Curr Genet (1991) 19:49–54

Current Genetics

Organization of the chloroplast genome in the red alga *Porphyra yezoensis*

M.S. Shivji

School of Fisheries, HF-10, University of Washington, Seattle, WA 98195, USA

Received April 20, 1990/August 27, 1990

Summary. A comprehensive assessment of the origin and evolution of plastids will require more information on the nature of plastid genomes from non-green algae. I have constructed a physical map of the chloroplast genome from the red alga *Porphyra yezoensis*. The 185 kb circular genome contains ribosomal RNA encoding inverted repeats (6.6 kb), and is divided into small and large single-copy regions of approxiamtely 16 kb and 156 kb respectively. The *Porphyra* genome contains several genes not found in higher plant chloroplasts. Genes encoding the pigmented, light-harvesting phycobiliproteins are organized relatively close to one another on the genome, and represent components of a multi-gene family. The phycocyanin biliprotein genes (*ppc*BA) map in two single-copy regions, suggesting either duplicated genes or a trans-splicing mechanism. In contrast to higher plants, the *tuf*A and *rbc*S genes are chloroplast-encoded in *Porphyra*, and *rbc*S is clustered with the *rbc*L gene, suggesting an operon type of arrangement. The *Porphyra* chloroplast genome is distinctive, also, in that part of it has sequence homology to plasmid-like DNA molecules which co-isolate with the chloroplast DNA.

Key words: Red-algal chloroplast DNA – Phycobiliprotein genes – Physical map

Introduction

The biochemical and ultrastructural diversity found in plastids of rhodophytes, chromophytes and green plants (green algae and higher plants) is postulated by some workers to be the result of a polyphyletic, symbiotic origin of chloroplasts (Whatley and Whatley 1981; Gray and Doolittle 1982). This is not a universally shared point of view however (Cavalier-Smith 1982, 1987), and the issue of monophyletic versus polyphyletic origins of plastids remains contentious. The utility of chloroplast DNA analysis for elucidating plastid evolution (Palmer 1986a), and the hypothesized role of red algal ancestors in the origin of chromophyte plastids (Gibbs 1981; Whatley 1981), suggest that studies on the red algal chloroplast genome may help resolve this issue. There is, unfortunately, a paucity of information on the nature of red algal chloroplast genomes, with only one species being physically characterized to date (Li and Cattolico 1987).

The gene content of red algal chloroplast geomes remains largely unknown. Circumstantial evidence indicates that the pigmented phycobiliproteins may be plastid-encoded in the red algae (Egelhoff and Grossmann 1983; Steinmuller et al. 1983). Valentin and Zetsche (1989) have recently demonstrated that the gene for the small subunit polypeptide of ribulose-1, 5-bisphosphate carboxylase/oxygenase is also chloroplast-encoded in the red algae. Localization of these genes on the chloroplast genome will be instructive in elucidating their regulation as well as their evolution.

I report here on the isolation and physical organization of chloroplast DNA from the economically important red alga *Porphyra yezoensis*. The genome maps as a circular, inverted repeat-containing molecule which carries several genes not found in higher plant chloroplasts. These include the phycobiliprotein genes, the gene for the small subunit polypeptide of ribulose-1, 5-bisphosphate carboxylase/oxygenase, and the gene for the polypeptide elongation factor EF-TU. In addition, part of the *Porphyra* chloroplast genome has sequence homology to some plasmid-like DNA molecules that co-isolate with chloroplast DNA

Materials and methods

Culture methods. Clonal, axenic *Porphyra yezoensis* conchocelis filaments were grown at 20 °C in f/2 medium (Guillard and Ryther 1962) under a photon fluence rate of 5–10 $\mu E/m^2/s$ (cool white fluorescent lamps).

Isolation of chloroplast DNA. Ten to twelve grams (damp weight) of conchocelis filaments were harvested by filtering through miracloth and rinsed with buffer A (0.7 M glucose, 10 mM Hepes, 20 mM

EDTA, 0.1% Bovine serum albumin, 1% Polyvinylpyrrolidone, 2 mM mercaptoethanol, pH 8.0). To break the cells, the filaments were frozen in liquid nitrogen and ground to a fine powder using a mortar and pestle. The powder was transferred to 100 ml of ice-cold buffer A and centrifuged at 3020 g at 4 °C for 15 min. All subsequent DNA isolation steps (unless otherwise noted) were performed at 0–4 °C. The pellet was resuspended in buffer B (buffer A minus Polyvinylpyrrolidone) and centrifuged once at 3020 g for 10 min, and twice at 270 g for 15 min each. The last two centrifugation steps removed most of the cell debris and nuclei. The supernatant was centrifuged at 3020 g for 10 min. The pellet, containing mostly chloroplasts plus some nuclei and mitochondria, was resuspended in cold (5 °C) 50 mM TRIS, 100 mM EDTA, pH 8.0 buffer. Proteinase-K (Sigma, St. Louis, Mo.), sodium dodecyl sulphate (SDS) and Hoechst 33 258 dye (Sigma) were added to 100 µg/ml, 2%, and 50 µg/ml final concentration, respectively. The suspension was mixed by inversion and incubated at 37 °C for 4 h. Fresh proteinase-K (100 µg/ml) was added 2 h into the incubation period. Solid cesium chloride (Gallard-Schlesinger Industries Inc., New York) was added and dissolved by inversion. The lysate was then centrifuged at 27 200 g for 15 min to remove remaining cell and membrane debris. The DNA containing supernatant was adjusted to a refractive index of 1.3985 and centrifuged in a fixed angle rotor for 24–30 h at 180 000 g at 20 °C. Gradients were fractionated, and individual DNA species pooled and re-centrifuged twice more, using the conditions described above. The final DNA fractions were extracted with NaCl-TE-(10 mM Tris, 1 mM EDTA, pH 8.0)-saturated isopropanol to remove the Hoechst dye, and dialyzed against TE for 2 days at 5 °C with at least four changes of buffer. The DNA solution was made 0.3 M in sodium acetate and precipitated with two volumes of ice-cold 95% ethanol.

Restriction enzyme analysis. Chloroplast DNA was digested with restriction endonucleases as recommended by the suppliers (Bethesda Research Laboratories, Gaithersburg, Md. and Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Analytical and preparative digests were electrophoretically separated through 0.37–0.6% horizontal agarose gels in TBE buffer (Maniatis et al. 1982). Analytical digests of DNA were transferred onto nylon membranes (Gene Screen Plus; New England Nuclear, Boston, Mass.) using protocols recommended by the manufacturer.

Cloning of chloroplast DNA. Porphyra chloroplast DNA was digested to completion with *Xho*I, shot-gun cloned into a pIC-7 plasmid vector (Marsh et al. 1984) and transformed into competent *E. coli* strain JM83 using standard methods (Maniatis et al. 1982). Plasmid DNA was purified using the boiling lysis method (Maniatis et al. 1982), and identity of chloroplast clones verified by gel electrophoresis, as well as by hybridization of clones back to *Xho*I digests of *Porphyra* chloroplast DNA.

Hybridization of radiolabelled probes. Uncloned *Xho*I chloroplast restriction fragments were purified from preparative gels by electroelution and labelled with ^{32}P-dATP by nick-translation (Maniatis et al. 1982) or the random primer method of Feinberg and Vogelstein (1983). The plasmid cloning vector alone (control), as well as recombinant vector containing chloroplast inserts, were labelled by nick-translation. Prehybridization and hybridization for homologous DNA probing was carried out at 65 °C in 1% SDS, 1 M NaCl. Blots were washed twice (with agitation) for 5 min each at room temperature in 2 × SSC, twice at 65 °C for 30 min each in 2 × SSC, 1% SDS, and twice for 30 min each at room temperature in 0.1 × SSC. Heterologous chloroplast, cyanelle, and cyanobacterial genes (Table 1) were labelled with ^{32}P-dATP and used as hybridization probes to determine the approximate location of ribosomal and protein coding genes on the *Porphyra* chloroplast genome. Prehybridization and hybridization conditions for heterologous ribosomal and *rbc*L genes were as described above. For other, less-conserverd heterologous genes, stringency conditions were reduced to 55 °C and the last 30 min washes in 0.1 × SSC not performed.

Mapping strategy. The physical map of *Porphyra* chloroplast DNA was constructed by using cloned *Xho*I fragments and gel-purified *Xho*I, *Bgl*I, *Bst*EII and *Eco*RI fragments to probe membrane-bound chloroplast DNA digested with *Xho*I, *Bgl*I, *Bst*EII, and *Eco*RI (single and double digests) (Palmer1986b). Mapping ambiguities were resolved by reciprocal restriction endonuclease digestions of gel-purified fragments, as well as by hybridization with heterologous gene probes.

Results

Isolation of chloroplast DNA

Three DNA species were resolved using cesium chloride – Hoechst 33 258 dye equilibrium density centrifugation. The least dense band was cleanly separated from the lower two bands (presumptive mitochondrial and nuclear DNA), and was found to be highly enriched for chloroplast DNA, based on positive hybridizations obtained with heterologous plastid-specific genes (Fig. 1). Maize mitochondrial genes (*cox*I, *cox*II) did not hybridize to this DNA fraction (data not shown). Some contamination by low molecular weight plasmid-like DNAs of unknown origin was evident, however, when undigested chloroplast DNA was electrophoretically separated through agarose gels (Fig. 2). Several plasmid-like molecules of differing copy number were resolved. Part of the main chloroplast genome (the 11.7 kb *Xho*I fragment) cross-hybridizes with two of these plasmid-like molecules under high stringency conditions (Fig. 3). Additional hy-

Table 1. Description of heterologous probes

Gene designation	Source	Insert size	Reference
*rbc*L	*O. luteus*	*Bam*HI/*Hin*dIII 700 bp	S. Newman (pers comm)
*rbc*S	*O. luteus*	*Eco*RI/*Ssp*I 647 bp	Boczar et al. (1989)
*psb*D	*C. reinhardtii*	*Eco*RI 2.6 kb	Rochaix et al. (1984)
16S rRNA	*O. luteus*	*Sma*I/*Eco*RI 2.0 kb	Delaney (1989)
23S rRNA	*O. luteus*	*Pvu*II 3.0 kb	Delaney (1989)
*pap*AB	*C. paradoxa*	*Bam*HI/*Eco*RI 1.9 kb	Bryant et al. (1985)
*ppe*BA	*Pseudoanabaena*	*Hin*dIII/*Eco*RI 1.1 kb	D. Bryant (pers comm)
*ppc*BA	*C. paradoxa*	*Eco*RI/*Pst*I 800 bp	Lemaux & Grossman (1984)
5′*tuf*A	*C. reinhardtii*	*Pst*I/*Eco*RI 379 bp	Baldauf & Palmer (1990)
3′*tuf*A	*C. reinhardtii*	*Eco*RI/*Ssp*I 762 bp	Baldauf & Palmer (1990)
*lpc*A, B, C	*F. diplosiphon*	*Hin*dIII 1.8 kb	Lomax et al. (1987)

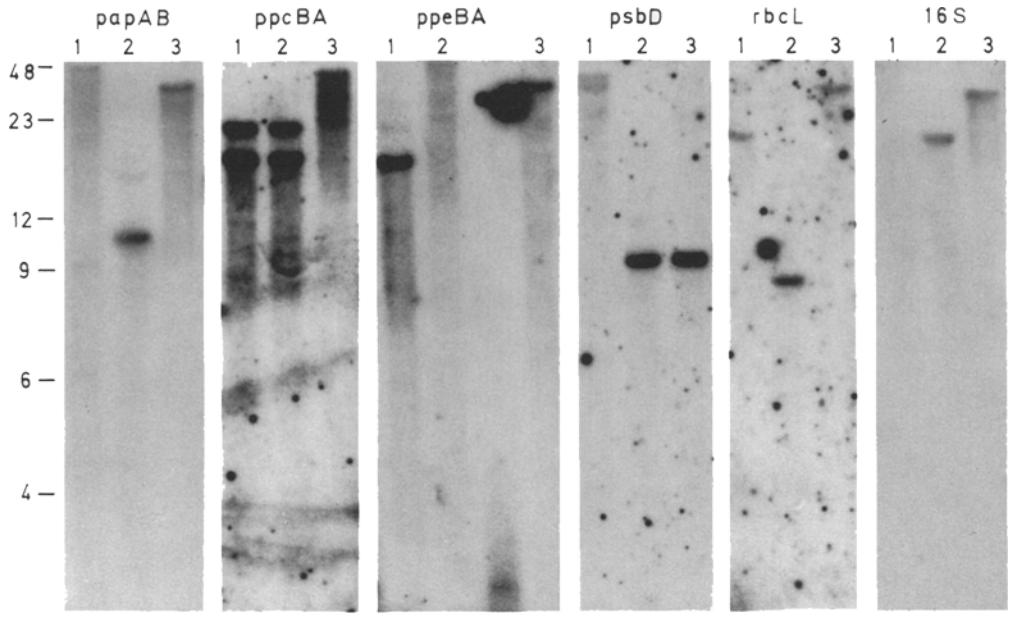

Fig. 1. Hybridization of heterologous genes to restriction enzyme-digested *Porphyra* chloroplast DNA. *pap*AB: *lane 1* *Xho*I, (*2*) *Bst*EII, (*3*) *Bgl*I. *ppc*BA: *lane 1* *Bst*EII, (*2*) *Bst*EII/*Xho*I, (*3*) *Xho*I. *ppe*BA: *lane 1* *Bst*EII, (*2*), *Xho*I, (*3*) *Bgl*I. *psb*D: *lane 1* *Bst*EIII, (*2*) *Bst*EII/*Xho*I, (*3*) *Xho*I. *rbc*L: *lane* (*1*) *Bst*EII, (*2*) *Bst*EII/*Xho*I, (*3*) *Xho*I. 16S: *lane 1* *Xho*I, (*2*) *Bst*EII, (*3*) *Bgl*I. Molecular size markers (in kb) are shown on the left

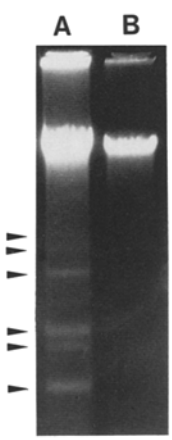

Fig. 2

Fig. 2. Plasmid-like DNA molecules co-isolating with *Porphyra* chloroplast DNA. *A*, undigested chloroplast DNA; *B*, undigested nuclear DNA, resolved on a 0.6% agarose gel. Plasmid-like DNAs are indicated by *arrows*

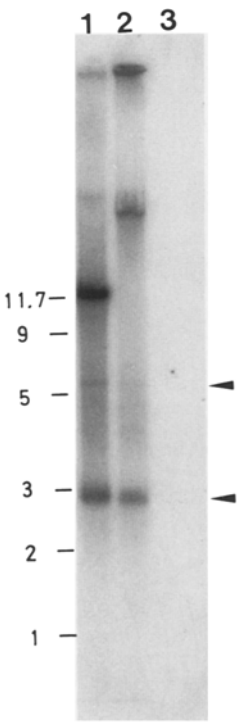

Fig. 3

Fig. 3. Hybridization of the *Porphyra* chloroplast DNA 11.7 kb *Xho*I fragment to plasmid-like molecules. *Lane 1*, *Xho*I-digested chloroplast DNA; *lane 2*, undigested chloroplast DNA; *lane 3*, *Xho*I-digested nuclear DNA. *Arrows* indicate cross-hybridizing plasmid-like molecules. Molecular size markers (in kb) are shown on the left

bridization data (not shown) indicate that the remaining plasmids have distinct sequences, and are not simply topological isomers of each other.

Restriction enzyme analysis and mapping

Restriction enzyme digest patterns of *Porphyra* chloroplast DNA are shown in Fig. 4. Summation of fragment sizes (excluding plasmid-like DNA bands) indicates a chloroplast genome size of approximately 185 kb.

Mapping data, using cloned and gel-purified *Xho*I fragments as probes, are summarized in Table 2. Hybridization ambiguities resulting from cross-contamination of high molecular weight gel-purified fragments were resolved by reciprocal hybridizations using gel-purified *Bst*EII fragments as probes (Table 2).

Initial experiments demonstrated that the chloroplast ribosomal and phycobiliprotein genes were all located within the 48 kb *Xho*I fragment. To map this region of the genome more precisely, gel-purified *Bst*EII 12 kb, 17 kb and 21 kb fragments were used as hybridization probes against chloroplast DNA digested with *Eco*RI, *Bst*EII and *Bst*EII/*Eco*RI (Table 2). To reduce hybridization ambiguities, the *Bst*EII fragments were used as probes after two cycles of gel purification.

Based on the above analyses, the chloroplast genome of *Porphyra yezoensis* maps a circular molecule. A composite restriction and gene map is shown in Fig. 5. The genome is divided into small (15–17 kb) and large (approximately 156 kb) single-copy regions by ribosomal DNA containing inverted repeats of approximately 6.6 kb. The 16S and 23S ribosomal RNA genes are organized with the 16S gene proximal, and 23S gene distal, to the small single-copy region. The small single-copy borders of the inverted repeat were estimated by gel purifying the *Eco*RI 7.5 kb/7.7 kb bands together (since they were too close to be separated), and using them to probe a

Table 2. Summary of restriction enzyme mapping hybridization data[a]

Probe fragment	Fragment number (size in kb)			
	*Xho*I	*Bst*EII	*Bgl*I	*Eco*RI
*Xho*I				
1(48)	1(48)	7(12) 6(17) 4(21)	7(7.6) 2(30) 1(35)	
2(34)	2(34)	5(19) 2(27)	11(1.8) 6(9.4) 4(18.5) 3(24) 1(35)	
3(25)	3(25)	12(2.3) 2(28)	10(2.7) 4(18.5) 1(35)	
4(11.7)*	4(11.7)	1(40)	9(3.0) 8(4.1) 2(30)	
5(10.7)	5(10.7)	8(9.5) 5(19)	3(24)	
6(9.6)	6(9.6)	1(40)	2(30)	
7(8.9)	7(8.9)	10(4.2) 9(6.5) 7(12)	1(35)	
8(7.6)	8(7.6)	1(40)	5(10)	
9(7.2)	9(7.2)	9(6.5) 8(9.5)	9(3.0) 2(24)	
10(5.2)	10(5.2)	1(40)	5(10) 1(35)	
11(4.4)	11(4.4)	8(9.5)	3(24)	
12(3.6)	12(3.6)	1(40) 2(28)	1(35)	
13(2.2)	13(2.2)	1(40)	1(35)	
14(1.4)	14(1.4)	2(28)	1(35)	
15(1.2)	15(1.2)	1(40)	2(30)	
16(0.6)	16(0.6)	9(6.5)	1(35)	
*Bst*EII				
1(40)	15(1.2)	1(40)	9(3.0)	
	13(2.2)		8(4.1)	
	12(3.6)		5(10)	
	10(5.2)		2(30)	
	8(7.6)		1(35)	
	6(9.6)			
	4(11.7)			
4(21)	1(48)			27(2.1) 13(5.6) 10(6.6) 7(7.5) 2(10.5)
6(17)	1(48)			34(0.6) 29(1.8) 24(2.6) 10(6.6) 2(10.5)
7(12)	1(48)			10(6.6) 4(9.05) 3(10)

[a] Hybridization signals due to contamination artifacts are not listed
* *Xho*I fragment 4(11.7) also hybridizes to an extrachromosomal "plasmid-like" molecule

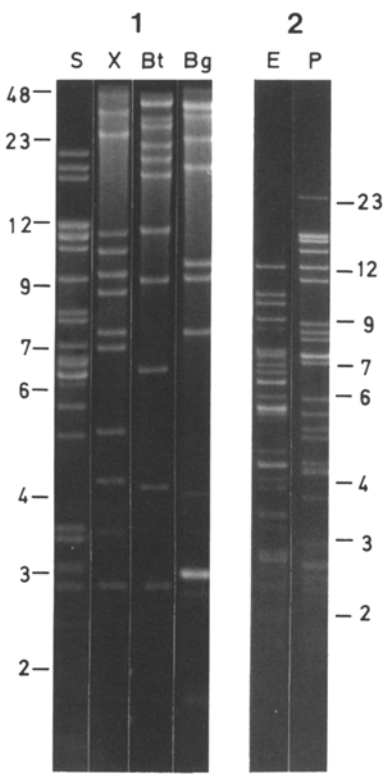

Fig. 4

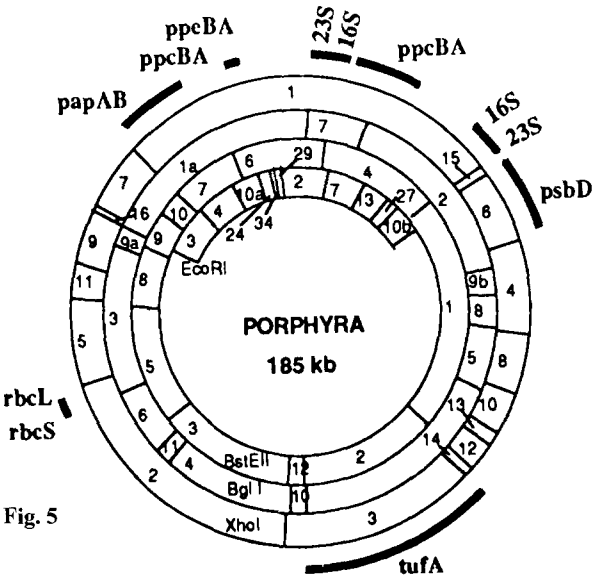

Fig. 5

Fig. 4. Digestion products of *Porphyra yezoensis* chloroplast DNA. *Panel 1,* 0.37% gel: (*S*) *Sst*I, (*X*) *Xho*I, (*Bt*) *Bst*EII, (*Bg*) *Bgl*I. *Panel 2,* 0.6% gel: (*E*) *Eco*RI, (*P*) *Pst*I. Molecular size markers (in kb) are shown beside each panel

Fig. 5. Physical and gene map of the *Porphyra yezoensis* chloroplast genome. Restriction fragments are numbered according to size, starting from the largest fragment. Genes mapped are shown outside of the circles as *thick lines*. Genes acronyms are as in Table 1

Porphyra chloroplast DNA *Eco*RI digest. No hybridization was obtained to the *Eco*RI 5.6 kb or 2.1 kb bands, indicating that the repeat did not extend into these small single-copy regions. The minimum size of the small single-copy region is therefore approximately 15.2 kb (*Eco*RI 7.5 kb + 5.6 kb + 2.1 kb). The maximum size cannot be much larger since both 16S genes and part of both 23S genes lie within the *Bst*EII 21 kb fragment. Assuming these ribosomal genes encompass at least 2 kb at each end of the *Bst*EII 21 kb fragment, the maximum size the small single-copy region can occupy is approximately 17 kb.

The large single-copy borders of the inverted repeat were estimated to lie within 3.3 kb of the terminal ends of the *Bst*EII 21 kb fragment, based on the following observation. The *Eco*RI site in the *Bst*EII 40 kb fragment flanking the *Bst*EII 21 kb fragment, is absent in the *Bst*EII 17 kb fragment flanking the other end. The absence of this *Eco*RI site indicates that each repeated region is confined within approximately 6.6 kb.

Protein gene mapping

The phycocyanin (*ppc*BA) subunit genes map in both the small, as well as large, single-copy regions where they appear clustered with the phycoerythrin (*ppe*BA) genes. The allophycocyanin (*pap*AB) subunit genes map in the large single-copy region, and are located within 18 kb of the phycoerythrin-phycocyanin gene cluster. All the phycobiliprotein genes are organized relatively close to one another on the genome (Fig. 5). No hybridization was obtained to *Porphyra* chloroplast or nuclear DNA when cyanobacterial, phycocyanin-associated, linker polypeptide genes (*lpc*A, *lpc*B, *lpc*C) were used as probes, even at low hybridization stringency (data not shown). Genes encoding the large (*rbc*L) and small (*rbc*S) subunit-polypeptides of ribulose-1, 5-bisphosphate carboxylase/oxygenase are clustered, and along with the *psb*D and *tuf*A genes, map to the large single-copy region (Fig. 5).

Discussion

I have isolated high molecular weight chloroplast DNA from the marine red alga *Porphyra yezoensis* by exploiting the fact that its chloroplast DNA differs appreciably in buoyant density from its nuclear DNA, in cesium chloride-Hoechst dye gradients. The method used results in the co-isolation of small, plasmid-like DNA molecules of unknown origin. Plasmid-like molecules have also been found to co-isolate with chloroplast DNA in the red alga *Gracilaria* (Goff and Coleman 1988). Sequence homology between part of the *Porphyra* chloroplast genome and some of the plasmid-like molecules, indicates that these plasmids may also exist as integrated forms. Such a phenomenon has been observed in the green algae *Chlamydomonas moewusii* (Turmel et al.1986) and *Acetabularia cliftonii* (Ebert et al. 1985), and bleached mutants of *Euglena gracilis* (Heizmann et al. 1982). The function of these molecules, however, remains unknown.

Although the overall structure of the *Porphyra* chloroplast genome is similar to most higher plant chloroplast genomes studied, several notable differences exist. First, the size of the inverted repeat is smaller than the typical higher plant inverted repeats of 10–28 kb (Palmer 1985a, b), and is possibly the result of a reduced spacer region between the 16S and 23S rRNA genes (T. Delaney, pers comm). In contrast to *Porphyra*, the chloroplast genome of the red alga *Griffithsia pacifica* lacks a rDNA-containing inverted repeat (Li and Cattolico 1987). More data on chloroplast genome structure in the red algae is necessary, however, before theories on the origin, antiquity, and phylogenetic utility of inverted repeats in this taxon can be formulated.

A second notable difference between *Porphyra* and higher plant chloroplast genomes is the orientation of their inverted repeats. The universal arrangement of rRNA genes in higher plants and several algae appears to be 23S gene proximal to, and 16S gene distal to, the small single-copy region (Palmer 1985a). Thus far, this order has been found to be reversed only in *Porphyra* and the cyanelle of *Cyanophora paradoxa* (Wasmann et al. 1987). The apparent rarity of this arrangement may provide a useful phylogenetic marker for studies on plastid evolution.

Third, the *Porphyra* chloroplast genome is relatively large compared to most higher plants (Palmer 1986a). Some of this increased sequence complexity can be accounted for by additional genes that either do not occur (phycobiliprotein genes), or are nuclear-encoded (*rbc*S, *tuf*A), in higher plants.

The phycobiliprotein genes in *Porphyra* are organized relatively close to one another on the chloroplast genome and represent the first known case of a chloroplast-encoded multigene family. The two different map positions for the phycocyanin genes in *Porphyra* raises the possibility of two active genes, a pseudogene, or the existence of a trans-splicing mechanism. Interestingly, the cyanobacteria *Fremyella diplosiphon* and *Anacystis nidulans* also contain clustered phycobiliprotein genes, and have two sets of transcriptionally active phycocyanin genes (Conley et al. 1986, 1988; Kalla et al. 1988). Since the red algal chloroplast is considered derived from cyanobacterial ancestors (Cavalier-Smith 1987), the clustered and duplicated phycobiliprotein genes in *Porphyra* may reflect an evolutionarily conserved arrangement.

Whether additional phycobilisome-complex polypeptide genes are chloroplast-encoded in *Porphyra* is not clear. If the linker polypeptides are indeed nuclear-encoded in red algae (Egelhoff and Grossman 1983), it is possible that their gene sequences are no longer sufficiently conserved to be detected by the cyanobacterial linker gene probes used in our study.

In green plants, genes encoding the large (*rbc*L) and small (*rbc*S) subunit polypeptides of ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) are located in the chloroplast and nucleus respectively (Akazawa et al. 1984). In contrast, both subunit genes are chloroplast-encoded and clustered in *Porphyra* and the rhodophytes *Porphyridium aerugineum* and *Antithamnion* sp. (Valentin and Zetsche 1989). Since co-transcription of the Rubisco genes occurs in the red alga *P. aerugineum* (Val-

entine and Zetsche 1989), it is likely that co-transcription occurs in *Porphyra* as well. The occurrence of a plastid-encoded and highly similar (at the sequence level) *rbc*S gene in rhodophytes (Valentin and Zetsche 1989), cryptomonads (Douglas and Durnford 1989), and chromophytes (Boczar et al. 1989), is consistent with the hypothesis that plastids of these groups are closely related (Gibbs 1981; Whatley and Whatley 1981; Ludwig and Gibbs 1989), and may indeed be monophyletic (sensu Cavalier-Smith 1982) in origin.

The polypeptide chain elongation factor, EF-Tu gene (*tuf*A) is chloroplast-encoded in green algae, but nuclear-encoded in higher plants (Baldauf and Palmer 1990). The fact that the *Chlamydomonas tuf*A gene hybridizes to the chloroplast genomes of *Porphyra,* and the chromophyte *O. luteus* (unpublished results) indicates that this gene is chloroplast-encoded in all three major algal groups, and that its transfer to the nuclear genome in higher plants must have been a relatively recent evolutionary event.

In conclusion, although the overall architecture of the red algal chloroplast genome is similar to higher plants, the gene content can be quite different. Whether such differences are indicative of a different ancestry for red and green plastids will require further information on genome structure and gene sequences from the red algae. In addition, the regulation of certain chloroplast genes, such as *rbc*S and *tuf*A, in the red algae is likely to be different from the regulation of their nuclear-encoded counterparts in higher plants. Such coding location differences underscore the need for more information on red algal chloroplast genomes.

Acknowledgements. I am grateful to Drs. B. Boczar, D. Bryant, T. Delaney, P. Lemaux, T. Lomax, S. Newman and J. Palmer for the heterologous gene probes used in this study. I thank Drs. J. Ammirati, A. Bendich, R. A. Cattolico and J. Matches for use of laboratory space and equipment. This work was supported by grants from the Washington Sea Grant Program and fellowships from the AK Foundation, NSERC (Canada), the Egtvedt Food Research Fellowship and the School of Fisheries.

References

Akazawa T, Takabe T, Kobayashi H (1984) TIBS 9:380–383
Baldauf SL, Palmer JD (1990) Nature 344:262–265
Boczar BA, Delaney T, Cattolico RA (1989) Proc Natl Acad Sci USA 86:4996–4999
Bryant DA, DeLorimer R, Lambert DH, Dubbs JM, Stirewalt VL, E Stevens Jr S, Porter RD, Tam J, Jay E (1985) Proc Natl Acad Sci USA 82:3242–3246
Cavalier-Smith T (1982) Biol J Linn Soc 17:289–306
Cavalier-Smith T (1987) The simultaneous symbiotic origin of mitochondria, chloroplasts and microbodies. Ann NY Acad Sci 503:55–71
Conley PB, Lemaux PG, Lomax TL, Grossmann A (1986) Proc Natl Acad Sci USA 83:3924–3928
Conley PB, Lemaux PG, Grossman AR (1988) J Mol Biol 199:447–465
Delaney TP (1989) PhD Dissertation, Univ of Washington, USA
Douglas SE, Durnford DG (1989) Plant Mol Biol 13:13–20
Ebert C, Tymms MJ, Schweiger HG (1985) Mol Gen Genet 200:187–192
Egelhoff T, Grossman A (1983) Proc Natl Acad Sci USA 80:3339–3343
Feinberg AP, Vogelstein B (1983) Anal Biochem 132:6–13
Gibbs SP (1981) Int Rev Cytol 72:49–99
Goff LJ, Coleman AW (1988) J Phycol 24:357–368
Gray MW, Doolittle WF (1982) Microbiol Reviews 46:1–42
Guillard RRL, Ryther J (1962) Can J Microbiol 8:229–239
Heizmann P, Ravel-Chapuis P, Nigon V (1982) Curr Genet 6:119–122
Kalla SR, Lind LK, Lidholm J, Gustafsson P (1988) J Bacteriol 170:2961–2970
Lemaux PG, Grossman AR (1984) Proc Natl Acad Sci USA 81:4100–4104
Li N, Cattolico RA (1987) Mol Gen Genet 209:343–351
Lomax TL, Conley PB, Schilling J, Grossman A (1987) J Bacteriol 169:2675–2684
Ludwig M, Gibbs SP (1989) J Cell Biol 108:875–884
Marsh JL, Erfle M, Wykes E (1984) Gene 32:481–485
Maniatis T, Fritsch EF, Sambrock J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York
Palmer JD (1985a) Evolution of chloroplast and mitochondrial DNA in plants and algae. In: R.J. MacIntyre (ed) Molecular Evolutionary Genetics. Plenum Press, New York, pp. 131–240
Palmer JD (1985b) Annu Rev Genet 19:325–354
Palmer JD (1986a) Chloroplast DNA and phylogenetic relationships. In: K.D. Dutta (ed) DNA Systematics, Vol II: Plants. CRC Press Inc., Florida, pp 63–80
Palmer JD (1986b) Methods Enzymol 118:167–187
Rochaix JD, Dron M, Rahire M, Malnoe P (1984) Plant Mol Biol 3:363–370
Steinmuller K, Kaling M, Zetsche K (1983) Planta 159:308–313
Turmel M, Bellamare G, Lee R, Lemieux C (1986) Plant Mol Biol 6:313–319
Valentin K, Zetsche K (1989) Curr Genet 16:203–209
Wasmann CC, Loeffelhardt W, Bohnert HJ (1987) Cyanelles: organization and molecular biology. In: P. Fay and C. Van Baalen (eds.). The Cyanobacteria. Elsevier Science Publishers, pp 303–324
Whatley JM (1981) Annu NY Acad Sci 361:154–165
Whatley JM, Whatley FR (1981) New Phytol 87:233–247

Communicated by K. Esser