Nucleotide Sequences of Chloroplast 5S Ribosomal Ribonucleic Acid in Flowering Plants

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Evidence for the sequence of duckweed (*Lemna minor*) chloroplast 5S rRNA was derived from the analysis of partial and complete enzymic digests of the ^{32}P-labelled molecule. The possible sequence of the chloroplast 5S rRNA from three other flowering plants was deduced by complete digestion with T_1 ribonuclease and comparison of the sequences of the oligonucleotide products with homologous sequences in the duckweed 5S rRNA. This analysis indicates that the chloroplast 5S rRNA species differ appreciably from their cytosol counterparts but bear a strong resemblance to one another and to the 5S rRNA species of prokaryotes. Structural features apparently common to all 5S rRNA molecules are also discussed.

Chloroplast nucleic acids are clearly different from those in the cytosol, which are of nuclear origin. For instance, the high-molecular-weight ribosomal RNA species of chloroplasts are smaller (Loening & Ingle, 1967), 5.8S rRNA is present in the cytosol but not the chloroplast ribosomes (Payne & Dyer, 1972), and *N*-formylmethionyl-tRNA rather than methionyl-tRNA initiates protein synthesis in chloroplasts (Burkard *et al.*, 1969; Leis & Keller, 1970). Also, in the broad bean (*Vicia faba*) the 5S rRNA of chloroplast ribosomes was found to be slightly larger than that of cytosol ribosomes (Dyer & Leech, 1968; Payne & Dyer, 1971).

A detailed comparison of the sequences of homologous chloroplast and cytosol nucleic acids should help resolve whether differences, such as those enumerated, are superficial or are indicative of differences in the way in which they function and have evolved. For a comparison of this type, the 5S RNA of ribosomes is particularly suitable, as it is relatively easy to prepare and sequence. Furthermore, the sequence of 5S rRNA from many different organisms is already known (Erdmann, 1978), which facilitates wide comparisons to determine which characters are common to all 5S rRNA species.

We have already studied the sequences of the cytosol 5S rRNA in flowering plants (Payne & Dyer, 1976), and in the present paper we show how the complete sequence of the chloroplast 5S rRNA of duckweed was derived. As far as we are aware this is the first complete sequence to be described for the ribosomal RNA of an organelle.

Materials and Methods

Materials different from, or in addition to, those mentioned in our previous publications (Payne *et al.*,

1973; Payne & Dyer, 1976) were obtained from the following sources: broad bean (*Vicia faba* cv. The Sutton) and dwarf bean (*Phaseolus vulgaris* cv. Masterpiece) seed from Charles Sharp, Sleaford, Lincs., U.K.; tobacco (*Nicotiana tabacum* cv. Turkish Samsum) plants were a gift from Dr. J. C. Gray, Botany Department, University of Cambridge, Cambridge, U.K.; the initial duckweed (*Lemna minor*) culture was a gift from Dr. C. J. Leaver, Botany Department, University of Edinburgh, Edinburgh, U.K.; U_2 ribonuclease (Sankyo Co.) was from Calbiochem., Bishops Stortford, Herts., U.K.; Kethoxal (Serva) from Uniscience Ltd., Cambridge CB5 8BA, U.K.; DEAE-cellulose thin-layer sheets (Polygram Cel 300 DEAE) from Camlab, Cambridge, U.K. and Cyanogum from BDH, Poole, Dorset, U.K. Fronds of the duckweed were grown in axenic culture at 27°C on 200 ml portions of half-strength Huntner's (1953) medium in 500 ml conical flasks. The fronds were illuminated by a mercury-vapour lamp (Philips 500W M.B.T.L. bulb) with an intensity of about 2000 lx. When the nucleic acids were to be labelled with ^{32}P, the non-radioactive phosphate was omitted from the medium and its pH was adjusted to 7.0 with 1 M-Tris, so that addition of the [^{32}P]P$_i$, which was in dilute HCl, did not lower the pH below 6.5. In each experiment, fronds were incubated for 6 days with 10, 20 or 30 mCi of [^{32}P]P$_i$ (1–1.5 mCi per flask, each of which contained about 400 fronds). To lower the specific radioactivity of rapidly labelled material, which contaminates the rRNA preparations, potassium phosphate, pH 7.0, was added to a concentration of 2.3 mM about 12 h before the fronds were harvested. All operations to this stage were carried out under aseptic conditions.

Broad-bean and dwarf-bean seeds were surface-sterilized by soaking them for 5 min in sodium

hypochlorite solution (5% available chloride). They were then immersed overnight in running tap water, rinsed with distilled water, sown in fine vermiculite and kept in the dark at 25°C. About 10 days later, apical segments were excised from the etiolated shoots. Broad-bean shoots were cut about 2 cm, and dwarf-bean shoots about 3 cm, below the plumular hook. In each experiment 40 of these apical segments were placed in individual 2 ml-capacity plastic vials that contained 0.5 ml of sterile water; subsequently, 0.5 mCi of $[^{32}P]P_i$ in 0.1 ml of water was added. The shoots were illuminated as described above for 3 days at 27°C; during this treatment, greening of the leaves occurred.

To label tobacco RNA, shoots of greenhouse-grown plants were excised 3 cm below the apical rosette of small leaves and about 20 of these apical segments were placed individually in plastic vials each with 1 mCi of $[^{32}P]P_i$. Further treatment was as described above.

Extraction and purification of chloroplast 5 S rRNA

The chloroplast 5S rRNA of duckweed, broad bean and dwarf bean was prepared from the large subunits of chloroplast ribosomes. As the tobacco RNA was degraded to an unacceptable extent during such a procedure, tobacco chloroplast 5S rRNA was prepared by fractionation of total RNA that had been extracted from the tissue with a phenol/detergent mixture.

Extraction and fractionation of ribosome subunits

About 5 g of fronds (or 10–15 g of leaves) were chilled for at least 1 h after collection, then homogenized in 8 vol. of ice-cold medium containing 50 mM-KCl/10 mM-MgCl$_2$/10 mM-dithiothreitol/50 mM-Tris/HCl (pH 7.8 at room temperature). The homogenate was adjusted to 4% with respect to Triton X-100 and centrifuged at 25000g (r_{av}. 7.4 cm) for 10 min at 5°C. To pellet the ribosomes, the supernatant was layered over 2 ml of 1 M-sucrose in the homogenization medium (dithiothreitol omitted) and centrifuged in a Spinco type-65 rotor at 229400g (r_{av}. 5.7 cm) for 120 min at 2°C. The ribosome pellet was suspended in a medium containing 3 mM-MgCl$_2$/50 mM-KCl/10 mM-Tris/HCl (pH 7.8 at room temperature). Under these conditions the chloroplast ribosomes are selectively dissociated into subunits, whereas the cytoplasmic ribosomes remain intact (Dyer & Koller, 1971). Portions (1 ml) of the suspension were layered on to discontinuous sucrose gradients prepared in the same medium. The gradients [which comprised six layers, with sucrose concen-
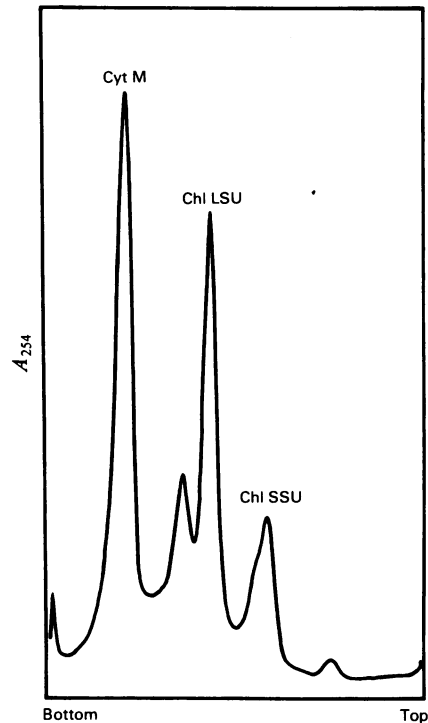

Fig. 1. *Fractionation by sucrose-density-gradient centrifugation of a ribosome preparation from duckweed*
Abbreviations used: Cyt M, cytoplasmic monosome; Chl LSU, chloroplast large subunit; Chl SSU, chloroplast small subunit.

trations of 34, 29, 24, 19, 14 and 10% (w/v) and volumes of 9, 8, 7, 5.5, 4 and 3 ml] were centrifuged in a Spinco type-SW 27 rotor at 58300g (r_{av}. 11.8 cm) for 16 h at 2°C. After centrifugation, the base of each tube was pierced and 50% sucrose (w/v) was injected to displace the gradient upwards through a coned cap with an outlet connected to a u.v. monitor. The A_{254} was measured and the chloroplast ribosomes large-subunit fraction collected. The fractionation of the ribosomal particles is shown in Fig. 1. The magnesium concentration was adjusted to 10 mM-MgCl$_2$ and the ribonucleoprotein was precipitated by storing for at least 1 h at 0°C after the addition of 0.7 vol. of ethanol (Falvey & Staelin, 1970). After washing thoroughly with 80% (v/v) ethanol, the RNA was extracted by resuspending the ribonucleoprotein in a medium containing 0.5% (w/v) sodium dodecyl sulphate/150 mM-sodium acetate (pH 6.0), and deproteinized by adding an equal volume of water-saturated phenol/cresol/8-hydroxyquinoline (1000:140:1, w/v/w). The RNA was precipitated from the aqueous layer by adding 2 vol. of ethanol and maintaining at −20°C overnight. The pelleted RNA was washed in 80% ethanol and dried *in vacuo*.

Table 1. *Analysis of oligonucleotides obtained by complete digestion of duckweed chloroplast 5S rRNA with T_1 or pancreatic A ribonuclease*

Sequencing methods: (a) position on fingerprint; digestion with (b) alkali, (c) alkali after removal of the terminal phosphates with alkaline phosphatase, (d) pancreatic A ribonuclease, (e) T_1 ribonuclease, (f) U_2 ribonuclease, (g) pancreatic A ribonuclease after carbodi-imide modification of the uracil residues, (h) U_2 ribonuclease after Kethoxal modification of guanine residues, (i) snake-venom phosphodiesterase, (j) snake-venom phosphodiesterase after removal of the 3'-terminal phosphate with alkaline phosphatase; partial digestion with (k) T_1 ribonuclease, (l) pancreatic A ribonuclease, (m) U_2 ribonuclease, (n) determination of oligonucleotide length (and of the fragments produced from it by T_1 ribonuclease digestion) by homochromatography in parallel with marker oligonucleotides of known length. Abbreviation used: n.d., not detected.

	Oligonucleotide	Molar yield — Experimental*	Molar yield — Predicted	Sequencing methods
T1	Gp	10.8	11	a, d
T2	C-Gp	3.1	3	a, d
T3	A-Gp	2.4	2	a, d
T4	A-C-Gp	1.7	2	a, d
T5	C-C-A-Gp	0.9	1	a, b, d
T6	U-Gp	3.1	3	a, d
T7	U-A-Gp	2.0	2	a, d
T8	C-U-C-Gp	0.9	1	a, d, g, j
T9	U-C-C-U-Gp	1.0	1	a, d, g, j
T10	U-C-C-U-A-Gp	1.0	1	a, d, g, j
T11	A-A-C-U-A-A-U-A-Gp	1.0	1	b, d
T12	A-A-C-U-U-Gp	0.9	1	d, f
T13	A-U-A-C-U-Gp	0.9	1	d, f
T16	U-U-A-A-A-C-U-C-U-A-C-U-Gp	0.9	1	d, f, g
T17	A-A-C-C-A-C-C-A-C-C-A-A-U-C-C-A-U-C-C-C-Gp	0.7	1	d, f, l
3'a	A-A-U$_{OH}$	n.d.	0.5	b, i
3'b	A-A$_{OH}$	n.d.	0.5	(n.d.)
5'a	pU-A-U-C-U-Gp	0.4	0.5	d, f, g
5'b	pA-U-C-U-Gp	0.4	0.5	d, j, g

	Oligonucleotide	Molar yield — Experimental†	Molar yield — Predicted	Sequencing methods
P1	Up	11.2	12	a, e
P2	Cp	12.5	15	a, e
P3	A-Cp	3.7	4	a, e
P5	A-A-Cp	0.9	1	a, b, e
P6	G-Cp	2.6	3	a, e
P7	A-Up	1.7	1.5	a, e
P9	G-A-Cp	1.7	2	a, e
P10	A-G-Cp	0.9	1	a, e
P11	G-A-A-Cp	0.9	1	a, e
P13	A-A-Up	3.1	3	a, b, e
P14	G-Up	1.1	1	a, e
P18	G-A-Up	0.7	1	a, e, j
P19	A-G-G-Cp	3.4	4	a, e
P20	G-G-Up	0.8	1	e, k, m
P23	A-G-A-G-G-A-A-Cp	0.9	1	b, e, h, n
P24	G-G-A-A-A-A-A-Up	0.3	1	b, e, j, h, k
P25	A-G-G-G-A-G-G-Up	0.5	0.5	b, e, i
3'a	A-G-A-A-U$_{OH}$	0.5	0.5	b, e, i
3'b	A-G-A-A$_{OH}$	0.5	0.5	a, b
5'a	pUp	0.5	0.5	b, e, i
5'b	pA-Up	0.4	0.5	a, b, c, j

* Expressed relative to the mean radioactivities found in spots U-Gp/6 and U-A-Gp/2. Average values from four experiments.

† Expressed relative to the mean radioactivities found in spots A-A-Up, G-A-Up and G-A-A-Cp. Average values from three experiments.

Extraction and fractionation of total RNA

Total RNA (but not DNA) was extracted from the ^{32}P-labelled tobacco leaves (about 10g fresh weight) by the sodium naphthalene-1,5-bis-sulphonate method of Hastings & Kirby (1966). The RNA derived by this method was dissolved in an ice-cold solution of 0.65M-NaCl in 0.02M-potassium acetate buffer, pH5.6, and the high-molecular-weight nucleic acid pelleted by high-speed centrifugation as described previously (Payne *et al.*, 1973). The supernatant was diluted with 2vol. of water, and the low-molecular-weight RNA that it contained was adsorbed on to a column (2cm × 2cm) of DEAE-cellulose. The column was washed with 0.2M-NaCl in 0.02M-potassium acetate buffer, pH5.6, and the RNA was eluted with 0.65M-NaCl in the same buffer. The RNA was precipitated by adding 2vol. of ethanol and storing overnight at −20°C.

Polyacrylamide-gel electrophoresis of low-molecular-weight RNA

RNA derived from the large subunits of chloroplast ribosomes was fractionated in 12% (w/v) slab gels made with Cyanogum in the 'E' buffer of Loening (1969). A high-resolution system of electrophoresis (Rubin, 1975) was used to fractionate the total low-molecular-weight RNA from tobacco.

In each case the strip of gel containing the chloroplast 5S rRNA was excised and fragmented by forcing it, in the presence of 0.65M-NaCl/0.02M-potassium acetate buffer, pH5.6, through a syringe fitted with a 21 G needle. Elution of the RNA was monitored by measuring Čerenkov radiation. At least 80% recovery was usually achieved. Polyacrylamide that eluted with the RNA was removed from the preparation by using a small DEAE-cellulose column (Payne *et al.*, 1973).

Digestion of RNA and analysis of products

Standard procedures were used for the complete digestion of chloroplast 5S rRNA, the fractionation of the products by two-dimensional electrophoresis, and for most further analyses of the primary digestion products (Brownlee, 1972). In a few instances the primary digestion products were fractionated on 'minifingerprints' (Volckaert *et al.*, 1976) to prepare the large oligonucleotides with less background
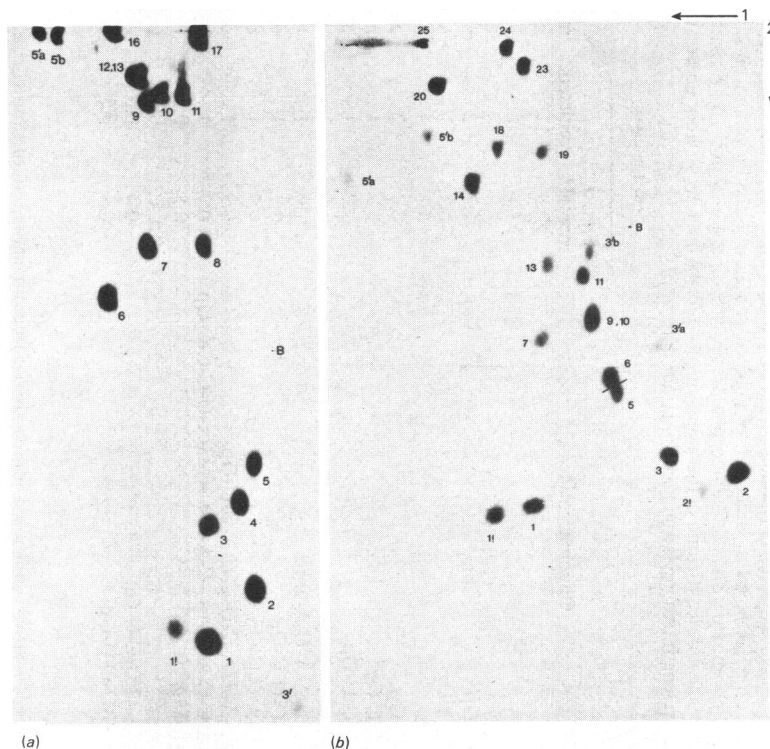

Fig. 2. *Radioautographs of the fractionated products from duckweed chloroplast 5S rRNA that had been digested with (a) ribonuclease T₁ and (b) pancreatic A ribonuclease*
Fractionation was by electrophoresis in cellulose acetate (direction 1) and on DEAE-cellulose paper (direction 2).

contamination than was obtained by using the standard procedure. Oligonucleotides, which had been treated with bacterial alkaline phosphatase, were separated from it by electrophoresis of the digestion mixture for 2h at 1.5kV on DEAE-paper in 7% (v/v) formic acid.

Information about the sequence of pancreatic A ribonuclease products P23 and P25 (see the Results) from duckweed 5S rRNA was obtained by using a method modified from that described by Min Jou & Fiers (1976). Guanine residues were modified with Kethoxal by dissolving each oligonucleotide in 30 μl of a solution containing 0.15 M-Kethoxal/1 mM-EDTA/0.1M-sodium acetate, pH 5.0, and incubating for 4h at 37°C. After the addition of 0.1 vol. of 20% (w/v) potassium acetate, the reaction mixtures were dried on to small pieces of Whatman 3MM paper, which were then thoroughly washed with ethanol to remove unchanged Kethoxal. The oligonucleotides were then eluted with water and freeze-dried. After this they were digested with 0.2 unit of U_2 ribonuclease in 20 μl of 1 mM-EDTA/50 mM-sodium acetate buffer, pH 4.5, for 16h at 37°C; modification of the guanine residues rendered the adjacent phosphodiester linkages resistant to cleavage. Further analysis was carried out as described by Min Jou & Fiers (1976).

Partial digestions with T_1 or pancreatic A ribonuclease were carried out (at 0°C for 30 min) with enzyme-to-substrate ratios of either 1:1000 or 1:2000 (w/v). The partial digestion products were fractionated by high-voltage electrophoresis on cellulose acetate followed by homochromatography on DEAE-cellulose thin layers with Homomix a (Brownlee, 1972). The partial products were eluted from the thin layers by the capillary-tube method of Volckaert et al. (1976), and the secondary and tertiary digestion products were analysed by the standard procedures.

Results

Derivation of the duckweed sequence

Oligonucleotides produced by complete digestion of duckweed 5S rRNA with T_1 ribonuclease and with pancreatic A ribonuclease are listed in Table 1 and radioautographs of the fractionated products are shown in Fig. 2.

The methods used for the derivation of the sequence of each oligonucleotide are given in the right-hand column of Table 1. By using these methods it was possible to deduce unequivocally the sequence of all the primary digestion products. The sequences of ribonuclease products T11 and P24 are revised from those given in a preliminary account of this work (Dyer & Bowman, 1976); that of product T17 was
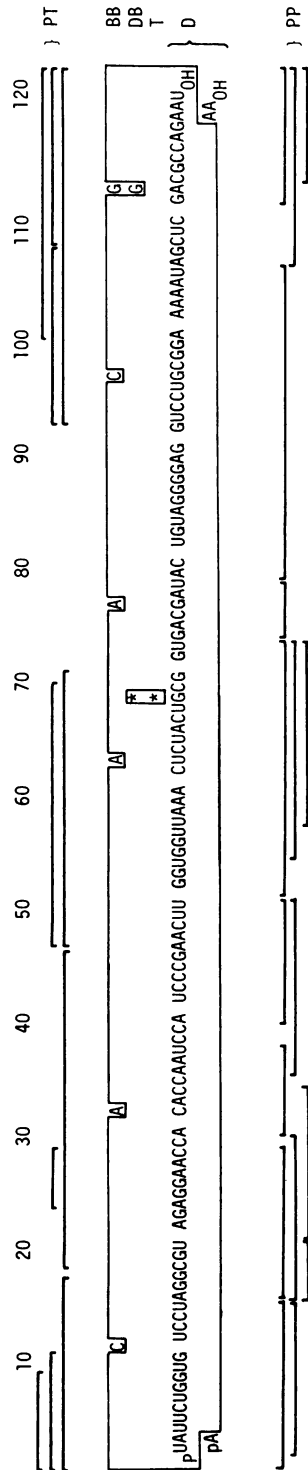
Fig. 3. The nucleotide sequence of chloroplast 5S rRNA from duckweed (D)

The sequence is compared with those of the chloroplast 5S rRNA from tobacco (T), dwarf bean (DB), and broad bean (BB). The heterogeneity of the terminal oligonucleotides in the duckweed molecule is also indicated. Asterisks denote gaps introduced to obtain maximum sequence homology and the brackets above and below the sequence indicate respectively the products of partial T_1 (PT) and partial pancreatic A (PP) ribonuclease digestion of the duckweed molecule.
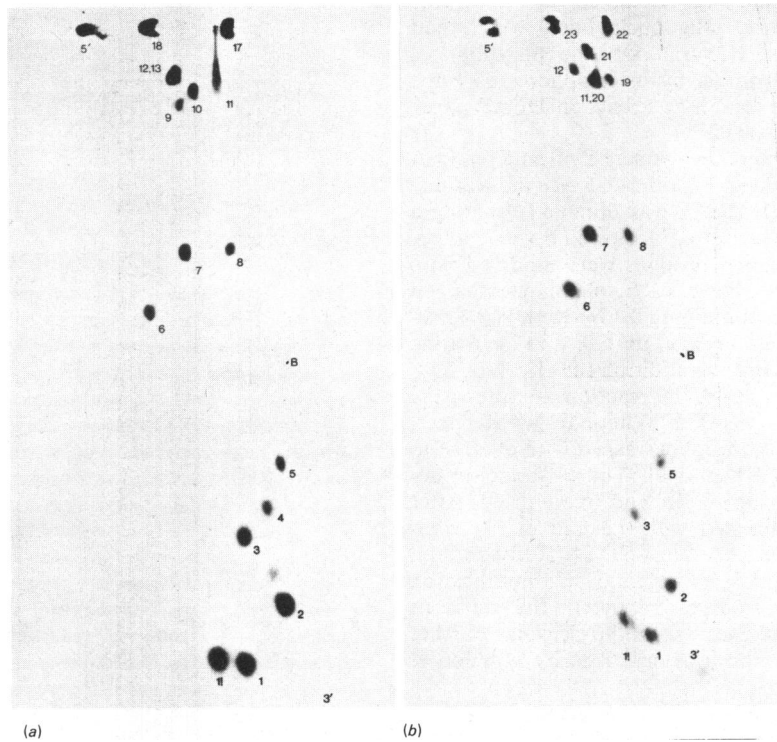
Fig. 4. *Radioautograph of the fractionated T_1 ribonuclease digestion products of chloroplast 5S rRNA from (a) dwarf bean and (b) broad bean*

Fractionation was as described in legend to Fig. 2. The split spots for the 5'-oligonucleotide and for product T23 of the broad-bean material are a result of a fractionation artefact.

finally established from the products of the partial ribonuclease A digestion of intact 5S rRNA. The terminal oligonucleotide AA_{OH} was never detected in T_1 ribonuclease digests, but its presence is inferred because the product $AGAA_{OH}$ was always present in pancreatic A ribonuclease digests of the molecule.

The experiments in which the 5S rRNA was partially digested gave sufficient overlapping fragments for the sequence of nucleotides in the molecule to be determined unambiguously, as shown in Fig. 3.

Sequence analysis of 5S rRNA from the broad bean, dwarf bean and tobacco

The products derived by T_1-ribonuclease digestion of chloroplast 5S rRNA from duckweed were compared with those derived by similar digestion of the chloroplast 5S rRNA from tobacco, dwarf bean and broad bean. The appreciable similarity between their RNA species may be assessed by comparing the radioatuographs of the fractionated products (Figs. 2 and 4). As the tobacco 5S rRNA radioautograph is

very similar to that of the dwarf bean and also shows that there was heavy contamination of the chloroplast RNA preparation by cytoplasmic 5S rRNA, it is not included.

Sequence analysis of the ribonuclease T_1 digestion products confirmed the evidence of the 'fingerprints', that the chloroplast 5S rRNA species of tobacco, dwarf bean and duckweed show few differences in the composition of their 'T_1' oligonucleotides. The broad-bean chloroplast 5S rRNA is the most dissimilar of the four examples. Those oligonucleotides that differ from the corresponding duckweed products are listed in Table 2. Such differences may be most readily explained by assuming that the base changes suggested in the right-hand column of Table 2 have taken place. The probable positions of diversity in the complete sequence are shown in Fig. 3. These designations are tentative of, course; it would be necessary to repeat the entire duckweed study separately with each plant in order to establish the differences with certainty.

The sequence presented here for the chloroplast 5S rRNA of duckweed is consistent with that deduced

Table 2. *Analysis of sequence variation in oligonucleotides produced by T_1 ribonuclease digestion of chloroplast 5S rRNA from duckweed, tobacco, dwarf bean and broad bean*

Corresponding fragment in duckweed	Plant		Composition of changed fragment	Inferred sequence of fragments	Probable base changes causing sequence difference
T9+T2 U-C-C-U-G-C-Gp	Broad bean	T19	(U2C4)Gp	U-C-C-U-C-C-Gp	G→C, T2 lost
T10 U-C-C-U-A-Gp	Broad bean	T20	(U2C3)A-Gp	C-U-C-C-U-A-Gp	C added
T4+T13 A-C-G-A-U-A-C-U-Gp	Broad bean	T21	(U-A-C2,A-A-U)Gp	A-C-A-A-U-A-C-U-Gp	G→A, T4 lost
T17 A-A-C-C-A-C-A-A-C-C-A-A-U-C-C-A-U-C-C-C-Gp	Broad bean	T22	(C6,A-C2,A-U,A-A-C,A-A-A-C)Gp	A-A-C-C-A-A-U,A-A-C-C-A-A-U-C-C-A-U-C-C-C-Gp	C→A
T16 U-U-A-A-A-C-U-C-U-A-C-U-U-G-C-Gp	Broad bean	T23	(U3,A-C2,A-A-A-C)Gp	U-U-A-A-A-C-A-C-U-C-U-A-C-U-U-Gp	U→A
T16+T2 U-U-A-A-A-C-U-C-U-A-C-I-G-C-Gp	Tobacco, Dwarf bean	T18	(U3,A-C,C2,A-A-C)Gp	U-U-A-A-A-C-U-C-U-A-C-U-C-Gp	G deleted, T2 lost
T4 A-C-Gp	Broad bean, Dwarf bean		T1+T2 G-C-Gp		A→G
3'a A-A-U_OH	Tobacco, Dwarf bean		3' A-A-U_OH		No heterogeneity
3'b A-A_OH, 5'a pU-A-U-U-C-U-Gp	Tobacco, Dwarf bean		3' A-A-U_OH		No heterogeneity
5'b pA-U-U-C-U-Gp	Broad bean, Dwarf bean		5' pU(A-U,U2C)Gp	pU-A-U-U-C-U-Gp	

for a cloned chloroplast 5S-rRNA gene from maize (T. A. Dyer & J. R. Bedbrook, unpublished work).

Discussion

When the low-molecular-weight RNA from the large subunit of chloroplast ribosomes is fractionated by polyacrylamide-gel electrophoresis, a 5S rRNA component and also other low-molecular-weight rRNA species are resolved. The latter have been the subject of a separate study [the following paper, Bowman & Dyer (1979)]. Here we discuss the nucleotide sequence of the chloroplast 5S rRNA of duckweed and relate it to that of three other flowering plants.

As mentioned in the introduction, the chloroplast 5S rRNA of broad bean is slightly larger than the comparable cytosol component. This seems to be true for other higher-plant chloroplast 5S rRNA species as well. From the sequence data presented here it may be deduced that, in duckweed, the chloroplast 5S rRNA molecule contains 121 nucleotides and has a mol.wt. of 39300, whereas the cytosol 5S rRNA of duckweed (Dyer & Bowman, 1976), like that of rye (*Secale cereale*), tomato (*Lycopersicum esculentum*), sunflower (*Helianthus annuus*), dwarf bean and broad bean (Payne & Dyer, 1976) has 118 nucleotides and a mol.wt. of 37800. Similarly, the molecular weights of the other chloroplast 5S rRNA species studied also exceed that of the cytosol 5S rRNA. Their base compositions differ only slightly from that of the duckweed chloroplast 5S rRNA, and the analyses indicate mol.wts. of 39600 for broad bean, 39700 for tobacco and 38700 for dwarf bean. Confirmation that these chloroplast 5S rRNA species are similar in size and larger than their cytosol counterparts (results not shown) was obtained by high-resolution polyacrylamide-gel electrophoresis by the method of Rubin (1975).

Fig. 5 shows that the nucleotide sequence of the chloroplast and cytosol 5S rRNA species from the same plant are very different. At equivalent positions in the two, about 50% of the residues are different. Furthermore, they have few long sequences of nucleotides in common. In contrast, the sequences of chloroplast 5S rRNA and that from *Anacystis nidulans*, a photosynthetic prokaryote (Corry *et al.*, 1974*a*), are remarkably alike, with long sequences in common, the proportion of differences between them at equivalent positions being only 25%. This degree of difference is less than is observed between the 5S rRNA of *A. nidulans* and that of other bacteria such as *Escherichia coli* (37%) and *Pseudomonas fluorescens* (41%) (Corry *et al.*, 1974*b*). Despite these differences, the 5S rRNA molecules are obviously all homologues as, even between the most dissimilar, the difference is much less than the 75% that would be expected if two molecules were unrelated random sequences
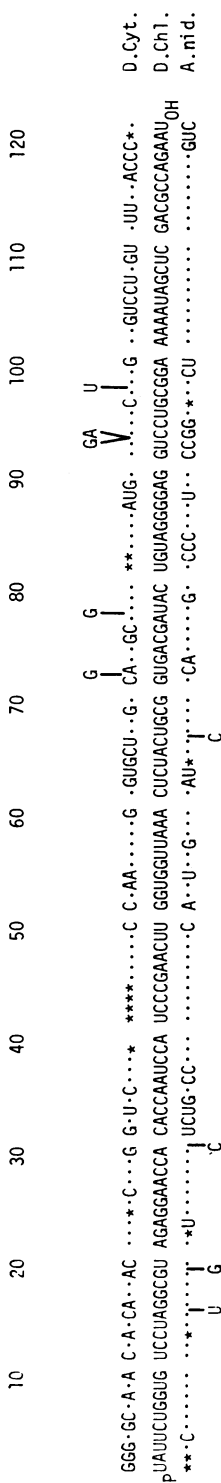
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            10        20        30        40        50        60        70        80        90       100       110       120
                                                          G         G     GA  U
                                                          |_        |_    |_  V
GGG·GC·A·A  C·A·CA··AC  ···*·C···G  G·U·C···*  ****······C  C·AA······G  ·GUGCU··G·  CA··GC····  **·····AUG·  ·V···C··.·G  ·GUCCU·GU  ·UU·ACCC·*.      D.Cyt.
pUAUUCUGGUG  UCCUAGGCGU  AGAGGAACCA  CACCAACCA  UCCCGAACUU  GGUGGUUAAA  CUCUACUGCG  GUGACCAUAC  UGUAGGGGAG  GUCCUGCGGA  AAAAUAGCUC  GACGCCAGAAU_OH   D.Chl.
                  U                    C
**·C······  ··*·|·····  ·*U······  |UCUG·CC···  ··········  A··U··G··  ·CA*·|····  ·CCC···G·  ·CCC···U·  ·CCGG·*··CU  ··········  ········GUC      A.nid.
              U  G                  C                                          C
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Fig. 5. *Comparison of the nucleotide sequence of 5S rRNA from duckweed chloroplasts (D. Chl.), duckweed cytosol (D. Cyt.) (Dyer & Bowman, 1976) and Anacystis nidulans (A. nid.) (Corry et al., 1974a)*

Dots represent nucleotides identical with those in the duckweed chloroplast 5S rRNA sequence and asterisks indicate gaps introduced to maximize sequence homology.

were compared. It has been suggested, though, that the eukaryote cytosol and prokaryote 5S rRNA species have diverged in function to some extent (Wrede & Erdmann, 1977).

From the comparison of the sequences of 5S rRNA from the chloroplast and cytosol of duckweed with that from the (blue–green alga) *A. nidulans*, it is possible to make some observations concerning the evolutionary relationship of these molecules and, by implication, the organisms and structures of which they are part.

The high degree of sequence similarity between the 5S rRNA of chloroplasts and that of the cyano-bacterium provides unequivocal proof that they both were derived from a common ancestral prokaryote. There is also considerable additional evidence to support such a concept (Phillips & Carr, 1977). This raises the question as to how the chloroplast evolved. Two main theories have been proposed to explain their origin (and that of mitochondria) within the eukaryotic cell. In one (the endosymbiont hypothesis), it is proposed that these organelles have evolved from prokaryotic cells that became incorporated into the cytosol of a protoeukaryote [see Margulis (1970) for a review]. In the other (the cluster-clone, episome or interfilial hypothesis), it is suggested that the sub-cellular structures originated by compartmentation and differentiation of the ancestral eukaryote cell, which had retained some features of the prokaryotes from which it, in turn, had evolved [see Bogorad (1975) for a review].

The endosymbiont hypothesis predicts that the RNA species of chloroplasts and prokaryotes will resemble one another more closely than will the RNA species from the chloroplast and cytosol. The reverse would be expected were the cluster-clone hypothesis correct. These predictions are based on two assumptions. Firstly, that the RNA species are indeed derived from a common ancestor, and, secondly, that the rate of nucleotide substitution has been the same in each (see Hori, 1975). On the basis of these assumptions, our results strongly support the endosymbiont hypothesis. However, although the first assumption is likely to be correct, the second may not be.

With the exception of the broad bean, there has been a slower rate of nucleotide change in the 5S rRNA of chloroplasts than in that of the cytosol in the plants that we studied (Table 3). Although only four species were compared, they represent widely differing groups. Duckweed is a monocotyledon, whereas the beans and tobacco are from quite unrelated families of dicotyledons. Furthermore, it seems probable that there has also been a relatively slow rate of nucleotide substitution in the 5S rRNA of cyanobacteria. Therefore the similarity of the chloroplast molecule to the 5S rRNA of these bacteria is not surprising. Nevertheless if the cluster-

Table 3. *Number of bases that differ between the chloroplast 5S rRNA and the cytosol 5S rRNA of four flowering plants*
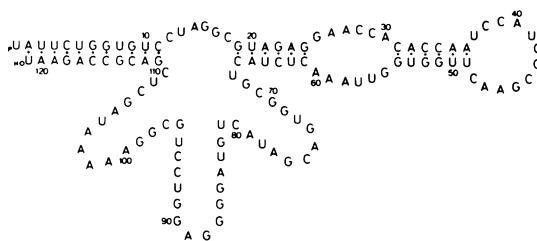
The cytosol sequences used in this comparison, with the exception of that for tobacco, were those described by Payne & Dyer (1976). However, the tobacco cytosol 5S rRNA gave, on digestion with T_1 ribonuclease, the same oligonucleotides as those that had been derived from tomato, and therefore the sequence of the cytosol 5S rRNA from these plants was assumed to be identical.

Plants compared	Number of bases different	
	Chloroplast	Cytosol
Duckweed/dwarf bean	2	8
Duckweed/tobacco	1	5
Duckweed/broad bean	6	8
Dwarf bean/tobacco	1	6
Dwarf bean/broad bean	6	4
Tobacco/broad bean	7	7

Fig. 6. *Possible secondary structure of chloroplast 5S rRNA*

clone hypothesis were correct, one would expect the 5S rRNA of the cytosol to resemble that of chloroplasts and cyanobacteria to a greater degree than it does that of other, non-photosynthetic, bacteria, even allowing for differences in nucleotide-substitution rates. This follows because the common progenitor of the plant cells and cyanobacteria would have diverged from the non-photosynthetic prokaryotes before the evolution of the photosynthetic process and of cytosol structures. As this expectation is not realized, our results still favour the endosymbiont hypothesis. This conclusion is also supported by a sequence analysis that has been made of chloroplast 16S and cytosol 18S rRNA of duckweed (C. R. Woese, T. A. Dyer & C. M. Bowman, unpublished work).

The lower rate of change in chloroplast rRNA compared with cytosol rRNA may be an indication that the chloroplast genome is more stable than the nuclear genome. Additional evidence of this comes from the study of the amino acid composition of ribulose 1,5-bisphosphate carboxylase. In this enzyme the large subunit, which is coded for by the chloroplast genome, has changed less in composition than has the small subunit, which is coded for by the nuclear genome (Takabe & Akazawa, 1975).

From the nucleotide sequence it is likely that chloroplast 5S rRNA shares several features of secondary structure with all 5S rRNA molecules. It can be folded as suggested by a recent universal model for this RNA (Fox & Woese, 1975a,b). This is shown in Fig. 6. In common with other 5S rRNA species, the nucleotides at the 5'-end of the molecule are complementary to those at the 3'-end, indicating that the two segments are base-paired *in situ*. As Fig. 6 shows, the model proposes that there are also base-paired regions in other parts of the molecule. In the 5S rRNA of duckweed, tobacco and dwarf

bean, the number of nucleotides in the paired regions may be greater than in most other 5S rRNA species. However, if the nucleotide composition shown in Fig. 3 for broad bean is correct, the number of possible base-pairs in this molecule is fewer than in the other chloroplast 5S rRNA species and therefore more typical of the general pattern.

The cytosol 5S rRNA molecule, although dissimilar in sequence from that of the chloroplast, may also be folded in the same way (Payne & Dyer, 1976). The comparison of these, and of other 5S RNA species, therefore indicates that widely differing nucleotide sequences may give rise to a molecule of similar shape. This shape may be essential for it to function and therefore probably only those nucleotide substitutions that do not change it are tolerated (Benhamou *et al.*, 1977).

There is, however, one region of secondary structure that apparently is not present in all 5S rRNA molecules. It has been suggested that most prokaryotic 5S rRNA molecules contain a further segment of base-pairing in the region of nucleotides 82–86 and 90–94 (Fox & Woese, 1975a). In the chloroplast 5S rRNA this is likely to be between nucleotides 81–85 and 92–96.

Another prokaryotic characteristic of chloroplast 5S rRNA is that, unlike cytosol 5S rRNA, it has a -CCGAAC- sequence of nucleotides in the segment of the molecule between nucleotides 40 and 50. Such a sequence has been found in all prokaryotic 5S rRNA species studied so far. In the model, these nucleotides are located in a single-stranded loop just preceding a base-paired segment that forms a stem to the loop. Some of these nucleotides may interact with the -RGTΨC- sequence in tRNA during protein synthesis (Erdmann, 1976). In the 5S rRNA of eukaryote cytosol ribosomes, the equivalent part of the molecule is heterogeneous in composition (Erdmann, 1978), and it has been suggested that in cytosol ribosomes, a -YGAAC- sequence in 5.8S rRNA may have the comparable tRNA-binding role (Wrede & Erdmann, 1977).

A third specific resemblance between the chloroplast and prokaryote 5S rRNA is the single phosphate residue at the 5'-end. This is what might be expected

if it were excised from an initial transcript that was the precursor of all the ribosomal RNA species (Ginsberg & Steitz, 1975) and is consistent with the observation that, in chloroplast DNA, the cistrons coding for 5S rRNA are near those coding for 23S rRNA (Bedbrook *et al.*, 1977; Whitfeld *et al.*, 1978). A feature of particular interest in the chloroplast 5S rRNA of duckweed is that two different oligonucleotides are obtained from each end of the molecule when it is digested enzymically. These are present in half-molar amounts (Table 1). From this it would seem either that the chloroplast population itself is heterogeneous or that there are two slightly different sites in chloroplast DNA where this RNA is synthesized. The latter is the more probable explanation, as hybridization results have indicated that there are two cistrons for high-molecular-weight rRNA per copy of chloroplast DNA (Thomas & Tewari, 1974). These cistrons have been shown, by restriction-enzyme 'mapping', to be in segments of the genome that are inverted repeats of one another (Bedbrook *et al.*, 1977). In contrast with duckweed, however, comparable heterogeneity was not detected in the chloroplast 5S rRNA of the other plants that were studied.

Despite the large amount of information that has now accumulated on the primary structure of 5S rRNA, much remains uncertain about its function and secondary and tertiary structure. The present study on a 5S rRNA molecule that differs widely in its origin from those already examined should help provide the data from which satisfactory generalizations can be made. Furthermore, a knowledge of the sequence of chloroplast 5S rRNA makes it possible to investigate the detailed organization of the genes from which it is transcribed.

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