the past decade of research in this area has revealed anything, it is that more data does not always lead to increased clarity. Detailed analyses of complete algal nuclear genome sequences have uncovered an unexpected degree of genome mosaicism, and there is as yet no clear consensus as to what it means. Distinguishing between *bona fide* past endosymbioses versus HGT-derived genomic footprints is a formidable challenge that will require a combination of further refinement and implementation of a priori testing procedures of the sort used by Stiller et al. (2009) and even more data from diverse primary and secondary plastid-bearing lineages. Fortunately, continued technological advances in DNA sequencing mean that virtually *any* eukaryote, no matter how large its genome, will become a viable target for whole genome and/or near-complete transcriptome sequencing in the very near future. Particularly important organisms and lineages include (a) the red algae, for which there is still only a single complete genome available (Matsuzaki et al. 2004), (b) the photosynthetic alveolate *Chromera* (Moore et al. 2008), (c) the plastid-lacking cryptomonad *Goniomonas* and other phagotrophs currently classified as Hacrobia (Okamoto et al. 2009), (d) various photosynthetic and nonphotosynthetic dinoflagellates, and (e) plastid-lacking lineages within the stramenopiles (e.g., bicosoecids). With so much new data on the horizon, it is difficult to predict which hypotheses will still be “in play” even a few years from now, especially considering that organisms are being discovered on a regular basis. One recent such example is the “rappemonads,” an as-yet uncultured lineage defined solely on the basis of environmental plastid rDNA operon sequencing and fluorescence in situ hybridization (Kim et al. 2010). Rappemonads are most closely related to, but are clearly distinct from, the

haptophytes, and constitute a genetically diverse lineage found in both marine and freshwater environments. It is sobering to consider that organisms that represent potentially important pieces of the endosymbiosis puzzle have escaped detection for decades.

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References

- Andersson JO, Roger AJ (2002) A cyanobacterial gene in nonphotosynthetic protists – an early chloroplast acquisition in eukaryotes? *Curr Biol* 12:115–119
- Archibald JM (2005) Jumping genes and shrinking genomes – probing the evolution of eukaryotic photosynthesis using genomics. *IUBMB Life* 57:539–547
- Archibald JM (2006) Algal genomics: examining the imprint of endosymbiosis. *Curr Biol* 16:R1033–R1035
- Archibald JM (2008) Plastid evolution: remnant algal genes in ciliates. *Curr Biol* 18:R663–R665
- Archibald JM (2009) The puzzle of plastid evolution. *Curr Biol* 19:R81–R88
- Archibald JM, Lane CE (2009) Going, going, not quite gone: nucleomorphs as a case study in nuclear genome reduction. *J Hered* 100:582–590
- Archibald JM, Rogers MB, Toop M, Ishida K, Keeling PJ (2003) Lateral gene transfer and the evolution of plastid-targeted proteins in the secondary plastid-containing alga *Bigeloviella natans*. *Proc Natl Acad Sci U S A* 100:7678–7683
- Baurain D, Brinkmann H, Petersen J, Rodriguez-Ezpeleta N, Stechmann A, Demoulin V, Roger AJ, Burger G, Lang BF, Philippe H (2010) Phylogenomic evidence for separate acquisition of plastids in cryptophytes, haptophytes, and stramenopiles. *Mol Biol Evol* 27:1698–1709
- Bhattacharya D, Archibald JM (2007) Response to Theissen and Martin: “the difference between endosymbionts and organelles”. *Curr Biol* 16:R1017–R1018
- Blanchard JL, Hicks JS (1999) The non-photosynthetic plastid in malarial parasites and other apicomplexans is derived from outside the green plastid lineage. *J Eukaryot Microbiol* 46:367–375
- Blankenship RE (1994) Protein structure, electron transfer and evolution of prokaryotic photosynthetic reaction centers. *Antonie van Leeuwenhoek* 65:311–329
- Bodl A (2005) Do plastid-related characters support the chromalveolate hypothesis? *J Phycol* 41:712–719
- Bodl A (2006) Did the peridinin plastid evolve through tertiary endosymbiosis? A hypothesis. *Eur J Phycol* 41:435–448
- Bodl A, Mackiewicz P, Stiller JW (2007) The intracellular cyanobacteria of *Paulinella chromatophora*: endosymbionts or organelles? *Trends Microbiol* 15:295–296
- Bodl A, Stiller JW, Mackiewicz P (2009) Chromalveolate plastids: direct descent or multiple endosymbioses? *Trends Ecol Evol* 24:119–121
- Bodl A, Mackiewicz P, Stiller JW (2010) Comparative genomic studies suggest that the cyanobacterial endosymbionts of the amoeba *Paulinella chromatophora* possess an import apparatus for nuclear-encoded proteins. *Plant Biol (Stuttg)* 12:639–649

- Burki F, Shalchian-Tabrizi K, Minge M, Skjaeveland Å, Nikolaev SI, Jakobsen KS, Pawlowski J (2007) Phylogenomics reshuffles the eukaryotic supergroups. *PLoS One* 8:e790
- Burki F, Shalchian-Tabrizi K, Pawlowski J (2008) Phylogenomics reveals a new 'megagroup' including most photosynthetic eukaryotes. *Biol Lett* 4:366–369
- Burki F, Inagaki Y, Brate J, Archibald JM, Keeling PJ, Cavalier-Smith T, Sakaguchi M, Hashimoto T, Horak A, Kuma K, Klaveness D, Jakobsen KS, Pawlowski J, Shalchian-Tabrizi K (2009) Large-scale phylogenomic analyses reveal that two enigmatic protist lineages, telonemia and centrohelioczoa, are related to photosynthetic chromalveolates. *Genome Biol Evol* 1:231–238
- Cavalier-Smith T (1999) Principles of protein and lipid targeting in secondary symbiogenesis: euglenoid, dinoflagellate, and sporozoan plastid origins and the eukaryote family tree. *J Eukaryot Microbiol* 46:347–366
- Cavalier-Smith T (2000) Membrane heredity and early chloroplast evolution. *Trends Plant Sci* 5:174–182
- Chesnick JM, Hooistra WH, Wellbrock U, Medlin LK (1997) Ribosomal RNA analysis indicates a benthic pennate diatom ancestry for the endosymbionts of the dinoflagellates *Peridinium foliaceum* and *Peridinium balticum* (Pyrrhophyta). *J Eukaryot Microbiol* 44:314–320
- Daugbjerg N, Andersen RA (1997) Phylogenetic analyses of the *rbcL* sequences from haptophytes and heterokont algae suggest their chloroplasts are unrelated. *Mol Biol Evol* 14:1242–1251
- Delwiche CF, Kuhsel M, Palmer JD (1995) Phylogenetic analysis of *tufA* sequences indicates a cyanobacterial origin of all plastids. *Mol Phylogenet Evol* 4:110–128
- Delwiche C, Andersen RA, Bhattacharya D, Mishler BD (2004) Algal evolution and the early radiation of green plants. In: Cracraft J, Donoghue MJ (eds) *Assembling the tree of life*. Oxford University Press, New York, pp 121–137
- Dodge JD (1969) Observations on the fine structure of the eyespot and associated organelles in the dinoflagellate *Glenodinium foliaceum*. *J Cell Sci* 5:479–493
- Douglas SE, Penny SL (1999) The plastid genome of the cryptophyte alga, *Guillardia theta*: complete sequence and conserved syntenic groups confirm its common ancestry with red algae. *J Mol Evol* 48:236–244
- Douglas SE, Murphy CA, Spencer DF, Gray MW (1991) Cryptomonad algae are evolutionary chimaeras of two phylogenetically distinct unicellular eukaryotes. *Nature* 350:148–151
- Douglas SE, Zauner S, Fraunholz M, Beaton M, Penny S, Deng L, Wu X, Reith M, Cavalier-Smith T, Maier U-G (2001) The highly reduced genome of an enslaved algal nucleus. *Nature* 410:1091–1096
- Durnford DG, Deane JA, Tan S, McFadden GI, Gantt E, Green BR (1999) A phylogenetic assessment of the eukaryotic light-harvesting antenna proteins, with implications for plastid evolution. *J Mol Evol* 48:59–68
- Elias M, Archibald JM (2009) Sizing up the genomic footprint of endosymbiosis. *Bioessays* 31:1273–1279
- Fast NM, Kissinger JC, Roos DS, Keeling PJ (2001) Nuclear-encoded, plastid-targeted genes suggest a single common origin for apicomplexan and dinoflagellate plastids. *Mol Biol Evol* 18:418–426
- Frommolt R, Werner S, Paulsen H, Goss R, Wilhelm C, Zauner S, Maier UG, Grossman AR, Bhattacharya D, Lohr M (2008) Ancient recruitment by chromists of green algal genes encoding enzymes for carotenoid biosynthesis. *Mol Biol Evol* 25:2653–2667
- Funes S, Davidson E, Reyes-Prieto A, Magallón S, Herion P, King MP, Gonzalez-Halphen D (2002) A green algal apicoplast ancestor. *Science* 298:2155
- Gibbs SP (1978) The chloroplasts of *Euglena* may have evolved from symbiotic green algae. *Can J Bot* 56:2883–2889
- Gilson PR, McFadden GI (1996) The miniaturized nuclear genome of a eukaryotic endosymbiont contains genes that overlap, genes that are cotranscribed, and the smallest known spliceosomal introns. *Proc Natl Acad Sci U S A* 93:7737–7742

- Gilson PR, Su V, Slamovits CH, Reith ME, Keeling PJ, McFadden GI (2006) Complete nucleotide sequence of the chlorarachniophyte nucleomorph: nature's smallest nucleus. *Proc Natl Acad Sci U S A* 103:9566–9571
- Glockner G, Rosenthal A, Valentin K (2000) The structure and gene repertoire of an ancient red algal plastid genome. *J Mol Evol* 51:382–390
- Gould SB, Waller RF, McFadden GI (2008) Plastid evolution. *Annu Rev Plant Biol* 59:491–517
- Graham LE, Wilcox LW (2000) *Algae*. Prentice-Hall, Upper Saddle River, NJ
- Green BR, Durnford DG (1996) The chlorophyll-carotenoid proteins of oxygenic photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol* 47:685–714
- Greenwood AD (1974) The Cryptophyta in relation to phylogeny and photosynthesis. In: Sanders JV, Goodchild DJ (eds) *Proceedings of the eighth international congress on electron microscopy*, vol 2, Canberra, Australia, pp 566–567
- Greenwood AD, Griffiths HB, Santore UJ (1977) Chloroplasts and cell compartments in Cryptophyceae. *Br Phycol J* 12:119
- Hackett JD, Maranda L, Yoon HS, Bhattacharya D (2003) Phylogenetic evidence for the cryptophyte origin of the plastid of Dinophysis (Dinophysiales, Dinophyceae). *J Phycol* 39:440–448
- Hackett JD, Anderson DM, Erdner DL, Bhattacharya D (2004) Dinoflagellates: a remarkable evolutionary experiment. *Am J Bot* 91:1523–1534
- Hackett JD, Yoon HS, Li S, Reyes-Prieto A, Rummele SE, Bhattacharya D (2007) Phylogenomic analysis supports the monophyly of cryptophytes and haptophytes and the association of rhizaria with chromalveolates. *Mol Biol Evol* 24:1702–1713
- Hampl V, Hug L, Leigh JW, Dacks JB, Lang BF, Simpson AG, Roger AJ (2009) Phylogenomic analyses support the monophyly of Excavata and resolve relationships among eukaryotic “supergroups”. *Proc Natl Acad Sci U S A* 106:3859–3864
- Helmchen TA, Bhattacharya D, Melkonian M (1995) Analyses of ribosomal RNA sequences from glaucocystophyte cyanelles provide new insights into the evolutionary relationships of plastids. *J Mol Evol* 41:203–210
- Hibberd DJ, Norris RE (1984) Cytology and ultrastructure of *Chlorarachnion reptans* (Chlorarachniophyta divisio nova, Chlorarachniophyceae classis nova). *J Phycol* 20:310–330
- Howe CJ, Barbrook AC, Nisbet RE, Lockhart PJ, Larkum AW (2008) The origin of plastids. *Philos Trans R Soc Lond B Biol Sci* 363:2675–2685
- Huang J, Mullapudi N, Lancot CA, Scott M, Abrahamsen MS, Kissinger JC (2004) Phylogenomic evidence supports past endosymbiosis, intracellular and horizontal gene transfer in *Cryptosporidium parvum*. *Genome Biol* 5:R88
- Imanian B, Keeling PJ (2007) The dinoflagellates *Durinskia baltica* and *Kryptoperidinium foliaceum* retain functionally overlapping mitochondria from two evolutionarily distinct lineages. *BMC Evol Biol* 7:172
- Imanian B, Carpenter KJ, Keeling PJ (2007) Mitochondrial genome of a tertiary endosymbiont retains genes for electron transport proteins. *J Eukaryot Microbiol* 54:146–153
- Inagaki Y, Dacks JB, Doolittle WF, Watanabe KI, Ohama T (2000) Evolutionary relationship between dinoflagellates bearing obligate diatom endosymbionts: insight into tertiary endosymbiosis. *Int J Syst Evol Microbiol* 50(Pt 6):2075–2081
- Ishida K, Cao Y, Hasegawa M, Okada N, Hara Y (1997) The origin of chlorarachniophyte plastids, as inferred from phylogenetic comparisons of amino acid sequences of EF-Tu. *J Mol Evol* 45:682–687
- Ishida K, Green BR, Cavalier-Smith T (1999) Diversification of a chimaeric algal group, the chlorarachniophytes: phylogeny of nuclear and nucleomorph small-subunit rRNA genes. *Mol Biol Evol* 16:321–331
- Janouskovec J, Horak A, Obornik M, Lukes J, Keeling PJ (2010) A common red algal origin of the apicomplexan, dinoflagellate, and heterokont plastids. *Proc Natl Acad Sci USA* 107:10949–10954
- Jarvis P, Soll J (2001) Toc, Tic, and chloroplast protein import. *Biochim Biophys Acta* 1541:64–79
- Keeling PJ (2008) Evolutionary biology: bridge over troublesome plastids. *Nature* 451:896–897

- Keeling PJ (2009) Chromalveolates and the evolution of plastids by secondary endosymbiosis. *J Eukaryot Microbiol* 56:1–8
- Keeling PJ, Palmer JD (2008) Horizontal gene transfer in eukaryotic evolution. *Nat Rev Genet* 9:605–618
- Khan H, Parks N, Kozera C, Curtis BA, Parsons BJ, Bowman S, Archibald JM (2007) Plastid genome sequence of the cryptophyte alga *Rhodomonas salina* CCMP1319: lateral transfer of putative DNA replication machinery and a test of chromist plastid phylogeny. *Mol Biol Evol* 24:1832–1842
- Kim E, Archibald JM (2009) Diversity and evolution of plastids and their genomes. In: Aronsson H, Sandelius AS (eds) *The chloroplast-interactions with the environment*. Springer, Berlin, pp 1–39
- Kim E, Graham LE (2008) EEF2 analysis challenges the monophyly of Archaeplastida and Chromalveolata. *PLoS One* 3:e2621
- Kim E, Harrison J, Sudek S, Jones MDM, Wilcox HM, Richards TA, Worden AZ, Archibald JM (2010) A new and diverse plastid-bearing branch on the eukaryotic tree of life. *Proc Natl Acad Sci U S A* 108:1496–1500
- Köhler S, Delwiche CF, Denny PW, Tilney LG, Webster P, Wilson RJM, Palmer JD, Roos DS (1997) A plastid of probable green algal origin in apicomplexan parasites. *Science* 275:1485–1489
- Lane CE, Archibald JM (2008) The eukaryotic tree of life: endosymbiosis takes its TOL. *Trends Ecol Evol* 23:268–275
- Lane CE, van den Heuvel K, Kozera C, Curtis BA, Parsons B, Bowman S, Archibald JM (2007) Nucleomorph genome of *Hemismelis andersenii* reveals complete intron loss and compaction as a driver of protein structure and function. *Proc Natl Acad Sci U S A* 104:19908–19913
- Larkum AW, Lockhart PJ, Howe CJ (2007) Shopping for plastids. *Trends Plant Sci* 12:189–195
- Lauterborn R (1895) Protozoenstudien II. *Paulinella chromatophora* nov. gen., nov. spec., ein beschalter Rhizopode des Subwassers mit blaugrunen chromatophorenartigen Einschlüssen. *Z Wiss Zool* 59:537–544
- Leander BS, Triemer RE, Farmer MA (2001) Character evolution in heterotrophic euglenids. *Eur J Protistol* 37:337–356
- Lim L, McFadden GI (2010) The evolution, metabolism and functions of the apicoplast. *Philos Trans R Soc Lond B Biol Sci* 365:749–763
- Longet D, Archibald JM, Keeling PJ, Pawlowski J (2003) Foraminifera and Cercozoa share a common origin according to RNA polymerase II hylogenies. *Int J Syst Evol Microbiol* 53:1735–1739
- Mackiewicz P, Bodyl A (2010) A hypothesis for import of the nuclear-encoded Psae protein of *Paulinella chromatophora* (Cercozoa, Rhizaria) into its cyanobacterial endosymbionts/plastids via the endomembrane system. *J Phycol* 46:847–859
- Marin B, Nowack ECM, Melkonian M (2005) A plastid in the making: evidence for a second primary endosymbiosis. *Protist* 156:425–432
- Martin W, Stoebe B, Goremykin V, Hansmann S, Hasegawa M, Kowallik KV (1998) Gene transfer to the nucleus and the evolution of chloroplasts. *Nature* 393:162–165
- Martin W, Rujan T, Richly E, Hansen A, Cornelsen S, Lins T, Leister D, Stoebe B, Hasegawa M, Penny D (2002) Evolutionary analysis of *Arabidopsis*, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. *Proc Natl Acad Sci U S A* 99:12246–12251
- Maruyama S, Misawa K, Iseki M, Watanabe M, Nozaki H (2008) Origins of a cyanobacterial 6-phosphogluconate dehydrogenase in plastid-lacking eukaryotes. *BMC Evol Biol* 8:151
- Maruyama S, Matsuzaki M, Misawa K, Nozaki H (2009) Cyanobacterial contribution to the genomes of the plastid-lacking protists. *BMC Evol Biol* 9:197
- Matsuzaki M, Misumi O, Shin IT, Maruyama S, Takahara M, Miyagishima SY, Mori T, Nishida K, Yagisawa F, Nishida K, Yoshida Y, Nishimura Y, Nakao S, Kobayashi T, Momoyama Y, Higashiyama T, Minoda A, Sano M, Nomoto H, Oishi K, Hayashi H, Ohta

- F, Nishizaka S, Haga S, Miura S, Morishita T, Kabeya Y, Terasawa K, Suzuki Y, Ishii Y, Asakawa S, Takano H, Ohta N, Kuroiwa H, Tanaka K, Shimizu N, Sugano S, Sato N, Nozaki H, Ogasawara N, Kohara Y, Kuroiwa T (2004) Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. *Nature* 428:653–657
- McEwan ML, Keeling PJ (2004) HSP90, tubulin and actin are retained in the tertiary endosymbiont genome of *Kryptoperidinium foliaceum*. *J Eukaryot Microbiol* 51:651–659
- McFadden GI (1999) Plastids and protein targeting. *J Eukaryot Microbiol* 46:339–346
- McFadden GI, van Dooren GG (2004) Evolution: red algal genome affirms a common origin of all plastids. *Curr Biol* 14:R514–R516
- McFadden GI, Gilson PR, Hofmann CJ, Adcock GJ, Maier UG (1994) Evidence that an amoeba acquired a chloroplast by retaining part of an engulfed eukaryotic alga. *Proc Natl Acad Sci USA* 91:3690–3694
- Melkonian M, Mollenhauer D (2005) Robert Lauterborn (1869–1952) and his *Paulinella chromatophora*. *Protist* 156:253–262
- Mereschkowsky C (1905) Über Natur und Ursprung der Chromatophoren im Pflanzenreiche. *Biol Centralbl* 25:593–604
- Minge MA, Shalchian-Tabrizi K, Torresen OK, Takishita K, Probert I, Inagaki Y, Klaveness D, Jakobsen KS (2010) A phylogenetic mosaic plastid proteome and unusual plastid-targeting signals in the green-colored dinoflagellate *Lepidodinium chlorophorum*. *BMC Evol Biol* 10:191
- Moore CE, Archibald JM (2009) Nucleomorph genomes. *Annu Rev Genet* 43:251–264
- Moore RB, Obornik M, Janouskovec J, Chrudimsky T, Vancova M, Green DH, Wright SW, Davies NW, Bolch CJ, Heimann K, Slapeta J, Hoegh-Guldberg O, Logsdon JM, Carter DA (2008) A photosynthetic alveolate closely related to apicomplexan parasites. *Nature* 452:900
- Moustafa A, Reyes-Prieto A, Bhattacharya D (2008) Chlamydiae has contributed at least 55 genes to *Plantae* with predominantly plastid functions. *PLoS One* 3:e2205
- Moustafa A, Beszteri B, Maier UG, Bowler C, Valentin K, Bhattacharya D (2009) Genomic footprints of a cryptic plastid endosymbiosis in diatoms. *Science* 324:1724–1726
- Müller KM, Oliveira MC, Sheath RG, Bhattacharya D (2001) Ribosomal DNA phylogeny of the Bangiophycidae (Rhodophyta) and the origin of secondary plastids. *Am J Bot* 88:1390–1400
- Nakayama T, Ishida K (2009) Another acquisition of a primary photosynthetic organelle is underway in *Paulinella chromatophora*. *Curr Biol* 19:R284–R285
- Nikolaev SI, Berney C, Fahmi JF, Bolivar I, Polet S, Mylnikov AP, Aleshin VV, Petrov NB, Pawlowski J (2004) The twilight of Heliozoa and rise of Rhizaria, an emerging supergroup of amoeboid eukaryotes. *Proc Natl Acad Sci U S A* 101:8066–8071
- Nowack ECM, Melkonian M, Glöckner G (2008) Chromatophore genome sequence of *Paulinella* sheds light on acquisition of photosynthesis by eukaryotes. *Curr Biol* 18:410–418
- Nowack EC, Vogel H, Groth M, Grossman AR, Melkonian M, Glöckner G (2011) Endosymbiotic gene transfer and transcriptional regulation of transferred genes in *Paulinella chromatophora*. *Mol Biol Evol* 28:407–422
- Nozaki H, Matsuzaki M, Takahara M, Misumi O, Kuroiwa H, Hasegawa M, Shin-i T, Kohara Y, Ogasawara N, Kuroiwa T (2003) The phylogenetic position of red algae revealed by multiple nuclear genes from mitochondria-containing eukaryotes and an alternative hypothesis on the origin of plastids. *J Mol Evol* 56:485–497
- Nozaki H, Takano H, Misumi O, Terasawa K, Matsuzaki M, Maruyama S, Nishida K, Yagisawa F, Yoshida Y, Fujiwara T, Takio S, Tamura K, Chung SJ, Nakamura S, Kuroiwa H, Tanaka K, Sato N, Kuroiwa T (2007) A 100%-complete sequence reveals unusually simple genomic features in the hot-spring red alga *Cyanidioschyzon merolae*. *BMC Biol* 5:28
- Okamoto N, Chantangsri C, Horak A, Leander BS, Keeling PJ (2009) Molecular phylogeny and description of the novel katablepharid *Roombia truncata* gen. et sp. nov., and establishment of the Hacrobia taxon nov. *PLoS One* 4(9):e7080
- Oliveira MC, Bhattacharya D (2000) Phylogeny of the Bangiophycidae (Rhodophyta) and the secondary endosymbiotic origin of algal plastids. *Am J Bot* 87:482–492

- Palmer JD (2003) The symbiotic birth and spread of plastids: how many times and whodunnit? *J Phycol* 39:4–11
- Parfrey LW, Grant J, Tekle YI, Lasek-Nesselquist E, Morrison HG, Sogin ML, Patterson DJ, Katz LA (2010) Broadly sampled multigene analyses yield a well-resolved eukaryotic tree of life. *Syst Biol* 59:518–533
- Park MG, Park JS, Kim M, Yih W (2008) Plastid dynamics during survival of *Dinophysis caudata* without its ciliate prey. *J Phycol* 44:1154–1163
- Patron NJ, Rogers MB, Keeling PJ (2004) Gene replacement of fructose-1,6-bisphosphate aldolase supports the hypothesis of a single photosynthetic ancestor of chromalveolates. *Eukaryot Cell* 3:1169–1175
- Patron NJ, Inagaki Y, Keeling PJ (2007) Multiple gene phylogenies support the monophyly of cryptomonad and haptophyte host lineages. *Curr Biol* 17:887–891
- Ralph SA, van Dooren GG, Waller RF, Crawford MJ, Fraunholz MJ, Foth BJ, Tonkin CJ, Roos DS, McFadden GI (2004) Tropical infectious diseases: metabolic maps and functions of the *Plasmodium falciparum* apicoplast. *Nat Rev Microbiol* 2:203–216
- Reyes-Prieto A, Hackett JD, Soares MB, Bonaldo MF, Bhattacharya D (2006) Cyanobacterial contribution to algal nuclear genomes is primarily limited to plastid functions. *Curr Biol* 16:2320–2325
- Reyes-Prieto A, Weber AP, Bhattacharya D (2007) The origin and establishment of the plastid in algae and plants. *Annu Rev Genet* 41:147–168
- Reyes-Prieto A, Moustafa A, Bhattacharya D (2008) Multiple genes of apparent algal origin suggest ciliates may once have been photosynthetic. *Curr Biol* 18:956–962
- Rice DW, Palmer JD (2006) An exceptional horizontal gene transfer in plastids: gene replacement by a distant bacterial paralog and evidence that haptophyte and cryptophyte plastids are sisters. *BMC Biol* 4:31
- Rodriguez-Ezpeleta N, Brinkmann H, Burey SC, Roure B, Burger G, Löffelhardt W, Bohnert HJ, Philippe H, Lang BF (2005) Monophyly of primary photosynthetic eukaryotes: green plants, red algae, and glaucophytes. *Curr Biol* 15:1325–1330
- Rodriguez-Ezpeleta N, Brinkmann H, Burger G, Roger AJ, Gray MW, Philippe H, Lang BF (2007a) Toward resolving the eukaryotic tree: the phylogenetic positions of jakobids and cercozoans. *Curr Biol* 17:1420–1425
- Rodriguez-Ezpeleta N, Brinkmann H, Roure B, Lartillot N, Lang BF, Philippe H (2007b) Detecting and overcoming systematic errors in genome-scale phylogenies. *Syst Biol* 56:389–399
- Rogers MB, Gilson PR, Su V, McFadden GI, Keeling PJ (2007) The complete chloroplast genome of the chlorarachniophyte *Bigelowiella natans*: evidence for independent origins of chlorarachniophyte and euglenid secondary endosymbionts. *Mol Biol Evol* 24:54–62
- Sanchez-Puerta MV, Delwiche CF (2008) A hypothesis for plastid evolution in chromalveolates. *J Phycol* 44:1097–1107
- Sanchez-Puerta MV, Bachvaroff TR, Delwiche CF (2007a) Sorting wheat from chaff in multi-gene analyses of chlorophyll c-containing plastids. *Mol Phylogenet Evol* 44:885–897
- Sanchez-Puerta MV, Lippmeier JC, Apt KE, Delwiche CF (2007b) Plastid genes in a non-photosynthetic dinoflagellate. *Protist* 158:105–117
- Schnepf E, Elbrächter M (1988) Cryptophycean-like double membrane-bound plastid chloroplast in the dinoflagellate, *Dinophysis* Ehrenb.: evolutionary, phylogenetic and toxicological implications. *Bot Acta* 101:196–203
- Slamovits CH, Keeling PJ (2008) Plastid-derived genes in the non-photosynthetic alveolate *Oxyrrhis marina*. *Mol Biol Evol* 25:1297–1306
- Stelter K, El-Sayed NM, Seeber F (2007) The expression of a plant-type ferredoxin redox system provides molecular evidence for a plastid in the early dinoflagellate *Perkinsus marinus*. *Protist* 158:119–130
- Stiller JW (2007) Plastid endosymbiosis, genome evolution and the origin of green plants. *Trends Plant Sci* 12:391–396

- Stiller JW, Hall BD (1997) The origin of red algae: implications for plastid evolution. *Proc Natl Acad Sci U S A* 94:4520–4525
- Stiller JW, Reel DC, Johnson JC (2003) A single origin of plastids revisited: convergent evolution in organellar genome content. *J Phycol* 39:95–105
- Stiller JW, Huang J, Ding Q, Tian J, Goodwillie C (2009) Are algal genes in nonphotosynthetic protists evidence of historical plastid endosymbioses? *BMC Genomics* 10:484
- Stoebe B, Kowallik KV (1999) Gene-cluster analysis in chloroplast genomics. *Trends Genet* 15:344–347
- Suzuki K, Miyagishima SY (2010) Eukaryotic and eubacterial contributions to the establishment of plastid proteome estimated by large-scale phylogenetic analyses. *Mol Biol Evol* 27:581–590
- Takishita K, Yamaguchi H, Maruyama T, Inagaki Y (2009) A hypothesis for the evolution of nuclear-encoded, plastid-targeted glyceraldehyde-3-phosphate dehydrogenase genes in "chromalveolate" members. *PLoS One* 4:e4737
- Taylor FJR (1974) Implications and extensions of the serial endosymbiosis theory of the origin of eukaryotes. *Taxon* 23:229–258
- Taylor FJR (1980) On dinoflagellate evolution. *Biosystems* 13:65–108
- Teles-Grilo ML, Tato-Costa J, Duarte SM, Maia A, Casal G, Azevedo C (2007) Is there a plastid in *Perkinsus atlanticus* (Phylum Perkinsozoa)? *Eur J Protistol* 43:163–167
- Tengs T, Dahlberg OJ, Shalchian-Tabrizi K, Klaveness D, Rudi K, Delwiche CF, Jakobsen KS (2000) Phylogenetic analyses indicate that the 19'hexanoyloxy-fucoxanthin-containing dinoflagellates have tertiary plastids of haptophyte origin. *Mol Biol Evol* 17:718–729
- Theissen U, Martin W (2006) The difference between organelles and endosymbionts. *Curr Biol* 16:R1016–R1017; author reply R1017–R1018
- Turmel M, Gagnon MC, O'Kelly CJ, Otis C, Lemieux C (2009) The chloroplast genomes of the green algae *Pyramimonas*, *Monomastix*, and *Pycnococcus* shed new light on the evolutionary history of prasinophytes and the origin of the secondary chloroplasts of euglenids. *Mol Biol Evol* 26:631–648
- Tyler BM, Tripathy S, Zhang X, Dehal P, Jiang RH, Aerts A, Arredondo FD, Baxter L, Bensasson D, Beynon JL, Chapman J, Damasceno CM, Dorrance AE, Dou D, Dickerman AW, Dubchak IL, Garbelotto M, Gijzen M, Gordon SG, Govers F, Grunwald NJ, Huang W, Ivors KL, Jones RW, Kamoun S, Krampis K, Lamour KH, Lee MK, McDonald WH, Medina M, Meijer HJ, Nordberg EK, Maclean Y, Ospina-Giraldo MD, Morris PF, Phuntumart V, Putnam NH, Rash S, Rose JK, Sakihama Y, Salamov AA, Savidor A, Scheuring CF, Smith BM, Sobral BW, Terry A, Torto-Alalibo TA, Win J, Xu Z, Zhang H, Grigoriev IV, Rokhsar DS, Boore JL (2006) *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Science* 313:1261–1266
- Waller RF, McFadden GI (2005) The apicoplast: a review of the derived plastid of apicomplexan parasites. *Curr Issues Mol Biol* 7:57–79
- Waller RF, Keeling PJ, van Dooren GG, McFadden GI (2003) Comment on "A green algal apicoplast ancestor". *Science* 301:49a
- Watanabe MM, Suda S, Inouye I, Sawaguchi I, Chihara M (1990) *Lepidodinium viride* gen et sp. nov. (Gymnodiniales, Dinophyta), a green dinoflagellate with a chlorophyll *a*- and *b*-containing endosymbiont. *J Phycol* 26:741–751
- Wisecaver JH, Hackett JD (2010) Transcriptome analysis reveals nuclear-encoded proteins for the maintenance of temporary plastids in the dinoflagellate *Dinophysis acuminata*. *BMC Genomics* 11:366
- Yoon HS, Hackett JD, Pinto G, Bhattacharya D (2002) The single, ancient origin of chromist plastids. *Proc Natl Acad Sci U S A* 99:15507–15512
- Yoon HS, Hackett JD, Ciniglia C, Pinto G, Bhattacharya D (2004) A molecular timeline for the origin of photosynthetic eukaryotes. *Mol Biol Evol* 21:809–818
- Yoon HS, Reyes-Prieto A, Melkonian M, Bhattacharya D (2006) Minimal plastid genome evolution in the *Paulinella* endosymbiont. *Curr Biol* 16:R670–R672

- Yoon HS, Nakayama T, Reyes-Prieto A, Andersen RA, Boo SM, Ishida K, Bhattacharya D (2009) A single origin of the photosynthetic organelle in different *Paulinella* lineages. BMC Evol Biol 9:98
- Zhang Z, Green BR, Cavalier-Smith T (1999) Single gene circles in dinoflagellate chloroplast genomes. Nature 400:155–159
- Zhang Z, Green BR, Cavalier-Smith T (2000) Phylogeny of ultra-rapidly evolving dinoflagellate chloroplast genes: a possible common origin for sporozoan and dinoflagellate plastids. J Mol Evol 51:26–40

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