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The genes of both subunits of ribulose-1,5-bisphosphate carboxylase constitute an operon on the plastome of a red alga

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Summary. Plastid (pt) DNA from the red alga *Porphyridium aerugineum* was purified by CsCl gradient centrifugation. An EcoRI library of the ptDNA was screened with a gene probe specific for the gene encoding the large subunit (LSU) of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco EC 4.1.1.39) from spinach. A 5.8 kb EcoRI clone containing the LSU gene (*rbcL*) was isolated and the DNA sequence of the *Porphyridium rbcL* gene and its flanking regions was determined. An open reading frame was found 130 bp downstream from the *rbcL* gene that shows homology to genes coding for the small subunit of Rubisco (*rbcS*) from higher plants and cyanobacteria. Both genes (*rbcL* + *rbcS*) are co-transcribed. Comparison of *rbcL* and *rbcS* sequences from *Porphyridium*, higher plants and cyanobacteria seems to reveal a remarkable evolutionary distance between the plastids of the red algae (rhodoplasts), chloroplasts and cyanobacteria.

Key words: Red algae – ptDNA – Ribulose-1,5-bisphosphate carboxylase – Plastid evolution

Introduction

The plastid DNA (ptDNA, plastome) of recent plants encodes only a limited number of plastid proteins, whereas the great majority are encoded by nuclear genes (Ohyama et al. 1986; Shinozaki et al. 1986). The hypothesis of an endosymbiotic origin of plastids (Margulis 1981) therefore implies that genes from the putative cyanobacteria-like endosymbionts have been transferred to the nucleus of the host. This raises the questions of whether the gene transfer has advanced to the same degree in all plants and whether the same set of genes has been transferred to the nucleus in all cases. Different patterns of such gene transfers in various plastid types may indicate a polyphyletic origin of the plastids.

Analyses of the ptDNA of more than 200 chlorophyll a- and b-containing plants (green algae and higher plants) reveals that these plastomes have a relatively uniform gene arrangement and coding capacity (Palmer 1985). This seems to indicate that the chloroplasts have a monophyletic origin.

Little information is available on the plastomes of chlorophyll a- and c-containing algae (brown algae and related taxa) (Kuhsel and Kowallik 1987; Linne v. Berg and Kowallik 1988). Kowallik and coworkers found that an intensive rearrangement of plastid genes had occurred of these algae compared in comparison with the situation present in the chloroplasts. The coding capacity of the analyzed plastomes (from *Dictyota dichotoma* and several species of the genus *Vaucheria*) is similar to those of the chloroplasts. DNA sequence homologies between ATP synthase genes (*atpA, atpE, atpF, atpH*) from the plastome of *Dictyota dichotoma* and from the plastomes of chloroplasts are discussed as evidence for a monophyletic origin of chloro- and phaeoplasts (K. Kowallik personal communication).

Almost nothing is known about the plastomes of phycobiliprotein-containing algae (Rhodophyceae, Cryptophyceae). The plastome of one red alga has been physically mapped (Li and Cattolico 1987), but no sequence data are available as yet.

In order to estimate the evolutionary position of the red algae and the rhodoplasts, we have analyzed the organization of the Rubisco genes in *Porphyridium aerugineum*. The genes for both subunits of Rubisco (*rbcL, rbcS*) constitute a single operon in cyanobacteria (Nierzwicki-Bauer et al. 1984, Shinozaki and Sugiura

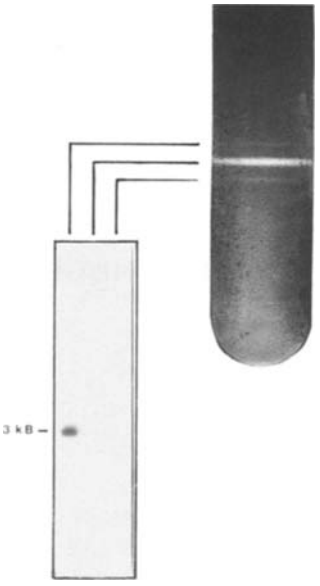

Fig. 1. Identification of ptDNA from *Porphyridium aerugineum*. Total cellular DNA from *Porphyridium* was separated by CsCl density gradient centrifugation. Three bands were visible after centrifugation. DNA from these bands was isolated, digested with EcoRI, electrophresed and blotted. The blots were then hybridized with a psbA-specific gene probe from spinach. Only a 3-kb fragment from the upermost band hybridized with this gene probe

1985). The same organization of the Rubisco genes was found on the organelle genome of *Cyanophora paradoxa*, an unicellular alga containing cyanelles (photosynthetic entities with a rudimentary cell wall resembling cyanobacteria; Starnes et al. 1985). In green algae and higher plants the *rbcL* gene resides on the plastome, whereas the *rbcS* genes are always located in the nuclear genome (Palmer 1985).

Sequence homologies between cyanobacterial and higher plant *rbcL* genes are in the range of 71%–77% at the nucleotide level and of 80%–85% at the amino acid level (Curtis and Haselkorn 1983; Shinozaki et al. 1983). This high degree of homology supports the hypothesis that chloroplasts and cyanobacteria are closely related (Hunt et al. 1985).

In the present paper we demonstrate that the Rubisco genes are located on the plastome where they are clearly organized in the form of an operon in the unicellular red alga *Porphyridium aerugineum* and probably have this form in the multicellular red alga *Antithamnion spec.* Sequencing analysis of the Rubisco genes from *Porphyridium* appears to indicate a remarkable evolutionary distance between rhodoplasts, chloroplasts and even cyanobacteria.

Materials and methods

Growth of algae and isolation of ptDNA. Axenic cultures of *Porphyridium aerugineum Geitler* were grown in 5-l fermenters as previously described (Steinmüller et al. 1983). Cells were harvested by centrifugation and lysed by treatment with 50 m*M* Tris/HCl, pH 7.5, 1% SDS, 50 m*M* EDTA and 500 µg Proteinase K/ml for 1 h at 50°C.

Antithamnion spec. Naegeli was cultured in 500-ml flasks containing seawater medium (Schlösser 1982). Algae threads were homogenized by grinding in a mortar in 50 m*M* Tris/HCl, pH 7.5, 50 m*M* EDTA and 1% SDS at 4°C.

Total cellular DNA was isolated from the lysed cells by phenolization and separation was accomplished by CsCl density gradient centrifugation in the presence of ethidium bromide. Three (*Porphyridium*, Fig. 1) or two (*Antithamnion*, data not shown) bands were visible after centrifugation. DNA from these bands was isolated, digested with EcoRI (*Porphyridium*) or BglII (*Antithamnion*), electrophoresed and blotted. The blots were hybridized with a plastid *psbA* gene probe from *spinach*. A 3-kb EcoRI fragment from the uppermost DNA band from *Porphyridium* (Fig. 1) and a 5-kb BglII fragment from the uppermost DNA band from *Antithamnion* (data not shown) hybridized to this gene probe. As the *psbA* gene is located on the plastome in all the plants analyzed so far (Palmer 1985), this indicates that the uppermost DNA bands are the ptDNAs.

Molecular cloning of the rbcL gene from Porphyridium aerugineum. PtDNA from *Porphyridium aerugineum* was digested with EcoRI and cloned in pUC18 using standard procedures (Maniatis et al. 1982). *E. coli* strain JM83 was used for transformation.

Since sequence homology between a spinach gene *rbcL* probe and the *Porphyridium rbcL* gene was too low for significant colony hybridization, we developed the following procedure for identifying *rbcL* clones: single clones were grown in 400 µl dYT-medium (10 g/l trypton, 5 g/l yeast extract, and 5 g/l NaCl) in 1.5 ml Eppendorf tubes under constant agitation (200 rpm) at 37° for 15–18 h. Ten cultures at a time were pooled (150 µl portions), and plasmids were isolated as described (Maniatis et al. 1982). The plasmids were digested with EcoRI, electrophoresed, blotted and hybridized (Maniatis et al. 1982) with a ^{32}P-labeled spinach *rbcL* gene probe. In order to minimize the hybridization of co-labeled traces of vector DNA with the cloning vector DNA, the labeled gene probe was co-precipitated with a large excess of unlabeled pUC18 DNA (5–10 µg), redissolved, heated to 95°C for 5 min and chilled on ice before being used for hybridization. A 5.8 kb EcoRI clone was identified that contains the whole *rbcL* gene and its flanking regions (see Fig. 2).

DNA Sequencing. Parts of the 5.8 kb EcoRI fragment were subcloned in pUC18 and sequenced by the dideoxy chain termination method (Sanger et al. 1977). The sequences of both strands of the DNA were determined and all restriction sites were crossed. Computer analysis was performed using the "Kroeger menue" (Kröger and Kröger-Block 1984).

Northern analysis. Total cellular RNA was isolated as described (Steinmüller et al. 1983). RNA (5–10 µg) was heat-denatured,

Fig. 2A. Nucleotide sequence of the *Porphyridium aerugineum* Rubisco operon. The predicted amino acid sequences of the *rbcL* and *rbcS* open reading frames (*boxes*) are indicated as well as the dyad symetry (*arrows*) of 27 bp of the proposed transcription terminator. Putative Shine Dalgano sequences are *underlined.* The source of DNA for sequencing was a 5.8-kb EcoRI clone. B *B* BamHI, *H* HindIII)

1A

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    1 ATCAACAATGATAATCGTACATATTTTTTAATATTAATGCATTACTTTAAAGAGTCCTAATGATACTGCCTTTAATCCAAGGAGGAATAC

   91 ATGGATCAATCCGTACAAGAGCGGACTAGAATTAAGAATGAACGTTATGAATCTGGTGTAATACCTTATGCTAAAATGGGTTACTGGGAT
      MetAspGlnSerValGlnGluArgThrArgIleLysAsnGluArgTyrGluSerGlyValIleProTyrAlaLysMetGlyTyrTrpAsp

  181 CCTGATTATGCTATTAAAGCAACAGATGTTTTAGCTCTTTTCCGTGTTACTCCACAACCTGGTGTAGACCCTGTTGAAGCAGCAGCTGCG
      ProAspTyrAlaIleLysAlaThrAspValLeuAlaLeuPheArgValThrProGlnProGlyValAspProValGluAlaAlaAlaAla

  271 ATTGCAGGTGAATCGTCTACAGCGACTTGGACTGTTGTATGGACAGACTTATTAACAGCTTGTGATTTATATCGTGCTAAAGCATATAGA
      IleAlaGlyGluSerSerThrAlaThrTrpThrValValTrpThrAspLeuLeuThrAlaCysAspLeuTyrArgAlaLysAlaTyrArg

  361 GTAGACCCTGTACCTAGCAGTCCTGATCAATATTTCGCATATATCGCTTATGATATTGACCTATTTGAAGAAGGTTCAATTGCTAACTTA
      ValAspProValProSerSerProAspGlnTyrPheAlaTyrIleAlaTyrAspIleAspLeuPheGluGluGlySerIleAlaAsnLeu

  451 ACAGCTTCAATCATCGGTAACGTATTCGGTTTCAAAGCAGTTAAAGCTCTTCGTCTAGAAGACATGCGTATCCCTGTAGCTTACTTAAAA
      ThrAlaSerIleIleGlyAsnValPheGlyPheLysAlaValLysAlaLeuArgLeuGluAspMetArgIleProValAlaTyrLeuLys

  541 ACATTCCAAGGTCCAGCTACTGGTGTTGTTGTAGAACGTGAACGTATGAACAACTTTGGTCGTCCTTTACTAGGTGCTACTGTAAAACCT
      ThrPheGlnGlyProAlaThrGlyValValValGluArgGluArgMetAsnAsnPheGlyArgProLeuLeuGlyAlaThrValLysPro

  631 AAATTAGGTTTATCTGGTAAAAACTATGGCCGTGTAGTTTACGAAGGTCTAAAAGGCGGTCTAGATTTCCTAAAAGATGACGAAAACATT
      LysLeuGlyLeuSerGlyLysAsnTyrGlyArgValValTyrGluGlyLeuLysGlyGlyLeuAspPheLeuLysAspAspGluAsnIle

  721 AACTCACAACCATTTATGCGTTGGAGAGAACGTTTCCTATTCTGTATGGAAGGTGTTAACCGTGCTTCAGCTTCAAGCGGTGAAGTAAAA
      AsnSerGlnProPheMetArgTrpArgGluArgPheLeuPheCysMetGluGlyValAsnArgAlaSerAlaSerSerGlyGluValLys

  811 GGACACTACCTAAACGTAACTGCTGCTACAATGGAAGATATGTACGAACGTGCAGAATTTTCTAAAGAAGTTGGTAGCATCATTTGTATG
      GlyHisTyrLeuAsnValThrAlaAlaThrMetGluAspMetTyrGluArgAlaGluPheSerLysGluValGlySerIleIleCysMet

  901 ATCGACTTAGTAATCGGTTATACTGCGATTCAATCAATGGCAAAATGGGCTCGTAAGAATGATATGATTCTTCACTTACACCGTGCAGGT
      IleAspLeuValIleGlyTyrThrAlaIleGlnSerMetAlaLysTrpAlaArgLysAsnAspMetIleLeuHisLeuHisArgAlaGly

  991 AACTCTACTTATTCTAGACAAAAGAACCATGGTATGAATTTCCGTGTAATTTGTAAGTGGATGCGTATGGCAGGTGTTGACCATATTCAC
      AsnSerThrTyrSerArgGlnLysAsnHisGlyMetAsnPheArgValIleCysLysTrpMetArgMetAlaGlyValAspHisIleHis

 1081 GCAGGTACTGTTGTAGGTAAACTAGAAGGGGATCCTCTAATGATTAAAGGTTTCTATAACACTCTTCTAGAGCCACATCTAAATATTAAC
      AlaGlyThrValValGlyLysLeuGluGlyAspProLeuMetIleLysGlyPheTyrAsnThrLeuLeuGluProHisLeuAsnIleAsn

 1171 CTTCCTGAAGGTCTATTCTTCGAACAAAACTGGGCTTCTTTAAGAAAAGTAATGCCAGTTGCTTCTGGTGGTATTCATTGTGGTCAAATG
      LeuProGluGlyLeuPhePheGluGlnAsnTrpAlaSerLeuArgLysValMetProValAlaSerGlyGlyIleHisCysGlyGlnMet

 1261 CACCAACTTATTGATTATCTAGGTGATGACGTTGTATTACAATTCGGTGGTGGTACTATTGGTCACCCAGATGGTATTCAAGCAGGTGCT
      HisGlnLeuIleAspTyrLeuGlyAspAspValValLeuGlnPheGlyGlyGlyThrIleGlyHisProAspGlyIleGlnAlaGlyAla

 1351 ACTGCAAACCGTGTTGCTTTAGAATCTATGCTTTTAGCTCGTAACGAAGGTCGTGATTATGTAGCTCAAGGTCCTCAAATCTTAAGAGAT
      ThrAlaAsnArgValAlaLeuGluSerMetLeuLeuAlaArgAsnGluGlyArgAspTyrValAlaGlnGlyProGlnIleLeuArgAsp

 1441 GCTGCTAAAACATGCGGACCTCTACAAACAGCTTTAGATTTATGGAAAGATATTTCATTCAACTATACATCTACTGATACTGCAGACTTC
      AlaAlaLysThrCysGlyProLeuGlnThrAlaLeuAspLeuTrpLysAspIleSerPheAsnTyrThrSerThrAspThrAlaAspPhe

 1531 GTACAAACTCCAACTGCAAACGTATAATTAAGTAGTAACAAACTGAGGTATTTCAAATTGATTTATCGAGGTTTTAAATTAAAATCTCGA
      ValGlnThrProThrAlaAsnVal-c-

 1622  AAACTTTTGTATATCAATAAATGTTACAATAATAATTTTATTAACCAATTTTAAAGAGGTAAGATCGTGAGACTAACACAAGGTACTTT
                                                                 MetArgLeuThrGlnGlyThrPhe

 1711 TTCATTTCTTCCAGACTTAACTGATGCTCAAATTCAAAAACAAGTTCAATATGCTGTTAGCAAAAAATGGGCTGTAAGTGTTGAATATAC
       SerPheLeuProAspLeuThrAspAlaGlnIleGlnLysGlnValGlnTyrAlaValSerLysLysTrpAlaValSerValGluTyrThr

 1801 AGATGATCCACATCCAAGAAACTCATTCTGGGAACTATGGGGCCTTCCTTTATTTGATGTTAAAGATGCATCAGCTTTAATGTATGAGAT
       AspAspProHisProArgAsnSerPheTrpGluLeuTrpGlyLeuProLeuPheAspValLysAspAlaSerAlaLeuMetTyrGluIle

 1891 TGCTGCTTGTCGTAAAGCAAAACCAAACTATTATATTAAAGTTAACGCATTTGATAATACAAGAGGTGTTGAATCTTGTTGTTTATCATT
       AlaAlaCysArgLysAlaLysProAsnTyrTyrIleLysValAsnAlaPheAspAsnThrArgGlyValGluSerCysCysLeuSerPhe

 1981 CATTATTAATCGTCCGATTAATGAGCCAGGTTTCCATTTAGAACGTCAAGAAGTTCAAGGTCGTAACATCCTTTACACTATCAAATCTTA
       IleIleAsnArgProIleAsnGluProGlyPheHisLeuGluArgGlnGluValGlnGlyArgAsnIleLeuTyrThrIleLysSerTyr

 2071 CGCTGTAAACAAACCAGAAGGTTCTCGTTACTAAAACGAAATAATATTCATATATAAAATTAGAAAACTAATTAGTTTTCTAATTTTATA
       AlaValAsnLysProGluGlySerArgTyr-c-

 2161 TATGAAACTTACTTATTAGATAGTATTATTTTTTCAAACTTAATTTATTATTATGAGCAAACTAGAGAATATTAAGAATAACACACTTGT
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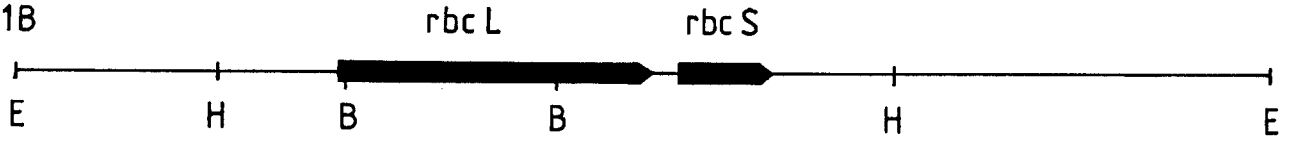
1B



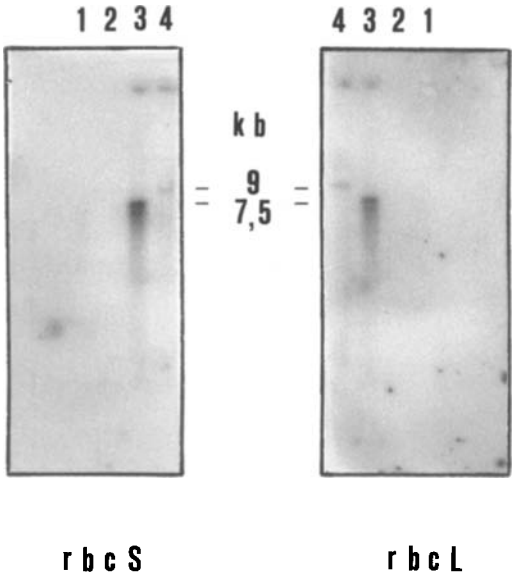


Fig. 3. Localization of the genes for both subunits of Rubisco (*rbcL, rbcS*) on the plastome of *Antithamnion spec.* Cellular DNA (lanes 1 and 2) and ptDNA (lanes 3 and 4) was digested with EcoRI (1 and 3) or BamHI (2 and 4), electrophoresed and blotted. The blots were then hybridized with *rbcS*- (*left*) or *rbcL*- (*right*) specific gene probes from *Porphyridium aerugineum* (see Fig. 4)

separated electrophoretically in 1.5% formaldehyde/agarose gels and transferred to nitrocellulose using standard procedures (Davis et al. 1986). Filters were hybridized to *rbcL*- and *rbcS*-specific gene probes from *Porphyridium aerugineum* labeled with ^{32}P. Hybridization and washing steps were carried out as described (Maniatis et al. 1982).

Results and discussion

Localization of the genes of both subunits of Rubisco on the plastome of Porphyridium aerugineum and Antithamnion spec.

In a previous work we demonstrated that both subunits of Rubisco are translated from poly-A$^-$mRNA in both algae in a cell-free translation system in their mature form (Steinmüller et al. 1983 and unpublished results). This suggests that both subunits are plastome encoded. To prove this assumption we tried to detect the genes coding for both subunits on the organelle genome by heterologous hybridization. We achieved weak hybridization signals with ptDNA from *Porphyridium* using a *rbcL* gene probe from spinach (data not shown). Nor did cellular DNA or ptDNA from both species hybridized with *rbcS* gene probes from maize, *Chlamydomonas reinhardii* or *Cyanophora paradoxa*. As both genes are closely adjacent in cyanobacteria we cloned and sequenced the *rbcL* gene and its flanking regions from *Porphyridium*. We found an open reading frame 130 bp downstream from the *rbcL* gene that shows 29%–37% homology to *rbcS* genes from cyanobacteria and higher plants at the amino acid level (Fig. 2, 5 and 6).The deduced relative molecular mass (M_r) of the SSU protein (15,900) corresponds to the M_r of the mature SSU of *Porphyridium Rubisco (15,400, Steinmüller et al. 1983).*

In further hybridization experiments rbcL- and *rbcS*-specific gene probes from *Porphyridium* were used to localize both genes on the plastome of the more advanced multicellular red alga *Antithamnion spec.* (Florideophycidae). Fig. 3 shows that a 9-kb BamHI fragment and a 7.5-kb EcoRI fragment of the ptDNA hybridize to both gene probes. This indicates that both Rubisco genes are located on the plastome of *Antithamnion spec.*, probably in the form of an operon.

Reith and Cattolico (1986) located both Rubisco genes on the plastome of *Olisthodiscus luteus* (a unicellular algae that belongs to the Chrysophyceae) by inhibitor analyses and immunological detection of the gene products expressed in *E. coli.*

The different coding sites of the *rbcS* genes in the red algae and Chrysophyceae compared with green algae and higher plants (plastome versus nuclear genome) is good evidence for a polyphyletic origin of chloroplasts, rhodoplasts and possibly phaeoplasts.

Co-transcription of both Rubisco genes in Porphyridium aerugineum

Both Rubisco genes are co-transcribed in the cyanobacteria and in the cyanelles of *Cyanophora paradoxa* (Nierzwicki-Bauer 1984; Shinozaki and Sugiura 1985; Starnes et al. 1985). The spacer between the closely adjacent *rbcL* and *rbcS* genes of *Porphyridium aerugineum* contains a "Shine Dalgano sequence" (SD sequence, Shine and Dalgarno 1975, GAGG, see Fig. 2) at an appropriate distance from the start codon of the *rbcS* gene. No promotor-like sequences could be detected upstream of the *rbcS* gene. As functional SD sequences have been found in several plastomes (Ruf and Kössel 1988), this structural feature indicates that both Rubisco genes are also co-transcribed in *Porphyridium aerugineum.* Figure 4 shows that *rbcL*- and *rbcS*-specific gene probes from *Porphyridium aerugineum* hybridize to the same mRNA which is approximately 2.5 kb in size. No hybridization signals with smaller RNAs could be detected.

RbcL-specific hybridization with a RNA of a similar size was found in *Antithamnion spec.* using a *rbcL* gene probe from *Porphyridium aerugineum* (Fig. 4), which suggests that the Rubisco genes are also co-transcribed in this species. No specific hybridization of the *Antithamnion* RNA with a *rbcS* gene probe (see Fig. 4) from

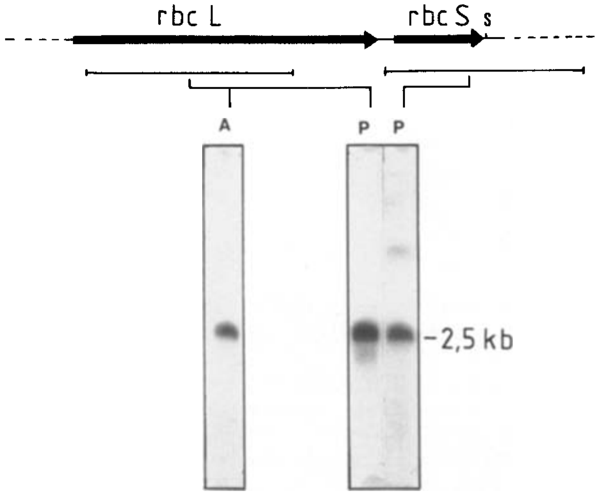


Fig. 4. Identification of *rbcL* and *rbcS* mRNAs. Total cellular RNA from *Porphyridium aerugineum* (*P*) and *Antithamnion spec.* (*A*) was electrophoresed and blotted. The blots were then hybridized with *rbcL* (1-kb BamHI fragment) or *rbcS*-(0.9-kb HindII/DraI fragment) specific gene probes from *Porphyridium aerugineum*. (*S* indicates the proposed transcription terminator, see Fig. 2)

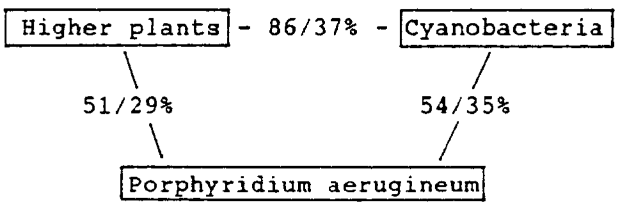


Fig. 5. Sequence homologies between Rubisco LSU/SSU proteins from higher plants (Bedbrook et al. 1980; Zurawski et al. 1981; Sugita et al 1987), cyanobacteria (Curtis and Haselkorn 1983; Shinozaki et al. 1983, Shinozaki and Sugiura 1983; Nierzwicki-Bauer et al. 1984) and *Porphyridium aerugineum*. (Deletions were excluded in the SSU proteins; see Fig. 6)

Porphyridium could be achieved, probably due to the smaller size of the gene and a lower sequence homology. Newsman and Cattolico (1988) found that both Rubisco genes are also co-transcribed into a single (> 6 kb) mRNA in *Olisthodiscus luteus*.

More data are necessary to evaluate whether the similarity in the organization of the Rubisco genes between red algae and Chrysophyceae is evidence of a phylogenetic relationship between the plastids of both taxa.

Sequence analysis

The high degree of homology between *rbcL* genes from chloroplasts and the cyanobacteria supports the hypothesis that chloroplasts and cyanobacteria are closely related (Hunt et al. 1985).

Similarities in the photosynthetic apparatus of recent cyanobacteria and rhodoplasts may indicate that cyanobacteria-like organisms were the direct ancestors of rhodoplasts. The hypothesis of a close relationship between cyanobacteria and rhodoplasts is also supported by sequence analyses of ferredoxin (Hunt et al. 1985). The comparison of partial sequences of the large (28s) cytoplasmic rRNA has led to the conclusion that the rhodophytes, chromophytes and chlorophytes emerged as three distinct groups late among the eucaryotes (Perasso et al. 1989).

To estimate the evolutionary relationship between rhodoplasts, cyanobacteria and chloroplasts more clearly, we sequenced the plastome-encoded Rubisco operon from *Porphyridium aerugineum* (Fig. 2). The sequences of the gene products (LSU and SSU of Rubisco) from *Porphyridium* have been compared to corresponding sequences from the cyanobacteria and

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1        MRLTQGTFSFLPDLTDAQIQKQVQYAVSKKWAVSVEYTDDPHPRN------------SFWELWGLPLFDVK
2    MQTLPKERRYETLSYLPPLTEVQIEKQAQYIRSQGYIPAVEFNEVSEPTE------------LYWTRWKLPWFGAK
3 MSMKTLPKERRFETFSYLPPLSDRQIAAQIEYMIEQGFHPLIEFNEHSNPEE------------FYWTMWKLPLFDCK
4     MQLRVERKFETFSYLPPLNDQQIARQLQYALSNGYSPAIEFSFTGKAED------------LVWTLWKLPLFGAQ
5 MQVWPPLGLKKFETLSYLPPLTTEQLLAEVNYLLVKGWIPPLEFEVKDGFVEHDKSPGYYDGRYRYWTMWKLPMFGGT
               * * ** *   *      *          *                       *  * ** *
                +      ++  + ++ +   +      +       +                       + + +
                       #       #     # #

 DASALMYEIAACRKAKPNYYIKVNAFDNTRGVESCCLSFIINRPINEPGFHLERQEVQGRNILYTIKSYAVNKPEGSRY
 TSREVLAEVQSCRSQYPGHYIGVVGFDNIK------------------------QCQILSFIVH--------KPS--RY
 SPQQVLDEVRECRSEYGDCYIRVAGFDNIK------------------------QCQTVSFIVH--------RKG--RY
 SPEEVLSEIQACKQQFPNAYIRVVAFDSIR------------------------QVQTLMFLVY--------KPL
 DPAQVVNEVKKAYPDAFVRFI---GFDNKR------------------------EVQCISFIAY--------KPA--GY
        *            *    **                                                   *
            ++   +  +  +    +                          +      +          ++   ++
 #                          # #                               # #        ##    #
```

Fig. 6. Comparison of the amino acid sequences of different SSU proteins from *Porphyridium aerugineum* (*1*), *Anabaena 7120* (*2*), *Anacystis nidulans* (*3*), *Cyanophora paradoxa* (*4*) and spinach (*5*). * identical residues; + identical residue between cyanobacteria and *Porphyridium*; # identical residue between spinach and *Porphyridium*; – gap introduced to give maximum homology)

higher plants (Fig. 5). We found homologies in the range of 51%–54% in the LSU protein between *Porphyridium*, higher plants and even cyanobacteria, whereas LSU homology between cyanobacteria and chloroplasts is about 86%. This demonstrates a larger evolutionary distance between rhodoplasts and recent cyanobacteria than between chloroplasts and cyanobacteria, which may be explained by one of the following: (1) the rhodoplasts branched off much earlier from the cyanobacterial phylogenetic tree than the chloroplasts. This explanation implies that the red algae are an older eucaryotic group than the green (chlorophyll *b*-containing) algae. (2) Different lines of cyanobacteria have led to rhodo- and chloroplasts.

A comparison of the SSU proteins supports the findings obtained with the LSU sequences, especially when structural features (insertions/deletions) are taken into account. The cyanobacterial SSU proteins contain 110–112 amino acids and have a relative molecular mass (M_r) of approximately 12,000 whereas the *Porphyridium* protein contains 138 amino acids and has a M_r of 15,900. SSU proteins of a similar M_r were found in some other red algae in our laboratory (*Cyanidium caldarium, Antithamnion spec., Porphyridium cruentum*; Kaling 1983). Figure 6 shows that the higher M_r is due to two insertions/deletions in the carboxy terminal region of the protein. SSU proteins from higher plants contain a characteristic insertion of 12 amino acids in the amino terminal region compared with the cyanobacterial and red algal proteins. Further sequence analyses of red algal *rbcS* genes are necessary in order to establish whether the insertions at the carboxy terminus of the *Porphyridium* SSU protein are typical of the red algae as a whole.

Sequence homologies between the different SSU proteins are presented in Fig. 5. As the higher plant *rbcS* genes are nuclear encoded whereas the *Porphyridium rbcS* gene is located in the plastome, different mutation rates in the nuclear and plastid genome must be taken into account when evaluating the degrees of homology. The homologies in the LSU proteins between *Porphyridium* and cyanobacteria (54%) are much lower than between cyanobacteria and higher plants (86%). In contrast, the homologies between the SSU proteins from *Porphyridium* and cyanobacteria (35%) are in the same range as those between higher plants and cyanobacteria (37%). This might be caused by a lower mutation rate in the plastome than in the nuclear genome.

To estimate whether the results found in *Porphyridium aerugineum* are typical of the red algae we are now sequencing the Rubisco operons from *Cyanidium caldarium* and *Antithamnion spec.* Further analyses of other plastid genes of red algae are in progress in our laboratory.

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Two recent studies (Douglas and Durnford (1989) Plant Mol Biol 13:13–20; Hwang & Tabita, Plant Mol Biol 13:69–79) demonstrate that in the Cryptomonade *Cryptomonas* Φ and in the Diatom *Cylindrotheca sp.* strain N1 the *rbcS* gene is located on the ptDNA closely adjacent to the *rbcL* gene, too. The SSU protein from both species has a M_r of approx. 15,000. The homology between the *Phorphyridium* and *Cryptomonas rbcS* genes is 72% on DNA and aa level respectively and both proteins are colinear except an insertion of one aa at position 115 in the *Cryptomonas* protein. Thus, the structural features of the *Porphyridium* SSU protein might therefore be typical not only for red algae but also for Cryptomonades. These data therefore strongly support the idea that the plastids of recent Cryptomonads derived from endosymbiotic unicellular red algae (like Porphyridium) as was previously suggested (Ludwig and Gibbs (1989), J Cell Biol 108:875–884