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MINIREVIEW

THE MESOZOIC RADIATION OF EUKARYOTIC ALGAE: THE PORTABLE PLASTID HYPOTHESIS[1]

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Although all chloroplasts appear to have been derived from a common ancestor, a major schism occurred early in the evolution of eukaryotic algae that gave rise to red and green photoautotrophic lineages. In Paleozoic and earlier times, the fossil record suggests that oceanic eukaryotic phytoplankton were dominated by the green (chl *b*-containing) algal line. However, following the end-Permian extinction, a diverse group of eukaryotic phytoplankton evolved from secondary symbiotic associations in the red (chl *c*-containing) line and subsequently rose to ecological prominence. In the contemporary oceans, red eukaryotic phytoplankton taxa continue to dominate marine pelagic food webs, whereas the green line is relegated to comparatively minor ecological and biogeochemical roles. To help elucidate why the oceans are not dominated by green taxa, we analyzed and compared whole plastid genomes in both the red and green lineages. Our results suggest that whereas all algal plastids retain a core set of genes, red plastids retain a complementary set of genes that potentially confer more capacity to autonomously express proteins regulating oxygenic photosynthetic and energy transduction pathways. We hypothesize that specific gene losses in the primary endosymbiotic green plastid reduced its portability for subsequent symbiotic associations. This corollary of the plastid "enslavement" hypothesis may have limited subsequent evolutionary advances in the green lineage while simultaneously providing a competitive advantage to the red lineage.

Key index words: **endosymbiosis; evolution; phytoplankton; plastid genome; RUBISCO**

In eukaryotic algae, all plastids are derived from a common photoautotrophic prokaryotic ancestor and were sequentially appropriated by various nonphotosynthetic host cells through primary, secondary, and even tertiary endosymbiotic events (Wolfe et al. 1994, Delwiche 1999, Tomitani et al. 1999). Based on biochemical and ultrastructural features of their plastids (van den Hoek et al. 1995), eukaryotic algae can be clustered into two major groups: a "green" and a "red" lineage. The green lineage comprises the Kingdom Viridiplantae, which includes all green algae (Chlorophyta) and higher plants (Streptophyta), all of which have primary endosymbionts. Two green algal phyla have secondary endosymbiotic plastids (euglenophytes and chlorarachniophytes), but both contain relatively few species. In the red lineage, the red algae per se (Rhodophyta), which have plastids derived from a primary endosymbiotic event, contain relatively few extant taxa and very few unicellular members. In contrast to the green lineage, most eukaryotic algal taxa that contain plastids from the red line are products of secondary or tertiary endosymbioses. These include the dinoflagellates (Dinophyta) and the Kingdom Chromista comprised, in part, of haptophytes, cryptophytes, and heterokonts (including diatoms, brown algae, and raphidophytes).

In Paleozoic and earlier eras, the fossil record of acritarchs is interpreted to contain taxa associated with the extant prasinophyte green algae, suggesting that members of the green lineage dominated eukaryotic phytoplankton communities in the oceans (Tappan 1980, Lipps 1993, Mendelson 1993). After the end-Permian extinction, however, several unicellular algal phyla with secondary red plastids rose to ecological prominence. Although the possibility that secondary symbiotic associations occurred in the red lineage before the end-Permian extinction cannot be excluded, the fossil evidence suggests that thecate di-

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noflagellates appeared in the early to mid-Triassic and intensively radiated in the Jurassic period (Bujak and Williams 1979, Fensome et al. 1996), the coccolithophorids (Haptophyta) evolved in the late Triassic and rose to ecological prominence in the Cretaceous (Roth 1987, Bown et al. 1992), and the diatoms emerged sometime in the Jurassic and rose to ecological prominence from the late Cretaceous (Lipps 1993, Harwood and Nikolaev 1995). These phyla continue to dominate the eukaryotic phytoplankton assemblages in the contemporary ocean. Although green eukaryotic phytoplankton have not been competitively excluded from the ocean (indeed, quantitative molecular analyses suggest that prasinophytes remain relatively abundant in picoplankton; Moon-Van Der Staay et al. 2001, Vaulot et al. 2002), green eukaryotic phytoplankton seldom dominate in contemporary pelagic marine ecosystems. For example, an analysis of HPLC-based pigments composition from the Joint Coastal Ocean Flux Study reveals that chl *c* is 3.4 times more abundant in the ocean than chl *b* on a molar basis (Falkowski, unpublished data). Why have red plastid-containing phyla become so ecologically successful over the past 250 million years?

Why plastids (or any other organelle) retain any genetic information at all is unclear (Allen and Raven 1996). It is clear, however, that no plastid is completely autonomous, that is, capable of growth and replication outside of the host cell. The reason is that after all symbiotic events, most protoplastid genes were transferred to the host cell nucleus (McFadden 1999). However, all plastids retain a set of genes that are essential for the physiological function of the organelle in oxygenic photosynthesis. Here we present an analysis of whole plastid genomes from various eukaryotic algal taxa in an attempt to understand what genetic features distinguish the two major plastid lineages from each other. From our analysis, we suggest that differences in gene composition between plastid genotypes could account, in part, for the ecological success of phytoplankton containing secondary red plastids.

In considering the relative ecological and evolutionary success of the red plastid phyla, we postulated that genetic information retained by plastids is critical in defining the potential fitness of the plastid in new endosymbiotic associations. This hypothesis suggests that after a major selection event, such as a change in redox chemistry of the ocean, red and green plastids effectively compete with each other for accommodation within new host cells. Assuming that, before the symbiotic association, the host cell did not contain photosynthetic or other genes retained within what would become a plastid, the selection and subsequent fitness of the plastid within the host must depend on the extent to which the host cell can support the genetically depleted organelle. Hence, the potential for "portability" of the plastid among host cells should increase with the amount of genetic information retained by the plastid. We call this the "portable plastid hypothesis."

CORE SET OF PLASTID GENES

At the time of this writing, we located 24 complete chloroplast genome sequences in public databases; 10 are from algae (Table 1). We do not include in our analysis the plastid genome of dinoflagellates, for which the current knowledge remains fragmentary at best (Boczar et al. 1991, Zhang et al. 1999, Barbrook and Howe 2000). Gene distributions in the nine photosynthetic algal plastid genomes (excluding the nonphotosynthetic *Astasia longa*) were established with the 190 identified and 66 hypothetical (*ycf*) protein-coding genes from the last update of plastid gene nomenclature and distribution (Stoebe et al. 1998) (Table 2).

The distribution reveals a set of approximately 50 core protein-coding genes retained in all taxa (Fig. 1). These genes are broadly clustered into three major functional domains: 1) genes encoding for the proton coupled ATP synthase (*atp* genes); 2) genes encoding for photosynthetic processes, including reaction center proteins (*psa*, *psb* genes), most of the electron transport components that connect the two photosystems (*pet* genes), and the large subunit of RUBISCO (*rbcL* genes); and 3) housekeeping genes that include the plastid ribosomal proteins (*rpl*, *rps* genes), RNA polymerase (*rpo* genes), and elongation factor (*tufA* gene). Only one biosynthetic gene, involved in chl synthesis (*chlI*), is retained in all photosynthetic algae; this gene was transferred to the nucleus in higher plants. In the heterotrophic *A. longa*, the plastid genome retained mostly housekeeping genes, losing all but one (*rbcLg*) gene involved in photosynthesis. Among glaucophytes, the third primary endosymbiotic phylum with a few, rare, extant species, *Cyanophora paradoxa* retains about 30 genes in common with all red plastids (rhodophytes, cryptophytes, and diatoms) and about 10 additional genes found only in rhodophytes but only 3 to 4 genes associated with green algal plastids (including the *rbcLg* gene coding for the type IB large subunit of RUBISCO).

TABLE 1. The algal plastid genomes available in databases.

Algal division, species, and reference	Number of protein-coding genes
Rhodophyta (primary red plastids)	
Porphyra purpurea (Reith and Munholland 1995)	203
Cyanidium caldarium (Glöckner et al. 2000)	199
Cryptophyta (secondary red plastids)	
Guillardia theta (Douglas and Penny 1999)	147
Bacillariophyta (secondary red plastids)	
Odontella sinensis (Kowallik et al. 1995)	139
Glaucophyta (primary plastids)	
Cyanophora paradoxa (Stirewalt et al. 1995)	136
Chlorophyta (primary green plastids)	
Mesotigma viride (Lemieux et al. 2000)	99
Nephroselmis olivacea (Turmel et al. 1999)	91
Chlorella vulgaris (Wakasugi et al. 1997)	78
Euglenophyta (secondary green plastids)	
Euglena gracilis (Hallick et al. 1993)	52
Astasia longa (Gockel and Hachtel 2000)	28

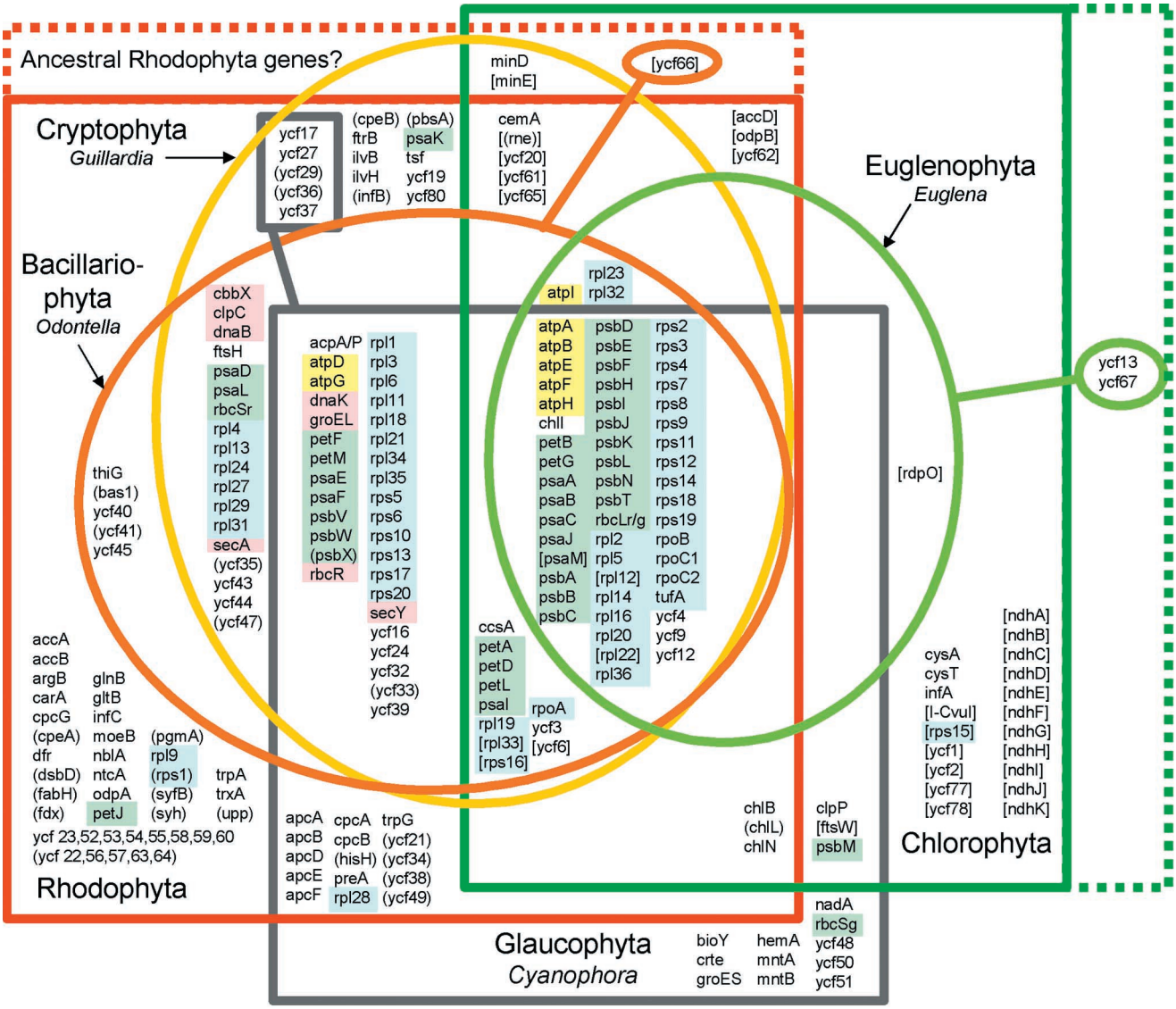

FIG. 1. Distribution of the 190 first identified and 66 hypothetical (*ycf*) protein-coding genes in the nine photosynthetic algal plastid genomes, using the last update of nomenclature and distribution of Stoebe et al. (1998). (See Table 2 for the summary of the gene nomenclature.) Primary endosymbiotic genomes (Glaucophyta, Rhodophyta, Chlorophyta, square boxes) are widely overlapping, in accordance with the monophyletic origin of all plastids. The secondary endosymbiotic genomes (Euglenophyta, Cryptophyta, Bacillariophyta, round boxes) are included in the primary lineages from which they emerged. In the secondary plastid genomes, *minD-minE* genes in cryptophytes and *ycf66* in diatoms are assumed to originate from their ancestral rhodophyte endosymbiont and euglenophyte *ycf13* and *ycf67* from their ancestral chlorophyte endosymbiont. On the whole, distribution of *ycf*s does not differ significantly from those of other protein-coding genes. Parentheses indicate genes not found in all rhodophyte plastid genomes; square brackets indicate genes not found in all chlorophyte plastid genomes. Genes involved in the three main plastid functions represented in the core set are highlighted: ATP synthase genes (yellow), photosynthetic processes (green), and housekeeping genes (blue). Genes involved in protein regulatory pathways (transcriptional and post-transcriptional regulation) in the complementary gene set of red plastids are shown in pink.

The strong similarity among numerous functional genes in all plastids, including the reaction centers and ATPase, and the corresponding concordance with extant cyanobacterial gene sequences strongly support a monophyletic origin of chloroplasts from a cyanobacterium-like ancestor (Douglas and Murphy 1994, Bhattacharya and Medlin 1995, 1998, Hess et al. 1995, Palenik and Swift 1996, Köhler et al. 1997). Phylogenetic analyses, using data from single plastid genes or nuclear genes coding for plastid proteins, suggest that the plastids of heterokonts, cryptophytes, haptophytes, and dinoflagellates were derived from rhodophytes (Chesnick et al. 1996, Bhattacharya and Medlin 1998, Durnford et al. 1999, Takishita et al. 1999, 2000, Takishita and Uchida 1999, Zhang et al. 1999). In the green lineage, euglenophyte and chlo-

TABLE 2. The protein-coding gene nomenclature in algal chloroplast genomes (adapted from Stoebe et al. 1998).

Gene code	Protein function
accA,B,D	Acetyl-CoA carboxylase
acpP	Acyl carrier protein
apcA,B,D,E,F	Allophycocyanin phycobilisome
argB	Acetylglutamate kinase
atpA,B,D,E,F,G,H,I	ATP synthase
bas1	Thiol-specific antioxidant protein
bioY	Biotin synthase
carA	Carbamoyl phosphate synthetase
cbbX	Red type Calvin cycle operon
ccsA	Heme attachment to plastid cytochrome *c*
cemA	Envelope membrane protein
chlB,I,L,N	Protochlorophyllide reductase
clpC/P	Caseinolytic-like protease (Clp)
cpcA,B,G	Phycocyanin phycobilisome
cpeA,B	Phycoerythrin
crtE	Geranylgeranyl pyrophosphate synthetase
cysA,T	Probable transport proteins
dfr	Drug sensory protein
dnaB	DNA-replication helicase
dnaK	Hsp 70-type chaperone
dsbD	Thiol:disulfide interchange protein
fabH	β-Ketoacyl-acyl carrier protein synthase III
fdx	2[4Fe-4S] ferredoxin
ftrB	Ferredoxin-thioredoxin reductase
ftsH,W	Division proteins
glnB	Nitrogen regulatory protein
gltB	Glutamate synthase (GOGAT)
groEL,ES	Chaperonins 60 and 10 kDa
hemA	5-Aminolevulinic acid synthase
hisH	Histidinol-phosphate aminotransferase
I-CvuI	DNA endonuclease
ilvB,H	Acetohydroxyacid synthase
infA,B,C	Translational initiation factors
minD,E	Homologues of bacterial cell division regulators
mntA,B	Manganese transport system proteins
moeB	Molybdopterin biosynthesis protein
nadA	Quinolinate synthase
nblA	Phycobilisome degradation protein
ndhA-J	NADH-plastoquinone oxidoreductase
ndhK	NADH-ubiquinone oxidoreductase
ntcA	Global nitrogen transcriptional regulator
odpA,B	Pyruvate dehydrogenase E1 component
pbsA	Heme oxygenase
petA,B,D,F,G,J,L,M	Photosystem electron transport proteins
pgmA	Phosphoglycerate mutase
preA	Prenyl transferase
psaA-M	PSI proteins
psbA-X	PSII proteins
rbcLg/r	RUBISCO large subunit, green and red forms
rbcR	RUBISCO operon transcriptional regulator
rbcSg/r	RUBISCO small subunit, green and red forms
rdpO	Probable reverse transcriptase
rne	RNAseE
rpl1-36	Large subunit ribosomal proteins
rpoA,B,C1,C2	RNA polymerase
rps1-20	Small subunit ribosomal proteins
secA,Y	Preprotein-translocase
syfB	Phenylalanine tRNA synthetase
syh	Histidine tRNA synthetase
thiG	Thiamine biosynthesis
trpA	Tryptophane synthase
trpG	Anthranilate synthase, glutamine amidotransferase
trxA	Thioredoxin
tsf	Translational elongation factor Ts
tufA	Translational elongation factor Tu
upp	Uracil phosphoribosyltransferase
ycf	Hypothetical proteins

rarachniophyte plastids appear to have been acquired from secondary endosymbiosis of chlorophytes (van de Peer et al. 1996, Bhattacharya and Medlin 1998, Palmer and Delwiche 1998, Turmel et al. 1999). Accordingly, the set of genes retained in secondary endosymbiotic plastids is almost totally included in the primary lineage from which they descended (Fig. 1). The only exceptions are a small number of genes retained in secondary plastids that may not be present in their related extant primary plastids: *minD* and *minE* in cryptophytes (Fig. 1), *ycf66* in diatoms, and *ycf13* and *ycf67* in euglenophytes. These genes are relics from ancestral primary plastids that were later lost.

GENE RETENTION AND LOSSES IN CHLOROPLAST GENOMES REFLECT EVOLUTIONARY PATTERNS

Whereas the presence of specific genes provides clues about the origin of plastids, plastid gene losses, which are effectively irreversible, also provide evolutionary information. There are 200 or fewer protein-coding genes in primary plastids. Assuming that the number of genes in the original primary ur-plastid was similar to that found in the extant cyanobacterium *Synechocystis* PCC6803 (3168 protein-coding genes; Kaneko et al. 1996), more than 93% of the ancestral endosymbiont genome was lost or transferred to the host nucleus in the primary plastid lineages (Fig. 2). Green plastids exhibit the most numerous gene losses, either specific losses or those in common with glaucophytes. Subsequently, patterns of gene losses occurred differently at phylum level radiations within primary lineages and at radiations accompanying secondary symbiotic events. Between 1% and 10% of the remaining plastid genes were lost at phylum level radiations within rhodophytes and chlorophytes. A larger number of the remaining primary plastid genes were lost at radiations linked to secondary symbiotic events. For example, with the divergence of cryptophyte and bacillariophyte plastids from the rhodoplasts, between 15% and 20% of the plastid genes were lost. Approximately 30% of the genes were lost at the divergence of euglenoid plastids from chloroplasts. This analysis suggests that endosymbiotic events resulted in relatively rapid and massive gene losses in plastids, whereas the radiations within phyla were accompanied by slower and more gradual genomic erosion.

We used the patterns of gene loss, inferred from a simple presence/absence analysis, to construct an evolutionary tree (Fig. 3). In this analysis, we implicitly assume that each gene retained in a plastid reflects an ancestral status, and hence, at least within a lineage, the more evolved a plastid, the more genes were lost. The patterns of gene loss clearly separate the red plastid lineage, with rhodophytes at the base of the cluster, from the green lineage. Further out of the red lineage are the secondary endosymbiotic algae: the cryptophytes and bacillariophytes. In the green lineage, the *Mesostigma* plastid appears ancestral within the chlorophytes, and the pattern of gene loss suggests that land plants diverged early from chloro-

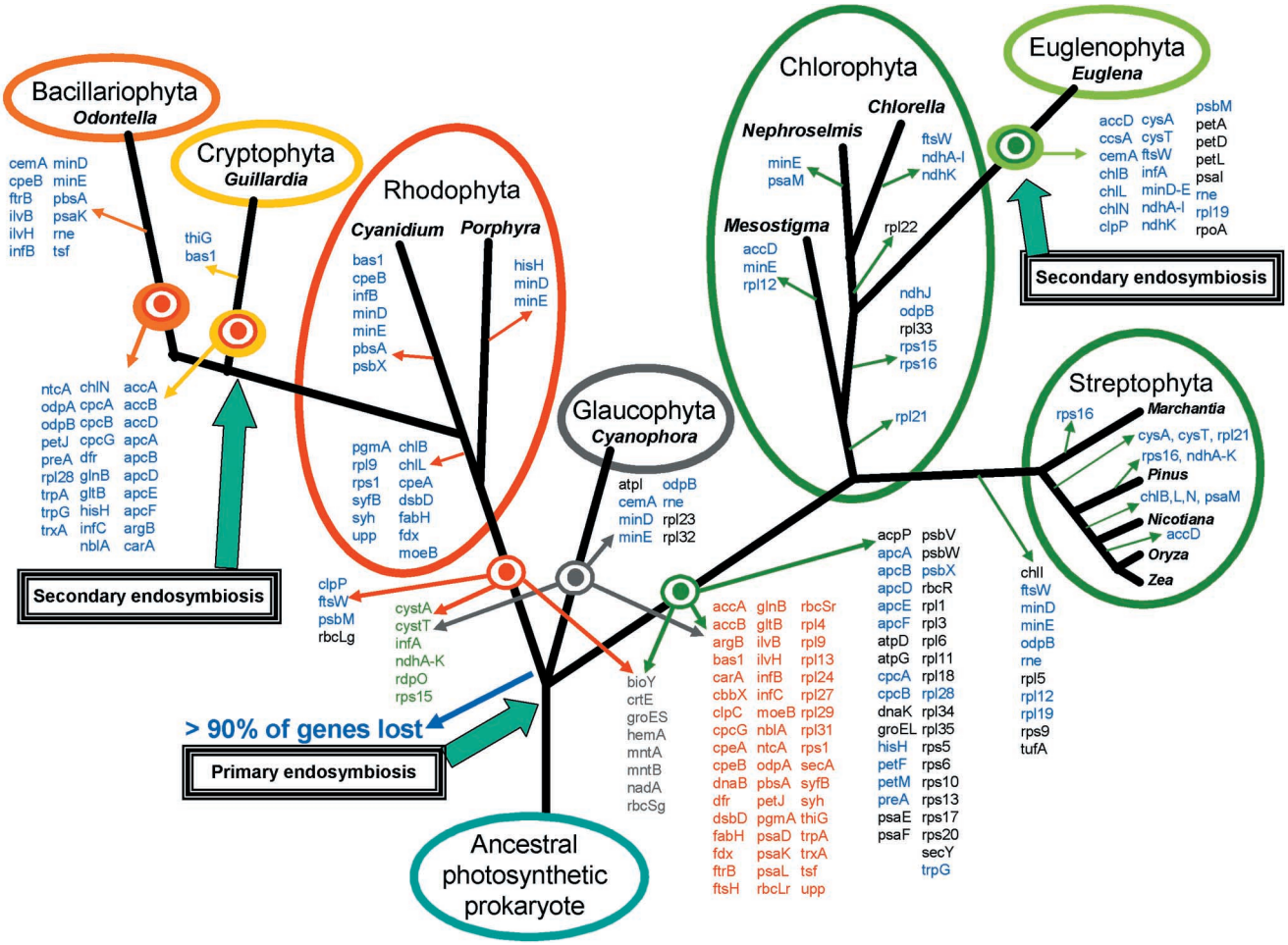

FIG. 2. Hypothetical patterns of gene losses at radiations of phytoplankton and plant plastids inferred from 190 identified protein-coding genes (Stoebe et al. 1998) in extant species plastid genomes. For clarity, the *ycf* losses, whose pattern is similar to identified protein-coding genes (from the gene distribution in Fig. 1), are not shown. From the ancestral photosynthetic eukaryote genome, radiations leading to primary phytoplankton lineages after the primary endosymbiosis are indicated by a single circle. Radiations due to secondary endosymbiotic events are indicated by a double circle. After primary endosymbiosis, gene losses are shown from a hypothetical ancestral plastid genome containing the whole set of genes found in extant plastids: genes specifically retained in the red primary plastids (i.e. lost in both green and glaucophyte plastids) are in red, and genes specifically retained by green and glaucophyte plastids are in green and gray, respectively. Other genes losses that occurred at a single radiation are in black, and those that occurred at several independent radiations are in blue.

phytes (Figs. 2 and 3). Our phylogenetic analysis (Fig. 3) points out the high degree of evolution in euglenophyte plastids, reflected by their remarkably reduced genomes (Table 1). The retention of *rpl22* in the center of the ribosomal protein cluster L2 in *Euglena* and *Astasia* suggests, however, that euglenophyte plastids radiated relatively early from chlorophytes (Fig. 2). Our analysis places the glaucophyte *Cyanophora* at the base of the green plastid cluster (Fig. 3). However, the plastid in this organism retains more genes (up to 53, that is, approximately 39% of its genome) in common with rhodophyte plastids (Fig. 1). Glaucophytes are generally considered a primitive primary endosymbiotic lineage; their plastids (cyanelles) have retained some of the structural, biochemical, and genetic features of the cyanobacterium-like ancestor and appear as intermediate between cyanobacteria and the red plastid lineage (van den Hoek et al. 1995, Ohta et al. 1997, Martin et al. 1998, Stoebe and Kowallik 1999).

On the whole, the evolutionary relationships inferred from gene composition of plastids, at least within the red and green plastid lineages, are surprisingly similar to those derived from concatenated plastid protein-coding gene sequences and corroborates that view of plastid phylogeny (Martin et al. 1998, 2002, Turmel et al. 1999, Lemieux et al. 2000).

NUMBER OF SECONDARY AND TERTIARY ENDOSYMBIOTIC EVENTS

Before we elaborate further on our portable plastid hypothesis, we need to summarize current knowledge about the number of secondary and tertiary endosym-

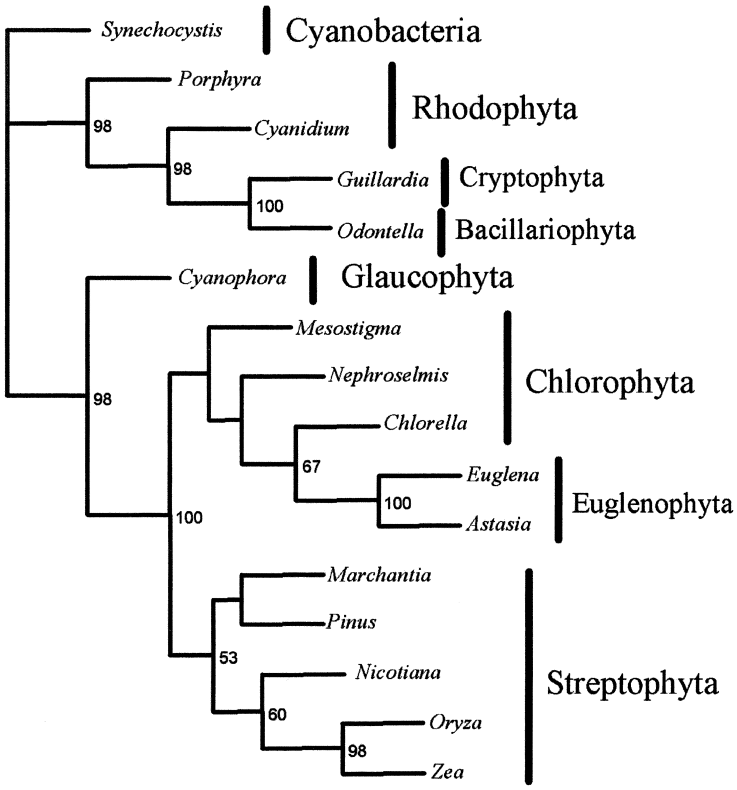

FIG. 3. Phylogenetic analysis of plastids inferred from gene presence and gene loss in plastid genomes, from the 190 identified and 66 hypothetical protein-coding genes of the list of Stoebe et al. (1998). The cyanobacterium *Synechocystis* sp. was used as an outgroup. Bootstrap values over 50% are shown at nodes. The consensus tree was inferred using the Camin-Sokal parsimony method in the PHYLIP 3.57 package (Felsenstein 1993) (program MIX, using the Jumble option to run the analysis 10 times for each of the 1000 bootstrapped data sets). Gene presence (reflecting an ancestral status) was scored 0, and gene absence (i.e. loss, reflecting an evolved status) was scored 1.

biotic events assumed to have occurred in the green and red lineages. Over the last decade, molecular phylogenetic analyses have been used in an attempt to resolve the evolutionary relationships between the main algal phyla. These analyses have been based largely on phylogenetic relationships between specific nuclear and plastid-encoded genes. A major concern is whether, within a lineage, a single host cell acquired its plastids before the divergence of the endosymbiotic associations into distinct clades or if various apoplastidic heterotrophic host cells, which were phylogenetically distinct, acquired plastids independently.

In the green plastid lineage, analyses of 18S rRNA genes (rDNA) clearly distinguished euglenophytes from chlorarachniophytes, and according to specific features of their plastids, the hypothesis of their emergence from two independent secondary events is well accepted (Delwiche 1999, McFadden 2001, Cavalier-Smith 2002b). In the red plastid lineage, analyses of 18S rDNA and GAPDH genes suggest no close phylogenetic relationship between the various secondary host cells that comprise the red line (Bhattacharya and Medlin 1998, Fast et al. 2001). Moreover, analyses of plastid-encoded genes (mostly 16S rRNA and *rbcL* genes) suggest an independent origin of several secondary red plastids within the ancestral rhodophytes (Bhattacharya and Medlin 1995, Takishita et al. 1999, 2000, Oliveira and Bhattacharya 2000, Müller et al. 2001, Martin et al. 2002). Consequently, one hypothesis suggests that eukaryotes in the red line arose from at least four independent secondary endosymbiotic events involving red algae (as illustrated in Delwiche 1999). An alternative hypothesis suggests that all phyla containing secondary red plastids arose from a single secondary endosymbiotic event that led to the chromalveolate lineage (Cavalier-Smith 1999, 2000, 2002a,b, McFadden 2001). This hypothesis is supported by phylogenetic analyses of nuclear genes, which distinguish several clades, including Alveolates (ciliates and apicomplexa, which include dinoflagellates) and Chromista (heterokonts and cryptophytes). Neither analysis included haptophytes (Baldauf et al. 2000, Fast et al. 2001).

Altogether, there appears to be twice as many secondary endosymbiotic events in the red line compared with the green line. Curiously, one phylum appears to be promiscuous in appropriating plastids. Although it is questioned whether the ecologically predominant peridinin-containing plastid dinoflagellates arose from a secondary or tertiary endosymbiotic event (Morden and Sherwood 2002, Yoon et al. 2002), the great variety of chloroplasts found within dinoflagellates clearly reveals that several independent endosymbiotic events occurred in this phylum. These events involved at least three types of secondary red plastid algal phyla (diatoms, haptophytes, and cryptophytes) and one from primary green plastid algae (prasinophytes) (Schnepf and Elbrächter 1999). It is unclear whether the green plastids of dinoflagellates are monophyletic or if they are secondary or tertiary endosymbionts.

"PORTABLE PLASTID" HYPOTHESIS

Why then have the disparate taxa within the red line become ecologically successful in the contemporary ocean, and what processes might have contributed to their increased competitive advantage in the Mesozoic ocean? It is clear from our whole plastid genome analysis that whatever the host cell, all red plastids retain a common set of genes that distinguishes this plastid type from all other plastids in eukaryotic algae (Fig. 1). Specifically, all algae with red plastid genomes exclusively retained a set of 14 genes. In addition, they have about 30 genes retained in glaucophytes (Fig. 1). These two groups of genes constitute a complementary core set in red plastids, 75% of which are related to the core set described earlier: 20 ribosomal proteins (*rpl* and *rps* genes), 2 ATPase genes, 9 genes related to photosynthesis (2 *pet*, 4 *psa* including *psaD* encoding for the ferredoxin docking protein in PSI, 3 *psb*), and the small subunit of RUBISCO (*rbcS*). Among the other genes, several encode for proteins involved in various processes of post-transcriptional regulation of plastid proteins, especially those encoded in the nucleus. For example, the translocase complex *secA/Y*, located in the thylakoid membrane, imports such proteins as plastocyanin or PSI protein Psa-N from the stroma into the thylakoid lumen (Kloesgen 1997, Dalbey and Robinson 1999). Several genes encode for stromal chaperones (*dnaK* and *groEL*) and chaperone-proteases (*clpC*) involved in shaping/folding, assembly, and activation of oligomeric protein complexes (Chen and Schnell 1999, Agarraberes and Dice 2001). Interestingly, the red plastids retained the Clp regulatory ATP-binding subunit gene, *clpC*, whereas green plastids retained the proteolytic subunit gene, *clpP*. Other genes encode for a DNA helicase (*dnaB*), a protein involved in organelle division (*fstH*), and an acyl carrier protein (*acpP* gene) involved in an early step of lipid synthesis. The *rbcR* and *cbbX* genes are transcriptional regulators. The latter is specifically involved in transcriptional regulation of the red-type RUBISCO gene operon, *rbcL-rbcS*, retained in all plastids in the red line. In contrast, in the green line, the transfer to the nucleus of the gene encoding the small subunit of RUBISCO (which is essential for the catalytic activity) ensures nuclear (i.e. host cells) control of RUBISCO expression (Rodermel 1999). The retention of both RUBISCO genes in the red plastid genomes ensured much greater autonomy in RUBISCO expression. Altogether, the complementary core set of genes in the red lineage suggests that compared with green plastids, red plastids have retained significantly more capacity to autonomously express proteins critical to maintaining and regulating oxygenic photosynthetic and energy transduction pathways.

We hypothesize that after the long and dramatic changes in ocean chemistry and circulation associated with the end-Permian extinction, gene retention in the red line increased their potential to be appropriated by a variety of new, phylogenetically diverse, heterotrophic host cells that were under strong selection to become autotrophic. A corollary of this hypothesis is that, in contrast, the transfer of some of key core plastid genes to the host nuclear genome decreased the portability of primary green plastids. Once these genes were lost from the plastid, a potential secondary host would have needed to retrieve them from the primary host nucleus. The probability that such an event occurred is difficult to quantify, but the fact that most extant species within the green algal lineages are primary endosymbionts suggests that it was rare; to wit, there are no known heterokonts, haptophytes, and cryptophytes with green plastids. The enigma, however, is how retrieving these few genes from the nucleus of potential green primary endosymbionts might have presented a problem to the secondary hosts that had successfully appropriated or discarded the other approximately 2000 nuclear-encoded plastid protein genes from red primary endosymbionts. Alternatively, to replace the gene lost in the plastid, the secondary host would have to obtain analogous nonplastid genes from elsewhere by lateral gene transfer: a documented example of lateral gene transfer is the α-proteobacterial origin of the form L_2 RUBISCO found in the peridinin-containing dinoflagellates (Morse et al. 1995, Whitney et al. 1995).

Why then are there green secondary endosymbiotic plastids in other algal taxa, the euglenophytes, chlorarachniophytes, and the green dinoflagellates? For the euglenophytes and chlorarachniophytes, the plastid phylogeny inferred from 16S rDNA suggests their plastids are derived early within the green plastid lineage (Bhattacharya and Medlin 1998). Indeed, to reconcile the plastid portability hypothesis with the occurrence of secondary symbionts in the green line requires that the secondary endosymbiotic events in the green line occurred *before* the transfer of some core and complementary genes to the primary host nucleus. Subsequent gene transfers from secondary green plastids paralleled those from primary green plastids (i.e. the transfer of the *rbcS* gene to the nucleus of euglenophytes).

POTENTIAL ROLE OF HOST CELLS IN THE ECOLOGICAL SUCCESS OF THE ENDOSYMBIOTIC ASSEMBLAGES

Although the modern dominant eukaryotic marine phytoplankton (diatoms, peridinin-containing dinoflagellates, haptophytes, cryptophytes) have rhodophyte-derived plastids, the rhodophytes themselves are rare in contemporary oceanic phytoplankton assemblages. This apparent paradox suggests that the ecological success of the secondary red symbiotic taxa is not solely a consequence of the composition of their plastids' genetic and functional potential but also involves the eukaryotic host cells, which may have provided new genetically determined fitness.

What genetic potential of the various eukaryotic hosts determined the ecological success of the endosymbiotic assemblages? One major attribute that sec-

ondary hosts have brought in to new phytoplankton phyla of the red line is armor, in the form of siliceous, calcareous, or cellulose based cell walls, or formation of polysaccharide exopolymers that permit aggregation of cells to form massive colonies or dramatically increase the seawater viscosity. These host-dependent properties afford protection against grazing pressure and may have conferred a critical ecological advantage to the secondary red plastid phytoplankters (Verity and Smetacek 1996). In contrast, the marine green plastid host cells are subjected to relatively heavy grazing pressure. Indeed, environments where green unicellular algae form blooms are lacustrine, including the Great Salt Lake. In such environments the diversity of grazers is low compared with the contemporary ocean. Most extant red and green primary endosymbiotic taxa (green and red macroalgae and land plants) greatly diverged with the emergence of multicellularity with parenchymatous tissue development. A similar evolutionary pathway only occurred one time in the various secondary endosymbiotic phyla to give rise to the brown macroalgae (Phaeophyceae). Curiously, the red plastid line appears never to have colonized terrestrial ecosystems.

CONCLUSIONS

Although all major classes of plastids are found in the modern ocean, *all* the dominant phytoplankton taxa are secondary red endosymbionts. Our whole plastid genome analysis suggests that plastid portability was a major factor that contributed to the ecological success of the red line. Plastid portability is based on the retention of functional photosynthetic and energy transducing genes in the organelle. One key gene appears to be the small subunit of RUBISCO. Although the transfer of these plastid genes to the host nucleus did not predestine the green lineage to extinction in the marine phytoplankton, we propose that it reduced the evolutionary plasticity of this lineage to evolve to rapidly changing ocean chemistry and redox conditions in the Mesozoic period. Our plastid portability hypothesis suggests two major factors were critical in determining the evolutionary trajectory of eukaryotic phytoplankton after the end-Permian extinction: 1) the loss of critical genes from plastid genomes during endosymbiotic events, which differentially affected the genetic and functional potential of plastids, and 2) the concurrent ecological fitness of the specific host cells, which afforded selective advantages under changing environmental conditions. The selection pressures brought about by the end-Permian extinction that led to the subsequent ecological success of the red lineage remains to be elucidated.

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