Algal Chloroplasts

Secondary article

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A great diversity of chloroplasts is found amongst the various algal groups. This diversity is the result of an intriguing evolutionary process that involved the acquisition of chloroplasts by different eukaryotic organisms.

Article Contents

Introduction

The chloroplast is one of a family of related biosynthetic organelles (termed plastids) found within the cells of plants, eukaryotic algae and certain protists. The primary role of the chloroplast is the fixation of atmospheric carbon through photosynthesis, but it is also the site of synthesis of many other important compounds including pigments, fatty acids, amino acids and nucleotides. Chloroplasts are distinguishable from other plastid types in that they contain chlorophyll and other pigments that are involved in light energy capture and dissipation. In higher plants, nonphotosynthetic plastids such as chromoplasts, amyloplasts and leucoplasts are found in nongreen tissue and fulfil various biosynthetic and storage functions. Within the algae, this level of plastid differentiation is not seen and one plastid type prevails. Typically, algae possess chloroplasts, although those heterotrophic algae that have lost photosynthetic function may retain a nonpigmented plastid. This is certainly the case for the apicomplexans, a group of parasitic protists that possess a plastid of unknown function.

The idea that chloroplasts evolved from photosynthetic prokaryotes was first proposed by Mereschowsky nearly one hundred years ago. Today, it is generally agreed that the original progenitor of all plastids was a cyanobacterium that was engulfed by a nonphotosynthetic eukaryote cell some 1–2 billion years ago, and was retained within the cell as an endosymbiont. The modern-day descendants of this eukaryote–prokaryote chimaera include all plants, together with the green and red algae. Other algal groups almost certainly acquired their chloroplasts 'second-hand' by engulfing other eukaryotic algae. It is this spread of the chloroplast during evolution that has resulted in the many different algal groups found today. Consequently, chloroplast characteristics play a central role in the identification and classification of this diverse group of organisms.

Diversity and Classification of Algae

The term 'alga' encompasses a large and rather heterogeneous collection of (mainly) photosynthetic organisms that are most commonly found in aquatic environments, and which lack the differentiated structures that define higher plants (roots, shoots, leaves, etc.). Indeed, the algae are often referred to as 'lower' or 'primitive' plants. Included within the algae are the prokaryotic cyanobacteria (formerly referred to as blue-green algae), together with a diverse collection of microscopic and macroscopic eukaryotes. Algal species can be unicellular, filamentous or multicellular and they range in size from the unicellular forms that are only a few micrometres in diameter to the giant *Laminaria* seaweeds that are tens of metres long. Algae have adapted to life in a wide range of environments. Aquatic algae are found in all water bodies – freshwater, seawater and brackish – whereas terrestrial algae are found in soils, rocks and snow throughout the world.

The principal feature that separates the eukaryotic algae from other members of the kingdom Protista is the presence of a chloroplast (although some algae possess nonphotosynthetic plastids and others have lost their plastid completely). All algal chloroplasts possess chlorophyll *a* as their primary photosynthetic pigment. However, the nature of the accessory pigments varies markedly between the different algal groups. This difference in pigmentation has long been used in the classification of algae, and allowed early phycologists to define species as belonging to the 'green algae', 'red algae', 'brown algae', etc. The system of classification was subsequently refined using ultrastructural characteristics of the cell, with particular emphasis on the chloroplast. The number of membranes surrounding the chloroplast, the presence and arrangement of chloroplast structures such as the eyespot and the pyrenoid body, and the type of carbohydrate reserve within the cell, all serve as diagnostic aids to classification. This has led to a classification system composed of ten major phyla as outlined in **Table 1**.

In the last twenty years, extensive DNA sequencing and the development of computational methods for comparing gene sequences, protein sequences or genome organization have led to a new discipline: molecular phylogenetics. This has proved to be a powerful tool in understanding the evolutionary relationships between the algae.

Table 1 The classification of the algae

Phylum (= Division)	Pigment content[a]	Number of chloroplast membranes	Storage product	Association of thylakoids
Kingdom Prokaryota				
Cyanophyta 1. Cyanobacteria	*a*, PB	Free-living prokaryotes with cell membrane bounded by peptidoglycan (PG) cell wall	Myxophycean starch	Concentric, unstacked
2. Oxychlorobacteria	*a*, *b*, (*c*)		Starch-like compound	Stack of 2–5
Kingdom Protista				
Glaucophyta	*a*, PB	2, separated by PG wall	Starch	Concentric, unstacked
Rhodophyta	*a*, PB	2	Floridean starch	Single, unstacked
Chlorophyta	*a*, *b*	2	Starch	Stacks of 2–6
Cryptophyta	*a*, *c*, PB[b]	4, nucleomorph	Starch	Paired stacks
Chlorarachniophyta	*a*, *b*	4, nucleomorph	Paramylon	Stacks of 1–3
Chromophyta (= Heterokontophyta)	*a*, *c*	4	Chrysolaminaran	Stacks of 3
Haptophyta (= Prymnesiophyta)	*a*, *c*	4	Chrysolaminaran	Stacks of 3
Euglenophyta	*a*, *b*	3	Paramylon	Stacks of 3
Dinophyta	*a*, *c*	3	Starch	Stacks of 3

[a]Major pigments are chlorophylls *a*, *b* and *c* and phycobilins (PB).
[b]Phycobilins are not contained within a phycobilisome.

Origins and Evolution of Algal Chloroplasts

Endosymbiosis – a landmark in evolution

Symbiosis and the establishment of the chloroplast organelle almost certainly began when a unicellular phagotrophic eukaryote engulfed an ancestral cyanobacterium. Instead of being digested as food, the cyanobacterium was retained in the cell as an endosymbiont (**Figure 1**). The eukaryote benefited from the carbohydrate generated by the photosynthetic activity of the bacterium, and the bacterium found itself in a nutrient-rich and protected niche. The growth and division of the bacterium allowed it to be inherited as a component of the eukaryotic cell. However, the autonomy of the bacterium was soon lost as selective pressures resulted in a dramatic reduction in the size and complexity of its genome. Of the several thousand bacterial genes, those no longer required for an intracellular existence (e.g. genes required for cell wall synthesis, motility and scavenging of nutrients) were rapidly lost. Additional genes were eliminated by a process of gene substitution in which host nuclear genes for key enzyme activities functionally replaced homologous bacterial genes. This probably involved the duplication of a nuclear gene and the redirecting of one of the gene products into the fledgling organelle, resulting ultimately in the loss of the corresponding bacterial gene. Finally, and perhaps most remarkably, there was a mass transfer of most of the remaining bacterial genes from the endosymbiont to the nucleus. This gene transfer almost certainly involved a three-step process that started with the escape of genetic material (DNA or messenger RNA) from the endosymbiont compartment into the nucleus such that gene copies were now present in both genomes. This was then followed by the establishment of the nuclear copy as a functionally expressed gene able to target its product back to the endosymbiont. The original, and now redundant, gene was then lost from the endosymbiont genome.

The nature of the driving force behind these gene substitution and gene transfer events is not known for certain, but is best explained by the genetics principle termed 'Müller's ratchet'. This proposes that deleterious mutations accumulate more rapidly in asexually propagated genes (e.g. chloroplast genes) than in sexually propagated ones (nuclear genes), since asexuality does not allow the combining of different alleles and the subsequent elimination of mutations through recombination and selection. Müller's ratchet is further exacerbated in the chloroplast since the photosynthetic process generates reactive oxygen species that can damage DNA. Selective pressures therefore ensured that a cyanobacterium that was once free-living and genetically autonomous lost most of its genes and became an enslaved organelle dependent on the host nucleus for the majority of the genetic information required for its biogenesis.

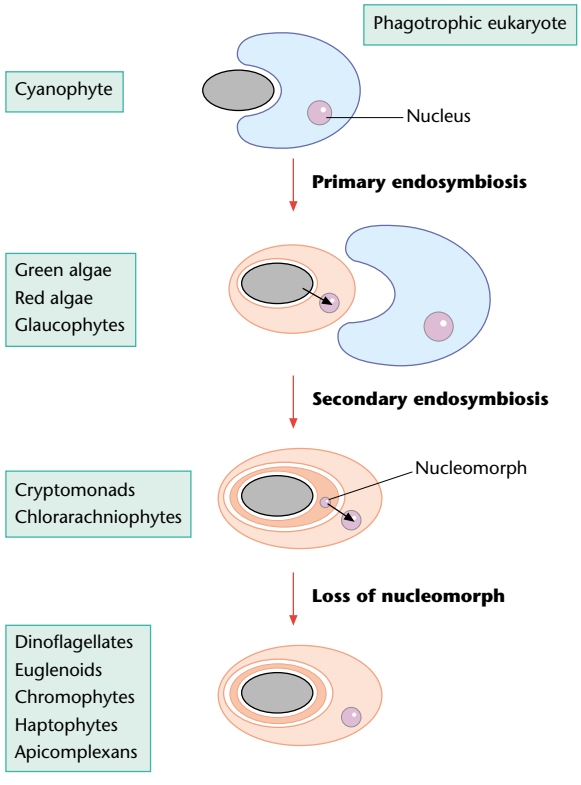

Figure 1 The evolution of chloroplasts by endosymbiosis. The primary chloroplasts arose through the capture and retention of a cyanobacterium by a phagotrophic eukaryote. Subsequent gene loss and transfer to the nucleus (arrowed) resulted in the evolution of the chloroplast organelle. This organelle spread to other eukaryotes by secondary endosymbiotic events in which eukaryotic algae were themselves engulfed. In two phyla the endosymbiont nucleus remains as a vestigial structure, the nucleomorph.

Three extant lineages possess primary plastids

As depicted in **Figure 1**, three major algal lineages have arisen as a result of the endosymbiotic process. In each case, the chloroplast is surrounded by two membranes and is defined as a primary plastid. The outer membrane is believed to have originated from the phagotrophic membrane of the host cell, whereas the inner membrane is derived from the bacterial plasma membrane. The first of these lineages encompasses the green algae (Chlorophyta) together with the land plants that have evolved from within the chlorophytes. Their chloroplasts have stacked thylakoids and light-harvesting complexes (LHC) that are integral to the thylakoid membrane. The LHC contains the pigments chlorophylls *a* and *b*, together with carotenoids. The second major group is the red algae (Rhodophyta) and includes both microscopic members and macroscopic seaweeds. The rhodophyte chloroplasts

resemble modern-day cyanobacteria in that they have unstacked thylakoids and an LHC complex (the phycobilisome) that is not integral to the membrane and has chlorophyll *a* and phycobilins as its light-harvesting components. The smallest and least well-characterized group is the glaucophytes (also termed glaucocystophytes). These algae have an unusual chloroplast termed a cyanelle. The cyanelle is similar to the red algae chloroplast in that it has unstacked thylakoids and phycobilisomes, but it also has a thin peptidoglycan wall between the two envelope membranes. This wall is structurally very similar to the cell wall of cyanobacteria, and for a long time it was assumed that glaucophytes possess a symbiotic cyanobacterium rather than a true chloroplast. However, recent molecular analysis has revealed that the cyanelle genome has also undergone the dramatic loss of size and gene content that defines the organelle. It therefore appears that the cell wall of the glaucophytes is an ancient feature that has been retained during the evolutionary process.

There has been considerable debate over whether all plastids are derived from the same prokaryotic ancestor (a 'monophyletic' origin) or from different ones (a 'polyphyletic' origin). Early arguments based mainly on the pigment content of the different algal groups favoured a polyphyletic origin. For example, the red algal chloroplast and the cyanelle were believed to have evolved from a cyanobacterium possessing chlorophyll *a* and phycobilins, whereas the chloroplast of green algae and plants arose independently from an unknown bacterial ancestor possessing chlorophyll *a* and *b*. The subsequent discovery of such bacteria, the oxychlorobacteria, strengthened this argument. However, more recent molecular phylogenetic analysis using genes from cyanobacteria, oxychlorobacteria and the three classes of primary plastid have strongly indicated a monophyletic origin in which all chloroplasts share a common ancestor. A plausible explanation for the pigment diversity amongst the chloroplasts is that the original cyanobacterial endosymbiont possessed all three pigments (Tomitani *et al.*, 1999). Evolution therefore resulted in the independent loss of either chlorophyll *b* (modern-day cyanobacteria, red algal chloroplasts and cyanelles) or phycobilins (oxychlorobacteria and chloroplasts of green algae and plants).

The only evidence for an algal chloroplast with a different ancestry comes from the photosynthetic amoeba *Paulinella chromatophora*. This organism possesses a cyanelle very similar to that found in glaucophytes such as *Cyanophora paradoxa*. However, the host cells of these two cyanelle-containing algae are clearly unrelated, suggesting that *Paulinella* acquired its cyanelle by an independent endosymbiosis. Unfortunately, this enigmatic amoeba has not yet been studied in detail and it is possible that it acquired its plastid by secondary endosymbiosis of a glaucophyte, as discussed in the next section.

Second-hand organelles: the evolution of complex plastids

Electron micrograph studies of other algal groups have revealed that their chloroplasts are surrounded by more than two membranes (typically, three or four). These are referred to as complex plastids and have almost certainly arisen by a process of secondary endosymbiosis (**Figure 1**). This involved a phagotrophic eukaryote engulfing a eukaryotic alga. In most cases, the phagotroph was probably nonphotosynthetic but there is evidence to suggest that some species may have evolved from phagotrophic algae that acquired a new chloroplast by secondary endosymbiosis and subsequently lost their original chloroplast. For those algae whose chloroplasts are surrounded by four membranes (**Table 1**), we can account for each membrane as follows: the inner two membranes are the double membrane of the endosymbiont's own chloroplast; the third membrane is derived from the plasma membrane of the endosymbiont; and the fourth membrane is the food vacuole membrane of the host. The outer two membranes are termed the chloroplast endoplasmic reticulum (CER) and are often associated with the host nuclear envelope. Following the incarceration of the alga, most of the cytosolic components except the chloroplast were gradually lost leaving a greatly reduced inter-membrane compartment (the periplastidal space) bounded by the CER. As with the primary plastids, the establishment of the endosymbiont was followed by a process of genome reduction. However, in the case of the complex plastids, gene loss or transfer to the host nucleus was principally from the endosymbiont nucleus (**Figure 1**).

The most compelling evidence for this evolutionary scenario is found in two groups of algae: the cryptomonads and the chlorarachniophytes. In each case, the periplastidal space contains both a vestigial nucleus termed the 'nucleomorph' and 80S ribosomes. It is clear that this genetic system is derived from the nucleo-cytosolic system of the eukaryotic endosymbiont. Despite the similarities between the cryptomonads and the chlorarachniophytes, biochemical and molecular studies clearly demonstrate that their chloroplasts each have an independent ancestry. The cryptomonad chloroplasts are similar to the red algae in that they have phycobilins and lack chlorophyll *b*. In contrast, the grass-green chloroplasts of the chlorarachniophytes have chlorophyll *b*, but not phycobilins. Molecular phylogenetic studies have since confirmed that the cryptophyte chloroplast is of red algal descent, whereas the chlorarachniophyte chloroplast is derived from a green alga, most closely related to the chlorophycean class that includes *Chlamydomonas* and *Chlorella*.

The reduction of the endosymbiont nuclear genome to that of the nucleomorph could be considered as an intermediate step towards its complete loss. This is indeed what is found in two other major algal groups: the chromophytes and the haptophytes. These groups are

often known collectively as the 'golden algae' since photosynthetic members possess various types of chlorophyll *c* together with the carotenoid fucoxanthin. Their chloroplasts have four membranes with the outer two strongly resembling the CER of the cryptomonads, but without a nucleomorph. Despite the lack of phycobilins within the chloroplasts, molecular studies have shown that, like the cryptomonads, the chloroplasts of the golden algae have a red algal ancestry.

A surprising new member to the four-membrane family of plastids has emerged in recent years. The apicomplexans are a group of obligate endoparasites of animals that include the malarial parasite *Plasmodium* and the opportunistic human pathogen *Toxoplasma*. The apicomplexans possess a nonpigmented plastid bounded by four membranes and containing a highly reduced plastid genome. Phylogenetic studies of this genome have confirmed its chloroplast origins and have indicated that it may be of green algal descent (Köhler *et al*., 1997). The plastid is widespread amongst the apicomplexans and appears to play an essential role within the cell. Although the nature of this essential function is currently unknown, the plastid represents a potentially useful target for therapeutic agents aimed at killing the parasite but not the animal host.

Two major groups of algae have complex plastids bounded by three membranes. These are the euglenoid algae and the peridinin-containing dinoflagellates. The outermost membrane is probably derived from the food vacuole of the host, but is not associated with the host nucleus. The mechanism by which a three-membrane chloroplast was acquired by a phagotrophic host is far from clear. It has been proposed that endosymbiosis involved the capture of an isolated chloroplast, rather than an intact alga. This is unlikely since the bulk of the genetic information required for the maintenance of the chloroplast in its new host would be missing, having already transferred to the nucleus of the original alga. A more likely explanation can be found in the feeding mechanism observed in some phagotrophic dinoflagellates and euglenoids. This involves the puncturing of the prey's plasma membrane and the sucking out of the cell contents. As a result, the cell membrane is not taken up and the cytoplasm of the ingested prey is surrounded only by the food vacuole membrane.

As with the other complex plastids, the euglenoid and dinoflagellate chloroplasts have evolved from independent secondary endosymbiotic events. The euglenoid chloroplasts contain chlorophyll *b* and are related to the green algae. In contrast, the peridinin-containing dinoflagellates appear to possess a chloroplast of red algal lineage. Until recently, this supposition was based only on the similarity of the chloroplast internal membrane organization to that of the golden algae, and the presence of chlorophyll *c*. The recent isolation of chloroplast genes from several dinoflagellates has now allowed a limited phylogenetic analysis which supports the grouping of this chloroplast within the red algal lineage (Zhang *et al*., 1999).

Within the Dinophyta are examples of other complex chloroplasts that were acquired by feeding on various other algal prey including chlorophytes, haptophytes and crytophytes, with the latter two involving tertiary endosymbiosis. One such example is found among the fucoxanthin-containing dinoflagellates of the genus *Gymnodinium* where the chloroplast is of haptophyte origin. What is remarkable about this process is that the genetic information for the chloroplast appears to have 'moved house' yet again, going from the haptophyte nucleus to the dinoflagellate nucleus.

It is clear that the chloroplast, originating probably from a single ancestor, has spread itself like an opportunistic parasite – taking up residence in a wide range of protist hosts. The value of a photosynthetic organelle is underlined by the finding that various ciliates and sea slugs are able to maintain isolated chloroplasts obtained from their algal prey as temporary intracellular organelles. Although these chloroplasts lack the genetic information required from their replication and inheritance, they are retained for a surprisingly long time. In the case of the sea slug *Elysia chlorotica*, the chloroplasts are maintained within the cytoplasm of the epithelial cells for many months, providing the animal with an important supply of carbohydrate (Mujer *et al*., 1996).

Chloroplast Genetics and Molecular Biology

Chloroplast genomes – going, going, gone?

We can quantify the extent of gene lost from the original endosymbiont by comparing genomic data from a modern-day cyanobacterium, *Synchecocystis* sp. 6803, with that from various algal and higher plant chloroplasts. As can be seen in **Table 2** there has been a severe reduction in both size and gene content during evolution. The genes that have been retained represent only some 5–10% of the total predicted to be necessary for the biogenesis of the organelle. Chloroplast genes can be classified under three major headings, based on function. The first group encompasses those genes required for the organelle's genetic system, such as genes for ribosomal RNAs, transfer RNA and protein subunits of the RNA polymerase and the ribosome. The second group of genes encodes components of the photosynthesis apparatus. These include the large subunit of the CO_2-fixing enzyme Rubisco together with the core subunits of the photosynthetic complexes. The third class includes genes for other aspects of plastid metabolism such as synthesis of fatty acids, pigments and amino acids. A comparison of the various chloroplast genomes reveals that the gene content is highly conserved.

Table 2 Comparison of sequenced plastid genomes

Phylum	Species	Genome size (kilobases)	No. of genes (protein/RNA)	Accession no.[a]
Cyanophyta	*Synechocystis* PCC6803	3573	3168/46	AB001339
Embryophyta (land plants)	*Zea mays*	140	70/34	X86563
Embryophyta (land plants)	*Epifagus virginiana*[b]	70	21/21	M81884
Chlorophyta	*Chlorella vulgaris*	151	77/34	AB001684
Chlorophyta	*Nephroselmis olivacea*	201	91/36	AF137379
Rhodophyta	*Porphyra purpurea*	191	212/39	U38804
Rhodophyta	*Cyanidium caldarium*	165	196/33	AF022186
Glaucophyta	*Cyanophora paradoxa*	136	149/40	U30821
Cryptophyta	*Guillardia theta*	122	137/36	AF041468
Chromophyta	*Odentella sinensis*	120	139/35	Z67753
Euglenophyta	*Euglena gracilis*	143	67/30	Z11874
Apicomplexa	*Plasmodium falciparum*[b]	35	30/27	X95275-6
Apicomplexa	*Toxoplasma gondii*[b]	35	28/27	U87145
Dinophyta	*Heterocapsa triquetra*	2.1–3.1 (minicircles)	7/2	AF130031-9
Dinophyta	*Amphidinium operculatum*	2.3–2.4 (minicircles)	5/0	AJ250262-6

[a]Accession number can be used to obtain annotated sequence via the Genbank DNA database (http://www.ncbi.nlm.nih.gov/).
[b]Nonphotosynthetic obligate parasites.

For example, almost all of the genes found on the green algal genomes are also found in the red algal and cyanelle genomes. This further supports the idea of a monophyletic origin for the chloroplast and also indicates that the process of gene loss occurred relatively early, prior to the emergence of the different algal lineages. This early 'founder' chloroplast probably had several hundred genes representing all three categories. The lineages that lead to the red algae and glaucophytes have retained most of these genes, whereas the green algae and plants have subsequently lost most of the biosynthetic genes, together with some members of the other two groups. Further gene losses are seen in those plastids of green algal descent that have lost photosynthetic function (**Table 2**). These include the plant parasite *Epifagus virginiana*, the euglenoid heterotroph *Astasia longa* and the apicomplexan animal parasites. In each case, all of the photosynthesis genes have been lost. This leaves a small genome containing a set of genes whose only apparent role is in gene expression. Why this genetic system is retained and what essential function it fulfils in plastid metabolism is far from clear, and is an area of much debate.

Recent molecular analysis of the chloroplast genomes of several dinoflagellates has revealed an even more curious situation in which further gene losses have resulted in only a handful of genes remaining within the chloroplast (**Table 2**). Furthermore, each of the dinoflagellate genes is maintained on its own minicircle of DNA. This contrasts with the highly conserved genome structure found in all other plastids, where the genes are all contained on a circular chromosome. The loss of most of the photosynthetic genes from the dinoflagellate is intriguing since it questions the current hypothesis for why chloroplast genes have been retained despite Müller's ratchet (Race *et al*., 1999). This hypothesis proposes that the genes for core components of the photosynthetic complexes need to be in the organelle so that their expression can be tightly coupled to electron transfer activity. This ensures the rapid synthesis of new components when needed, and thereby minimizes photo-oxidative damage due to a defective electron transfer chain. It is possible that the remaining few photosynthetic genes of the dinoflagellate genome represent those genes that absolutely cannot be uncoupled from this regulatory system. Alternatively, we may be seeing the last act in the complete transfer of all chloroplast genes to the nucleus. A further search among the many dinoflagellate species may therefore reveal a chloroplast without a genome.

Nucleomorph molecular biology – Bonsai chromosomes

Secondary endosymbiosis created a eukaryote-within-a-eukaryote in which the majority of the genes required for chloroplast biogenesis were in the endosymbiont nucleus. This entrapped nuclear genome was now subject to the same selective pressure as the original cyanobacterial genome – namely loss or substitution of redundant genes,

and transfer to the nucleus of genes required for chloroplast biogenesis. This can be seen by comparing the size of the chlorarachniophyte nucleomorph genome (380 kb) with the nuclear genome of a green alga such as *Chlamydomonas* (~70 000 kb). Selective pressures have also acted on the genes that have remained in the nucleomorphs of both chlorarachniophytes and cryptophytes (McFadden *et al.*, 1997). In both cases, chromosome number has been reduced to three and the genes are densely packed with intergenic spaces reduced to a minimum and some genes actually overlapping. The chlorarachniophyte genes have retained introns but these are remarkably small (18–20 bp), whereas introns have been completely lost in the cryptophyte genes. Like the chloroplast genome, the nucleomorph genome appears to contain primarily genes encoding components required for gene expression and protein degradation within the periplastidal space. In addition, several chloroplast proteins are encoded in the nucleomorph. The presence of these genes provides the *raison d'être* for the nucleomorph genetic system. It is also possible that the unusual gene structure that has resulted from the compaction of the nucleomorph genome now prevents any further transfer of these genes to the nucleus. Nonetheless, the complete loss of the nuclear genome of the secondary endosymbiont has clearly occurred in those algal groups possessing complex chloroplasts without a nucleomorph.

Genetic manipulation of chloroplast genomes

The genetic manipulation of the chloroplast genome has proved particularly challenging for two reasons. Firstly, the multiple membranes of the cell and its chloroplast present a significant barrier to DNA delivery into the organelle compartment. Secondly, the polyploid nature of the chloroplast genome (typically 50–100 copies per chloroplast), and the presence of multiple chloroplasts per cell in many species, requires a strong selection strategy to recover a stable transgenic cell in which all copies of the genome have been modified. Despite these problems, chloroplast transformation has been established for *Chlamydomonas* and for several higher plants. As shown in **Figure 2**, DNA can be delivered into the organelle using the biolistic transformation method in which cells or tissue are bombarded at high velocity with DNA-coated gold microparticles. Once inside the chloroplast, the DNA is able to replace the target region of the genome via two homologous recombination events. Selection for the modified genome is achieved using a marker gene that confers spectinomycin resistance. Reverse genetic studies of chloroplast genes in *Chlamydomonas* have proved particularly valuable in determining the function of previously unidentified genes and in understanding the role of the many chloroplast-encoded proteins in photosynthesis (Rochaix, 1997). Chloroplast transformation of the red alga *Porphyridium* spp. has been reported recently.

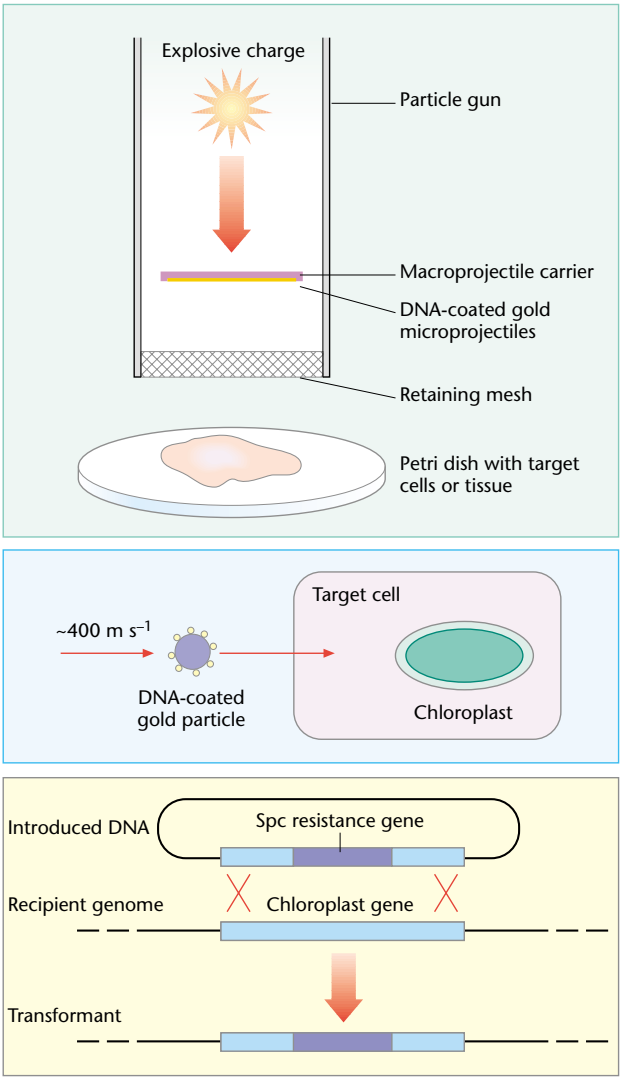

Figure 2 Biolistic transformation of the chloroplast. The genetic engineering of the chloroplast genome can be achieved using the biolistic process in which DNA is delivered into the organelle compartment using a particle gun. The DNA is coated onto gold microparticles that are fired at the target cells or tissue. Recombination results in the integration of the DNA into the genome. The bottom panel illustrates the disruption of a chloroplast gene using a selectable marker conferring spectinomycin (Spc) resistance (Rochaix, 1997).

Future gene disruption studies in this organism should allow the investigation of the many genes that are present in the chloroplast genomes of red algal descent but absent from those of green algae and plants.

Protein Transport in the Chloroplast

The mass transfer of genes from the primary endosymbiont to the nucleus presented a major logistical problem to the

evolving cell. The gene products that were previously synthesized within the endosymbiont were now being synthesized on the host's 80S ribosomes in the cytosol and therefore needed to be re-imported into the chloroplast. This required the evolution of a new machinery for protein import across the two envelope membranes of the chloroplast, since it is unlikely that the free-living cyanobacterial progenitor possessed any mechanisms for protein import. In addition, targeting information needed to be attached to each protein to ensure that it was recognized by this import machinery. Recent studies of the translocon complex responsible for chloroplast protein import in higher plants suggest that several components of this complex may have evolved from cyanobacterial membrane proteins that had other physiological functions. These proteins, together with several membrane proteins from the host, appear to have been modified to fulfil the novel function of chloroplast protein import (Reumann and Keegstra, 1999). Proteins destined for the chloroplast have an N-terminal presequence of some 30–100 residues that is recognized by the translocon complex, and cleaved from the protein once it is inside the chloroplast (**Figure 3**). This presequence, termed the transit peptide, is similar to (and may have evolved from) the presequences responsible for targeting proteins into the mitochondrion. This system of protein recognition and import appears to have evolved early and uniquely during chloroplast evolution since isolated chloroplasts from plants are able to import precursor proteins from various algal groups including chlorophytes, rhodophytes, glaucophytes and chromophytes.

In complex chloroplasts where additional membranes surround the chloroplast, the process of protein targeting from cytosol to chloroplast interior is even more elaborate. Those proteins whose genes have been retained in the nucleomorph genome, and therefore have only to cross the inner two membranes, have presequences that resemble typical transit peptides. In contrast, proteins whose genes have transposed for a second time and now reside in the host nucleus have to cross one or more additional membranes in which the outermost membrane is part of the host's endoplasmic reticulum (ER). These proteins have an extra N-terminal extension that functions as an ER targeting signal. The import process is tightly coupled to translation such that the protein is targeted across the outermost membrane as it is being synthesized on membrane-associated ribosomes. Once across the membrane, the signal peptide is removed by a peptidase (**Figure 3**). It is unclear at present how these proteins cross the second membrane of the CER into the periplastidal space, although several mechanisms have been proposed (Kroth and Strotmann, 1999). Studies of protein import in *Euglena* reveal that transport across the three membranes surrounding the euglenoid chloroplast also requires a signal peptide and involves the cotranslational import of the protein into ER (Sulli and Schwartzbach, 1996). Recent

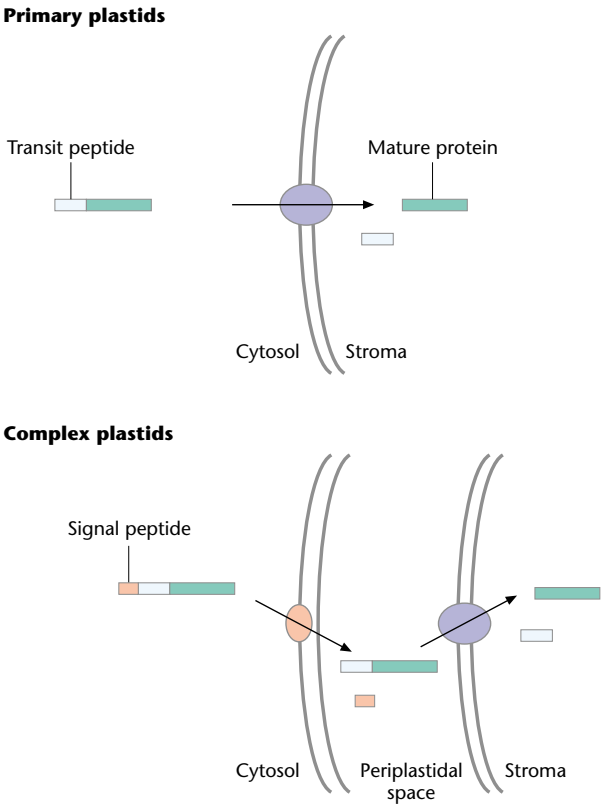

Figure 3 Protein targeting into the chloroplast. In primary plastids, proteins synthesized in the cytosol are transported across the outer and inner membranes by a translocon complex (blue) that recognizes the transit peptide. This peptide is then removed by a stromal peptidase. In complex plastids, an additional peptide signal directs the protein across the outermost membrane as it is being translated on membrane-bound ribosomes. This signal peptide is cleaved by a signal peptidase. How proteins cross the inner membrane of the CER is not known.

developments in the nuclear transformation of various algal groups including chlorophytes, rhodophytes and apicomplexans are now allowing a detailed molecular dissection of chloroplast protein trafficking *in vivo* using reporter proteins such as green fluorescent protein (see Striepen, 2001).

Summary

Chloroplasts and other plastids are the products of a cyanobacterial journey through evolutionary time. Starting from a free-living entity, the cyanobacterium has invaded and spread through the eukaryotic world in a series of endosymbiotic events. Along its journey it has collected additional membranes and modified its pigment content, but has handed over the bulk of its genetic burden to the host. In return it has provided a site for biosynthesis and storage of carbohydrates and other important

macromolecules, and thereby ensured its own survival even when photosynthetic function was no longer required. The study of algal chloroplasts not only provides powerful insights into the evolutionary process but also allows us to make sense of the vast array of algal species that populate our planet.

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