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A 16 kb small single-copy region separates the plastid DNA inverted repeat of the unicellular red alga *Cyanidium caldarium*: physical mapping of the IR-flanking regions and nucleotide sequences of the *psbD-psbC*, *rps16*, 5S rRNA and *rpl21* genes

Udo Maid und Klaus Zetsche*
Institut für Pflanzenphysiologie der Justus Liebig Universität, Heinrich Buff Ring 58, D-63 Giessen, Germany (author for correspondence)*

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Abstract

The four inverted repeat (IR) flanking regions of the *Cyanidium caldarium* plastid DNA were cloned. Southern blotting, transcript and sequence analyses of the border regions revealed the *psbD-psbC* operon and the *rps16* gene within the large single-copy region upstream of the 16S rDNA gene and the *rpl21* gene downstream of the 5S rDNA within the 16 kb small single-copy region. The size of the IR is ca. 5 kb. The nucleotide sequences of the *psbD-psbC*, *rps16*, *rpl21* and 5S rRNA genes with the corresponding alignments and physical maps of the regions are presented. Northern analysis revealed a less complex *psbD-psbC* transcription pattern than has been found in higher plants. Comparisons to other red algal data point to structural diversity within red algal plastid DNA.

Introduction

The plastid genomes of different higher-plant taxa show remarkable structural conformity. With the exception of some legume species and certain conifers [25] they all have a duplicated area in inverse orientation. These inverted repeats (IR), containing at least the genes for the ribosomal RNAs, are of different size so that a different number of additional genes like *rpl2* or *rbcL* are duplicated too [23]. Hence chloroplast genome size depends to a large extent on the size of the IR. Algal plastid DNAs are less uniform with respect to existence and size of the IR. Different *Euglena* strains possess up to five complete rRNA operons [10, cf. 26] in direct repeats, recombination events destroyed the normally found rRNA order in *Chlamydomonas reinhardtii* [4] and *Chlorella ellipsoidea* [33, 34]; *Codium fragile* lacks the IR [20]. Such differences may be useful characteristics to reconstruct the phylogenetic relationships of the various plant lineages.

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Database under the accession number X62578.

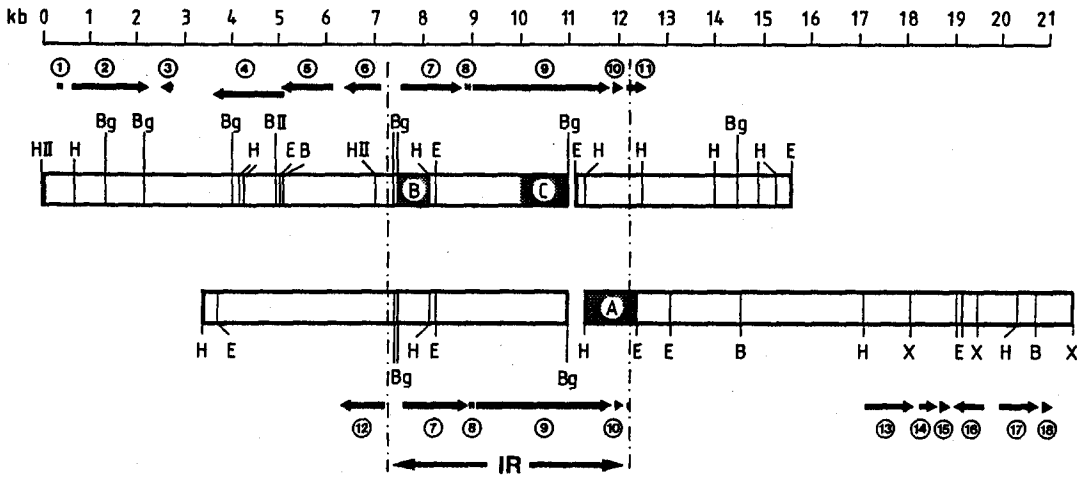

Fig. 1A. Physical map of the *C. caldarium* plastid inverted repeat and flanking regions. Numbered arrows represent open reading frames: 1, $tRNA^{Gln}$ and $tRNA^{Arg}$; 2, *groEL*; 3, *rps16*; 4, *psbC*; 5, *psbD*; 6, *ompR'*; 7, 16S rRNA; 8, $tRNA^{Ile}$ and $tRNA^{Ala}$; 9, 23S rRNA; 10, 5S rRNA; 11, *rpl21*; 12, UDP-acetylglucosamine-acyltransferase; 13, *psbA*; 14–18, phycobiliproteins and linkers. Abbreviations: B, *Bam* HI; Bg, *Bgl* II; E, *Eco* RI; H, *Hind* III; HII, *Hind* II; X, *Xba* I; kb, kilobase pairs; IR, inverted repeat. Shaded areas represent the DNA fragments A, B, and C, which were used for Southern hybridizations (Table 1).

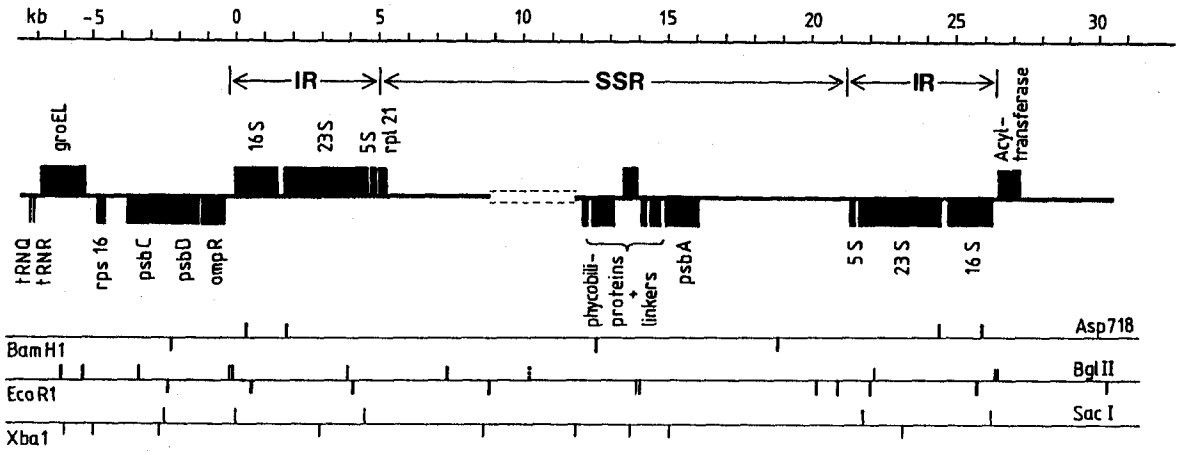

Fig. 1B. Physical map of the *C. caldarium* plastid DNA area containing the small single-copy region (SSR), the inverted repeat (IR) and parts of the large single-copy region (LSR), reconstructed from the cloned fragments shown in Fig. 1A and the Southern hybridizations listed in Table 1. Dotted lines represent the area which is not yet cloned. Genes represented by black boxes above the bold line are transcribed clockwise and those below the bold line counterclockwise.

Recently controversial data concerning the plastid DNA structure of red algae were published. Li and Cattolico [13] presented a physical map of *Griffithsia pacifica* lacking an IR whereas Shivji [25] found a small IR in the case of *Porphyra yezoensis*. In our laboratory we analysed the plastid genomes of three red algal species: *Antithamnion* sp. (multicellular), *Porphyridium aerugineum* and *Cyanidium caldarium* (syn. *Galdieria sulphuraria*) (both unicellular), to get an impression of the gene content and the range of structural genome diversity within the red algae. In this paper we present a physical map of the *C. caldarium* small single-copy region (SSR) and

IR with the flanking parts of the large single-copy region (LSR), a total of about 34 kb. Up to now, sequence and transcript analyses revealed 20 genes and open reading frames, respectively. Sequences of the *psbD-psbC* operon and the genes for 5S rRNA, *rps16* and *rp121* including alignments to bacterial and higher-plant counterparts are shown. Our data do not agree with either the *Griffithsia* or the *Porphyra* plastid DNA map, suggesting at least an isolated position of the unicellular *Cyanidium caldarium*. The data could also point to a large structural diversity within red algal plastid DNA as well.

Materials and methods

Cloning and sequencing of the *C. caldarium* plastid DNA was done as described [16]. Note that the genus was revised and *C. caldarium* Geitler, strain 14-1-1 isolated by Allen [1] was designated as *Galdieria sulphuraria* [22].

Southern blotting and colony hybridization for genomic walking and northern analysis used either digoxygenin-dUTP or α-^{32}P-dATP-labelled probes, using the random-primed labelling kits from Boehringer Mannheim according to standard procedures.

Computing of the nucleotide sequence data was done with the DNASIS/PROSIS software (Pharmacia) using the EMBL/SWISSPROT database, the KROEGER MENUE [12], and the alignment software CLUSTAL [8].

Results and discussion

Previously we were able to clone the 23S rDNA 3′-end/5S rDNA by genomic walking from the plastid encoded *psbA* gene of *C. caldarium* [16]. As it was not possible to clone further overlapping fragments (maybe due to interactions between the clones 23S rDNA with the *Escherichia coli* JM 83 host genetic apparatus), we cloned a heterologous rDNA probe from *Cyanophora paradoxa* cyanelle DNA [2] to identify the 16S rRNA coding regions of the *C. caldarium* plastom by colony-hybridization. A 4 kb *Bgl* II fragment was in agreement with the southern blot pattern probed with the homologous 23S rDNA 3′-end (data not shown). From this 4 kb *Bgl* II fragment we started again with genomic walking which enabled us to identify the two IR-flanking regions upstream of the 16S rDNA. The second 5S rDNA downstream region was identified with the homologous 5S rDNA probe mentioned above. All fragments were proved by comparison to Southern blot patterns of *C. caldarium* plastid DNA restriction digests and by sequencing. The observed Southern-hybridization signals of the cloned plastid DNA probes A, B and C (shaded in Fig. 1A) are listed in Table 1. They enabled us to localize the small single-copy region (SSR) between the two 5S rDNA portions of the IR. The

Table 1. Fragment sizes of southern blot hybridization patterns using the cloned *C. caldarium* plastid DNA-fragments **A**, **B** and **C** (shaded in Fig. 1A) and restriction digests of enriched *C. caldarium* plastid DNA. The position and size of the small single-copy region was deduced from the restriction sites of the cloned fragments and the hybridization patterns of probes A and C. The two *Eco* RI and *Hind* III fragments hybridizing with fragment B contain the two IR/large single-copy borders. The two IR/small single-copy borders are situated within the two *Eco* RI and *Hind* III fragments hybridizing with fragment A.

DNA fragment (see Fig. 1A)	Enzyme	Size of detected fragments(s)
A	*Asp* 718	23
	Bam HI	14 + >23
	Bgl II	3.5 + 12
	Dra I	2.2
	Eco RI	1.1 + 4.6
	Hinc II	12 + 13
	Hind III	1 + 5.5
	Pst I	20
	Sac I	4.3 + 18
	Xba I	5.7 + 8
	Bam HI/*Eco* RI	1.1 + 4.6
	Xba I/*Eco* RI	1.1 + 4.3
B	*Ava* I	10 + 13
	Eco RI	3 + 4.5
	Hind III	3.6 + 4.5
C	*Asp* 718/*Bam* HI	5.3 + 11
	Ava I/*Bam* HI	4.5 + 7.5

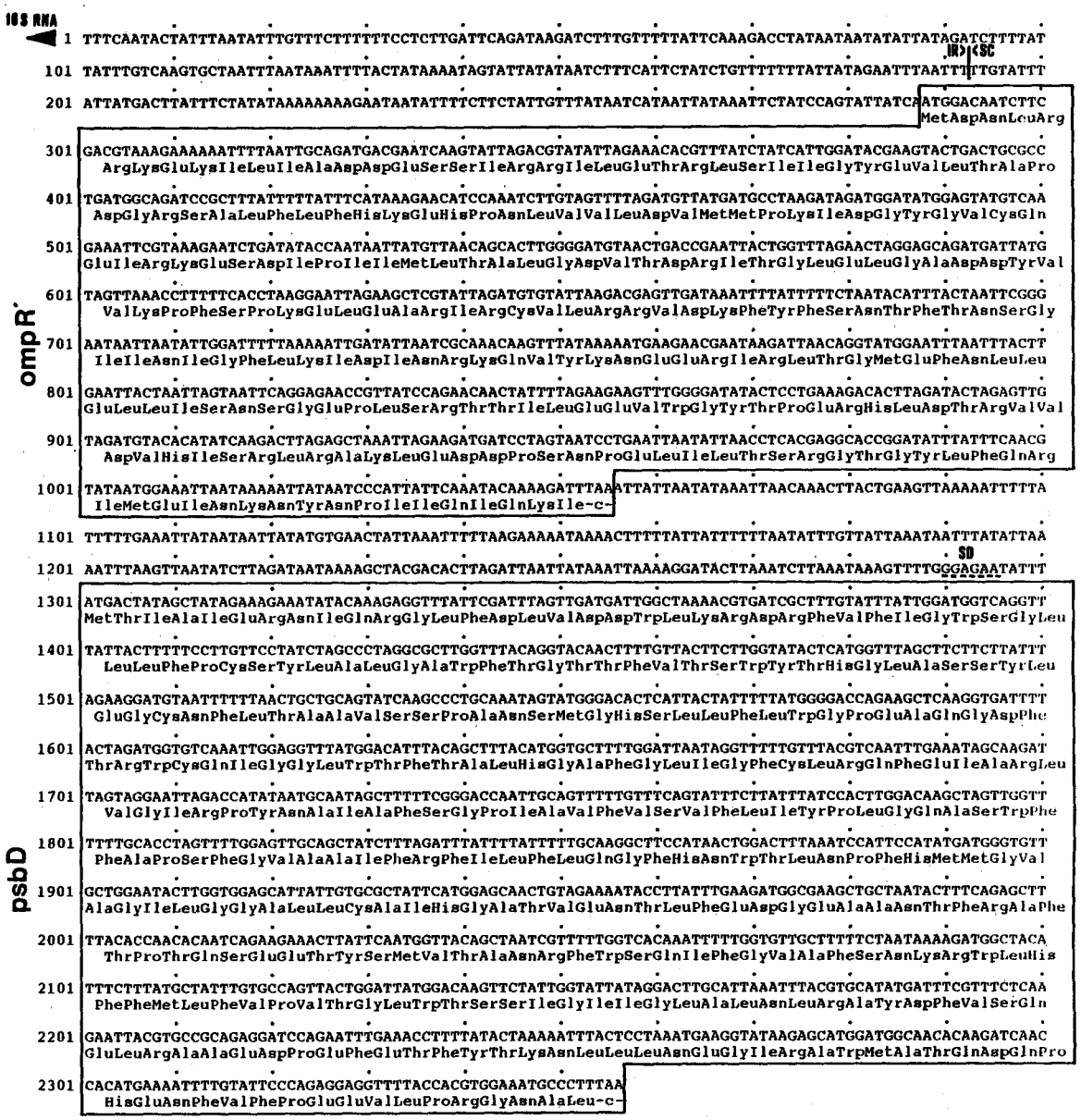

Fig. 2A. Nucleotide sequence of one *C. caldarium* 16S rDNA upstream region encoding an *ompR* homologue [9], the *psbD-psbC* operon and *rps16* (boxed). Nucleotide position one is the first nucleotide upstream to the 16S rDNA (arrowhead). The inverted repeat (IR) border is marked. Putative Shine-Dalgarno sequences (SD) are marked by dashed lines and the *psbC* downstream IR by arrows.

cloned areas represent about 34 kb of the plastid genome with a small (less than 0.1 kb) gap within the 23S rDNA gene and a gap of some 3 kb within the SSR. Fig. 1A shows a restriction map of the areas with the hitherto detected coding areas. Fig. 1B summarizes the results of the Southern analyses with respect to the orientation of the IR and the SSR. As we know about additional genes like *rbcS*, *ompR* and phycobiliprotein genes [9, 11, 28a, 28b, 29, 30; Glöckner, unpublished

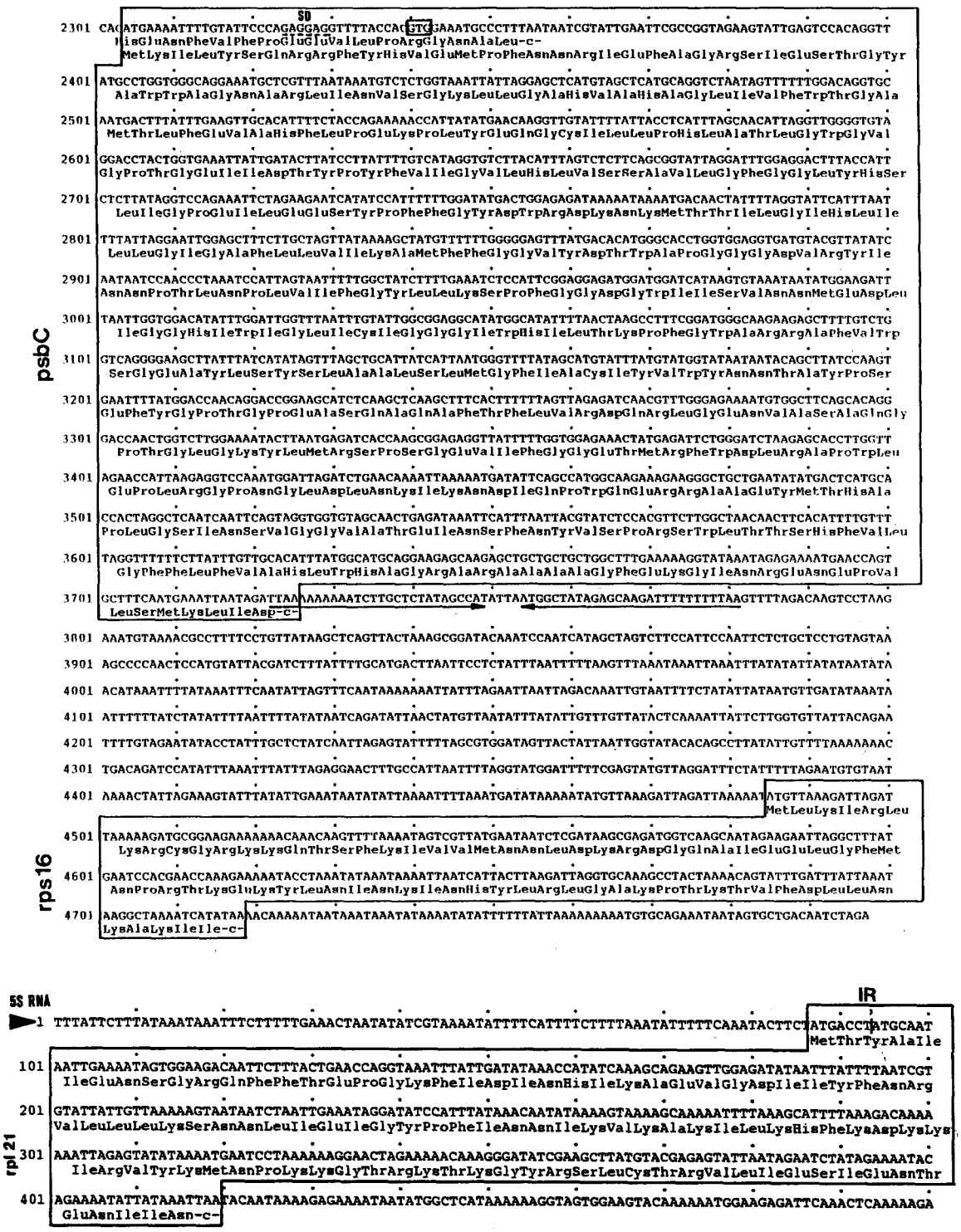

Fig. 2A. (Continued) (top).

Fig. 2B (bottom). Nucleotide sequence of one *C. caldarium* plastid 5S rRNA downstream region encoding ribosomal protein L21 (boxed). The inverted repeat border is marked.

results; K. Valentin *et al.*, submitted] on the rhodoplast genome compared to the higher-plant plastids, we are interested in the coding capacity of the *C. caldarium* plastid genome which is similar to the size reported for *Porphyra yezoensis* (A. Emich, unpublished results). Our previous results revealed a tendency to dense arrangement of genes and short intergenic regions [18]. Up to now sequence analyses of the IR and surrounding areas detected the following genes (Fig. 1).

Within the IR. 16S rRNA [17], 23S rRNA, 5S rRNA, $tRNA^{Ala}$ (UGC) and $tRNA^{Ile}$ (GAU) [18].

Upstream of the 16S rRNA, within the LSR. psbD, psbC, rps16, an *ompR* homologue [9], *groEL* (homologue to the nuclear-encoded Rubisco subunit-binding protein of higher plants), $tRNA^{Gln}$ (UUG), $tRNA^{Arg}$ (ACG) and an equivalent to a bacterial UDP-acetylglucosamine-acyltransferase gene. The latter four will be published elsewere [19].

Downstream of the 5S rRNA, within the SSR. rpl21, psbA [16] and five genes for phycobiliproteins and related linker proteins (K. Valentin *et al.*, submitted).

The psbD-psbC *operon.* This operon, coding for the D2 protein of the photosystem II (PSII) reaction centre and the 43 kDa chlorophyll-binding protein of PSII, is located 1.3 kb upstream of the 16S rRNA gene. It is transcribed from the opposite strand with a transcript size of about 3.5 kb (Fig. 3). With total cellular RNA from cells grown at 40 °C, the northern blot displays a single cotranscript without smaller signals which may represent processed transcripts or point to different promoters as they were reported for the *psbD-*

Table 2. **A.** Percentage of conserved amino acid residues of the ribosomal proteins S16 and L21 deduced from the nucleotide sequence. **B.** Percentage of conserved amino acid residues of the deduced *psbD/psbC* gene products.

A[1]

	S16				L21		
	Sa	Nt	Ec		So	Mp	Ec
Cc	43	42	37	Cc	32	37	25
Ec	39	33		Ec	31	28	
Nt	85			Mp	29		

B[2]

	Os	Sc	Nt	So	Mp	Cr	Sy
Cc	87/83	87/83	86/83	86/83	88/83	86/82	88/nd
Sy	88/nd	86/nd	85/nd	86/nd	86/nd	86/nd	
Cr	93/85	93/85	92/86	93/86	94/87		
Mp	97/95	97/94	97/96	97/95			
So	97/96	97/96	98/98				
Nt	98/97	97/96					
Sc	99/98						

[1] Abbreviations and references: Cc, *Cyanidium caldarium* (pt); Ec, *Escherichia coli* (EMBL X01818); Mp, *Marchantia polymorpha* (pt, EMBL X04465); Nt, *Nicotiana tabacum* (pt, EMBL X03415); Sa, *Sinapis alba* (pt, EMBL X13609); So, *Spinacia oleracea* (nc, [21]); nc, nucleus-encoded; pt, plastid-encoded.

[2] Abbreviations and references: Cc, *Cyanidium caldarium*; Cr, *Chlamydomonas reinhardtii* (EMBL X04147/X13879); Mp, *Marchantia polymorpha* (EMBL X04465); Nt, *Nicotiana tabacum* (EMBL Z00044); Os, *Oryza sativa* (EMBL X15901); Sc, *Secale cereale* (EMBL X13366); So, *Spinacia oleracea* (EMBL X01724); Sy, *Synechococcus* sp. (EMBL M20815); nd, not determined.

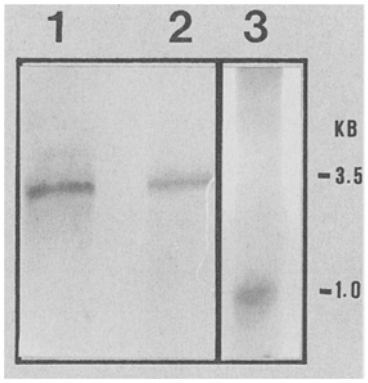

Fig. 3. Northern blots of total cellular *C. caldarium* RNA probed with α-^{32}P-dATP-labelled DNA fragments of *psbD* gene (internal, 1) *psbC* gene (internal, 2) and *rps16* gene (3) with 0.3 kb upstream area. Filters were washed three times at 50 °C for 20 min in 0.5 × SSC, 0.5% SDS. Exposure time was 2 h for lanes 1 + 2 and 8 h for lane 3.

psbC operon from tobacco [35] and barley [6]. The *C. caldarium* transcription pattern seems to be less complex, even though it still has to be tested whether the transcription pattern changes depending on culture conditions. As in other organisms, the coding regions of the two genes overlap by 50 bp. This situation is found in higher plant *psbD-psbC* operons too. A possible Shine-Dalgarno sequence is present only upstream of the *psbD* start codon. However, there is a possible Shine-Dalgarno sequence with a GTG translation initiation site downstream of the *psbC* ATG codon. This second initiation site was postulated in the case of the tobacco *psbC* gene [35]. Figures 2A and 2B show the nucleotide sequence with the two reading frames. Downstream of the *psbC* gene there is a 30 bp IR. Such relatively small repeat structures are thought to work as processing signals of the mRNA rather than as transcription terminators [24]. Table 2 shows the percentage of identical amino acids for both deduced proteins with homologous proteins of other organisms. On the higher-plant plastid DNAs a gene for tRNA^{Ser} was found downstream the *psbC* gene on the opposite strand [3, 35]. On the *C. caldarium* plastid genome *psbC* is followed by the *rps16* gene in the same direction.

rps16. This gene for the S16 ribosomal protein of the 30S ribosomal subunit is encoded 0.7 kb downstream of the *psbD-psbC* operon on the same strand. As in other plants it is not part of a ribo-

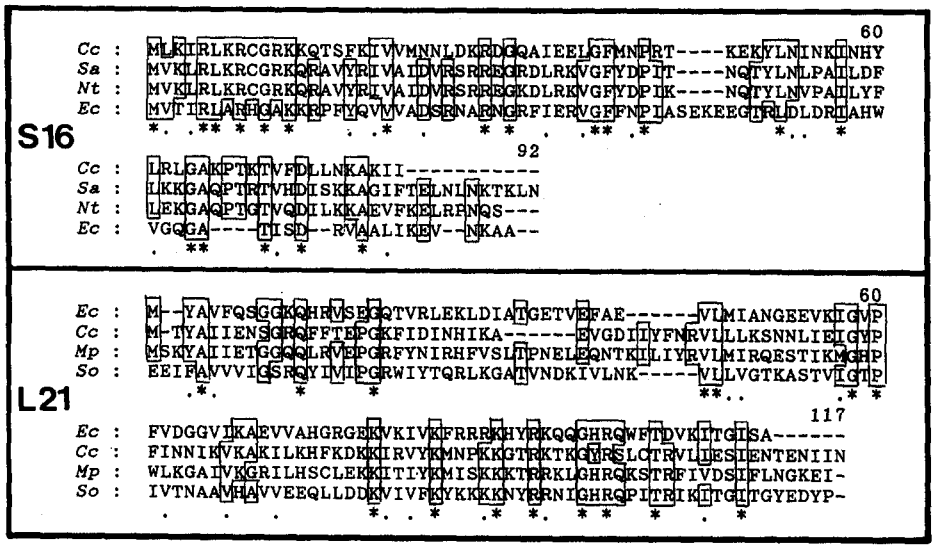

Fig. 4. Amino acid sequence alignment of *C. caldarium* plastid-encoded ribosomal proteins S16 and L21 with corresponding proteins from *E. coli* and plants (EMBL/SWISSPROT database and [21]). Abbreviations: Cc, *C. caldarium*; Ec, *Escherichia coli*; Mp, *Marchantia polymorpha*; Nt, *Nicotiana tabacum*; Sa, *Sinapis alba*; So, *Spinacia oleracea*. Asterisks mark identical, and dots mark homologous residues. Residues conserved in at least three of four cases are boxed.

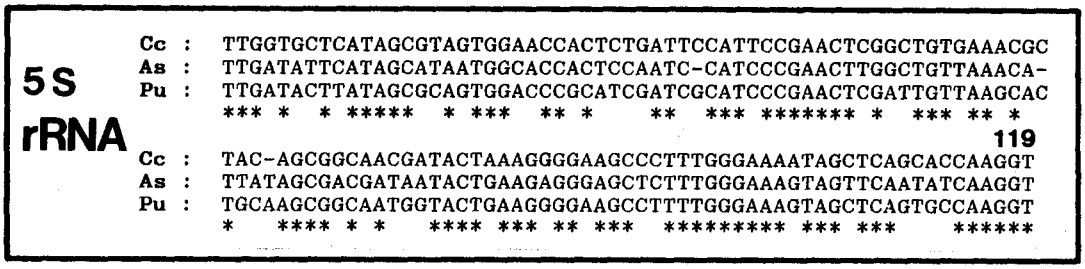

Fig. 5. Nucleotide sequence alignment of red algal plastid 5S rDNA. Abbreviations: As, *Antithamnion* sp.; Cc, *Cyanidium caldarium*; Pu, *Porphyra umbilicalis* [31]. Asterisks mark identical positions.

somal protein operon. However, there is a small (294 bp) open reading frame which ends 20 bp upstream of the *rps16* start codon. This ORF could not be identified by comparison to EMBL data bank up to now. A DNA probe containing both the ORF and the *rps16* gene hybridizes to a transcript of 1.0 kb (Fig. 3), so both genes may be cotranscribed. Within the plants investigated so far, there are examples for nuclear- as well as for plastid-encoded *rps16* genes (for review see [14]). Figure 2 shows the nucleotide sequence with the deduced amino acid sequence which is aligned with other S16 proteins in Fig. 4.

rpl21. The start codon for the gene of the L21 protein of the 50S ribosomal subunit is located 84 bp downstream of the 5S rDNA and 7 bp within the IR. Hence there is a very short *rpl21* pseudogene (Fig. 1, 2A, 2B). The *rpl21* gene is plastid-encoded in *Marchantia polymorpha* whereas it was not found on the plastid genomes of higher plants [21]. An amino acid alignment with the currently available corresponding *E. coli*, *M. polymorpha*, and *S. oleracea* proteins is shown in Fig. 4. The spinach L21 nucleus-encoded plastid sequence was deduced from a cDNA clone. Its remarkably low homology to the plastid-encoded liverwort L21 protein gave rise to the hypothesis that the spinach plastid L21 protein is derived from a mitochondrial gene [21]. As is shown in Table 2, both *C. caldarium* ribosomal protein genes investigated show merely slightly higher homologies to their chloroplast counterparts than to those of *E. coli*. More data from red algal and cyanobacterial ribosomal protein genes are necessary to interpret these results.

5S rRNA. This small and therefore easy-to-sequence rRNA gene is frequently used to establish phylogenetic trees [27, 31] because of its high degree of conservation throughout all organisms. Nevertheless its taxonomic significance is not uncontradicted because of the insufficient length of the gene [7]. We compared the *C. caldarium* 5S rRNA with its counterpart from the multicellular red alga *Antithamnion* sp. and from *Porphyra umbilicalis* [31]. Figure 5 shows the nucleotide alignment. 64% of the nucleotide positions are conserved within the compared species.

Discussion

The data presented are the first detailed sequence analyses of an IR on a plastid genome of a red alga. They show remarkably dense arrangement of genes throughout the investigated areas. None of the genes contains introns. The IR region of some 5 kb is limited to the rRNA operon. However, within the SSR there is a 4.5 kb region between the *rpl21* pseudogene and the *psbA* gene (Fig. 1) from which no transcript could be detected in northern blot experiments. Sequence analyses of this region revealed relatively long (more than 600 bp) reading frames without initiation codons or Shine-Dalgarno sequences (U. Kessler, unpublished results). These interrupted reading frames may represent relics of former genes which have been destroyed by size alterations of the IR region, as is the case for the *rpl21* pseudogene.

The gene localization of the *C. caldarium* plastom shows striking differences when compared to

the situation in higher plants. All these aforesaid features allow additional genes to be encoded on the red algal plastid genome, which may be transferred to the nucleus in the case of higher plants (ribosomal proteins, *groEL*) or may be reduced due to a loss of function (phycobiliproteins). Hence the red algal plastid genome organization may represent a more ancient situation with more similarities to (cyano-)bacterial genomes. It is noteworthy that the *psbD* amino acid sequence homology is almost identical between *C. caldarium* and the cyanobacterium *Synechococcus* and between *C. caldarium* and higher plants (Table 2), pointing to similar evolutionary distances between the recent cyanobacteria and the plastids of red algae and higher plants. The same discrepancy was found in the case of the red algal *psbA* gene [16, 32] which shows on the one hand the cyanobacterial carboxy terminus with a seven amino acid insertion, and on the other higher amino acid homologies to *psbA* genes from higher plants. This situation is probably due to different rates of point mutations affecting the amino acid sequence and larger insertion/deletion events reordering the whole plastid genome.

Our data do not agree with the previously published physical maps of the red algae *Griffithsia pacifica* (no IR) and *Porphyra yezoensis* (SSR adjacent to the 16S rDNA portion of the IR); instead, they show remarkable resemblance between the chloroplast genome of *C. caldarium* and those of the cryptophyta [5, 15] with respect to the position of the SSR and the *psbA* gene. It would be of great interest to know whether these differences represent a general plastid DNA diversity throughout the red algae or require assignment of the unicellular *C. caldarium* to a more isolated position within the red algal group.

Acknowledgements

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References

1. Allen MB: Studies with *Cyanidium caldarium*, an anomalously pigmented chlorophyte. Arch Microbiol 32: 270–277 (1959).
2. Bohnert HJ, Michalowski C, Bevacqua S, Mucke H, Löffelhardt W: Cyanelle DNA from *Cyanophora paradoxa*. Physical mapping and location of protein coding regions. Mol Gen Genet 201: 565–574 (1985).
3. Bukharov AA, Kolosov VL, Klezovich ON, Zolotarev AS: Nucleotide sequence of rye chloroplast DNA fragment, comprising *psbD*, *psbC* and *trnS*. Nucl Acids Res 17: 798 (1989).
4. Dron M, Rahire M, Rochaix J-D: Sequence of the chloroplast 16S rRNA gene and its surrounding regions of *Chlamydomonas reinhardii*. Nucl Acids Res 10: 7609–7620 (1982).
5. Douglas SE: Physical mapping of the plastid genome from the chlorophyll *c*-containing alga, *Cryptomonas* Φ. Curr Genet 14: 591–598 (1988).
6. Gamble PE, Berends Sexton T, Mullet JE: Light dependent changes in *psbD* and *psbC* transcripts of barley chloroplasts: accumulation of two transcripts maintains *psbD* and *psbC* translation capability in mature chloroplasts. EMBO J 7: 1289–1297 (1988).
7. Halanych KM: 5S ribosomal RNA sequences inappropriate for phylogenetic reconstruction. Mol Biol Evol 8: 249–253 (1991).
8. Higgins DG, Sharp PM: CLUSTAL: a package for performing multiple sequence alignments on a microcomputer. Gene 73: 237–244 (1988).
9. Kessler U, Maid U, Zetsche K: An equivalent to bacterial *ompR* genes is encoded on the plastid genome of red algae. Plant Mol Biol (in press).
10. Koller B, Delius H: A chloroplast DNA of *Euglena gracilis* with five complete rRNA operons and two extra 16S rRNA genes. Mol Gen Genet 188: 305–308 (1982).
11. Kostrzewa M, Valentin K, Maid U, Radetzky R, Zetsche K: Structure of the rubisco operon from the multicellular red alga *Antithamnion* spec. Curr Genet 18: 465–469 (1990).
12. Kröger M, Kröger-Block A: Extension of a flexible computer program for handling DNA sequence data. Nucl Acids Res 12: 113–123 (1984).
13. Li N, Cattolico RA: Chloroplast genome organization in the red alga *Griffithsia pacifica*. Mol Gen Genet 209: 343–351 (1987).
14. Mache R: Chloroplast ribosomal proteins and their genes. Plant Sci 72: 1–12 (1990).
15. Maerz M, Wolters J, Hofmann CJB, Sitte P, Maier U-G: Plastid DNA from *Pyrenomonas salina* (Cryptophyceae): physical map, genes and evolutionary implications. Curr Genet 21: 73–81 (1992).
16. Maid U, Valentin K, Zetsche K: The *psbA* gene from a red alga resembles those from Cyanobacteria and Cyanelles. Curr Genet 17: 255–259 (1990).

17. Maid U, Zetsche K: Nucleotide sequence of the plastid 16S rRNA gene of the red alga *Cyanidium caldarium*. Nucl Acids Res 18: 3996 (1990).
18. Maid U, Zetsche K: Structural features of the plastid ribosomal RNA operons of two red algae: *Antithamnion* sp. and *Cyanidium caldarium*. Plant Mol Biol 16: 537–546 (1991).
19. Maid U, Steinmüller R, Zetsche K: Structure and expression of a plastid encoded *groEL* homologous heat shock gene in a thermophilic unicellular red alga. Curr Genet (in press).
20. Manhart JR, Kelly K, Dudock BS, Palmer JD: Unusual characteristics of *Codium fragile* chloroplast DNA revealed by physical and gene mapping. Mol Gen Genet 216: 417–421 (1989).
21. Martin W, Lagrange T, Li YF, Bisanz-Seyer C, Mache R: Hypothesis for the evolutionary origin of the chloroplast ribosomal protein L21 of spinach. Curr Genet 18: 553–556 (1990).
22. Merola A, Castaldo R, De Luca P, Gambardella R, Musacchio A, Taddei R: Revision of *Cyanidium caldarium*. Three species of acidophilic algae. Giorn Bot Ital 115: 189–195 (1981).
23. Palmer JD: Comparative organization of chloroplast genomes. Annu Rev Genet 19: 325–354 (1985).
24. Schuster G, Gruissem W: Chloroplast mRNA 3′ end processing requires a nuclear encoded RNA-binding protein. EMBO J 10: 1493–1502 (1991).
25. Shivji MS: Organization of the chloroplast genome in the red alga *Porphyra yezoensis*. Curr Genet 19: 49–54 (1991).
26. Siemeister G, Hachtel W: Organization and nucleotide sequence of ribosomal RNA genes on an circular 73 kb DNA from the colourless flagellate *Astasia longa*. Curr Genet 17: 433–438 (1990).
27. Specht T, Wolters J, Erdmann A: Compilation of 5S rRNA and 5S rRNA sequences. Nucl Acids Res 18(suppl.): 2215–2236 (1990).
28a. Strauss SH, Palmer JD, Howe GT, Doerksen AH: Chloroplast genomes of two conifers lack a large inverted repeat and are extensively rearranged. Proc Natl Acad Sci USA 85: 3898–3902 (1988).
28b. Valentin K, Zetsche K: The genes of both subunits of ribulose-1,5-bisphosphate carboxylase constitute an operon on the plastome of a red alga. Curr Genet 16: 203–209 (1989).
29. Valentin K, Zetsche K: Structure of the Rubisco operon from the unicellular red alga *Cyanidium caldarium* – evidence of a polyphyletic origin of the plastids. Mol Gen Genet 222: 425–430 (1990).
30. Valentin K, Zetsche K: Rubisco genes indicate a close phylogenetic relationship between the plastids of chromophyta and rhodophyta. Plant Mol Biol 15: 575–584 (1990).
31. Van den Eynde H, de Baere R, de Roeck E, van de Peer Y, Vandenberghe A, Willekens P, de Wachter R: The 5S ribosomal RNA sequences of a red algal rhodoplast and a gymnosperm chloroplast. Implications for the evolution of plastids and cyanobacteria. J Mol Evol 30: 463–476 (1990).
32. Winhauer T, Jäger S, Valentin K, Zetsche K: Structural similarities between *psbA* genes from red and brown algae. Curr Genet 20: 177–180 (1991).
33. Yamada T: Repetitive sequence-mediated rearrangements in *Chlorella ellipsoidea* chloroplast DNA: completion of nucleotide sequence of the large inverted repeat. Curr Genet 19: 139–147 (1991).
34. Yamada T, Shimaji M: Peculiar feature of the organization of rRNA genes of the *Chlorella* chloroplast DNA. Nucl Acids Res 14: 3827–3839 (1986).
35. Yao WB, Meng BY, Tanaka M, Sugiura M: An additional promotor within the protein-coding region of the *psbD-psbC* gene cluster in tobacco chloroplast DNA. Nucl Acids Res 17: 9583–9591 (1989).
36. Zurawski G, Bottomley W, Whitfeld R: Junctions of the large single copy region and the inverted repeats in *Spinacia oleracea* and *Nicotiana debneyi* chloroplast DNA: sequence of the genes for $tRNA^{His}$ and the ribosomal proteins S19 and L2. Nucl Acids Res 12: 6547–6558 (1984).