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Physical mapping of the plastid genome from the chlorophyll *c*-containing alga, *Cryptomonas Φ*

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Summary. A physical map of the circular plastid genome of *Cryptomonas* Φ has been constructed using the enzymes *Sac*I, *Bam*HI, *Sma*I, *Sal*I, *Pst*I and *Xho*I. In addition, fine-structural mapping of the inverted repeat region has been performed using *Ava*I, *Bgl*II, *Eco*RI and *Xba*I. The inverted repeat is very small, encompassing no more than 6 kb and containing only genes for the rRNAs. It divides the plastid genome into a small single-copy region of 12–13 kb which contains genes for phycoerythrin and the 32 kd photosystem II polypeptide, and a large single-copy region of 93–94 kb, giving a total size of 118 kb. The genes for the large subunit of ribulose-1,5-bisphosphate carboxylase (Rubisco) and the beta subunit of ATP synthase CF_1 are encoded in the large single-copy region. The evolutionary significance of the organization of this plastid genome, the first presented from the chlorophyll *c*-, phycobiliprotein-containing group of algae, is discussed.

Key words: Plastid genome evolution – Algae – Cryptomonad – Inverted repeat

Introduction

The cryptomonads are an interesting group of algae in that they share characteristics of both chromophyte and rhodophyte lineages. Although placed in the chromophyte group (Whatley and Whatley 1981; Whatley 1983; Palmer 1985), their unique pigment composition and plastid ultrastructure has caused their evolutionary position to be the subject of debate (Cavalier-Smith 1986). In order to gain further insight into the possible origin of this group of algae, the plastid genome has been mapped and compared with those of other plants and algae.

All plants and algae contain chlorophyll *a*, but in the three major algal lineages, the accessory photosynthetic pigments differ. Chlorophytes contain chlorophyll *b*, rhodophytes contain phycobiliproteins and chromophytes contain chlorophylls *c*. The cryptomonad algae contain both chlorophyll *c* and phycobiliproteins and thus share characteristics of both of the latter two groups.

The number of membranes surrounding the plastid is also significantly different in the three major lines and this has led to the proposition (Whatley and Whatley 1981) that plastids arose polyphyletically, in some cases from eukaryotic endosymbionts. Chlorophyte plastids have two outer membranes which surround the stacked thylakoids. Rhodophyte plastids also possess two membranes but the thylakoids are unstacked. The plastids of chromophytes usually have four outer membranes surrounding thylakoids arranged in stacks of two or three. In the case of cryptomonad plastids, there are four membranes, the outermost of which is continuous with the nuclear membrane (Gibbs 1962). These plastids are characterized by the presence of a nucleomorph, 80S ribosomes and starch granules in the periplastidal space (Greenwood et al. 1977). These are though to represent the nucleus and cytoplasm of an ancestral endosymbiotic red alga (Whatley et al. 1979; Gibbs 1983). The nucleomorph has been shown to contain nucleic acid (Gillott and Gibbs 1980; Ludwig and Gibbs 1985) and to undergo a simple form of division (Morrall and Greenwood 1982; McKerracher and Gibbs 1982). Its function in the cell is unknown although it has been proposed to be responsible for the production of ribosomes and other components of the outer chloroplast compartment (Greenwood et al. 1977).

Plastid genome structure has been a useful tool in tracing phylogenetic relationships among land plants

because of the high degree of conservation of this molecule (Palmer 1985). Studies of chloroplast genomes from over 250 angiosperms and nonangiosperms (Palmer 1985) revealed that the plastid DNA exists as a large population of homogeneous circles. These plastid genomes vary in size between 120–217 kb depending on the plant, but most are between 120–160 kb. The plastid DNA of all but one group contains a duplicated segment of 20–30 kb which exists as an inverted repeat and contains the two copies of the rRNA genes. Exceptions occur in certain members of the Fabaceae where there is no inverted repeat structure and only one copy of the rRNA genes (Koller and Delius 1980; Palmer and Thompson 1981).

The plastid genomes of land plants are highly conserved not only in structure, but also in functional arrangement. The location of specific genes in the large single-copy region, small single-copy region and inverted repeat, and the clustering of genes in groups, e.g., *atp*B/*atp*E, *psa*A/*psa*B, *psb*D/*psb*C are the same among widely divergent genera (Palmer 1985). Similarly, the partitioning of genes between nuclear and plastid genomes is shared among land plants.

Almost all of these traits have been found to be highly variable among the limited number of algae which have been investigated (Cattolico 1985, 1986). Compared to the land plant literature, there is a relative paucity of information on algal chloroplast genome structure, particularly in the chlorophyll *c* lineage. This study was carried out to investigate the physical structure and organization of the plastid genome from a cryptomonad alga in order to determine how it relates to that of land plants and other algae, and to ascertain whether plastid genome organization will be as useful a taxonomic probe in the algae as in the land plants.

Materials and methods

Cryptomonas Φ derives from the Culture Collection of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine. It was maintained in modified SWM-3 media (McLachlan 1973) at 20 °C under cool-white fluorescent lights (Sylvania VHO). Carboys (20 l) were inoculated with 100 ml of exponential-phase cells and the cultures harvested by centrifugation after 4–5 days of growth. The light intensity was kept at 50 μE m^{-2} s^{-1} when cultures were freshly inoculated and at low cell density. After two days, when the culture was denser, the light was increased to 100 μE m^{-2} s^{-1}.

Plastid DNA was prepared from total DNA separated on Hoechst 33258-cesium chloride density gradients. Approximately 5 g of cells were suspended in 20 ml of lysis buffer (2% Sarkosyl, 100 mM NaCl, 10 mM EDTA, 20 mM Tris, pH 7.6), proteinase K was added to 10 mg/ml and the mixture incubated at 37 °C for 1 h with occasional gently swirling. Cell debris was removed by centrifugation in a JA17 rotor (Beckman) for 10 min at 3,000 g and 4 °C. Protein and cell debris were removed by two phenol:chloroform:isoamyl alcohol (25:24:1) extractions and one chloroform:isoamyl alcohol (24:1) extraction (Maniatis et al. 1982). Cesium chloride and Hoechst 33258 dye (Polysciences) were added as described by Szeto et al. (1981) and the DNA banded by equilibrium density centrifugation at 184,000 g for 38 h and 66,200 g for 2 h in a Type 70. Rotor (Beckman) at 20 °C (Anet and Strayer 1969). The gradient was fractionated by upward displacement using an Isco fractionator (Model 184) and Fluorinert (Isco) as the displacement fluid. Fractions (200 μl) were extracted three times with isopropanol equilibrated with a saturated NaCl solution, diluted two-fold with water and precipitated at −20 °C using 2 vol. of ethanol. The DNA precipitates were collected by centrifugation for 30 min at 12,000 g and 4 °C. A second precipitation using 0.5 vol. of 7.5 M ammonium acetate and 0.56 vol. of isopropanol (Owen and Borman 1987) was performed to facilitate removal of contaminating RNA and polysaccharides.

Restriction endonucleases were purchased from a variety of sources and used according to the manufacturer's specifications except that 4 mM spermidine was included to improve digestion (Bouché 1981). DNA fragments were resolved on 0.4%–0.8% agarose gels (11 x 14 cm) and transferred to Zeta Probe membranes (BioRad) by the method of Rigaud et al. (1987). Hybridisation conditions were as described in the Zeta Probe instructions. Labelled bacteriophage lambda DNA was added to identify the markers.

The probes used in this study were purified from vector DNA by restriction endonuclease digestion followed by gel electrophoresis in 0.4% low-melting-point agarose. Aliquots containing 25 ng of DNA were labelled with 25 μCi of [α^{32}P]-dCTP (3,000 Ci/mmol; New England Nuclear, Wilmington, Delaware) using the oligonucleotide method of Feinberg and Vogelstein (1983). Probes were purified from unincorporated nucleotides by passage over a 1-ml column of Sephadex G-50 (Maniatis et al. 1982). The probe used to locate the rRNA genes consisted of the entire *rrn*B operon and 1.5 kb of additional 3′-flanking sequences from *Anacystis nidulans* on a 6.3 kb *Eco*RI/*Bam*HI fragment (Douglas and Doolittle 1984). The 16S rRNA gene probe was a 1.0 kb *Eco*RI/*Hind*III fragment from this clone which contained 960 bp of the 16S rRNA gene and 43 bp of 16S–23S rDNA spacer sequence. The 23S rRNA gene probe was composed of two *Hind*III fragments of 880 and 440 bp from the *rrn*B operon which contained internal sequences starting 727 bp from the 5′ end and ending 850 bp from the 3′ end of the gene. The *psb*A probe was a 1.2 kb *Eco*RI/*Pst*I fragment from pea chloroplast (Palmer et al. 1982) which contained gene-internal sequences from the 3′-end of the gene. The probe for the large subunit of Rubisco was a 0.9 kb *Eco*RV/*Hind*III fragment from *Anabaena* 7120 which contained gene-internal sequences (Curtis and Haselkorn 1983). The Rubisco small subunit probe was a 1.0 kb *Xba*I/*Hind*III fragment which contained the entire *rbc*S, 100 bp of 5′-flanking sequence and 400 bp of 3′-flanking sequence from *Anabaena* 7120 (Nierzwicki-Bauer et al. 1984). The phycoerythrin gene was located using a 1.08 kb *Xho*I fragment which contained *cpe*B from *Synechococcus* 7803 (Newman and Carr, unpublished). The gene for the beta subunit of ATP synthase was located using a 0.72 kb *Eco*RI/*Sac*I fragment containing 290 bp of the 5′-terminus of *atp*B and 440 bp of 5′-flanking sequence from spinach (Zurawski et al. 1982).

Total digests of plastid DNA were ligated into pUC18 (Viera and Messing 1982) and cloned into *Escherichia coli* TBI using standard methods (Maniatis et al. 1982). Clones were screened using the in-well lysis method (Sekar 1987), those with desirable inserts amplified and the plasmid DNA purified on cesium chloride density gradients (Garger et al. 1983).

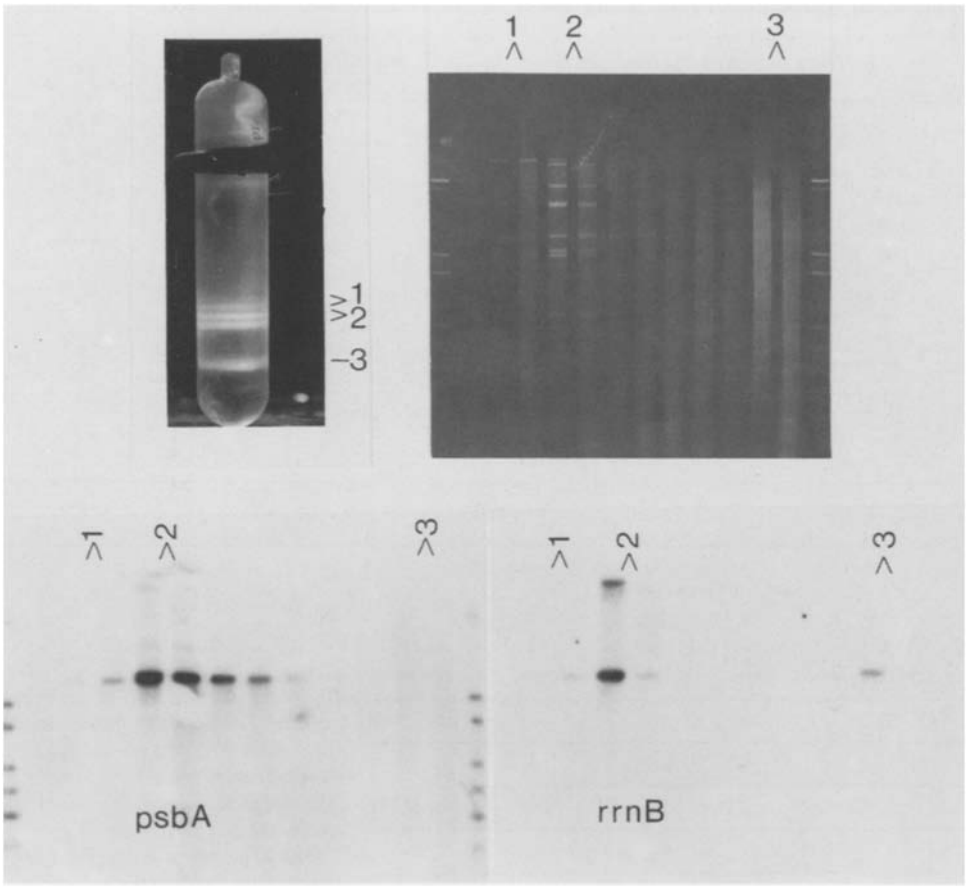

Fig. 1. Restriction endonuclease analysis of fractions from Hoechst 33258-cesium chloride gradient. The fractions represent mitochondrial (*1*), plastid (*2*) and nuclear (*3*) DNA. Aliquots from every second 200 µl fraction were digested with 10 units of *Bam*HI for 12 h at 37 °C, the DNA fragments resolved on a 0.4% agarose gel, transferred to Zeta Probe and hybridised with *psb*A and *rrn*B probes. The markers are lambda DNA digested with *Sty*I (23.58, 20.0, 7.7, 6.2, 4.3, 3.4, 2.7 and 1.89 kb)

Results

After cesium chloride density gradient centrifugation in the presence of Hoechst 33258 dye, four minor bands were visible above the main band of nuclear DNA (Fig. 1). The bands were identified by comparing their positions in the gradient relative to the volume of the collected fractions, and by hybridisation of the DNA in those fractions with specific probes. After *Bam*HI digestion of the fractions, the plastid DNA was identified by its ability to hybridise with the *psb*A probe from pea. The *rrn*B probe from *Anacystis nidulans* detected both plastid and nuclear rRNA genes on fragments of similar size. The two least dense fractions from the gradient contained two physical forms of a DNA species of low complexity presumed to be of mitochondrial origin. This DNA could not be eliminated from the plastid DNA preparations but its contribution to restriction analysis (visible as submolar bands) could be accounted for by performing a series of digestions of fractions from the gradient using different restriction endonucleases.

Fragments obtained by restriction with *Bam*HI, *Sal*I, *Sac*I, *Pst*I, *Sma*I and combinations of these enzymes were cloned into pUC18. Since the largest fragment which could be cloned was 15 kb, fragments from several restriction endonuclease digestions were used to construct the clone bank. The fragments were identified by size, restriction endonuclease digestion patterns and by hybridisation experiments with restriction endonuclease-digested plastid DNA.

A linear representation of the circular genome is shown in Fig. 2. The sizes of the fragments resulting from single restriction endonuclease digestions are indicated on the map. The results of restriction endonuclease double-digestions of the plastid DNA are summarized in Table 1. The molecular weight of the plastid genome (118 kb) was determined from an average of values from these single and double digests. The map of the plastid genome was determined from

Table 1. Sizes of fragments of *Cryptomonas* Φ plastid DNA produced by double-digestion with restriction endonucleases

*Sac*I/*Sma*I	*Sac*I/*Xho*I	*Sac*I/*Sal*I	*Sac*I/*Bam*HI	*Sma*I/*Bam*HI	*Sma*/*Xho*I	*Sma*I/*Sal*I
31.0	25.0	24.0	21.0	23.0	25.0	24.0
22.5	24.0	12.0	15.5	21.0	23.0	22.5
17.0 (d)	17.0	11.5 (t)	12.0	15.0	22.5	18.0
12.0	12.0	8.5	10.0 (d)	14.0	17.5	17.0
5.3	8.8	7.0	8.2	8.2	9.8	11.0
4.2	7.7	6.2	8.0	7.7	8.8	9.8
2.6	7.0	5.5	7.7	7.5	8.5	5.0
2.5	6.2	5.0 (d)	6.6	7.2	4.2	4.2
1.2 (d)	4.0	3.4	6.2	7.0		3.4
0.8	2.6 (d)	2.6	4.2	2.8		1.9 (d)
		1.9 (d)	2.6	2.6		
			1.8 (d)	1.5 (d)		
			1.2			
117.3	116.9	117.5	116.8	119.0	119.3	118.7

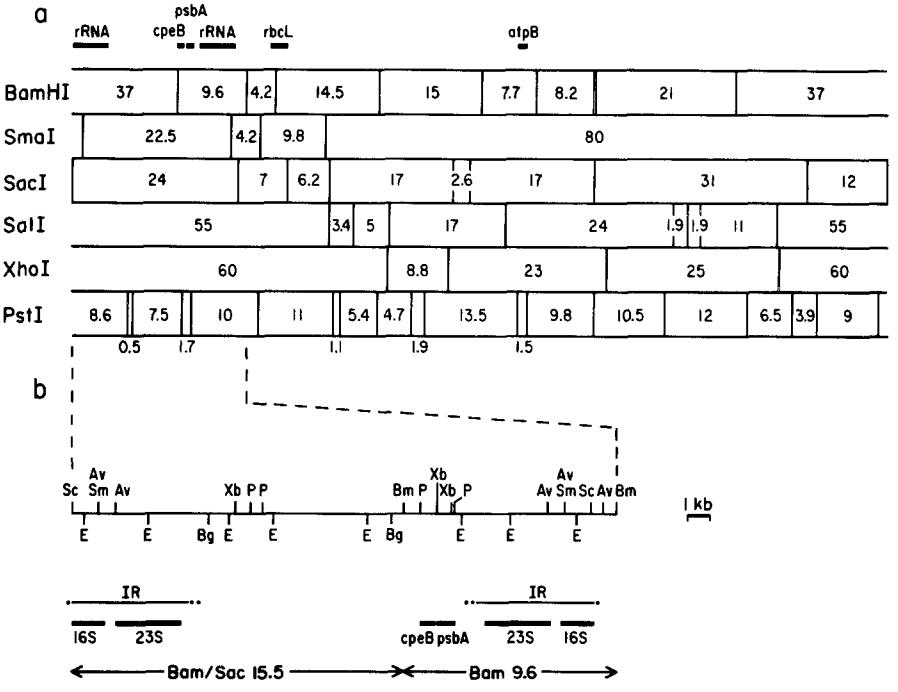

Fig. 2. **a** Physical map of the circular plastid genome of *Cryptomonas* Φ. The sizes of the fragments in kb are indicated inside the *boxes*. **b** Fine-structure map of the inverted repeat region of the plastid genome. The map was generated by restriction endonuclease digestions of two clones (Sac/Bam15.5 and Bam/Sac Bam9.6). The locations of the 16S and 23S rRNA genes as well as *psb*A, *cpe*B, *atp*B and *rbc*L are shown by *solid boxes*. The restriction endonucleases used are: Av, *Ava*I; Bg, *Bgl*II; Bm, *Bam*HI; E, *Eco*RI; P, *Pst*I; Sc, *Sac*I; Sm, *Sma*I; Xb, *Xba*I. There are two additional *Eco*RI sites between the two most internal *Eco*RI sites, and seven additional *Bgl*II sites between the two *Bgl*II sites, giving rise to many small fragments which have not been mapped

these data and from restriction and cross-hybridisation analysis of individual cloned fragments representing at least 90% of the genome. The inverted repeat is approximately 5.5 kb. Two clones spanning the inverted repeat region (Bam/Sac15.5 and Bam9.6) were used to map this region and determine the orientation of the rRNA genes (Fig. 2b). A conserved *Sma*I (*Ava*I) site is found 160 bp from the 3′ end of the 16S rRNA gene in most organisms, and the hybridisation data presented here confirm that this site is conserved in *Cryptomonas* Φ.

Table 2 shows the results of restriction endonuclease digestions and hybridisations of clones Bam/Sac15.5 and Bam9.6. Both 16S and 23S rRNA probes hybridised to the 6.2 kb *Bgl*II, 7.5 kb *Xba*I and 8.1 kb *Pst*I fragments of Bam/Sac15.5, localising both genes to the left side of the map. The 16S probe hybridised strongly to the 1.2 kb *Sac*I/*Sma*I fragment and weakly to the 14.3 kb *Sma*I/*Bam*HI fragment (which contains the 3′-terminal 160 bp of 16S rRNA gene) of Bam/Sac15.5. This 14.3 kb fragment showed intense hybridisation to the 23S rRNA gene. When *Ava*I was

Table 2. Restriction fragments hybridising to 16S and 23S rRNA gene probes

Clone	Fragment	16S rRNA	23S rRNA
Bam/*Sac*15.5	*Bgl*II	6.2	6.2
	*Xba*I	7.5	7.5
	*Pst*I	8.1	8.1
	*Eco*RI	3.0	3.7, 3.0
	*Ava*I	1.2, 0.8	13.5
	*Sma*I	14.3, 1.2	14.3
*Bam*9.6	*Pst*I	7.3	7.3
	*Eco*RI	3.0	3.0, 2.3
	*Ava*I	2.5, 0.8	6.3
	*Sma*I	7.1, 2.5	7.1

Table 3. Restriction fragments hybridising to *atp*B, *rbc*L, *psb*A and *cpe*B probes

Fragment	*atp*B	*rbc*L	*psb*A	*cpe*B
*Sac*I	?	7.0	24.0	24.0
*Bam*HI/*Sac*I	7.7	4.2, 1.8	8.2	8.2
*Bam*HI	7.7	14.5, 4.2	9.6	9.6
*Bam*HI/*Sal*I	4.3	8.0, 4.2	9.6	9.6
*Sal*I	24.0	55.0	55.0	55.0
*Eco*RI	13.5	7.0	4.5	4.5
*Pst*I	1.5	11.0	1.7	1.7
*Xba*I	19.0	4.5	0.8	9.0
*Ava*I	23.5	10.0	20.0	20.0
*Bgl*II	2.3	28.0	12.0	12.0

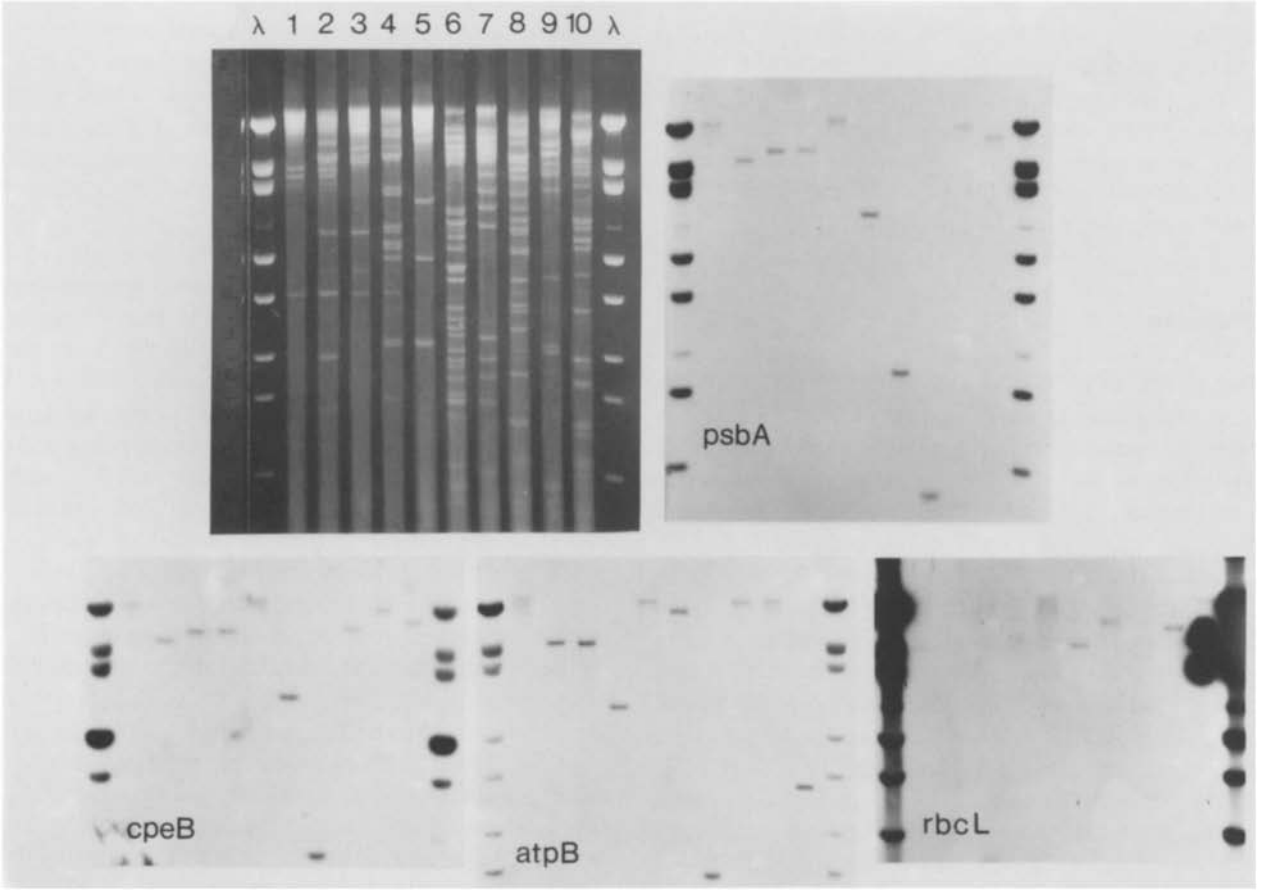

Fig. 3. Hybridisation analysis of restriction endonuclease-digested plastid DNA from *Cryptomonas* Φ. The digests were resolved on 0.4% agarose gels. Lane 1 *Sac*I; 2 *Sac*I/*Bam*HI; 3 *Bam*HI; 4 *Bam*HI/*Sal*I; 5 *Sal*I; 6 *Eco*RI; 7 *Pst*I; 8 *Xba*I; 9 *Ava*I; 10 *Bgl*II; λ. Lambda DNA digested with *Sty*I (see Fig. 1). The probes used are indicated on the autoradiograms

used to digest the clone, the 14.3 kb fragment was reduced to a 13.5 kb fragment still showing an intense signal with the 23S probe, and a 0.8 kb fragment which hybridised to the 16S probe only. When *Eco*RI was used to digest the clone, a 3.0 kb fragment showed a strong signal with both the 16S and 23S probes, but a 3.7 kb fragment hybridised only to the 23S probe. The orientation of the other copy of rRNA genes was determined by restriction endonuclease analysis of the Bam9.6 clone. When this clone was digested with

*Sma*I, a 2.5 kb fragment was produced which hybridised to the 16S probe only, and a 7.1 kb fragment which hybridised to both 16S and 23S probes. *Ava*I rediced the size of the 7.1 kb fragment by 0.8 kb and this hybridised to both 16S and 23S probes. *Ava*I reduced the other copy of rRNA genes, a 3.0 kb *Eco*RI fragment hybridised to both 16S and 23S probes. In addition, a 2.3 kb *Eco*RI fragment hybridised to the 23S probe.

The limits of the inverted repeat are found not less than 0.6 kb from the *Sac*I sites at the 5′ end of the rRNA operons, since the restriction sites on either side of these sites quickly diverge. For instance, an *Ava*I site is present 0.6 kb from the right hand *Sac*I site, followed by a *Bam*HI site another 0.5 kb away. Neither of these sites is present at the corresponding positions at the end of the other inverted repeat. At the 3′ end of the rRNA operons, the limits are located within the *Eco*RI fragments of 2.3 and 3.7 kb. This gives a maximum size for the inverted repeat of 6 kb.

The locations of some of the genes on the plastid genome were determined by hybridisation studies with heterologous probes. Figure 3 and Table 3 show the results of probing the *Cryptomonas* Φ plastid genome with *psb*A, *cpe*B, *rbc*L and *atp*B.

Discussion

The plastid DNA of the alga *Cryptomonas* Φ appears to exist as a homogenous population of circular molecules. No evidence of DNA rearrangements or linearity was observed from restriction endonuclease digestion and the only sub-molar bands which were produced could be attributed to the mitochondrial component. Organelle DNAs were isolated from total cellular DNA by density gradient centrifugation because we found organelle separation impossible. This difficulty may stem from the tendency of mitochondria to be in close approximation or even ramify into the plastid of photosynthetic organisms grown under low-light conditions (Montes and Bradbeer 1976). Hoechst 33258 dye (bisbenzamide) was particularly useful in this procedure since, even in high salt conditions, it binds preferentially to AT-rich regions of the DNA (Manuelidis 1977) and enhances the separation of DNA components of slightly different base compositions. The gradient relaxation technique (Anet and Strayer 1969) allowed very good separation of the DNAs in a relatively short centrifugation time. Slow fractionation of the gradient by upward displacement allowed the organellar DNAs to be separated from the nuclear DNA very well. Side-puncture of the tube and withdrawal of DNA bands was not as effective. Due to the gentle lysis procedure used, only partial extraction of the nuclear DNA was achieved, and the high relative yield of plastid DNA could be purified by a single gradient centrifugation.

As can be seen in Fig. 1, the putative mitochondrial DNA component of the total DNA from the gradient is readily apparent. An estimate of the complexity of this DNA was made by performing a series of similar analyses with different restriction enzymes. At approximately 50 kb, it is intermediate in size between the relatively small green algal and the (sometimes) enormous land plant mitochondrial genomes (Palmer 1985).

The plastid genome, at 118 kb, is smaller than most land plant plastid genomes (average 150 kb) and similar to those of three chromophyte algae, *Dictyota dichotoma* (Kuhsel and Kowallik 1985), *Vaucheria* spp. (Linne von Berg et al. 1982) and *Ochromonas danica* (Cattolico, unpublished). However, plastid genomes from three other chromophyte algae, *Pylaiella littoralis, Sphacelaria* sp. and *Olisthodiscus luteus*, are considerably larger (Dalmon et al. 1983; Reith and Cattolico 1986). The plastid genomes of the rhodophyte algae *Griffisthia pacifica* and *Porphyra yezoensis*, most green algae and *Euglena gracilis* (see Cattolico 1986) are also larger.

The plastid genome of *Cryptomonas* Φ contains an inverted repeat which divides the plastid genome into small and large single-copy regions. The inverted repeat is small (5.5–6 kb), containing only enough room for the rRNA genes. Ribosomal RNA-containing inverted repeats have been demonstrated in nearly all land plants and most algae. However, in *Euglena gracilis* the rRNA gene clusters are found as tandem repeats (Rawson et al. 1978) and only once copy exists in two rhodophyte algae, *Griffisthia pacifica* (Li and Cattolico 1987) and *Porphyra yezoensis* (Cattolico personal communication). Preliminary studies in our laboratory indicate, however, that an inverted repeat is present in the red alga *Palmaria palmata* (Douglas and van der Meer, unpublished). In the chromophyte alga, *Dictyota dichotoma*, the inverted repeat is 4.7 kb (Kuhsel and Kowallik 1987). However, the plastid DNA of two other chromophyte algae, *Olisthodiscus luteus* and *Ochromonas danica*, contain much larger inverted repeats (Reith and Cattolico 1986; Cattolico unpublished). Thus, it appears that the presence or absence of inverted repeats and the size of these repeats is highly variable among the algae, even within the Rhodophyta or Chromophyta. Additional information from other members of these groups is needed.

Fine-structural mapping of the inverted repeat has allowed the order of the rRNA genes to be determined. Unlike the situation in most green algae, where the 23S rRNA genes are adjacent to the large single-copy region, they are adjacent to the small single-copy region in *Cryptomonas* Φ. This feature is shared with two

chromophyte algae, *Olisthodiscus luteus* (Reith and Cattolico 1986) and *Dictyota dichotoma* (Kuhsel and Kowallik 1987), and land plants (Palmer 1985).

The size of the spacer region between the 16S and 23S rRNA genes, like those of the chromophytes, *Olisthodiscus luteus* (Delaney and Cattolico, submitted) and *Pylaiella littoralis* (Markowicz et al. 1988), the green alga *Chlamydomonas reinhardtii* (Schneider and Rochaix 1986), and *Euglena gracilis* (Graf et al. 1980) is small (no more than 500 bp), and it appears likely that if there are tRNA genes present, they will not contain introns. Introns have been demonstrated in the spacer tRNAs from the land plants *Zea mays, Nicotiana tabacum* and *Marchantia polymorpha*, but not in any of those from the "primitive" group of algae (see Markowicz et al. 1988). Similarly, there is not room for the unusual structures found in the spacer region of the chloroplast genome of the green alga, *Chlorella ellipsoidea* (Yamada and Shimaji 1986). No information is yet available for the Rhodophyta.

The small single-copy region (12–13 kb) is the smallest yet reported. Those of the chromophytes *Olisthodiscus luteus* and *Dictyota dichotoma* are 32 kb and 42 kb, respectively (Reith and Cattolico 1986; Kuhsel and Kowallik 1987). The small single-copy region of the green alga, *Chlorella ellipsoidea*, is 29.5 kb (Yamada 1983) but in *Chlamydomonas reinhardtii* and *C. eugametos*, the inverted repeat divides the plastid genome into two regions of nearly equal size (Lemieux et al. 1985b). It will be of interest to see to what extent the coding capacity of this region has been rearranged or reduced in *Cryptomonas* Φ relative to other algae.

The presence or absence of introns in *Cryptomonas* Φ plastid genes remains to be determined. In *Euglena gracilis, rbc*L is interrupted by nine introns (Koller et al. 1984). Sequencing studies are underway to investigate the coding arrangement of this gene in *Cryptomonas* Φ. The gene for the 32 kd photosystem II polypeptide of the green alga, *Chlamydomonas smithii* contains three introns (see Cattolico 1986), that of *C. reinhardtii* four (Erikson et al. 1984) and that of *E. gracilis* five (Johanningmeier and Hallick 1987). In *Cryptomonas* Φ, a 1.8 kb *Pst*I fragment contains both *psb*A and *cpe*B, and therefore it is unlikely that either of these genes contain large introns.

The position of *psb*A just downstream of the rRNA gene cluster but outside of the inverted repeat distinguishes this chloroplast genome from those of the chromophyte alga, *Olisthodiscus luteus*, and the green algae, *Chlamydomonas reinhardtii, C. moewusii* and *C. eugamatos* (Reith and Cattolico 1986; see Lemieux et al. 1985a, b), where the gene is located on the inverted repeat. Similarly, *rbc*L is also not found on the inverted repeat, but in the large single-copy region. This feature is shared with the chromophyte alga, *Dictyota dichotoma* (Kuhsel and Kowallik 1987). The gene for the beta subunit of ATP synthase is also found in the large-single copy region as in land plants and green algae (Palmer 1985).

In all plants and green algae examined so far, the small subunit of Rubisco is encoded by a multigene family in the nucleus and the large subunit is encoded in the plastid. However, in at least one chromophyte, *Olisthodiscus luteus*, the genes for both subunits reside on the plastid genome adjacent to one another (Reith and Cattolico 1986), as is probably the case the rhodophytes *Cyanidium caldarium* and *Porphyridium aerugineum* (Steinmuller et al. 1983). In *Cryptomonas* Φ, studies with the *rbc*S probe were unsuccessful and sequencing studies of the *rbc*L region are being conducted to determine if the small subunit gene is present on this part of the plastid genome. The partitioning of genes between the plastid, nucleomorph and nucleus has important implications for the evolution of the cryptomonad algae (Cavalier-Smith 1986).

Although information is scarce on plastid genome structure and organization among the Chromophyta and Rhodophyta, the data which is emerging does not indicate the same degree of conservation that is seen in the land plants. Plastid genome characteristics such as size, presence of inverted repeats, gene content of the inverted repeats and small and large single-copy regions, and clustering of genes seem to be as diverse as the number of organisms investigated. The plastid genome of *Cryptomonas* Φ shares characteristics from both the Chromophyta and Rhodophyta, a finding not inconsistent with its hypothesized evolution from a primitive red alga. Further mapping studies and sequence analysis of specific genes from *Cryptomonas* Φ are currently underway to investigate additional features shared among the plastids of algae. It will be of interest to see how other plastid genes and gene clusters are arranged in this group of phycobiliprotein/chlorophyll *c*-containing algae.

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