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The central part of the cyanelle rDNA unit of *Cyanophora paradoxa*: Sequence comparison with chloroplasts and cyanobacteria

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Abstract

The 287-bp spacer and the flanking 3′-end of the 16S- and 5′-end of the 23S-rRNA genes of the cyanelles from *Cyanophora paradoxa* have been sequenced and compared with the corresponding regions of cyanobacteria and chloroplasts. The spacer contains the uninterrupted genes for $tRNA^{ile}$ and $tRNA^{ala}$. All coding regions show high homology to their prokaryotic counterparts. At the 3′-end of the 16S-rDNA a CCTCCTTT sequence has been identified which is complementary to putative ribosome binding sites observed immediately upstream of the coding region of cyanelle protein genes.

Introduction

Cyanelles, the photosynthetic organelles from the biflagellated protist *Cyanophora paradoxa*, possess dual properties: the genome size of chloroplasts and a peptidoglycan-containing rudimentary cell wall pointing to their origin from endosymbiotic cyanobacteria. It appears justified to view the cyanelles from this organism as a model for an evolutionarily intermediate stage between cyanobacteria and chloroplasts [23].

As in most chloroplast DNAs, two inverted repeat segments have been identified, which are 10 kb in size in cyanelles, coding for the 16S- and 23S-rRNAs. Within the spacer, genes for $tRNA^{ile}$ and $tRNA^{ala}$ – a common feature found in chloroplast and prokaryotic rDNA units – have been revealed by homologous hybridization [11]. Heterologous hybridization experiments have shown a considerable sequence homology to the maize and spinach counterparts for these tRNAs.

To get a more precise estimation of the relationship between cyanelles and chloroplasts or cyanelles and cyanobacteria, respectively, the spacer region, the 3′-end of the 16S-, and the 5′-end of the 23S-rDNA have been sequenced and compared with the corresponding sequences of *Anacystis nidulans* [10, 21, 24] and the chloroplasts from *Euglena gracilis* [7, 8], *Chlamydomonas reinhardii* [5, 15, 16], *Chlorella ellipsoidea* [25, 26], *Marchantia polymorpha* [14], *Zea mays* [6, 9, 17], and *Nicotiana tabacum* [19]. This nucleotide sequence comparison is important in view of the variable spacer sizes and the presence of introns in the tRNAs among higher plants and algae (Table 1). In algal chloroplast rDNA units thus far studied, no introns in tRNA genes have been found, in spite of the large size of *Chlamydomonas* and *Chlorella* spacer segments. In both algae, however, introns in the 23S-rDNA have been reported [15, 26]. *Chlorella* chloroplast DNA appears to lack the $tRNA^{ala}$ gene and the 23S-gene is in opposite orientation to the 16S-gene [25].

Table 1. Comparison of chloroplast and cyanobacterial rDNA spacers

Organism	Spacer size (bp)	$tRNA^{ile}$	$tRNA^{ala}$	Introns	
				$tRNA^{ile}$	$tRNA^{ala}$
Maize	2408	+	+	+	+
Tobacco	2079	+	+	+	+
Euglena	260	+	+	–	–
Chlorella	4842	+	–	–	
Chlamydomonas	1805	+	+	–	–
Marchantia	2308	+	+	+	+
Anacystis	395	+	+	–	–

Materials and methods

The strain *Cyanophora paradoxa* LB 555 UTEX was obtained from the culture collection of algae of the University of Texas at Austin. The cyanelles and cyanelle DNA were isolated as described [2] and the Sma-4/5 fragment [2] was cloned into pUC 18 with *E. coli* 71-18 as host cell. From this approximately 950-bp insert several subclones have been produced, again in pUC 18. Plasmid DNA was prepared from the subclones according to Birnboim and Doly [1]. Plasmid DNA was sequenced directly using the supercoil DNA sequencing method [4]. For two regions where no subclones in the opposite direction could be obtained, oligonucleotides were synthesized: a 17mer (positions 178–195) and a 15mer (positions 557–571).

Results and discussion

The approximately 950-bp Sma-4/5 fragment contains parts of the 16S- and 23S-rDNA [2] and the $tRNA^{ile}$ and $tRNA^{ala}$ genes [11]. Figure 1 shows the restriction map of this fragment and the sequencing strategy used. Both strands were sequenced except

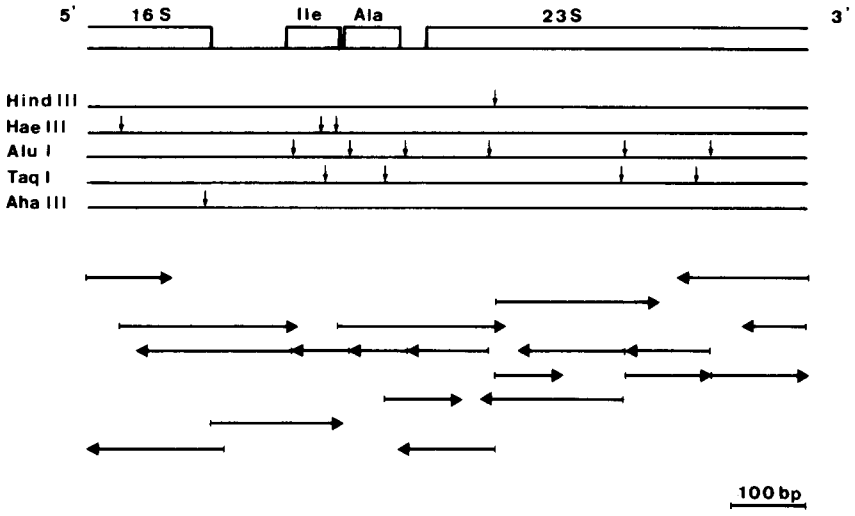

Fig. 1. Restriction map and outline of the sequencing strategy for the cyanelle Sma-4/5 fragment of *Cyanophora paradoxa*. The $tRNA^{ile}$ and $tRNA^{ala}$ genes and the 3′-end of 16S- and 5′-end of 23S-rDNA are indicated.

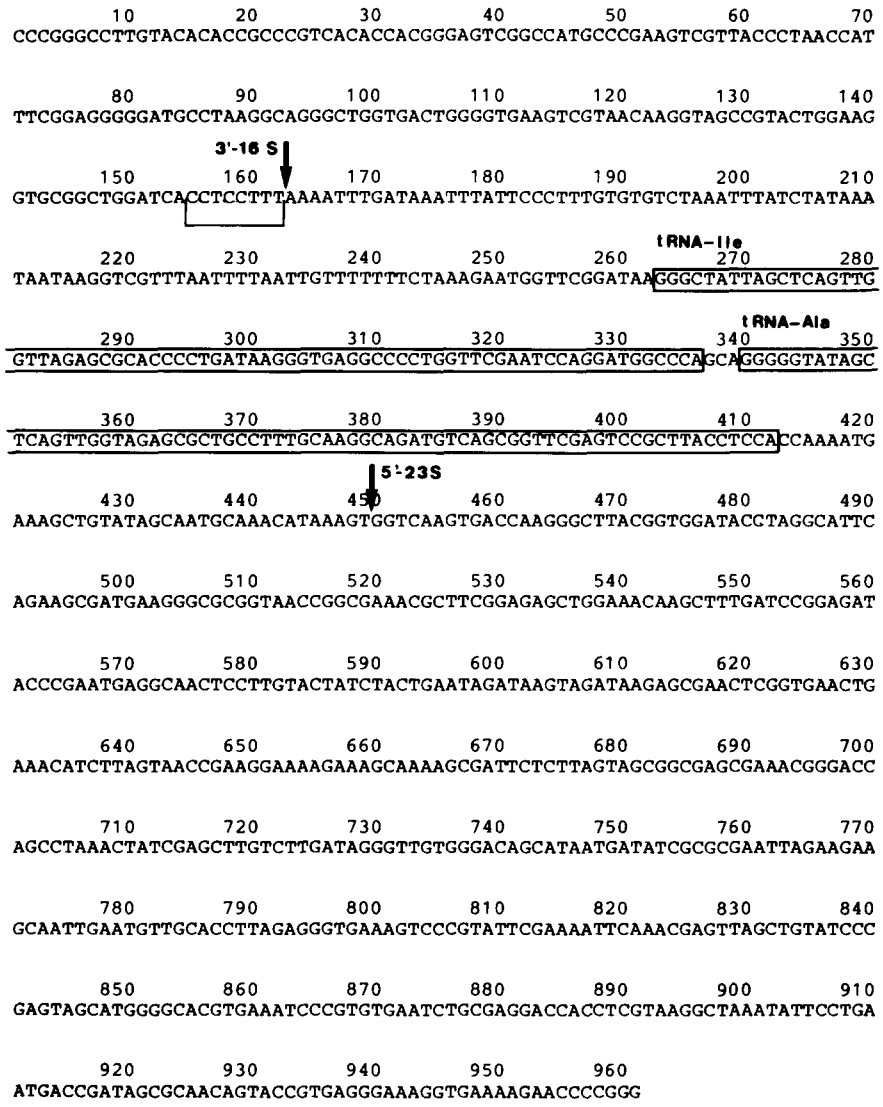

Fig. 2. Nucleotide sequence of the noncoding (RNA-like) strand of the cyanelle ribosomal spacer and flanking regions (Sma-4/5 fragment). The genes for $tRNA^{ile}$ and $tRNA^{ala}$ are framed. The 3′-end of 16S- and the 5′-end of 23S-rDNA are marked by arrows. The region at the 3′-end of 16S-rDNA complementary to Shine-Dalgarno sequences of cyanelle protein genes is bracketed.

for a small 7-bp region where only one strand was sequenced 4 times independently. The size of the total fragment was determined as 961 bp. Figure 2 shows the noncoding (RNA-like) strand of this SmaI fragment. Based on the homology with the corresponding genes of *Anacystis nidulans* the 3′-end of the 16S-rDNA, the 5′-end of the 23S-rDNA and the two tRNA genes were located. This determined the size of the spacer as 287 bp. This size is similar to that of *Anacystis nidulans* and *Euglena gracilis* (Table 1). The spacer tRNA genes do not contain introns, as they have been found until now only in higher plants (Table 1). Notable is the very short distance, only three basepairs, between the two tRNA genes. Table 2 gives a comparison of the sequence homology of the rRNA and tRNA genes of the spacer region between cyanelles, prokaryotic and eukaryotic algae and plants. No homology was found

Table 2. Percentage of homology between the cyanelle genes and the corresponding genes of cyanobacteria and chloroplasts

Organism	3′-16S (162 bp)	t-RNA^{ile}	t-RNA^{ala}	5′-23S (512 bp)
Anacystis	93%	94.5%	92%	79%
Euglena	83%	84%	97%	69%*
Chlamydomonas	85%	73%	96%	80%
Chlorella	n.d.	90.5%	-	75%
Marchantia	89.5%	88%	93%	76%
Maize	88%	89%	90%	75%
Tobacco	89.5%	89%	90%	75%

n.d. = not determined.

* The *Euglena* sequence (391 bp) is from Orozco, Dubbs, Karabin and Hallick (unpublished) as cited in Rochaix and Darlix (1982).

when the intergenic regions were compared. The cloverleaf structure of the two tRNAs is shown in Fig. 3. In analogy to chloroplast tRNAs the 3′-terminal CCA is not encoded by the cyanelle DNA.

For the first time partial sequence information was obtained for the large ribosomal RNAs from cyanelles of *C. paradoxa*. Cyanelle 5S-rRNA has been sequenced recently and found to be most closely related to that from the cyanobacterium *Synechococcus lividus* [13]. A pyrimidine-rich region at the 3′-end of the 16S-rDNA was found complementary to putative ribosome binding sites (Shine-Dalgarno sequences) [18] which have been reported upstream of several analyzed cyanelle protein genes. Among those are the genes for α- and β-phycocyanin (GGAG) [12], β-allophycocyanin (AAGG), α-allophycocyanin (AAAG) [3, 12], the β subunit for the ATP synthase (GAGG) [22] and the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (GGAG) [22]. These four different 4-bp sequence motives match the CCTCCTTT sequence at the 3′-end of 16S-rDNA (Fig. 2). This is not the case for the small subunit gene of ribulose-1,5-bisphosphate carboxylase/oxygenase [20, 22], that is cotranscribed with the large subunit gene [20].

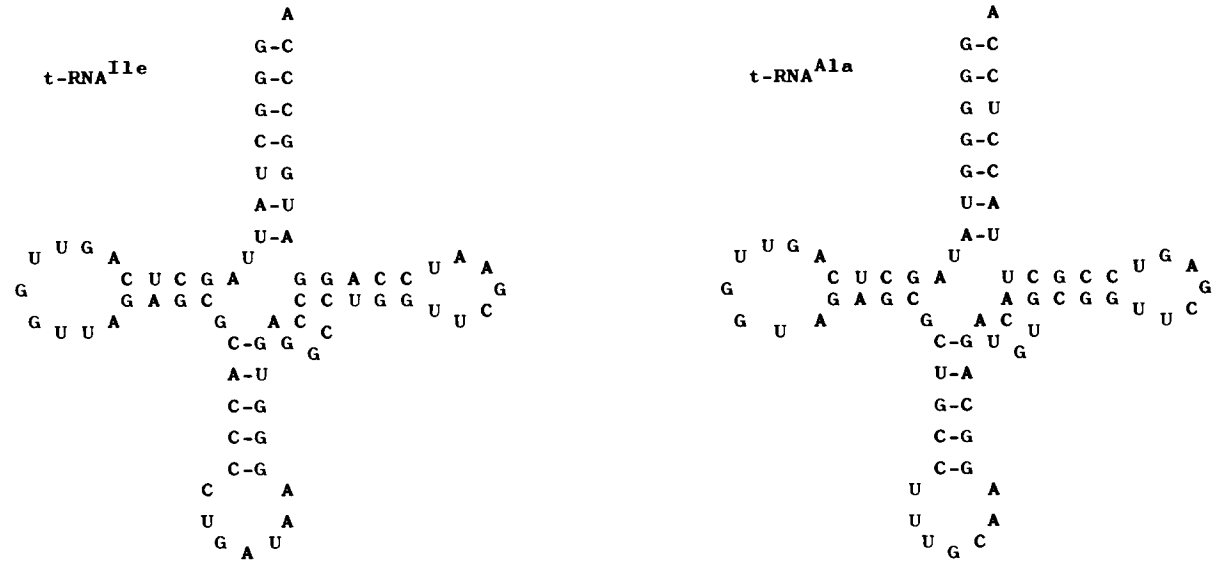

Fig. 3. Cloverleaf structure of tRNA^{ile} and tRNA^{ala} from *C. paradoxa* cyanelles.

We have compared the homology of the sequenced region of the cyanelle 16S-rDNA gene (162 bp) and the sequenced region of the cyanelle 23S-rRNA gene (512 bp) with the corresponding regions of these genes from several other species (Table 2). Among the 16S-rRNA genes the homology of the cyanelle gene is highest (93%) with the *Anacystis* counterpart, with values ranging from 83% (*Euglena*) to 89.5% (*Marchantia*, tobacco). Similar values were obtained when the 23S-rRNA genes were compared. The highest homology is found with *Anacystis* (79%) and *Chlamydomonas* (80%) while sequence homology of approximately 75% is observed in comparisons with the other species. The data presented here lend further support to the notion that cyanelles are a unique evolutionary link between chloroplast and free-living cyanobacteria in an organism where the evolution of plastids has taken a separate route.

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