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Review

Changing concepts of a plant: current knowledge on plant diversity and evolution

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Abstract From a phylogenetic perspective, most plant biodiversity lie in the algae, which comprise nine divisions distinct in cell architecture. In the past decade or so, molecular phylogenies have revealed that many algal divisions are only distantly related, and belong to five different supergroups of eukaryotes. The scattered and distant distributions of algae are interpreted as the result of separate endosymbioses that occurred in various lineages of eukaryotes. Endosymbiosis is a major driving force of algal evolution and diversification, and therefore, is a key process in understanding plant evolution. In this paper, the process of plastid acquisition via endosymbiosis is considered, focusing on the evolution of protein transport machinery, which is indispensable for establishing new lineages of algae, and simultaneous lateral gene transfer from the symbiont to the host nucleus. The current understanding of protein transport diversity is reviewed in relation to the membrane topologies of plastids. The endosymbioses found in various algae and protists are natural occurrences in plant evolution and diversification, and exhibit intermediate stages of plastid acquisition. The conditions necessary for plastid establishment are also considered, using several examples of ongoing endosymbioses.

Key words: Algae, diversity, endosymbiosis, evolution, protists.

Our understanding of the tree of life has changed over the centuries. Biology originally relegated all living things to either Plantae (L. *planta*) or Animalia (L. *anima* "breath, soul"). However, this view changed dramatically beginning with the invention of the microscope and subsequent discoveries of previously "invisible" organisms (microorganisms). German zoologist Ernst Haeckel (1866) recognized microscopic organisms as the third group of life, the Protista (Gk. *protos* "first"). He included in this group all unicellular organisms, such as bacteria and protozoa. Developments in the field of pathology showed that bacteria (Gk. *bakterion* "small stick"), which cause many types of infectious diseases, are smaller than protists and lack distinct intracellular structures. Their distinctive prokaryotic nature was confirmed with the aid of an electron microscope in the mid-1990s, and prokaryotes were classified into another kingdom, Monera (L. *moneron* "single"). Many present-day textbooks classify life using the five-kingdom system, which is dividing into one prokaryotic kingdom, Monera, and four eukaryotic kingdoms, Protista (or Protoctista, Gr. *kystos* "to establish, first builder"), Fungi (L. *fungus* "fungus"), Plantae, and Animalia (Whittaker 1959, 1969; Whittaker and Margulis 1978; Margulis and Schwartz 1998). This system is based on the ecological roles of animals (phagotrophic), plants (autotrophic), and fungi (absorption). The Protista/Protoctista are an assemblage of principally unicellular organisms that cannot be classified into any of the three other eukaryotic kingdoms; i.e., the Protista/Protoctista are defined by the process of elimination. This group has long been regarded a collection of distantly related eukaryotes, including algae and various non-photosynthesizing organisms.

Ultrastructural data on algae and heterotrophic protists that have accumulated since the 1960s have clearly revealed dozens of distinct algal and protistan groups (Patterson 1999). At the same time, developments in the field of molecular cell biology coupled with a rapid accumulation of both gene sequences and complete genomes of various organisms have provided revolutionary views of life phylogenies, especially of algae and protists. Ultrastructural evidence and molecular phylogenies together have played a major role in creating an overall picture of the phylogeny and classification of eukaryotes as a whole. Algae, or eukaryotic autotrophs, are found in several different groups of eukaryotes, yet molecular phylogenies as well as biochemical and physiological studies clearly indicate a single origin of plastids. To address this apparent contradiction, the concept of secondary endosymbiosis, which occurs between heterotrophic protists and eukaryotic algae, has been introduced. This paper

This article can be found at http://www.jspcm 6.jp/

reviews the recent advances in phylogenetic studies and life classifications, with a focus on eukaryotic algae.

The development of molecular techniques to analyze cell functions and metabolism of various groups of algae has greatly advanced our understanding of plastid evolution. Plastids are formerly independent photosynthesizing organisms that were integrated into eukaryotic cells via a temporary symbiont, eventually becoming a permanent organelle. This process of endosymbiosis requires genetic integration between the host and symbiont; vast amount of genes are transferred to the host nucleus, and protein transport machinery is established to transport products back into the plastid. The symbiont's cell cycle should also be controlled to transmit newly acquired cell components to descendants. Algal lineages that are the result of secondary endosymbioses are particularly important to understand the diversification and evolution of algae. Here, the process of plastid establishment will be reviewed by introducing examples of probable intermediate stages of plastid acquisition.

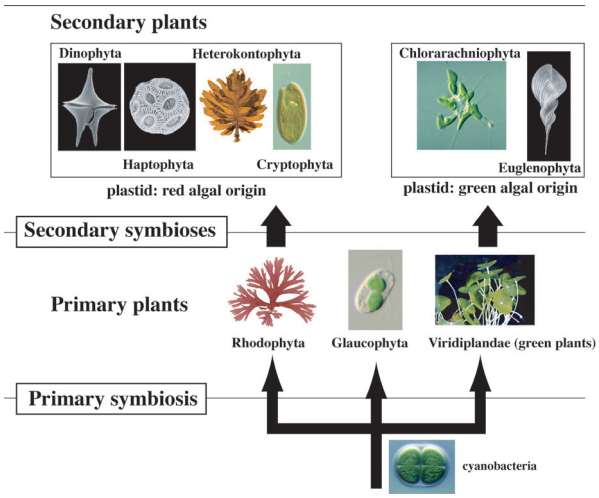

Figure 1. Nine divisions/phyla of eukaryotic algae. Three are designated as primary plants, as they are thought to be descendants of the first eukaryotic plant that acquired plastid via primary endosymbiosis of a cyanobacterium. The six other divisions are secondary plants: two became autotrophs by engulfing green algae, while the rest four by engulfing red algae.

Plants as polyphyletic taxa

Photosynthesizing eukaryotes comprise nine groups of division- or phylum-level taxa: Glaucophyta, Rhodophyta, Viridiplantae (Chlorophyta+land plants), Cryptophyta, Heterokontophyta, Haptophyta, Dinophyta, Euglenophyta, and Chlorarachniophyta. They are distinguished by differences in the pigment composition of the photosynthetic antenna system, and cell architecture, which includes cell coverings, and mitochondrial cristae, flagella, and flagellar apparatuses (Figure 1).

Oxygenic photosynthesis is the most important and remarkable cell function, because it produces organic matter using solar energy, and ultimately supports nearly the entire biosphere of present-day Earth. Oxygenic photosynthesis first appeared in cyanobacteria about three billion years ago, and eventually resulted in an aerobic atmosphere, which had irreversible effects on the evolution of life (e.g. Condie and Sloan 1978). The process of photosynthesis in land plants and eukaryotic algae is basically the same as that of cyanobacteria: both comprise two photosystems linked in series by the cytochrome b_6/f complex, and the proteins comprising the reaction center of both photosystems and the core antenna are undoubtedly homologous between the cyanobacteria and eukaryotic autotrophs. That is, oxygenic photosynthesis was derived from a common origin, and today is a characteristic of organisms from both of the two empires of life, Prokaryota and Eukarya (*sensu* Mayr 1998; Cavalier-Smith 2002) or two of the three domains of life, Bacteria and Eukarya (*sensu* Woese 1990). Based on this fact, it is believed that eukaryotic autotrophs evolved from a single endosymbiotic event, during which a heterotrophic eukaryotic protist engulfed and enslaved a symbiotic cyanobacterium (McFadden 2001; review in Archibald and Keeling 2004). This endosymbiosis is believed to be the sole origin of all plastids in eukaryotic autotrophs (algae and land plants).

It is still uncertain which group of protists was the host of this evolutionary event. However, it is generally believed, based on molecular phylogenies of multiple protein genes, that the Rhodophyta and Chlorophyta (green plants) are sisters and direct descendants of the first eukaryotic plant. Hence, these are termed "primary plants" (Delwiche and Palmer 1997; Martin et al. 1998; Delwiche 1999; Gray et al. 1999; Moreira et al. 2000; c.f., Stiller and Hall 1997 and Nozaki et al. 2003, 2004). The endosymbiosis of cyanobacterium is called primary endosymbiosis (Figure 1). The Glaucophyta possess a plastid resembling a cyanobacterium. This plastid was once thought to be a cyanobacterial non-plastid symbiont called a "cyanelle" harbored within the host cell, because it has chlorophyll *a*, phycobilisome, carboxysome, and a peptideglycan wall between the two membranes as do cyanobacteria (Hall and Klaus 1963; Pfanzagl et al. 1996). However, molecular analyses revealed that the cyanelle genome is only 135.6 kb, which is only 1/26 the size of the *Synechocystis* genome (Löffelhardt et al. 1997) and falls in the range of plastid genomes of most algae and land plants. Several multigene trees have suggested that glaucophytes form a monophyletic group with the red and green algae (Baldauf et al. 2000; Moreira et al. 2000). These groups all have plastids with only a double membrane that is not bound by any extra membranes. They also have a flat mitochondrial cristae

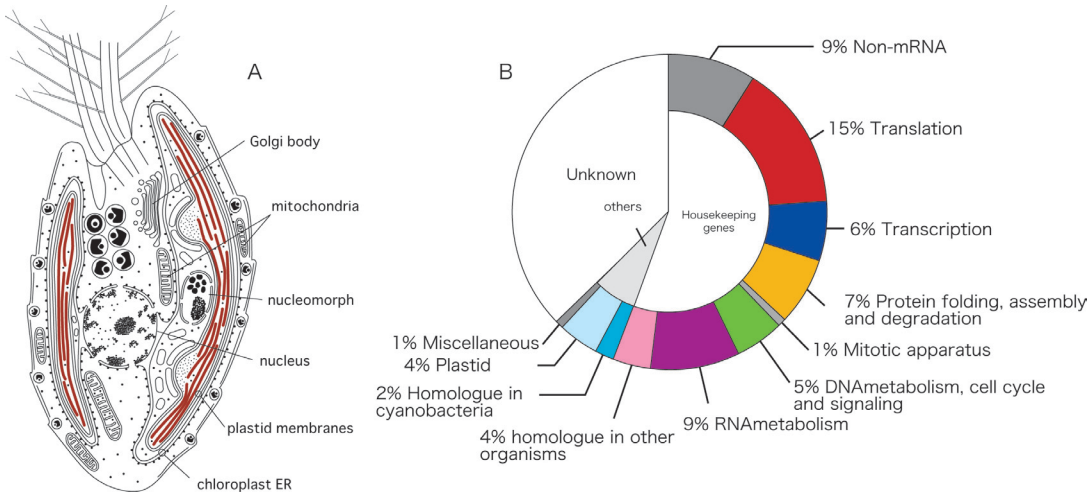

Figure 2. Cryptophyta. A. Cell architecture. B. Composition of the nucleomorph genome of *Guillardia theta.* 245 out of 464 estimated genes are housekeeping genes. (drawn after Douglas et al. 2001)

in common, which may support their monophyletic relationship.

Cryptophytes and chlorarachniophytes: missing-link algae in plant diversification

Six other recognized groups of eukaryotic algae are not direct descendants of the first endosymbiosis. Called "secondary plants," it is believed that they became plants by engulfing eukaryotic algae (primary plants, not cyanobacteria), enslaving them, and eventually integrating them as plastids. This type of endosymbiosis is generally known and accepted as "secondary endosymbiosis" (e.g., McFadden 2001; review in Archibald and Keeling 2004). The concept of secondary endosymbiosis arose from early ultrastructural studies on cryptophyte and chlorarachniophyte algae (Greenwood 1974; Hibberd and Norris 1984). It is fortuitous that these two groups of algae have persisted for millions of years. Had they not existed today, secondary endosymbiotic theory would have taken much more time to be recognized and accepted. In both types of algae, the plastid is contained in two membrane-bound compartments, and an unusual organelle bound by a perforated double membrane is present between the outer two membranes and the plastid double membranes. This organelle was thought to be a relic nucleus of symbiotic eukaryotic algae and was thus named nucleomorph (Figure 2; Greenwood 1974; Hibberd and Norris1984). Later cytological studies proved the presence of DNA in the nucleomorph, and showed that it divides amitotically prior to cytokinesis of the cell (McKerracher and Gibbs 1982; Ludwig and Gibbs 1985). Molecular phylogenetic studies suggested that the nucleomorph of the cryptophytes is truly a vestigial nucleus phylogenetically related to the Rhodophyta, or red algae (Douglas et al. 1991), while that of chlorarachniophytes is related to the chlorophytes, or green algae (McFadden et al. 1994; Gilson and McFadden 1996; Van de Peer et al. 1996; Ishida et al. 1997). The complete nucleomorph genome of the cryptophyte *Guillardia theta* has been determined. It is only 551 kb (1/220 the size of *Arabidopsis thaliana* and 1/6 that of the cyanobacterium *Synechocystis*), coding less than 500 genes, most of which are categorized into housekeeping genes, as shown in Figure 2 (Zauner et al. 2000; Douglas et al. 2001). Although the other four algal groups, Heterokontophyta, Haptophyta, Dinophyta, and Euglenophyta lack nucleomorphs, they are believed to have originated via secondary endosymbiosis, but to have subsequently lost their symbiont nucleus (e.g., McFadden 2001; review in Archibald and Keeling 2004). All six secondary plants have plastids bound by one or two extra membranes. The discoveries of secondary endosymbiotic events are some of the most important advances in plant science and evolutionary biology in the 20th century, and they have led to a new evolutionary view of plant diversification and a reconsideration of eukaryote classification.

Apicoplast: a vestigial plastid of a malaria parasite

In 1996, McFadden et al presented an exciting report that the malaria parasite *Plasmodium* contained a vestigial plastid, a four-membrane-bound organelle in its cytoplasm without distinctive intrastructures. It was soon confirmed that it was universally present in members of the protistan group Apicomplexa and thus was called an apicoplast. The apicoplast genome has been determined in several members of apicomplexans. It typically has circular DNA (although linear in *Toxoplasma gondonii*) 35 kb in size. Malaria parasites lack photosynthetic ability and genes related to photosynthesis, and the apicoplast is used for fatty acid and isoplenoid metabolism (Gardner et al. 2002). Thus, malaria parasites are algae that switched to a parasitic lifestyle,

losing photosynthetic ability but retaining the ability to metabolize fatty acids. Although the origin of apicoplasts is controversial (Kšhler 1997), it is generally believed to be a secondary plastid of red algal origin.

Plants in the global tree of eukaryotes

Ultrastructural and molecular data have begun to create an overall picture of life's relationships. Our understanding of algal and protistan taxa, in particular, has quickly improved and has helped develop a more accurate framework of phylogenies and eukaryote classification. Molecular phylogenies have succeeded in demonstrating the presence of large monophyletic groups of eukaryotes. To date, about a half dozen eukaryotic supergroups have been identified (e.g., Cavalier-Smith 2000; Arisue et al. 2002; Baldauf 2003; Keeling 2004), and these supergroups can be categorized into two (unikonts and bikonts) or three (Amoebozoa, opisthokonts, and bikonts) very large assemblages, as shown in Figure 3 (Simpson and Roger, 2002; Cavalier-Smith 2004). In this overall view of eukaryotes, algae are distributed among five supergroups of bikonts, including Plantae (Chlorophyta, Rhodophyta, and Glaucophyta), stramenopiles (such as Heterokontophyta), alveolates (Dinophyta and Apicomplexa), Rhizalia (Chlorarachniophyta), and Excavates (Euglenophyta). Nevertheless, it should be noted that the position of the Cryptophyta and Haptophyta is still not clear in these global trees of life.

The Plantae are synonymous with the primary plants so that only Glaucophyta, Rhodophyta, and Viridiplantae should be classified as plants in a strict phylogenetic sense. All other algal groups are not plants, phylogenetically, but rather different lineages that acquired plastids secondarily. One of the most important issues remaining to be resolved in current phylogenetic studies of eukaryotes is the accurate number of secondary endosymbioses that generated the seven secondary plants. In the 1990 s, most molecular trees (e.g., 18S rDNA, actin, beta-tubulin) of eukaryotes suggested different origins of secondary plants, and it was widely believed that six independent secondary endosymbioses occurred to generate all of the secondary plants (two green algal endosymbioses to generate Euglenophyta and Chlorarachniophyta, and four red algal endosymbioses that resulted in Cryptophyta, Heterokontophyta, Haptophyta, and Dinophyta). Later, Apicomplexa was added as a fifth group of secondary plant with a red algal origin. This interpretation, however, has recently been challenged by several studies.

Cavalier-Smith (1999, 2000) suggested a common origin of euglenophyte and chlorarachniophyte plastids. Recently, it has been suggested that a number of plastid-related genes are present in trypanosomes (a relative of euglenoids) and members of Euglenozoa and

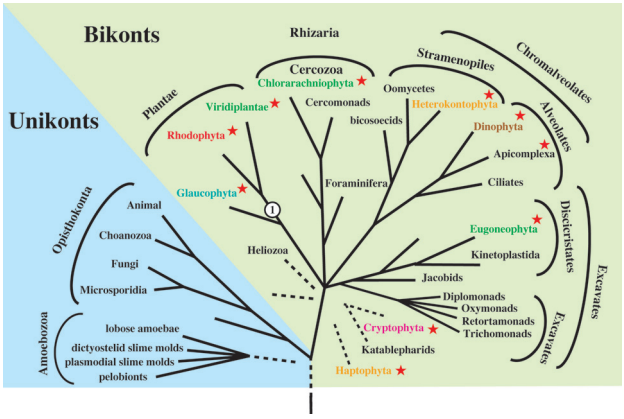

Figure 3. A global tree of eukaryotes drawn after Baldauf 2003 with some modifications. Eukaryotes can be classified into about a dozen of supergroups. Stars indicate distribution of photosynthesis. Arabic number 1 in a circle indicates the position where primary endosymbiosis occurred.

discicristates (Hannaert et al. 2003), and it has been postulated that the position of plastid acquisition lies at the base of the discicristates. However, molecular data have suggested that the discicristates form a larger supergroup (excavates), while chlorarachniophytes fall in the assemblage of Cercozoa and possibly in the larger group, Rhizalia. Euglenophytes and chlorarachniophytes have never formed a monophyletic group. Thus, it is generally believed that the secondary endosymbioses that generated euglenoids and chlorarachniophytes were independent evolutionary events.

The situation is much more complex in algae possessing plastids of red algal origin. All of the taxa lacking photosynthetic ability, except for Apicomplexa, have chlorophyll *c*. Of these, cryptophytes, haptophytes, and heterokontophytes have in common four-membrane-bound plastids with ribosomes attached to the outermost membrane, and tubular hairs on one of two flagella that are assumed to have been lost in the haptophytes. Cavalier-Smith (1981, 1986) proposed a new kingdom, Chromista, for these algae. He interpreted the acquisition of these characters as very rare evolutionary events that must have occurred simultaneously (see original articles for his arguments) in order for these groups to have a common origin. Cavalier-Smith (1999) expanded his chromistan hypothesis to include members of Chromista (cryptophytes, heterokontophytes, and haptophytes) and alveolates (dinoflagellates, apicomplexans, and ciliates) as a monophyletic group. The plastids in these groups were acquired via a single common secondary endosymbiosis of a red alga. This expansion of the hypothesis, called the chromalveolate hypothesis, was based mainly on 18S rDNA trees that showed a sister relationship between the stramenopiles (heterokontophytes) and alveolates (Van de Peer et al. 1996; Van de Peer and De Wachter 1997; Van de Peer et

al. 2000), using a more detailed phylogenetic analysis (Arisue et al. 2002). Data supporting the chromistan or chromalveolate hypotheses have been published for the last several years. Analyses of the GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene first supported the chromistan hypothesis. In plants, two copies of GAPDH are present, one expressed in cytosol and one targeting the plastid. Although the plastid-targeted GAPDH is usually of cyanobacterial origin, that of these algal groups were derived from a duplication of the cytosolic GAPDH (Fast et al. 2001). Analyses of the FBA (fructose 1–6 bisphosphate aldolase) gene also suggest a single origin of chromoalveolates and their plastids (Patron et al. 2004; Rogers and Keeling 2004). There are two types of FBA, classes I and II. The plastid-targeted FBA from algae possessing plastids of red algal origin is class II, while that of red and green algae is class I. The phylogenetic tree of plastid-encoded genes also suggests a single origin of cryptophyte, heterokontophyte, and haptophyte plastids (Yoon et al. 2002). Harper and Keeling (2004) analyzed chromalveolates using six concatenated genes. Their trees strongly suggested monophyly of heterokontophytes and alveolates and very weakly supported monophyly of cryptophytes and haptophytes. All of these recent works position the secondary endosymbiosis that generated all algal lineages in the stramenopiles and alveolates at the stem of the chromalveolates. It should be noted once again, however, that neither cryptophytes nor haptophytes have ever been confidently positioned in the chromalveolate clade in trees of nuclear-encoded genes.

An even more complicated concept of "plants" has been proposed by Nozaki et al (2003, 2004). They presented a phylogenetic tree of concatenated protein genes in which red algae were positioned at the base of the bikonts. Based on this, they proposed a novel scenario of plastid origin (Figure 4), suggesting the possibility that a primary endosymbiosis occurred at the stem of the bikonts. They defined this largest assemblage of eukaryotes as Plantae. They suggested that the presence of cyanobacterial genes (Andersson and Roger 2002) and plastid-related genes in *Trypanosoma* (Hannaert et al 2003) would support their concept of Plantae.

Together, all of these recent hypotheses raise a big question. If the ancestor of the chromalveolates were photosynthetic (as suggested by Cavalier-Smith (1999) and others), heterotrophic groups of the stramenopiles and alveolates (such as oomycetes, bicosoecids flagellates, and ciliates) all should have lost plastids secondarily. If the primary endosymbiosis occurred at the stem of the bikont lineage (as suggested by Nozaki et al. (2003, 2004), many more lineages now depending on heterotrophic nutrition should have lost plastids

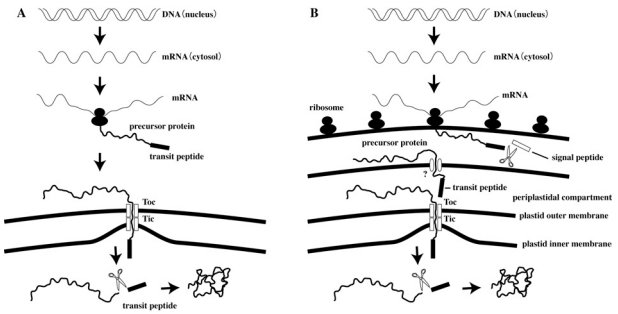

Figure 4. A diagram of the Plantae proposed by Nozaki et al. (2003, 2004). Plantae are nearly equivalent to bikonts *sensu* Cavalier-Smith, and include various heterotrophic eukaryotic groups that are assumed to have been lost plastids secondarily. 1. primary endosymbiosis, 2, 3. secondary endosymbioses that resulted in secondary plants, Euglenophyta and Heterokontophyta.

secondarily and independently.

In various algal lineages, many taxa are known to have lost their photosynthetic ability secondarily (e.g., *Polytomella* and *Prototheca* in green algae, *Astasia* in euglenophytes, *Chilomonas* in cryptophytes and *Ciliophrys* in heterokonts). As far as we know, these heterotrophic algae retain vestigial plastids as leucoplasts, while similar structures have never been reported from, e.g., the oomycete pseudofungal parasite *Phytophthora*, the kinetoplastid parasite *Trypanosoma*, and the ciliate *Tetrahymena*. These facts suggest that plastids do not easily disappear, even though photosynthetic function can be discarded. Heterotrophic members of the chromalveolates and other bikont lineages should be reexamined in this respect. Debate on the number of secondary endosymbioses and the position of the primary endosymbiosis in the universal tree is not yet settled.

Membrane topology of the plastids and protein transport machinery

A majority (85–90%) of plastid genes were transferred to the nuclear genome during the primary symbiotic event (Martin and Herrmann 1998; Martin et al. 1998, 2002). The driving forces of this large-scale gene transfer are unknown, but the avoidance of Muller's ratchet situation (accumulation of deleterious mutations due to a lack of recombination and a genetic bottleneck) is suggested (Moran 1996; Martin et al. 1998). Each gene should have acquired an N-terminal extension called a transit peptide, which acts like a transport tag and is necessary to send products back to the plastid (Fuks and Schnell 1997; review in Keegstra and Cline 1999).

Comparative genomics of *Arabidopsis* and *Synechocystis* (Kaneko et al. 1996) (as the ancestor of the chloroplast) and molecular plant cell biology have provided valuable insights into understanding protein import machinery of the plastid, and have allowed an interpretation of membrane topology of the primary and

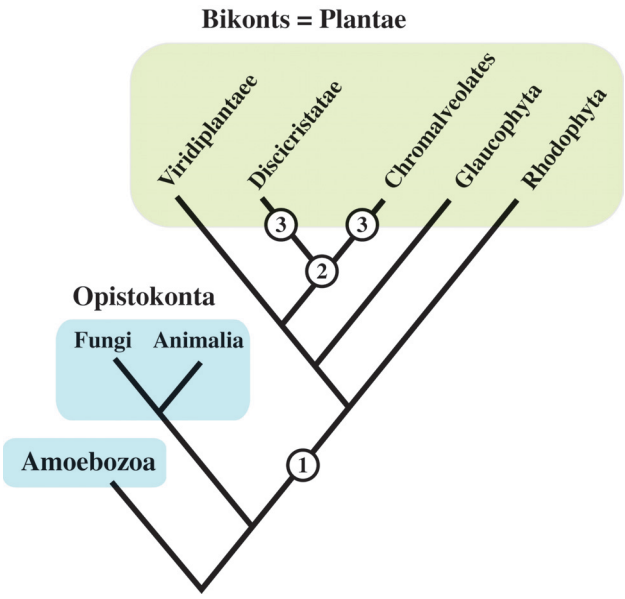

Figure 5. Protein transport machinery. A. Protein transport from nuclear encoded gene to plastid stroma. Precursor protein translated in cytosol has a transit peptide. Toc and Tic channels embedded in the plastid membranes recognize transit peptide and import precursor protein into stroma. Transit peptide is removed and functional protein is formed. B. Protein transport of heterokontophyte algae possessing four membrane-bounded complex plastid. Translation proceeds on ribosomes attached to the outermost membrane (rER). How it passes across the second membrane is unclear, though the presence of a copy of Toc is suggested. Precursor protein then passes across the plastid double membrane.

secondary plants (Martin et al. 2002). The Toc complex, the translocon of the outer envelope of the chloroplast, is embedded in the outer membrane, while the Tic complex, the translocon of the inner envelope of the plastid, is in the inner membrane. Nuclear-encoded plastid proteins are translated in the cytosol, and precursor proteins are targeted to the plastid stroma. Various genes of plastid-targeted protein translocators have been cloned. Toc75 is one such protein, and it is a major component of the Toc channel in the chloroplast outer membrane. A protein homologous to Toc75 was identified in *Synechocystis* (Bölter et al. 1998; Settles and Martienssen 1998; Reumann et al. 1999, 2005). Interestingly, this protein, SynToc75, acts as a secretion translocator in *Synechocystis*, which suggests that a translocator used for export in cyanobacteria was changed to an import translocator during the process of gene transfer to the nucleus (Figure 5). It is also suggested that the origin of the transit peptide is the substrate of SynToc75. It is now accepted that the plastid double membranes are homologous with the double membranes of cyanobacteria (McFadden 1999; Bruce 2000; Jarvis and Soll 2001). Transit peptides have been found in all primary plants, and nuclear-encoded proteins in glaucophytes and red algae can both be imported into isolated pea chloroplasts, which implies that the plastids of these algae and land plants have fundamentally the same protein import machinery and thus that they arose from a single endosymbiotic event (primary endosymbiosis).

In the secondary plants, chloroplast-targeted nuclear-encoded genes should have extra tag-like extensions to import precursor proteins into chloroplast stroma across one or two extra membranes. A signal peptide-like N-terminal extension is found in heterokont algae such as diatoms, raphidophytes, and brown algae (Grossman et al. 1990; Bhaya and Grossman 1991; Ishida et al. 2000) and cryptophytes (Liaud et al. 1997). Both diatoms and cryptophytes have transit peptides, and when signal peptides are removed, precursor proteins can be imported into isolated pea chloroplasts (Lang et al. 1998; Wastl and Maier 2000), which indicates that the translocons of the plastid double membranes are functionally comparable. The process of importing precursor proteins into complex plastids is presently interpreted as shown in Figure 5B The mechanisms of protein import of other secondary plants are not sufficiently understood, but it is suggested that the Golgi apparatus is involved (Sulli and Schwartzbach, 1996; Sechwartz et al. 1998; Sulli et al. 1999; van Dooren et al. 2000, 2001).

Plastid acquisition via secondary endosymbiosis and half-plant/half-predator model

To integrate engulfed prey into the complex eukaryotic cell as a plastid requires many steps, including lateral gene transfer from the symbiont to the host nucleus and the establishment of protein transport machinery to send products of these genes back to the symbiont. These evolutionary processes reduce symbiont autonomy and the host gradually enslaves and integrates the symbiont as an organelle. The most critical and indispensable steps are the establishment of a morphological and functional association and synchronization of the cell cycle between the host and the symbiont. These steps may be summarized as shown in Figure 6. Endosymbiosis may be initiated when a phagotrophic protist engulfs an alga as prey. The protist fails, however, to digest the alga and thus accidentally retains it within the cell (Figure 6, Stage I), like a toothpick that cannot be swallowed (stuck-in-the-throat model by van Dooren (2001)). The temporary symbiont then becomes a persistent symbiont when synchronization of the cell cycle is established between the host and the symbiont. During this process (Figure 6, Stage II), lateral gene transfer from the symbiont nucleus to the host nucleus occurs, which results in decreased symbiont autonomy and increased control by the host. The symbiont's organelles are then erased one by one, but a relic symbiont nucleus is retained for a period of time as a nucleomorph (Figure 6, Stage III) until control by the host is fully established (Figure 6, Stage IV).

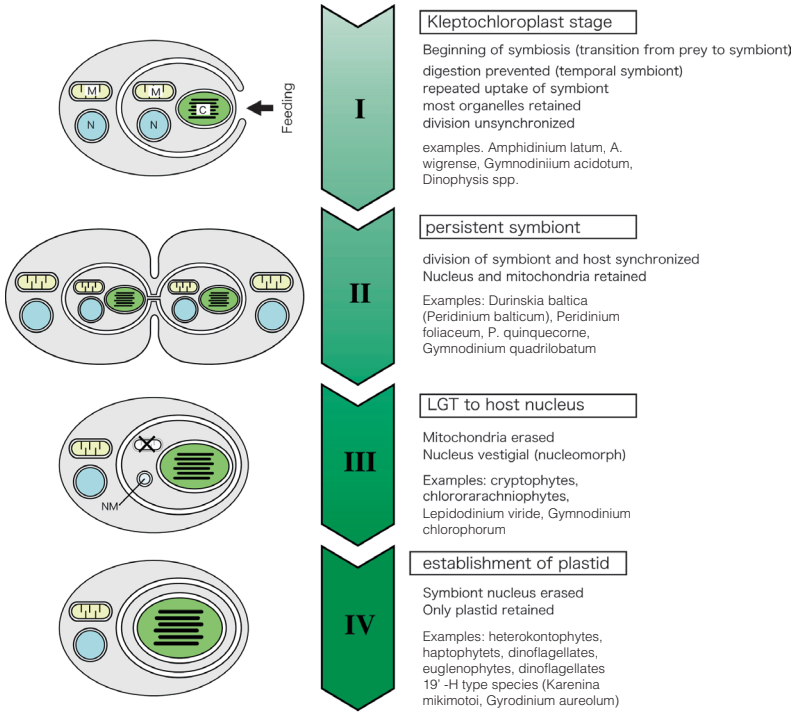

Figure 6. Postulated processes of plastid acquisition. Host cell increases control to symbiont by lateral gene transfer and synchronization of cell cycle. Symbiont nucleus becomes vestigial (nucleomorph) and eventually erased. C. chloroplast, M, mitochondria, N. nucleus, NM. nucleomorph.

We have recently discovered a flagellate protist belonging to a new phylum of eukaryote, Katablepharidophyta/Kathablepharida (Okamoto and Inouye 2005a) that appears to be in an early stage of plastid acquisition. This protist, tentatively named Hatena ("enigmatic" in Japanese), has a green endosymbiont of *Nephroselmis* sp. (Prasinophyceae, Viridiplantae). The symbiont retains its nucleus, mitochondria, plastid, and sometimes its Golgi apparatus, but completely lacks flagella, a cytoskeleton, and an endomembrane system. The plastid is well developed, ten times larger than that of the biggest free-living *Nephroselmis*. Interestingly, the symbiont has a single eyespot that is always, without exception, situated at the apex of the host cell, suggesting that morphological and functional association is partly established between the host and symbiont. An unusual phenomenon observed in Hatena is that the symbiont is inherited by one particular daughter cell by host cell division, while the other daughter cell becomes colorless, i.e., a cell lacking the symbiont is produced during every cell division in this organism. The colorless cell is able to feed on algal prey, because it has a complex feeding apparatus that is undoubtedly homologous with that of phagotrophic katablepharids (Okamoto and Inouye 2005a). Importantly, the feeding apparatus in the pigmented cell is absent; in its place is the symbiont's eyespot (Okamoto and Inouye 2005b). It is therefore apparent that endosymbiosis causes drastic changes to both the host and the symbiont. This unusual life cycle may reflect an early stage of secondary endosymbiosis and could be expressed as a "half-plant/half-predator model." This stage may be intermediate between stages I and II in Figure 6. Although the *Nephroselmis* symbiont may not be persistent, it is obvious that both morphological and functional associations between the host and symbiont have already begun to some extent. Such intermediate stages reflect steps of integration from temporary symbiont to the plastid. It is obvious that the process of plastid acquisition requires drastic changes not only to the symbiont but also to the host organism. To understand these intermediate stages of plastid acquisition at a molecular and cell level would lead to breakthroughs in the study of evolutionary plant biology.

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