Plastid Evolution

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Abstract

The ancestors of modern cyanobacteria invented O_2-generating photosynthesis some 3.6 billion years ago. The conversion of water and CO_2 into energy-rich sugars and O_2 slowly transformed the planet, eventually creating the biosphere as we know it today. Eukaryotes didn't invent photosynthesis; they co-opted it from prokaryotes by engulfing and stably integrating a photoautotrophic prokaryote in a process known as primary endosymbiosis. After approximately a billion of years of coevolution, the eukaryotic host and its endosymbiont have achieved an extraordinary level of integration and have spawned a bewildering array of primary producers that now underpin life on land and in the water. No partnership has been more important to life on earth. Secondary endosymbioses have created additional autotrophic eukaryotic lineages that include key organisms in the marine environment. Some of these organisms have subsequently reverted to heterotrophic lifestyles, becoming significant pathogens, microscopic predators, and consumers. We review the origins, integration, and functions of the different plastid types with special emphasis on their biochemical abilities, transfer of genes to the host, and the back supply of proteins to the endosymbiont.

Contents

INTRODUCTION

In nature the counterpart of chaos is not cosmos, but evolution. The spark of life was initially a chemical one, leading to the synthesis of the first molecules. Some of these persisted and evolved in a precellular period, perhaps similar to that described in the model of the RNA world, leading to the first prokaryotic life approximately 3.5 to 4 billion years ago (48, 74). The invention of oxygenic photosynthesis by prokaryotic cyanobacteria approximately 500 million years later was the next major achievement of biological evolution. It had a major impact on the earth by enriching the atmosphere with O_2 to a level that transformed the geochemistry of the planet.

The first molecular carbon skeletons typical of cyanobacteria can be identified in strata from approximately 2.75 billion years ago (15). At the same time a novel mineral known as hematite (Fe_2O_3), which can form only in the presence of a minimum critical concentration of oxygen, began to appear. These geological indices testify to an ever-increasing concentration of atmospheric oxygen due to photosynthetic activity. Photosynthesis was also the evolutionary trigger for the sweeping diversification of O_2-dependent life. Indeed, oxygen has become critical for most living things, acting as an acceptor for the electrons released from carbon-carbon bonds that were ultimately created using energy captured by photosynthesis. Thus, a byproduct of photosynthesis (oxygen) became an essential component for the burning of the sugars produced by photosynthesis. The balance of the biosphere was born.

Nineteenth century microscopists (Sachs, Altmann, and Schimper) recognized the semi-autonomous nature and bacterial-like staining properties of chloroplasts (then known as chlorophyll bodies) and mitochondria (then known as cell granules) (4, 106), but it took another 15 years before Mereschkowsky synthesized these observations into the theory that chloroplasts are derived from cyanobacteria (81, 109). Margulis later formalized the Theory of Endosymbiosis, which posits that plastids and mitochondria of eukaryotic cells derive from bacterial endosymbionts (71).

FROM FREEDOM TO SLAVERY: OUTLINING ENDOSYMBIOTIC STEPS

As far as we know, all eukaryotes have mitochondria (or modified, anaerobic forms of mitochondria known as hydrogenosomes or mitosomes), and the establishment of this partnership is generally regarded as integral to the origin of eukaryotes (123). The acquisition of plastids by eukaryotes occurred later, after the establishment of a diversity of heterotrophic eukaryotic lineages, one of which adopted a cyanobacterium-like endosymbiont to acquire photosynthesis and become autotrophic. We refer to an initial plastid-creating endosymbiosis as the primary endosymbiosis. Secondary (or eukaryotic) endosymbiosis refers to subsequent endosymbiotic events in which the progeny

of the primary endosymbiotic partnership become endosymbionts within other heterotrophic eukaryotes, thus transferring the captured cyanobacterial symbiont laterally among eukaryotes. Subsequently, the progeny of these secondary endosymbiotic partnerships have become endosymbionts in other eukaryotes, creating tertiary endosymbioses, to weave an extraordinarily complex set of endosymbiotic relationships of cells within cells within cells within cells (**Figure 1**). In this review we examine the cell biology of these endosymbiotic events and examine how the various compartments and genomes of these extraordinary chimeras cooperate as a single cell, albeit one made up of parts from multiple individual cells.

Primary Endosymbiosis

The endosymbiotic integration of a free-living, cyanobacterial-like prokaryote into a eukaryotic host produced three major autotrophic lineages: the glaucophytes, the green algae (and their descendants, the plants), and the red algae (2, 46) (**Figure 1**). Plastids in these primary endosymbionts are characterized by having two bounding membranes, which are derived from the two membranes (plasma membrane and outer membrane) of the Gram-negative cyanobacterium (17, 20). If a phagocytotic membrane surrounded the symbiont when it was first internalized by the host, it has disappeared without a trace (20). The main lines of evidence supporting homology between the outer envelope membrane and the outer membrane of a cyanobacterium are (*a*) the presence of galactolipids (52), (*b*) the presence of β-barrel proteins in both membranes (110), and (*c*) the occurrence of peptidoglycan (or rudiments of peptidoglycan synthesis machinery) beneath these membranes (117).

Phylogenetic analyses suggest that the glaucophytes were the first primary endosymbiotic lineage to diverge, some 550 mya, and that the red and green algae diverged later (75, 82, 103). Plants, which probably diverged from their green algal ancestors approximately 400 to 475 mya (36), subsequently conquered the terrestrial environment, paving the way for animals to follow them onto land. In accordance with this sequence, plastids in the glaucophytes (which are sometimes referred to as cyanelles but are definitely plastids) most resemble their cyanobacterial ancestors in that they retain a peptidoglycan, wall-like layer between the inner and outer envelope membranes (57). Additionally, the thylakoids inside the glaucophyte plastid stroma are studded with phycobilisomes that are identical to those of cyanobacteria, and the composition of the oxygen-evolving enhancer complex is also very similar to that of free-living cyanobacteria (117). Rhodophyte plastids also use phycobilins in protein-based light harvesting antenna (phycobilisomes), but their plastids have apparently lost the peptidoglycan wall (31). The green algal/plant lineage plastids are the most derived in the primary endosymbiosis lineage. Phycobilisomes were replaced by chlorophyll *b* embedded in thylakoid membranes, and a rich panoply of accessory pigments developed to capture light and protect the photosynthetic apparatus from the unfiltered terrestrial light (80).

Generally, primary plastids have undergone major modification during their tenure in the eukaryotic host; reduction of their genome's coding capacity is one of the more conspicuous attenuations. The genome of the cyanobacterium *Anabaena* sp. PCC 7120 has 5366 protein-encoding genes, and other cyanobacteria possess similar numbers of genes (53). In contrast, the most gene-rich plastid reported to date, that of the red alga *Porphyra purpurea*, encodes a paltry 251 genes (99), and the plastids of the parasitic plant *Epifagus virginiana* harbor a mere 42 genes (132). Thus, most of the original genetic material of the endosymbiont was clearly either lost or transferred to the host genome during their coevolution. Selection likely favored the initial loss of genetic material by the endosymbiont because it turned the

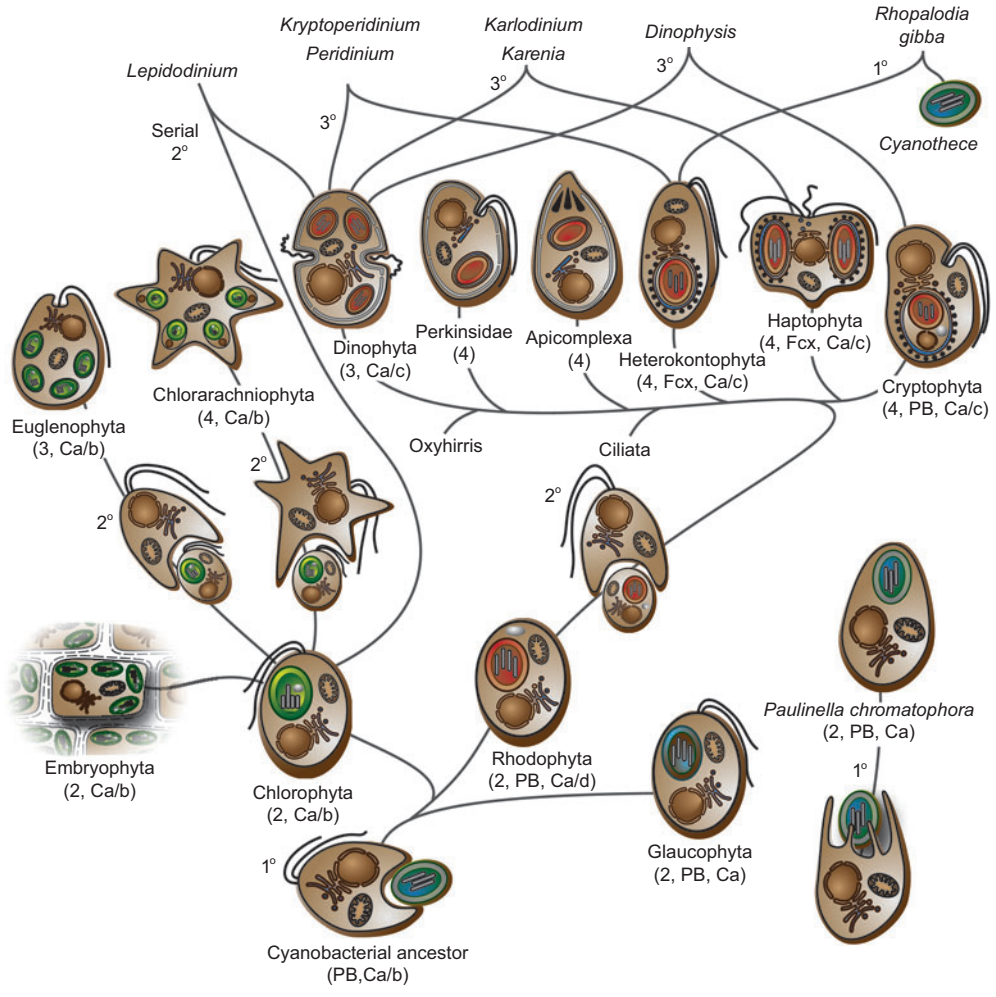


Figure 1

A schematic representation of plastid evolution. Engulfment of a cyanobacterial ancestor and subsequent reduction to a primary plastid (1°) by a eukaryotic host initially led to the formation of three lineages with primary plastids: the chlorophytes, and land plants, rhodophytes and glaucophytes. The subsequent uptake of a green or a red alga by independent hosts to form secondary endosymbioses (2°) resulted in euglenophytes, chlorarachniophytes, and the monophyletic chromalveolates. Chromalveolates, which represent the association of chromists (Heterokontophyta, Haptophyta, and Cryptophyta) and the Alveolata (Apicomplexa, Perkinsidae, Dinophyta, Ciliata), unite an extremely diverse array of protists and not all authors accept the grouping. Different Dinophyta have replaced their original secondary plastid with a green alga either by serial secondary endosymbiosis (*Lepidodinium*) or even tertiary endosymbioses (3°); e.g., *Karlodinium* harbors a tertiary plastid of haptophyte origin. The heterokontophyte *Rhopalodia gibba* engulfed a cyanobacterial *Cyanothece* species and reduced it to so-called spheroid bodies, which are not used for photosynthesis, but rather act in N_2-fixation. The plastid organelles were apparently lost in the case of the ciliates and the dinoflagellate *Oxyhirris*. A possible nascent primary endosymbiosis (1°) is represented by *Paulinella chromatophora*, although whether this endosymbiont is a true plastid organelle remains uncertain. The number of membranes surrounding the plastid and the photosynthetic pigments is shown in parentheses. PB, phycobilin proteins; Fcx, fucoxanthin; C*a/b/c/d*, chlorophyll *a/b/c/d*.

prokaryote-eukaryote consortium into an obligate symbiotic relationship. However, we now know that a concerted and ongoing transfer of genes from endosymbiont to host has radically depleted the endosymbiont's gene catalog. Much of this intracellular gene transfer was likely achieved prior to the divergence of the three primary endosymbiotic lineages because they share a similar residue of common genes (75).

This transfer of genetic material mandated the development of a mechanism to return the gene product to the organelle. We discuss this problem in detail below, but some general concepts can be outlined now. Host-encoded proteins destined for the plastid are typically translated as precursor proteins bearing an N-terminal topogenic signal that is recognized by a proteinaceous receptor, which is either soluble in the cytoplasm or bound to the outer plastid membrane. After recognition, the precursor is subsequently translocated across the plastid envelope by a suite of translocation machineries spanning the two bounding membranes. The preprotein is pulled into the plastid and the topogenic signal is proteolytically removed to yield the mature protein.

The protein import mechanism probably evolved early on in the conversion of the endosymbiont into an organelle and no doubt facilitated the relocation of genes from the endosymbiont to the host. Transferred genes would need to acquire expression and topogenic signals for the gene product to be returned to the organelle. Rhodophtye and green algal/plant plastid-protein-targeting machineries appear to be fairly similar (79); although virtually nothing is known about the translocation machinery in glaucophytes, we predict that it is also similar because these plastids have also relinquished so many of their genes to the host genome (75).

Eukaryotic Endosymbiosis

The current consensus of molecular phylogeny recognizes six eukaryotic superclusters: Opisthokonta, Amoebozoa, Plantae (Archaeplastida), Chromalveolata, Rhizaria, and Excavata (2, 54). The Plantae supercluster embraces the three lines (glaucophytes, rhodophytes, and chlorophytes) with primary endosymbiotic (two membrane) plastids and their monophyly is consistent with a common origin for their plastids (103). However, plastids also occur in the Chromalveolata, Rhizaria, and Excavata, and all these multi-membrane plastids are derived from secondary endosymbioses (**Figure 1**). These events created a variety of eukaryotic-eukaryotic chimeras referred to as meta-algae (22). Secondary or complex plastids are derived from eukaryotic, primary plastid-containing endosymbionts and have undergone reduction during their tenure in the secondary host. The degree of reduction varies; sometimes it is relatively minor, such as in the partially integrated secondary endosymbionts of *Hatena* (85), and sometimes it is extensive, such as in the case of euglenoids in the Excavata where the only trace of the eukaryotic endosymbiont is an extra (third) membrane around the plastid (130). Two important intermediate stages in the secondary endosymbiont reduction process are represented by cryptophytes and chlorarachniophytes, in which a very reduced endosymbiont nucleus, cytoplasm, and plasma membrane can still be identified. The remnant nucleus, known as the nucleomorph, is located inside the periplastidial compartment (the former endosymbiont's cytosol), and the overall topology allows us to rationalize the presence of four membranes around related plastids in chromalveolates, in which the endosymbiont nucleus has completely disappeared. Reduction forces have obviously been at work in these endosymbionts because the great majority of the endosymbiont nuclear genes have been transferred to the host nucleus and most of the cytoplasmatic features, other than a small collection of ribosomes, have been lost (27, 33).

Secondary endosymbioses introduced plastids into heterotrophic lineages, and

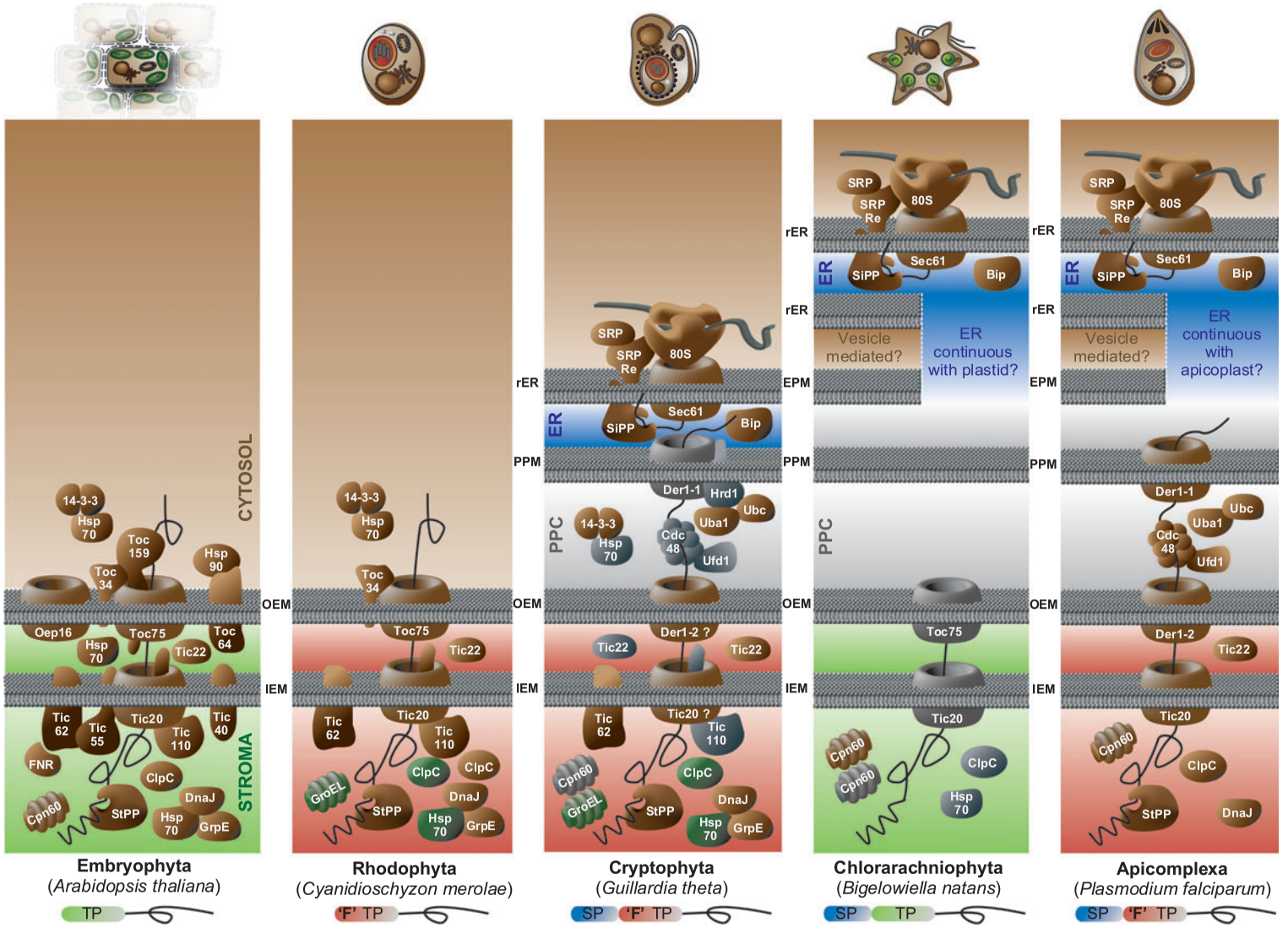
Nucleomorph: the former nucleus of the eukaryotic endosymbiont; lost in most secondary algae, but still present in a highly reduced form in cryptophytes and chlorarachniophytes

much energy has been focused on establishing how many separate times a eukaryotic symbiont has been integrated into a previously nonphotosynthetic lineage. The environmental and commercial importance of the lineages created, not to mention the importance of these events as drivers of eukaryotic diversity, make this a particularly fascinating question. The antiquity of these events and the reduction processes that have occurred in the ensuing millennia also make the question a difficult one to resolve. The most parsimonious hypothesis, put forward by Cavalier-Smith, invokes only two secondary endosymbioses: one involving a green alga leading to the Cabozoa (which unites euglenophytes and chlorarachniophytes) and one involving a red alga that created the Chromalveolata (which unites cryptophytes, haptophytes, heterokontophytes, dinoflagellates, perkinsids, apicomplexa, and the plastid-lacking ciliates) (22). Interestingly, no examples of a glaucophyte secondary endosymbiont are known. Various lines of evidence now refute the Cabozoa hypothesis (7, 10, 33, 66, 104) and it is now clear that two separate acquisitions of green algal endosymbionts created the euglenophytes and chlorarachniophytes independently. The veracity of the chromalveolate hypothesis remains uncertain, and whether or not the chromalveolates are derived from a single or multiple secondary endosymbioses of separate red algal endosymbionts is still much debated. The chromalveolate hypothesis finds some support from molecular phylogenies (44, 86), and some unusual recruitments of enzymes to the endosymbiont shared by chromalveolates also lend credence to a single secondary endosymbiotic event (9, 30, 43, 44, 88). It was argued early on that the mechanism of how proteins are targeted from the host to the complex plastid would give insight into the endosymbiont's ancestry (22), and recent insights into this process (see **Figure 2** and below) are congruent with the chromalveolate scenario. Drawn together, these different analyses support the idea of a monophyletic origin for chromalveolates from a single red

Chromalveolate hypothesis: monophyly of the chromists (Cryptophyta, Haptophyta and Heterokontophyta) and Alveolata (Dinophyta, Ciliata and Apicomplexa); also, the common ancestor contained a complex plastid derived from a red alga that is retained in several of these lineages

Figure 2

Models of the machineries that import nuclear-encoded plastid proteins for select primary and secondary plastids. Nuclear-encoded factors are brown, plastid-encoded factors are green, and nucleomorph-encoded factors are gray. Organisms with primary plastids (green algae, plants, and rhodophytes) share core components of the import apparatus, although land plants apparently have a more elaborate set of receptors (Toc159 and Toc64) in the outer envelope membrane (OEM) and other participating factors (Tic55 and Tic40) in the inner envelope membrane (IEM). These factors, together with Tic62, might be involved in redox-regulated import. Oep16 imports protochlorophyllide oxidoreductase A from the cytosol in *Arabidopsis* independently of the canonical Toc system. A more complicated import pathway is necessary for secondary plastids, as in the case of the cryptophytes, which are surrounded by additional membranes, namely the periplastidial membrane (PPM) and rough endoplasmatic reticulum (rER). In cryptophytes preproteins are cotranslationally inserted into the ER via the Sec61 complex and the signal peptide (SP) is cleaved by the lumenal signal peptide peptidase (SiPP). The remaining transit peptide (TP) mediates translocation across the remaining three membranes, before being cleaved by the stromal processing peptidase (StPP) inside the stroma, similar to primary plastids. Whether the secondary plastid of *P. falciparum* and chlorarachniophytes is actually located within part of the ER, as in cryptophytes and other chromists, is uncertain. Morphology obviously has a significant impact on the actual import pathway and machinery necessary. Proposed models for complex plastids are mostly inferred from genome data mining and lack experimental proof. Tic20 and Der1-2 have not yet been identified in cryptophytes (*question marks*) but genes that encode proteins believed to be targeted into the plastid are present in other chromalveolates for which full genome sequence is available. PPC, periplastidial compartment; EPM, epiplastid membrane. Topogenic signals for stromal targeting are displayed beneath the organisms' names. The F-motif, which is apparently critical for stromal targeting, occurs in plastids with red algal origin.



Eukaryotic endosymbiosis: all events in which the engulfed organism that was reduced to an organelle was a eukaryote

Endosymbiont metabolic replacement: replacement of an existing host-cell metabolic pathway with one acquired with the endosymbiont

alga endosymbiont. However, some analyses with genes encoding cytosolic host proteins do not support the chromalveolate hypothesis (86, 120), suggesting that the spread of a single red algal endosymbiont among the chromalveolate branch may have occurred by subsequent tertiary endosymbioses. Furthermore, the clustering of genes representing Rhizaria together with the Chromalveolata in a recent report by Hackett and colleagues (40) reminds us that definite proof for the monophyletic origin of chromalveolates has not been found.

One further aspect of eukaryotic endosymbiosis is tertiary, maybe even quaternary, and serial secondary endosymbiosis. Tertiary endosymbiosis is the uptake of a secondary endosymbiosis-derived alga by a eukaryote, and serial secondary endosymbiosis is the replacement of an original complex plastid with a new, primary endosymbiosis–derived alga. Select dinoflagellate algal lineages represent the best-studied cases of these higher order endosymbiotic events, and independent cases are represented by the genera *Lepidodinium*, *Kryptoperidinium*, *Karlodinium*, and *Dinophysis* (for detailed description of these unusual dinoflagellates lineages see References 39, 46, and 54) (**Figure 1**). In each case, the host dinoflagellate previously contained a secondary plastid, so these new endosymbionts represent organelle replacements. The mechanisms for organelle reduction and integration are likely the same for secondary endosymbionts; however, in cases of organelle replacement even transferred genes from the first plastid can contribute to the integration of these new recruits (49, 91).

Nature's Playground: The Evolution Continues

Plastid loss and reversion to obligate heterotrophy. A fascinating but often overlooked element of endosymbiotic theory concerns organelle reduction and loss. In a sense, all endosymbiotic organelles are products of massive reduction of the metabolic complexity and capabilities of the ancestral free-living symbiont. But there is a tendency to regard functional organelles as having reached a stable suite of core metabolic functions—in the case of plastids photosynthesis is considered the cornerstone of organellar function (see below for summary of plastid biochemical functions). Despite this mindset, an extensive number of lineages have independently advanced their plastids a further rung on the ladder of reduction by losing their photosynthetic capability (21). Parasitic plants and apicomplexan parasites such as the malaria parasites are notable examples; many other protists have also lost the ability to perform photosynthesis but retained their further reduced plastids (e.g., the euglenid *Astasia* and the dinoflagellate *Crypthecodinium*). These nonphotosynthetic plastids apparently still provide essential services to the host cells—for instance, fatty acid synthesis, isoprenoid synthesis, and heme synthesis in the case of the malaria parasites (96). Most of these additional plastid pathways have likely replaced equivalent host cell pathways that occurred in the ancestral host cell prior to plastid acquisition (endosymbiont metabolic replacement). Why a plastid pathway should replace an existing host cell pathway is unclear, although it is quite conceivable that chance has played a role in the elimination of any one of the duplicated pathways after endosymbiotic merger. In any case, fixation of the plastid copy of any essential metabolic pathway would commit a cell to plastid retention even if photosynthesis was subsequently abandoned. What, then, is the likelihood of such a cell achieving complete plastid loss?

To date, members of at least six major eukaryotic lineages may have achieved outright plastid loss: ciliates, the apicomplexan (e.g., *Cryptosporidium*), dinoflagellates (several lineages), heterokontophytes (e.g., oomycetes), trypanosomatids, and cryptophytes (*Goniomonas*). However, for several of these lineages such claims have inspired lively debate. The case for plastid loss in alveolates (i.e., ciliates, apicomplexans, and

dinoflagellates) largely hinges on acceptance of the chromalveolata hypothesis, which unites alveolates with chromists (heterokontophytes, cryptophytes, and haptophytes) and proposes plastid origin in a common ancestor (22). If this hypothesis is correct, ciliates and basal lineages of apicomplexans (*Cryptosporidium* and gregarines) and dinoflagellates (e.g., *Oxyrrhis*, *Amoebophyra*, *Noctiluca*) that all lack plastids must have independently lost these organelles (1, 107, 121, 134). Challenging this scenario is the lack of strong phylogenetic evidence for the monophyly of chromalveolates host cells (40, 86, 120). Thus, an alternative explanation for plastid occurrence in alveolates is that apicomplexans and dinoflagellates independently gained their plastids, and that ciliates and the basal members of each ancestrally lacked a plastid. The dinoflagellate lineage, however, may represent an independent case for plastid loss, because several nonphotosynthetic groups are apparently scattered throughout photosynthetic dinoflagellates [according to small subunit rRNA phylogenies and plate tabulation data (107)], implying several independent losses. However, loss of photosynthesis may not always imply plastid loss, and the recent demonstration that at least one such taxon (*Crypthecodinium*) retains a nonphotosynthetic plastid indicates that plastid loss should be more closely examined in this group (108).

In contrast to the conspicuous photosynthetic members of the heterokontophytes such as kelp and diatoms, many members (e.g., thraustochytrids or oomycetes) are nonphotosynthetic (23). Oomycetes are well known plant pathogens, responsible for significant historical events such as the Irish potato famine. Most nonphotosynthetic heterokontophytes fall into basal clades (23), and therefore again the question of plastid loss hinges on whether heterokontophytes share a common plastid with other major lineages (i.e., other chromists, or indeed all chromalveolates) or whether a plastid was independently acquired within the heterokontophyte radiation. Trypanosomatids are a heterotrophic group of parasites whose sister relationship to the euglenids (many of which are photosynthetic) has inspired suggestion that these parasites also once contained a plastid but have since lost it (41). However strong evidence for secondary plastids as a recent gain in the euglenoid lineages (66), along with the rebuttal of the Cabozoa hypothesis (see above), undermines the case for plastid loss in trypanosomatids.

Perhaps the strongest case for plastid loss occurs in the Cryptophyta. Most cryptophytes are photosynthetic, although some have apparently lost photosynthesis but retain a relict plastid (113). Conversely, *Goniomonas* is a basal heterotrophic cryptophyte that apparently lacks a plastid (77). Recent phylogenies based on molecular data have strongly identified haptophytes as the sister lineage to cryptophytes (40, 86), and a gene replacement of plastid-encoded rpl36 is uniquely shared by these taxa, implying they share a common plastid (55, 101). Thus, reasonable evidence exists that the common ancestor of cryptophytes and haptophytes contained a plastid, and therefore *Goniomonas* has lost its plastid.

Although further cases of plastid loss will likely be substantiated as global phylogenies develop better resolution, a case for plastid loss in cryptophytes at least looks well supported. How then, is a eukaryote able to reverse the endosymbiotic process—particularly, how can endosymbiont metabolic replacement be reversed? Two scenarios are possible. One is that the plastid is lost relatively early in endosymbiont integration, before endosymbiont metabolic replacement occurs. Cavalier-Smith (19) has suggested that this accounts for why plastidless taxa are often basal to photosynthetic lineages; they represent the period before a cell starts to rationalize its own biochemistry and rely on elements of the symbiont's biochemistry. If the chromalveolate hypothesis is correct, then only after the major lineages diverged did the plastid become essential beyond photosynthesis, because most of the major lineages have plastidless basal members. A second scenario

is that through heterotrophy a cell can satisfy its requirement for the macromolecules it had come to rely upon from the plastid. Many secondarily nonphotosynthetic groups are either predators or highly adapted parasites, with access to a rich supply of macromolecules. If this diet can satisfy the need for fatty acids, isoprenoids, and heme, for instance, a cell might be well on the way to making its plastid obsolete.

In either case, if a plastid is lost from a eukaryotic lineage, would evidence of the plastid's tenancy remain? The many hundreds to thousands of nucleus-encoded plastid genes would initially be present, but without a function these would likely degrade quite rapidly. However, any plastid genes that had come to fulfill an alternative, nonplastid-localized function would remain useful even after plastid loss. Such genes, referred to as EGT (endosymbiont gene transfers) have been estimated to represent between 10% of nucleus-encoded genes derived from the plastid in glaucophytes and 50% in plants (73, 100). This amounts to ~150 and ~2250 genes in total in the two groups, respectively. Thus, loss of a plastid could conceivably leave a conspicuous footprint if these genes remained useful in the absence of the plastid. In the oomycetes one such plastid-derived gene (*gnd*) has been hailed as evidence for the former plastid in this lineage (6). However, to form a compelling case for a former plastid we should anticipate a large collection of such genes when the annotation of oomycete genomes is complete. Conversely, ciliates have revealed no such plastid footprint; the complete sequence of *Tetrahymena thermophila* was recently investigated for just such relict plastid genes (29). It will be very interesting to undertake the same analysis for *Goniomonas* in pursuit of a better understanding of the process of plastid loss.

Symbioses in progress. In addition to full-fledged plastid organelles, numerous organisms demonstrate that endosymbiosis is a continual driving force in evolution. Here we present four interesting cases of organisms at various points of negotiation of these cellular marriage contracts.

Rhopalodia gibba is a diatom that hosts both a secondary red algal–derived plastid and a novel cyanobacterial symbiont (**Figure 1**) known as the spheroid body (32). Unlike cyanobacterial-derived plastids, which are typically photosynthetic, the spheroid body has apparently lost this ability. However, the spheroid body has retained another core cyanobacterial function, the ability to fix nitrogen, which it performs for its diatom host during the day (93). Spheroid bodies are inherited vertically from one generation to the next, and their numbers are regulated in the host cell, which implies a high level of host control over the endosymbiont. As yet no evidence for spheroid body genes in the diatom host has been found, so whether the final stage of organelle integration has been achieved in this case remains undetermined. However, the spheroid bodies have clearly suffered gene loss, and they are most likely incapable of again living outside the host (93). Nitrogen fixation is another of the innovations specific to prokaryotes, so it is noteworthy that endosymbiotic capture again has played a role in extending such fundamental capabilities to eukaryotes.

A further independent example of primary endosymbiosis is seen in the freshwater amoeba *Paulinella chromatophora*, which also hosts a cyanobacterium-like symbiont (56). In this case photosynthesis is retained, and *P. chromatophora* accordingly has converted from heterotrophy to autotrophy. The *Paulinella* endosymbiont, referred to as a cyanelle, occurs in the cytoplasm without any additional bounding membranes, and symbiont numbers are strictly regulated, again suggesting a higher level of host-symbiont interaction (133). Attempts to culture the symbiont separate from the host have thus far been unsuccessful, but analysis of the *Paulinella* cyanelle genome reveals neither obvious gene loss nor transfer of genes to the host, so it appears that there has been relatively little genetic response to this union so

far (133). The *Paulinella* symbionts are thus enigmatic; however, these symbionts potentially represent a second case of primary endosymbiosis enabling photosynthetic capture. Fortunately, *Paulinella chromatophora* has symbiont-lacking sister species (*P. indentata* and *P. ovalis*), which offers the possibility of understanding how this organism can acquire a permanent prokaryotic endosymbiont in a process reminiscent of the origin of plastids.

Nascent secondary endosymbioses are also in evidence. The enigmatic flagellate *Hatena arenicola* harbors a quasi-permanent prasinophyte-like endosymbiont (related to the genus *Nephroselmis*), which exhibits substantial structural modification when within the host. Permanent integration of this photosynthetic symbiont is apparently pending because division of the symbiont is not yet coordinated with that of the host (85). Nevertheless, a degree of integration has apparently occurred, because host cell division results in one daughter cell inheriting the endosymbiont while the other daughter cell is left without a symbiont and presumably sources a new symbiont from the environment.

Perhaps one of the more startling cases of endosymbiosis in progress is the plastid theft performed by the sea slug *Elysia chlorotica*. This sea slug feeds upon algae and can salvage the plastids from their diet of *Vaucheria litorea* and maintain them, generating photosynthate that can nourish the animal for many months. The plastids, which are arrayed in specially generated diverticulae of the slug gut to be exposed to incoming light, remain transcriptionally and translationally active for up to nine months (83). Circumstantial evidence suggests that the plastids even receive proteins synthesized by the sea slug (42). If substantiated, this would implicate horizontal transfer of a gene from the alga to the sea slug, which by one definition would make this captured plastid an animal organelle. However, these plastids are unable to divide, and are not passed on from one slug generation to another, nor do they occur in the animal's germ line. In fact, stolen plastids such as these could probably never achieve permanent endosymbiont status because many essential plastid genes would have been left behind when the algal nucleus was digested. Sea slug plastids are thus examples of kleptoplastids: photosynthetic organelles stolen from another organism but not permanently acquired.

PREPROTEIN TARGETING

Intracellular gene relocation is dependent on the existence of a system to reimport the gene product back to the compartment of origin. Given the massive scale of plastid-to-nucleus gene relocation, this system must recognize and sort a large number of plastid-destined proteins from all other proteins synthesized in the cytoplasm. Elements of this system, including proteins of the import apparatus embedded in the plastid membranes and some features of plastid precursor proteins, are shared throughout phototrophic eukaryotes and reflect the common origin of primary plastids. Core elements of the system shared by red algae and green algae/plants clearly arose early, prior to the diversification of the primary endosymbiont-containing lineage (79). In plants additional elements such as extra receptors and redox-sensing components of plastid protein import clearly have arisen to optimize this system and also facilitate the biogenesis of different plastid types (e.g., amyloplasts, chromoplasts, and chloroplasts) (14). Great insight has now been achieved into the complex and sophisticated plastid protein import machinery of plants, the details of which are reviewed elsewhere (14, 38, 115). Here, we confine ourselves to a synopsis of the primary plastid import system and give more focus to the less well-understood machinery for protein import into secondary plastids with multiple bounding membranes.

Targeting to Primary Plastids

Primary plastids contain three distinct sets of membrane: the outer envelope membrane

(OEM), the inner envelope membrane (IEM), and the thylakoid membranes, which thus create three separate compartments (intermembrane space, stroma, and thylakoid lumen). Proteins can therefore be targeted to six regions within plastids: three membranes and three soluble compartments. Dedicated translocation machineries and peptide targeting information within the nuclear-encoded plastid proteins are used in concert to achieve these targeting feats (**Figure 2**).

The majority of proteins is targeted to the plastid posttranslationally, facilitated by an N-terminal transit peptide extension. This peptide leader can vary in length from approximately 20 to 150 amino acids and no primary sequence consensus or common secondary structure has been identified in the large collections of transit peptides known from different plants. General characteristics include hydrophobicity at the extreme N terminus, enrichment of hydroxylated amino acids, and a depletion in acidic residues that leads to a positive charge, particularly toward the N terminus (16, 89, 95). Some transit peptides are phosphorylated by an ATP-dependent cytosolic kinase, which leads to an interaction with a guidance complex; these peptides are then preferred for import (76). After translocation, transit peptides are cleaved off by the stromal processing peptidase, which belongs to the M16 family of metallopeptidases, releasing the mature protein into the stroma (102).

Transport of the majority of preproteins across the two envelope membranes is the job of the Toc (translocator of the outer chloroplast membrane) and Tic (translocator of the inner chloroplast membrane) machineries. These two apparatuses comprise multiple soluble and membrane-bound proteins named for their molecular masses (see **Figure 2**). Toc75 is the main translocation pore in the outer membrane (28) and together with Toc 33/34 and 159 makes up the Toc core (111, 112). Other Toc components apparently have subsidiary roles; for example, Toc64 is implicated in plastid protein recognition and delivery to the Toc pore. In plants such as *Arabidopsis* multigene families encode different (partially redundant) isoforms of Toc components and differential isoform expression probably generates import complexes tailored to particular plastid states in different tissues (12, 51). Conversely, the haploid moss *Physcomitrella patens* appears to lack these isoforms, and thus is emerging as a superior model for gene knockout studies of Toc/Tic function (47). Interestingly, in the genome of the red alga *Cyanidioschyzon merolae*, only Toc34 and Toc75 have thus far been identified (**Figure 2**), which might mean that major receptor components of the outer membrane (e.g., Toc 159 and 64) of land plant plastids are specific to this green lineage, and that red-specific Toc receptors await discovery (79).

The core of the Tic complex includes Tic20, Tic22, and Tic110 (**Figure 2**). Tic22 is a soluble protein in the intermembrane space and is thought to be the first Tic component to interact with incoming precursors (59). Either or both of the two membrane proteins Tic20 and Tic110 could be involved in pore formation but details are unclear (50, 60, 122). Tic110 also interacts with the chaperone Hsp93 (ClpC) inside the stroma (3). A similar function is proposed for Tic40, because it possesses a conserved domain known from Hsp70 cochaperones (24). Several reports suggest the other Tic components of land plants (Tic32, Tic55, and Tic62) might be involved in the redox-regulated import of preproteins (13, 61). The presence of homologs for Tic20, Tic22, Tic62, and Tic110 in the genome of the red alga *C. merolae*, combined with the apparent absence of Tic32 and 55, might suggest that the former are essential and the latter dispensable for functional plastid import (79), but experimental confirmation is needed. Finally, on the stromal side of the membrane chaperones such as GroEL and CplC interact with the Tic complex to receive the imported proteins and fold them, after cleavage of the transit peptide, to their mature conformation (125). However, some proteins require further targeting and the

thylakoid membranes contain no less than three independent sets of protein translocation machineries for this purpose: the signal recognition particle–dependent (Albino3) pathway, the Tat (twin arginine translocon) pathway, and the Sec-dependent pathway. In addition, spontaneous insertion of proteins into thylakoid membranes is also known to occur, thus offering at least four alternative routes into the membrane or lumen of thylakoids (38).

Alternative, Toc/Tic-independent routes to plastids are also becoming apparent (60, 97). For instance, the outer envelope protein 16 (Oep16)—a homolog of bacterial preprotein and amino acid transporters—serves as the translocase for one plastid protein, NADPH:protochlorophyllide oxidoreductase A (98). Another noncanonical import pathway through the outer envelope membrane was recently revealed with the identification of nuclear-encoded plastid proteins possessing N-terminal signal peptides rather than the standard plastid transit peptides (94, 126). These plastid proteins traverse the endoplasmic reticulum (ER), and most likely also traverse the Golgi apparatus where they are glycosylated, and are subsequently targeted to the outer envelope membrane of the plastid (94, 126). The details of this alternate route remain mysterious.

Targeting Into and Within Secondary Plastids

Translocation of precursor proteins to secondary plastids must surmount additional obstacles in the form of extra bounding membranes, which also creates additional compartments that have their own specific proteomes. Three membranes surround dinoflagellate and euglenophyte complex plastids, whereas cryptophyte, heterokontophyte, haptophytes, apicomplexan, and chlorarachniophyte plastids are surrounded by four membranes (22). Independent origins of secondary plastids have resulted in distinct targeting solutions to these advanced trafficking needs; however, remarkably, some unifying principals have emerged. Virtually all known complex plastid preproteins encoded in the nucleus possess an N-terminal topogenic signal composed of at least two parts: a signal peptide and a transit peptide. The ubiquity of what appears to be a canonical signal peptide in complex plastid-targeted proteins is consistent with the outermost membrane being a component of the host cell's endomembrane system, apparently derived from the formative phagocytic event (22). The signal peptide mediates cotranslational import into the ER lumen, where signal peptidase removes the signal peptide to expose the transit peptide, which is responsible for targeting across the remaining membranes. An unusual elaboration of this bipartite leader occurs in the two cases of complex plastids surrounded by three membranes: dinoflagellates and euglenoids. Here an additional signal, a hydrophobic membrane anchor, is embedded in the transit peptide region of most plastid proteins (84, 87, 118). Thus, insertion of preproteins into the ER lumen in these taxa is apparently delayed; plastid preproteins are anchored to endomembranes until they are delivered to the plastid and the complete plastid import is enabled.

In heterokonts, haptophytes, and cryptophytes the outer plastid membrane is continuous with the host rough ER and thus is studded with ribosomes (18). Plastid proteins encoded in the nucleus have therefore already passed through the first of four membranes after cotranslational insertion into the ER lumen by the N-terminal signal peptide (**Figure 2**). However, in other complex plastid systems no such continuity of plastids and ER is apparent, so plastid preproteins must be delivered from the ER lumen to the outer plastid membranes [termed epiplastid membranes (EPM) in **Figure 2**], presumably by vesicular traffic (84, 118). After signal peptide cleavage, plastid proteins must be distinguished from secretory proteins; mutagenesis experiments in several complex plastid systems indicate that the transit peptide is responsible for this discrimination (34, 62, 129).

Epiplastid membrane (EPM): the outermost membrane surrounding complex plastids

Periplastidial compartment (PPC): the reduced cytosol of the engulfed alga; harbors the nucleomorph and 80S ribosomes in cryptophytes and chlorarachniophytes

Periplastidial membrane (PPM): represents the former cytoplasmic membrane of the endosymbiont and is the second outermost membrane in four-membrane-bounded plastids

The signal peptide and transit peptide thus act sequentially to mediate targeting into the stroma of complex plastids.

At least for some complex plastids, proteins are also targeted into the periplastidial compartment (PPC); these proteins also utilize a bipartite leader. The most N-terminal residue of the transit peptide is critical in dictating whether a protein travels all the way through the three innermost plastid membranes to the stroma or whether it stops in the PPC after traversing only the periplastidial membrane (35, 116). In the cryptophyte *Guillardia theta* and the heterokontophyte *Phaeodactylum tricornutum*, this +1 transit peptide residue is typically a phenylalanine for stromal proteins (in a few exceptions other aromatic amino acids fulfill this role). In the absence of this phenylalanine the preprotein accumulates in the PPC (34, 37). In other chromalveolates an aromatic amino acid–based motif (F-motif) is also a conspicuous feature of the N terminus of transit peptides, and likely plays a role in correct stromal targeting (35, 58, 90). Interestingly, this F-motif also occurs in the transit peptides of plastid-targeted proteins of glaucophytes and rhodophytes (117), which suggests that the F-motif could be an ancient targeting element for plastid import. Thus, in complex plastids the role of this F-motif has apparently been extended to discriminate between proteins that are required to be targeted fully into the plastid stroma and those that must be halted in the PPC. The corollary is that the remainder of the transit peptide is sufficient for targeting across the periplastidial membrane (PPM) (**Figure 2**). Curiously, the F-motif does not occur in transit peptides of the green algal/plant lineage, and thus this ancient targeting signal has apparently been abandoned here. This might explain the apparent need for extra receptors like Toc159 and 64 in these plastids (**Figure 2**).

The second step of protein trafficking into complex plastids (the translocation from the ER lumen into the PPC, see **Figure 2**) presumably requires a translocon in the PPM. Termed the Top translocon (22), this hypothetical membrane transporter and its intriguing evolutionary pedigree may recently have been identified. The nucleomorph of the cryptophyte *G. theta* encodes ERAD (ER-associated degradation) components, including a Der1p (degradation in the ER) membrane translocon able to complement ERAD-deficient yeast (116). Because no ER is present inside the periplastidial space, the location of this nucleomorph-encoded ERAD machinery was intriguing. Preliminary immunolocalization studies suggest that the Der1p translocon is located in the PPM of the cryptophyte's complex plastid (116), leading to speculation that it could be the long sought after Top translocon. Further credence for this hypothesis comes from the identification of an extra set of ERAD machinery (distinct from the canonical host ER ERAD machinery) that is apparently targeted to the complex plastids of other chromalveolates such as diatoms and *Plasmodium* (116). Because these plastids have lost all traces of the endosymbiont cytoplasm it is highly plausible that this ERAD machinery could localize to the periplastidial membrane and have a role in translocating transit peptide–bearing preproteins into the complex plastids. The ERAD-derived translocon is proposed to recognize the transit peptide (which might resemble an unfolded protein similar to the normal ERAD substrate) and to pull the precursor proteins out of the ER and into the periplastidial compartment (**Figure 2**).

Although the role of this ERAD apparatus in targeting proteins to complex plastids remains to be substantiated, it provides tantalizing support for the chromalveolate hypothesis. As alluded to above, solving the "protein-import problem" was a major hurdle in the establishment of secondary endosymbionts (18). The apparent co-option of a normally ER-based protein translocation system into plastid transport by cryptophytes, heterokontophytes, and Apicomplexa is suggestive of a common origin for the plastids. Similarly, use of the F-motif to discriminate between periplastidial and stromal-directed

proteins by cryptophytes, diatoms, and perhaps even apicomplexa is also congruent with a common origin for their plastids.

BIOCHEMICAL PATHWAYS

The union of a heterotroph and an autotroph in an endosymbiotic partnership amalgamates two suites of metabolic pathways into a single organism (131). The driver for the union is typically believed to be the acquisition of photosynthesis by the host. Thus, both primary and secondary endosymbioses likely converted heterotrophs into phototrophs. Some serial secondary endosymbioses and tertiary endosymbioses may have simply exchanged one photoautotrophic endosymbiont for another, but in general we can frame the question in terms of heterotroph + autotroph = new autotroph. From a metabolic perspective this fusion creates interesting possibilities. Autotrophs are typically self-sufficient metabolically; some require vitamins, but in general they synthesize everything they need from scratch. Conversely, heterotrophs have access to a range of preformed macromolecules in their diet and are able to salvage various building blocks from these macromolecules and utilize them in their metabolism. Thus, as a general principle, the endosymbiosis likely introduced extra metabolic capability beyond just photosynthesis to the host's repertoire. A key challenge is to unravel which pathways were introduced into the amalgam from the endosymbiont. As discussed above, the host can become dependent on endosymbiont pathways other than photosynthesis, and this dependency can impact plastid persistence should the organism subsequently revert to a totally heterotrophic lifestyle.

What do we know about the metabolic repertoires of the original hosts and endosymbionts? For the hosts we can say very little. We have a relatively poor understanding of the nature of the host for the primary endosymbiosis, and, similarly, we are largely ignorant of the host's affinities for the three known secondary endosymbioses (euglenophytes, chlorarachniophytes, and chromalveolates). It is thus rather difficult to speculate on what kind of metabolisms these hosts could have had at the outset of the endosymbiotic relationship. However, we are in a better position to hypothesize about the metabolic repertoire of the endosymbionts. For primary endosymbiosis we can postulate that the endosymbiont had a suite of metabolic capabilities similar to those in modern-day cyanobacteria. For secondary endosymbioses we can assume that the endosymbionts had a metabolic potential similar to that in the modern representatives of red or green algae as appropriate.

Weeden (131) was the first to ponder from a metabolic perspective the consequences of fusing an endosymbiont and host. He recognized that the endosymbiont introduced novel pathways into the host and he outlined how amino acid, heme, and starch pathways were inducted into hosts via endosymbiosis. We have subsequently learned that cells have also exercised considerable creativity during these metabolic mergers, and complex amalgams of host and symbiont pathways have also been the fruits of these partnerships.

Starch Synthesis

Excess photosynthate is generally stored as glucan polymers. Plants and green algae store starch (α-1,4 glucan) in the plastid, whereas red algae store starch in the cytosol (127). On the basis of these localities of starch synthesis, red algae were assumed to utilize a host-derived glucan synthesis mechanism whereas the green algae and plants were assumed to employ a system derived from the endosymbiotic ancestor of the plastid (**Figure 3**). The starting points for each of these pathways—UDP-glucose precursors for red algae and ADP-glucose precursors for green algae and plants—also reflect the dichotomy between eukaryotic and prokaryotic glucan pathways. However, in reality both host and symbiont proteins have been recruited in starch synthesis in both red and green algae, and only

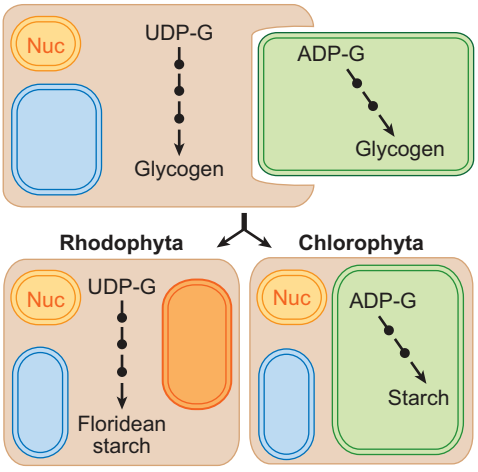


Figure 3

Schematic representation of starch synthesis before and after primary endosymbiosis. Nuc, nucleus; UDP-G, uridine-diphosphate glucose; ADP-G, adenosine-diphosphate glucose.

the localities, either cytosolic or organellar, have been derived from either host or symbiont (87). Why red algae retained the site of host glucan storage whereas green algae (and their descendants) adopted the endosymbiont storage site remains unknown (**Figure 4**).

In secondary endosymbiosis glucan storage distribution differs: Sometimes it is in the host, and sometimes it is in the endosymbiont. For instance, euglenoids store paramylon (β-1,3 glucan) in the cytosol, although their endosymbiont is thought to have been a green alga that presumably stored starch in the plastid (26, 128). Chlorarachniophytes and heterokontophytes also store β-1,3 glucans in the secondary host cytosol and have abandoned the glucan storage systems of the green and red algal endosymbionts, respectively (78). Conversely, cryptophytes store starch in the PPC (remnant endosymbiont cytoplasm), thus conserving the endosymbiont glycan storage system of the red algal endosymbiont (34). Dinoflagellates also store starch; however, rather than in a PPC, storage occurs in the host cytoplasm, implying relocation of this pathway from the red algal cytosol to that of the host (25). Thus, the storage of surplus photosynthate in either the host or endosymbiont compartments has taken a range of alternatives in both primary and secondary endosymbiotic partnerships.

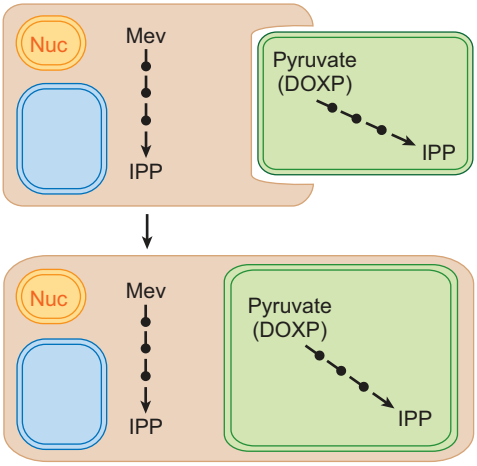


Figure 4

Schematic representation of the isopentenyl diphosphate (IPP) synthesis pathway before and after primary endosymbiosis. Mev, mevalonate; DOXP, 1-deoxy-D-xylulose 5-phosphate.

Isopentenyl Diphosphate (Isoprenoid Precursor) Synthesis

Isopentenyl diphosphate (IPP) is a building block for terpenes, sterols, carotenoids, and isoprenoids that are important components of a diverse range of cellular molecules such as chlorophylls and quinones. IPP synthesis was only recently discovered to occur in plastids (68). In plants the cytosol harbors the canonical mevalonate pathway for IPP synthesis (8), and for many years it was presumed that this was the sole source of isoprenoid precursors in plants. Given the extensive use of isoprene subunits in plants for secondary metabolites such as terpenes, chlorophylls, ubiquinol, prenylated proteins, and isopentyl tRNAs, it is sobering to reflect that a plastid-based, nonmevalonate, deoxyxylulose (DOXP) pathway for IPP synthesis was overlooked, or at least unrecognized, by plant physiologists. However, once it emerged that bacteria synthesize IPP from pyruvate and glyceraldehyde 3-phosphate and not from mevalonate like

eukaryotes, it was a simple step to identify a DOXP pathway in plastids (105). Indeed, the discovery of the plastid DOXP pathway reconciled some previously incongruous precursor incorporation and inhibitor data (67). Synthesis of IPP in plastids also simplifies the delivery of these entities to isopentenylate tRNAs for plastid translation and to isoprene chains for chlorophyll production (63).

In plants, IPP synthesis occurs in both the host compartment (cytosol) and the endosymbiont compartment (plastid). The two different pathways coexist and are even integrated to an extent (65), but their differences are congruent with one (acetate/mevalonate) being derived from the host and the other (DOXP) being introduced with the cyanobacterial symbiont (**Figure 4**). Exactly why both pathways persist is not known but the requirement for products in both the host and endosymbiont compartments perhaps necessitated the retention of two pathways.

Heme Synthesis

The synthesis of the tetrapyrroles that act as temporary electron carriers in various redox reactions is reminiscent of IPP synthesis in that there are two very different pathways that begin with different substrates and utilize some, but not all, different enzymes. Many plastid-lacking eukaryotes utilize the so-called C4 or Shemin pathway, which commences by fusing succinyl-CoA and glycine to create δ-aminolevulinic acid (ALA), courtesy of aminolevulinic acid synthase (ALAS) (**Figure 5**). In animals and yeast, ALAS (114) is located in the mitochondrion and ALA is then exported to the cytosol where a series of steps convert it to coproporphinobilogen III (CPIII). CPIII is then routed back into the mitochondrion by a recently identified transporter for the last four steps to eventually produce heme (**Figure 5**).

Cyanobacteria have a different initial substrate, commencing C-5–type heme synthesis from glutamyl-tRNA rather than succinyl-CoA and glycine (5) (**Figure 5**). Glu-tRNA

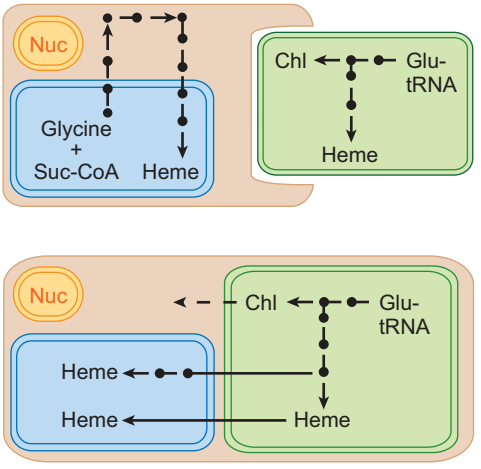


Figure 5

Schematic representation of heme synthesis before and after primary endosymbiosis. Nuc, nucleus; Glu tRNA, glutamyl tRNA; Suc-CoA, succinyl-Coenzyme A; Chl, chlorophyll.

reductase followed by Glu-SA aminomutase convert the aminoacylated tRNA to ALA. Steps from ALA to heme are then identical in cyanobacteria and the Shemin pathway, and the enzymes involved are homologous. However, in cyanobacteria the pathway forks at protoporphinobilogen IX. One branch leads to heme as per the Shemin pathway, but the other branch involves the addition of Mg^{2+} to protoporphinobilogen IX to generate chlorophyll (124).

Because the original host for primary endosymbiosis likely had aerobically respiring mitochondria, we can assume it had a Shemin pathway to generate heme for its cytochromes. The acquisition of a cyanobacterial endosymbiont almost certainly introduced the glutamyl-tRNA–based pathway into the first eukaryotic autotrophs. At the outset this organism would have had two heme synthesis pathways: a Shemin pathway in the mitochondrion/cytosol and a cyanobacterial-like C5 pathway in the endosymbiont (**Figure 5**). To carry on photosynthesis, the endosymbiont likely continued to synthesize chlorophyll from glutamyl-tRNA; indeed, plant plastids still synthesize chlorophyll entirely within the

plastid using a pathway homologous to that of cyanobacteria (124). Interestingly, plants have disposed of the early portions of the Shemin pathway and do not use glycine or ALAS to commence heme synthesis (92). Rather, they export protoporphinobilogen IX from the plastid to the mitochondrion, which then performs the last two steps of heme synthesis using enzymes homologous to those of the animal/yeast Shemin pathway (**Figure 5**). It is noteworthy that plastids also complete the conversion of protoporphinobilogen IX to heme independently for the benefit of furnishing the prosthetic group for their own cytochromes.

For heme we thus see a picture of two pathways rationalized into one pathway that forks three ways: chlorophyll and heme synthesis from glutamyl-tRNA in the plastid, and heme synthesis in the mitochondrion commencing not from its original ALAS but from plastid-synthesised ALA (**Figure 5**).

Aromatic Amino Acid Synthesis

The essential amino acids are a necessary part of the animal diet because we lack a pathway to synthesize tryptophan, phenylalanine, and tyrosine. Autotrophs lack a diet and must synthesize these and all 17 other amino acids. In plants the shikimate pathway located in the plastid synthesizes the precursors for the aromatic amino acids. There are two versions of the shikimate pathway: a prokaryotic-style version, which is what occurs in the plant plastid, and a cytosolic-based version with different enzymes, as occurs in fungi (45). In plants the plastid has clearly retained its ancestral ability to synthesize tryptophan, phenylalanine, and tyrosine and supplies these amino acids to the cytosol (host) (**Figure 6**). Whether or not the original host possessed a shikimate pathway prior to primary endosymbiosis remains unclear. If it did, all traces are now lost and the plastid bears sole responsibility for this task in members of the red algae, green algae, and plants.

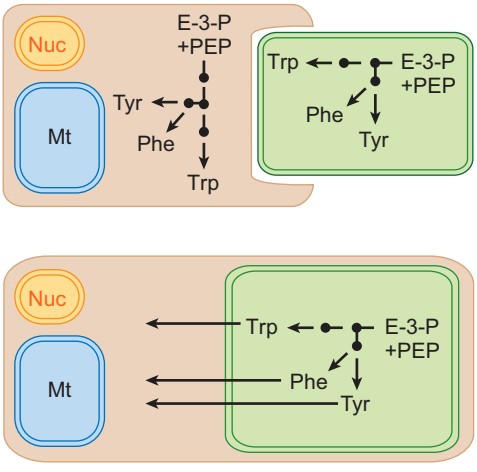


Figure 6

Schematic representation of aromatic amino acid synthesis before and after primary endosymbiosis. Nuc, nucleus; Mt, mitochondrion; E-3-P, erythrose-3-phosphate; PEP, phosphoenolpyruvate; Tyr, tyrosine; Phe, phenylalanine; Trp, tryptophan.

Fe-S Clusters

Fe-S clusters are important prosthetic groups of various metalloproteins that participate in redox reactions, sensing of iron and oxygen, and catalysis (69). The Fe atom in Fe-S clusters is able to take up an electron reversibly, thus providing the required electron carrier capacity. Fe-S–containing proteins are pervasive in life and ancient; well-known examples include the ferredoxins, NADH dehydrogenase, and Coenzyme Q. The clusters contain different numbers of iron and sulfur depending on cluster type and are coordinated into the protein through cysteinyl ligands. Synthesis and insertion or removal of Fe-S clusters into and out of proteins is managed by a number of enzymes, not all of which have been identified. At least three different systems for Fe-S cluster formation and insertion [iron sulfur cluster (ISC), nitrogen fixation (NIF), and mobilization of sulfur (Suf)] are known thus far. Plastids harbor a Suf-type Fe-S cluster formation system homologous to that of cyanobacteria (11, 119). Suf system–generated Fe-S clusters are probably incorporated into a range of plastid proteins

including ferredoxin and the Rieske iron sulfur protein.

The original host of the primary plastid endosymbiont almost certainly contained an Fe-S cluster formation system, but it was probably not cytosolic. Eukaryotes all appear to form Fe-S clusters, but the initial steps are mitochondrial and typically utilize the ISC system (64). An export machinery translocates the Fe-S cluster into the cytosol where machinery exists to insert the cluster into apoproteins. This mitochondrial-based synthesis was likely obtained with the α-proteobacterial endosymbiont that gave rise to the mitochondrion (70). The host cell for primary endosymbiosis likely had this system, and indeed, plants utilize an ISC system in their mitochondria and a Suf system in the plastid (11).

SUMMARY POINTS

1. All plastids ultimately arose from a single prokaryotic endosymbiosis, where a cyanobacterium was engulfed and retained by a eukaryotic cell.
2. Eukaryotic endosymbiosis has occurred multiple times, and this process has lead to the spread of plastids throughout a great diversity of eukaryotes.
3. Further endosymbiotic events continue to occur in nature, and a wide continuum exists between temporary symbiotic relationships, stable interdependent partnerships, and those that are intimately integrated at a molecular-genetic level.
4. Pivotal to the integration of plastids was the establishment of protein delivery systems that enabled a shift of genetic control from the organelle to the host nucleus. Prokaryotic endosymbiosis required the generation of a novel protein import system, whereas eukaryotic endosymbionts have co-opted and adapted existing protein translocation systems to achieve this task for complex plastids.
5. Eukaryotes have gained numerous metabolic capabilities through endosymbiosis, including but not restricted to photosynthesis. Some of these capabilities were unique prokaryotic inventions, and have thus extended the capabilities of eukaryotes. Others represented duplications of existing host pathways, and in many cases rationalization of redundancy has generated novel chimeric pathways in eukaryotes.

FUTURE ISSUES

In recent years the broadening of genome sequencing programs has encompassed a greater diversity of plastid-bearing eukaryotes, and we are now seeing great advances in our understanding of plastids. This diversity includes the molecular integration of plastid genomes with those of the host, the mechanisms and trafficking routes of transferred gene products on their return journey to the plastid, and the metabolic integration and trade between plastids and their diverse hosts. Several key research directions now present themselves and are conceivably within greater reach than ever before. (1) Although the phylogenetic affinities between the major eukaryotic lineages are begining to slowly resolve into focus, considerable controversy continues to surround the question of how many endosymbiotic events have generated the plastid diversity observed in eukaryotes—notably with respect to the plastids of the Chromalveolates. Resolution of these issues

is critical to our interpretation of evolution of the diversity we see among plastids in this group, and our understanding of the frequency and mechanisms of plastid loss. (2) Although our understanding of protein targeting to primary plastids has reached a relatively advanced state, equivalent insight into the mechanisms for targeting proteins to complex plastids lags behind. These details may be vital in tackling some of the more insidious complex plastid-bearing eukaryotes such as the apicomplexan parasites, where plastid-targeted pathways offer tantalizing possibilities as drug targets for diseases such as malaria. (3) Genomic analyses have presented some insights into the broader suite of metabolic functions of plastids beyond photosynthesis; however, little is known about delivery of the products of these pathways to the host cell, or vice versa. Although this presents one of the larger challenges to plastid researchers, the realization of this goal is necessary to provide a full appreciation of the significance of plastid gain in eukaryotes through endosymbiosis.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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Annual Review of
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Contents

Indexes

Errata

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