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

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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-tofive 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 transsplicing 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-

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. Cvanidium 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, 1993 a, b). For the synthesis of the long PCR



Fig. 1. Restriction enzyme analysis of *P. purpurea* light-band DNA with the enzymes EcoRI (*lane 1*), PstI (*lane 2*), SacI (*lane 3*), KpnI (*lane 4*), SalI (*lane 5*) and SalI + KpnI (*lane 6*). Size markers are in kb. Presumptive mitochondrial DNA bands are indicated by a *dot* to the right of the band

products shown in Fig. 3 B, 0.4 units of Hot Tub Polymerase (Amersham) and the reaction buffer provided by the supplier were used in a 50- μ l 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-GGTGCCTTGGGAT-GGGTGC; #5-ACTAAATCCTGGATCTCTGCAG; #6-CTGC-AGGAGATCCAGATTTAGT. 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.

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 λ ZAPII 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): *SalI*, *KpnI*, *SacI*, *PstI*

and EcoRI. 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 EcoRI, SacI, SalI 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 EcoRI 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 EcoRI, yields a total of approximately 35 kb for the putative *P. purpurea* mitochondrial genome. The EcoRI fragments sum to approximately 60 kb, apparently due to incomplete digestion at one site, possibly because of partial methylation or microheterogeneity. The largest EcoRI 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 EcoRI size estimate. In addition, there do not appear to be any Sall restriction sites in this genome as shown by the presence of a high-molecularweight smear in the SalI lane and identical hybridization patterns in the KpnI and KpnI + SalI 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 repeats, except in *Euglena* where one-to-five tandem copies of the rRNA operon are present (Hallick and Buetow 1989).

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 singlecopy 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, EcoRI cuts twice to produce three rRNA operon-containing fragments (Fig. 2). Consequently, the EcoRI 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 EcoRI fragments containing the



Fig. 3A, 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

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 $C \leftrightarrow T$ or $A \leftrightarrow G$ substitutions. In the 23S rRNA sequence (Fig. 5), there is $T \leftrightarrow A$ substitution at position 15, while at position 677, a C \leftrightarrow 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.

	-190	-180	-170	-160	-150	-140
A	TTTTAGTATAT			-ATTTIGAAT.		
в	AACTATTTAG	CACAAAAAAA	ATATTTTAGT	TAATTTTAAG	TATAGACTT	ГТААТТААА
	-130	-120	-110	-100	-90	-80
~	ATTTTCAAA	AAAGGGGTTG	ACAAACTIGC		GIARICIACI	
B	ATGACTITITT.	AAAAAGGTTG	ACAAG-TTGA	TTATTAAAAG /	AGTAACCTCT	TTACATAT
	-70	-60	-50	-40	-30	-20
A		GCTCGTATGT.				
в	AGCGAATTTAA	AAAATCATTA'	TAAAGCAATT	TTTTAAGTGT	PTTTCTGAAA'	FCTTAATTT
2	-10	1 cmcclamacci		20 #G&#CO#COC</th><th>30 Caccameraa</th><th>40 Comedecem</th></tr><tr><th>n</th><th></th><th>CIGG AIACC</th><th>NCOGRGRGITI</th><th>IGAICCIGGC</th><th>CASSAIGAA</th><th>.001000001</th></tr><tr><th>в</th><th>ATCTTTTAATA</th><th>CTGG ATACC</th><th>ACGGAGAGTT 165 rRNA</th><th>TGATCCTGGC</th><th>ICAGGATGAA</th><th>CCTGGCGGT</th></tr><tr><th></th><th>50</th><th>60</th><th>70</th><th>80</th><th>9.0</th><th>100</th></tr><tr><th>A</th><th>ATGCTTAACAC</th><th>ATGCAAGTCG</th><th>AACGAAAGTT</th><th>TGTAAAAACT</th><th>TAGTGGCGG</th><th>ACGGGTGAG</th></tr><tr><th>в</th><th>ATGCTTAACAC</th><th>ATGCAAGTCG</th><th>AACGAAAGTT</th><th>TGTAAGAACT</th><th>TAGTGGCGG</th><th>ACGGGTGAG</th></tr><tr><th></th><th>110</th><th>120</th><th>130</th><th>140</th><th>150</th><th>160</th></tr><tr><th>A</th><td>TAACACGTGAG</td><td>AATCTACCTT</td><td>TAGGAAAGGC.</td><td>ATAACAGTTG</td><td>BAAACGACTG</td><td>CTAAAGCCT</td></tr><tr><th>в</th><th>TAACACGTGAG</th><th>AATCTACCTT</th><th>PAGGAAAGGC.</th><th>ATAACAGTTG</th><th>BAAACGACTG</th><th>TAAAGCCT</th></tr><tr><th></th><th>170</th><th>180</th><th>190</th><th>200</th><th>210</th><th>220</th></tr><tr><th>A</th><th>CATATGCTGCA</th><th>AAGTGAAAAA</th><th>GAGAAATCTG</th><th>CCTAAAGATG</th><th>AGCTCGCGCC</th><th>FGATTAGCT</th></tr><tr><th>в</th><th>TATATGCTGCA</th><th>алстсалала</th><th>GAGAAATCTG</th><th>CCTGAAGATG</th><th>AGCTCGCGCC</th><th>IGATTAGCT</th></tr><tr><th></th><th>230</th><th>240</th><th>250</th><th>260</th><th>270</th><th>280</th></tr><tr><th>A</th><th>AGTIGGTAAGG</th><th>TAACIGCITA</th><th>CAAGGCAAC</th><th>GATCAGTAGC</th><th>I'GG1-1-1'GAGA</th><th>JGACGACCA</th></tr><tr><th>в</th><th>AGTTGGTAAGG</th><th>TAACTGCTTA</th><th>CCAAGGCAAC</th><th>GATCAGTAGC'</th><th>rggtttgaga</th><th>JGACGACCA</th></tr><tr><th></th><th>290</th><th>300</th><th>310</th><th>320</th><th>330</th><th>340</th></tr><tr><th>A</th><th>GULACAUIGGG</th><th>ACTGAGACAC</th><th>GCCCAGACT</th><th>CCTACGGGAG</th><th>SCAGCAG16G</th><th>JGAATTTTC</th></tr><tr><th>в</th><th>GCCACACTGGG</th><th>ACTGAGACAC</th><th>GGCCCAGACT</th><th>CCTACGGGAG</th><th>GCAGCAGTGG</th><th>JGAATTTTC</th></tr><tr><th></th><th>350</th><th>360</th><th>370</th><th>380</th><th>390</th><th>400</th></tr><tr><th>n</th><th>CGCAAIGGGCG</th><th>AAAGCUIGAU</th><th>GGAGCAATAC</th><th>COCGIGAGGG</th><th></th><th>310001101</th></tr><tr><th>в</th><th>UGCAATGGGCG</th><th>AAAGCCTGAC</th><th>GGAGCAATAC</th><th>CGCGTGAGGG</th><th>ATGAAGGCCC</th><th>JIGGGTTGT</th></tr><tr><th>a</th><th>410</th><th>420</th><th>430</th><th>440 CCTACCTAAA</th><th>450</th><th>460</th></tr><tr><th>-</th><th></th><th></th><th></th><th></th><th></th><th></th></tr><tr><th>в</th><th>AAACCTCTTTT</th><th>CTTAGGGAAG</th><th>AAGATCTGAC</th><th>GGTACCTAAG</th><th>GAATAAGCAT</th><th>CGGCTAACT</th></tr><tr><th>A</th><th>470 CCGTGCCAGCA</th><th>480 GCCGCGGTAA</th><th>490 TACGGAGGAT</th><th>500 GCAAGCOTTA</th><th>510 FCCGGAATCA</th><th>520 CTGGGCGTA</th></tr><tr><th>в</th><td>CCGTACCAGCA</td><td>GCCGCGGTAA</td><td>TACGGAGGAT</td><td>GCAAGCGTTA</td><td>ICCGGAATCA</td><td>CTGGGCGTA</td></tr><tr><th></th><th>530</th><th>540</th><th>550</th><th>560</th><th>570</th><th>590</th></tr><tr><th>A</th><th>AAGCGTCTGTA</th><th>GGTTGCTTAA</th><th>TAAGTCTGCT</th><th>GTTAAAGATT</th><th>GGGCTTAAC</th><th>CCCAAAGCA</th></tr><tr><th>в</th><th>AAGCGTCTGTA</th><th>GGTTGCTTAA</th><th>TAAGTCTGCT</th><th>GTTAAAGATT</th><th>GGGCTCAAC</th><th>CCCAAAGCA</th></tr><tr><th></th><th>590</th><th>600</th><th>610</th><th>620</th><th>630</th><th></th></tr><tr><th>A</th><th>GCAGTGGAAAC</th><th>TGTTAAGCTA</th><th>GAGTATGGTA</th><th>AGGGTAAAGG</th><th>GAATTC</th><th></th></tr><tr><th>в</th><th>GCAGTGGAAAC</th><th>TGTTAAGCTA</th><th>GAGTATGGTA</th><th>AGGGTAAAGG</th><th>GAATTC</th><th></th></tr></tbody></table>		

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 16s and 5S rRNAs suggests that the promotor and transcriptionterminator 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

	10	20	30	40	50	60
A	GAATTCTAACCTTG.	AAAGCCGTT	ATCCGGCCAAG	GAACAGTTCO	CAGGTAGGCA	GTTTGAC
в	GANTTOTALCOTTO	TAAGCCGTT	AMCCGGCCA A/	2628202699900	ACCTACCA	ማማማስረ እር
2			AI GOOGGILA	Sonnandi i di	MOOTAGGEA	31110AC
	70	80	90	100	110	120
A	TGGGGCGGTCGCCT	CCTAAAAAG	TAACGGAGGC	JIGCAAAGGTI	PCTCTCAGGC	IGGTCGG
в	TGGGGCGGTCGCCT	CCTAAAAAG	TAACGGAGGC	TGCAAAGGT	ICTCTCAGGC	TGGTCGG
	130	140	150	160	170	180
A	AAATCAGTCGTAGA	GIGIAAAGG	CATAAGAGAGG	CITGACTGIGA	AGACCTACAA	GTCGAAC
в	AAATCAGTCGTAGA	GTGTAAAGG	CATAAGAGAG	CTTGACTGTG	GACCTACAA	GTCGAAC
	100	200	210			
A	AGAGACGAAAGTCG	200 CCTTAGTG	210 ATCCGGCGGT3		230 AGGGCCGTCG	240
B	AGAGACGAAAGTCG	SCCTTAGTG	ATCCGGCGGT	ACCGAGTGGA	AGGGCCGTCG	CTCAACG
	250	260	270	280	290	300
A	GATAAAAGTTACTC	TAGGGATAA	CAGGCTGATCI	CCCCCAAGAC	TTCACATCG	ACGGGGA
~	()					
Б	GATAAAAGTTACIC	IAGGGATAA	CAGGCIGAICI	ICCCCCARGAG	FICACATUG.	ACGGGGA
	310	320	330	340	350	360
A	GGTTTGGCACCTCG	ATGTCGGCT	CATCGCATCCI	rgggggggtad	TACGTCCCA	AGGGTTG
в	GGTTTTGGCACCTCG	ATCTCCCCT	CATCOCATCC	RAGAGAGE	TACGTCCCA	ACCOUNC
-	30111000100100		enregenree	COOGCOOTAC	JINCOLCCON	1999119
	370	380	390	400	410	420
A	GGUTGTICGCCCAT	GAAAGCGGT	ACGCGAGCTG	JG1 ICAGAACG	FICGIGAGAC	AGTTCGG
в	GGCTGTTCGCCCAT	GAAAGCGGT	ACGCGAGCTG	GTTCAGAACO	TCGTGAGAC	AGTTCGG
A	43V TCCATATCCGCTCT	440 AGGCGTTAG	450	460 നേനന്ന് മോട	4/0 [TAGTACCAG	480
	1001111100001011	1000011110	NOTHI CHOR	John Ticree	INGIACOAG	nooneeo
в	TCCATATCCGGTGT.	AGGCGTTAG	AGTATTGAGAG	GATTTCTCCI	TAGTACGAG	AGGACCG
	490	500	510	520	530	540
A	GGAGAGACGCACCT	CTGGTGTAC	CAGTTATCGT	GCCAACGGTA	ACCTOGGT	AGCTAAG
_						
в	GGAGAGACGCACCT	CIGGIGIAC	CAGTTATTGTC	GCCAACGGTAA	ACGCTGGGT	AGCTAAG
	550	560	570	580	590	600
A	TACGGGAAGGATAA	CCGCTGAAA	GCATCTAAGT	GGAAGCCAAG	CTCAAGATG	AGTACTC
в	TACCCADACCATA					a cima cimo
5	INCOGRAMOGAIAA	CCGCIGNAN	GCATCIAAGI	GGAAGCCAA	CICAAGAIG	MGIACIC
	610	620	630	640	650	660
A	610 TCATTGTTTTAAAC	620 Aagtaaggt	630 CACGGCAAGAG	640 CTAGCCGTTAT	650 FATAGGTATC	660 AAGTATA
А В	610 TCATTGTTTTAAAC. TCATTGTTTTAAAC.	620 AAGTAAGGT AAGTAAGGT	630 CACGGCAAGAG CACGGCAAGAG	640 Stageogttat Stageogttat	650 FATAGGTATC. FATAGGTATC.	660 AAGTATA AAGTATA
A B	610 TCATTGTTTTAAAC. TCATTGTTTTAAAC.	620 AAGTAAGGT AAGTAAGGT	630 CACGGCAAGAG CACGGCAAGAG	640 CTAGCCGTTAT CTAGCCGTTAT	650 FATAGGTATC. FATAGGTATC.	660 AAGTATA AAGTATA
A B A	610 TCATTGTTTTAAAC. TCATTGTTTTAAAC. 670 AGTGCAGTAATGTA	620 AAGTAAGGT AAGTAAGGT 680 CTCAGCTGA	630 CACGGCAAGAG CACGGCAAGAG 690 GATATACTAAG	640 CTAGCCGTTAT CTAGCCGTTAT 700 CAGACCGAGGG	650 FATAGGTATC, FATAGGTATC, 710 ACTTGACTAA	660 AAGTATA AAGTATA 720
A B A	610 TCATTGTTTTTAAAC. TCATTGTTTTTAAAC. 670 AGTGCAGTAATGTA	620 AAGTAAGGT AAGTAAGGT 680 CTCAGCTGA	630 CACGGCAAGAG CACGGCAAGAG 690 GATATACTAAG	640 TAGCCGTTAT TAGCCGTTAT 700 CAGACCGAGGJ	650 FATAGGTATC. FATAGGTATC. 710 ACTTGACTAA	550 RAGTATA AAGTATA 720 TATTTGT
A B A B	610 TCATTGTTTTAAAC. TCATTGTTTTAAAC. 670 AGTGCAGTAATGTA AGTGCAGTAATGTA	620 AAGTAAGGT AAGTAAGGT 680 CTCAGCTGA TTAAGCTGA	630 CACGGCAAGAG CACGGCAAGAG 690 GATATACTAAG GATATACTAAG	640 TAGCCGTTAT TAGCCGTTAT 700 CAGACCGAGGA CAGACCGAGGA	650 FATAGGTATC. FATAGGTATC. 710 ACTTGACTAA	550 AAGTATA AAGTATA 720 TATTTGT TATTTGT
A B A B	610 TCATTGTTTTAAAC. TCATTGTTTTAAAC. 670 AGTGCAGTAATGTA AGTGCAGTAATGTA	620 AAGTAAGGT AAGTAAGGT 680 CTCAGCTGA TTAAGCTGA	630 CACGGCAAGAG CACGGCAAGAG 690 GATATACTAAG GATATACTAAG	640 TTAGCCGTTAT TTAGCCGTTAT 700 CAGACCGAGGA CAGACCGAGGA 2	650 FATAGGTATC. TATAGGTATC. 710 ACTTGACTAA ACTTGACTAA 23S1RNA—>	550 AAGTATA AAGTATA 720 TATTTGT TATTTGT
A B A B	610 TCATTGTTTTAAAC. TCATTGTTTTTAAAC. 670 AGTGCAGTAATGTA AGTGCAGTAATGTA 730	620 AAGTAAGGT AAGTAAGGT 680 CTCAGCTGA TTAAGCTGA 740	630 CACGGCAAGAG CACGGCAAGAG 690 GATATACTAAG GATATACTAAG 750	640 TAGCCGTTAT TAGCCGTTAT 700 CAGACCGAGGA CAGACCGAGGA 2 760	650 FATAGGTATC, FATAGGTATC, 710 ACTTGACTAA ACTTGACTAA 23SFRNA—> 770	550 AAGTATA AAGTATA 720 TATTTGT TATTTGT 780
A A B A	610 ТСАТТСТРТТАААС. ТСАТТСТТТТАААС. 670 АСТССАСТААТСТА АСТССАСТААТСТА 730 ТСТССТТСТААААТ.	620 AAGTAAGGT AAGTAAGGT 680 CTCAGCTGA TTAAGCTGA 740 AACCTTTTA	630 CACGGCAAGAG CACGGCAAGAG 690 GATATACTAAG GATATACTAAG 750 GGTTTA TGCT	640 CTAGCCGTTAT CTAGCCGTTAT 700 CAGACCGAGGA CAGACCGAGGA 2 760 FTTGGTGCCAA	650 FATAGGTATC, 710 ACTTGACTAA ACTTGACTAA 33STRNA—> 770 ATAGCACAGT ¹	560 AAGTATA AAGTATA 720 TATTTGT TATTTGT 780 GGAACCAC
A B A B A B	610 TCATTGTFTTAAAC. TCATTGTFTTAAAC. 670 AGTGCAGTAATGTA AGTGCAGTAATGTA 730 TGTGCTTGTAAAAAT.	620 AAGTAAGGT AAGTAAGGT 680 CTCAGCTGA TTAAGCTGA 740 AACCTTTTA	630 CACGGCAAGAG CACGGCAAGAG 690 GATATACTAAC GATATACTAAC GGTTTA TGCT GGTTTA TGCT	640 TTAGCCGTTAT TAGCCGTTAT 700 CAGACCGAGG CAGACCGAGG 2 760 FTTGGTGCCAI	650 FATAGGTATC, TATAGGTATC, 710 ACTTGACTAA 23 STRNA—> 770 ATAGCACAGT	AAGTATA AAGTATA 720 TATTTGT TATTTGT TATTTGT 780 GGAACCAC
A B B A B	610 ТСАТТСТРТТАААС. ССАТТСТРТТАААС. 670 АСТССАСТААТСТА АСТССАСТААТСТА СТСССТССТАСАААТ. ТСТССТТСТАААААТ.	620 ААСТААССТ ААСТААССТ 680 СТСАССТСА ТТААССТСА 740 ААССТТТТА ААССТТТТА	630 CACGGCAAGAG CACGGCAAGAG GATATACTAAC GATATACTAAC GGTTTA TGCT SGTTTA TGCT SGTTTA TGCT	640 TTAGCCGTTAT TAGCCGTTAT 700 CAGACCGAGGJ CAGACCGAGGJ CAGACCGAGGJ 760 TTTGGTGCCAJ TTTGGTGCCAJ	650 FATAGGTATC, PATAGGTATC, 710 ACTTGACTAA ACTTGACTAA 23 STRNA—> 770 ATAGCACAGT ATAGCACAGT	AAGTATA AAGTATA 720 TATTTGT TATTTGT 780 GGAACCAC
A B A B A B	610 ТСАТТСТТТАААС. ТСАТТСТТТТАААС. 670 АСТССАСТААТСТА АСТССАСТААТСТА 730 ТСТССТТСТАААААТ. ТСТАСТТАТСААААТ.	620 AAGTAAGGT AAGTAAGGT 680 CTCAGCTGA TTAAGCTGA 740 AACCTTTTA AACCTTTTA	630 CACGGCAAGAC CACGGCAAGAC GATATACTAAC GATATACTAAC GGTTTA GGTTTA GGTTTA TGCT	640 TTAGCCGTTAT TAGCCGTTAT 700 CAGACCGAGGJ CAGACCGAGGJ CAGACCGAGGG 760 TTTGGTGCCAJ TTTGGTGCCAJ SSTRNA	650 FATAGGTATC, 710 ACTTGACTAA ACTTGACTAA 23 STRNA—> 770 ATAGCACAGT ATAGCACAGT	AAGTATA AAGTATA 720 TATTTGT TATTTGT GGAACCAC GGAACCAC
A B A B A B	610 TCATTGTTTTAAAC. TCATTGTTTTAAAC. 670 AGTGCAGTAATGTA AGTGCAGTAATGTA TGTGCTTGTAAAAT. TGTACTTATGAAAT. 790 ACCGATCCATCTCG	620 AAGTAAGGT 680 CTCAGCTGA TTAAGCTGA 740 AACCTTTTTA AACCTTTTTA 800 AACTGGTT	630 CACGGCAAGAG CACGGCAAGAG GATATACTAAC GATATACTAAC GGTTTA TGCT 30 GGTTTA TGCT 810 GTTAA AGCTT	640 TTAGCCGTTAT TTAGCCGTTAT 700 CAGACCGAGGI CAGACCGAGGI 2 760 TTTGGTGCCAJ TTTGGTGCCAJ SISTRNA 820 TBCCCCCAAC	650 FATAGGTATC. TATAGGTATC. 710 ACTTGACTAA 33STRNA—> 770 ATAGCACAGT ATAGCACAGT 830 830	AAGTATA AAGTATA 720 TATTTGT TATTTGT TATTTGT GGAACCAC GGAACCAC 840
A B A B A B	610 TCATTGTFTTAAAC. TCATTGTFTTAAAC. 670 AGTGCAGTAATGTA AGTGCAGTAATGTA TGTGCCTTGTAAAAT. TGTACTTATGAAAT. 790 ACCGATCCCG	620 AAGTAAGGT AAGTAAGGT 680 CTCAGCTGA TTAAGCTGA 740 AACCTTTTA AACCTTTTA 800 AACTCGGTT	630 CACGGCAAGAG CACGGCAAGAG GATATACTAAC GATATACTAAC GGTTTAA TGCT GGTTTAA TGCT 810 GTTAAATGCTC	640 TTAGCCGTTAT TTAGCCGTTAT 700 CAGACCGAGGI CAGACCGAGGI 2 760 FTTGGTGCCAJ STAGCGCCAJ STAGCGGCAAC	650 FATAGGTATC. 710 ACTTGACTAA ACTTGACTAA 33SFRNA—> 770 ATAGCACAGT ATAGCACAGT 830 BAATACTTAA	AAGTATA AAGTATA AAGTATA 720 TATTTGT TATTTGT 780 GGAACCAC GGAACCAC GGAACCAC 840 GGGGCAG
А В А В А В А В	610 ТСАТТБТРТТАААС. ТСАТТБТТТТАААС. 670 Адтбсадтаатдта адтбсадтаатдта адтбсадтаатдта тдтосттбтаааат. тдтасттатбааат. 790 асседатссатстс. асседасссатстс.	620 AAGTAAGGT AAGTAAGGT 680 CTCAGCTGA TTAAGCTGA AACCTTTTA AACCTTTTA 800 AACTCGGTT AACTCGGTT	630 CACGGCAAGAG (CACGGCAAGAG 690 GATATACTAAC GATATACTAAC GGTTTAA TGCT GGTTTAA TGCT 310 GTTAAATGCTC GTTAAATGCTC GTTAAATGCTC	640 TTAGCCGTTAT TAGCCGTTAT 700 CAGACCGAGGJ CAGACCGAGGJ CAGACCGAGGJ TTGGTGCCAJ STRNA 820 TTAGCGGCAAC STAGCGGCAAC	650 FATAGGTATC. TATAGGTATC. 710 ACTTGACTAA 33SIRNA> 770 ATAGCACAGT 830 SAATACTTAA SAATACTTAA	AAGTATA AAGTATA AAGTATA 720 TATTTGT TATTTGT TATTTGT 3GAACCAC GGGAACCAC GGGAACCAC GGGAACCAC GGGGCAG GGGGCAG
A B A B A B A B	610 ТСАТТСТТТАЛАС. ТСАТТСТТТТАЛАС. 670 АСТССАСТАЛТАТАЛАС. 730 ТСТССТТСТАЛАЛАТ. ТСТССТТСТАЛАЛАТ. ТСТАСТТАТСАЛАЛАТ. 790 АСССАТССАТСТСС, АСССАТСТСС,	620 AAGTAAGGT AAGTAAGGT 680 CTCAGCTGA TTAAGCTGA AACCTTTTA AACCTTTTTA 800 AACTCGGTT AACTCGGTT	630 CACGGCAAGAG CACGGCAAGAG 690 GATATACTAAC GATATACTAAC GGTTTAA GGTTTAA GGTTTAAATGCTC GTTAAAACGCTC	640 TTAGCCGTTAT TAGCCGTTAT 700 CAGACCGAGGJ CAGACCGAGGJ CAGACCGAGGJ CAGACCGAGGJ TTTGGTGCCAJ STAGCGGCAAC STAGCGGCAAC	650 FATAGGTATC. TATAGGTATC. 710 ACTTGACTAA 23 STRNA	AGGATATA AAGTATA 720 TATTTGT TATTTGT 780 GGAACCAC GGAACCAC GGAACCAC 840 GGGGCAG GGGGCAG
A B A B A B A B	610 TCATTGTTTAAAC. TCATTGTTTTAAAC. 670 AGTGCAGTAATGTA AGTGCAGTAATGTA TGTGCTTGTAAAAT. TGTGCTTGTAAAAT. TGTACTTATGAAAT. 790 ACCGATCCATCTCG. 850	620 AAGTAAGGT AAGTAAGGT CTCAGCTGA TTAAGCTGA 740 AACCTTTTA AACCTTTTA AACCTTTTA AACCTCGGTT AACTCGGTT 860	CACGGCAAGAG CACGGCAAGAG CACGGCAAGAG GATATACTAAC GATATACTAAC GGTTTAA GGTTTAA GTTAAAGCTY B10 GTTAAAGCTY GTTAAACGCTY 870	640 TTAGCCGTTAT TTAGCCGTTAT 700 CAGACCGAGGI CAGACCGAGGI CAGACCGAGGI TTTGGTGCCCAJ TTTGGTGCCCAJ SSITRNA 820 STAGCGGCAAC STAGCGGCAAC 880	650 FATAGGTATC. 710 710 710 710 710 710 710 710	AAGTATA AAGTATA 720 TATTTGT TATTTGT 3GAACCAC GGGAACCAC GGGGCAG 3GGGCAG 900
A B A B A B A B	610 TCATTGTTTTAAAC. TCATTGTTTTAAAC. 670 AGTGCAGTAATGTA AGTGCAGTAATGTA TGTGCTTGTAAAAT. TGTGCTTGTAAAAT. TGTACTTATGAAAT. ACCGATCCATCTCG. ACCGACCCATCTCG. 850 CCTTTGGAAAAGT.	620 AAGTAAGGT AAGTAAGGT TCAGCTGA TTAAGCTGA 740 AACCTTTTA AACCTTTTA 800 AACTCGGTT AACTCGGTT 860 AGCTCAGTG	630 CACGGCAAGAC CACGGCAAGAC GATATACTAAC GATATACTAAC GGTTTA TGCT GGTTTA TGCT S10 GTTAAATGCTC GTTAAACGCTC 810 GTTAAACGCTC	640 TTAGCCGTTAT TTAGCCGTTAT 700 CAGACCGAGGI CAGACCGAGGI 2 760 TTTGGTGCCAJ TTGGTGCCAJ SISTRNA 820 STAGCGGCAAC STAGCGGCAAC 880 TTG <u>TTAAATJ</u>	650 FATAGGTATC. 710 CTTGACTAA 33 STRNA	AAGTATA AAGTATA AAGTATA 720 TATTTGT TATTTGT 3GAACCAC GGAACCAC GGAACCAC 3GGAACCAC 3GGAACCAC 3GGAACCAC 3GGAACCAC 3GGAACCAC 3GGAACCAC 3GGACCAC 3GGACCAC
A B A B A B A B A B	610 TCATTGTFTTAAAC. TCATTGTFTTAAAC. 670 AGTGCAGTAATGTA AGTGCAGTAATGTA TGTGCCTGTAAAAT. TGTACTTATGAAAT. 790 ACCGATCCATCTCG. ACCGACCCATCTCG. 850 CCCTTTGGAAAAGT.	620 AAGTAAGGT AAGTAAGGT CTCAGCTGA TTAAGCTGA 740 AACCTTTTA AACCTTTTA 800 AACTCGGTT 860 AGCTCAGTG	CACGGCAAGAC CACGGCAAGAC CACGGCAAGAC GATATACTAAC GATATACTAAC GGTTTAA TGCT GGTTTAA TGCT GGTTAAATGCTC GTTAAATGCTC GTTAAACGCTTAC 810 GTTAAATGCTC CCAAAGC TAC	640 TTAGCCGTTAT TAGCCGTTAT 700 CAGACCGAGGI CAGACCGAGGI CAGACCGAGGI TTTGGTGCCAJ STAGCGCCAJ STAGCGCCAAC STAGCGGCAAC STAGCGGCAAC STAGCGGCAAC	650 FATAGGTATC. 710 ACTTGACTAA ACTTGACTAA 33SIRNA—> 770 ATAGCACAGT 830 SAATACTTAA SAATACTTAA 890 ATAATTAATT	AAGTATA AAGTATA AAGTATA 720 TATTTGT TATTTGT TATTTGT 3GGAACCAC GGAACCAC GGAACCAC GGGACCAC GGGGCAG GGGGCAG 900 TAATCCAC
A B A B A B A B A B	610 TCATTGTFTTAAAC. TCATTGTFTTAAAC. 670 AGTGCAGTAATGTA AGTGCAGTAATGTA TGTGCTTGTAAAAT. TGTGCTTGTAAAAT. 790 ACCGATCCATCTCG. ACCGACCCATCTCG. 850 CCCTTTGGGAAAGT.	620 AAGTAAGGT AAGTAAGGT 680 CTCAGCTGA TTAAGCTGA 740 AACCTTTTA AACCTTTTA 800 AACTCGGTT 860 AGCTCAGTG AGCTCAGTG SS	630 CACGGCAAGAG (CACGGCAAGAG 690 GATATACTAAC GATATACTAAC GGTTTAA TGCT GGTTTAA TGCT GGTTAAATGCTC GTTAAATGCTC GTTAAATGCTC GTTAAAGC TAT CCAAAGC TAT	640 TTAGCCGTTAT TAGCCGTTAT 700 CAGACCGAGGI CAGACCGAGGI CAGACCGAGGI TTGGTGCCAJ 55FRNA 820 STAGCGCCAAC STAGCGGCAAC STAGCGGCAAC STAGCGGCAAC	650 FATAGGTATC. 710 ACTTGACTAA ACTTGACTAA 33 SIRNA> 770 ATAGCACAGT 830 SAATACTTAA SAATACTTAA 890 ATAATTAATT CAAAG	AAGTATA AAGTATA 720 TATTTGT TATTTGT TATTTGT GGAACCAC GGAACCAC GGGGCAG GGGGCAG GGGGCAG 900 TAATCCAC
A B A B A B A B A B	610 ТСАТТСТТТТАААС. ТСАТТСТТТТАААС. 670 АСТССАСТАТСТАТАААС. 730 ТСТССТТСТАТААААТ. ТСТАСТТАТСААААТ. 790 АСССАТСТСС, АСССАТСТСС, СССТТТССААААСТ. СТСТТТСССАААСТ.	620 AAGTAAGGT AAGTAAGGT CTCASCTGA TTAAGCTGA TTAAGCTGA AACCTTTTTA AACCTTTTTA AACTCGGTT AACTCGGTT 860 AGCTCAGTG SS	630 CACGGCAAGAC CACGGCAAGAC GATATACTAAC GATATACTAAC GGTTTAA GGTTTAA GGTTAAAGCCTC GTTAAAGCCTC 870 CCAAAGC TAT CCAAAGC TAT	640 TTAGCCGTTAT TTAGCCGTTAT 700 CAGACCGAGGA CAGACCGAGGA CAGACCGAGGA 2 700 1 TTGGTGCCCAA 5 5 1 1 1 2 2 1 1 1 2 2 1 1 1 1 1 1 1 1 1 1 1 1 1	650 FATAGGTATC. 710 ACTTGACTAA ACTTGACTAA 335rRNA> 770 ATAGCACAGT ATAGCACAGT 830 SAATACTTAA 830 SAATACTTAA 830 SAATACTTAA 830 SAATACTTAA 830 SAATACTTAA 830 SAATACTTAA	AGGTATA AAGTATA 720 TATTTGT TATTTGT 780 GGAACCAC GGAACCAC GGAACCAC GGGGCAG 900 TAATCCAC
A B A B A B A B A B	610 TCATTGTTTAAAC. TCATTGTTTTAAAC. AGTGCAGTAATGTAA AGTGCAGTAATGTAA AGTGCAGTAATGTAA TGTGCTTGTAAAAT. TGTACTTATGAAAAT. TGTACTTATGAAAAT. ACCGATCCATCTCG. ACCGATCCATCTCG. 850 CCCTTTGGAAAAGT. CTCTTTGGGAAAAGT.	620 AAGTAAGGT AAGTAAGGT CTCAGCTGA TTCAGCTGA TTAAGCTGA AACCTTTTA AACCTTTTA AACCTCTGGTT AACTCGGTT AACTCGGTT AGCTCAGTG SS 920 TATTTCAGT	CACGGCAAGAC CACGGCAAGAC CACGGCAAGAC GATATACTAAC GATATACTAAC GGTTTAATCCTAAC GGTTTAATGCTC GGTTAAACGCTC GTTAAACGCTC CCAAAGC TRNA—S 930	640 TTAGCCGTTAT 700 CAGACCGAGGI CAGACCGAGGI 2 760 TTTGGTGCCCAJ TTGGTGCCCAJ TTGGTGCCCAJ SIRNA 820 STAGCGGCAAC STAGCGGCAAC 880 TTTGTTAAATA 880 TTTGTTAAATA	650 FATAGGTATC. TATAGGTATC. 710 ACTTGACTAA 33 STRNA	AAGTATA AAGTATA 720 TATTTGT TATTTGT 3GAACCAC 3GGACCAC 3GGGCAG 3GGGCAG 3GGGCAG 900 TAATCCAC
A B A B A B A B A B A B	610 TCATTGTTTTAAAC. TCATTGTTTTAAAC. AGTGCAGTAATGTAA AGTGCAGTAATGTAA AGTGCAGTAATGTAA TGTGCTTGTAAAAT. TGTACTTATGAAAAT. TGTACTTATGAAAAT. ACCGATCCATCTCG. ACCGACCCATCTCG. 850 CCCTTTGGAAAAGT. CTCTTTGGGAAAGT. GTCTTATGCTTG	620 AAGTAAGGT AAGTAAGGT TTAAGCTGA TTAAGCTGA 740 AACCTTTTA AACCTTTTA AACCTTTTA AACTCGGTT AACTCGGTT 860 AGCTCAGTG SS 920 TATTTGTGA	630 CACGGCAAGAC CACGGCAAGAC GATATACTAAC GATATACTAAC GGTTTA TGCT GGTTTA TGCT GGTTTAATGCTC GTTAAATGCTC GTTAAACGCTC CCAAAGC TAT CCAAAGC TAT CCAAAGC TAT	640 TTAGCCGTTAT TTAGCCGTTAT 700 CAGACCGAGGI CAGACCGAGGI 2 760 TTTGGTGCCAJ TTGGTGCCAJ TTGGTGCCAJ SJERNA 820 STAGCGGCAAC STAGCGCCAAC STAGCGGCAAC 880 TTTGTTAAAAAC 880 TTTGTTAAAAAC	650 FATAGGTATC. 710 CTTGACTAA 33 SIRNA	AAGTATA AAGTATA 720 TATTTGT TATTTGT 3GAACCAC GGAACCAC GGGAACCAC GGGACCAC GGGACCAC GGGACCAC GGGGCAG 900 TAATCCAC AC
ABABABABABAB	610 TCATTGTTTTAAAC. TCATTGTTTTAAAC. AGTGCAGTAATGTAA AGTGCAGTAATGTAA AGTGCAGTAATGTAA TGTGCCTGTAAAAT. TGTACTTATGAAAAT. TGTACTTATGAAAAT. ACCGATCCATCTG. ACCGACCCATCTCG. ACCGACCCATCTCG. 850 CCCTTTGGAAAAGT. CTCTTTGGGAAAGT. GTCTTATGCTTG GTCTTAGATGTAAG.	620 AAGTAAGGT AAGTAAGGT CTCAGCTGA TTAAGCTGA 740 AACCTTTTA AACCTTTTA AACCTTTTA AACTCGGTT 860 AGCTCAGTG AGCTCAGTG 55 920 TATTTGTGA AATTTATTA	630 CACGGCAAGAC CACGGCAAGAC GATATACTAAC GATATACTAAC GGTTTAA TGCT GGTTTAA TGCT GGTTAAATGCTC GTTAAATGCTC GTTAAATGCTC GTTAAACGCT CCAAAGC TAT FRNA—> 930 ATACAAGCATJ A-ATATACAAJ	640 TTAGCCGTTAT TTAGCCGTTAT 700 CAGACCGAGGI CAGACCGAGGI CAGACCGAGGI TTTGGTGCCAJ SSERNA 820 STAGCGCAAC STAGCGCAAC STAGCGCAAC STAGCGCAAC STAGCGCAAC STAGCGCAAC	650 FATAGGTATC. 710 ACTTGACTAA ACTTGACTAA 3351RNA—> 770 ATAGCACAGT 830 SAATACTTAA 830 SAATACTTAA 830 ATAATTAATT CAAGATAT 950 TTAAATTAATT 950 TTAAATTAATT	860 860 AAGTATA 720 TATTGT 720 TATTGT 780 GGAACCAC 660 GGGAACCAC 840 GGGGCAG 900 TATTCCAC 840 GGGGCAG 900 TATCCAC A TTAACCAC A
AB ABAB ABAB AB	610 TCATTGTTTAAAC. TCATTGTTTAAAC. 670 AGTGCAGTAATGTAA AGTGCAGTAATGTAA AGTGCAGTAATGTAA TGTGCTTGTAAAAAT. TGTGCTTGTAAAAAT. TGTACTTATGAAAAAT. TGTACTTATGAAAAAT. 790 ACCGATCCATCTCG. ACCGATCCATCTCG. 850 CCCTTTGGAAAAGT. CTCTTTGGAAAAGT. GTCTTATGCTTG GTCTTATGCTTG	620 AAGTAAGGT AAGTAAGGT 680 CTCAGCTGA TTAAGCTGA 740 AACCTTTTA AACCTTTTA 800 AACTCGGTT 860 AGCTCAGTG AGCTCAGTG 55 920 TATTTGTGA AATTTATTA	630 CACGGCAAGAG CACGGCAAGAG 690 GATATACTAAC GATATACTAAC GGTTTA TGCT GGTTTA TGCT GGTTAAAGCCTA S10 GTTAAAGCCTA GTTAAAGCCTA CCAAAGC TAT CCAAAGC TAT CCAAAGC TAT	640 TTAGCCGTTAT TAGCCGTTAT 700 CAGACCGAGGI CAGACCGAGGI CAGACCGAGGI TTGGTGCCAJ STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC 880 TTGTTAAATA AACTTTTT-AT	650 650 FATAGGTATC. 710 ACTTGACTAA 33 STRNA-> 770 ATAGCACAGT ATAGCACAGT 830 SAATACTTAA 830 830 830 830 830 830 830 830	AGGATATA AAGTATA 720 TATTTGT TATTTGT 780 GGAACCAC GGAACCAC GGAACCAC GGGACCAC GGGGCAG 900 TAATCCAC AC AC
AB ABAB ABAB AB	610 TCATTGTTTAAAC. TCATTGTTTTAAAC. AGTGCAGTAATGTAA AGTGCAGTAATGTAA AGTGCAGTAATGTAA AGTGCAGTAATGTAA TGTGCTTGTAAAAAT. TGTACTTATGAAAAT. TGTACTTATGAAAAT. ACCGATCCATCTCG. ACCGATCCATCTCG. ACCGATCCATCTCG. ACCGATCCATCTCG. CCCTTTGGAAAAGT. GTCTTAGATGTAAG. 910	ACTACACTA AAGTAAGGT AAGTAAGGT CTCAGCTGA TTAAGCTGA AACCTTTTA AACCTTTTA AACCTTTTA AACCGGTT AACTCGGTT AACTCGGTT AGCTCAGTG SS 920 TATTTGTGA AATTTATTA 980	CACGGCAAGAC CACGGCAAGAC CACGGCAAGAC GATATACTAAC GATATACTAAC GGTTTAA GGTTTAA GGTTAAAGCTC GTTAAAGCTC CCAAAGC TRAAAGCTTA CCAAAGC TRAAAGCTTA CCAAAGC TAAAGCATA AAATATACAAA 990	640 TTAGCCGTTAT TTAGCCGTTAT TTAGCCGTTAT 700 CAGACCGAGGA CAGACCGAGGA TTTGGTGGCCAA TTTGGTGCCCAA 820 TTAGCGGCAAC STAGCGGCAAC STAGCGGCAAC STAGCGGCAAC STAGCGGCAAC STAGCGGCAAC STAGCGGCAAC TTGTTAAAAAC AAGACGTGGAT AACTTTTTT-AT 1000	650 FATAGGTATC. FATAGGTATC. TATAGGTATC. ACTTGACTAA 23 STRNA	AGGATA AAGTATA 720 TATTTGT 780 3GAACCAC 3GGAACCAC 3GGAACCAC 3GGGCAG 3GGGCAG 900 TAATCCAC 4 TTAACCAC A TTAAC 1020
ABABABABABA	610 TCATTGTTTTAAAC. TCATTGTTTTAAAC. AGTGCAGTAATGTAA AGTGCAGTAATGTAA AGTGCCAGTAATGTAA TGTGCTTGTAAAAT. TGTGCTTGTAAAAT. TGTGCTTGTAAAAT. TGTACTTATGAAAAT. TGTACTTATGAAAAT. ACCGATCCATCTCG. ACCGACCCATCTCG. CCCTTTGGAAAAGT. GTCTTATGCTTG GTCTTAGATGTAAG. 910 GTCTTAGTTAGATGTAAG. 970 TTCTATTTGTTAT	620 AAGTAAGGT AAGTAAGGT TTAAGCTGA TTAAGCTGA AACCTTTTA AACCTTTTA AACCTTTTA AACTCGGTT AACTCGGTT AACTCGGTT 860 AGCTCAGTG 55 920 TATTTGTGA AATTTATTA 980 CTTTATTC	CACGGCAAGAC CACGGCAAGAC CACGGCAAGAC GATATACTAAC GATATACTAAC GGTTTA TGCT GGTTTA TGCT GGTTAAATGCTC GTTAAATGCTC GTTAAACGCT CCAAAGC TAT CCAAAGC TAT CCAAAGC TAT S30 ATACAAGCAT7 A-ATATACAAC	640 TTAGCCGTTAT TTAGCCGTTAT 700 CAGACCGAGGI 200 TTGGTGCCAJ TTGGTGCCAJ TTGGTGCCAJ TTGGTGCCAJ TTGGTGCCAJ TTGGTGCCAJ TTGGTGCCAJ TTGGTGCAA 880 TTGTTAAATA 820 STAGCGGCAAC STAGCGCCAAC 940 AAGCCTGGAJ AACTTTTT-AJ 1000 TAAATACCTAJ	650 FATAGGTATC. FATAGGTATC. TTAGGTATC. TTAGGTATC. TTAGGTATC. 3357RNA	AAGTATA AAGTATA 720 TATTAGT TATTTGT GGAACCAC GGGGCAG 900 TATTCCAC
ABABABABABABAB	610 TCATTGTTTTAAAC. TCATTGTTTTAAAC. AGTGCAGTAATGTAA AGTGCAGTAATGTAA AGTGCAGTAATGTAA TGTGCTTGTAAAAT. TGTACTTATGAAAAT. TGTACTTATGAAAAT. ACCGATCCATCTCG. ACCGACCCATCTCG. ACCGACCCATCTCG. CCCTTTGGAAAAGT. GTCTTAGTGGAAAAGT. GTCTTAGTGTAGTGTAAG. 970 TTCTATTTTGTTAT	620 AAGTAAGGT AAGTAAGGT TTAAGCTGA TTAAGCTGA 740 AACCTTTTA AACCTTTTA AACCTTTTA 800 AACTCGGTT 860 AGCTCAGTG SS 920 TATTTGTGA AATTTATTA 980 CTTTATTCT	630 CACGGCAAGAC CACGGCAAGAC GATATACTAAC GATATACTAAC GGTTTAA TGCT GGTTTAA TGCT GGTTTAA TGCT GGTTAAAGCTT GTTAAAGCTT CCAAAGC TAT CCAAAGC TAT CCAAAGC TAT S30 ATACAAGCAT A ATATACAAJ 930 GCTTATTGTT GCTTATTGTT	640 TTAGCCGTTAT TTAGCCGTTAT 700 CAGACCGAGGI CAGACCGAGGI 2 760 FTTGGTGCCAI 2 760 FTTGGTGCCAI 2 760 FTTGGTGCCAI 2 55FRNA 820 5TAGCGCAAC 5TAGCGCCAAC 880 FTTGTTAAATI 880 FTTGTTAAATI 940 AACTFTTT-AT 1000 FANATCCTAI	650 FATAGGTATC. TATAGGTATC. 710 ACTTGACTAA 33SIRNA	AAGTATA AAGTATA 720 TATTTGT TATTTGT TATTTGT GGAACCAC GGAACCAC GGGACCAC GGGACCAC GGGACCAC GGGACCAC GGGACCAC GGGACCAC GGGGCAG 900 TATTCCAC
A B A B A B A B A B	610 ТСАТТБТТТАААС. ТСАТТБТТТАААС. 670 АБТБСАБТАЛБТА. АБТБСАБТАЛБТА. ТБТСАТТБТТАААС. 730 ТБТССТТБТААААТ. ТБТССТТБТААААТ. ТБТССТТБТААААТ. 790 АССБАТССАТСТСБ. АССБАТССАТСТСБ. АССБАСССАТСТСБ. СССТТТББААААБТ. СТСТТТББААААБТ. СТСТТТББАААБТ. СТСТТТББАААБТ. СТСТТТББАААБТ. СТСТТТББАТАТБАТТТТББТТА. Э?0 ТТСТАТТТТБТТАТТСТТТ ТТАААТТАСАТТА.	620 AAGTAAGGT AAGTAAGGT CTCAGCTGA TTAAGCTGA TTAAGCTGA AACCTTTTA AACCTTTTA AACTCGGTT AACTCGGTT 860 AGCTCAGTG AGCTCAGTG SS 920 TATTTGTGA AATTTATTA 980 CTTTATTCT AACTCTAAGAT	630 CACGGCAAGAC CACGGCAAGAC GATATACTAAC GATATACTAAC GGTTTA TGCT GGTTTA TGCT GGTTTA TGCT GGTTAAAGCC TA CCAAAGC TAT CCAAAGC TAT CCAAAGC TAT CCAAAGC TAT SACACCAT ATACAAGCAT ATACAAGCAT ACATATACAAG GCTTATTGT GCTT-TTTGA	640 TTAGCCGTTAT 700 CAGACCGAGGJ CAGACCGAGGJ CAGACCGAGGJ CAGACCGAGGJ TTTGGTGCCAJ SISIRNA 820 STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCGCCAAC STAGCCCCAA STAGCCCCAA STAGCCCCAAC STAGCCCCAAC STAGCCCCAAC STAGCCCCAAC STAGCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCAAC STAGCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCCCAAC STAGCCCCCCAAC STAGCCCCCAAC STAGCCCCCAAC STAGCCCCCCAAC STAGCCCCCCCAAC STAGCCCCCCCCCCCCCCAAC STAGCCCCCCCAAC STAGCCCCCCCCCCCCCCCCCCCCCCCCCCAAC STAGCCCCCCCCCCCCCCCCCAAC STAGCCCCCCCCCCCCCCCCAAC STAGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	650 FATAGGTATC. 710 ACTTGACTAA ACTTGACTAA ACTTGACTAA ACTTGACTAA ACTTGACTAA ACTTGACACAGT ATAGCACAGT 830 SAATACTTAA 830 ATAATAATTAATT 200 ATAATAATTAATT 1010 ATAATATTTA	AAGTATA 720 TATTTGT 720 TATTTGT TATTTGT TATTTGT GGAACCAC GGAACCAC GGGGCAG 900 TATACCAC GGGGCAG 900 TATACCAC AC <ac< td=""> <ac< td=""> 1020 CATCAAA TGGCAGA</ac<></ac<>
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A B A B A B A B A B A B	610 TCATTGTTTAAAC. TCATTGTTTAAAC. AGTGCAGTAATGTAA AGTGCAGTAATGTAA AGTGCAGTAATGTAA AGTGCAGTAATGTAA TGTGCTTGTAAAAAT. TGTACTTATGAAAAT. TGTACTTATGAAAAT. ACCGATCCATCTCG. ACCGATCCATCTCG. ACCGATCCATCTCG. CCCTTTGGAAAAGT. CTCTTTGGGAAAAGT. GTCTTAATGCTTG GTCTTAGATGTAAAG. TTCTATTTTGTTAT TTAAATTACACTAG TCTTAACGACTTTT	620 AAGTAAGGT AAGTAAGGT TTAAGCTGA TTAAGCTGA AACCTTTTA AACCTTTTA AACCTTTTA AACCTCGGTT AACTCGGTT 860 AGCTCAGTG 55 920 TATTGTGGA AGCTCAGTG 55 920 TATTGTGGA AGCTCAGTG 55 920 TATTGTGGA AGCTCAGTG 55 920 TATTGTGGA AGCTCAGTG 55 920 TATTGTGGA AGCTCAGTG 55 920 TATTGTGGA AGCTCAGTG 1040 CAAAAGATT	630 CACGGCAAGAG CACGGCAAGAG GATATACTAAC GATATACTAAC GGTTTA TGCT GGTTTA TGCT GGTTAAAGCTTA GTTAAAGCTTA CCAAAGC TAT CCAAAGC TAT CCACAAGC TAT CCACAAGC TAT CCACAAGC TAT CCACAAGC TAT CCACAAGC TAT CCACACACAT	640 TTAGCCGTTAT TTAGCCGTTAT TTAGCCGTTAT 700 CAGACCGAGGA CAGACCGAGGA TTTGGTGGCCAA TTTGGTGCCCAA TTTGGTGCCAA TTTGGTGCGCAAC TTTGGTAAAAAC 880 TTTGTTAAAAAC	650 FATAGGTATC. FATAGGTATC. 710 ACTTGACTAA 23 STRNA	AAGTATA 720 TATTTGT TATTTGT TATTTGT 3GAACCAC GGGGCAG 900 TATTCCAC AC 1020 CATCAAA 1020 CATCAAA 1080 IGACTTGT
A B A B A B A B A B A B	610 TCATTGTTTTAAAC. TCATTGTTTTAAAC. AGTGCAGTAATGTAA AGTGCAGTAATGTAA AGTGCAGTAATGTAA AGTGCAGTAATGTAA TGTGCTTGTAAAAAT. TGTACTTATGAAAAT. TGTACTTATGAAAAAT. TGTACTTAGAAAAAT. TGTACTTTGGAAAAAGT. GTCTTAGAGACAAAGT. GTCTTAGATGTAAAG 910 GTCTTATGCTTG GTCTTAGATGTAAAG 970 TTCTATTTTGTTAAG	620 AAGTAAGGT AAGTAAGGT TTAAGCTGA 740 AACCTTTTA AACCTTTTA AACCTTTTA AACCCTTTTA AACTCGGTT 860 AGCTCAGTG 55 920 TATTTAGTGA AATTTATTA 980 CTTTATTCT AACTCAGAT 1040 CAAAAGATT SAAATAAAA	630 CACGGCAAGAG CACGGCAAGAG CACGGCAAGAG GATATACTAAC GATATACTAAC GATATACTAAC GGTTTA TGCT GGTTTA TGCT GGTTAAATGCTC CCAAAGC TAT CCAAAGC TAT CCAAAGC TAT CCAAAGC TAT S30 ATACAAGCATT ATATACAAG GGTTATATGT GCTT-TTTGA	640 CTAGCCGTTAI CTAGCCGTTAI 700 CAGACCGAGGI CAGACCGAGGI 2 760 PTTGGTGCCAJ PTTGGTGCCAJ PTTGGTGCCAJ SSIRNA 820 STAGCGGCAAC STAGCGGCAAC STAGCGGCAAC 940 AAGTCTTTTT-AT 1000 FAAATCCTAJ 1060 CCCATTTCCTJ	650 FATAGGTATC. TATAGGTATC. TATAGGTATC. TIO ACTTGACTAA 33 STRNA	860 860 860 860 860 860 174775 720 1747757 720 1747757 780 3GAACCAC 3GAACCAC 3GAACCAC 3GGACCAC 3GGACCAC 3GGGCAG 3GGGCAG 900 TAATCCAC

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. 6A, 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

ichlL →

AGTGCCAAAGCTATATGATAAAACA-TTT-76 bp-ATGAAAC

5s rrna \rightarrow

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 tran-



script 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

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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 onehalf 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 singlecopy 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 PstI 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 EcoRI fragment that is internal to the repeat. As seen in Fig. 7, only the two expected PstI

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 singlecopy 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. vezoensis (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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References

- Bancroft I, Wolk CP, Oren EV (1989) J Bacteriol 171: 5940-5948 Bird CJ, McLachlan JL (1992) Seaweed flora of the maritimes, 1,
- Rhodophyta the red algae. Biopress, Bristol, England
- Boyen C, Somerville CC, Le Gall Y, Kloareg B, Loiseaux-de Goër S (1991) J Phycol 27:11
- Bryant DA, Stirewalt VL (1990) FEBS Lett 259:273-280
- Carbon P, Ehresmann C, Ehresmann B, Ebel J-P (1979) Eur J Biochem 100: 399-410
- Douglas SE (1992) Bio Systems 28:57-68
- Douglas SE, Turner S (1991) J Mol Evol 33:267-273
- Dryden SC, Kaplan S (1990) Nucleic Acids Res 18:7267-7277
- Gray MW (1989) Trends Genet 5:294-299
- Gray MW (1991) Origin and evolution of plastid genomes and genes. In: Bogorad L, Vasil IK (eds) Cell culture and somatic cell genetics of plants, vol 7A. The molecular biology of plastids. Academic Press, San Diego, California, pp 303-330
- Gray MW, Doolittle WF (1982) Microbiol Rev 46:1-42
- Hallick RB, Buetow DE (1989) Chloroplast DNA. In: Buetow DE (ed) The biology of *Euglena*, vol IV. Academic Press, New York, pp 351–414
- Hiratsuka J, Shimada H, Whittier R, Ishibashi T, Sakamoto M, Mori M, Kondo C, Honji Y, Sun C, Meng B, Li Y, Kanno A, Nishizawa Y, Harai A, Shinozaki K, Suguira M (1989) Mol Gen Genet 217:185-194
- Kainz P, Schmiedlechner A, Strack HB (1992) Anal Biochem 202:46-49

Karabin GD, Narita JO, Dodd JR, Hallick RB (1983) J Biol Chem 258: 14790-14796

Kessler U, Maid U, Zetsche K (1992) Plant Mol Biol 18:777-780

- Kourilsky P (1986) Trends Genet 2:60-63
- Lambert DH, Bryant DA, Stirewalt VL, Dubbs JM, Stevens SE, Jr, Porter RD (1985) J Bacteriol 164:659-664
- Li N, Cattolico RA (1987) Mol Gen Genet 209:343-351
- Lindstrom SC, Cole KM (1992) Can J Bot 70:1355-1363
- Loiseaux-de Goër SL, Markowicz Y, Dalmon J, Audren H (1988) Curr Genet 14:155-162
- Maid U, Zetsche K (1992) Plant Mol Biol 19:1001-1010
- Maid U, Steinmüller R, Zetsche K (1992) Curr Genet 21: 521-525
- Neumann-Spallart C, Brandtner M, Kraus M, Jakowitsch J, Bayer MG, Maier TL, Schenk HEA, Löffelhardt W (1990) FEBS Lett 268:55-58
- Newton KJ (1988) Annu Rev Plant Physiol Plant Mol Biol 39: 503– 532
- Ohyama K, Fukuzawa H, Kohchi T, Shirai H, Sano T, Sano S, Umesano K, Shiki Y, Takeuchi M, Chang Z, Aota S, Inokuchi M, Chang Z, Aota S, Inokuchi H, Ozeki H (1986) Nature 322:572-574
- Palmer JD (1985) Annu Rev Genet 19: 325-354
- Palmer JD (1991) Plastid chromosomes: structure and evolution. In: Bogard L, Vasil IK (eds) Cell culture and somatic cell genetics of plants, vol 7A. The molecular biology of plastids. Academic Press, San Diego, California, pp 5-53
- Reith M (1992) Plant Mol Biol 18:773-775
- Reith M (1993) Plant Mol Biol 21:185-189
- Reith M, Munholland J (1991) FEBS Lett 294:116-120
- Reith M, Munholland J (1993a) Curr Genet 23:59-65
- Reith M, Munholland J (1993b) Plant Cell 5:465-475
- Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chunwongse J, Obokata J, Yamaguchi-Shinozaki, Ohto C, Torazawa K, Meng BY, Sugita M, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Kato A, Tohdoh N, Shimada H, Suguira M (1986) EMBO J 5:2043-2049
- Shivji MS (1991) Curr Genet 19:49-54
- Shivji MS, Li N, Cattolico RA (1992) Mol Gen Genet 232:65-73
- Tomioka N, Shinozaki K, Suguira M (1981) Mol Gen Genet 184:359-363
- Valentin K. Zetsche K (1990) Mol Gen Genet 220:425-430
- Wolfe KH, Morden CW, Palmer JD (1992) Proc Natl Acad Sci USA 89: 10648 – 10652
- Communicated by R.W. Lee