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The 5S ribosomal RNAs of *Paracoccus denitrificans* and *Prochloron*

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ABSTRACT

The nucleotide sequences of the 5S rRNAs of *Paracoccus denitrificans* and *Prochloron* sp. are pGUCUGGUGGCCAAAGCACGAGCAAAACACCCGAUCCCAUCCCGAACUCGG CCGUUAAGUGCCGUAGCGCCAAUGGUACUGCGUCAAAAGACGUGGGAGAGUAGGUCACCGCCAGACC$_{OH}$ and UUCCUGGUGUCUCUAGCGCUUUGGAACCACUUCGAUUCCAUCCCGAACUCGAUUGUGAAACUUUGCUGCGGCUA AGAUACUUGCUGGGUUGCUGGCUGGGAAAAUAGCUCGAUGCCAGGAUU$_{OH}$, respectively. Specific phylogenetic relationships of *P. denitrificans* with purple non-sulphur bacteria, and of *Prochloron* with cyanobacteria are demonstrated, and unique features of potential secondary structure are described.

INTRODUCTION

The notion that mitochondria and plastids descend from once free-living prokaryotes is nearly a century old [1] but only in the last decade have we been able to obtain molecular evidence with which to test it, and identified contemporary prokaryotes whose resemblance to putative organellar ancestors demands specific application of such tests.

Partial or complete sequence analyses of 16S and 18S ribosomal RNAs (rRNAs) indicate only a general eubacterial ancestry for mitochondria [2]. However a specific origin of mitochondria from nonphotosynthetic derivatives (such as *Paracoccus denitrificans*) of the Rhodospirillaceae (purple non-sulphur bacteria) has been claimed on the basis of physiological and sequence data on the components of the respiratory chain [3-5].

Plastids can plausibly be derived from oxygen-evolving photosynthetic prokaryotes [1]. Of these we know two kinds, cyanobacteria and prochlorophytes (members of the genus *Prochloron*). The former contain phycobiliproteins and lack chlorophyll *b*, as do the plastids of rhodophytes and cryptomonads, which are thought to be their descendants [5-7]. The latter lack phycobiliproteins but contain chlorophyll *b*, and a specific prochlorophytan ancestry for the similarly pigmented plastids of chlorophytes and plants has

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been suggested on this basis [8].

Sequences are available for the 5S rRNAs of one mitochondrion [9], several plastids [10], and one cyanobacterium [11]. Sequences of the 5S rRNAs of *Paracoccus denitrificans* and *Prochloron* are required to test predictions made by the specific evolutionary hypotheses discussed above. Here we present these sequences.

MATERIALS AND METHODS

Preparation of unlabelled 5S rRNAs

Paracoccus denitrificans (ATCC no. 13543) was cultured for two days at 26°C in 500 ml of rich medium (4 g Difco Neopeptone, 10 g Difco yeast extract, 10 g glucose and 10 g K_2HPO_4 in 1 liter, pH 6.8). Cells were harvested by centrifugation (approx. 3 ml yield) resuspended in 30 ml of buffer (10 mM each of Tris-HCl, $MgCl_2$ and NaCl, pH 7.6) and lysed at 1270 kg/cm^2 in a French pressure cell. The lysate was made 35 units/ml in Worthington deoxyribonuclease (ribonuclease-free) and incubated at 1°C for 2 hr, and then made 0.2 mg/ml in Boehringer Mannheim proteinase K and incubated at 20°C for 1.5 hr. The lysate was extracted with a phenol/m-cresol mixture [12]. RNA was precipitated with two volumes of ethanol and that fraction soluble in 3 M NaCl at 4°C (5S and tRNA) was isolated.

Prochloron sp. cells were obtained from colonies of its symbiotic host, the giant didemnid ascidian *Lissoclinum patella* (collected at low-tide level on reef-flat sand between Kamori Island and Koror, Palau, Western Caroline Islands, June 1981). The harvested colonies were held (and remained healthy for several days) in constantly running sea water (30°C) at the Micronesian Mariculture Demonstration Centre at Koror. Colonies were cleaned of gravel and attached macroscopic organisms, rinsed in cooled, buffered (40 mM Tris-HCl, pH 8.4) sea water, and squeezed by hand to express the *Prochloron* cells. The algae were received in an equal volume of the buffered sea water; this neutralized the acids liberated by the bruised ascidians and thereby maintained the pH high enough to keep the algal cells alive and green. The cells were washed twice with cool, buffered sea water and concentrated by centrifugation. Microscopic examination revealed much less than 1% contamination by ascidian and bacterial cells. Resuspended cells (3 g in 10 ml buffered sea water) were lysed by a French pressure cell directly into an equal volume of phenol and RNA was extracted and fractionated as above.

Salt-soluble *P. denitrificans* and *Prochloron* RNAs were dissolved to 8-10 mg/ml in loading buffer [13] and resolved on 20 x 20 x 0.3 cm 10% poly-

acrylamide slab gels [14]. Bands containing 5S rRNA were identified by UV-absorbance, and RNA was eluted from gel slices by electrophoresis [15].

Terminal labelling of RNA

5S rRNAs (5-10 µg) were labelled at 5'- or 3'-termini as described previously [15], following the protocols of Donis-Keller *et al.* [14] and Peattie [13] respectively. For 3'-labelling, the pCp:RNA ratio was 2:1, and the reaction mixtures were 20 µM in ATP. Labelled RNAs were resolved on 33 x 40 x 0.15 cm, 7 M urea, 6% polyacrylamide gels (acrylamide:bis-acrylamide, 19:1), located by autoradiography and eluted as above. Identification of labelled terminal nucleotides was as described [15].

Sequencing of terminally labelled RNAs

The partial chemical degradation method of Peattie [13] was used with 3'-labelled RNA. The partial ribonuclease digestion method of Donis-Keller *et al.* [14], supplemented by the use of ribonuclease Phy M [16], was used for both 3'- and 5'-labelled RNA. Ribonucleases T_1 and U_2 were obtained from P.L Biochemicals, Phy M was a gift of H. Donis-Keller.

Analysis of *in vivo*-labelled RNA

P. denitrificans was grown in the presence of [^{32}P]-orthophosphate and *in vivo*-labelled 5S rRNA was isolated from 50S ribosomal subunits [17]. Ribonuclease T_1 digests were prepared and analyzed by a modification [18,19] of the methods of Sanger *et al.* [20] and Brownlee [21].

RESULTS

The data used to derive the nucleotide sequences of the 5S rRNAs have been provided to the editor and referees and are available from the Dalhousie authors.

DISCUSSION

Structural considerations

The nucleotide sequences of the 5S rRNAs of *P. denitrificans* and *Prochloron* are shown in Fig. 1A and B respectively, arranged in potential secondary structure models which resemble the modification of the Fox and Woese model [22] recently proposed by Garrett *et al.* [23]. Several unusual features of these arrangements are worthy of note: (i) helix II in both models is considerably shorter than in other eubacteria (most prokaryotes [23] and eukaryotes [24] have 8 base pairs in this region); (ii) a base-pair has been deleted (relative to other eubacterial 5S rRNAs [23]) from helix IV of *P. denitrificans* 5S; (iii) *Prochloron* 5S has the potential to form 4 base-

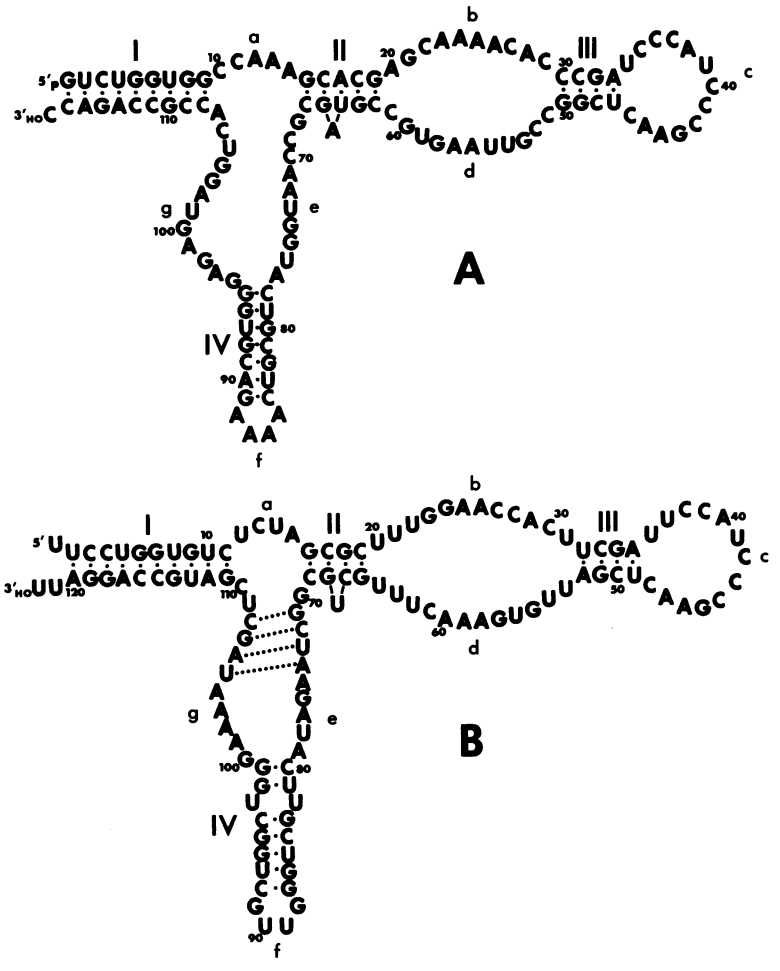

FIGURE 1. Nucleotide sequences of the 5S rRNAs of (A) *Paracoccus denitrificans* and (B) *Prochloron* sp. arranged in secondary structure models which closely resemble the recent revision of the Fox and Woese [22] model proposed by Garrett *et al.* [23] for prokaryotic 5S rRNAs. Dotted lines between nominally single-stranded regions *e* and *g* of *Prochloron* 5S indicate a potential fifth helical region which is not part of the Garrett *et al.* model.

pairs between residues which are considered part of single-stranded regions *e* and *g* in minimal models of eubacterial 5S secondary structure [22,23] (but which are indeed base-paired in current models of eukaryotic 5S structure [23-25]). The potential to form this helix in *Prochloron* lends credence to recent hypotheses that eubacterial 5S rRNA *does* generally exhibit helical structure in

this region [25-27].

Phylogenetic considerations

Fig. 2 shows the 5S rRNAs of *P. denitrificans*, *Prochloron*, wheat mitochondrion, spinach chloroplast and nine diverse eubacteria aligned to minimize the number of differences in all comparisons. Differences between pairs of sequences were counted and are expressed below as a percent of the total number of nucleotide positions considered in each such comparison.

Prochloron 5S rRNA is much more similar to the 5S of the cyanobacterium *Anacystis nidulans* (25% difference) than either are to any of the other nine eubacterial 5S rRNAs (mean percent differences of other eubacterium:*Prochloron* or *A. nidulans* comparisons are 46 ± SD 3.3 and 47 ± SD 3.7, respectively; minimums of 41% and 42% for comparisons of *B. stearothermophilus* and *E. coli* to *Prochloron* and *A. nidulans*). Both *Prochloron* and *A. nidulans* show more similarity to spinach chloroplast (32% and 34% difference, respectively) than chloroplast does to most other eubacteria (mean percent difference of other chloroplast:eubacterium comparisons is 44 ± SD 6.2). Thus, the common ancestor of all three photosynthetic lineages may have been either *Prochloron*-like or cyanobacterial and we conclude that a *specific* relationship between chloroplasts and *Prochloron*, although entirely possible, has not been demonstrated.

An unexpected value (32% difference) was obtained for the *Clostridium pasteurianum:*chloroplast comparison; however *C. pasteurianum* does not show an unusually close affiliation to either *A. nidulans* or *Prochloron* (46% and 47% differences, respectively), and we conclude that the *C. pasteurianum* and chloroplast 5S rRNAs are spuriously similar. Such irregularities are expected when a substantial fraction of the nucleotide positions being compared are highly variable, as is the case for eubacterial 5S rRNA [28]. Observed similarities at highly variable positions are as likely to reflect convergent changes as true geneological relationships. The accumulation of more 5S sequences from the cyanobacteria-*Prochloron*-chloroplast group should substantiate the preceding argument and will help to elucidate relationships within this group.

Wheat mitochondrion 5S bears more similarity to *P. denitrificans* 5S (46% difference) than to any of the other ten eubacteria in Fig. 2 (mean percent difference 55 ± SD 3.0, minimum of 51% for *Prochloron:*mitochondrion); however, acceptance of the spurious nature of the *C. pasteurianum:*chloroplast comparison (see above) casts doubt on the significance of this *P. denitrificans:*mitochondrion similarity. *P. denitrificans* 5S *is* much more similar (21% difference) to the recently published 5S of *Rhodospirillum*

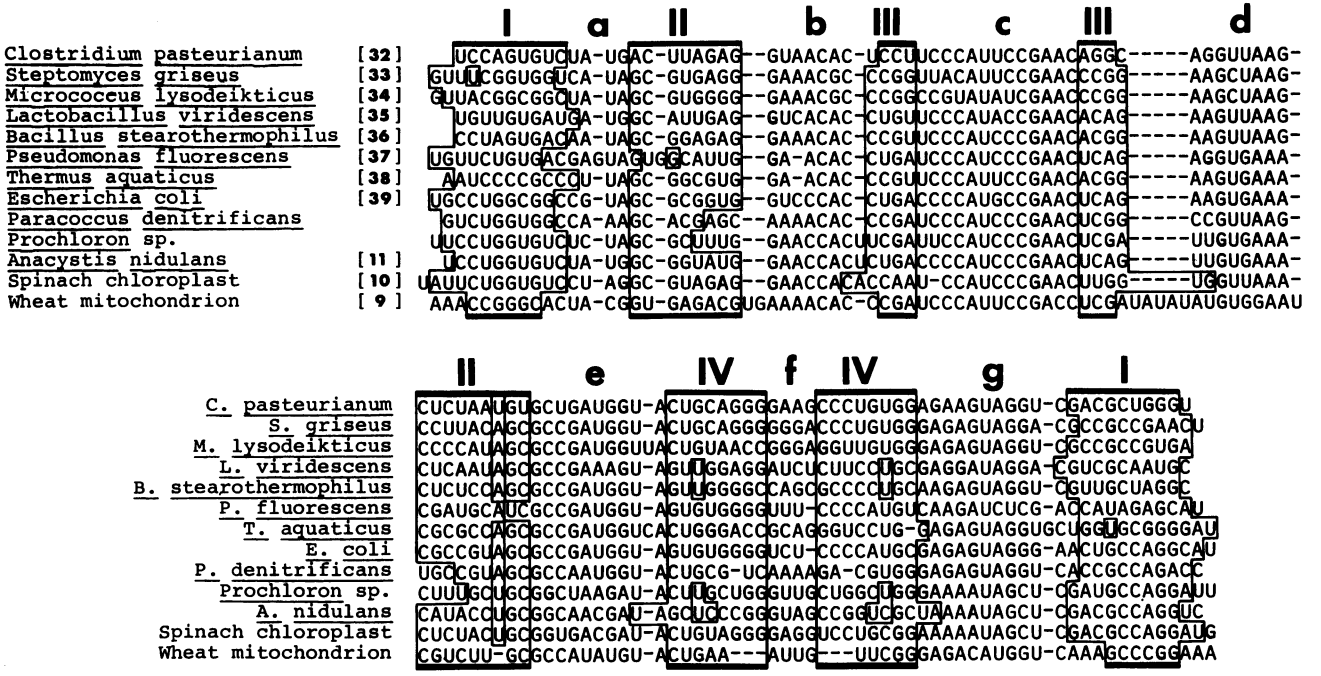

FIGURE 2. Eubacterial and organellar 5S rRNAs aligned to minimize the number of differences between sequences. Solid lines enclose those regions involved in helical regions as per the model of Garrett *et al.* [23] (see Fig. 1)

rubrum [29] than either are to the other eubacterial 5S rRNAs (mean percent differences for such *P. denitrificans* and *R. rubrum* comparisons are 42 ± SD 5.3 and 41 ± SD 5.4, respectively). This observation is consistent with data demonstrating that *P. denitrificans* and members of the Rhodospirillaceae are similar in rRNA processing [17], rRNA nucleotide sequences [17,30) and c-type cytochrome amino acid sequences [31], and strongly supports the hypothesis that the former lineage was derived from the latter by the loss of photosynthetic capability [4].

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NOTE ADDED 16S rRNA sequence data support the finding that *Prochloron* is more closely related to cyanobacteria than to plant chloroplasts (E. Seewaldt and E. Stackebrandt, Nature, in press).