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Structure and organization of rhodophyte and chromophyte plastid genomes: implications for the ancestry of plastids

M.S. Shivji*, N. Li[1,], and R.A. Cattolico[2]**

School of Fisheries, HF-10, [1] Department of Botany, KB-15 and [2] Departments of Botany and Oceanography, University of Washington, Seattle, WA 98195, USA

Summary. Plastid genomes of two rhodophytes (*Porphyra yezoensis* and *Griffithsia pacifica*) and two chromophytes (*Olisthodiscus luteus* and *Ochromonas danica*) were compared with one another and with green plants in terms of overall structure, gene complement and organization. The rhodophyte genomes are moderately colinear in terms of gene organization, and are distinguished by three rearrangements that can most simply be explained by transpositions and a large (approximately 40 kb) inversion. *Porphyra* contains two loci for *ppcBA* and *Griffithsia* has two loci for *rpoA*. Although there is little similarity in gene organization between the rhodophytes and consensus green plant genome, certain gene clusters found in green plants appear to be conserved in the rhodophytes. The chromophytes *Olisthodiscus* and *Ochromonas* contain relatively large plastid inverted repeats that encode several photosynthetic genes in addition to the rRNA genes. With the exception of *rbcS*, the plastid gene complement in *Olisthodiscus* is similar to that of green plants, at least for the subset of genes tested. The *Ochromonas* genome, in contrast, appears unusual in that several of the green plant gene probes hybridizing to *Olisthodiscus* DNA did not detect similar sequences in *Ochromonas* DNA. Gene organization within the chromophytes is scrambled relative to each other and to green plants, despite the presence of putatively stabilizing inverted repeats. However, some gene clusters conserved in green plants and rhodophytes are also present in the chromophytes. Comparison of the entire rhodophyte, chromophyte and green plant plastid genomes suggests that despite diferences in gene organization, there remain overall similarities in architecture, gene content, and gene sequences among in three lineages. These similarities are discussed with reference to the ancestry of the different plastid types.

Offprint requests to: M. Shivji

Present address: * Tetra Tech, Inc., 11820 Northup Way, Suite 100E, Bellevue, WA 98005, USA

Present address: ** USDA/ARS/PS1, Plant Molecular Biology Laboratory, Bldg 006, BARC-west, Beltsville, MD 20705, USA

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Introduction

Photosynthetic eukaryotes are divided into three main lineages, i.e. the green plants [the chlorophyll *b*-containing higher plants (includes all seed plants, mosses, ferns and liverworts) and green algae], the rhodophytes (algae containing phycobilin pigments) and the chromophytes (algal groups whose members usually contain chlorophyll *c*), based primarily on the diverse physical and biochemical characteristics of their plastids.

Although the origin of all plastids from endosymbiotic, photosynthetic eubacteria is widely accepted (Gray 1989), controversy is now focused on whether the morphologically and biochemically distinct plastids characteristic of the three plant lineages share a common ancestor, or were derived from different eubacterial ancestors (i.e., whether the plastids have monophyletic or polyphyletic origins) (Raven 1970; Cavalier-Smith 1982, 1987; Gray 1989; Kowallik 1989; Valentin and Zetsche 1990a).

Phylogenetic analysis of nucleic acid sequences has been proposed as one approach to resolve the issue of monophyly versus polyphyly of organelles (Gray 1988; Turner et al. 1989). There are, however, numerous methodological difficulties associated with the construction and interpretation of sequence-based phylogenetic trees, resulting in vigorous debate as to which genes and which tree construction methods provide the most accurate phylogenies (Mishler et al. 1988; Gouy and Li 1990; Lake 1990; Rohlf et al. 1990; Van de Peer et al. 1990). An additional complication with this approach results from the common use of single gene sequences to infer phylogenetic relationships. This practice suffers from the arguable assumption that evolution of a single gene accurately represents evolution of the whole genome (Gray 1988).

In view of the above difficulties, we have undertaken a complementary approach to gain a better understanding of plastid genome origin and evolution. This approach entails a comparative analysis of the structural and organizational characteristics of whole plastid genomes from the three plant lineages. The advantage of this approach lies in the use of characteristics of entire plastid genomes (as opposed to characteristics of single genes) as the basis for inferring the evolutionary history of these organelles.

Although structural aspects of green plant (mostly higher plant) plastid genomes have been extensively investigated (reviewed in Sugiura 1989), plastid genomes of the evolutionarily distant and ecologically distinct rhodophytes and chromophytes have received very little attention. We report here on a comparative analysis of the structural and organizational characteristics of plastid genomes from two rhodophytes (*Porphyra yezoensis* and *Griffithsia pacifica*), and two chromophytes [*Ochromonas danica* and *Olisthodiscus luteus* (also known as *Heterosigma akashiwo* (Hara et al. 1985) or *Heterosigma luteus* (Coleman and Goff 1991) – taxonomic nomenclature for this organism is under revision)]. The genomes have been analyzed with the following questions in mind. First, what is the gene complement and organization of plastid genomes in the rhodophyte and chromophyte algae? Second, can plastid genome characteristics in the three plant lineages provide clues about the ancestry of their plastids?

Materials and methods

DNA Isolation and analysis. Plastid DNAs from the rhodophytes and chromophytes were isolated using CsCl gradient ultracentrifugation, as described elsewhere (Li and Cattolico 1987; Delaney and Cattolico 1989; Li 1989; Shivji 1991). Plasmid DNAs containing heterologous gene probes were isolated using the boiling lysis method described in Maniatis et al. (1982). Plastid DNAs from the four algae were digested with restriction endonucleases used to construct physical maps of these plastid genomes (see Reith and Cattolico 1986; Li and Cattolico 1987; Li 1989; Shivji 1991). Digested DNAs were transferred to GeneScreen Plus (Du Pont, Boston, Mass.) nylon filters following the recommendations of the manufacturer. Heterologous gene probes were labeled with ^{32}P by the random primer method of Feinberg and Vogelstein (1983). Nylon filters were prehybridized and hybridized in 1% SDS and 1 M NaCl at 55° C. Filters were washed twice in 2 × SSC (0.3 M NaCl, 3 mM sodium citrate) at room temperature for 5 min each, and twice in 2 × SSC, 1% SDS at 55° C for 30 min each prior to autoradiography.

A description of the heterologous plastid and cyanobacterial genes used to probe the rhodophyte and chromophyte plastid DNAs is given in Table 1. Control experiments using the plasmid cloning vectors were performed initially to preclude the possibility that hybridization signals were due to similarities between the plasmid vector and plastid DNAs.

Results and discussion

Comparative organization of the rhodophyte genomes

Restriction and gene maps for the plastid genomes of *Porphyra yezoensis* and *Griffithsia pacifica* are shown in Fig. 1. Both rhodophyte genomes are circular molecules, similar in size, and contain the same complement of identified genes. Both genomes are also relatively large compared to plastid genomes of most higher plants. This larger size cannot be accounted for by expansion of the inverted repeats as is the case in some higher plants (Palmer et al. 1987), because *Griffithsia* lacks such repeats and *Porphyra* contains only a small repeat (Li and Cattolico 1987; Shivji 1991). The additional sequence complexity in the rhodophytes can be accounted for by the presence of genes which are absent from the higher plant plastid genome. In this study the phycobiliprotein, Rubisco small subunit, and elongation factor EF-Tu genes have been identified.

Although genome size and identified gene complement appear to be very similar in *Porphyra* and *Griffithsia*, some notable differences exist between the two genomes. First, *Porphyra* differs from *Griffithsia* in containing rRNA-encoding inverted repeats (Shivji 1991). the sequence complexity of the two genomes, however, is approximately the same. Second, the numbers of loci coding for *ppcBA* and *rpoA* differ in the two rohodophytes. The two loci for *ppcBA* in *Porphyra* and for *rpoA* in *Griffithsia* may both represent active genes; alternatively, one of the loci could be a pseudogene. Another possibility is that *ppcBA* in *Porphyra* and *rpoA* in *Griffithsia* are split genes whose transcripts are *trans*-spliced. Although our data do not distinguish among these possibilities, examples of the latter two possibilities have been found in other plastid genomes (Choquet et al. 1988; Markowicz et al. 1988). The possibility that both *ppcBA* loci in *Porphyra* are active is suggested by the occurrence of two or more active, differentially regulated phycocyanin loci in some cyanobacteria (Mazel et al. 1988). All other genes mapped (excluding the 16S and 23S rRNA genes) on the two rhodophyte plastid genomes occur as single copy genes.

The occurrence of *rbcS* in rhodophyte plastids appears to be a general characteristic of this algal division (Kostrzewa et al. 1990; Valentin and Zetsche 1990a). With the exception of *rbcS*, *tufA* and the phycobilin genes, the eighteen genes mapped on the *Porphyra* and *Griffithsia* genomes are also common to green plant plastids. In addition, some of the gene clusters found in green plants (e.g., *psaA-psaB*, *psbC-psbD*, *petB-petD*) are potentially conserved in the rhodophytes.

Gene organization on the two rhodophyte genomes presents a mixed picture. The genes are moderately rearranged relative to each other, but still retain some colinearity (Fig. 2). Three major rearrangements distinguish the *Porphyra* and *Griffithsia* genomes. The simplest explanation for two of the rearrangements, involving *psbB* and the *petA*–*psbA* cluster, appears to be transposition of these regions. Transposition events, although uncommon, are not without precedent in plastid genomes

Table 1. Description of gene probes

Gene designation	Source	Gene product	Insert	Reference
psaA	Spinach	68 kDa photosystem I apoprotein I	*Kpn*I – *Bam*HI 1100 bp	H. Bohnert (pers. comm.)
psaB	Spinach	68 kDa photosystem I apoprotein II	*Bam*HI 1600 bp	H. Bohnert (pers. comm.)
psbA	Pea	32 kDa photosystem II D1 protein	*Eco*RI – *Pst*I 532 bp	Oishi et al. (1984)
psbB	*Cyanophora paradoxa*	51 kDa photosystem II protein	*Pst*I 1300 bp	D. Bryant (pers. comm.)
psbC	Spinach	44 kDa photosystem II protein	*Bam*HI – *Pst*I 367 bp	Alt et al. (1984)
psbD	*Chlamydomonas*	34 kDa photosystem II D2 protein	*Eco*RI 2600 bp	Rochaix et al. (1984)
psbE/F	*C. paradoxa*	9 kDa/4 kDa photosystem II Cyt b-559 proteins	*Eco*RI 750 bp	D. Bryant (pers. comm.)
petA	Pea	cytochrome *f*	*Hin*dIII – *Bam*HI 900 bp	Willey et al. (1984)
petB	Spinach	cytochrome *b6*	*Eco*RI 2380 bp	H. Bohnert (pers. comm.)
petD	Spinach	subunit IV	*Bam*HI 400 bp	H. Bohnert (pers. comm.)
atpA	Spinach	58 kDa α subunit of ATP synthase complex	*Hin*dIII – *Sal*I 1024 bp	Hudson et al. (1987)
atpB	Pea	57 kDa β subunit of ATP synthase complex	*Pst*I – *Xba*I 1171 bp	Zurawski et al. (1986)
atpE	Spinach	14 kDa ε subunit of ATP synthase complex	*Eco*RI – *Xba*I 420 bp	Zurawski et al. (1982)
tufA	*Chlamydomonas*	polypeptide elongation factor EF-Tu	*Eco*RI – *Ssp*I 762 bp	Baldauf and Palmer (1990)
rpoA	Spinach	α subunit of RNA polymerase	*Xba*I 1040 bp	Sijben-Muller et al. (1986)
rpoB	Tobacco	β subunit of RNA polymerase	*Bam*HI 1063 bp	Ohme et al. (1986)
ppeBA	*Pseudanabaena*	$\beta\alpha$ subunits of phycoerythrin	*Hin*dIII – *Eco*RI 1100 bp	D. Bryant (pers. comm.)
apcAB	*C. paradoxa*	$\alpha\beta$ subunits of allophycocyanin	*Bam*HI – *Eco*RI 1900 bp	Bryant et al. (1985)
ppcBA	*C. paradoxa*	$\beta\alpha$ subunits of phycocyanin	*Eco*RI – *Pst*I 800 bp	Lemaux et al (1984)
rbcS	*Olisthodiscus luteus*	small subunit of ribulose bisphosphate carboxylase	*Eco*RI – *Ssp*I 647 bp	Boczar et al. (1989)

(Palmer et al. 1987). Plasmid-like molecules with homology to the plastid genome occur in *Porphyra* (Shivji 1991). This observation prompts the speculation that these molecules may be transposable elements, providing a possible mechanism for the observed gene rearrangements.

The third major rearrangement that distinguishes the two rhodophyte plastid genomes is a reversal of gene order in a region spanning approximately 40 kb. This reversal can most simply be explained by invoking a large inversion in one of the genomes relative to the other (Fig. 2). The inversion has its end-points approximately in the vicinity of (but excluding) the *ppeBA-ppcBA* and *psaA-psaB-tufA* gene clusters. Hybridization data suggest that gene order within this inversion remains co-linear. Although inversions are the most common rearrangements in plastid genomes (Palmer et al. 1988), large inversions such as the one distinguishing the two red algae are relatively rare, and therefore provide valuable phylogenetic characters. Because phylogenetic relationships within the red algae remain controversial (Gabrielson et al. 1985), determination of the distribution of this inversion among other red algal taxa may prove useful for evolutionary studies.

The question of which of the two rhodophyte plastid genomes is closer to (or represents) the ancestral genome condition is also of evolutionary interest. If we apply the general rules that have been proposed to influence genome evolution in higher plant plastids (Palmer 1985), the occurrence of an inverted repeat in *Porphyra* should represent an ancestral condition, and the lack of an inverted repeat in *Griffithsia* a derived condition. Such a scenario is consistent with traditional red algal taxonomic schemes, which place *Porphyra* in the more "primitive" sub-class Bangiophycidae, and *Griffithsia* in the more "advanced" sub-class Florideophycidae (Bold and Wynne 1985). Recent studies by Boyen et al. (1991) show that an inverted repeat is absent from the plastid genome of the rhodophyte *Chondrus crispus* (Florideophycidae). Interestingly, the *Chondrus* genome contains a 16S/23S

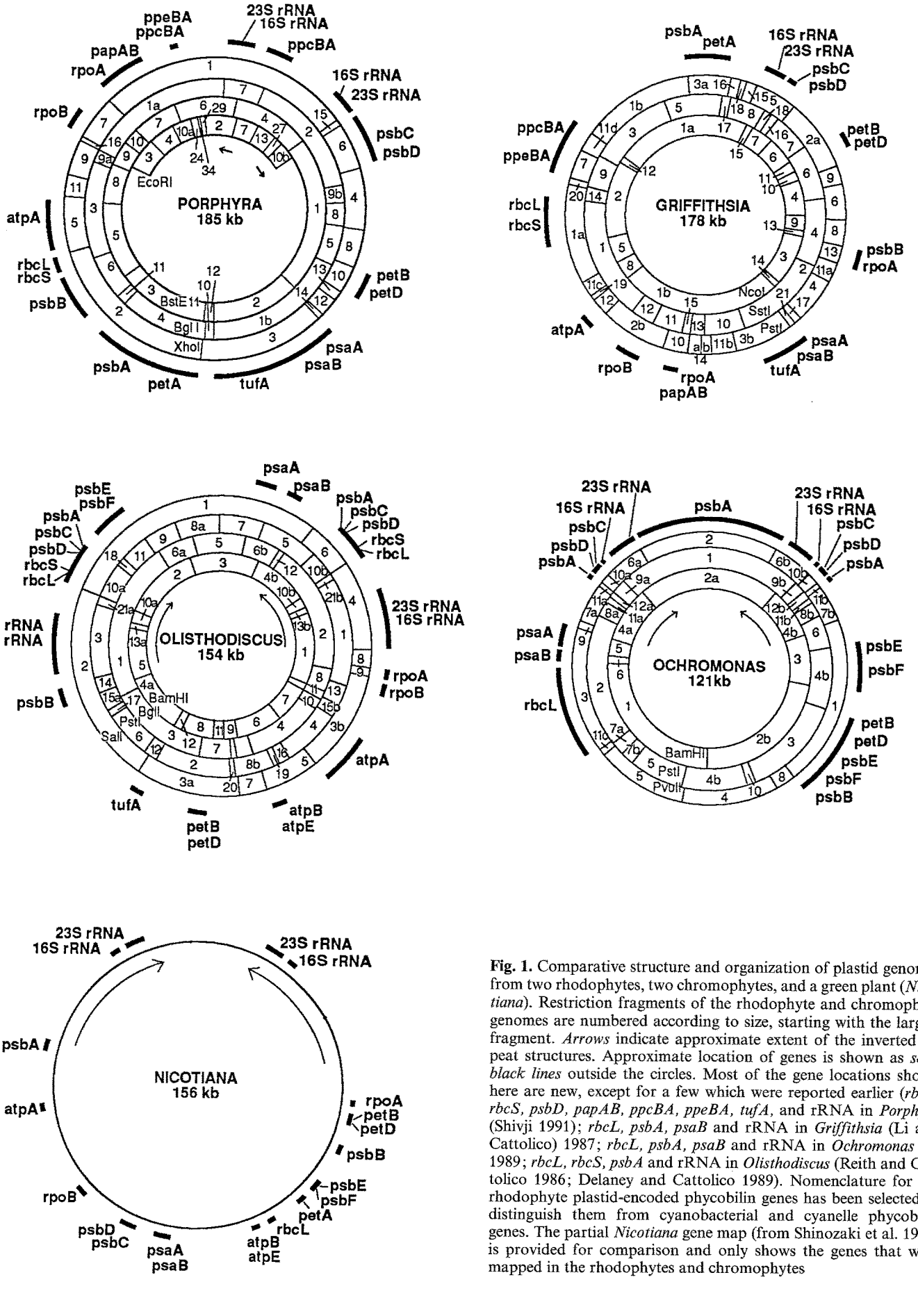

Fig. 1. Comparative structure and organization of plastid genomes from two rhodophytes, two chromophytes, and a green plant (*Nicotiana*). Restriction fragments of the rhodophyte and chromophyte genomes are numbered according to size, starting with the largest fragment. *Arrows* indicate approximate extent of the inverted repeat structures. Approximate location of genes is shown as *solid black lines* outside the circles. Most of the gene locations shown here are new, except for a few which were reported earlier (*rbcL, rbcS, psbD, papAB, ppcBA, ppeBA, tufA,* and rRNA in *Porphyra* (Shivji 1991); *rbcL, psbA, psaB* and rRNA in *Griffithsia* (Li and Cattolico) 1987; *rbcL, psbA, psaB* and rRNA in *Ochromonas* (Li 1989; *rbcL, rbcS, psbA* and rRNA in *Olisthodiscus* (Reith and Cattolico 1986; Delaney and Cattolico 1989). Nomenclature for the rhodophyte plastid-encoded phycobilin genes has been selected to distinguish them from cyanobacterial and cyanelle phycobilin genes. The partial *Nicotiana* gene map (from Shinozaki et al. 1988) is provided for comparison and only shows the genes that were mapped in the rhodophytes and chromophytes

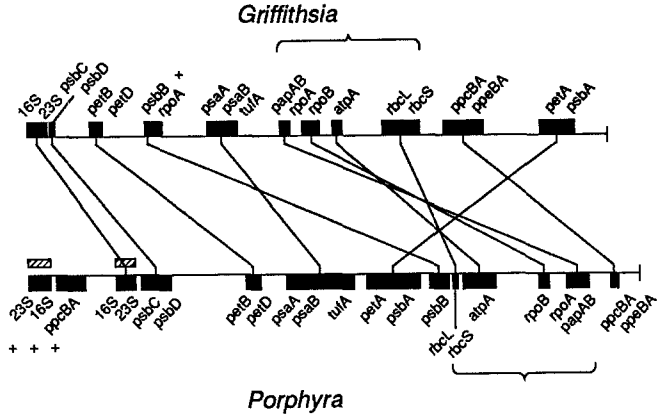

Fig. 2. Comparison of plastid gene order in *Porphyra* and *Griffithsia*. *Brackets* indicate approximate extent of the inversion. Putative additional loci for specific genes are denoted by the + symbol. *Hatched bars* indicate approximate extent of the rRNA encoding inverted repeat in *Porphyra*.

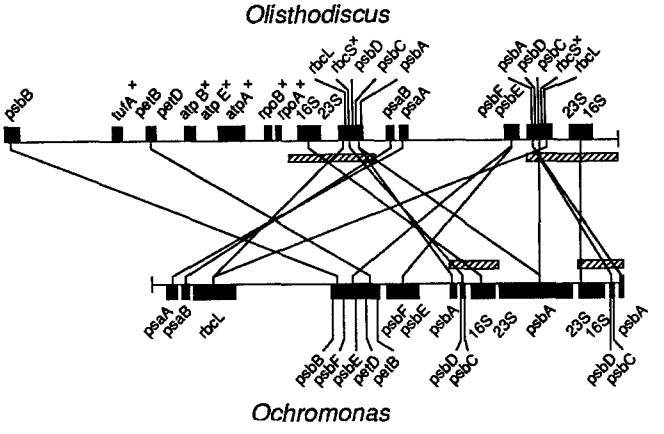

Fig. 3. Comparison of plastid gene orders in *Olisthodiscus* and *Ochromonas*. *Hatched bars* indicate approximate extent of rRNA encoding inverted repeat structures. The + symbol denotes the genes that failed to hybridize to the *Ochromonas* plastid genome

rRNA operon, as well as an independent 16S rRNA gene.

Comparative organization of the chromophyte genomes

Restriction and gene maps of the plastid genomes of *O. luteus* and *O. danica* are shown in Fig. 1. Both chromophyte genomes have a similar overall architecture in terms of circularity and presence of asymmetrically positioned, rRNA-encoding inverted repeats. As in green plant plastid genomes, the two chromophyte inverted repeats are organized with their 23S rRNA genes proximal and the 16S rRNA genes distal to the small single-copy regions. Other similarities between the two chromophyte genomes include clustering and duplication of the *psbA*, *psbC*, and *psbD* genes, due to their occurrence on the inverted repeat. Gene clusters (e.g., *psbC-psbD*, *petB-petD*, *psbE-psbF*, *psaA-psaB*) thought to be co-transcribed in higher plants (Palmer et al. 1988) also appear to be present in these chromophytes.

Characteristics that distinguish between the two chromophyte genomes include differences in genome size, inverted repeat size (*Olisthodiscus*, 22 kb; *Ochromonas*, 15 kb), gene organization and possibly gene content. Other than the preservation of certain gene clusters mentioned above, the two chromophyte genomes are highly rearranged relative to each other (Fig. 3). This observation is in contrast to the remarkable conservation in gene organization found in most green plants, including evolutionarily diverse lineages such as angiosperms, ferns, mosses and liverworts (Sugiura 1989).

Although *Olisthodiscus* plastid DNA contains a subset of genes which are similar to those of green plants, the *Ochromonas* genome may be unusual in this respect. In contrast to *Olisthodiscus*, no hybridization signal was obtained from *Ochromonas* DNA when *rbcS*, *rpoA*, *rpoB*, *atpA*, *atpB*, *atpE* and *tufA* were used as probes under the hybridization conditions described. Lack of hybridization signals suggests either absence of these genes from the *Ochromonas* plastid genome, or a degree of sequence divergence that precludes their detection by heterologous probes. Interestingly, the same regions of the *rpoA*, *rpoB*, *atpE* and *tufA* gene probes used in our study also failed to hybridize to plastid DNA from the green alga *Spirogyra maxima* (Manhart et al. 1990). It is also notable that a *petA* gene probe derived from pea, which hybridized to both rhodophyte plastid DNAs, failed to hybridize to either of the chromophyte DNAs. Manhart et al. (1989) have also reported failure of a *petA* gene from pea to hybridize to plastid DNA from the green alga *Codium fragile*.

The failure of the *Olisthodiscus rbcS* gene to hybridize to *Ochromonas* DNA (despite repeated trials) was surprising, particularly because the same probe hybridized to both *Griffithsia* and *Porphyra* DNA as well as to plastid DNA from the chromophyte *Cylindrotheca* sp. (Hwang and Tabita 1989), several cryptomonads (Chesnick et al. 1991), and dinoflagellates (J. Chesnick, T. Nadeau and R.A. Cattolico, unpublished data). Analysis of plastid *rbcS* genes from chromophytes and rhodophytes reveals a high degree of sequence similarity between these groups (Valentin and Zetsche 1990b). In addition, recent sequencing studies (K. Viehmann, M. Kostrzewa and K. Zetsche, personal communication) have shown that *rbcS* occurs on the *Ochromonas* plastid genome immediately 3' to the *rbcL* gene. We have no explanation for the absence of *rbcS* hybridization in our studies.

Plastid genomes of six other chromophyte genera i.e., *Dictyota dichotoma*, *Odontella sinensis*, *Coscinodiscus granii*, *Vaucheria* sp., (reviewed in Kowallik 1989), *Pylaiella littoralis* (Loiseaux-de Goër et al. 1988), and *Cryptomonas* ϕ (Douglas 1988), have now been physically mapped. All the chromophyte genomes studied (including *Olisthodiscus* and *Ochromonas*) fall within the 118–150 kb size range, and are characterized by asymmetrically positioned rRNA-encoding inverted repeats that divide the plastid genome into small and large single-copy sections. Several of the gene clusters conserved in green plant plastid genomes (e.g., *psbC-psbD*, *psaA-psaB*, *petB-petD*) also occur in all these chromophytes.

There is little similarity in gene organization among the chromophytes studied. In addition, both *Olisthodiscus* and *Ochromonas* differ from the other chromophytes in possessing relatively large inverted repeats that contain several photosynthetic genes in addition to rRNA genes. The mechanism and evolutionary significance of such rearrangements is uncertain. The extensive conservation in the organization of plastid genomes of higher plants has been proposed to result from stability conferred on the genome by the inverted repeat (Palmer 1985; Manhart et al. 1990). The chromophytes, however, present an enigmatic picture in that their genomes are highly rearranged despite possessing inverted repeats. The evolutionary constraints operating to preserve the characteristic genome organization of plastids in higher plants appear to be absent (or different) in the case of the ecologically distinct chromophytes. The rearrangements in the chromophytes are complex, and do not indicate any obvious trend in plastid genome evolution.

Plastid ancestry

One of the more interesting and controversial issues in plastid evolution concerns the ancestry of plastids in the three plant lineages: did these diverse plastids have monophyletic or polyphyletic origins from eubacterial ancestors (Gray 1989)? This issue is complicated by the hypothesis that certain algal groups possessing three to four membranes around their plastid (i.e. most chromophytes) were derived by a secondary endosymbiosis between a eukaryotic, non-photosynthetic host cell and a eukaryotic alga (Gibbs 1981; Cavalier-Smith 1986). Even if this hypothesis were correct, however, this putative secondary endosymbiotic event cannot be interpreted as a true polyphyletic origin for plastids, since it involved the uptake of a pre-existing plastid (Cavalier-Smith 1982).

The use of plastid gene sequences to resolve the monophyly versus polyphyly debate has resulted in an ambiguous picture. For example, analysis of 16S rRNA sequences from *Porphyra*, *Griffithsia*, *Olisthodiscus* and *Ochromonas* (Delaney 1989) and *Pylaiella* (Assali et al. 1990) indicates that the rhodophytes and chromophytes form a sister group with green plants and cyanobacteria. In contrast, analysis of *rbcL* gene sequences from the rhodophytes *Porphyridium aerugineum* and *Cyanidium caldarium*, and the chromophytes *Cryptomonas* ϕ, *Ectocarpus siliculosus*, *Olisthodiscus*, and *Pylaiella* indicates the rhodophytes and chromophytes are more closely related to the β-purple bacteria than to the green plants and cyanobacteria (Assali et al. 1990; Douglas et al. 1990; Valentin and Zetsche 1990b; Hardison et al. 1991). The latter observations have prompted the suggestion that plastids arose polyphyletically (Douglas et al. 1990; Valentin and Zetsche 1990b). Obviously, the 16S rRNA and *rbcL* phylogenetic schemes cannot both be accurate reflections of the true phylogeny. Such discrepancies underscore the shortcomings sometimes associated with construction of phylogenetic trees using only one or two sequences, and with interpreting trees derived by different tree construction methods. Conflicting phylogenies resulting from analysis of different genes have also been noted in other organisms (Turner et al. 1989; Morden and Golden 1989; Miller et al. 1990).

The presence in rhodophytes (Valentin and Zetsche 1990a) and chromophytes (Assali et al. 1990; Douglas et al. 1990; Valentin and Zetsche 1990b; Hardison et al. 1991) of *rbcL* sequences that are more similar to *rbcL* sequences from β-purple bacteria than cyanobacteria or green plants is intriguing. However, in view of the contradictory phylogenetic relationships predicted by analysis of different gene sequences, alternative possibilities (in addition to polyphyletic origins) must be considered in attempts to explain the origins of the divergent gene sequences in the rhodophyte and chromophyte plastid genomes. These possibilities include different rates of sequence evolution in different parts of the plastid genome (Palmer 1985), and/or lateral gene transfer. Although lateral gene transfer into the plastid genome has not yet been demonstrated, as it has for the nucleus and mitochondrion, there is no compelling reason to exclude its existence. The successful artificial transfer of genes into the plastid genome (Svab et al. 1990), the natural transfer of both single-stranded DNA and naked RNA into plastids (Schoelz and Zaitlin 1989; Groning et al. 1990) and the discovery of a reverse transcriptase-like sequence in the plastid genome of a green alga (Kück 1989), all suggest the distinct possibility that lateral gene transfer (via DNA or RNA) does indeed occur. Cavalier-Smith (1989) and Assali et al. (1990) have also suggested lateral gene transfer as a possible explanation for the apparently different phylogenetic origins of the *Olisthodiscus* and *Pylaiella* 16S rRNA and *rbcL* genes. These alternative possibilities caution against interpretation of the divergent *rbcL* sequences as evidence for polyphyletic origins of the whole plastid genome.

In a complementary approach to gain insight into the question of plastid ancestry, we have compared features of whole plastid genomes from the three plant groups. The data reveal similarities in overall genome architecture and characteristics among the three plastid lineages (Fig. 1). For example, both rhodophyte and chromophyte genomes are circular, falling within the size range exhibited by green plant genomes (Sugiura 1989). All three plastid lineages contain the "signature", asymmetrically positioned, rRNA-encoding, inverted repeats that divide the genomes into large and small single-copy regions. Several genes are conserved (*Ochromonas* excepted), as judged by positive hybridization with gene probes derived from green plants and cyanobacteria. Moreover, the physical arrangement of certain gene clusters (e.g., *psbC-psbD*, *psaA-psaB*, *petB-petD* and *psbE-psbF*) appears to be conserved in all three lineages.

Can the pronounced similarities in overall plastid genome architecture, selected gene sequences, and organization of some gene clusters in the rhodophytes, chromophytes and green plants be reasonably interpreted as supportive of the monophyletic origin hypothesis? A primary assumption of the endosymbiont hypothesis is that the contemporary plastid genome is a remnant of a much

larger eubacterial endosymbiont genome, which was greatly reduced in size during its evolutionary conversion into a plastid. Given this assumption, the overall conservation in plastid genome features is difficult to reconcile with polyphyletic origins, because it would require a significant amount of convergent evolution to produce such similar plastid genomes among extant taxa that had originated from different ancestors. The alternative hypothesis, i.e., that such genome similarities already existed in the eubacterial ancestors of plastids is also unlikely, for two reasons. First, this hypothesis requires deliberate preservation of these similarities during a conversion process in which the different endosymbiont genomes were undergoing major structural and size modifications. The selective forces required to ensure the retention of such similarities are unclear. Second, there is no indication that the "signature" rRNA-encoding inverted repeats of plastid genomes are common in photosynthetic eubacteria (Bancroft et al. 1989; Dryden and Kaplan 1990).

In conclusion, the similarities in overall plastid genome characteristics, the monophyletic clustering of plastids indicated by analyses of 16S rRNA sequences (Delaney 1989; Turner et al. 1989; Assali et al. 1990), and the unexpected discovery of both chlorophylls *b* and *c* in the primitive green alga *Mantoniella* (Wilhelm 1987), are all consistent with the hypothesis (Cavalier-Smith 1982; Kowallik 1989) of a common ancestry for the three plastid lineages. On the other hand, the occurrence of lateral gene transfer into plastids is still uncertain and only about 15% of the rhodophyte and chromophyte plastid genes have been identified (primarily by hybridization using probes from higher plants and cyanobacteria). In addition, fundamental differences in plastid gene transcription control (A. Reynolds, E. McConaughy and R.A. Cattolico, unpublished data) and gene product function (Newman and Cattolico 1990) have been found among the plant lineages. Whether these differences truly reflect polyphyletic plastid origins or plastid diversification following a common ancestry remains unresolved. Resolution of the question of plastid ancestry will most likely require that plastid genome architecture and gene expression characteristics be considered together with analysis of sequence data from much larger portions of the rhodophyte and chromophyte plastid genomes.

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