What makes a chloroplast? Reconstructing the establishment of photosynthetic symbioses

Richard G. Dorrell* and Christopher J. Howe

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK

*Author for correspondence (rd357@cam.ac.uk)

Summary

Earth is populated by an extraordinary diversity of photosynthetic eukaryotes. Many eukaryotic lineages contain chloroplasts, obtained through the endosymbiosis of a wide range of photosynthetic prokaryotes or eukaryotes, and a wide variety of otherwise non-photosynthetic species form transient associations with photosynthetic symbionts. Chloroplast lineages are likely to be derived from pre-existing transient symbioses, but it is as yet poorly understood what steps are required for the establishment of permanent chloroplasts from photosynthetic symbionts. In the past decade, several species that contain relatively recently acquired chloroplasts, such as the rhizarian *Paulinella chromatophora*, and non-photosynthetic taxa that maintain photosynthetic symbionts, such as the sacoglossan sea slug *Elysia*, the ciliate *Myrionecta rubra* and the dinoflagellate *Dinophysis*, have emerged as potential model organisms in the study of chloroplast establishment. In this Commentary, we compare recent molecular insights into the maintenance of chloroplasts and photosynthetic symbionts from these lineages, and others that might represent the early stages of chloroplast establishment. We emphasise the importance in the establishment of chloroplasts of gene transfer events that minimise oxidative stress acting on the symbiont. We conclude by assessing whether chloroplast establishment is facilitated in some lineages by a mosaic of genes, derived from multiple symbiotic associations, encoded in the host nucleus.

Key words: Chloroplast, Photosymbiont, Kleptoplast, Endosymbiotic gene transfer

Introduction

Chloroplasts arose through the symbiotic integration of two organisms, a eukaryotic host and a free-living photosynthetic prokaryote, in a process termed endosymbiosis (reviewed by Howe et al., 2008a). This process has generated an extraordinary diversity of extant photosynthetic eukaryotic lineages (Fig. 1). The first chloroplasts are believed to have originated through the endosymbiotic acquisition of a cyanobacterium by an ancestor of the group known as the archaeplastids (Fig. 1). It is widely assumed that this primary endosymbiosis was a unique event, although the reliability of the inference has been questioned (Howe et al., 2008a), and there is recent evidence, discussed below, of an independent primary endosymbiotic acquisition of a cyanobacterium by the rhizarian amoeba *Paulinella chromatophora* (Marin et al., 2005). Other major photosynthetic eukaryotic lineages (e.g. diatoms and haptophytes) have arisen subsequently through similar endosymbiotic events. However, in these lineages, the host has taken up a free-living photosynthetic eukaryote (e.g. red or green alga), in a process termed secondary or tertiary endosymbiosis (Fig. 1) (reviewed by Dorrell and Smith, 2011; Kim and Archibald, 2009). In most cases, the host lineage is believed to have originally been non-photosynthetic, but some examples are known in which a previously photosynthetic eukaryote acquired a new chloroplast lineage by serial replacement of the original chloroplast. Such serial endosymbioses gave rise to green algal-, haptophyte- and diatom-derived chloroplasts in dinoflagellates, which ancestrally contained a red-algal-derived chloroplast (Gabrielsen et al., 2011; Imanian et al., 2010; Kim and Archibald, 2009; Minge et al., 2010).

Although the chloroplast lineages listed above are permanently retained by their host, many otherwise non-photosynthetic eukaryotes are known to harbour transient internal photosynthetic symbionts, with varying degrees of stability (Fig. 1) (Johnson, 2011b; Stoecker et al., 2009). In some lineages, the entire cells of prokaryotic or eukaryotic photosynthetic symbionts are retained, which we refer to here as 'photosymbionts'. In other cases, the host specifically harvests and preserves chloroplasts from photosynthetic prey, generating structures termed 'kleptoplasts'. It is interesting that, whereas so many eukaryotic lineages acquire photosymbionts or kleptoplasts, fewer have acquired permanent chloroplasts (Fig. 1).

Permanent chloroplasts provide a multitude of beneficial functions for photosynthetic eukaryotes, including carbon fixation, assimilation of ammonia into amino acids, assembly of iron-sulphur complexes, biosynthesis of aromatic amino acids and phenolic compounds, and dissipation of excess mitochondrial reducing potential (Balk and Lobreaux, 2005; Herrmann and Weaver, 1999; Hoefnagel et al., 1998; Weber and Flugge, 2002). Presumably, the most significant barrier to endosymbiotic establishment of permanent chloroplasts is the sheer complexity of the process. Most importantly, a wealth of processes required for chloroplast function in plants, including gene expression, stress signalling and chloroplast division, are dependent on the expression of genes located in the host nucleus (reviewed by Barkan, 2011; Beck, 2005; Miyagishima, 2011), and many of these are likely to be necessary for the establishment of permanent chloroplasts. Many of the genes encoding these
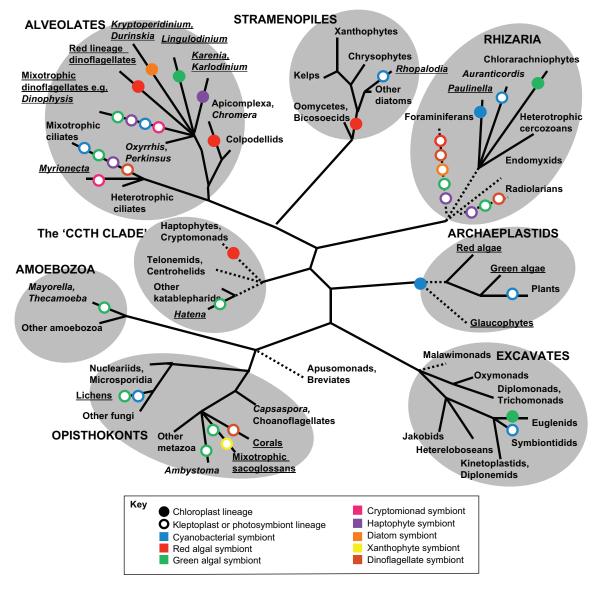
Fig. 1. The distribution of photosynthesis across the eukaryotes. A tree of the eukaryotes, based on information published previously (Dorrell and Smith, 2011; Stoecker et al., 2009), that shows the distribution and phylogenetic origins of permanent chloroplast lineages (solid circles), and kleptoplasts and photosymbionts (open circles), which are found in each branch of the tree of eukaryotes. The colour of each circle corresponds to the photosynthetic prokaryotic or eukaryotic lineage from which the chloroplast is derived; multiple rings on each branch correspond to different symbiont lineages found in different host taxa. For clarity, some phylogenetic relationships have been condensed; branch positions that are currently under debate are shown by broken lines. Branches are grouped into higher-order phylogenetic affiliations, as demarcated by a shaded ellipse, which is named in block capitals. Taxa mentioned elsewhere in this paper are underlined.

factors are derived from the symbiont and have been transferred to the host nucleus at some point during the process of endosymbiosis.

Studies of archaeplastid chloroplasts have provided some insights into the original features of the symbiont and the host: for example, that the symbiont was able to fix nitrogen (Deusch et al., 2008), and starch biosynthesis occurred in the host cytoplasm (Ball et al., 2011). However, the primary chloroplasts of archaeplastids and many secondary chloroplast lineages are ancient, having been acquired potentially over a billion years ago (Fig. 2) (Berney and Pawlowski, 2006; Parfrey et al., 2011) and it is therefore difficult to identify definitively which of these features were actually involved in the initial act of endosymbiosis. By contrast, several chloroplast lineages are

believed to have been acquired more recently, and studies of these lineages, as well as of photosymbionts and kleptoplasts, might help us understand the process of chloroplast establishment. In the past few years, several taxa have been characterised at a molecular level that may illuminate our understanding of the processes underpinning chloroplast establishment (supplementary material Table S1), and we first summarise these lineages briefly. We stress the importance of coordinating cellular processes in the host and symbiont, and consider the role of gene transfer from the symbiont to the host. We finally consider whether a mosaic of information from multiple different organisms may be responsible for the establishment of serial endosymbiotic associations. A glossary of relevant terms is shown in Box 1.
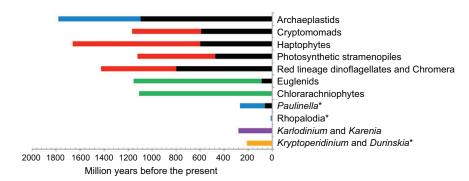
Fig. 2. A timeline of the origins of permanent chloroplasts across the eukaryotes. This timeline shows the range of dates over which chloroplast endosymbiotic events are believed to have occurred as coloured bars for different photosynthetic taxa. The colour of each bar corresponds to the origin of the respective chloroplast lineage as shown in Fig. 1. The range of each bar extends from the earliest date at which the taxon is believed to have diverged from its closest relatives that do not possess the given chloroplast lineage to the latest date at which the taxon is believed to have radiated; black regions correspond to dates after which the taxon is believed to have radiated (i.e. definitely after endosymbiosis has occurred). Divergence dates are taken from molecular estimates calibrated against fossil records (Parfrey et al., 2011) for all taxa apart from those asterisked; the divergence dates for *Paulinella*, *Rhopalodia*, *Kryptoperidinium* and *Durinskia*, as well as the radiation date of *Paulinella* are as given previously [(Berney and Pawlowski, 2006; Nakayama et al., 2011; Nowack et al., 2008), respectively]. Where alternative estimates are provided in one publication, the most extreme values are displayed here.

Novel chloroplasts, photosymbionts and kleptoplasts

One of the most notable examples of a recent chloroplast acquisition, representing a primary endosymbiosis independent of that in the archaeplastids, is that in the rhizarian *Paulinella chromatophora*. This organism contains stably transmitted cyanobacteria-like photosynthetic organelles termed 'chromatophores' (supplementary material Table S1). Sequence analysis of the *Paulinella* chromatophore genome confirms its cyanobacterial ancestry, and divergence estimates between different *Paulinella* isolates, and between *Paulinella* and close nonphotosynthetic relatives (Berney and Pawlowski, 2006; Marin et al., 2005; Nowack et al., 2008), indicate that the chromatophore was acquired 60 to 200 million years ago (Fig. 2). Another example that might represent an independent primary endosymbiosis is found in a group of diatoms, the Rhopalodiaceans. These also contain cyanobacterial-like structures, known as spheroid bodies, which might be involved in nitrogen fixation (Prechtl et al., 2004) and were probably acquired as recently as 12 million years ago (Nakayama et al., 2011) (Fig. 2). Many dinoflagellates, such as *Karlodinium*, *Karenia*, *Kryptoperidinium*, *Durinskia* and *Lepidodinium*, contain serial chloroplast lineages from a range of algal sources different from the ancestral red-algal-derived chloroplasts (supplementary material Table S1). These dinoflagellates must have acquired their serial chloroplasts following their divergence from other dinoflagellates, which is estimated to have occurred ~200 million years ago (see Fig. 1) (Berney and Pawlowski, 2006; Parfrey et al., 2011).

Several taxa harbouring photosymbionts or kleptoplasts are known to be able to retain their symbionts for extended periods (supplementary material Table S1). Corals represent one of the most striking examples of photosymbiont acquisition, in which certain strains of the dinoflagellate genus *Symbiodinium* can be stably acquired for the lifetime of the host (DeSalvo et al., 2010). The most dramatic kleptoplast association known to date occurs in the sacoglossan sea slug *Elysia chlorotica*, which can retain photosynthetically active kleptoplasts, derived from ingested xanthophyte algae, for up to 10 months (Rumpho et al., 2011).

Other closely related species in the genera *Elysia* and *Plakobranchus* are likewise able to sustain ingested kleptoplasts (Curtis et al., 2010; Haendeler et al., 2009). Other taxa harbouring kleptoplast lineages, such as the ciliate *Myrionecta rubra* and the dinoflagellate genus *Dinophysis*, are able to retain their symbionts over multiple generations of host cell division (Johnson, 2011a; Stoecker et al., 2009). The long-term retention of photosynthetic symbionts might be underpinned in these lineages by components of the host cell machinery. If so, this machinery might be analogous to that required for permanent chloroplast retention in photosynthetic eukaryotes.

How to avoid immediate destruction?

Photosynthetic symbioses are presumably derived from initial trophic interactions between a heterotrophic host and photosynthetic prey. The first barrier to the establishment of photosynthetic symbionts is likely to be their degradation or expulsion by the host. For example, although the sacoglossan *Elysia clarki* is able to sequester intact kleptoplasts from the green algae *Bryopsis plumata* and *Penicillus capitatus* for up to four months, *Elysia patina*, which feeds on the same algal sources, does not retain intact kleptoplasts in its digestive tracts (Curtis et al., 2007; Curtis et al., 2010). Even hosts that are able to maintain long-term symbionts can be selective in what they retain. For example, coral polyps typically take up multiple strains of the dinoflagellate genus *Symbiodinium*, but these are gradually reduced in number until often only one or two are identifiable in adults (DeSalvo et al., 2010; Voolstra et al., 2009). The potential for the early termination of possible symbiotic relationships clearly exists and must be avoided.

Recent expressed sequence tag (EST) and microarray studies have suggested that the induction of symbiosis between coral larvae and *Symbiodinium* cells induces significant changes in the host transcriptome. However, far greater transcriptomic changes are observed in coral larvae that are incubated with non-symbiotic prey, such as the brine shrimp *Acropora*, or with lines of *Symbiodinium* believed to be unable to enter symbiosis. These changes include the differential upregulation of genes involved in secondary metabolism, cytoskeletal remodelling and apoptosis

Box 1. Glossary

- **Archaeplastids.** A monophyletic group consisting of green algae and plants, red algae and glaucophytes. All of the archaeplastid lineages harbour primary chloroplasts.
- **Ciliates.** A clade of non-photosynthetic protists, closely related to dinoflagellates. Some ciliates such as *Myrionecta* retain kleptoplasts.
- **Cyanobacterium.** Oxygenic photosynthetic bacteria. The primary chloroplasts of archaeplastid lineages and of *Paulinella* are believed to have been derived from free-living cyanobacteria.
- **Dinoflagellates.** One of the most species-rich groups of algae. Some dinoflagellates are acquired as photosymbionts by corals. Different dinoflagellate lineages harbour different types of chloroplasts, and dinoflagellates are the only group of eukaryotes known to have undergone tertiary and serial endosymbiosis.
- **Endosymbiosis.** The process by which chloroplasts are believed to have originated, in which a free-living organism is uptaken by endocytosis, then converted into a stable symbiont. Primary endosymbiosis involves a prokaryotic symbiont, secondary endosymbiosis involves a eukaryotic symbiont containing a primary chloroplast and tertiary endosymbiosis involves a eukaryotic symbiont containing a secondary chloroplast.
- **Endosymbiotic gene transfer.** The relocation of genetic material from a symbiont to the nucleus of its host.
- **Establishment.** The transition of a photosynthetic symbiont to become a chloroplast.
- **Green algae.** A diverse group of photosynthetic eukaryotes, ranging from single-celled and colonial algae to complex multicellular organisms and including land plants. Secondary chloroplasts derived from green algae are present in several other algal lineages (e.g. the dinoflagellate *Lepidodinium*).
- **Haptophytes.** A group of algae harbouring secondary red-algal-derived chloroplasts; haptophyte shells form the principal components of chalk. Haptophytes have been taken up as tertiary chloroplasts by some dinoflagellates (e.g. *Karenia* and *Karlodinium*).
- **Kleptoplast.** A photosynthetic symbiont, specifically derived from a eukaryote, where only the chloroplasts of the symbiont are retained by the host.
- **Peripheral photosystem subunit.** Components of photosystems that are not part of the core photosynthetic machinery.
- **Photodamage.** The destruction or impairment of the photosynthetic machinery as a result of the light reactions of photosynthesis. Photodamage can occur either becuase of the direct activity of light or through the production of reactive oxygen species.
- **Photosynthetic symbiont.** A photosynthetic organism that is taken up by a non-photosynthetic host in a non-permanent symbiosis. Photosynthetic symbionts are divided in the text into two categories: kleptoplasts and photosymbionts.
- **Photosymbiont.** A photosynthetic symbiont where the whole of the symbiont cell is retained by the host.
- **Photosystem.** Multi-protein transmembrane complexes involved in driving photosynthetic electron transport. Photosystem II uses light to split water into oxygen, protons and electrons; photosystem I uses light to excite free electrons so that they can be used to reduce $NADP^+$.
- **Phylogenetic mosaic.** Something within an organism that is dependent on the expression of genes with a variety of different phylogenetic affinities, consistent with them having potentially been acquired from multiple distinct sources.
- **Red algae.** The second major archaeplastid lineage, in addition to green algae, containing single-celled and multicellular lineages. They differ from green algae in their light-harvesting pigments. Secondary chloroplasts derived from red algae are also contained in many other groups of algae (e.g. diatoms and haptophytes).
- **Retention.** The ability of a host to maintain a stable chloroplast or symbiont without destruction.
- **Rhizarians.** A diverse group of amoeboid and amoebo-flagellate protists. The only known photosynthetic lineages are chlorarachniophytes and *Paulinella*.
- **Sacoglossans.** A group of aquatic molluscs that feed by sucking the cytoplasmic contents of filamentous algae and seaweed. Several species, most notably *Elysia chlorotica*, retain kleptoplasts derived from their algal prey.
- **Xanthophytes.** Also referred to as yellow–green algae; members of the stramenopiles (i.e. close relatives of diatoms and kelps). The sacoglossan *Elysia chlorotica* harvests kleptoplasts from the xanthophyte *Vaucheria litorea*.

(Schnitzler and Weis, 2010; Voolstra et al., 2009; Yuyama et al., 2011). One of the earliest steps in the coral symbioses might therefore be the repression (or at least avoiding the induction) of genes that are involved in the degradation or expulsion of endocytosed prey (Voolstra et al., 2009).

Following uptake, the symbiont must continue dividing for it to persist through multiple host cell generations. In addition, the symbiont must be partitioned into both daughter cells during host cell division. The processes required for the replication and partitioning of chloroplasts (reviewed by Miyagishima, 2011) have yet to be extensively studied other than in archaeplastids, but appear to rely on the integration of the chloroplast division apparatus with components of the host cytoskeleton. Some components of these pathways are probably not established until late in endosymbiosis, as studies of some relatively stable symbioses suggest that symbiont division ceases after uptake, or that symbionts are only partitioned into one daughter cell following host cell division (Johnson et al., 2006; Minnhagen et al., 2008; Minnhagen et al., 2011; Okamoto and Inouye, 2006). However, the consistent subcellular distributions of many photosynthetic symbionts within host cells – for example the stellate structures formed by multiple kleptoplasts in *Dinophysis*, or the sublamellar distribution of kleptoplasts in *Myrionecta* (Garcia-Cuetos et al., 2010) – suggest that partitioning of symbionts by the host cytoskeleton might commence early in the process of chloroplast establishment.

Playing with fire – the need to avoid photodamage

Even if symbionts are not digested by the host and are capable of proliferating in the host cell environment, they might not be permanently retained. A newly acquired chloroplast is potentially a very dangerous thing for an inexperienced host. In addition to the desired products of photosynthesis, photosystems might generate reactive oxygen species that damage key components of the chloroplast photosynthetic machinery, thus reducing the ability of the chloroplast to function (Murata et al., 2007; Nishiyama et al., 2006). If this damage accumulates at a faster rate than it is repaired, the chloroplast might be degraded and the damage could extend beyond the chloroplast to the rest of the cell.

Although photodamage typically occurs under high-light conditions, it can also occur under low-light conditions, typically in organisms that are subjected to environmental stresses that reduce their capacity for photosynthesis or that impede the repair of damaged photosystems (Murata et al., 2007;

Nishiyama et al., 2006). It is therefore very likely that any photosynthetic symbiont will accumulate photodamage at some point during symbiosis. Over time, transient symbioses typically show a decline in photosynthetic performance (Park et al., 2008), loss of pigmentation (Garcia-Cuetos et al., 2010) and structural remodelling of symbiont thylakoids (Curtis et al., 2010), ultimately leading to degradation. These are consistent with progressive damage of the symbiont photosynthetic machinery, as would occur under oxidative stress.

The ability of some symbionts to persist for extended periods, despite the risk of oxidative stress, is likely to be dependent on three factors. First, the most stable symbionts will presumably be those that can remain intact for the longest time without support from their native cellular environment. For example, isolated chloroplasts of *Vaucheria litorea*, which are acquired as relatively stable kleptoplasts by the sea slug *Elysia chlorotica*, appear to have substantially longer lifespans than isolated spinach chloroplasts (Green et al., 2005; Rumpho et al., 2009). Second, hosts might specifically harvest factors from the symbiont that extend chloroplast lifespan. This process has been best characterised in the ciliate *Myrionecta rubra*, which, as well as acquiring kleptoplasts, retains the nuclei of its cryptomonad prey (Johnson et al., 2007). These nuclei remain transcriptionally active during symbiosis and might protect or repair damaged kleptoplasts (Johnson et al., 2007). Thirdly, the host might utilise endogenous metabolic and cellular pathways to maintain the symbiont. Hosts can minimise photodamage to the symbiont by synthesising light-protective compounds, such as mycosporine-like amino acids, which are produced in symbiotic lichens, corals and ciliates (Carreto and Carignan, 2011). Alternatively, stress might be minimised by the behaviour of hosts. For example, the sea slug *Elysia timida* avoids bright light sources and contracts under high photosynthetic flux, shading its kleptoplasts (Jesus et al., 2010; Schmitt and Waegele, 2011). These processes might allow host lineages to minimise oxidative stress, ultimately extending symbiont lifespan.

Gene transfer to the host nucleus

The genomes of extant chloroplasts are very different from those of their closest free-living relatives. Primary chloroplast genomes contain far fewer genes than cyanobacteria, ranging up to 250 in red algae, compared with a few thousand in cyanobacteria (Green, 2011; Howe et al., 2008b; Reith and Munholland, 1995). Gene loss also occurs during the acquisition of chloroplasts from eukaryotic sources, although the extent of this loss appears to vary between different lineages. The unusual genomes of red-algal-derived chloroplasts within many dinoflagellates probably have no more than 20 genes, so might have lost over 200 (Howe et al., 2008b). The haptophyte-derived chloroplasts of the dinoflagellate *Karlodinium veneficum* have lost over forty genes following endosymbiosis (Gabrielsen et al., 2011), whereas the diatom-derived chloroplasts of the dinoflagellates *Kryptoperidinium foliaceum* and *Durinskia baltica* have only lost three (Imanian et al., 2010). With a very few known exceptions, the nuclei and/or mitochondria of eukaryotic symbionts are not retained along with the secondary and tertiary chloroplasts (Archibald and Lane, 2009; Imanian et al., 2010; Johnson et al., 2007). The establishment of eukaryotic symbionts therefore implicitly involves the loss of extensive symbiont genetic information.

Genes lost from chloroplast lineages could either be lost completely or could be relocated to the host nucleus in a process termed endosymbiotic gene transfer (Fig. 3). This is believed to occur principally through the direct movement of DNA, following organelle degradation (Hanekamp and Thorsness, 1996; Sheppard et al., 2008; Thorsness et al., 1993). Alternative mechanisms have been proposed, such as transfer via RNA intermediates (reviewed by Kleine et al., 2009), but these are probably not responsible for the majority of examples (Kleine et al., 2009; Sheppard et al., 2011). Whatever the mechanism, a much greater rate of gene transfer is observed in taxa containing multiple chloroplasts per cell (Lister et al., 2003; Martin, 2003; Smith et al., 2011; Stegemann et al., 2003). This would suggest that immediately following gene transfer, host cells contain copies of the transferred gene in two subcellular compartments: in the nucleus and in the remaining chloroplasts that have not been degraded (Fig. 3). Although there is an extensive body of evidence for the presence of duplicated chloroplast DNA in the nuclei of photosynthetic eukaryotes (Richly and Leister, 2004; Smith et al., 2011) very few of these duplicated sequences appear to be functionally expressed (Jiroutova et al., 2010; Richly and Leister, 2004). It therefore appears that one of the most significant barriers to gene transfer is the functional incorporation of the relocated gene into the host cell machinery (Fig. 3). For chloroplast-derived genes, this will require the acquisition of elements such as nuclear promoters (Lloyd and Timmis, 2011) and the import of the gene products into the chloroplast through a complex protein translocation machinery (Fig. 3) (Jarvis, 2008).

However, in addition to chloroplast-derived genes, host cells undergoing secondary or tertiary endosymbiosis might acquire genes from the nuclei of their symbionts. These could be much more readily integrated into the host cell machinery, as they would already contain most of the elements that would allow them to be expressed in the host nucleus. If these genes encode proteins that are targeted to the chloroplast of the symbiont, they might additionally contain some of the sequence elements required for protein import.

Chloroplast genome reduction is likely to commence early in primary endosymbiosis. Extensive organelle genome reduction and rearrangement have been observed in the cyanobacterial-derived symbionts of *Paulinella chromatophora* (Nowack et al., 2008; Reyes-Prieto et al., 2010) and *Rhopalodia gibba* (Kneip et al., 2008). Of the genes lost from the former, over 30 have been identified as transferred to the nucleus (Nakayama and Ishida, 2009; Nowack et al., 2011) (supplementary material Table S2) and there is evidence for the import of the products of at least some of these genes into the chromatophore (Nowack and Grossman, 2012; Bodyl et al., 2010). With the exception of genes from the unusual red-algal-derived chloroplasts of some dinoflagellates, the transfer of genes from chloroplasts to the nucleus seems to be much more rare in secondary and tertiary chloroplasts, photosymbionts and kleptoplasts. For example, none of the genes believed to have been lost from the chloroplast of *Karlodinium veneficum* has yet been recovered from the nucleus of any serial dinoflagellate lineage (supplementary material Table S2) (Gabrielsen et al., 2011).

By contrast, transfers to the host from the nucleus of the precursors of secondary or tertiary chloroplasts and kleptoplasts are more common. For example, extensive gene transfer events have been identified in the serial dinoflagellates *Karlodinium*
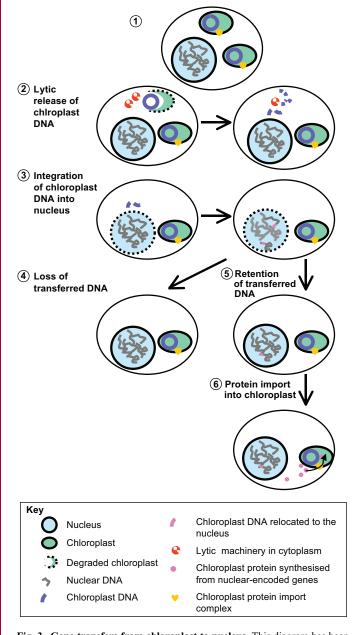
Key

Nucleus		Chloroplast DNA relocated to the nucleus	
Chloroplast		Lytic machinery in cytoplasm	
Degraded chloroplast		Chloroplast protein synthesised from nuclear-encoded genes	
Nuclear DNA		Chloroplast protein import complex	
Chloroplast DNA			

Fig. 3. Gene transfers from chloroplast to nucleus. This diagram has been adapted from information in previous publications (Jarvis, 2008; Kleine et al., 2009; Martin, 2003) and shows the different steps involved in the relocation of a gene from a chloroplast to the nucleus [denoted (1) to (6)]. Chloroplast DNA is released into the cytoplasm by chloroplast lysis, during which most of the chloroplast DNA might be degraded (2). Some DNA escapes degradation, traverses the nuclear envelope, either directly or during mitosis, and recombines with nuclear DNA, integrating into the nuclear genome (3). In the majority of cases, the transferred DNA is not retained permanently by the host nucleus and is lost over subsequent host cell generations (4). However, some transferred genes might be integrated into the host cell and retained, while chloroplast copies are lost (5). Proteins synthesised from nuclear genes might be imported into the chloroplast through a complex protein targeting and translocation machinery (6).

veneficum and *Karenia brevis*, which contain haptophyte-derived chloroplasts, and *Lepidodinium chlorophorum*, which contains green-algal-derived chloroplasts (supplementary material Table

S2) (Ishida and Green, 2002; Minge et al., 2010; Nosenko et al., 2006; Patron et al., 2006). All of these transfers are of genes that are typically nucleus-encoded in photosynthetic eukaryotes (supplementary material Table S2) (reviewed in Green, 2011). Preliminary evidence has also emerged for similar nuclear-to-nuclear gene transfer events in several kleptoplast-harbouring species, which suggests that gene transfer commences before chloroplast establishment. A number of sequences have been identified in *Elysia chlorotica* that are believed to represent genes transferred to the host from the nuclei of the *Vaucheria* kleptoplasts (supplementary material Table S2) (Pelletreau et al., 2011; Rumpho et al., 2011), and similar potential transferred genes have been identified in other kleptoplast-harbouring species, such as in *Elysia crispata* (Pierce et al., 2003) and *Dinophysis acuminata* (Wisecaver and Hackett, 2010).

The significance of gene transfer events in transient photosynthetic symbioses remains controversial. Although it is entirely possible that some of the putatively transferred genes identified in *Elysia chlorotica* represent lateral contaminants from remnant algal cells, several of these genes have been amplified by direct PCR of DNA from larvae apparently lacking symbionts, suggesting that algal genes have indeed been transferred to the host nucleus (Pierce et al., 2010). However, the low levels of transcripts from transferred genes observed in some studies of kleptoplast associations suggest that even if gene transfers occur, the expression of transferred genes is not essential for long-term symbiont retention (Pelletreau et al., 2011; Waegele et al., 2011). Although this might be true for some taxa, the majority of existing surveys of gene transfer events in kleptoplast associations are based on partial EST assemblies, hence they are unlikely to recover all the genes transferred to the nucleus. Furthermore, to minimise contamination from symbiont nucleic acids, many of the transcriptomic datasets obtained thus far are from symbiont-starved cultures (Rumpho et al., 2009; Schwartz et al., 2010; Wisecaver and Hackett, 2010) and might therefore have missed genes that are expressed only under non-starvation conditions. Further exploration of the nuclear genomes of kleptoplast harbouring taxa might therefore identify greater numbers of transferred genes.

Despite the current uncertainty over whether gene transfer is explicitly required for long-term symbiont retention, we argue that extensive gene transfer events must occur before the establishment of permanent chloroplasts. If gene transfer is dependent on symbiont lysis, transfer should occur freely in taxa harbouring transient symbionts, which can frequently lyse and be replaced by fresh symbionts. In the case of secondary and tertiary endosymbioses, one would also expect large-scale nuclear-to-nuclear gene transfers to occur before chloroplast establishment, given that – as detailed above – most taxa harbouring secondary or tertiary chloroplasts do not retain the symbiont nucleus. In particular, any genes located in the symbiont nucleus that were essential for chloroplast maintenance would have to be transferred to the host to allow the establishment of permanent chloroplasts.

Are certain types of genes preferentially transferred?
Given that the initial integration of DNA into the host nucleus is likely to be spontaneous and random (Fig. 3), the earliest transferred genes might be an essentially random subset of those contained within the symbiont. However, if certain types of genes are either more readily incorporated into the host or have a

Table 1. The evolutionary distribution and functions of subunits of photosystems I and II

Subunit	Gene transfers[a]	Subunit function[b]						Gene localisation[c]	Cyanobacterial mutant[d]
		A	B	C	D	E	F		
Photosystem I									
PsaA, PsaB[e]		■	■		■			green	black
PsaC			■	■		■		green	grey
PsaD					■			cyan	black
PsaE	Pf; Pm; Kv			■				cyan	grey
PsaF		■						cyan	
PsaG					■			cyan	N/A
PsaH						■		cyan	N/A
PsaI	Pf				■			green	grey
PsaJ					■			green	
PsaK	Pm				■			cyan	
PsaL					■	■		cyan	black
PsaM					■			cyan	grey
PsaN		■						cyan	N/A
PsaO					■	■		cyan	N/A
PsaP					■			cyan	N/A
Photosystem II									
PsbA			■	■	■			green	black
PsbB					■			green	black
PsbC					■			green	black
PsbD			■		■			green	black
PsbE			■					green	black
PsbF			■					green	black
PsbH					■			green	grey
PsbI					■			green	grey
PsbJ					■			green	grey
PsbK					■			green	grey
PsbL						■		green	black
PsbM	Da					■		cyan	
PsbN	Pm				■			cyan	
PsbO	Ec; Kb; Lc	■						cyan	grey
PsbR	Lc					■		cyan	N/A
PsbS	Et			■		■		cyan	N/A
PsbT						■		green	
PsbU, PsbQ[e]	Kb; Kv	■						cyan	
PsbV, PsbP[e]	Lc	■						cyan	grey
PsbW						■		cyan	N/A
PsbX					■			cyan	
PsbY					■			cyan	
PsbZ					■			cyan	

[a]Genes that have been identified from the nuclei of lineages harbouring long-term kleptoplasts or recently acquired chloroplasts (see supplementary material Tables S1 and S2). Each taxon is named in abbreviated form. Taxa harbouring kleptoplasts: Da, *Dinophysis acuminata*; Ec, *Elysia chlorotica*; Et, *Elysia timida*. Taxa harbouring recently acquired primary chloroplasts: Pf, *Paulinella chromatophora* FK01; Pm, *Paulinella chromatophora* M0880a. Taxa harbouring recently acquired secondary or tertiary chloroplasts: Kb, *Karenia brevis*; Kv, *Karlodinium veneficum*; Lc, *Lepidodinium chlorophorum*.

[b]Simplified functional roles of each subunit (Busch and Hippler, 2011; Chitnis, 2001; Nelson and Yocum, 2006; Shi and Schroder, 2004). A, interactions with electron donors (plastocyanin and alternatives for PSI; the M complex for PSII); B, electron transport through photosystems; C, interactions with electron acceptors (ferredoxin for PSI; plastoquinone for PSII); D, pigment binding; E, complex assembly and multimerisation; F, non-photochemical quenching.

[c]The typical subcellular localisation of each gene in photosynthetic eukaryotes, as described previously (Green, 2011; Howe et al., 2008b). Subunits shaded in blue are typically encoded by nuclear genes; subunits shaded in green are typically encoded by chloroplast genes.

[d]The deletion mutant phenotype for *Synechocystis* (Chauvat et al., 1989; Chitnis, 2001; Jansson et al., 1987; Komenda and Barber, 1995; Shen et al., 1998; Shi and Schroder, 2004; Smart et al., 1991). Mutants of subunits shaded in black are unable to grow photoautotrophically; mutants of subunits shaded in grey have impaired photosynthetic growth; unshaded subunits have negligible mutant phenotypes. Data are not available for subunits marked N/A.

[e]PsaA and PsaB are functionally similar proteins, and hence are listed as one entry. In the cases of PsbP, PsbQ, PsbU and PsbV, different genes are recorded to have orthologous functions in cyanobacteria and eukaryotes; information for these genes is listed together in one row, with the localisation of the eukaryotic genes (PsbU, PsbV) and the mutant phenotype of the prokaryotic genes (PsbP, PsbQ) shown.

selectively beneficial function in chloroplast establishment, they might be preferentially retained by the host following transfer. As noted above, the earliest genes that appear to be transferred in secondary and tertiary endosymbioses are those from the symbiont nucleus, consistent with them being able to be incorporated more swiftly into the host cell machinery. However, remarkably similar patterns of gene transfer appear to occur early in primary endosymbiosis. Of the over 30 genes recognized to date as having been relocated from the *Paulinella* chromatophore to the nucleus (supplementary material Table S2) (Nakayama and Ishida, 2009; Nowack et al., 2011), only one (photosystem I subunit I) is typically retained in other primary chloroplast genomes (Green, 2011) and at least one gene (photosystem I subunit E) is also known to have been relocated to the nucleus of *Karlodinium veneficum* (Patron et al., 2006). The convergent transfer of similar sets of genes to the hosts of both prokaryotic and eukaryotic symbionts would suggest that certain genes are preferentially transferred early in endosymbiosis, potentially because they have specific functional roles in chloroplast establishment.

The earliest transferred genes are distributed across a range of functional categories. For example, *Elysia chlorotica* is believed to have acquired genes encoding components of photosystem II (*psbO*), light-harvesting complexes (*lhcv1-4*), the Calvin cycle (phosphoribulokinase) and the chlorophyll biosynthesis pathway (chlorophyll synthase) (supplementary material Table S2) (Pierce et al., 2010; Rumpho et al., 2009; Rumpho et al., 2008; Schwartz et al., 2010). Certain classes of genes have been identified more frequently than one might expect if gene transfer were random. A particularly well-characterised example is the peripheral subunits of photosystems I and II. Unlike any other functional category, genes for peripheral photosystem subunits have been found in the nuclei of *Paulinella*, serial dinoflagellates and multiple taxa harbouring kleptoplasts (Table 1). Furthermore, photosystem genes have been identified in kleptoplast-harbouring species for which there is no other evidence of gene acquisition from the symbiont nucleus, for example, the *psbM* gene in the nucleus of *Dinophysis acuminata* and a candidate *psbS* in *Elysia timida* (Waegele et al., 2011; Wisecaver and Hackett, 2010).

The subunits of photosystems I and II that are typically nuclear-encoded have multiple functional roles and accordingly the products of the transferred genes do not seem to have a single conserved biochemical function, with functions ranging instead from pigment binding (PsaK and PsbN) to stabilising the donors and acceptors of photosystem electron transport (PsaE and PsbO) and effecting non-photochemical quenching (PsbS) (Table 1) (Busch and Hippler, 2011; Nelson and Yocum, 2006). Despite this diversity of function, very few of the transferred subunits appear to be essential for photosynthesis (Table 1). Deletion mutants of *Synechocystis* for *psbM*, *psaE*, *psaK* or *psaI* typically show only limited differences in growth rate or photosynthetic electron transport compared to wild-type lines in replete medium and under low-light irradiance (Bentley et al., 2008; Chitnis et al., 1989a; Naithani et al., 2000; Xu et al., 1995). By contrast, the subunits of photosystem I and II that are typically encoded in chloroplast genomes (such as PsaA, -B, or -C and PsbA) are essential for photosynthesis, as analogous deletion mutants are unable to grow photoautotrophically (Gong et al., 2003; Jansson et al., 1987; Smart et al., 1991). The only significant exception to this is PsaD, a factor involved in binding ferredoxin to photosystem I. It is encoded in the nucleus of most eukaryotes

and exhibits a non-photoautotrophic mutant phenotype in *Synechocystis* (Barth et al., 1998; Chitnis et al., 1989b). However, *psaD* is not known to be nuclear either in kleptoplast-harbouring species or in *Paulinella.* The photosystem subunit genes that are typically relocated to the host are therefore not rate limiting for photosynthesis under standard physiological conditions.

Instead, many of the subunits whose genes are frequently transferred appear to be important for efficient photosynthesis under stress conditions. *Synechocystis* deletion mutants for the transferred subunits exhibit increased sensitivity to high light (*psbO*), high temperature (*psaI*, *psbU*) and nutrient deprivation conditions (*psbP*, *psbU*) (Balint et al., 2006; Komenda and Barber, 1995; Nishiyama et al., 1999; Shen et al., 1997; Thornton et al., 2004; Xu et al., 1995). In addition, lines lacking these subunits upregulate enzymes involved in scavenging reactive oxygen species, suggesting that their absence elevates sensitivity to oxidative stress (Balint et al., 2006; Jeanjean et al., 2008).

The peripheral subunits of photosystems I and II therefore might play an important role in altering oxidative stress in symbionts. They would not only themselves be extremely vulnerable to damage from oxidative stress, as they form part of the complexes from which reactive oxygen species are generated, but their damage would theoretically increase the amount of reactive oxygen species produced by the symbiont (Fig. 4A). The acquisition of genes encoding the peripheral subunits of photosystems I and II by the host could have a significant impact on symbiont stability. Transferring the symbiont copy of the gene to the host nucleus not only allows it to be retained (if it would otherwise be lost with the symbiont nucleus) but also allows continuing production of functional protein even if the symbiont itself were physiologically compromised by photodamage (Fig. 4B).

It has been suggested that genes whose products affect the redox poise of the chloroplast are preferentially retained in chloroplast genomes to allow rapid redox regulation of expression (Allen, 1993; Allen, 2003). Our proposal is not inconsistent with this because it is unlikely that the transferred subunits would need to be specifically redox regulated, as their relative dispensability for photosynthetic function implies that over- or mis-expression would not affect photosystem activity (Fig. 4B). Instead, the host would only have to ensure that they were expressed at a sufficient level to ensure the efficient function of the chloroplast photosynthetic machinery. We argue that the earliest genes transferred in endosymbiosis are not essential for chloroplast function but assist in avoiding oxidative stress that would otherwise prevent long-term chloroplast retention.

Chloroplasts as mosaics

One largely outstanding question is whether the genes involved in chloroplast maintenance are derived from single or multiple phylogenetic sources. This is particularly pertinent to instances of serial endosymbiosis. Host lineages undergoing serial endosymbiosis might already possess genes for components of the chloroplast proteome, retained from their original chloroplasts, and these could be utilised to maintain the new chloroplast lineages. Red-algal-derived nuclear genes for chloroplast-targeted proteins have been identified in each of *Karenia brevis*, *Karlodinium veneficum*, *Lepidodinium chlorophorum* and *Dinophysis acuminata*, in addition to nuclear

genes in diatoms and other taxa harbouring red-algal-derived chloroplasts might point to a cryptic green algal symbiosis in these lineages (Dorrell and Smith, 2011; Moustafa et al., 2009). It must be noted that the precise evolutionary origin of many of these genes remains unproven and controversial. However, it is theoretically possible that hosts could, over their evolutionary history, accumulate what has been termed a 'shopping bag' of genes from multiple symbiotic associations, which would facilitate chloroplast retention (Howe et al., 2008a; Larkum et al., 2007).

Conclusions

The establishment of permanent chloroplasts from prior photosynthetic symbioses almost certainly requires major evolutionary innovations, both within the host and in the symbiont. Recent molecular studies of potential model organisms, such as *Elysia chlorotica* and *Paulinella chromatophora*, have led to a more detailed understanding of the processes underpinning chloroplast establishment. The availability of a genome sequence for at least one lineage harbouring an early endosymbiotic intermediate would be invaluable for identifying and quantifying the extent and timing of gene transfer events in endosymbiosis. More extensive molecular and cellular information from these taxa might additionally help resolve the questions of how chloroplast protein translocation systems and division machineries originate, and whether photosystem genes and others involved in minimising oxidative stress are indeed preferentially relocated to the host early in chloroplast establishment.

Greater exploration of taxa that have undergone serial endosymbiosis or serial symbiont replacement will help elucidate the impact of mosaic symbiotic associations on chloroplast establishment. The identification of several new lineages of dinoflagellate, in addition to *Dinophysis*, that can maintain long-term photosymbionts or kleptoplasts (Escalera et al., 2011; Gast et al., 2007; Yamaguchi et al., 2011) should provide a significant opportunity to expand our understanding of which genes derived from prior endosymbiotic associations are recruited in the establishment of new symbionts. At a taxonomically broader level, more rigorous exploration of the phylogenetic derivation of chloroplast-targeted genes that are nuclear-encoded in photosynthetic taxa not known to have undergone serial endosymbiosis might cast further light on the validity of the 'shopping bag' model and indicate whether chloroplasts arise by the symbiotic integration of multiple organisms – that is, the host, the symbiont and the genetic remnants of prior symbionts.

References

Allen, J. F. (1993). Control of gene expression by redox potential and the requirement for chloroplast and mitochondrial genomes. *J. Theor. Biol.* **165**, 609-631.
Allen, J. F. (2003). The function of genomes in bioenergetic organelles. *Philos. Trans. R. Soc. Biol.* **358**, 19-37.
Archibald, J. M. and Lane, C. E. (2009). Going, going, not quite gone: nucleomorphs as a case study in nuclear genome reduction. *J. Heredity* **100**, 582-590.
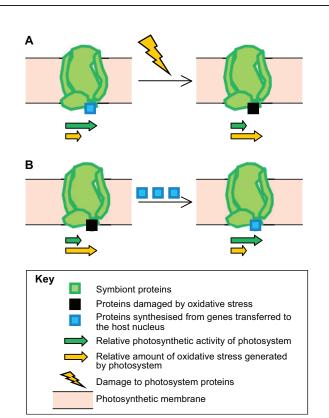

Fig. 4. A theoretical model for minimising oxidative stress through nucleus-encoded peripheral photosystem subunits. This diagram shows the potential advantage of relocating genes that encode peripheral subunits of photosystems I and II for chloroplast viability, using a stylised depiction of a transmembrane photosystem complex. Coloured arrows beneath the photosystem complex depict the relative biochemical activity of each photosystem. (**A**) In addition to photosynthesis, the activity of any photosystem will generate low levels of reactive oxygen species. The loss or damage of a peripheral photosystem subunit by oxidative species will yield a defective photosystem, augmenting the oxidative stress generated through photosynthesis (right-hand side). (**B**) Defective photosystems might be repaired by peripheral subunits synthesised from nuclear-encoded genes, reducing the amount of reactive oxygen species produced by the symbiont. Excess subunits are not incorporated into active photosystem complexes and therefore do not affect the physiology of the symbiont.

genes that were probably acquired with their tertiary haptophyte or green algal chloroplasts (Minge et al., 2010; Nosenko et al., 2006; Patron et al., 2006; Wisecaver and Hackett, 2010). These red-algal-derived genes might have accelerated the establishment of new chloroplast lineages.

If extensive gene transfer commences before chloroplast establishment, a mosaic chloroplast proteome might underpin an even wider range of endosymbioses. Nuclear genes for chloroplast-targeted proteins of multiple phylogenetic affinities have been identified in many taxa that are not known to have undergone serial endosymbiosis, including *Paulinella chromatophora*, the chlorarachniophyte *Bigelowiella natans*, the euglenid *Euglena gracilis* (Archibald et al., 2003; Maruyama et al., 2011; Nowack et al., 2011) and, notably, *Dinophysis acuminata*, which appears to have acquired at least two genes for chloroplast-targeted proteins from a haptophyte lineage donor (Wisecaver and Hackett, 2010). On a larger scale, the identification of extensive numbers of green-algal-derived

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.

Balint, I., Bhattacharya, J., Perelman, A., Schatz, D., Moskovitz, Y., Keren, N. and Schwarz, R. (2006). Inactivation of the extrinsic subunit of photosystem II, PsbU, in *Synechococcus* PCC 7942 results in elevated resistance to oxidative stress. *FEBS Lett.* **580**, 2117-2122.

Balk, J. and Lobreaux, S. (2005). Biogenesis of iron-sulfur proteins in plants. *Trends Plant Sci.* **10**, 324-331.

Ball, S., Colleoni, C., Cenci, U., Raj, J. N. and Tirtiaux, C. (2011). The evolution of glycogen and starch metabolism in eukaryotes gives molecular clues to understand the establishment of plastid endosymbiosis. *J. Exp. Bot.* **62**, 1775-1801.

Barkan, A. (2011). Expression of plastid genes: organelle-specific elaborations on a prokaryotic scaffold. *Plant Physiol.* **155**, 1520-1532.

Barth, P., Lagoutte, B. and Sétif, P. (1998). Ferredoxin reduction by photosystem I from *Synechocystis* sp. PCC 6803: toward an understanding of the respective roles of subunits PsaD and PsaE in ferredoxin binding. *Biochemistry.* **37**, 16233-16241.

Beck, C. F. (2005). Signaling pathways from the chloroplast to the nucleus. *Planta* **222**, 743-756.

Bentley, F. K., Luo, H., Dilbeck, P., Burnap, R. L. and Eaton-Rye, J. J. (2008). Effects of Inactivating psbM and psbT on Photodamage and Assembly of Photosystem II in *Synechocystis* sp PCC 6803. *Biochemistry.* **47**, 11637-11646.

Berney, C. and Pawlowski, J. (2006). A molecular time-scale for eukaryote evolution recalibrated with the continuous microfossil record. *Proc. R. Soc. B. Biol. Sci.* **273**, 1867-1872.

Bodyl, A., Mackiewicz, P. and Stiller, J. W. (2010). Comparative genomic studies suggest that the cyanobacterial endosymbionts of the amoeba *Paulinella chromatophora* possess an import apparatus for nuclear-encoded proteins. *Plant Biol.* **12**, 639-649.

Busch, A. and Hippler, M. (2011). The structure and function of eukaryotic photosystem I. *Biochim. Biophys. Acta Bioenerget.* **1807**, 864-877.

Carreto, J. I. and Carignan, M. O. (2011). Mycosporine-like amino acids: relevant secondary metabolites. chemical and ecological aspects. *Mar. Drugs* **9**, 387-446.

Chauvat, F., Rouet, P., Bottin, H. and Boussac, A. (1989). Mutagenesis by random cloning of an *Escherichia coli* kanamycin resistance geen into the genome of the cyanobacterium *Synechocystis* PCC 803: selection of mutants defective in photosynthesis. *Mol. Gen. Genet.* **216**, 51-59.

Chitnis, P. R. (2001). Photosystem I: Function and Physiology. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52**, 593-626.

Chitnis, P. R., Reilly, P. A., Miedel, M. C. and Nelson, N. (1989a). Structure and targeted mutagenesis of the gene encoding 8-kDa subunit of photosystem I from the cyanobacterium *Synechocystis* sp. PCC 6803. *J. Biol. Chem.* **264**, 18374-18380.

Chitnis, P. R., Reilly, P. A. and Nelson, N. (1989b). Insertional inactivation of the gene encoding subunit II of photosystem I from the cyanobacterium *Synechocystis* sp. PCC 6803. *J. Biol. Chem.* **264**, 18381-18385.

Curtis, N. E., Pierce, S. K., Massey, S. E., Schwartz, J. A. and Maugel, T. K. (2007). Newly metamorphosed *Elysia clarki* juveniles feed on and sequester chloroplasts from algal species different from those utilized by adult slugs. *Mar. Biol.* **150**, 797-806.

Curtis, N. E., Schwartz, J. A. and Pierce, S. K. (2010). Ultrastructure of sequestered chloroplasts in sacoglossan gastropods with differing abilities for plastid uptake and maintenance. *Invert. Biol.* **129**, 297-308.

DeSalvo, M. K., Sunagawa, S., Fisher, P. L., Voolstra, C. R., Iglesias-Prieto, R. and Medina, M. (2010). Coral host transcriptomic states are correlated with *Symbiodinium* genotypes. *Mol. Ecol.* **19**, 1174-1186.

Deusch, O., Landan, G., Roettger, M., Gruenheit, N., Kowallik, K. V., Allen, J. F., Martin, W. and Dagan, T. (2008). Genes of cyanobacterial origin in plant nuclear genomes point to a heterocyst-forming plastid ancestor. *Mol. Biol. Evol.* **25**, 748-761.

Dorrell, R. G. and Smith, A. G. (2011). Do red and green make brown? perspectives on plastid acquisitions within the chromalveolates. *Eukaryot. Cell* **10**, 856-868.

Escalera, L., Reguera, B., Takishita, K., Yoshimatsu, S., Koike, K. and Koike, K. (2011). Cyanobacterial endosymbionts in the benthic dinoflagellate *Sinophysis canaliculata* (Dinophysiales, Dinophyceae). *Protist* **162**, 304-314.

Gabrielsen, T. M., Minge, M. A., Espelund, M., Tooming-Klunderud, A., Patil, V., Nederbragt, A. J., Otis, C., Turmel, M., Shalchian-Tabrizi, K., Lemieux, C. et al. (2011). Genome evolution of a tertiary dinoflagellate plastid. *PLoS ONE* **6**, e19132.

Garcia-Cuetos, L., Moestrup, O., Hansen, P. J. and Daugbjerg, N. (2010). The toxic dinoflagellate *Dinophysis acuminata* harbors permanent chloroplasts of cryptomonad origin, not kleptochloroplasts. *Harmful Algae* **9**, 25-38.

Gast, R. J., Moran, D. M., Dennett, M. R. and Caron, D. A. (2007). Kleptoplasty in an Antarctic dinoflagellate: caught in evolutionary transition? *Env. Microbiol.* **9**, 39-45.

Gong, X. M., Agalarov, R., Brettel, K. and Carmeli, C. (2003). Control of electron transport in photosystem I by the iron-sulfur cluster FX in response to intra- and intersubunit interactions. *J. Biol. Chem.* **278**, 19141-19150.

Green, B. R. (2011). Chloroplast genomes of photosynthetic eukaryotes. *Plant J.* **66**, 34-44.

Green, B. J., Fox, T. C. and Rumpho, M. E. (2005). Stability of isolated algal chloroplasts that participate in a unique mollusc/kleptoplast association. *Symbiosis* **40**, 31-40.

Haendeler, K., Grzymbowski, Y. P., Krug, P. J. and Waegele, H. (2009). Functional chloroplasts in metazoan cells - a unique evolutionary strategy in animal life. *Front. Zool.* **6**, 28.

Hanekamp, T. and Thorsness, P. E. (1996). Inactivation of YME2/RNA12, which encodes an integral inner mitochondrial membrane protein, causes increased escape of DNA from mitochondria to the nucleus in *Saccharomyces cerevisiae. Mol. Cell. Biol.* **16**, 2764-2771.

Herrmann, K. M. and Weaver, L. M. (1999). The shikimate pathway. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 473-503.

Hoefnagel, M. H. N., Atkin, O. K. and Wiskich, J. T. (1998). Interdependence between chloroplasts and mitochondria in the light and the dark. *Biochim. Biophys. Acta Bioenerget.* **1366**, 235-255.

Howe, C. J., Barbrook, A. C., Nisbet, R. E. R., Lockhart, P. J. and Larkum, A. W. D. (2008a). The origin of plastids. *Philos. Trans. R. Soc. Biol.* **363**, 2675-2685.

Howe, C. J., Nisbet, R. E. R. and Barbrook, A. C. (2008b). The remarkable chloroplast genome of dinoflagellates. *J. Exp. Bot.* **59**, 1035-1045.

Imanian, B., Pombert, J. F. and Keeling, P. J. (2010). The complete plastid genomes of the two 'dinotoms' *Durinskia baltica* and *Kryptoperidinium foliaceum. PLoS ONE* **5**, e10711.

Ishida, K. and Green, B. R. (2002). Second- and third-hand chloroplasts in dinoflagellates: phylogeny of oxygen-evolving enhancer 1 (PsbO) protein reveals replacement of a nuclear-encoded plastid gene by that of a haptophyte tertiary endosymbiont. *Proc. Natl. Acad. Sci. USA* **99**, 9294-9299.

Jansson, C., Debus, R. J., Osiewacz, H. D., Gurevitz, M. and McIntosh, L. (1987). Construction of an Obligate Photoheterotrophic Mutant of the Cyanobacterium *Synechocystis* 6803: Inactivation of the psbA Gene Family. *Plant Physiol.* **85**, 1021-1025.

Jarvis, P. (2008). Targeting of nucleus-encoded proteins to chloroplasts in plants. *New Phytol.* **179**, 257-285.

Jeanjean, R., Latifi, A., Matthijs, H. C. P. and Havaux, M. (2008). The PsaE subunit of photosystem I prevents light-induced formation of reduced oxygen species in the cyanobacterium *Synechocystis* sp PCC 6803. *Biochim. Biophys. Acta Bioenerget.* **1777**, 308-316.

Jesus, B., Ventura, P. and Calado, G. (2010). Behaviour and a functional xanthophyll cycle enhance photo-regulation mechanisms in the solar-powered sea slug *Elysia timida* (Risso, 1818). *J. Exp. Mar. Biol. Ecol.* **395**, 98-105.

Jiroutova, K., Koreny, L., Bowler, C. and Obornik, M. (2010). A gene in the process of endosymbiotic transfer. *PLoS ONE* **5**, e13234.

Johnson, M. D. (2011a). Acquired phototrophy in ciliates: a review of cellular interactions and structural adaptations. *J. Eukaryot. Microbiol.* **58**, 185-195.

Johnson, M. D. (2011b). The acquisition of phototrophy: adaptive strategies of hosting endosymbionts and organelles. *Photosynthesis Res.* **107**, 117-132.

Johnson, M. D., Tengs, T., Oldach, D. and Stoecker, D. K. (2006). Sequestration, performance, and functional control of cryptophyte plastids in the ciliate *Myrionecta rubra* (Ciliophora). *J. Phycol.* **42**, 1235-1246.

Johnson, M. D., Oldach, D., Delwiche, C. F. and Stoecker, D. K. (2007). Retention of transcriptionally active cryptophyte nuclei by the ciliate *Myrionecta rubra. Nature* **445**, 426-428.

Kim, E. and Archibald, J. M. (2009). Diversity and evolution of plastids and their genomes. *Plant Cell Monogr.* **13**, 1-39.

Kleine, T., Maier, U. G. and Leister, D. (2009). DNA transfer from organelles to the nucleus: the idiosyncratic genetics of endosymbiosis. *Annu. Rev. Plant Biol.* **60**, 115-138.

Kneip, C., Voss, C., Lockhart, P. J. and Maier, U. G. (2008). The cyanobacterial endosymbiont of the unicellular algae *Rhopalodia gibba* shows reductive genome evolution. *BMC Evol. Biol.* **8**, 30.

Komenda, J. and Barber, J. (1995). Comparison of psbO and psbH deletion mutants of *Synechocystis* PCC 6803 indicates that degradation of D1 protein is regulated by the QB site and dependent on protein synthesis. *Biochemistry.* **34**, 9625-9631.

Larkum, A. W. D., Lockhart, P. J. and Howe, C. J. (2007). Shopping for plastids. *Trends Plant Sci.* **12**, 189-195.

Lister, D. L., Bateman, J. M., Purton, S. and Howe, C. J. (2003). DNA transfer from chloroplast to nucleus is much rarer in *Chlamydomonas* than in tobacco. *Gene* **316**, 33-38.

Lloyd, A. H. and Timmis, J. N. (2011). The origin and characterization of new nuclear genes originating from a cytoplasmic organellar genome. *Mol. Biol. Evol.* **28**, 2019-2028.

Marin, B., Nowack, E. C. M. and Melkonian, M. (2005). A plastid in the making: Evidence for a second primary endosymbiosis. *Protist* **156**, 425-432.

Martin, W. (2003). Gene transfer from organelles to the nucleus: Frequent and in big chunks. *Proc. Natl. Acad. Sci. USA* **100**, 8612-8614.

Maruyama, S., Suzaki, T., Weber, A. P. M., Archibald, J. M. and Nozaki, H. (2011). Eukaryote-to-eukaryote gene transfer gives rise to genome mosaicism in euglenids. *BMC Evol. Biol.* **11**, 105.

Minge, M. A., Shalchian-Tabrizi, K., Torresen, O. K., Takishita, K., Probert, I., Inagaki, Y., Klaveness, D. and Jakobsen, K. S. (2010). A phylogenetic mosaic plastid proteome and unusual plastid-targeting signals in the green-colored dinoflagellate *Lepidodinium chlorophorum. BMC Evol. Biol.* **10**, 191.

Minnhagen, S., Carvalho, W. F., Salomon, P. S. and Janson, S. (2008). Chloroplast DNA content in *Dinophysis* (Dinophyceae) from different cell cycle stages is consistent with kleptoplasty. *Env. Microbiol.* **10**, 2411-2417.

Minnhagen, S., Kim, M., Salomon, P. S., Yih, W., Graneli, E. and Park, M. G. (2011). Active uptake of kleptoplastids by *Dinophysis caudata* from its ciliate prey *Myrionecta rubra. Aquat. Microb. Ecol.* **62**, 99-108.

Miyagishima, S. (2011). Mechanism of Plastid Division: From a Bacterium to an Organelle. *Plant Physiol.* **155**, 1533-1544.

Moustafa, A., Beszteri, B., Maier, U. G., Bowler, C., Valentin, K. and Bhattacharya, D. (2009). Genomic footprints of a cryptic plastid endosymbiosis in diatoms. *Science* **324**, 1724-1726.

Murata, N., Takahashi, S., Nishiyama, Y. and Allakhverdiev, S. I. (2007). Photoinhibition of photosystem II under environmental stress. *Biochim. Biophys. Acta.* **1767**, 414-421.

Naithani, S., Hou, J. M. and Chitnis, P. R. (2000). Targeted inactivation of the psaK1, psaK2 and psaM genes encoding subunits of Photosystem I in the cyanobacterium *Synechocystis* sp PCC 6803. *Photosynthesis Res.* **63**, 225-236.

Nakayama, T. and Ishida, K. (2009). Another acquisition of a primary photosynthetic organelle is underway in *Paulinella chromatophora*. *Curr. Biol.* **19**, 284-285.

Nakayama, T., Ikegami, Y., Nakayama, T., Ishida, K.-i., Inagaki, Y. and Inouye, I. (2011). Spheroid bodies in rhopalodiacean diatoms were derived from a single endosymbiotic cyanobacterium. *J. Plant Res.* **124**, 93-97.

Nelson, N. and Yocum, C. F. (2006). Structure and function of photosystems I and II. *Annu. Rev. Plant Biol.* **57**, 521-565.

Nishiyama, Y., Los, D. A. and Murata, N. (1999). PsbU, a protein associated with photosystem II, is required for the acquisition of cellular thermotolerance in *Synechococcus* species PCC 7002. *Plant Physiol.* **120**, 301-308.

Nishiyama, Y., Allakhverdiev, S. I. and Murata, N. (2006). A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II. *Biochim. Biophys. Acta.* **1757**, 742-749.

Nosenko, T., Lidie, K. L., Van Dolah, F. M., Lindquist, E., Cheng, J. F. and Bhattacharya, D. (2006). Chimeric plastid proteome in the florida "red tide" dinoflagellate *Karenia brevis*. *Mol. Biol. Evol.* **23**, 2026-2038.

Nowack, E. C. M. and Grossman, A. R. (2012). Trafficking of protein into the recently established photosynthetic organelles of *Paulinella chromatophora*. *Proc. Natl. Acad. Sci. USA,* **109**, 5340-5345.

Nowack, E. C. M., Melkonian, M. and Gloeckner, G. (2008). Chromatophore genome sequence of *Paulinella* sheds light on acquisition of photosynthesis by eukaryotes. *Curr. Biol.* **18**, 410-418.

Nowack, E. C. M., Vogel, H., Groth, M., Grossman, A. R., Melkonian, M. and Glockner, G. (2011). Endosymbiotic gene transfer and transcriptional regulation of transferred genes in *Paulinella chromatophora*. *Mol. Biol. Evol.* **28**, 407-422.

Okamoto, N. and Inouye, I. (2006). *Hatena arenicola* gen. et sp nov., a katablepharid undergoing probable plastid acquisition. *Protist* **157**, 401-419.

Parfrey, L. W., Lahr, D. J., Knoll, A. H. and Katz, L. A. (2011). Estimating the timing of early eukaryotic diversification with multigene molecular clocks. *Proc. Natl. Acad. Sci. USA* **108**, 13624-13629.

Park, M. G., Park, J. S., Kim, M. and Yih, W. (2008). Plastid dynamics during survival of *Dinophysis caudata* without its ciliate prey. *J. Phycol.* **44**, 1154-1163.

Patron, N. J., Waller, R. F. and Keeling, P. J. (2006). A tertiary plastid uses genes from two endosymbionts. *J. Mol. Biol.* **357**, 1373-1382.

Pelletreau, K. N., Bhattacharya, D., Price, D. C., Worful, J. M., Moustafa, A. and Rumpho, M. E. (2011). Sea slug kleptoplasty and plastid maintenance in a metazoan. *Plant Physiol.* **155**, 1561-1565.

Pierce, S. K., Massey, S. E., Hanten, J. J. and Curtis, N. E. (2003). Horizontal transfer of functional nuclear genes between multicellular organisms. *Biol. Bull.* **204**, 237-240.

Pierce, S. K., Curtis, N. E. and Schwartz, J. A. (2010). Chlorophyll synthesis by a sea slug (*Elysia chlorotica*). *Integrat. Comparat. Biol.* **50**, 137.

Prechtl, 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.

Reith, M. and Munholland, J. (1995). Complete nucleotide sequence of the *Porphyra purpurea* chloroplast genome. *Plant Mol. Biol. Rep.* **13**, 333-335.

Reyes-Prieto, A., Yoon, H. S., Moustafa, A., Yang, E. C., Andersen, R. A., Boo, S. M., Nakayama, T., Ishida, K. and Bhattacharya, D. (2010). Differential gene retention in plastids of common recent origin. *Mol. Biol. Evol.* **27**, 1530-1537.

Richly, E. and Leister, D. (2004). NUPTs in sequenced eukaryotes and their genomic organization in relation to NUMTs. *Mol. Biol. Evol.* **21**, 1972-1980.

Rumpho, M. E., Worful, J. M., Lee, J., Kannan, K., Tyler, M. S., Bhattacharya, D., Moustafa, A. and Manhart, J. R. (2008). Horizontal gene transfer of the algal nuclear gene psbO to the photosynthetic sea slug *Elysia chlorotica*. *Proc. Natl. Acad. Sci. USA* **105**, 17867-17871.

Rumpho, M. E., Pochareddy, S., Worful, J. M., Summer, E. J., Bhattacharya, D., Pelletreau, K. N., Tyler, M. S., Lee, J., Manhart, J. R. and Soule, K. M. (2009). Molecular characterization of the calvin cycle enzyme phosphoribulokinase in the stramenopile alga *Vaucheria litorea* and the plastid hosting mollusc *Elysia chlorotica*. *Mol. Plant* **2**, 1384-1396.

Rumpho, M. E., Pelletreau, K. N., Moustafa, A. and Bhattacharya, D. (2011). The making of a photosynthetic animal. *J. Exp. Biol.* **214**, 303-311.

Schmitt, V. and Waegele, H. (2011). Behavioural adapatations in relation to long-term retention of endosymbiotic chloroplasts in the sea slug *Elysia timida* (Opisthobranchia, Sacoglossa). *Thalass.* **27**, 225-238.

Schnitzler, C. E. and Weis, V. M. (2010). Coral larvae exhibit few measurable transcriptional changes during the onset of coral-dinoflagellate endosymbiosis. *Mar. Genom.* **3**, 107-116.

Schwartz, J. A., Curtis, N. E. and Pierce, S. K. (2010). Using algal transcriptome sequences to identify transferred genes in the sea slug, *Elysia chlorotica*. *Evol. Biol.* **37**, 29-37.

Shen, J. R., Ikeuchi, M. and Inoue, Y. (1997). Analysis of the psbU gene encoding the 12-kDa extrinsic protein of photosystem II and studies on its role by deletion mutagenesis in *Synechocystis* sp PCC 6803. *J. Biol. Chem.* **272**, 17821-17826.

Shen, J. R., Qian, M., Inoue, Y. and Burnap, R. L. (1998). Functional characterization of *Synechocystis* sp. PCC 6803 Delta psbU and Delta psbV mutants reveals important roles of cytochrome c-550 in cyanobacterial oxygen evolution. *Biochemistry* **37**, 1551-1558.

Sheppard, A. E., Ayliffe, M. A., Blatch, L., Day, A., Delaney, S. K., Khairul-Fahmy, N., Li, Y., Madesis, P., Pryor, A. J. and Timmis, J. N. (2008). Transfer of plastid DNA to the nucleus is elevated during male gametogenesis in tobacco. *Plant Physiol.* **148**, 328-336.

Sheppard, A. E., Madesis, P., Lloyd, A. H., Day, A., Ayliffe, M. A. and Timmis, J. N. (2011). Introducing an RNA editing requirement into a plastid-localised transgene reduces but does not eliminate functional gene transfer to the nucleus. *Plant Mol. Biol.* **76**, 299-309.

Shi, L. X. and Schroder, W. P. (2004). The low molecular mass subunits of the photosynthetic supracomplex, photosystem II. *Biochim. Biophys. Acta Bioenerget.* **1608**, 75-96.

Smart, L. B., Anderson, S. L. and McIntosh, L. (1991). Targeted genetic inactivation of the photosystem I reaction center in the cyanobacterium *Synechocystis* sp. PCC 6803. *EMBO J.* **10**, 3289-3296.

Smith, D. R., Crosby, K. and Lee, R. W. (2011). Correlation between nuclear plastid DNA abundance and plastid number supports the limited transfer window hypothesis. *Genom. Biol. Evol.* **3**, 365-371.

Stegemann, S., Hartmann, S., Ruf, S. and Bock, R. (2003). High-frequency gene transfer from the chloroplast genome to the nucleus. *Proc. Natl. Acad. Sci. USA* **100**, 8828-8833.

Stoecker, D. K., Johnson, M. D., de Vargas, C. and Not, F. (2009). Acquired phototrophy in aquatic protists. *Aquat. Microb. Ecol.* **57**, 279-310.

Sunagawa, S., Wilson, E. C., Thaler, M., Smith, M. L., Caruso, C., Pringle, J. R., Weis, V. M., Medina, M. and Schwarz, J. A. (2009). Generation and analysis of transcriptomic resources for a model system on the rise: the sea anemone *Aiptasia pallida* and its dinoflagellate endosymbiont. *BMC Genom.* **10**, 258.

Thornton, L. E., Ohkawa, H., Roose, J. L., Kashino, Y., Keren, N. and Pakrasi, H. B. (2004). Homologs of plant PsbP and PsbQ proteins are necessary for regulation of photosystem II activity in the cyanobacterium *Synechocystis* 6803. *Plant Cell* **16**, 2164-2175.

Thorsness, P. E., White, K. H. and Fox, T. D. (1993). Inactivation of Yme1, a member of the FtsH-Sec18-Pas1-Cdc48 family of putative ATPase-encoding genes, causes increased escape of DNA from mitochondria in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13**, 5418-5426.

Voolstra, C. R., Schwarz, J. A., Schnetzer, J., Sunagawa, S., Desalvo, M. K., Szmant, A. M., Coffroth, M. A. and Medina, M. (2009). The host transcriptome remains unaltered during the establishment of coral-algal symbioses. *Mol. Ecol.* **18**, 1823-1833.

Waegele, H., Deusch, O., Haendeler, K., Martin, R., Schmitt, V., Christa, G., Pinzger, B., Gould, S. B., Dagan, T., Klussmann-Kolb, A. et al. (2011). Transcriptomic evidence that longevity of acquired plastids in the photosynthetic slugs *Elysia timida* and *Plakobranchus ocellatus* does not entail lateral transfer of algal nuclear genes. *Mol. Biol. Evol.* **28**, 699-706.

Weber, A. and Flugge, U. I. (2002). Interaction of cytosolic and plastidic nitrogen metabolism in plants. *J. Exp. Bot.* **53**, 865-874.

Wisecaver, J. H. and Hackett, J. D. (2010). Transcriptome analysis reveals nuclear-encoded proteins for the maintenance of temporary plastids in the dinoflagellate *Dinophysis acuminata*. *BMC Genom.* **11**, 366.

Xu, Q., Hoppe, D., Chitnis, V. P., Odom, W. R., Guikema, J. A. and Chitnis, P. R. (1995). Mutational analysis of photosystem I polypeptides in the cyanobacterium *Synechocystis* sp. PCC 6803. Targeted inactivation of psaI reveals the function of psaI in the structural organization of psaL. *J. Biol. Chem.* **270**, 16243-16250.

Yamaguchi, H., Nakayama, T., Kai, A. and Inouye, I. (2011). Taxonomy and phylogeny of a new kleptoplastidal dinoflagellate, *Gymnodinium myriopyrenoides* sp nov (Gymnodiniales, Dinophyceae), and its cryptophyte symbiont. *Protist* **162**, 650-667.

Yokoyama, A., Takahashi, F., Kataoka, H., Hara, Y. and Nozaki, H. (2011). Evolutionary analyses of the nuclear-encoded photosynthetic gene psbO from tertiary plastid-conctaining algae in Dinophyta. *J. Phycol.* **47**, 407-414.

Yuyama, I., Watanabe, T. and Takei, Y. (2011). Profiling differential gene expression of symbiotic and aposymbiotic corals using a high coverage gene expression profiling (HiCEP) analysis. *Mar. Biotechnol.* **13**, 32-40.