

The Puzzle of Plastid Evolution

Review

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A comprehensive understanding of the origin and spread of plastids remains an important yet elusive goal in the field of eukaryotic evolution. Combined with the discovery of new photosynthetic and non-photosynthetic protist lineages, the results of recent taxonomically broad phylogenomic studies suggest that a re-shuffling of higher-level eukaryote systematics is in order. Consequently, new models of plastid evolution involving ancient secondary and tertiary endosymbioses are needed to explain the full spectrum of photosynthetic eukaryotes.

Introduction

Our planet is teeming with photosynthetic life. The textbook version of how this came to be is relatively straightforward: oxygenic photosynthesis first evolved in the ancestors of modern cyanobacteria more than two billion years ago [1] and their light-harvesting capabilities were subsequently exploited by eukaryotic (nucleus-containing) cells through the process of endosymbiosis [2,3]. Co-evolving with their opportunistic hosts, these intracellular cyanobacteria were eventually transformed into *bona fide* organelles — plastids — ultimately giving rise to the plants and algae that surround us today. Easy, right?

The basic outline of this evolutionary scenario is correct, but the reality is much, much more complicated. Photosynthetic eukaryotes are astonishingly diverse in form and function, a fact that complicates efforts to discern their evolutionary history. Eukaryotic phototrophs can be macroscopic (e.g., land plants, seaweed) or microscopic (e.g., the unicellular green alga *Chlamydomonas*), sessile or motile (or both), and given a bit of sunlight, they thrive in virtually any habitat imaginable, terrestrial and aquatic, from the equator to the poles. This vast diversity actually makes sense when one considers that the term ‘algae’ can be applied to organisms that are not specifically related to one another. In addition to simple vertical inheritance, plastids have on multiple occasions spread laterally between distantly related groups of eukaryotes. Having evolved ~one billion years in the past [4], today’s plastids weave a tangled web across a very large fraction of the eukaryotic tree. Consequently, large sections of the puzzle of plastid evolution remain unassembled.

This article focuses on the latest advances in our understanding of the origin and spread of plastids. In particular, the merits and shortcomings of competing hypotheses about the evolution of plastids are discussed in light of a flood of new molecular, biochemical, genomic and phylogenomic data. Progress has been swift, but there are still many questions that need to be answered, and many newly discovered protist lineages that need to be investigated, before it can be

said that the evolution of eukaryotic photosynthesis is understood with confidence.

Primary Plastids

Unlike the origin of mitochondria, the details of which are still debated [5], there is no longer any doubt that plastids are derived from once free-living cyanobacteria and that the host cell was a full-blown eukaryote with a nucleus, cytoskeleton and mitochondrion. The so-called ‘primary’ endosymbiotic origin of plastids (Figure 1A) can be considered the ‘launch point’ for eukaryotic photosynthesis in the sense that all canonical plastids appear to be derived from this pivotal event, either directly or indirectly. Primary plastids are characterized by the presence of two membranes, both of which are cyanobacterial in nature [2], and are found in red algae, glaucophyte algae and green algae, the latter group being the unicellular lineage that gave rise to land plants. This tripartite assemblage is referred to as the *Plantae* or *Archaeplastida* [6] (Figure 2).

The evidence for and against the singular origin of primary plastids has been reviewed extensively elsewhere (e.g., [3,7,8] and references therein). For the purposes of this review, I will simply note that many researchers are reasonably convinced that primary plastids evolved only once, in the common ancestor of green, red and glaucophyte algae (Figure 2; see refs [3,7] for review), recognizing that the technical and conceptual challenges associated with inferring such ancient evolutionary events are considerable [8]. Indeed, there is much debate as to whether red, green and glaucophyte algae really are each other’s closest relatives to the exclusion of all other eukaryotes (e.g., [9–13]) and if they are not, how the evolution of their plastids should be interpreted [7,12,14].

What can be said with confidence is that intracellular (or endosymbiotic) gene transfer (EGT) was a major factor in the integration of the cyanobacterial progenitor of the plastid and its eukaryotic host [15,16]. While plastid genomes rarely encode more than ~200 proteins, a thousand or more nucleus-encoded proteins — many but not all of which are demonstrably cyanobacterial in origin — are needed to service a fully functional plastid. The bulk of these proteins are translated on cytoplasmic ribosomes and targeted to the plastid post-translationally by a dedicated protein import apparatus [2,17] whose evolution has been touted as the defining feature of an endosymbiont-turned-organelle [18,19]. The nuclear genomes of primary-plastid-bearing eukaryotes thus possess hundreds of endosymbiont-derived genes, many encoding plastid-targeted proteins as well as others that have evolved non-plastid, host-associated functions [15,16,20]. This endosymbiotic ‘footprint’ becomes significant when one considers the pervasiveness of plastid loss in eukaryotic evolution, and whether plastid-/algal-derived genes in the nuclear genomes of non-photosynthetic eukaryotes are reliable indicators of a photosynthetic ancestry (below).

As ancient as the primary endosymbiotic origin of plastids was, it is worth noting that several instances of ‘recent’ cyanobacterium–eukaryote endosymbioses are known, for example, in the testate amoeba *Paulinella chromatophora* [21] and the diatom *Rhopalodia gibba* [22]. Such examples

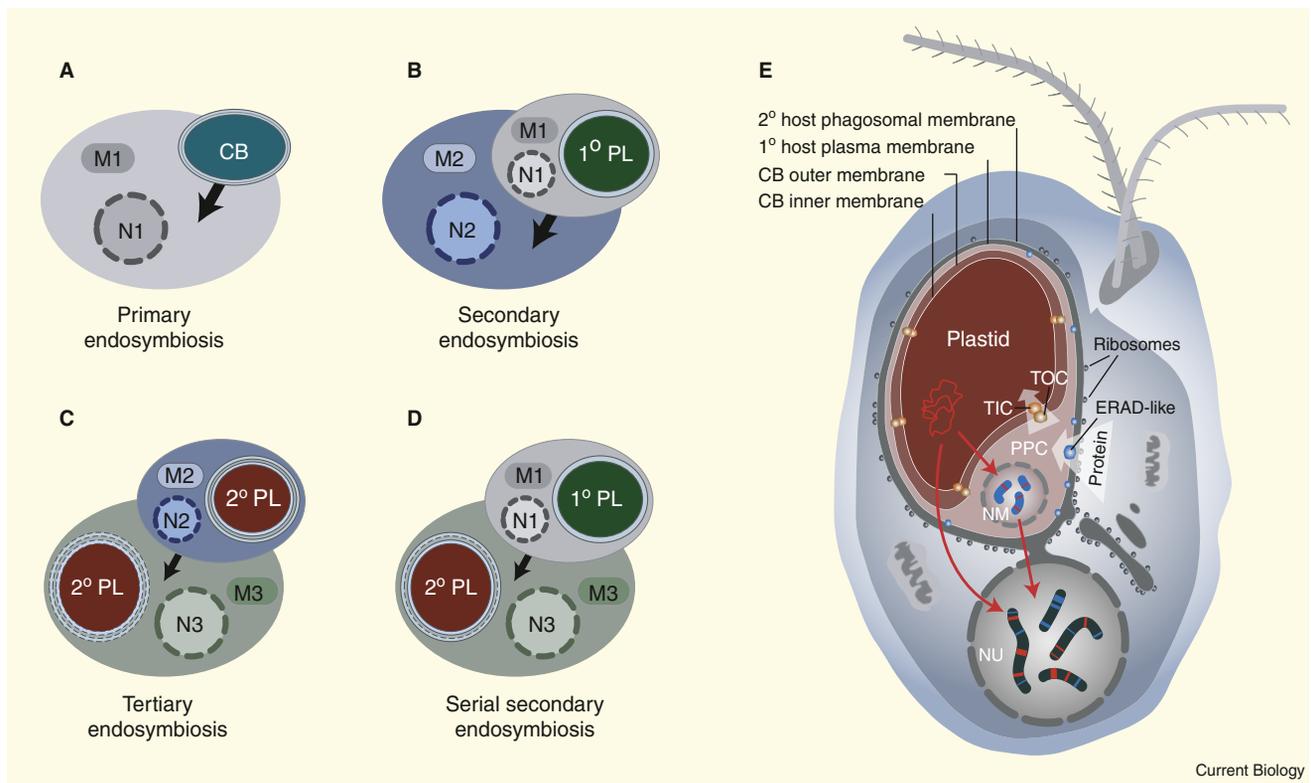


Figure 1. Plastid evolution by primary, secondary, and tertiary endosymbiosis.

(A) A cartoon depicting the primary endosymbiotic origin of plastids through the uptake of a double-membrane-bound cyanobacterium by a non-photosynthetic host eukaryote. (B) Secondary endosymbiosis involves the engulfment of a primary-plastid-containing eukaryote by a second, non-photosynthetic eukaryote. All known primary plastids are surrounded by two membranes and, in the case of glaucophyte algae, a layer of peptidoglycan. (C) Tertiary endosymbiosis occurs when a secondary-plastid-containing endosymbiont is taken up by a eukaryotic host, which may or may not itself possess a plastid. (D) Serial secondary endosymbiosis between a secondary-plastid-containing eukaryotic host and an endosymbiont with a primary plastid. (E) Endosymbiotic gene transfer and protein targeting in secondary-plastid-containing algae. Diagram shows basic cellular structure of a cryptophyte alga with a four-membrane-bound plastid of red-algal origin and a nucleomorph. Plastid and plastid-derived DNA is in red, nucleomorph and nucleomorph-derived DNA is blue, and host nuclear DNA (mitochondrial DNA has been omitted for simplicity). As in haptophytes and heterokonts, the outermost plastid membrane of the cryptophyte plastid is continuous with the host cell endomembrane system and is studded with ribosomes. In most secondary-plastid-containing algae, the nucleomorph-to-host-nucleus gene transfer process has gone to completion. See text for further discussion. Abbreviations: CB, cyanobacterium; M, mitochondrion; NU, host nucleus; PL, plastid; NM, nucleomorph; PPC, periplastid compartment; ERAD, endoplasmic reticulum-associated protein degradation machinery; TIC and TOC, translocons of the inner and outer chloroplast membrane, respectively.

do not directly bear on the diversification of canonical plastids, but they are potentially informative in that they may provide clues as to the molecular and cellular events that led to their establishment [23]. Whether the term ‘endosymbiont’ or ‘organelle’ is more appropriate in these cases is debatable and depends in large part on one’s definition of organelle [19,24,25].

Secondary and Tertiary Plastids: Who’s Got’em, Where’d They Get’em?

Speculation that photosynthesis has spread laterally across the eukaryotic tree dates back to the 1970s (e.g., [26,27]). A Canadian, Sarah Gibbs, first noted that the chlorophyll-*a+b*-pigmented plastid of the common lab alga *Euglena* was clearly green algal in nature, yet the ultrastructure of the host organism “...could not be more unlike green algae” [26,28]. Coupled with the presence of supernumerary plastid membranes, incongruence between the evolution of the plastid and the host cell in which it resides is now seen as the red flag of ‘secondary’ endosymbiosis, i.e., the movement of plastids from one eukaryote to another (Figure 1B). In the

case of *Euglena*, its plastid is indeed derived from a green alga [29], which was engulfed by a non-photosynthetic relative of the euglenids, an important protist group belonging to the eukaryotic ‘supergroup’ Excavata [30] (Figure 2).

The chlorarachniophytes are a group of rhizarian amoeboid flagellate algae that also possess green algal plastids of secondary endosymbiotic origin (Figure 2). In this case there is no doubt as to the mechanism of organelle capture: unlike *Euglena*, chlorarachniophyte cells still possess the nucleus of the green algal endosymbiont that came in with the plastid in a vestigial form referred to as a ‘nucleomorph’ [31–33]. Euglenid and chlorarachniophyte plastids possess three and four plastid membranes, respectively, in contrast to the two membranes that envelop all known primary plastids.

No fewer than six algal lineages are known to harbor red-algal-derived plastids: these include the cryptophytes (which also possess a nucleomorph [34,35]), haptophytes, plastid-bearing stramenopiles (e.g., diatoms and kelp), apicomplexans, dinoflagellates and *Chromera velia* (Figure 2). The case for red algal plastids in cryptophytes, haptophytes and stramenopiles is clear-cut (e.g., [34,36–38]), but the

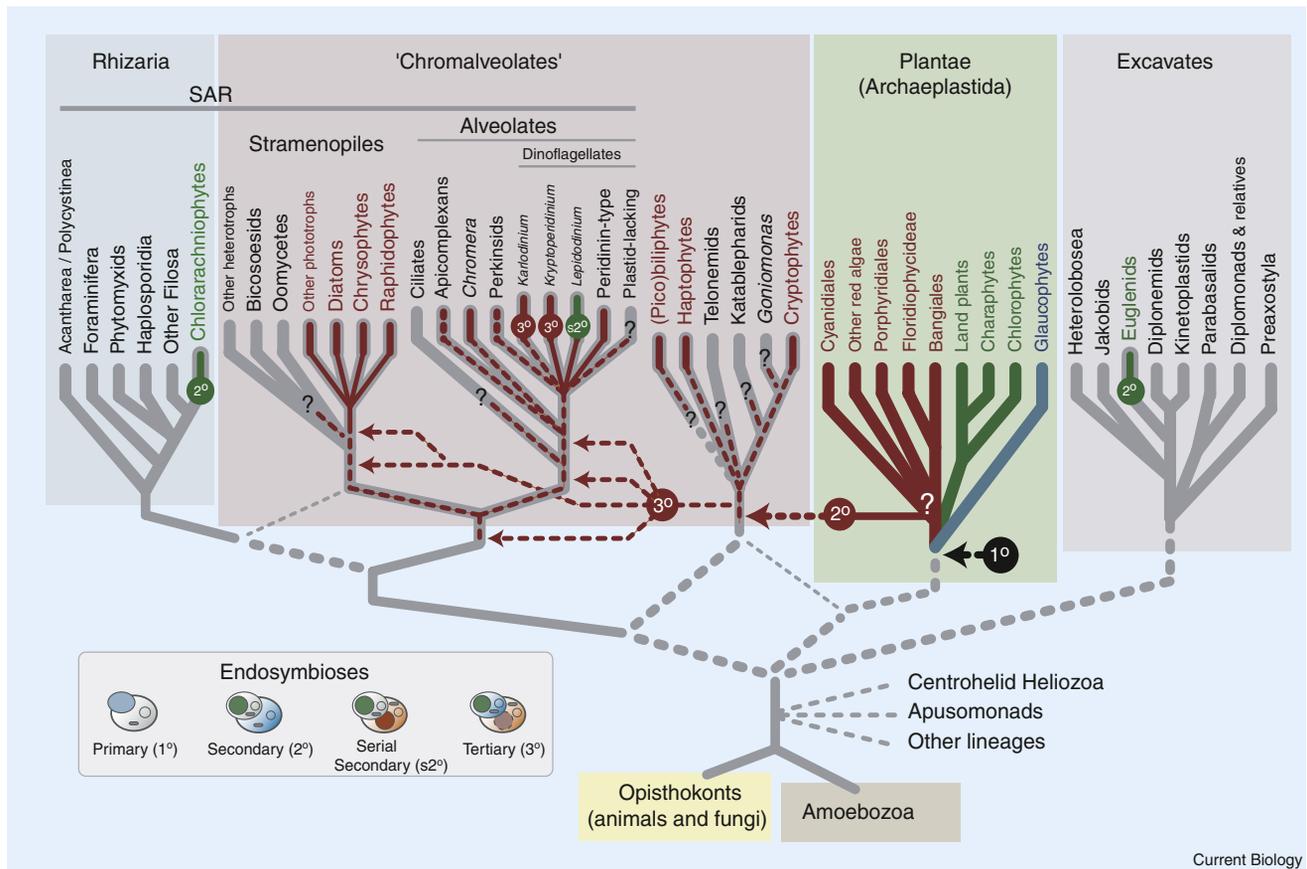


Figure 2. Hypothesis for the origin and spread of photosynthesis in eukaryotes.

The diagram shows six ‘supergroups’ of eukaryotes, with emphasis on lineages with one or more plastid-bearing groups. Several lineages of unknown affinity are also indicated. The tree topology is a consensus based on published phylogenetic and phylogenomic analyses as of August 2008, as well as consideration of discrete characters such as lateral gene transfers, endosymbiotic gene replacements, plastid pigmentation, etc. Branch lengths are not significant. Possible secondary, tertiary and serial secondary endosymbioses involving red and green algal endosymbionts are color-coded, based on the hypothesis of Sanchez-Puerta and Delwiche [66]. Dashed lines indicate uncertainty in organismal relationships and plastid movement. Question marks (?) indicate uncertainty about the presence of a plastid and/or photosynthetic ancestry, as well as a lack of knowledge about the source of the red secondary plastid in a common ancestor of cryptophytes and haptophytes. Refer to the text for discussion. Abbreviation: SAR, stramenopiles, alveolates and Rhizaria.

situation is more complicated for apicomplexans and dinoflagellates [39,40]. Apicomplexans are a medically important group of secondarily non-photosynthetic parasites (e.g., the malaria pathogen *Plasmodium*), many of which possess a remnant plastid with 4 membranes, retained because they are the site of essential cellular processes such as fatty acid and isoprenoid biosynthesis [41]. Apicomplexan plastids still possess a genome, but it is quite reduced, and its genes are highly derived and thus difficult to place accurately in molecular phylogenies.

The dinoflagellates are a relative of apicomplexans (Figure 2) and are one of the most remarkable algal lineages known. Only ~50% of known dinoflagellate species are actually photosynthetic, but of those that are, most possess a three-membrane-bound, peridinin-pigmented plastid [42]. Other dinoflagellates possess what is referred to as a ‘tertiary’ plastid, i.e., an organelle derived from the uptake of a secondary plastid-containing alga (Figure 1C). These include plastids that have been taken from cryptophytes [43,44], haptophytes [45] and stramenopiles [46,47]. Still others possess ‘serial secondary’ plastids (Figure 1D), such as *Lepidodinium*, which harbors a plastid of prasinophyte green algal ancestry [48,49]. The degree of host–endosymbiont

integration in these organisms varies considerably: the peridinin plastids are severely reduced and reveal few clues as to their origin, while the tertiary ‘plastids’ of *Kryptoperidinium* and related species are clearly a very recent acquisition from a diatom and still possess a nucleus and mitochondria [50–53]. A putative remnant of the original peridinin plastid is retained in the host cell as an ‘eye spot’ [46]. Finally, a newly discovered alga dubbed *Chromera velia* possesses a plastid of apparent red algal origin. As will be elaborated upon below, this organism represents a potentially important link between the dinoflagellate and apicomplexan plastids [54].

Endosymbiotic Gene Transfer and Protein Re-Targeting: Barriers to Secondary Plastid Evolution

One of the most intriguing aspects of secondary and tertiary endosymbiosis is the fate of the endosymbiont nucleus and the essential genes it harbors. Most secondary- and tertiary-plastid-containing organisms have completely done away with the primary algal nucleus that accompanied the plastid. Consequently, the hundreds of plastid genes that moved from the original cyanobacterial endosymbiont to the host nucleus during primary endosymbiosis (Figure 1A) must have moved again, this time from the primary host nucleus

to that of the secondary host (Figure 1E). Complete nuclear genome sequences from secondary-plastid-containing organisms such as the diatom *Thalassiosira* [55] and the apicomplexan *Plasmodium* [56] confirm that this is indeed the case. Even in the nucleomorph-containing cryptophytes and chlorarachniophytes, the coding capacity of the nucleomorph genome is extremely limited [31], and their plastids rely heavily on secondary host nucleus-encoded proteins [57,58].

The significance of these observations is that for secondary endosymbiosis to give rise to a secondary plastid, an additional protein import pathway must evolve. Primary plastids are characterized by the presence of the 'TOC/TIC' import machinery, a pair of multi-protein translocons that direct the movement of nucleus-encoded proteins from the cytoplasm across the outer and inner plastid membranes [2]. These translocons recognize transit peptides that are present on the amino termini of plastid-targeted proteins [59]. Secondary-plastid-containing algae have built upon this pre-existing system: in addition to a transit peptide, their nucleus-encoded, plastid-targeted proteins possess a signal peptide which directs them to the host cell's endomembrane system, where (in some organisms at least) a newly discovered endosymbiont-derived translocon with homology to the endoplasmic reticulum-associated degradation (ERAD) machinery guides them through to the remnant cytosol of the engulfed primary alga (the periplastid compartment or PPC; Figure 1E) [2,60]. Once in the PPC, these proteins shuttle to the plastid via the TOC/TIC system, as in primary plastids. It is not enough to simply transfer endosymbiont genes to the secondary host nuclear genome. Each of the many hundreds of transferred genes must 'acquire' a coding sequence sufficient to produce a signal peptide that can be recognized by the nascent protein import apparatus. This gene-transfer/protein-re-import process represents a potentially formidable barrier to the establishment of a secondary plastid and is the rationale behind the 'chromalveolate hypothesis' of Cavalier-Smith [61], without doubt the most controversial modern scheme put forth to explain the tempo and mode of plastid evolution.

Plastid Loss and the Genetic Legacy of Endosymbiosis

Despite years of effort, the question of exactly how many endosymbioses have given rise to the known diversity of photosynthetic eukaryotes remains unanswered. An absolute minimum of two independent secondary endosymbioses must be inferred given that examples of both green and red secondary plastids are known, and in the case of green secondary plastids, the general consensus is that the euglenid and chlorarachniophyte plastids are of independent origin [62,63]. It has been suggested that the plastids in these two groups might be the product of a single, ancient endosymbiotic event in their common ancestor [61], but given that the host cell components of these two lineages belong to two different eukaryotic supergroups dominated by non-photosynthetic, plastid-lacking organisms (Figure 2; see below), this view is considered by most to be untenable (e.g., [64]). This raises the minimum number of secondary endosymbioses to three, but beyond this, all bets are off.

The chromalveolate hypothesis [61] represents an ambitious attempt to unite the full spectrum of red secondary-plastid-derived algae by postulating a single red algal acquisition in the common ancestor of 'chromists' (cryptophytes, haptophytes and photosynthetic stramenopiles)

and alveolates (apicomplexans, dinoflagellates and ciliates). Inspired by intriguing ultrastructural and biochemical similarities shared among chromist taxa and some dinoflagellates (reviewed in [65,66]), the main assumptions of the chromalveolate hypothesis are that secondary endosymbiosis is very difficult and thus rare, and that the absence of plastids and/or photosynthesis in many 'chromalveolate' species is a derived feature. The challenge of testing the hypothesis amounts to assessing the degree of congruence between plastid- and host-associated features for the lineages in question, and deciding whether the data are more consistent with a single, ancient engulfment of a red alga in the common ancestor of all 'chromalveolate' taxa, or multiple independent endosymbioses involving different eukaryotic hosts and distinct (though potentially closely related) red algal endosymbionts. This has proven to be exceedingly difficult, as it is often unclear how conclusions drawn from different types of information (e.g., cell biological, biochemical, molecular phylogenetic) should be weighed relative to one another.

One prediction under the 'chromalveolate' model of plastid evolution is that non-photosynthetic lineages that are specifically related to photosynthetic groups might still possess plastids, as seen in apicomplexans [67]. This prediction has in fact been borne out several times in recent years. For example, an expressed sequence tag survey of the 'basal' non-photosynthetic dinoflagellate *Oxyrrhis* identified eight nuclear genes encoding proteins with plastid-related functions, at least four of which possess amino-terminal extensions reminiscent of plastid-targeting signals [68]. Similar results have been obtained for another non-photosynthetic dinoflagellate, *Crypthecodinium* [69], suggesting that both species probably possess hitherto unidentified plastids, as has just been uncovered in the dinoflagellate-like parasite *Perkinsus* [70]. These results are consistent with the possibility that dinoflagellates evolved from photosynthetic ancestors, perhaps together with apicomplexans.

That apicomplexans and dinoflagellates may have shared a photosynthetic common ancestor appears even more plausible with the recent discovery of *Chromera velia*, a tiny, unassuming alga found living in the coral at the bottom of Sydney Harbor, Australia [54]. *C. velia* is a photosynthetic unicell with a chlorophyll-*a*-pigmented plastid surrounded by four membranes (like most secondary-plastid-containing algae) and is characterized by the presence of cortical alveoli — membranous sacs underneath the cell surface — the hallmark feature for which the alveolates are named. Preliminary gene sequence analyses confirm that the host component of *C. velia* is indeed an alveolate and effectively settles the debate about the origin of the apicomplexan plastid: the 'apicoplast' is very likely derived from a red algal secondary endosymbiont [54,71]. Furthermore, the data are consistent with the idea that *C. velia* is the closest known free-living relative of apicomplexans: if confirmed, this opens up a possible window on the evolution of parasitism in these medically important organisms [72]. *C. velia* seems to occupy a pivotal position in alveolate evolution, pushing the probable origin of the apicomplexan and peridinin-containing dinoflagellate plastids back to their common ancestor.

Of course, the chromalveolate hypothesis predicts that this plastid goes still further back in time. Consistent with this idea is the fact that alveolates and stramenopiles show affinity for one another in host nuclear phylogenies (e.g., [73]), and the phylogenetic distribution of rare endosymbiotic

gene replacements involving nuclear genes for plastid proteins is interpreted by many [74–76] (though not all [77]) researchers as evidence in favor of the chromalveolate model, i.e., that chromist plastids (including those of stramenopiles) share a common origin with those of alveolates. This takes us back to the thorn in the side of the chromalveolate hypothesis: the existence of non-photosynthetic and assumed-to-be plastid-lacking sister groups to photosynthetic ‘chromalveolate’ taxa. These include the cryptomonad *Goniomonas*, many dinoflagellates, heterotrophic stramenopiles such as oomycetes, and most glaringly, the ciliates (Figure 2), a diverse, exclusively non-photosynthetic, and very well-studied protist group that is without doubt specifically related to dinoflagellates and apicomplexans [78]. As discussed above, several key heterotrophic dinoflagellate lineages now appear to have plastids after all [68,69], and given the essential biochemical role that non-photosynthetic plastids often play for their hosts [41], it is important to consider whether there are in fact any convincing cases of outright plastid loss.

The most compelling example thus far is in the apicomplexan *Cryptosporidium*, whose genome sequence revealed no evidence for the existence of plastid-targeted proteins in the organism [79] but did uncover a handful of plastid-/endosymbiont-derived genes [80]. Given its phylogenetic position within apicomplexans, it seems reasonable to conclude that outright plastid loss has indeed occurred in *Cryptosporidium*. A similar conclusion was drawn from the presence of plastid/cyanobacterial genes in the genome sequence of the plastid-lacking oomycete plant pathogen *Phytophthora* [81], and most recently, in the genome of the ciliates *Tetrahymena* and *Paramecium* [82]. As for *Cryptosporidium*, these results are interesting in that they bespeak a possible photosynthetic ancestry for both lineages, although it is also possible that the genes in question were acquired more recently by lateral gene transfer (LGT), a scenario that is not difficult to envision for ciliates, which make a living ingesting other organisms and sometimes harbor algal endosymbionts (e.g., [83]). Given increasing recognition of the prevalence of LGT in eukaryotic evolution [84] and the fact that LGT has been documented in both oomycetes [85] and ciliates [86], critics of the chromalveolate hypothesis are not likely to be impressed by the existence of small numbers of plastid/cyanobacterial genes in the nuclear genomes of plastid-lacking ‘chromalveolate’ taxa. What is needed in these cases is a much better sense of the ‘signal-to-noise’ ratio, i.e., how the genes of putative plastid/cyanobacterial ancestry stack up against non-cyanobacterial genes clearly acquired by LGT [87].

A New Phylogenomic Framework for Inferring the Spread of Plastids

Are ‘chromalveolates’ as a whole truly a natural (i.e., monophyletic) group? The last two years have seen intense activity in the application of ‘phylogenomics’ to broad-scale eukaryote systematics, and the answer to this question based on multiple analyses of datasets containing 100 or more genes seems to be ‘No.’ Figure 2 represents a consensus topology of the tree of eukaryotes based on the latest phylogenomic studies of nuclear loci [10,88–91], highlighting the increasingly popular (though much debated) notion of six eukaryotic ‘supergroups’ (see [30,92,93] for recent discussion).

From the perspective of deep plastid evolution, one of the most important phylogenomic advances has been the

realization that cryptophytes and haptophytes are specifically related to one another [88–91]. This result (a) unites two of the three original chromist groups [61], (b) argues for plastid loss in the basal cryptomonad *Goniomonas*, and (c) is consistent with the presence of a non-cyanobacterial *rpL36* gene in the plastid genome of both cryptophytes and haptophytes, presumed to be the product of a rare plastid LGT in their common ancestor [94]. Nevertheless, three other eukaryotic groups warrant discussion as potentially important pieces of the cryptophyte–haptophyte portion of the plastid puzzle. Foremost among them are the picobiliphytes (or biliphytes), an as-yet uncultured photosynthetic group that appears to be related to cryptophytes on the basis of nuclear ribosomal DNA phylogenies [95,96]. Intriguingly, picobiliphytes may possess a nucleomorph [96], as in cryptophytes: assuming they can be tamed in the lab, detailed experiments will be necessary to ascertain the origin of the picobiliphyte plastid and how it relates to those of cryptophytes and haptophytes. The remaining two groups are the telonemids and katablepharids, both of which are non-photosynthetic protists with firm phylogenetic and ultrastructural connections to cryptophytes [97,98]. With picobiliphytes, katablepharids and telonemids now in the mix (Figure 2), the cryptophyte–haptophyte clade becomes a complex assemblage of photosynthetic and non-photosynthetic taxa, reminiscent of the situation in stramenopiles.

In a twist few would have predicted, the most up-to-date phylogenomic analyses also show that stramenopiles and alveolates appear to share a common ancestor with Rhizaria — the supergroup to which the green secondary-plastid-containing chlorarachniophytes belong — to the exclusion of the haptophyte–cryptophyte group (Figure 2). The branching order of stramenopiles and alveolates relative to Rhizaria is not yet clear, but the monophyly of these three groups is consistent and strongly supported [88–90]. Some analyses place the haptophyte–cryptophyte clade as sister to the primary-plastid-containing Plantae [89] (see below), while others place cryptophytes–haptophytes basal to the ‘SAR’ group (i.e., stramenopiles, alveolates and Rhizaria [88,90]).

Taking into account these new and unexpected phylogenetic relationships, the movement of plastids depicted in Figure 2 is based on the recent hypothesis of Sanchez-Puerta and Delwiche [66]. A single primary endosymbiotic origin of plastids is evoked in the common ancestor of Plantae (the possibility of an even earlier primary endosymbiosis has not been discussed here but should be recognized; e.g., [11,99]) and an as-yet unidentified (and possibly extinct) lineage of red algae donated a plastid to the ancestor of cryptophytes and haptophytes by secondary endosymbiosis (Figure 2). Subsequently, this plastid spread from a cryptophyte–haptophyte ancestor to the stramenopile–alveolate lineage by tertiary endosymbiosis, prior to the lateral transfer of the non-cyanobacterial *rpL36* gene seen in the plastid genomes of cryptophytes and haptophytes [94]. This could have been a single tertiary event in a stramenopile–alveolate ancestor or two separate events, one at some point during stramenopile evolution and another at the base of alveolates or subsequent to the divergence of ciliates (Figure 2). Varying degrees of plastid loss and/or loss of photosynthesis must be proposed. Two independent green algal secondary endosymbioses are proposed in Rhizaria and Excavates, and tertiary and serial secondary endosymbiosis is frequent and ongoing in the dinoflagellates [42]. As emphasized by

Sanchez-Puerta and Delwiche [66], tertiary endosymbiosis has already been evoked to account for incongruence between host- and plastid-associated features in 'chromalveolates' (e.g., [77,100–103]) and other recipients of the original red algal 'chromalveolate' plastid should not be discounted (e.g., [103]).

The possibility of a truly ancient red algal secondary endosymbiosis in a putative 'chromalveolate'–rhizarian ancestor has been discussed [88,90,104], although it is problematic in that even more extensive plastid loss must be invoked. It also becomes completely untenable if the haptophyte–cryptophyte clade shares a common ancestor with Plantae, as suggested recently [89]. This result warrants close scrutiny, as ancient, cryptic eukaryote–eukaryote endosymbiotic gene replacements have the potential to greatly mislead phylogenomic studies of secondary- and tertiary-plastid-containing algae [104]. Coupled with well-known phylogenetic artifacts such as long branch attraction, gene replacements involving the red algal endosymbiont genome as donor and the ancestral cryptophyte–haptophyte genome as recipient could in part explain the cryptophyte–haptophyte–Plantae topology observed in the 135 gene phylogenies of Burki *et al.* [89].

Prospectus

So, is it time to dispense with the 'chromalveolate' concept? The model for plastid spread discussed above is certainly cause for re-evaluation of the principle of parsimony as applied to the perceived evolutionary stability of plastid membrane number and topology (e.g., the 'chloroplast endoplasmic reticulum' of chromists [61]), as well as the difficulty with which a protein import system can be established in the context of secondary and tertiary endosymbiosis [66]. Although there is still much to learn, our present understanding of the plastid protein import mechanism used in 'chromalveolate' taxa, chlorarachniophytes and euglenids [2] reveals that the host cell's endomembrane system has been 'hijacked' multiple times independently. Conversely, it also seems unnecessarily complex and premature to evoke a separate secondary or tertiary endosymbiosis to explain the presence of plastids in each and every photosynthetic lineage, given the steady rate at which remnant plastids are being discovered in heterotrophic taxa [68–70]. We certainly do not know enough about the relative difficulty of plastid gain versus plastid loss to be able to confidently distinguish between three, four, five or six endosymbiotic events based on parsimony alone, but if the evolution of a secondary plastid were utterly trivial, one might expect to see many more plastid-bearing photosynthetic groups nested within large and predominantly heterotrophic lineages (e.g., ciliates, Rhizaria, Amoebozoa) than we actually do, given the obvious advantage of photosynthesis and the propensity for eukaryotes to ingest other eukaryotes. For the time being, it seems reasonable to conclude that the rationale underlying the chromalveolate hypothesis is sound and some of its predictions have proven to be correct: endosymbiosis is an extremely complex process, outright plastid loss is possible [79–81] and some of the key 'chromalveolate' taxa are specifically related to one another (e.g., cryptophytes and haptophytes [88–91]).

How the puzzle of plastid evolution progresses from here depends on the results of current and future nuclear and organellar genome sequencing projects from diverse phototrophs such as *Chromera velia* [71], cryptophytes,

chlorarachniophytes, haptophytes, and additional red algae, as well as heterotrophic protists such as katablepharids and telonemids. Integrated with the results of ultrastructural studies and increasingly detailed biochemical analysis of the similarities and differences in the plastid protein import machinery (e.g., [60]), these genomes should provide the data with which to speculate with increased certainty on the original recipient of the primordial red algal secondary plastid, and the directionality and frequency of tertiary plastid flow among the ancestors of modern-day photosynthetic eukaryotes.

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References

1. Kaufman, A.J., Johnston, D.T., Farquhar, J., Masterson, A.L., Lyons, T.W., Bates, S., Anbar, A.D., Arnold, G.L., Garvin, J., and Buick, R. (2007). Late Archean biospheric oxygenation and atmospheric evolution. *Science* 317, 1900–1903.
2. Gould, S.B., Waller, R.F., and McFadden, G.I. (2008). Plastid evolution. *Annu. Rev. Plant Biol.* 59, 491–517.
3. Reyes-Prieto, A., Weber, A.P., and Bhattacharya, D. (2007). The origin and establishment of the plastid in algae and plants. *Annu. Rev. Genet.* 41, 147–168.
4. Yoon, H.S., Hackett, J.D., Ciniglia, C., Pinto, G., and Bhattacharya, D. (2004). A molecular timeline for the origin of photosynthetic eukaryotes. *Mol. Biol. Evol.* 21, 809–818.
5. Embley, T.M., and Martin, W. (2006). Eukaryotic evolution, changes and challenges. *Nature* 440, 623–630.
6. Adl, S.M., Simpson, A.G., Farmer, M.A., Andersen, R.A., Anderson, O.R., Barta, J.R., Bowser, S.S., Brugerolle, G., Fensome, R.A., Fredericq, S., *et al.* (2005). The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J. Eukaryot. Microbiol.* 52, 399–451.
7. Palmer, J.D. (2003). The symbiotic birth and spread of plastids: how many times and whodunnit? *J. Phycol.* 39, 4–11.
8. Howe, C.J., Barbrook, A.C., Nisbet, R.E., Lockhart, P.J., and Larkum, A.W. (2008). The origin of plastids. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363, 2675–2685.
9. Rodriguez-Ezpeleta, N., Brinkmann, H., Burey, S.C., Roure, B., Burger, G., Löffelhardt, W., Bohnert, H.J., Philippe, H., and Lang, B.F. (2005). Monophyly of primary photosynthetic eukaryotes: green plants, red algae, and glaucophytes. *Curr. Biol.* 15, 1325–1330.
10. Rodriguez-Ezpeleta, N., Brinkmann, H., Burger, G., Roger, A.J., Gray, M.W., Philippe, H., and Lang, B.F. (2007). Toward resolving the eukaryotic tree: the phylogenetic positions of jakobids and cercozoans. *Curr. Biol.* 17, 1420–1425.
11. Nozaki, H., Iseki, M., Hasegawa, M., Misawa, K., Nakada, T., Sasaki, N., and Watanabe, M. (2007). Phylogeny of primary photosynthetic eukaryotes as deduced from slowly evolving nuclear genes. *Mol. Biol. Evol.* 24, 1592–1595.
12. Stiller, J.W. (2007). Plastid endosymbiosis, genome evolution and the origin of green plants. *Trends Plant Sci.* 12, 391–396.
13. Kim, E., and Graham, L.E. (2008). EEF2 analysis challenges the monophyly of Archaeplastida and Chromalveolata. *PLoS ONE* 3, e2621.
14. Stiller, J.W., Reel, D.C., and Johnson, J.C. (2003). A single origin of plastids revisited: convergent evolution in organellar genome content. *J. Phycol.* 39, 95–105.
15. Martin, W., Rujan, T., Richly, E., Hansen, A., Cornelsen, S., Lins, T., Leister, D., Stoebe, B., Hasegawa, M., and 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. USA* 99, 12246–12251.
16. Timmis, J.N., Ayliffe, M.A., Huang, C.Y., and Martin, W. (2004). Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. *Nat. Rev. Genet.* 5, 123–135.
17. Soll, J., and Schleiff, E. (2004). Protein import into chloroplasts. *Nat. Rev. Mol. Cell Biol.* 5, 198–208.

18. Cavalier-Smith, T., and Lee, J.J. (1985). Protozoa as hosts for endosymbioses and the conversion of symbionts into organelles. *J. Protozool.* **32**, 376–379.
19. Theissen, U., and Martin, W. (2006). The difference between organelles and endosymbionts. *Curr. Biol.* **16**, R1016–R1017.
20. Reyes-Prieto, A., Hackett, J.D., Soares, M.B., Bonaldo, M.F., and Bhattacharya, D. (2006). Cyanobacterial contribution to algal nuclear genomes is primarily limited to plastid functions. *Curr. Biol.* **16**, 2320–2325.
21. Nowack, E.C.M., Melkonian, M., and Glöckner, G. (2008). Chromatophore genome sequence of *Paulinella* sheds light on acquisition of photosynthesis by eukaryotes. *Curr. Biol.* **18**, 410–418.
22. Prechtel, J., Kneip, C., Lockhart, P., Wenderoth, K., and Maier, U.G. (2004). Intracellular spheroid bodies of *Rhopalodia gibba* have nitrogen-fixing apparatus of cyanobacterial origin. *Mol. Biol. Evol.* **21**, 1477–1481.
23. Bhattacharya, D., Archibald, J.M., Weber, A.P., and Reyes-Prieto, A. (2007). How do endosymbionts become organelles? Understanding early events in plastid evolution. *BioEssays* **29**, 1239–1246.
24. Bhattacharya, D., and Archibald, J.M. (2007). Response to Theissen and Martin: “The difference between endosymbionts and organelles”. *Curr. Biol.* **16**, R1017–R1018.
25. Bodyl, A., Mackiewicz, P., and Stiller, J.W. (2007). The intracellular cyanobacteria of *Paulinella chromatophora*: endosymbionts or organelles? *Trends Microbiol.* **15**, 295–296.
26. Gibbs, S.P. (1978). The chloroplasts of *Euglena* may have evolved from symbiotic green algae. *Can. J. Bot.* **56**, 2883–2889.
27. Taylor, F.J.R. (1974). Implications and extensions of the serial endosymbiosis theory of the origin of eukaryotes. *Taxon* **23**, 229–258.
28. Gibbs, S.P. (2006). Looking at life: from binoculars to the electron microscope. *Annu. Rev. Plant Biol.* **57**, 1–17.
29. Hallick, R.B., Hong, L., Drager, R.G., Favreau, M.R., Monfort, A., Orsat, B., Spielmann, A., and Stutz, E. (1993). Complete sequence of *Euglena gracilis* chloroplast DNA. *Nucleic Acids Res.* **21**, 3537–3544.
30. Keeling, P.J., Burger, G., Dumford, D.G., Lang, B.F., Lee, R.W., Pearlman, R.E., Roger, A.J., and Gray, M.W. (2005). The tree of eukaryotes. *Trends Ecol. Evol.* **20**, 670–676.
31. Archibald, J.M. (2007). Nucleomorph genomes: structure, function, origin and evolution. *BioEssays* **29**, 392–402.
32. Gilson, P.R., Su, V., Slamovits, C.H., Reith, M.E., Keeling, P.J., and McFadden, G.I. (2006). Complete nucleotide sequence of the chlorarachniophyte nucleomorph: nature’s smallest nucleus. *Proc. Natl. Acad. Sci. USA* **103**, 9566–9571.
33. Hibberd, D.J., and Norris, R.E. (1984). Cytology and ultrastructure of *Chlorarachnion reptans* (Chlorarachniophyta divisio nova, Chlorarachniophyceae classis nova). *J. Phycol.* **20**, 310–330.
34. Douglas, S.E., Zauner, S., Fraunholz, M., Beaton, M., Penny, S., Deng, L., Wu, X., Reith, M., Cavalier-Smith, T., and Maier, U.-G. (2001). The highly reduced genome of an enslaved algal nucleus. *Nature* **410**, 1091–1096.
35. Lane, C.E., van den Heuvel, K., Kozera, C., Curtis, B.A., Parsons, B., Bowman, S., and Archibald, J.M. (2007). Nucleomorph genome of *Hemiselmis anderseni* reveals complete intron loss and compaction as a driver of protein structure and function. *Proc. Natl. Acad. Sci. USA* **104**, 19908–19913.
36. Douglas, S.E., and Penny, S.L. (1999). The plastid genome of the cryptophyte alga, *Guillardia theta*: complete sequence and conserved synteny groups confirm its common ancestry with red algae. *J. Mol. Evol.* **48**, 236–244.
37. Sanchez-Puerta, M.V., Bachvaroff, T.R., and Delwiche, C.F. (2007). Sorting wheat from chaff in multi-gene analyses of chlorophyll c-containing plastids. *Mol. Phylogenet. Evol.* **44**, 885–897.
38. Yoon, H.S., Hackett, J.D., Pinto, G., and Bhattacharya, D. (2002). The single, ancient origin of chromist plastids. *Proc. Natl. Acad. Sci. USA* **99**, 15507–15512.
39. Funes, S., Davidson, E., Reyes-Prieto, A., Magallón, S., Herion, P., King, M.P., and Gonzalez-Halphen, D. (2002). A green algal apicoplast ancestor. *Science* **298**, 2155.
40. Waller, R.F., Keeling, P.J., van Dooren, G.G., and McFadden, G.I. (2003). Comment on “A green algal apicoplast ancestor”. *Science* **301**, 49a.
41. Ralph, S.A., van Dooren, G.G., Waller, R.F., Crawford, M.J., Fraunholz, M.J., Foth, B.J., Tonkin, C.J., Roos, D.S., and McFadden, G.I. (2004). Tropical infectious diseases: metabolic maps and functions of the *Plasmodium falciparum* apicoplast. *Nat. Rev. Microbiol.* **2**, 203–216.
42. Hackett, J.D., Anderson, D.M., Erdner, D.L., and Bhattacharya, D. (2004). Dinoflagellates: A remarkable evolutionary experiment. *Am. J. Bot.* **91**, 1523–1534.
43. Hackett, J.D., Maranda, L., Yoon, H.S., and Bhattacharya, D. (2003). Phylogenetic evidence for the cryptophyte origin of the plastid of *Dinophysis* (Dinophysiales, Dinophyceae). *J. Phycol.* **39**, 440–448.
44. Schnepf, E., and Elbrächter, M. (1988). Cryptophyte-like double membrane-bound plastid chloroplast in the dinoflagellate, *Dinophysis Ehrenb.*: evolutionary, phylogenetic and toxicological implications. *Botanica Acta.* **101**, 196–203.
45. Tengs, T., Dahlberg, O.J., Shalchian-Tabrizi, K., Klaveness, D., Rudi, K., Delwiche, C.F., and Jakobsen, K.S. (2000). Phylogenetic analyses indicate that the 19’hexanoyloxy-fucoxanthin-containing dinoflagellates have tertiary plastids of haptophyte origin. *Mol. Biol. Evol.* **17**, 718–729.
46. Dodge, J.D. (1969). Observations on the fine structure of the eyespot and associated organelles in the dinoflagellate *Glenodinium foliaceum*. *J. Cell Sci.* **5**, 479–493.
47. Inagaki, Y., Dacks, J.B., Doolittle, W.F., Watanabe, K.I., and Ohama, T. (2000). Evolutionary relationship between dinoflagellates bearing obligate diatom endosymbionts: insight into tertiary endosymbiosis. *Int. J. Syst. Evol. Microbiol.* **50**, 2075–2081.
48. Watanabe, M.M., Suda, S., Inouye, I., Sawaguchi, I., and 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.
49. Hansen, G., Botes, L., and De Salas, M. (2007). Ultrastructure and large subunit rDNA sequence of *Lepidodinium viride* reveal a close relationship to *Lepidodinium chlorophorum* comb. nov. (= *Gymnodinium chlorophorum*). *Phycol. Res.* **55**, 25–41.
50. Imanian, B., and Keeling, P.J. (2007). The dinoflagellates *Durinskia baltica* and *Kryptoperidinium foliaceum* retain functionally overlapping mitochondria from two evolutionarily distinct lineages. *BMC Evol. Biol.* **7**, 172.
51. McEwan, M.L., and Keeling, P.J. (2004). HSP90, tubulin and actin are retained in the tertiary endosymbiont genome of *Kryptoperidinium foliaceum*. *J. Eukaryot. Microbiol.* **51**, 651–659.
52. Chesnick, J.M., Hooistra, W.H., Wellbrock, U., and Medlin, L.K. (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.
53. Schnepf, E., and Elbrächter, M. (1999). Dinophyte chloroplasts and phylogeny—a review. *Grana* **38**, 81–97.
54. Moore, R.B., Obornik, M., Janouskovec, J., Chrudimsky, T., Vancova, M., Green, D.H., Wright, S.W., Davies, N.W., Bolch, C.J., Heimann, K., et al. (2008). A photosynthetic alveolate closely related to apicomplexan parasites. *Nature* **451**, 959–963.
55. Armbrust, E.V., Berges, J.A., Bowler, C., Green, B.R., Martinez, D., Putnam, N.H., Zhou, S., Allen, A.E., Apt, K.E., Bechner, M., et al. (2004). The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. *Science* **306**, 79–86.
56. Gardner, M.J., Hall, N., Fung, E., White, O., Berriman, M., Hyman, R.W., Carlton, J.M., Pain, A., Nelson, K.E., Bowman, S., et al. (2002). Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* **419**, 498–511.
57. Archibald, J.M., Rogers, M.B., Toop, M., Ishida, K., and Keeling, P.J. (2003). Lateral gene transfer and the evolution of plastid-targeted proteins in the secondary plastid-containing alga *Bigelowiella natans*. *Proc. Natl. Acad. Sci. USA* **100**, 7678–7683.
58. Deane, J.A., Fraunholz, M., Su, V., Maier, U.G., Martin, W., Dumford, D.G., and McFadden, G.I. (2000). Evidence for nucleomorph to host nucleus gene transfer: light-harvesting complex proteins from cryptomonads and chlorarachniophytes. *Protist* **151**, 239–252.
59. Bruce, B.D. (2000). Chloroplast transit peptides: structure, function and evolution. *Trends Cell Biol.* **10**, 440–447.
60. Sommer, M.S., Gould, S.B., Lehmann, P., Gruber, A., Przyborski, J.M., and Maier, U.G. (2007). Der1-mediated preprotein import into the periplastid compartment of chromalveolates? *Mol. Biol. Evol.* **24**, 918–928.
61. 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.
62. Rogers, M.B., Gilson, P.R., Su, V., McFadden, G.I., and Keeling, P.J. (2007). The complete chloroplast genome of the chlorarachniophyte *Bigelowiella natans*: evidence for independent origins of chlorarachniophyte and euglenoid secondary endosymbionts. *Mol. Biol. Evol.* **24**, 54–62.
63. Takahashi, F., Okabe, Y., Nakada, T., Sekimoto, H., Ito, M., Kataoka, H., and Nozaki, H. (2007). Origins of the secondary plastids of Euglenophyta and Chlorarachniophyta as revealed by an analysis of the plastid-targeting, nuclear-encoded gene *psbO*. *J. Phycol.* **43**, 1302–1309.
64. Leander, B.S. (2004). Did trypanosomatid parasites have photosynthetic ancestors? *Trends Microbiol.* **12**, 251–258.
65. Archibald, J.M., and Keeling, P.J. (2002). Recycled plastids: a green movement in eukaryotic evolution. *Trends Genet.* **18**, 577–584.
66. Sanchez-Puerta, M.V., and Delwiche, C.F. (2008). A hypothesis for plastid evolution in chromalveolates. *J. Phycol.* **44**, 1097–1107.
67. Waller, R.F., and McFadden, G.I. (2005). The apicoplast: a review of the derived plastid of apicomplexan parasites. *Curr. Issues Mol. Biol.* **7**, 57–79.
68. Slamovits, C.H., and Keeling, P.J. (2008). Plastid-derived genes in the non-photosynthetic alveolate *Oxyrrhis marina*. *Mol. Biol. Evol.* **25**, 1297–1306.
69. Sanchez-Puerta, M.V., Lippmeier, J.C., Apt, K.E., and Delwiche, C.F. (2007). Plastid genes in a non-photosynthetic dinoflagellate. *Protist* **158**, 105–117.

70. Teles-Griolo, M.L., Tato-Costa, J., Duarte, S.M., Maia, A., Casal, G., and Azevedo, C. (2007). Is there a plastid in *Perkinsus atlanticus* (Phylum Perkinsozoa)? *Eur. J. Protistol.* **43**, 163–167.
71. Obornik, M., Janouskovec, J., Chrudimsky, T., and Lukes, J. (2009). Evolution of the apicoplast and its host: to autotrophy and back again. *Int. J. Parasitol.* **39**, 1–12.
72. Okamoto, N., and McFadden, G.I. (2008). The mother of all parasites. *Future Microbiol.* **3**, 391–395.
73. Baldauf, S.L., Roger, A.J., Wenk-Siefert, I., and Doolittle, W.F. (2000). A kingdom-level phylogeny of eukaryotes based on combined protein data. *Science* **290**, 972–977.
74. Fast, N.M., Kissinger, J.C., Roos, D.S., and Keeling, P.J. (2001). Nuclear-encoded, plastid-targeted genes suggest a single common origin for apicomplexan and dinoflagellate plastids. *Mol. Biol. Evol.* **18**, 418–426.
75. Harper, J.T., and Keeling, P.J. (2003). Nucleus-encoded, plastid-targeted glyceraldehyde-3-phosphate dehydrogenase (GAPDH) indicates a single origin for chromalveolate plastids. *Mol. Biol. Evol.* **20**, 1730–1735.
76. Patron, N.J., Rogers, M.B., and Keeling, P.J. (2004). Gene replacement of fructose-1,6-bisphosphate aldolase supports the hypothesis of a single photosynthetic ancestor of chromalveolates. *Eukaryot. Cell* **3**, 1169–1175.
77. Bodyl, A. (2005). Do plastid-related characters support the chromalveolate hypothesis? *J. Phycol.* **41**, 712–719.
78. Fast, N.M., Xue, L., Bingham, S., and Keeling, P.J. (2002). Re-examining alveolate evolution using multiple protein molecular phylogenies. *J. Eukaryot. Microbiol.* **49**, 30–37.
79. Abrahamsen, M.S., Templeton, T.J., Enomoto, S., Abrahamsen, J.E., Zhu, G., Lancto, C.A., Deng, M., Liu, C., Widmer, G., Tzipori, S., et al. (2004). Complete genome sequence of the apicomplexan, *Cryptosporidium parvum*. *Science* **304**, 441–445.
80. Huang, J., Mullapudi, N., Lancto, C.A., Scott, M., Abrahamsen, M.S., and Kissinger, J.C. (2004). Phylogenomic evidence supports past endosymbiosis, intracellular and horizontal gene transfer in *Cryptosporidium parvum*. *Genome Biol.* **5**, R88.
81. Tyler, B.M., Tripathy, S., Zhang, X., Dehal, P., Jiang, R.H., Aerts, A., Arredondo, F.D., Baxter, L., Bensasson, D., Beynon, J.L., et al. (2006). *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Science* **313**, 1261–1266.
82. Reyes-Prieto, A., Moustafa, A., and Bhattacharya, D. (2008). Multiple genes of apparent algal origin suggest ciliates may once have been photosynthetic. *Curr. Biol.* **18**, 956–962.
83. Summerer, M., Sonntag, B., and Sommeruga, R. (2007). An experimental test of the symbiosis specificity between the ciliate *Paramecium bursaria* and strains of the unicellular green alga *Chlorella*. *Environ. Microbiol.* **9**, 2117–2122.
84. Keeling, P.J., and Palmer, J.D. (2008). Horizontal gene transfer in eukaryotic evolution. *Nat. Rev. Genet.* **9**, 605–618.
85. Richards, T.A., Dacks, J.B., Jenkinson, J.M., Thornton, C.R., and Talbot, N.J. (2006). Evolution of filamentous plant pathogens: gene exchange across eukaryotic kingdoms. *Curr. Biol.* **16**, 1857–1864.
86. Ricard, G., McEwan, N.R., Dutilh, B.E., Jouany, J.P., Macheboeuf, D., Mitsuori, M., McIntosh, F.M., Michalowski, T., Nagamine, T., Nelson, N., et al. (2006). Horizontal gene transfer from Bacteria to rumen Ciliates indicates adaptation to their anaerobic, carbohydrates-rich environment. *BMC Genomics* **7**, 22.
87. Archibald, J.M. (2008). Plastid evolution: remnant algal genes in ciliates. *Curr. Biol.* **18**, R663–R665.
88. Burki, F., Shalchian-Tabrizi, K., Minge, M., Skjaeveland, Å., Nikolaev, S.I., Jakobsen, K.S., and Pawlowski, J. (2007). Phylogenomics reshuffles the eukaryotic supergroups. *PLoS One* **8**, e790.
89. Burki, F., Shalchian-Tabrizi, K., and Pawlowski, J. (2008). Phylogenomics reveals a new 'megagroup' including most photosynthetic eukaryotes. *Biol. Lett.* **4**, 366–369.
90. Hackett, J.D., Yoon, H.S., Li, S., Reyes-Prieto, A., Rummele, S.E., and 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.
91. Patron, N.J., Inagaki, Y., and Keeling, P.J. (2007). Multiple gene phylogenies support the monophyly of cryptomonad and haptophyte host lineages. *Curr. Biol.* **17**, 887–891.
92. Parfrey, L.W., Barbero, E., Lasser, E., Dunthorn, M., Bhattacharya, D., Patterson, D.J., and Katz, L.A. (2006). Evaluating support for the current classification of eukaryotic diversity. *PLoS Genet.* **2**, e220.
93. Yoon, H.S., Grant, J., Tekle, Y.I., Wu, M., Chaon, B.C., Cole, J.C., Logsdon, J.M., Jr., Patterson, D.J., Bhattacharya, D., and Katz, L.A. (2008). Broadly sampled multigene trees of eukaryotes. *BMC Evol. Biol.* **8**, 14.
94. Rice, D.W., and Palmer, J.D. (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.
95. Cuvelier, M.L., Ortiz, A., Kim, E., Moehlig, H., Richardson, D.E., Heidelberg, J.F., Archibald, J.M., and Worden, A.Z. (2008). Widespread distribution of a unique marine protistan lineage. *Environ. Microbiol.* **10**, 1621–1634.
96. Not, F., Valentin, K., Romari, K., Lovejoy, C., Massana, R., Tobe, K., Vaulot, D., and Medlin, L.K. (2007). Picobiliphytes: a marine picoplanktonic algal group with unknown affinities to other eukaryotes. *Science* **315**, 253–255.
97. Okamoto, N., and Inouye, I. (2005). The katablepharids are a distant sister group of the Cryptophyta: A proposal for Katablepharidophyta Divisio Nova/Katablepharida Phylum Novum based on SSU rDNA and beta-tubulin phylogeny. *Protist* **156**, 163–179.
98. Shalchian-Tabrizi, K., Eikrem, W., Klaveness, D., Vaulot, D., Minge, M.A., Le Gall, F., Romari, K., Thronsdon, J., Botnen, A., Massana, R., et al. (2006). *Telonemia*, a new protist phylum with affinity to chromist lineages. *Proc. Biol. Sci.* **273**, 1833–1842.
99. Maruyama, S., Misawa, K., Iseki, M., Watanabe, M., and Nozaki, H. (2008). Origins of a cyanobacterial 6-phosphogluconate dehydrogenase in plastid-lacking eukaryotes. *BMC Evol. Biol.* **8**, 151.
100. Bachvaroff, T.R., Sanchez Puerta, M.V., and Delwiche, C.F. (2005). Chlorophyll *c*-containing plastid relationships based on analyses of a multigene data set with all four chromalveolate lineages. *Mol. Biol. Evol.* **22**, 1772–1782.
101. Bodyl, A. (2006). Did the peridinin plastid evolve through tertiary endosymbiosis? A hypothesis. *Eur. J. Phycol.* **41**, 435–448.
102. Petersen, J., Teich, R., Brinkmann, H., and Cerff, R. (2006). A "green" phosphoribulokinase in complex algae with red plastids: evidence for a single secondary endosymbiosis leading to haptophytes, cryptophytes, heterokonts, and dinoflagellates. *J. Mol. Evol.* **62**, 143–157.
103. Teich, R., Zauner, S., Baurain, D., Brinkmann, H., and Petersen, J. (2007). Origin and distribution of Calvin cycle fructose and sedoheptulose bisphosphatases in plantae and complex algae: a single secondary origin of complex red plastids and subsequent propagation via tertiary endosymbioses. *Protist* **158**, 263–276.
104. Lane, C.E., and Archibald, J.M. (2008). The eukaryotic tree of life: endosymbiosis takes its TOL. *Trends Ecol. Evol.* **23**, 268–275.