4.5S Ribonucleic Acid, a Novel Ribosome Component in the Chloroplasts of Flowering Plants

By Catherine M. BOWMAN and Tristan A. DYER

Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, U.K.

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A species of low-molecular-weight ribosomal RNA, referred to as '4.5S rRNA', was found in addition to 5S rRNA in the large subunit of chloroplast ribosomes of a wide range of flowering plants. It was shown by sequence analysis that several variants of this RNA may occur in a plant. Furthermore, although in most flowering plants the predominant variant contains about 100 nucleotides, in the broad bean it has less than 80. It seems, therefore, to be much more diverse in size and sequence than the other ribosomal RNA species. Like 5S rRNA, it does not contain modified nucleotides and it is also unusual in having an unphosphorylated 5'-end. It is apparently neither a homologue of cytosol 5.8S rRNA nor a fragment of 23S rRNA.

Various features of ribosomal RNA are apparently constant in all living organisms. For example, in every ribosome there are two molecules of high-molecular-weight RNA, one of which is about twice the size of the other. Low-molecular-weight rRNA is also present; nearly all types of ribosome contain 5S rRNA, and the cytosol ribosomes of eukaryotes also contain 5.8S rRNA, which is hydrogen-bonded to the larger of the two high-molecular-weight RNA species. Deviations from these norms are infrequent. Consequently, when detected, they are of particular interest.

We describe here some characteristics of a novel species of low-molecular-weight RNA that occurs in addition to the rRNA in the chloroplast ribosomes of flowering plants. It was initially observed independently by ourselves (Dyer & Bowman, 1976; Dyer *et al.*, 1977) and by Whitfield *et al.* (1977), and has subsequently been described in greater detail by Whitfield *et al.* (1978). Hartley (1979) has also studied its synthesis in spinach (*Spinacia oleracea*).

Materials and Methods

Materials

The sources of all the materials referred to in the present paper are described in the preceding paper (Dyer & Bowman, 1979).

Methods

Growth of plants and incorporation of ^{32}P into their RNA in vivo. The culture of duckweed (*Lemna minor*) fronds and the growing of broad-bean (*Vicia faba*), dwarf-bean (*Phaseolus vulgaris*) and tobacco (*Nicotiana tabacum*) plants for labelling *in vivo* of their nucleic acids with ^{32}P are described in the preceding paper (Dyer & Bowman, 1979). For experiments not

involving the use of radioactivity, plants were grown in a peat-based compost in a greenhouse and were used 2–3 weeks after germination.

Extraction and purification of 4.5S rRNA. The chloroplast 4.5S rRNA of all the plants studied, with the exception of tobacco, was prepared from the large subunits of chloroplast ribosomes, as described in the preceding paper (Dyer & Bowman, 1979). About 5g of duckweed fronds or 10–15g of young leaves was used for each preparation. Because the tobacco rRNA was degraded to an unacceptable extent during ribosome extraction (see Fig. 2*d* below), 4.5S rRNA from this plant was prepared by fractionation of the RNA extracted by the sodium naphthalene-1,5-bis-sulphonate method of Hasting & Kirby (1966) and purified, again as described in the preceding paper (Dyer & Bowman, 1979).

Polyacrylamide-gel electrophoresis of low-molecular-weight RNA. For the preparation of RNA for sequence analysis, radioactive RNA derived from the large subunits of chloroplast ribosomes was fractionated by electrophoresis in slab gels made with 12% (w/v) Cyanogum in the 'E' buffer of Loening (1969) (see Fig. 1 below). The high-resolution electrophoresis system of Rubin (1975) was used to fractionate the total low-molecular-weight RNA from tobacco (Fig. 1*c* below). The 4.5S rRNA bands were extracted from strips of gel and purified on a column of DEAE-cellulose (Payne *et al.*, 1973). Non-radioactive low-molecular-weight RNA from the large subunit of chloroplast ribosomes was fractionated on cylindrical gels of 10% polyacrylamide (Loening, 1969) and the gels scanned at 260nm in a modified Hilger-Gilford spectrophotometer.

Digestion of RNA and analysis of the products. Standard procedures were used for the complete digestion of chloroplast 4.5S rRNA, for the fractionation of the products by two-dimensional

electrophoresis and for most further analysis of the primary digestion products as indicated in Table 1 below (Brownlee, 1972). Limited digestion with U_2 ribonuclease (0.1 unit/ml for 1 h at 37°C) was necessary to deduce the sequence of T_1 ribonuclease oligonucleotide products T20 and T21 and pancreatic A ribonuclease oligonucleotide product P22. The 5'-terminal nucleotide of T7, P14, P15, P19 and P20 was identified by digesting each with snake-venom phosphodiesterase after the 3'-terminal phosphate had been removed with alkaline phosphatase as described in the preceding paper (Dyer & Bowman, 1979).

Results

Occurrence of 4.5S rRNA

During the routine preparation of 5S rRNA from the large subunit of chloroplast ribosomes, we found an additional low-molecular-weight RNA component. Its presence is clear from the radioautograph of ^{32}P -labelled chloroplast low-molecular-weight rRNA fractionated by polyacrylamide-gel electrophoresis (Fig. 1a). The larger of the two major components was identified as chloroplast 5S rRNA and the smaller, which is described here in some detail, is referred to as '4.5S rRNA'.

The distribution of 4.5S rRNA was studied in a wide range of unrelated plants. It could be clearly distinguished in ribosomal RNA preparations from rye (*Secale cereale*), dwarf bean, duckweed, pea (*Pisum sativum*) and broad bean (Fig. 2), but was not

obvious in a preparation from tobacco (Fig. 2d). Furthermore, in pea (Fig. 2e) and broad bean (Fig. 2f) the 4.5S rRNA is smaller in size than in the other

Fig. 1. Radioautographs of ^{32}P -labelled low-molecular-weight RNA fractionated by electrophoresis in polyacrylamide slab gels

RNA from the large subunit of chloroplast ribosomes was from (a) duckweed and (b) broad bean; (c) total RNA from tobacco.

Fig. 2. Fractionation of RNA from the large subunit of chloroplast ribosomes by electrophoresis in 10% (w/v) polyacrylamide gels (a) Rye; (b) dwarf bean; (c) duckweed; (d) tobacco; (e) pea; (f) broad bean. Unlabelled arrows indicate putative minor 4.5S RNA components.

Fig. 3. Radioautographs of the fractionated ribonuclease digestion products of duckweed 4.5S rRNA species (a) 4.5S rRNA₁ digested to completion with T₁ ribonuclease; (b) 4.5S rRNA digested to completion with pancreatic A ribonuclease; (c) 4.5S rRNA₂ digested to completion with T₁ ribonuclease; (d) 4.5S rRNA₃ digested to completion with T₁ ribonuclease. Fractionation was by electrophoresis on cellulose acetate (direction 1) and on DEAE-cellulose paper (direction 2).

plants examined. In these species it could be mistaken for tRNA, which was bound to the ribosomes.

The low yield of 4.5S rRNA relative to 5S rRNA from tobacco (Fig. 2d and Table 4 below) was probably due to high endogenous activities of nuclease, which caused the degradation of this RNA species during ribosome extraction. When total RNA was extracted directly from tobacco leaves rather than from ribosomes, by using a phenol/detergent mixture, substantially greater amounts of 4.5S rRNA were obtained (Fig. 1c). This result indicates that, although a ribosomal component, the 4.5S rRNA is much more vulnerable to enzymic attack than is the 5S rRNA.

RNA comparable in size to the 4.5S rRNA of rye, dwarf bean, sunflower (*Helianthus annuus*) and duckweed was also distinguishable in the fractionated preparations of total low-molecular-weight RNA from several other flowering plants. These included mung bean (*Phaseolus aureus*), arum lily (*Zantedeschia aethiopica*), bluebell (*Endymion non-scriptus*), broccoli (*Brassica oleracea*), cucumber (*Cumcumis sativus*), cress (*Lepidum sativum*), buttercup (*Ranunculus acris*), spinach, wheat (*Triticum aestivum*), maize (*Zea mays*) and goose-grass (*Galium aparine*). It was not found in similar preparations from a blue-green alga (*Anabaena* sp.), liverwort, moss, or

fern (*Adiantum* sp.), but may be present in the club moss *Selaginella kraussiana*. From this it seems probable that 4.5S rRNA is present in the chloroplast ribosomes of all flowering plants.

In duckweed, two additional low-molecular-weight components are present in small amounts in RNA prepared from chloroplast ribosomes (Fig. 1, bands 2 and 3, and Fig. 2c). These both migrate faster during electrophoresis than does the predominant 4.5S component, and, as we show below, they are both variants of the 4.5S rRNA. They are accordingly referred to as '4.5S rRNA₂' and '4.5S rRNA₃'. It is unlikely that the minor components arise through breakdown of the major component during the preparation of the RNA, for in preparations where degradation of RNA has occurred, all the variants are decreased in amount. Minor components, corresponding in size to those of duckweed, also occur in preparations from dwarf bean (Fig. 2b), and there is also some indication of additional material in rye chloroplast ribosomes (Fig. 2a).

The 4.5S rRNA of broad bean corresponds in electrophoretic mobility to the 4.5S rRNA₃ of duckweed (Fig. 1) and is therefore much smaller than the main component of most other flowering plants (Figs. 1b and 2f). No minor components were resolved for broad bean by the methods of electro-

Fig. 4. Radioautographs of the fractionated products of 4.5S rRNA species after digestion to completion with T_1 ribonuclease (a) Dwarf bean; (b) tobacco; (c) broad bean. Fractionation was as described in the legend to Fig. 3.

Table 1. Analysis of oligonucleotides obtained by complete digestion of duckweed chloroplast 4.5S rRNA with *T₁* or pancreatic *A* ribonuclease. Sequencing methods: (a) position on 'fingerprints'; digestion with (b) alkali, (c) pancreatic *A* ribonuclease, (d) *T₁* ribonuclease, (e) *U₂* ribonuclease, (f) pancreatic *A* ribonuclease after carbodi-imide modification of the uridine residues, (g) snake-venom phosphodiesterase, (h) snake-venom phosphodiesterase after removal of the 3'-terminal phosphate with alkaline phosphatase; (i) partial digestion with *U₂* ribonuclease. Abbreviation used: n.d., not detected.

Oligonucleotide	Molar yield		Sequencing methods	Oligonucleotide	Molar yield		Sequencing methods†
	Experimental*	Theoretical			Experimental	Theoretical†	
T1 Gp	3.9	4	a	P1 Up	8.5	7	a, d
T3 C-C-GP	0.9	1	a, b, c	P2 Cp	7.0	6	a, d
T4 A-Gp	3.9	4	a, c	P3 A-Cp	2.2	2	a, d
T5 C-A-Gp	1.7	2	a, c	P4 A-A-Cp	1.0	1	a, d
T6 A-C-Gp	0.8	1	a, c	P6 G-Cp	1.8	2	a, d
T7 A-C-C-Gp	0.8	1	a, c, h	P7 A-Up	4.4	4	a, d
T8 A-A-Gp	1.0	1	a, c	P9 A-G-Cp	1.0	1	a, d
T9 C-A-A-Gp	1.1	1	a, c	P10 A-G-A-Cp	1.1	1	a, d
T10 U-Gp	3.6	4	a, c	P11 A-A-G-A-Cp	1.0	1	a, d
T11 C-U-Gp	1.0	1	a, c, f	P12 G-Up	3.7	4	a, d
T12 A-U-Gp	1.0	1	a, c	P13 G-G-Cp	1.0	1	a, d
T13 U-C-A-C-Gp	0.8	1	c, e, f	P14 G-A-G-Cp	0.9	1	a, d, h
T14 U-A-A-Gp	1.0	1	c	P15 G-A-G-A-Cp	1.0	1	a, d, h
T15 U-C-A-A-Gp	1.1	1	c, f	P16 G-A-Up	1.8	2	a, a, h
T16 A-U-A-Gp	1.0	1	a, c	P17 A-G-Up	1.0	1	a, d
T17 U-A-U-Gp	1.0	1	a, c, e	P18 A-A-G-Up	1.2	1	d
T18 A-U-U-U-Gp	1.0	1	a, b, c, e	P19 A-G-G-Up	1.3	1	d, h
T19 A-C-A-U-C-C-U-A-A-C-A-Gp	0.7	1	c, e, f	P20 A-A-G-G-Up	0.9	1	d, h
T20 U-U-U-A-U-C-A-U-U-A-C-Gp	0.5	1	b, c, e, i	P21 G-G-A-A-G-Up	1.2	1	b, d, i
3' A-A-C ₀	0.6	1	a, b, c, g	P22 G-A-G-A-G-A-Up	0.9	1	b, d, i
5' (n.d.)	—	—	a, b, c, g	3' G-A-A-C ₀ H	0.5	1	a, b, d, g
				5' (n.d.)	—	—	

* Expressed relative to the mean radioactivities found in spots CCG, AAG, CUG and AUG. Average values from four experiments.

† Expressed relative to the mean radioactivities found in spots AAC, AGC and GGC. Average values from four experiments.

phoresis described here. However, when the material was fractionated by a high-resolution method of electrophoresis (Rubin, 1975), by using a long gradient gel, the 4.5S rRNA was separated into two closely associated components that were present in approximately equal amounts (results not shown). This suggests that the broad-bean 4.5S rRNA is also heterogeneous. In pea, in addition to 4.5S rRNA, which is similar in size to that of broad bean, there is other low-molecular-weight material that fractionates as a shoulder at the leading edge of the 5S rRNA (Fig. 2e); Whitfield *et al.* (1978) have suggested that this is a 4.5S RNA component of pea chloroplast ribosomes.

Sequence analysis of 4.5S rRNA

The T₁ and pancreatic A ribonuclease 'fingerprints'

of 4.5S rRNA from duckweed are shown in Figs. 3(a) and 3(b) respectively. These 'fingerprints' are completely unlike those produced by similar digestion of chloroplast 5S rRNA [the preceding paper (Dyer & Bowman (1979))] or tRNA (e.g. Chakraborty, 1975). The sequence of each oligonucleotide is given in Table 1. The main features of the molecule that are evident from this analysis are as follows: duckweed 4.5S rRNA contains 103 nucleotides with 29 guanosine, 32 adenosine, 19 cytosine and 23 uridine residues and it has a molecular weight that is calculated to be 33 600. No unusual nucleotides were detected, and it appears that the 5'-terminal residue is not phosphorylated.

T₁ ribonuclease 'fingerprints' of the minor duckweed 4.5S rRNA components show that these are both related in sequence to the main 4.5S rRNA (Figs. 3c and 3d), although 4.5S rRNA₃ clearly

Table 2. Catalogue of oligonucleotides produced by digestion of chloroplast 4.5S rRNA from duckweed, tobacco, dwarf bean and broad bean to completion with T₁ ribonuclease

Oligonucleotide	Duckweed, 4.5S rRNA ₁	Duckweed, 4.5S rRNA ₂	Duckweed, 4.5S rRNA ₃ *	Tobacco	Dwarf bean	Broad bean
T1 Gp	3.9	4.2	+	7.6	5.4	2.9
T2 C-Gp	—	—	—	1.2	1.1	—
T3 C-C-Gp	0.9	1.0	—	0.8	0.8	—
T4 A-Gp	3.9	2.8	+	3.0	2.7	1.2
T5 C-A-Gp	1.9	1.8	+	1.0	1.9	1.4
T6 A-C-Gp	1.0	0.9	—	1.0	1.4	—
T7 A-C-C-Gp	0.8	0.9	+	0.6	0.7	—
T8 A-A-Gp	1.0	1.0	+	1.7	2.3	1.0
T9 C-A-A-Gp	1.1	1.0	—	—	—	—
T10 U-Gp	3.7	3.5	+	3.5	4.2	3.0
T11 C-U-Gp	1.1	1.0	+	1.0	1.2	1.0
T12 A-U-Gp	1.0	1.0	+	1.1	1.0	—
T13 U-C-U-A-Gp	0.8	1.0	—	0.7	0.7	—
T14 U-A-A-Gp	1.0	1.1	—	—	—	—
T15 U-C-A-A-Gp	1.1	0.9	+	1.1	1.2	0.9
T16 A-U-A-Gp	1.0	1.0	+	0.9	1.1	—
T17 U-A-U-Gp	1.0	1.2	—	1.0	1.1	1.0
T18 A-U-U-Gp	1.2	—	+	—	—	—
T19 A-C-A-U-C-C-U-A-A-C-A-Gp	0.7	0.9	+	—	—	—
T20 U-U-U-A-U-C-A-U-U-A-C-Gp	0.5	0.9	—	0.9	—	—
T21 U-U-A-Gp	—	—	—	—	0.9	—
T22 (U ₂ ,A-C)Gp	—	—	—	1.0	1.1	1.1
T23 (U ₂ ,C ₃ ,A-A-C,A-U)A-Gp	—	—	—	0.9	0.9	—
T24 (U ₅ ,C ₂ ,A-A-U ₂)Gp	—	—	—	—	1.0	—
T25 (C ₂ ,A-C,A-U)A-Gp	—	—	—	—	—	0.9
T26 (U ₁ ,A-A-U)Gp	—	—	—	—	—	1.0
T27 (U ₁ ,C ₃ ,A-U ₁ ,A-A-C)A-Gp	—	—	—	—	—	0.7
T28 (U ₂ ,C ₁ ,A-U ₁ ,A-A-C)A-Gp	—	—	—	—	—	0.6
T29 U-A-Gp	—	—	—	1.1	—	—
3' A-A-G _{OH}	0.6	0.6	+	0.7	0.9	0.6

* The oligonucleotides from duckweed 4.5S rRNA₃ were not quantified; only their presence (+) or absence (—) is indicated.

contained some tRNA, as shown by the presence of a large number of fragments in small amounts.

The composition of the three 4.5S rRNA bands from duckweed is compared in Table 2. Oligonucleotide A-U-U-U-Gp (T18) does not occur in digests of 4.5S rRNA₂ and one fewer molecule of A-Gp is obtained than from 4.5S rRNA₁. The 4.5S rRNA is therefore 96 nucleotides long.

Of the 21 nucleotides obtained from 4.5S rRNA₁, nine were absent from digests of 4.5S rRNA₃. It was not possible to quantify the oligonucleotides of 4.5S rRNA₃ because of tRNA contamination, but the results indicate that it contains about 63 nucleotides.

As the oligonucleotide A-A-C_{OH} is obtained on T₁ ribonuclease digestion of each, all three variants of 4.5S rRNA in duckweed have the same sequence of nucleotides at the 3'-end. Therefore the simplest explanation of their difference in size is that some of the residues present at the 5'-end of 4.5S rRNA₁ are absent from the other two. Although this is probably true for 4.5S rRNA₃, it does not appear to be so for 4.5S rRNA₂, as neither of the oligonucleotides missing from this occur at the 5'-end of 4.5S rRNA₁ (T. A. Dyer, J. R. Bedbrook & C. M. Bowman, unpublished work).

The T₁ ribonuclease 'fingerprints' of 4.5S rRNA of dwarf bean, tobacco and broad bean are shown in Figs. 4(a), 4(b) and 4(c) respectively. It is clear from these that the RNA species from dwarf bean and tobacco are very similar in composition to that of duckweed (Fig. 4). The similarity between the smaller molecule from broad bean and the others is less obvious. However, on further analysis by digestion of the T₁ ribonuclease oligonucleotides with pancreatic A ribonuclease it was found that many identical oligonucleotides are obtained from all the 4.5S rRNA

species (Table 2), and the differences can in many instances be explained as being the results of a single base change {e.g. oligonucleotide T19 [A-C-A-U-C-C-U-A-A-C-A-G_p] to oligonucleotide T23 [(G)-C-A-U-C-C-U-A-A-C-A-G_p]}. Interestingly, too, most of the oligonucleotides missing from 4.5S rRNA₃ of duckweed are also missing from the broad-bean 4.5S rRNA molecule.

As common features, all the 4.5S rRNA species have the same A-A-C_{OH} sequence at the 3'-end of the molecule; the 5'-end is not phosphorylated and apparently none contains any modified nucleotides.

From the four species of 4.5S rRNA compared, 29 different T₁ ribonuclease oligonucleotides were obtained (Table 2). Only one out of 20 oligonucleotides that occurred in digests of the tobacco 4.5S rRNA was not also obtained from at least one other 4.5S rRNA. Two unique fragments were derived from dwarf bean and four from duckweed and broad bean. As the number of small oligonucleotides that the RNA species have in common differs as well, it is difficult to establish the actual number of residues that are different in the molecules. The results do indicate, however, that the 4.5S rRNA species have been less conserved in sequence than the chloroplast 5S rRNA of the same plants [the preceding paper, Dyer & Bowman (1979)] and that some segments are particularly labile, especially the part from which oligonucleotides T20-T24 are derived.

Size and quantity of 4.5S tRNA

Estimates of the molecular weight of 4.5S rRNA species were made from their electrophoretic mobility relative to marker RNA species and from their oligonucleotide composition (Table 3). The results indicate that 4.5S rRNA occurs predominantly in two size classes, the larger of which contains slightly

Table 3. Comparison of size, molecular weight, and molar yield of 4.5S rRNA from several species of higher plant

Plant material	No. of nucleotides	Derivation of molecular weight		Molar yield†	
		Base composition*	Electrophoretic mobility§		
Duckweed	4.5S rRNA ₁	103	33 596	35 000	0.99
	4.5S rRNA ₂	96	31 327	30 500	0.26
Tobacco	101	33 007	33 200		0.09‡
Dwarf bean	103	33 588	34 900		0.74
Broad bean	72	25 261	25 300		0.73
Pea			25 900		0.84
Rye			33 400		0.79
Sunflower			33 400		0.94
Spinach			33 800		0.65

* Determined from data in Table 2.

† Molar yields were estimated relative to the yield of 5S rRNA.

‡ Sample degraded.

§ Determined from electrophoretic mobility in 10% (w/v) polyacrylamide gels.

in excess of 100 nucleotides, as shown by both methods of molecular-weight estimation. Values for the smaller size class cannot at present be assigned with the same degree of certainty. From their electrophoretic mobility it seems that they contain about 77 nucleotides. A similar value is derived from the oligonucleotide composition of broad-bean 4.5S rRNA. However, if the number of residues in the oligonucleotides missing from duckweed 4.5S rRNA₃ is subtracted from the number occurring in 4.5S rRNA, a value of 63 is obtained. At this stage the discrepancy between the values derived by the two methods is most easily explained as being the result of anomalous behaviour of the 4.5S rRNA during electrophoresis owing to some feature of its configuration.

The molecular yield of the major 4.5S rRNA species relative to 5S rRNA is in general slightly lower than unity in most of the plants examined, but, owing to the occurrence of minor variants as well, it is probable that there is one molecule of 4.5S rRNA per chloroplast ribosome.

Discussion

We show here that chloroplast ribosomes of flowering plants contain a low-molecular-weight species of RNA in addition to 5S rRNA. The main characteristic of this additional ribosomal component seems to be its heterogeneity in size and composition. For example, in duckweed (which we investigated most thoroughly) there appear to be three variants of this component. The major one contains 103 nucleotides and comprises 70–80% of the total yield of this RNA. The two smaller, less-prevalent, variants contain 96 and about 63 nucleotides respectively. The size of the major component also differs in different types of flowering plants. However, in most it is intermediate in size between 5S rRNA and tRNA and therefore we (Dyer & Bowman, 1976) and others (Whitfield *et al.*, 1977; Bohnert *et al.*, 1976) have called it '4.5S RNA'.

Other RNA species comparable in size with the major chloroplast 4.5S rRNA component have been described. These include the 4.5S RNA of *Escherichia coli*, which contains 108 nucleotides (Griffin, 1975), some of the precursors of tRNA (e.g. Knapp *et al.*, 1978) and the small nuclear 4.5S RNA species of many eukaryotic cells (Weinberg, 1973). However, these RNA species have little in common with the chloroplast 4.5S molecule apart from their size. Sequence analysis shows that their primary structure is completely different. The feature that distinguishes the chloroplast 4.5S RNA most clearly from these molecules that are superficially similar to it is its location in ribosomes.

Of the small rRNA species that have been described chloroplast 4.5S RNA most resembles the 5.8S RNA

of eukaryote cytosol ribosomes in that both RNA species are part of the large ribosomal subunit. However, there are substantial differences between them that indicate that they are not homologues; for instance: (1) the 4.5S RNA is much smaller than 5.8S RNA, which contains about 157 nucleotides in flowering plants (Payne & Dyer, 1972); (2) it is not as highly conserved in sequence; (3) it does not appear to be hydrogen-bonded to the largest ribosomal RNA (Whitfield *et al.*, 1978) as is the 5.8S RNA (Pene *et al.*, 1968; Payne & Dyer, 1972); (4) it does not contain any modified nucleotides, whereas 5.8S RNA in flowering plants has several (Woledge *et al.*, 1974); and (5) its 5'-end is not phosphorylated, whereas that of 5.8S RNA is (Woledge *et al.*, 1974).

Could the chloroplast 4.5S rRNA then be a fragment of 23S rRNA? Its unphosphorylated 5'-end does suggest that it is a product of ribonuclease digestion and it is known that 23S rRNA is degraded to some extent in most flowering plants (Leaver & Ingle, 1971). There are several reasons why this explanation is unlikely: (1) the 4.5S RNA is present in near equimolar amounts with the other ribosomal RNA species whereas variable proportions of the 23S RNA may be fragmented; (2) radioactive label appears in 4.5S RNA at a rate similar to that incorporated into 23S RNA at a stage when label in the known 23S RNA fragments is not detectable (Whitfield *et al.*, 1978); (3) there comparatively little competition between the 4.5S and 23S RNA for hybridization with chloroplast DNA (Hartley, 1979); and (4) the 23S RNA appears to be highly conserved in sequence (T. A. Dyer, unpublished work), but the 4.5S RNA is not. Nevertheless, until the complete sequence of chloroplast 23S RNA is known, the possibility that the 4.5S RNA is part of the 23S RNA cannot be dismissed completely.

The greater heterogeneity in the size and sequence of chloroplast 4.5S RNA in comparison with other ribosomal RNA species seems to preclude its having a function in the mature ribosome, and suggests that its derivation is particularly unusual. It possibly corresponds to transcribed spacer RNA, which is normally destroyed during the maturation of ribosomal RNA from the much larger precursor molecules (Perry, 1976). It appears that 4.5S rRNA is synthesized as a segment of a large RNA molecule that is also the putative precursor to the chloroplast 16S and 23S RNA species (Hartley, 1979). In spinach this has a mol.wt. of 2700000 (Hartley & Ellis, 1973) and contains approx. 7400 nucleotides. Of these, 4500 are probably the 16S and 23S RNA sequences. Sequences of tRNA, and perhaps 5S RNA too, may occur in the segments that contain the remaining 2900 nucleotides. Though this extra RNA have an important function in assembling ribosomal components (Perry, 1976), most of it is apparently destroyed during the assembly process.

An intermediate precursor to the chloroplast 23S RNA has also been detected in spinach, and this contains about 600 more nucleotides than the mature molecule. This competes with the 4.5S RNA for hybridization to chloroplast DNA and is therefore probably the precursor of the 4.5S RNA as well (Hartley, 1979). Hybridization of the 4.5S and 23S RNA species to fragments of chloroplast DNA digested with restriction enzymes indicates that the 4.5S RNA is located to the 3'-side of 23S RNA in the primary transcript (Whitfield *et al.*, 1978; T. A. Dyer & J. R. Bedbrook, unpublished work).

If the 4.5S RNA is transcribed spacer, why then has it been conserved? Perhaps its presence reflects some unique features in the organization of chloroplast ribosomal genes and/or of the way in which the precursor to the chloroplast ribosomal RNA is processed.

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