Plastid Origin and Evolution

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Advanced article

Article Contents

Online posting date: 15th November 2011

Plastids (or chloroplasts in plants) are organelles within which photosynthesis takes place in eukaryotes. The origin of the widespread plastid traces back to a cyanobacterium that was engulfed and retained by a heterotrophic protist through a process termed primary endosymbiosis. Subsequent (serial) events of endosymbiosis, involving red and green algae and potentially other eukaryotes, yielded the so-called 'complex' plastids found in photosynthetic taxa such as diatoms, dinoflagellates and euglenids. The field of plastid research also includes nonphotosynthetic organelles (apicoplasts) within the parasitic apicomplexans and the temporary sequestration ('theft') of plastids by heterotrophic organisms (kleptoplasty) such as the sea slug *Elysia chlorotica*. The gain and loss of plastids, and nonlineal gene transfer (associated with endosymbiosis) are key aspects of algal evolution that have decisive impacts on inference of their phylogenetic positions in the tree of life. Deciphering plastid origin therefore provides general insights into the evolution of eukaryote lineages.

Introduction

One of the landmark evolutionary events in the history of life is the origin of oxygenic photosynthesis (photoautotrophy), whereby organisms were able to harness light energy to produce biochemical energy that is utilised across all metabolic processes. Perhaps more importantly, widespread photosynthesis led to oxygenation of the atmosphere, laying the foundation for the evolution of diverse and abundant life forms that support extant ecosystems. Cyanobacteria were the first dominant photosynthetic organisms that emerged ca. 2.8 billion years ago (Olson, 2006), followed by the evolution of eukaryotic algae ca. 1.5 billion years ago (Yoon *et al.*, 2004) and finally by the rise of plants ca. 500 million years ago (Taylor, 1988).

Photosynthetic reactions occur within the cytosol in prokaryotes. In eukaryotes, however, the reaction takes place in the organelle, plastid (e.g. chloroplast in plants). The plastid also houses many other reactions that are essential for growth and development in algae and plants; for example, the biosynthesis of fatty acids, biosynthesis of amino acids and carbohydrate storage. **See also**: Cyanobacteria; Evolution of Photosynthesis; Photosynthesis; Photosynthesis: The Calvin Cycle; Photosynthetic Carbon Metabolism; Plant Chloroplasts and Other Plastids

Various forms of plastids have been found in eukaryotes, from the most 'simple' two-membrane (primary) plastid, the more 'complex' three- and four-membrane-bound (secondary and some tertiary) plastids, pigment-lacking nonphotosynthetic plastids (e.g. apicoplasts), to the non-permanent, 'stolen' plastids in a number of heterotrophic organisms (a phenomenon known as kleptoplasty). These diverse forms differ with respect to how the plastid originated in the 'host' cell and in the case of kleptoplasts, their level of genetic integration and dependence on host cell metabolism. Unfortunately, the frequency of complex plastid transfers between related eukaryote groups such as 'chromalveolates' (e.g. diatoms, haptophytes, oomycetes and dinoflagellates) is difficult if not impossible to divine, resulting in an incomplete understanding of this process. Given that endosymbiosis involves the wholesale transfer of genetic material between lineages, plastid evolution is one of the key complicating factors in understanding eukaryote genome evolution. Therefore, deciphering the origin of plastids significantly enhances our understanding of the basis of photosynthesis in plants, our primary food source on the planet, and its broader impact on eukaryote evolution.

eLS subject area: Evolution & Diversity of Life

How to cite:
Chan, Cheong Xin; and Bhattacharya, Debashish (November 2011) Plastid Origin and Evolution. In: eLS. John Wiley & Sons, Ltd: Chichester.
DOI: 10.1002/9780470015902.a0023639

Primary Plastids and Endosymbiosis

The simplest forms of plastids, the primary plastids, are found exclusively (except for *Paulinella*, see below) within

the eukaryote supergroup Plantae (or Archaeplastida) (Rodríguez-Ezpeleta *et al*., 2005) that comprises the glaucophyte algae (Glaucophyta), red algae (Rhodophyta), as well as green algae and plants (collectively known as Viridiplantae). The Plantae plastid, bound by two membranes, originated from a cyanobacterium that was engulfed and retained by a free-living, unicellular heterotrophic protist, in a process termed primary endosymbiosis (Margulis, 1970; Kutschera and Niklas, 2005). Rather than being digested, the captured cell in the food vacuole became a permanent endosymbiont and ultimately a functional organelle that has been vertically transmitted to subsequent generations for over a billion years (**Figure 1**). **See also**: Endosymbionts; Glaucocystophytes; Green Algae; Plant Biodiversity; Red Algae

The idea of endosymbiosis was based on the observation of the symbiotic relationship between fungi and algae in lichens, made by the Russian biologist, Konstantin Mereschkowsky (Mereschkowsky, 1905). He championed the idea that complex cells are derived from the symbiotic

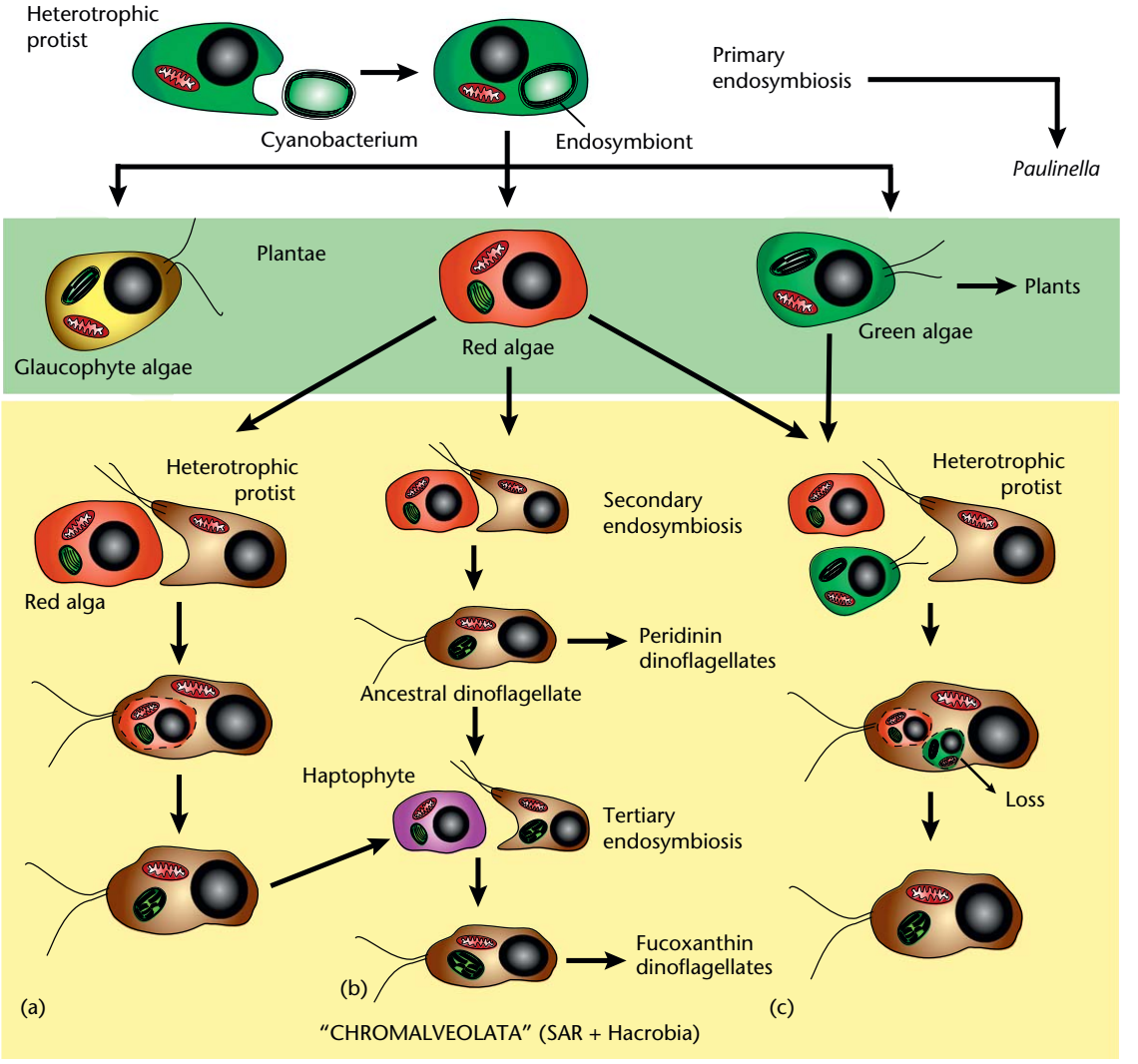

Figure 1 Current understanding of plastid origins in eukaryotes. Primary cyanobacterial endosymbiosis is thought to have given rise to the plastid shared by all Plantae lineages. An independent primary endosymbiosis gave rise to the plastid in the photosynthetic species of *Paulinella* (Rhizaria). Subsequent events of endosymbiosis, involving a red algal cell are implicated in the origin of plastids in chromalveolates (lineages that bear the complex chlorophyll *a*+*c* plastids). Three proposed evolutionary histories of chromalveolate plastid origin based on current literature are shown: (a) a single secondary red algal endosymbiosis that gave rise to the plastid in most chromalveolate lineages, according to the chromalveolate hypothesis; (b) secondary red algal endosymbiosis giving rise to the plastid in peridinin-type dinoflagellates, with a tertiary haptophyte endosymbiosis giving rise to the plastid in fucoxanthin-type dinoflagellates; (c) secondary red algal endosymbiosis that was potentially preceded by a cryptic green algal endosymbiosis, giving rise to the ancestral chromalveolate plastid but a genome with hundreds of genes of both green and red algal origin.

relationship between more simple forms (i.e. symbiogenesis). The term 'endosymbiosis' (Greek origin: *endo* 'within'; *syn* 'with'; *biosis* 'living') refers to the phenomenon of an organism living within another, and was extended by Ivan Wallin (an American biologist) to explain the origin of mitochondria in eukaryotes. In the 1960s, Lynn Margulis established the endosymbiotic hypothesis as an explanation for the origin of both mitochondria and plastids in eukaryotes based on evidence from palaeontological, biochemical and cytological sources (Margulis, 1970). Whereas her idea was first treated with much scepticism, nowadays endosymbiosis is a widely accepted explanation for the provenance of organelles.

The hypothesis that algal plastids originated from cyanobacteria (via endosymbiosis) has been rigorously tested in the past two decades, in parallel with the availability of molecular data, and the rapid advancement of techniques in molecular and computational biology. The cyanobacterial derivation of genes encoded in primary plastid genomes and clear evidence of gene transfer from the endosymbiont genome to the 'host' nucleus have been demonstrated in numerous studies, (e.g. Rodríguez-Ezpeleta *et al*., 2005), in addition to other independent lines of evidence based on protein transport and plastid biochemistry, (e.g. Reyes-Prieto and Bhattacharya, 2007). From the perspective of palaeontology, the limited and controversial fossil record of early algal forms has been a hindrance, but most scientists accept the *Bangiomorpha pubescens* fossil as indicative existence of sexual red algae ca. 1.2 billion years ago (Butterfield *et al*., 1990), providing a minimal estimate for the timing of the primary endosymbiotic event. Molecular clock analyses often agree with the *Bangiomorpha* data with the age of the primary plastid estimated at ca. 1.5 billion years old (Yoon *et al*., 2004), although dating such an ancient event based on molecular methods remains controversial. Extant primary plastids contain either chlorophyll *a* only (glaucophyte and red algae), or chlorophylls *a* and *b* (the green algae and plants). **See also**: Algal Chloroplasts; Chlorophyll: Structure and Function; Chlorophylls

Remarkably, in addition to Plantae, primary cyanobacterial endosymbiosis has also occurred in the photosynthetic lineages of *Paulinella*, filose amoebae within the disparate grouping of Rhizaria. The simple plastids found in *Paulinella chromatophora* are derived from an independent primary endosymbiosis involving a member of the alpha-cyanobacterium clade (Yoon *et al*., 2006; Nowack *et al*., 2008). Nevertheless, in comparison to the ca. 100–200 kbp plastid genome size in Plantae, the *Paulinella* plastid genome is nearly 1 Mbp in size, suggesting a clear instance of 'work in progress' with regard to outright gene loss and endosymbiotic gene transfer (EGT) to the amoeba nucleus. Assuming plastid genome reduction is correlated with the time of endosymbiosis, the *Paulinella* plastid is estimated to be ca. 60 million years old (Nowack *et al*., 2008), far more recent than what is observed in the ancient Plantae lineages. **See also**: Chloroplast Genome

The *Paulinella* plastid traces its origin to *Prochlorococcus* or *Synechococcus* type cells (Yoon *et al*., 2006). Also known as cyanelles or chromatophores, these plastids lie free in the cytoplasm, unbounded by a vacuolar membrane (i.e. characteristics of an organelle), but retain cyanobacterial features such as peptidoglycan and carboxysomes (Johnson *et al*., 1988). Interestingly, the closely related, phagotrophic sister lineage, *Paulinella ovalis*, although lacking a plastid, is an active predator of cyanobacteria that are commonly localised within food vacuoles (Johnson *et al*., 1988). A recent analysis of individual *P. ovalis* cells captured in the wild using single-cell genomics reveals the presence of genes derived from *Prochlorococcus* and *Synechococcoccus* lineages in the nuclear genome of *P. ovalis* cells; that is, these cyanobacteria were likely to have been ingested as food and are the source of the transferred nuclear genes. These results provide an intriguing and apparently direct link between the cyanobacterial diet of the phagotrophic lineage and the primary plastid in the (sister) photoautotrophic *Paulinella* species (Bhattacharya D, unpublished data).

The surprising discovery in *P. ovalis* is supported by recent analyses of *P. chromatophora* nuclear genes (Nowack *et al*., 2008; Reyes-Prieto *et al*., 2010) that show clear evidence of EGT from alpha-cyanobacteria, as well as unambiguous support for genome reduction (and hence significant decay in the genetic potential) of the cyanobacterial endosymbiont; that is, to ca. one-third of the genome size of the putative cyanobacterial donor. A recent comparative analysis (Reyes-Prieto *et al*., 2010) demonstrates a high degree of synteny between the plastid genomes of two photosynthetic species of *Paulinella* and the genome of their putative cyanobacterial donor. The same study also reveals that these two plastid genomes differ by only five inversions and a single translocation, with the vast majority undergoing purifying selection pressure (K_a/K_s ratios < 1) and a small number of lineage-specific gene loss. These data provide direct evidence that preservation of gene order and differential gene loss are key characteristics of plastid genome evolution.

Secondary (and Tertiary) Plastids

With the exception of the Plantae and *Paulinella*, plastids found in other photosynthetic eukaryotes such as brown algae, diatoms, dinoflagellates, ciliates and euglenids possess a more-complex membrane structure than the primary plastids, usually bounded by three or four membranes. These plastids also differ from primary plastids in that they contain chlorophylls $a + c$, or chlorophylls $a + b$ (euglenids, chlorarachniophytes) instead of only chlorophyll *a*. The chlorophyll $a + c$ lineages are often collectively referred to as the chromalveolates (Cavalier-Smith, 1999) although this group has recently been shown to be paraphyletic in the tree of life (Hackett *et al*., 2007; Baurain *et al*., 2010). The additional membrane layers in complex plastids have been attributed to additional endosymbiosis event(s) involving

an already plastid-bearing, eukaryote endosymbiont, instead of a cyanobacterium. **See also**: Chlorarachniophytes; Chromista; Ciliophora; Dinoflagellates; Euglena; Phytoplankton

As originally conceived by Cavalier-Smith (1999), Chromalveolata comprised cryptophytes, haptophytes, stramenopiles and Alveolata (apicomplexans, ciliates and dinoflagellates). The cryptophyte cells contain a remnant of red algal cell known as the nucleomorph within which extensive gene loss has been documented (Moore and Archibald, 2009), whereas the nucleus of the red algal endosymbiont in the other chromalveolates is absent, suggesting the genes that are necessary to ensure a fully functional plastid have been transferred to the host nucleus. The Chromalveolata is now known to include members of another supergroup, Rhizaria as well as a number of poorly studied heterotrophic lineages such as telonemids, katablepharids and picobiliphytes (Not *et al*., 2007; Yoon *et al*., 2011) and the putatively photosynthetic rappemonads (Kim *et al*., 2011). These data, therefore, invalidate the original chromalveolate hypothesis. An intriguing aspect of the original theory that remains of high biological interest is the supposition that chromalveolates ancestrally shared a single red algal secondary endosymbiont. **See also**: Alveolates; Chromista

Phylogenetic analysis of plastid-encoded genes in most chromalveolates shows a red algal origin of the organelle (Khan and Archibald, 2008) (see **Figure 1a**); regardless of whether it originated via a single red algal endosymbiosis as suggested by Cavalier-Smith or potentially by serial endosymbioses in different lineages, that is, one chromalveolate engulfs another, followed by plastid replacement. Such an alternative to the chromalveolate hypothesis is the independent acquisition hypothesis (also known as serial eukaryote–eukaryote endosymbiosis, or the serial EEE; Baurain *et al*., 2010), which suggests that the origin of the secondary plastids in different groups of chromalveolates, for example, haptophytes, cryptophytes and stramenopiles, is the result of independent, serial endosymbioses involving unicellular eukaryotes, not necessarily red algae. Under this scenario, the invocation of massive gene loss events or the subsequent loss of the red algal endosymbiont is unnecessary to explain the non-plastid containing lineages of chromalveolates such as ciliates.

One approach to help unravel the complex history of plastid evolution in chromalveolates is by searching nuclear genomes for genes of endosymbiont origin that have plastid-related or other functions. This sort of gene movement is explained by intracellular gene relocation (i.e. EGT). Because the majority of plastid proteins are encoded in the nucleus (only ca. 120–200 are plastid-encoded), nuclear genes can provide insights into past endosymbiosis via phylogenetic 'footprints' of EGT events, even if the plastid has long been lost or replaced. For example, a phylogenomic analysis of ciliate nuclear genes turned up over a dozen genes of algal origin in *Tetrahymena* and *Paramecium* that suggest a photosynthetic past for these currently plastid-lacking taxa (Reyes-Prieto *et al*., 2008). Similarly, dozens of algal genes have been identified in plastid-lacking oomycetes that support a photosynthetic ancestry for stramenopiles (Tyler *et al*., 2006).

Additional endosymbioses likely involving another eukaryote endosymbiont has been implicated in the dinoflagellates (**Figure 1b**), particularly among those lineages bearing the fucoxanthin pigment, which include the common species that causes 'red tides' in the ocean, *Karenia* and *Karlodinium*. The plastid genomes of dinoflagellates are however highly reduced with substantial gene transfer from the endosymbiont to the host nucleus (Hackett *et al*., 2004). Plastid-targeted proteins in many dinoflagellates trace their origin not only to red algae as expected, but also to other unicellular eukaryotes such as excavates, haptophytes and other chromalveolates (Ishida and Green, 2002; Yoon *et al*., 2005). A preliminary analysis of $>30\,000$ unique genes from the peridinin-containing dinoflagellate, *Alexandrium tamarense* reveal a high level of nonlineal gene history (due to endosymbiotic or horizontal gene transfer (HGT)) involving other eukaryote lineages (Bhattacharya D, unpublished data). The complicated evolutionary histories of dinoflagellate genes demonstrate that resolving the eukaryote tree of life is a daunting task, even with the accumulation of significant genome data from understudied protists. **See also**: Dinoflagellates; Universal Tree of Life

Therefore, among photosynthetic chromalveolates, it is possible that due to serial endosymbioses and recurrent HGTs, nuclear-encoded plastid-targeted proteins represent phylogenetically distinct collections derived from different genetic sources. Under this scenario, the observation that diatoms (and most chromalveolates) contain a red algal plastid does not conflict with the hypothesis that they may once have harboured other endosymbionts that were later replaced by the red algal-derived plastid. This notion gained significant support with a recent study of proteins from two diatoms, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* (Moustafa *et al*., 2009), of which the genomes are completed sequenced. This work provided strong evidence of gene sharing between the lineages of diatoms and green algae, specifically of the ancient diverged prasinophytes, in hundreds of genes (see **Figure 1c**). The massive transfer of green algal genes in diatoms indicates a likely cryptic endosymbiosis event involving a green algal cell, in a concurrent manner with, or predating the canonical red algal capture, during the early evolution of chromalveolates. Similarly, the chlorarachniophyte amoeba *Bigelowiella natans* harbours a green algal-derived plastid and therefore contains a large number of green algal-derived nuclear genes to support the function of the plastid (Archibald *et al*., 2003). This genome however also encodes a significant number of red algal-derived, plastid-targeted proteins that again suggest a more complex history of gene transfer in chlorarachniophytes than would be expected based solely on current plastid identity. **See also**: Chlorarachniophytes; Diatoms

Nonphotosynthetic Plastids

The plastids described thus far are permanent organelles within eukaryotes that are needed for proper function of photosynthesis (among other processes). Apicoplasts in the parasitic Apicomplexa are a unique type of plastids that lack photosynthetic functions and are found in most apicomplexan lineages, including *Plasmodium*, the causative agent of malaria. Whereas the organelle and the corresponding circular, organellar DNA have long been observed in the apicomplexans, they were not recognised as a plastid until the 1990s, when the organelle genomes (ca. 35 kbp in size) were unambiguously found to be highly similar to other algal plastid genomes, particularly with respect to genome architecture, rearrangement and gene content, with specific loss of genes that encode photosynthetic functions (Gleeson, 2000). Irrespective of photosynthetic functions, apicoplasts are crucial organelles for other important biological processes normally occurring in a plastid such as biosynthesis of fatty acids, isoprenoids, iron–sulfur clusters and haem (Ralph *et al*., 2004; Lim and McFadden, 2010). **See also**: Apicomplexa; *Plasmodium*

Previous studies based on molecular and microscopic (ultrastructural) data have demonstrated the presence of the unique organelles of apicoplasts in most apicomplexan species with the possible exception in *Cryptosporidium* (Zhu *et al*., 2000). The high degree of similarity shared by the apicoplast genomes, for example, the near identical gene order and content in the genomes between *Plasmodium falciparum* and *Toxoplasma gondii*, suggests a single common origin of these organelles among apicomplexan lineages.

Most apicoplasts are surrounded by four membranes with the exception of *Plasmodium* that has three bounding membranes (Hopkins *et al*., 1999), suggesting that these are complex plastids. Similar to their sister lineages of dinoflagellates, apicomplexans possess a eukaryote-type nuclear-encoded, plastid-targeted glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, that is distinct from the cyanobacterium-type gene copy found among the Plantae lineages (Fast *et al*., 2001). The finding suggests the displacement of the cyanobacterial gene copy via gene duplication before the divergence of dinoflagellates and apicomplexans, therefore lending support to the idea that apicoplasts are derived from secondary plastids, similar to the other chromalveolates. Phylogenetic analysis of plastid-targeted genes (Fast *et al*., 2001) and a structural analysis of the apicoplast (Blanchard and Hicks, 1999) in apicomplexans have indicated the likely red algal origin of the apicoplast. The architecture of the apicoplast genome is consistent with a red algal ancestry (Blanchard and Hicks, 1999), although green algal-derived genes have also been documented (Funes *et al*., 2002). These organelles have subsequently lost their photosynthetic capability likely due to the transition to obligate parasitism. The evolutionary history of apicoplasts was significantly clarified with the recent discovery of their photosynthetic relative, the coral-endosymbiont *Chromera velia* (Moore *et al*., 2008) and its relative *Chromerida* spp. CCMP3155. Studies of these taxa indicate an algal ancestry for apicoplasts as well as for the progenitor of dinoflagellates plus apicomplexans (Moore *et al*., 2008; Janouškovec *et al*., 2010). **See also**: Apicomplexa; Parasitism: the Variety of Parasites

Finally, it should be noted that mixotrophic (Stoecker, 1999) or nonphotosynthetic (plastid-lacking or with a relic plastid) dinoflagellates (or closely related taxa) are widely known, e.g. *Noctiluca*, *Pfiesteria*, *Gymnodinium*, *Protoperidinium* and some *Dinophysis* species (Jeong, 1999). However, it is only recently that the genomic footprint of a past plastid (i.e. endosymbiont) was described in the heterotroph or *Crypthecodinium cohnii* (Dauvillée *et al*., 2009), the early-branching dinoflagellate (plastid-lacking) *Oxyrrhis marina* (Slamovits and Keeling, 2008) and the bivalve-parasite *Perkinsus marinus* (Matsuzaki *et al*., 2008). **See also**: Dinoflagellates

Plastid Theft

We know that deciphering plastid origin and its evolution, particularly of the secondary plastids, is complicated by instances of nonlineal gene transfer resulting from multiple (and/or series) of endosymbioses. An interesting phenomenon that could further complicate our understanding of plastid evolution is kleptoplasty, the 'stealing', or rather sequestering, of plastids by heterotrophic eukaryotes, in which the host retains the functional plastids temporarily within the cell. First described by Trench (1969), kleptoplasty was observed between the sacoglossan mollusc (i.e. the sea slug), *Elysia chlorotica* and its algal prey, *Vaucheria litorea*.

Similar heterotroph–autotroph kleptoplasty has also been observed between the heterotrophic and autotrophic dinoflagellates (Gast *et al*., 2007), the ciliates and the cryptomonad algae (Johnson *et al*., 2007), as well as between a number of foraminifera species and the diatoms (Bernhard and Bowser, 1999). Whereas the host sequestered only the plastids from preys in many cases, there are instances where the whole algal cell was maintained within the host (see Rumpho *et al*., 2011 for review). The sequestered plastids assume photosynthetic functions for periods ranging from a few days, for example, in the dinoflagellates (Gast *et al*., 2007), to almost a year, for example, in the sea slug *E. chlorotica* (Rumpho *et al*., 2011). **See also**: Cryptomonads; Foraminifera

To ensure long-term plastid function in the sea slug, a straightforward hypothesis is that extensive HGT occurred from the algal nucleus to the heterotrophic host genome. A large number of algal-derived genes related to photosynthetic functions have been described in the sea slug (Pierce *et al*., 2009), however, thus far there is no empirical evidence that suggest massive HGT from the algal source into the host genome is necessary to ensure plastid functions (Rumpho *et al*., 2011).

Plastid Origin and Eukaryote Evolution

Plastid origin and evolution significantly contributes to our understanding of eukaryote evolution in general. For instance, the existing Plantae supergroup, consisting of glaucophyte, red and green algae (including plants), is partly based on the presence of the simple, primary plastids in these lineages. Phylogenetic analysis of plastid genes and nuclear genes that encode plastid-targeted proteins (e.g. Rodríguez-Ezpeleta *et al*., 2005), have demonstrated strong support for Plantae monophyly. In a recent phylogenomic analysis with the addition of novel red algal gene data from the mesophilic taxa, the unicellular *Porphyridium cruentum* and multicellular coralline alga, *Calliarthron tuberculosum* (Chan *et al*., 2011), ca. 50% of the examined gene phylogenies show strong support for red-green monophyly; that is, supporting vertical inheritance under the assumption of Plantae hypothesis, and interestingly, the other 50% of the phylogenies show evidence of nonlineal gene sharing between the red algae and other eukaryote and prokaryote taxa. Such findings suggest a complicated evolutionary history of red algal genes, partly owing to endosymbiotic affiliations with many chromalveolate lineages, such as the diatoms and dinoflagellates. **See also**: Algae: Phylogeny and Evolution; Horizontal Gene Transfer in Evolution; Protozoan Evolution and Phylogeny

In light of Plantae evolution, separating the chromalveolate lineages from their plastid sources is far from resolved. Nevertheless, the advancement of genome technologies and the availability of gene data from understudied taxa in recent years are shedding new insights into our understanding of eukaryote evolution. For instance, based on microscopic analysis, a novel taxon, the Picobiliphyta, was recently described as a plastid-bearing lineage that is closely related to the cryptophytes within the chromalveolates. The picobiliphyte plastid was thought to contain phycobiliproteins, most likely derived from a cryptophyte endosymbiont (Not *et al*., 2007). However, in a recent analysis based on the single-cell genomic approach of three individual wild-caught picobiliphyte cells, no evidence of plastid genes or nuclear genes encoding plastid-targeted proteins was found (Yoon *et al*., 2011). In this case, not only did single-cell genomics provide a snapshot in time of the organisms with respect to biotic interactions, the approach also suggested that picobiliphytes are likely to be heterotrophic, and that the observed phycobiliprotein-containing plastids within the cell are unlikely to be permanent; for example, resulting from kleptoplasty or simply prey.

Concluding Remarks

The origin of primary plastids via endosymbiosis involving a cyanobacterium is well established but the origins of secondary plastids remain controversial. With the advancement of genome technologies in recent years; for example, next-generation sequencing, metagenomics and single-cell genomic approaches, the enrichment of novel data from understudied taxa will significantly enhance our understanding of eukaryote evolution, from both the physiological and ecological viewpoints. Whereas additional studies and biochemical validation where possible are needed to better test existing hypotheses about the evolutionary origins of plastids in eukaryotes, genome data from glaucophyte algae, the photosynthetic *Paulinella* species or the massive genomes of dinoflagellates provide excellent opportunities to study the fundamental forces associated with endosymbiosis, EGT and protein transport, and to elucidate its role in organellogenesis. **See also**: Genomics of Algae; Nuclear-encoded Protein Import into Chloroplasts: Methods

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