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The ribosomal RNA repeats are non-identical and directly oriented in the chloroplast genome of the red alga *Porphyra purpurea*

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Abstract. A detailed restriction map of the chloroplast genome of the red alga *Porphyra purpurea* has been constructed. Southern hybridization experiments with cloned or gel-purified restriction fragments and PCR products indicate that the *P. purpurea* chloroplast genome is approximately 188 kb in size. This circular molecule contains two rRNA-encoding repeats (approximately 4.9 kb) that separate the genome into single-copy regions of 34 kb and 144 kb. Interestingly, these repeats are arranged in a direct orientation. In addition, DNA sequencing of the ends of both repeats revealed that the two rRNA repeats are not identical. No intramolecular recombination between the repeats can be detected. We discuss the possibility that the chloroplast genome of *P. purpurea* is organized like that of the ancestral chloroplast.

Key words: Chloroplast genome – Direct repeats – Restriction map – Rhodophyte

Introduction

Although the endosymbiont theory for the origin of chloroplasts is well accepted (Gray and Doolittle 1982; Gray 1989), many questions still remain as to how the diversity of present-day chloroplasts has arisen. Among the most hotly debated of these questions is whether chloroplasts are monophyletic or polyphyletic in origin; that is, was there one, or more than one, prokaryotic, endosymbiotic ancestor of present-day chloroplasts (see Gray 1991 for review)? Recently, molecular biological data on chloroplast genes and genomes have been used to try to resolve this argument. Many phylogenetic trees based on single gene sequences have been constructed and used to support one or the other hypothesis (e.g., Valentin and Zetsche 1990; Douglas and Turner 1991). None of these trees can be interpreted as unequivocally eliminating either hypothesis, although the majority have been interpreted as supporting a monophyletic origin. As suggested by several authors (Gray 1991; Palmer 1991; Shivji et al. 1992), comparisons of the organization and gene content of diverse chloroplast genomes, rather than individual genes, may be more useful in determining chloroplast ancestry.

At present, only land-plant chloroplast genomes have been comprehensively studied with the complete sequencing of the chloroplast genomes of tobacco (Shinozaki et al. 1986), rice (Hiratsuka et al. 1989), the liverwort *Marchantia polymorpha* (Ohyama et al. 1986) and the non-photosynthetic plant *Epifagus virginiana* (Wolfe et al. 1992). Photosynthetic land-plant chloroplast genomes are generally similar in gene content, organization and size, with most in the range of 120–160 kb (see Palmer 1991 for review). The most striking feature of land-plant chloroplast genomes is the rRNA-containing inverted repeat structure. These repeats usually contain protein-encoding genes in addition to the rRNA operon and the length of the repeat, while usually 20 to 30 kb, can vary from 10 to 76 kb. In several land-plant lineages, however, one of the repeats has been lost. Among chlorophyte algae (those containing chlorophylls *a* and *b*), chloroplast genomes are much more varied in both size (ranging from 89 to approximately 400 kb) and organization (inverted repeats in some species, no repeats in others, and one-to-five tandem repeats in *Euglena*; see Palmer 1991). Extensive studies of several chlorophyte chloroplast genomes indicate more substantial genome rearrangements than occur in land-plant chloroplast genomes, as indicated by the breakup of several ancestral operons, the introduction of introns, and gene scrambling in the form of trans-splicing of genes. It appears that chlorophyte chloroplast genomes, but not necessarily their genes, are evolving under more relaxed constraints, and thus more rapidly, than those of land plants.

The chloroplast genomes of chromophyte algae (chlorophyll *a*/*c*-containing) tend to be slightly smaller than those of land plants, with most ranging from 115 to 150 kb in size (see Palmer 1991). At least one chromophyte, *Pylaiella littoralis*, has a chloroplast genome com-

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posed of two circular molecules that are 133 and 58 kb in size (Loiseaux-de Goër et al. 1988). All known chromophyte chloroplast genomes contain inverted repeats, although the size of the repeats tends to be smaller (5–22 kb) than those of land-plant chloroplasts. With one notable exception, gene identification in chromophyte chloroplast genomes has relied primarily on heterologous hybridization and thus knowledge of chloroplast gene content in these species is rudimentary. The exception is the chloroplast genome of *Cryptomonas* Φ, where more than 60 genes have been mapped, primarily through DNA sequencing (Douglas 1992).

Analyses of chloroplast genomes from chlorophyll *a*/phycobilisome-containing algae (Rhodophyta and Glaucophyta) are also fragmentary, with significant amounts of information only from the chloroplast genomes of *Cyanophora paradoxa* (e.g., Lambert et al. 1985; Bryant and Stirewalt 1990; Neumann-Spallart et al. 1990) and, to a much lesser extent, *Cyanidium caldarium* (Kessler et al. 1992; Maid and Zetsche 1992; Maid et al. 1992). Both of these organisms are distinct from the main rhodophyte group and are often classified in a separate taxon, the Glaucophyceae. True rhodophytes are usually placed in two subclasses, the more primitive Bangiophycidae and the more advanced Floridiophycidae. Chloroplast genome maps, each with about 20 genes localized, are available from one member of each of these two rhodophyte classes: *Porphyra yezoensis*, Bangiophycidae, and *Griffithsia pacifica*, Floridiophycidae (Li and Cattolico 1987; Shivji 1991; Shivji et al. 1992). These chloroplast genomes are estimated to be 185 and 178 kb in size, respectively, with only one rRNA operon present in the *G. pacifica* chloroplast genome while two small (< 7 kb) rRNA repeats, organized in an inverted fashion, are found in *P. yezoensis*. Preliminary studies on the chloroplast genome of *Chondrus crispus*, Floridiophycidae (Boyen et al. 1991) indicate that it is similar in size (198 kb) to other rhodophyte chloroplast genomes, but that it contains both one complete rRNA operon and an additional copy of the 16S rRNA gene.

Recently, we have detected a number of genes in the chloroplast genome of the rhodophyte, *Porphyra purpurea*, that are absent from the chloroplast genomes of land plants (Reith and Munholland 1991, 1983a, b; Reith 1992, 1993). This alga was originally referred to as *P. umbilicalis*, but a reinvestigation of its taxonomy (C. Bird, J. Munholland and M. Reith, unpublished results) suggests that *P. purpurea* is more appropriate (Bird and McLachlan 1992; Lindstrom and Cole 1992). In order to characterize this chloroplast genome more thoroughly and to understand its position in the evolution of chloroplasts, a detailed restriction map has been constructed and is presented in this communication. Unexpectedly, the chloroplast genome of *P. purpurea* is organized differently from any known chloroplast genome.

Material and methods

Plant material and methods for DNA purification, cloning, Southern hybridization, standard polymerase chain reaction (PCR) experiments and DNA sequencing were as described previously (Reith and Munholland 1991, 1993a, b). For the synthesis of the long PCR products shown in Fig. 3B, 0.4 units of Hot Tub Polymerase (Amersham) and the reaction buffer provided by the supplier were used in a 50-μl reaction as described (Kainz et al. 1992). The primers employed were based on sequences from cloned regions of the *P. purpurea* chloroplast genome. Their sequences are: #1-CGGGATTATTGGAGCCAATGG; #2-GCATACCGCCAGCGTTC; #3-GCACCCATCCCAAGGCACC; #4-GGTGCCTTGGGATGGGTGC; #5-ACTAAATCCTGGATCTCTGCAG; #6-CTGCAGGAGATCCAGATTTAGT. Cycle parameters were 30 cycles of 30 s at 94° C, 10 min at 65° C, except for reactions containing primer 2 which used 30 s at 94° C, 30 s at 55° C, 10 min at 65° C because the shorter length of primer 2 required a lower annealing temperature.

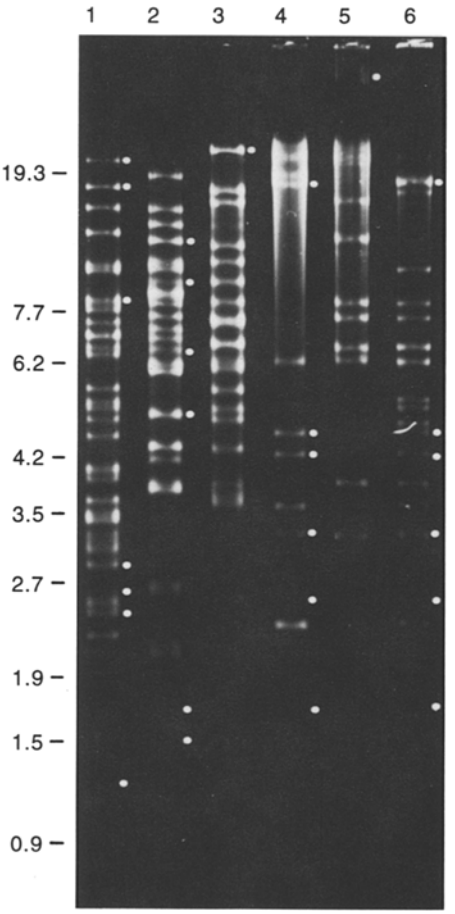


Fig. 1. Restriction enzyme analysis of *P. purpurea* light-band DNA with the enzymes *Eco*RI (*lane 1*), *Pst*I (*lane 2*), *Sac*I (*lane 3*), *Kpn*I (*lane 4*), *Sal*I (*lane 5*) and *Sal*I + *Kpn*I (*lane 6*). Size markers are in kb. Presumptive mitochondrial DNA bands are indicated by a *dot* to the right of the band

Results

Physical mapping

Total DNA from *P. purpurea* was separated into light and heavy bands by centrifugation through CsCl – Hoeschst 33258 gradients. Restriction digestion of the two bands indicated that the light band contained low-complexity DNA indicative of organellar DNA while the heavy band contained nuclear DNA. A clone bank of *Eco*RI-digested light band DNA was established in the vector λZAPII and used in the mapping of the *P. purpurea* chloroplast genome. Initially, 25 randomly selected clones were hy-

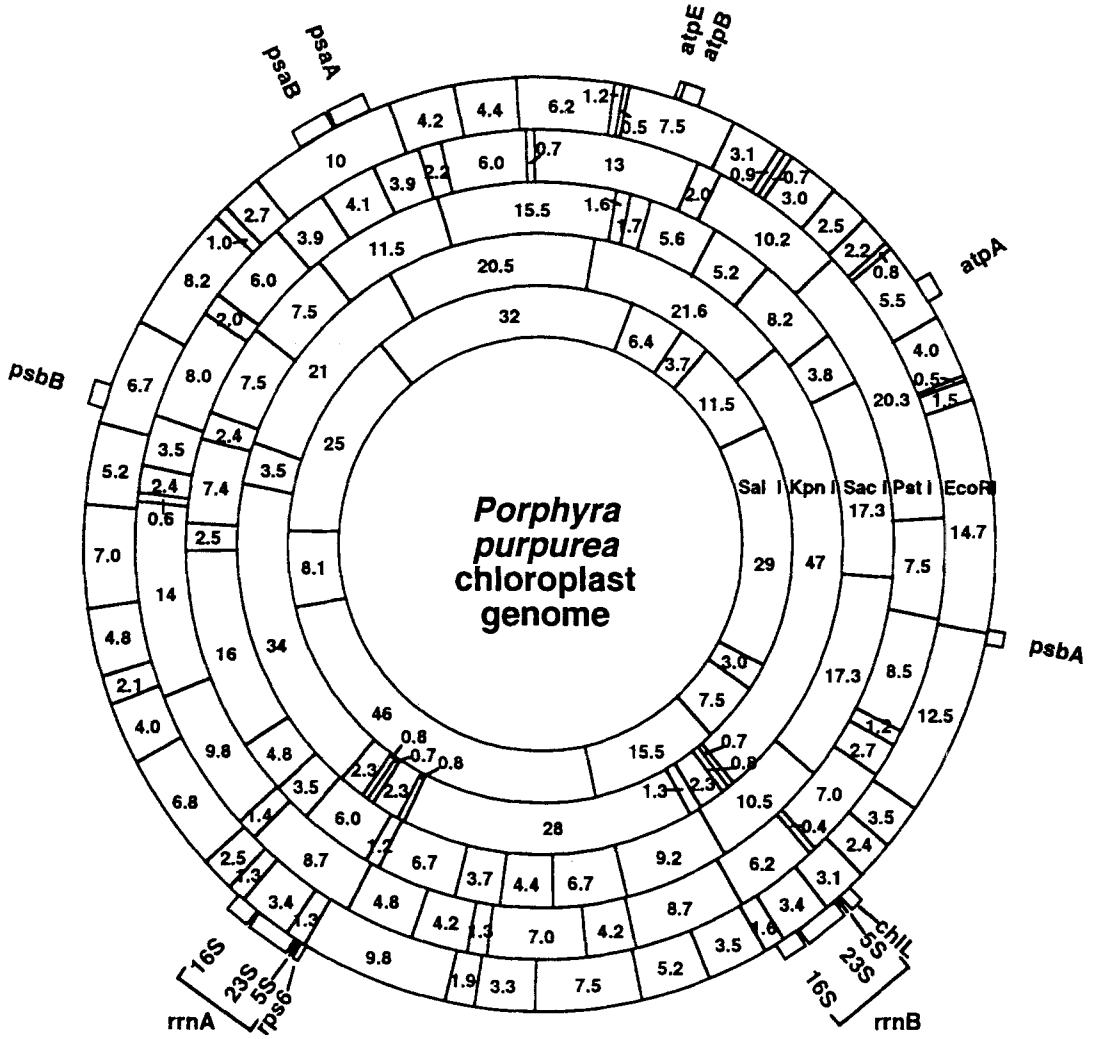


Fig. 2. Physical map of the *P. purpurea* chloroplast genome. *Numbers* indicate restriction fragment lengths in kb. Restriction enzyme sites mapped are (from the innermost circle): *Sal*I, *Kpn*I, *Sac*I, *Pst*I and *Eco*RI. A few genes are indicated for reference. See Reith and Munholland (1993b) for details on gene mapping

bridized to Southern blots of *P. purpurea* light band DNA digested with restriction enzymes as in Fig. 1. Both ends of the unique clones identified in this fashion were then sequenced. Additionally, selected *Eco*RI, *Sac*I, *Sal*I light-band DNA fragments were gel-purified and used as probes. Where appropriate, these fragments were also used for further screening of the clone bank. Newly selected clones were then checked by Southern hybridization and sequencing of both ends. As the mapping progressed, the DNA-sequence data were used to make oligonucleotide primers for PCR experiments that confirmed the orientation of fragments and/or generated further probes between mapped clones for Southern hybridization and clone-bank screening. The DNA-sequence data were also used in database searches to identify genes encoded on this DNA molecule. A large number of known, chloroplast DNA-encoded genes were identified through this process (see Reith and Munholland, 1993b), establishing that the mapped genome is from the chloroplast. Clones of all the chloroplast *Eco*RI fragments have now been isolated. The final map of the *P. purpurea* chloroplast genome shown in Fig. 2 has been completely confirmed through PCR experiments.

Not all of the restriction fragments found in digests of *P. purpurea* light-band DNA are accounted for in the map shown in Fig. 2. The DNA fragments marked with dots in Fig. 1 do not physically map to the chloroplast genome (M. Reith and J. Munholland, unpublished results). Preliminary DNA-sequence data (G. Burger, M. Reith and F. Lang, unpublished results) indicate that these fragments encode mitochondrial genes. Summation of restriction fragment sizes from each restriction digest, except for *Eco*RI, yields a total of approximately 35 kb for the putative *P. purpurea* mitochondrial genome. The *Eco*RI fragments sum to approximately 60 kb, apparently due to incomplete digestion at one site, possibly because of partial methylation or microheterogeneity. The largest *Eco*RI fragment (25 kb) hybridizes to the second (17 kb) and third (8 kb) fragments (M. Reith and J. Munholland, unpublished results) and thus accounts for the extra 25 kb in the *Eco*RI size estimate. In addition, there do not appear to be any *Sal*I restriction sites in this ge-

nome as shown by the presence of a high-molecular-weight smear in the *Sal*I lane and identical hybridization patterns in the *Kpn*I and *Kpn*I + *Sal*I digestions (lanes 4 and 6, Fig. 1).

The chloroplast genome of *P. purpurea* is approximately 188 kb in length. It is organized as a circular molecule and contains two repeats encoding the rRNA operon (designated *rrnA* and *rrnB* in Fig. 2). No other repeated DNA sequences were detected during the construction of the restriction map. The repeats separate the remainder of the genome into two single-copy regions of approximately 144 and 34 kb. The repeats are short (approximately 4.9 kb) and are oriented as direct repeats. To our knowledge, this is the first report of a chloroplast genome with the repeat regions organized in this fashion; all other repeat-containing chloroplast genomes have inverted repeats, except in *Euglena* where one-to-five tandem copies of the rRNA operon are present (Hallick and Buetow 1989).

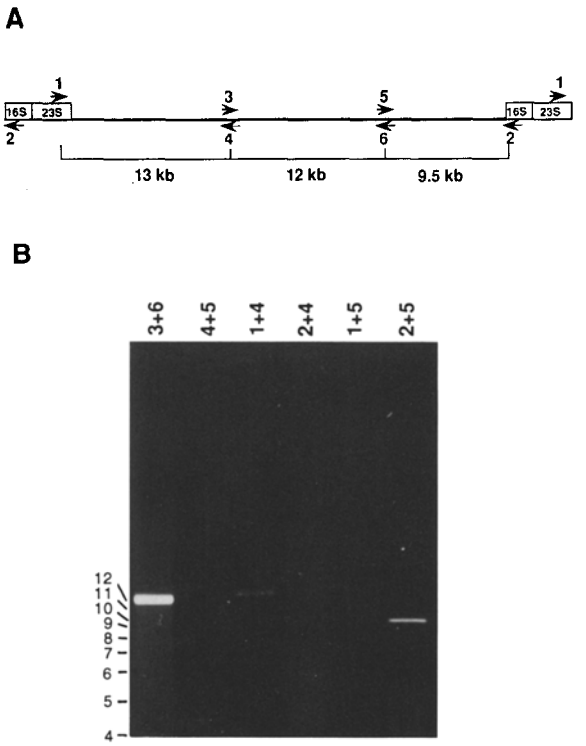


Fig. 3 A, B. Orientation of rRNA repeats in the *P. purpurea* chloroplast genome. **A** Experimental strategy. *Numbered arrows* represent the position and orientation of the primers used. **B** Results of PCR experiments. Primer combinations used are indicated at the top of each lane. Size markers are in kb

Direct repeats

To verify that the repeats are in fact organized as direct repeats, PCR experiments were performed across the entire small single-copy region. To accomplish this, Hot Tub DNA polymerase (Amersham), which can synthesize PCR products up to 15 kb in length (Kainz et al. 1992), and three pairs of oligonucleotide primers were used. The strategy of these experiements is described in Fig. 3A. Two of the primer pairs are exact complements of each other. Primers 3 and 4 are located approximately 13 kb from the *rrnA* operon while primers 5 and 6 are approximately 25 kb from this operon. Primers 1 and 2 located at the 23S and 16S ends of the repeat regions, respectively, and both are oriented with their 3′ termini directed out of the repeat region. To establish the orientation of the central primer pairs, PCR experiments using either primers 3 and 6 or 4 and 5 were done. As can be seen in Fig. 3B, only the reaction with primers 3 and 6 resulted in a product of the expected length (approximately 12 kb). Primers 4 and 5 were than separately paired with either primer 1 or 2 to determine the orientation of the rRNA repeats relative to the center of the small single-copy region. Primer 4 (oriented toward *rrnA*) generated the expected product (approximately 13 kb) when paired with primer 1, while primer 5 only produced the expected 9.5 kb band when paired with primer 2 (Fig. 3B). These results confirm the direct orientation of the rRNA repeats in the *P. purpurea* chloroplast genome.

Non-identical repeats

In each rRNA repeat, *Eco*RI cuts twice to produce three rRNA operon-containing fragments (Fig. 2). Consequently, the *Eco*RI fragments containing the 5′ and 3′ ends of each repeat are easily distinguished. In order to investigate how far outside the 16S and 5S rRNA genes the repeats extended, we sequenced the appropriate regions of the four cloned *Eco*RI fragments containing the 5′ ends of the rRNA repeats (1.3 and 1.6 kb) and 3′ ends (1.3 and 3.1 kb). The aligned sequences from *rrnA* and *rrnB* are shown in Fig. 4 for the 5′ end of the repeat and Fig. 5 for the 3′ end. At both ends of the rRNA repeat, the DNA sequences diverge within a few base pairs of the ends of the mature rRNA, which were determined by alignment with other sequences. The conserved sequences outside the mature rRNAs are probably required for the appropriate processing of the primary transcript into the mature rRNAs. More interestingly, it is apparent that a small percentage of nucleotides differ within the coding regions of the rRNAs. In the 632 bp of the 16S rRNA gene sequenced, there are seven nucleotide changes while there are five differences in 713 bp of the 23S rRNA gene. Four substitutions can also be seen in the 5S rRNA gene (121 bp) as well as three changes in the 36 bp 23S–5S spacer region. All but 2 of the 19 differences detected are C↔T or A↔G substitutions. In the 23S rRNA sequence (Fig. 5), there is T↔A substitution at position 15, while at position 677, a C↔A change occurs. Five of the changes in the 16S rRNA gene (positions 82, 166, 200, 395 and 470, Fig. 4), two in the 23S rRNA gene (positions 511 and 675, Fig. 5), and two in the 5S rRNA gene (positions 810 and 842, Fig. 5) occur in base-paired regions of these rRNAs, but none of these substitutions disrupt the complementary interactions in these helices. At these positions, either a G residue is paired with a C in one rRNA version, but with a U in the other, or a U is paired with either an A or a G.

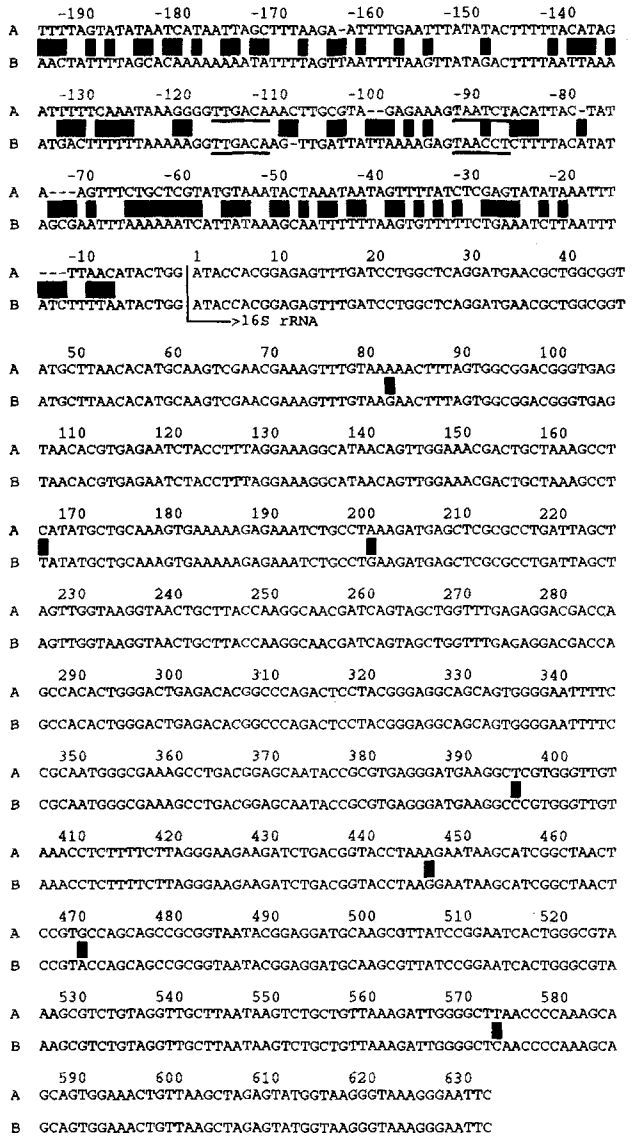


Fig. 4. DNA sequences at the 5′ end of the rRNA repeat regions. *A* sequence from the *rrnA* operon. *B* sequence from the *rrnB* operon. Nucleotides that differ between the two sequences are indicated with a *black box* between the two sequences. Putative promotor regions are *underlined*. Numbering begins from the first position of the mature 16S rRNA. These sequences have been deposited in the Genbank DNA sequence database under accession numbers L07257 and L07258

The observation that the sequences of the repeats deverge within a few bases of the ends of the mature 16*s* and 5S rRNAs suggests that the promotor and transcription-terminator elements might differ between the two repeats. Approximately 75 bp upstream of the 5′ end of the 16S rRNA gene is a region of relatively-high sequence conservation (69% identical between positions −125 to −75). Within this region are sequences similar to the canonical −35 and −10 regions of *E. coli* promoters (positions −117 to −112 and −92 to −87, respectively in Fig. 4). The region of similarity extends from just upstream of the presumptive −35 segment to just downstream from where the primary transcript would be expected to start. While the presumptive −35 region is identical for each repeat, the −10 regions differ at one

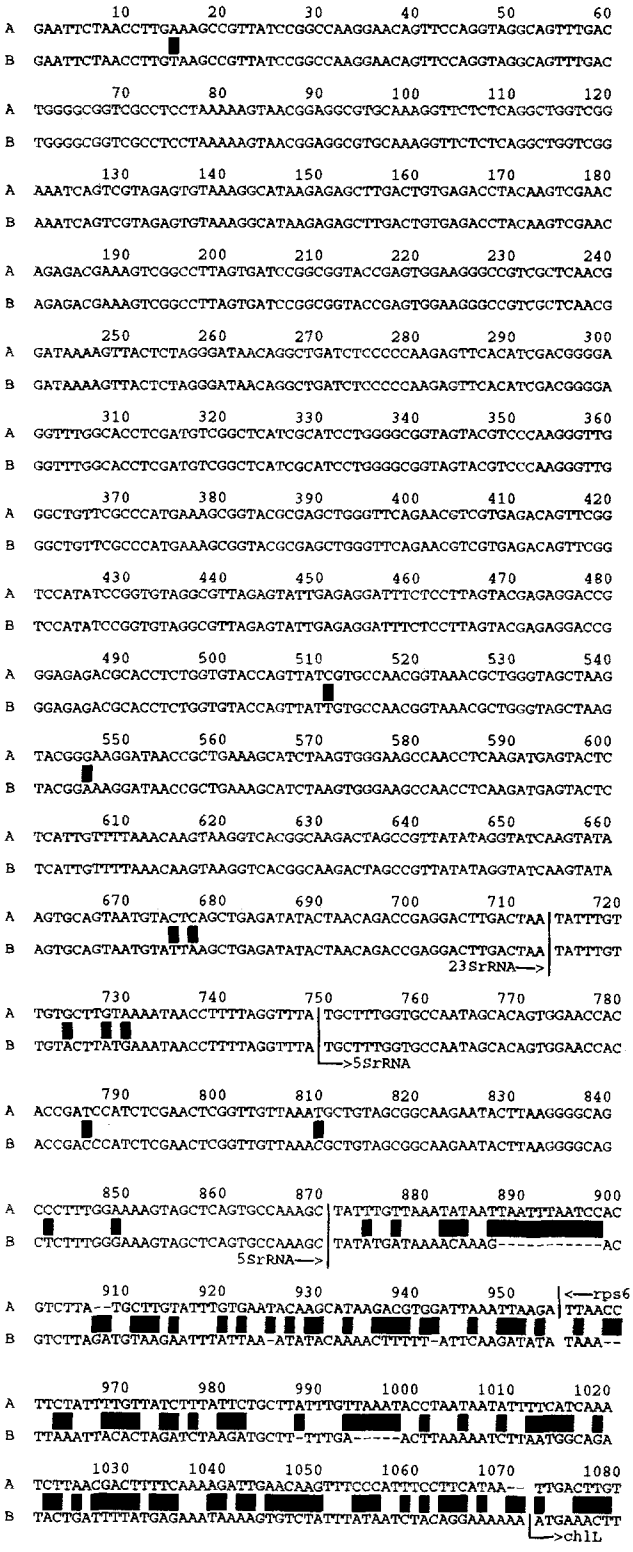
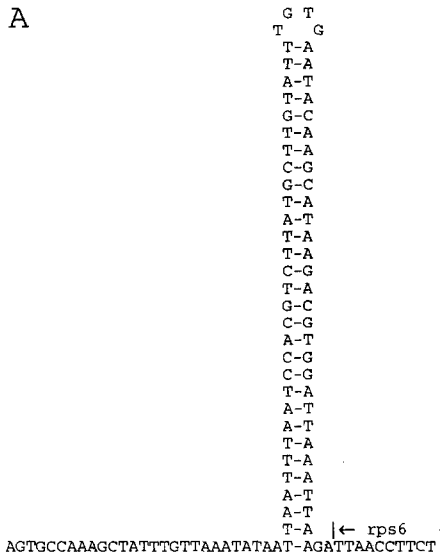


Fig. 5. DNA sequences at the 3′ end of the rRNA repeat regions. *A* sequence from the *rrnA* operon. *B* sequence from the *rrnB* operon. Nucleotides that differ between the two sequences are indicated with a *black box* between the two sequences. The ends of genes are indicated by *vertical lines*. These sequences have been deposited in the Genbank DNA sequence database under accession numbers L07259 and L07260



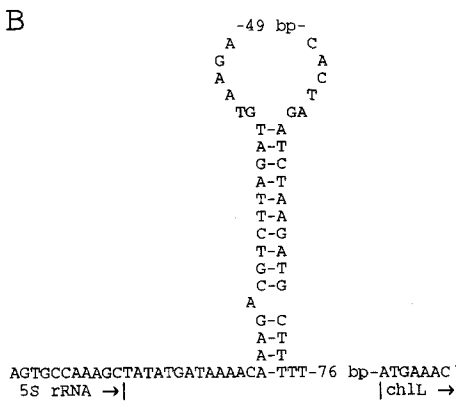


Fig. 6 A, B. Potential secondary structures of the stem-loop regions at the 3′ ends of the *rrnA* (**A**) and *rrnB* (**B**) operons. The ends of genes are indicated by the *vertical lines*

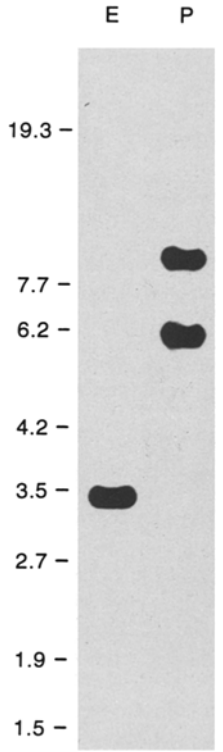


Fig. 7. Test for recombination between the rRNA repeats. Southern hybridization of *P. purpurea* light-band DNA, digested with *Eco*RI (*E*) or *Pst*I (*P*), with an rRNA repeat-internal probe. Size markers are in kb

position. In addition, the spacing between the two regions differs by one nucleotide (17 vs 18 bp) in the two repeats. Approximately 20 bp upstream of the −35 region is another segment of high sequences conservation (74% identical over 31 positions). This region may also play a role in controlling the transcription of the rRNA operons.

At the 3′ ends of the repeats, the sequence similarity disappears approximately 11 bases downstream from the 3′ end of the 5S rRNA gene. The regions downstream from both repeats can be folded into stem-and-loop structures, although the two structures are very different (Fig. 6). The stem/loop following *rrnA* has a 30 base stem region with a loop of only four bases while the structure following *rrnB* consists of a 14 base stem and 61 base loop. The *rrnA* stem/loop structure is more typical of a strong rho-independent terminator than the *rrnB* structure. This may be due to the orientation of the genes following the repeats. The gene downstream from *rrnA* (*rps6*) is oriented in the opposite direction (Fig. 6) while the gene downstream from *rrnB* (*chlL*) is oriented in the same direction. Termination of the primary rRNA transcript is probably much more critical after *rrnA* and thus requires a strong termination signal that can probably be used for the termination of both the *rrnA* and *rps6* transcripts.

Absence of recombination between repeats

In inverted repeat-containing chloroplast genomes, intramolecular recombination between the repeat regions results in two populations of molecules that differ only in the orientation of the small single-copy region (see Palmer 1985). This "flip-flop" isomerization can be detected by hybridizing repeat-internal restriction fragments to restriction digests performed with enzymes that do not cut within the repeat region. When this isomerization occurs in inverted repeat-containing genomes, four rather than two restriction fragments hybridize and the molar concentration of each of these fragments is one-half that of the other restriction fragments. In the case of a genome where the repeats are directly oriented, recombination would result in the loss of the small single-copy region (assuming the origin of replication is the large single-copy region) and the generation of a third, intermediate-sized, repeat-containing fragment. The stoichiometry of this third fragment, as well as those of the fragments from the small single-copy region, would depend on the percentage of genomes in which recombination has occurred. In order to test whether intramolecular recombination occurs in the *P. purpurea* chloroplast genome, a *Pst*I digest, which results in two fragments (8.7 and 6.2 kb) that span the repeat region (Fig. 2), was hybridized with a 3.4 kb *Eco*RI fragment that is internal to the repeat. As seen in Fig. 7, only the two expected *Pst*I

fragments hybridize, demonstrating that intramolecular recombination does not occur in the *P. purpurea* chloroplast genome.

Discussion

Extensive analyses by Southern hybridization, PCR and DNA sequencing have resulted in the construction of a detailed physical map of the *P. purpurea* chloroplast genome. This genome is an approximately 188 kb circular molecule, typical of the size estimated for other rhodophytes, but larger than the *C. paradoxa* cyanelle genome and the chloroplast genomes from most land plants, chromophytes, and some green algae. On the other hand, the size of the rRNA repeat, which is similar to those from *P. yezoensis*, *Cryptomonas* Φ and several chromophytes, is smaller than that of land plants, green algae and *C. paradoxa*. This results in a much-larger coding capacity for the *P. purpurea* chloroplast genome (Reith and Munholland 1993 b).

A unique feature of this chloroplast genome is the organization of the rRNA repeats in a direct, nontandem orientation. This type of genome organization has not been previously characterized in any chloroplast genome. Most chloroplast genomes studied contained inverted repeats that undergo intramolecular recombination resulting in two populations of the chloroplast genome that differ in the orientation of the small single-copy region. Plant mitochondrial genomes, which contain both direct and inverted repeats, also undergo recombination between each type of repeat (see Newton 1988, for review). However, recombination between the direct repeats results in a complex population of subgenomic molecules that are derived from the master genome. By testing for recombination between the direct repeats of the *P. purpurea* chloroplast genome (Fig. 7), we have shown that subgenomic molecules are not present. This observation indicates that intramolecular recombination is rare or absent in the *P. purpurea* chloroplast genome, perhaps because the loss of essential genes in the small single-copy region is deleterious.

A second unusual feature of the *P. purpurea* chloroplast genome is the presence of non-identical rRNA operons. In all known chloroplast genomes containing inverted repeats, the rRNA operons are identical. Only in *E. gracilis*, in which the rRNA repeats are arranged tandemly, have differences been detected between the individual repeats (Karabin et al. 1983). In chloroplast genomes containing inverted repeats, there appears to be a copy-correction mechanism to ensure the identity of the repeats. That both the recombination and copy-correction systems are absent in the *P. purpurea* chloroplast genome (and presumably in *E. gracilis* as well) suggests that these two processes may be functionally releated. That is, formation of a heteroduplex through recombination may be necessary for the copy-correction process to occur. This would be similar to the gene-conversion process first observed in ascomycete fungi (Kourilsky 1986). Such a process might also explain the mechanism for the expansion of the inverted repeat region seen in some angiosperm chloroplasts if the gene conversion process continued past the original ends of the repeat.

Evolutionary implications

If one assumes that chloroplasts arose monophyletically [as we present evidence for elsewhere (Reith and Munholland 1993 b)], the presence of direct, non-identical repeats in a primitive rhodophyte may have significant implications for the organization of the ancestral chloroplast genome. Based on the available data on the arrangement of chloroplast genomes, the most parsimonious interpretation of the organization of the ancestral chloroplast genome would be that it had rRNA operons organized as inverted repeats, and that at various times in the evolution of chloroplasts one of the repeats was inverted (to create direct repeats) or was lost. However, even among inverted repeat-containing chloroplast genomes, the rRNA repeats vary in orientation and location relative to the small single-copy region (see Palmer 1991), suggesting that there may not have been a common, inverted repeat-containing ancestor of all chloroplasts. This has led Palmer (1991) to suggest that the ancestral chloroplast may have contained only one copy of the rRNA operon that was later independently duplicated in each chloroplast lineage, even though two cyanobacteria, *Anacystis nidulans* (Tomioka et al. 1981) and *Anabaena* sp. (Bancroft et al. 1998), are known to have two rRNA operons.

Our data suggest a third alternative: that the ancestral chloroplast had two direct, non-identical rRNA repeats that have been maintained in lower rhodophytes. During the establishment of chlorophyte, chromophyte and glaucophyte chloroplasts, inversions occurred to reorganize these genomes into the inverted repeat type. Subsequently, in at least the chlorophyte and rhodophyte lineages, one of the repeats has been lost in some species. Thus, the direct organization of the rRNA repeats and the absence of copy correction or repeat expansion would be ancestral characteristics under this scenario. The observations that, in eubacteria, the rRNA repeats are limited to the rRNA genes and and associated tRNA genes and that, in at least *E. coli* (Carbon et al. 1979) and *Rhodobacter sphaeroides* (Dryden ana Kaplan 1990), the rRNA repeats are non-identical, provide support for this hypothesis. Unfortunately, there is very little data on the sequence and orientation of the rRNA operons in cyanobacteria, making it difficult to determine whether these characteristics reflect the genome organization of the progenitors of chloroplasts and are thus ancestral. An additional problem with this hypothesis is the scarcity of data on rhodophyte chloroplast genome organization and the seemingly contradictory organization of the inverted repeat-containing *P. yezoensis* chloroplast genome. As discussed previously (Reith and Munholland 1993 b), only three rhodophyte chloroplast genomes have been mapped and the chloroplast gene map of *P. yezoensis* (Shivji 1991) is vastly different from those of either of the other two known rhodophytes, *P. purpurea* or *G. pacifica*, which have fairly similar gene orders. This suggests either

an unusual amount of reorganization of the *P. yezoensis* chloroplast genome or problems with the gene map. Further analysis of the organization of cyanobacterial and algal, particularly rhodophyte, chloroplast genomes will be necessary to better understand the probable organization of the ancestral chloroplast genome and thus the evolution of chloroplasts.

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