Nucleotide Sequence Analysis of the Cytoplasmic 5S Ribosomal Ribonucleic Acid from Five Species of Flowering Plants

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Broad-bean 5S rRNA labelled with ^{32}P was digested separately with T_1 and pancreatic A ribonucleases and the resulting oligonucleotides (20 and 18 respectively) were fractionated by two-dimensional electrophoresis. The oligonucleotides were analysed further and 32 of them have been completely sequenced. They were compared with those of 5S rRNA from dwarf bean, sunflower, tomato and rye. Sequence differences were found at both the 3'- and 5'-termini and at up to nine other positions. Most base substitutions were transitions between C and U. In common with other 5S rRNA species that have been analysed the ends of the molecule in each plant species have complementary sequences.

Although 5S rRNA is an integral component of eukaryotic 80S ribosomes (Bachvaroff & Tongur, 1966) neither its function nor secondary structure is known. A comparison of its nucleotide sequence in a range of eukaryotes should reveal which parts of the molecule (a) differ little (and are therefore probably involved in its functioning) and (b) are complementary to one another. The complete sequences of rat, mouse, rabbit, human and marsupial 5S rRNA species are probably identical (Forget & Weissman, 1967; Williamson & Brownlee, 1969; Averner & Pace, 1972) and out of a total of 120 nucleotide residues there are only about four base differences between mammalian and chicken 5S rRNA species (G. G. Brownlee, personal communication) and eight between mammalian and *Xenopus laevis* (Brownlee *et al.*, 1972). Recently, the nucleotide sequence of 5S rRNA from the yeast *Saccharomyces carlsbergensis* was reported by Hindley & Page (1972). It has many sequence differences from mammalian 5S rRNA, yet about 60% of the molecule is homologous. Further analysis of these results plus a nucleotide sequence of 5S rRNA from a eukaryotic organism distantly related to both mammals and fungi, such as a flowering plant, should yield valuable information on the secondary structure of the molecule.

In the present paper we describe partial sequence analysis of broad-bean 5S rRNA and show that within the flowering plants, as in animals, there is little variation in the sequence of the molecule.

Materials and Methods

Seeds of tomato (*Lycopersicum esculentum*), sunflower (*Helianthus annuus*) and dwarf bean (*Phaseolus vulgaris*) were obtained from Thompson and Morgan Ltd., Ipswich, Suffolk, U.K., and broad bean (*Vicia*

faba) from Carters Seeds Ltd., Llangollen, Wales, U.K. Embryos isolated from rye grain (*Secale cereale*, obtained from Nunns, Corn and Coal Co. Ltd., Woodbridge, Suffolk, U.K.) by the method of Johnston & Stern (1957) were provided by Miss S. Sen.

Pancreatic A ribonuclease, snake-venom phosphodiesterase and *Escherichia coli* alkaline phosphatase (Worthington Biochemical Corp.) were obtained from Cambrian Chemicals Ltd., Croydon, Surrey, U.K., and T_1 ribonuclease (Sankyo Co.) from Calbiochem Ltd., London W.1, U.K. Cellulose acetate strips (Schleicher und Schüll) measuring 75 cm $\times$ 3 cm were obtained from Anderman and Co. Ltd., London S.E.1, U.K., and *N*-cyclohexyl-*N'*-[2-(4-morpholinyl)-ethyl]carbodi-imide] toluene-*p*-sulphonate (Fluka A.G.) from Fluorochem Ltd., Glossop, Derbyshire, U.K. Carrier-free [^{32}P]P_i was purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and ribonuclease U₂ was kindly provided by Dr. G. G. Brownlee, Medical Research Council Laboratory of Molecular Biology, Cambridge, U.K.

Growth of plants and the incorporation of [^{32}P]P_i in vivo

Seeds were soaked on moist cotton-wool for approx. 48 h and then grown in water culture. Seedlings whose roots were between 3 and 6 cm long were grown in 100 ml of tap-water containing 80 μg of chloramphenicol/ml and either 50 or 100 μCi of [^{32}P]P_i/ml. Air was bubbled through the solution and the incubation proceeded for either 24 or 48 h.

For incorporation of radioactivity into rye nucleic acids, ten 0.1 ml portions of a solution of [^{32}P]P_i (10 mCi/ml) were dried on different areas of a Petri dish. Approximately 130 unimbibed rye embryos were germinated on one of these areas in 1 ml of 2% (w/v)

Fig. 1. Fractionation of low-molecular-weight RNA from broad-bean roots

The ^{32}P -labelled RNA was subjected to electrophoresis in 12.5% (w/v) polyacrylamide gels at 80V (about 5mA/gel) for 16h. The vertical, dashed lines show where the gel was cut to recover the 5S rRNA.

sucrose and 50 μg of chloramphenicol/ml. At about 5h intervals, embryos and solution were transferred to other areas of ^{32}P and RNA was extracted after 48h of germination.

Extraction and purification of 5S RNA

RNA (but not DNA) was extracted from roots and embryos by the sodium naphthalene-1,5-disulphonate method of Hastings & Kirby (1966) as described previously (Payne & Dyer, 1971). The RNA precipitate obtained was dissolved in 0.65M-NaCl, 0.02M-sodium acetate buffer (pH5.6) and centrifuged at $3 \times 10^7 g_{av.}$ (usually $2.73 \times 10^5 g_{av.}$ for 110min) at 5°C. Low-molecular-weight RNA was precipitated from the supernatant with two volumes of ethanol and subsequently fractionated by electrophoresis in 15cm $\times$ 0.65cm cylinders of 12.5% (w/v) polyacrylamide gel (Loening, 1969) with a 2.6% spacer. Gels were scanned at 260nm with a modified Hilger-Gilford spectrophotometer. Fig. 1 shows a typical fractionation of root low-molecular-weight RNA.

The region of the gel which contained the 5S RNA was cut out and homogenized in 10ml of 0.2M-NaCl, 0.02M-potassium acetate buffer, pH5.6. After 1h with occasional shaking the polyacrylamide was pelleted by centrifugation and the supernatant loaded on to a 2cm $\times$ 0.4cm column of DEAE-cellulose (Payne & Dyer, 1971). The polyacrylamide was washed twice more with the buffer and the washings were passed

through the column. The column was washed with a further 20ml of buffer to remove soluble acrylamide and 5S RNA was eluted in a volume of 1.5ml with 0.65M-NaCl, 0.02M-potassium acetate, pH5.6. Purified, unlabelled *E. coli* high-molecular-weight RNA was added, if necessary, to make the weight of RNA up to 40 μg and then RNA was precipitated by adding 2vol. of ethanol and by storing overnight at -20°C .

Digestion of RNA and fractionation of oligonucleotides

For complete digestion with T_1 or pancreatic A ribonuclease, 40 μg of RNA were incubated for 30min at 37°C in 5 μl of 1mM-EDTA, 10mM-Tris-HCl (pH7.4) and enzyme at a concentration of 1mg/ml for T_1 ribonuclease and 0.1mg/ml for pancreatic A ribonuclease. The digests were fractionated by electrophoresis on cellulose acetate in 7M-urea (pH3.5) followed by electrophoresis on DEAE-cellulose paper in 7% (v/v) formic acid as described by Sanger & Brownlee (1967). The oligonucleotides were located by radioautography and eluted for further analysis with 30% (v/v) triethylamine carbonate, pH10, which contained 20mg of hydrolysed yeast RNA/ml (Sanger & Brownlee, 1967).

Analysis of oligonucleotides

The oligonucleotides were further digested with T_1 or pancreatic A ribonuclease for 30min at 37°C in 10 μl of 1mM-EDTA and 10mM-Tris-HCl, pH7.4, containing 0.1mg of enzyme/ml. The products were fractionated by electrophoresis on DEAE-cellulose paper at pH3.5 (Sanger & Brownlee, 1967).

Ribonuclease U_2 digestion of oligonucleotides from T_1 ribonuclease fingerprints was carried out for 16h at 37°C in 10 μl of 2mM-EDTA, 50mM-sodium acetate, pH4.5, containing 1.0 unit of enzyme/ml. The products were fractionated by electrophoresis on DEAE-cellulose paper at pH3.5 and identified by their position relative to the blue dye (Xylene Cyanol F.F.) or upon subsequent digestion with pancreatic A ribonuclease. The relative mobilities of some of the products are shown in Fig. 2. Separation at this pH is at least as informative as the commonly used pH1.9 system (Barrell, 1971).

Additional sequence information was obtained by reacting some of the T_1 ribonuclease oligonucleotides with *N*-cyclohexyl-*N'*-[2-(4-morpholinyl)ethyl]carbodi-imide] toluene-*p*-sulphonate before pancreatic A ribonuclease digestion (Brownlee, 1972).

Results

Sequence analysis of broad-bean 5S rRNA

When purified 5S rRNA was digested to completion with ribonuclease T_1 and fractionated in two dimensions, a consistent 'fingerprint' pattern of oligonucleotides was obtained (Plate 1). All the digestion

EXPLANATION OF PLATE 1

Radioautograph of fractionated products from broad-bean 5S rRNA after digestion with (a) ribonuclease T₁ and (b) pancreatic A ribonuclease

Fractionation was by electrophoresis in cellulose acetate (direction 1) and on DEAE-cellulose paper with 7% (v/v) formic acid (direction 2). Sequence analyses of the oligonucleotides are given in Tables 1 and 2. The inset of Plate 1(a) shows a particularly clear separation of the large oligonucleotides.

EXPLANATION OF PLATE 2

Radioautograph of fractionated T_1 -ribonuclease digestion products of 5S rRNA from (a) dwarf bean, (b) sunflower, (c) tomato and (d) rye. The sequences of oligonucleotides not found in broad-bean 5S rRNA are given in Table 3.

P. I. PAYNE, M. J. CORRY AND T. A. DYER

Fig. 2. Fractionation of ribonuclease U_2 products of ribonuclease T_1 oligonucleotides on DEAE-cellulose paper in 5% (v/v) acetic acid, 0.5% pyridine, pH 3.5

The R_B values refer to the mobility of fragments relative to the blue dye (Xylene Cyanol FF indicated by B at 1.0).

products were resolved and Table 1 summarizes the further analysis of the fragments. All but oligonucleotides T_{14} , T_{16} , T_{17} and T_{19} and the 3'- and 5'-termini could be completely sequenced by separate digestion with pancreatic A ribonuclease, U_2 ribonuclease and pancreatic A ribonuclease after modification of the oligonucleotides with *N*-cyclohexyl-*N'*-[2-(4-morpholinyl)ethyl]carbodi-imide] toluene-*p*-sulphonate.

After alkaline hydrolysis, the 5'-oligonucleotide gave Gp and a fragment which migrated slightly slower than Up at pH 3.5. When digested first with alkaline phosphatase and then in 0.2M-NaOH, only one fragment, Ap, was detected. The sequence at the 5'-end is therefore pA-G-.

The 3'-oligonucleotide yielded an extra molecule of uridine monophosphate compared with alkaline

hydrolysis when digested to completion with snake venom phosphodiesterase. The 3'-terminal nucleoside is therefore U_{OH} and the sequence of the oligonucleotide is C-A-C-C-U- U_{OH} (Table 1).

The complete sequences of oligonucleotides T_{14} , T_{16} , T_{17} and T_{19} have not been established (Table 2).

A radioautograph of a pancreatic A ribonuclease 'fingerprint' is shown in Plate 1(b). All but oligonucleotides P_{18} , P_{21} and P_{22} were sequenced by T_1 ribonuclease analysis (Table 2). In one experiment, a high enzyme/substrate ratio was used and the additional product A-A-G-Up was detected in small amounts and presumably arose by the action of a contaminating ribonuclease. It could only be derived from oligonucleotide P_{22} , whose sequence is therefore G-G-G-A-A-G-Up. As yet the complete sequences of P_{18} and P_{21} have not been established.

Sequence analysis of 5S rRNA from dwarf bean, sunflower, tomato and rye

Ribonuclease T_1 'fingerprints' of 5S rRNA from dwarf bean, sunflower, tomato and rye (Plate 2) are similar, both to each other and to the corresponding 'fingerprint' from the broad bean (Plate 1a).

Non-terminal sequence changes. Excluding the 3'- and 5'-oligonucleotides, up to five fragments were isolated that do not occur in broad-bean 5S rRNA (Table 3). Homologous sequences were deduced by first comparing the 'fingerprints' that differed least. For instance, oligonucleotide T_{14} [(U,C)C-U-U-Gp] found in the broad-bean 'fingerprint' (Plate 1a) is absent from the dwarf bean (Plate 2a), which has an additional fragment, T_{22} [(U_2 ,C₃)Gp]. Analysis of pancreatic A ribonuclease digestion products suggests a U $\leftrightarrow$ C transition, where transition is a pyr/pyr or pur/pur change and a transversion is a pur/pyr change.

There are two differences between the broad-bean and sunflower 'fingerprints' (Plate 2b). In common with the dwarf bean, the sunflower has T_{22} instead of T_{14} and as a result of another U $\leftrightarrow$ C transition contains T_{21} instead of T_{17} (Table 3).

The tomato 'fingerprint' (Plate 2c) has three oligonucleotide spots not found for the broad bean. Two arise from the T_{14}/T_{22} and T_{17}/T_{21} changes already described and the third is T_{23} . Secondary digestion of T_{23} shows that it is homologous to the broad-bean oligonucleotide T_{18} (C-A-C-U-A-A-U-Gp) and has arisen by another C $\leftrightarrow$ U transition. As the trinucleotide A-A-Up is replaced by A-A-Cp in the tomato pancreatic A ribonuclease 'fingerprint' (not shown) the sequence of T_{23} is C-A-C-U-A-A-C-Gp (Table 3). A further difference between tomato and broad bean is the presence of two A-A-Gp sequences (T_4) instead of one. The corresponding sequence in the pancreatic A 'fingerprint' is G-A-A-G-Up and as spot P_{17} contains 1 mol less of A-G-Up and spot T_{20} (C-A-Gp) is lost,

Table 1. Analysis of oligonucleotides obtained by complete digestion of broad-bean 5S rRNA with ribonuclease T_1
Underlined bases have been modified by *N*-cyclohexyl-*N'*-[2-(4-morpholinyl)ethylcarbodi-imide] toluene-*p*-sulphonate treatment.

Fragment	Pancreatic A ribonuclease products	U_1 ribonuclease products	<i>N</i> -Cyclohexyl- <i>N'</i> -[2-(4- morpholinyl)ethylcarbodi- imide] toluene- <i>p</i> -sulphonate-- pancreatic A products	Sequence deduced	Molar ratio	
					Experimental*	Predicted
T ₁	Gp	—	—	Gp	—	—
T ₂	Cp, Gp	C-Gp	—	C-Gp	3.8	3-4
T ₃	A-Gp	Ap, Gp	—	A-Gp	2.6	2-3
T ₄	A-A-Gp	Ap, A-Ap, Gp	—	A-A-Gp	1.0	1
T ₅	A-Cp, Cp ₂ , Gp	C-Ap, C-C-Gp	—	C-A-C-C-Gp	0.8	1
T ₆	Up, Gp	U-Gp	—	U-Gp	4.6	4-5
T ₇	A-Up, Gp	Ap, U-Gp	—	A-U-Gp	1.2	1
T ₈	Up, A-Gp	U-Ap, Gp	—	U-A-Gp	1.1	1
T ₁₀	Up ₂ , Gp	U-U-Gp	—	U-U-Gp	0.9	1
T ₁₁	Cp, Up ₂ , Gp	—	U-U-Gp	C-U-U-Gp	1.2	1
T ₁₂	Up, U-Up, A-A-Gp	U-U-Ap, Ap, Gp	—	U-U-A-A-Gp	1.0	1
T ₁₃	Up ₂ , A-Cp, A-Gp	U-Ap, (C, U)Ap, Gp	—	U-A-C-U-A-Gp	0.8	1
T ₁₄	Up ₃ , Cp ₂ , Gp	—	U-U-Gp	(U, C)C-U-U-Gp	0.7	1
T ₁₅	(A-Up) ₂ , A-Cp, Cp ₂ , A-Gp	Ap, Gp, U-Ap, (C, U)Ap, C-C-Ap	A-U-A-Cp(A, U, C)A-Gp	A-U-A-C-C-A-U-C-A-Gp	0.6	1
T ₁₆	(A-Up) ₂ , Cp ₃ , A-Gp	Ap, Gp, (C, U)Ap, (U, C ₂)Ap	—	A-U-C-A-U-C-C-A-Gp or A-U-C-C-A-U-C-A-Gp	0.8	1
T ₁₇	A-Cp, Cp ₃ , Up ₂ , Gp	Ap, fragment at origin	U-Cp, UGp, C-U-Gp	A-C(C, U)C-C-U-Gp	0.7	1
T ₁₈	A-A-Up, A-Cp, Cp, Up, Gp	Ap, C-Ap, U-Gp, (C, U)Ap	(U ₂ , A ₂)Gp	C-A-C-U-A-U-Gp	0.7	1
T ₁₉	A-A-Cp, Cp ₂ , Up, Gp	Ap, A-Ap, (C ₃ , U)Gp	U-Cp	A-A-C(C, U)C-Gp	0.7	1
T ₂₀	Cp, A-Gp	C-Ap, Gp	—	C-A-Gp	1.0	1
5'	resistant	—	—	? Up and Cp absent	0.8	1
3'	Cp ₂ , A-Cp, Up	C-Ap + unknown fragment	U-X _{OH}	C-A-C-C-U-X _{OH}	0.4	1

* Expressed relative to the mean radioactivities found in the five trinucleotide spots U-U-Gp, A-U-Gp, U-A-Gp, A-A-Gp and C-A-Gp.

Table 2. Analysis of oligonucleotides obtained by complete digestion of broad-bean 5S rRNA with pancreatic A ribonuclease

Fragment	T ₁ ribonuclease	Sequence deduced	Molar ratio	
			Experimental*	Predicted
P ₁	Cp	Cp	—	—
P ₂	A-Cp	A-Cp	4.7	4-5
P ₃	Up	Up	—	—
P ₄	Gp,Cp	G-Cp	5.1	5
P ₆	A-Up	A-Up	2.1	2
P ₇	Gp,A-Cp	G-A-Cp	0.9	1
P ₈	A-Gp,Cp	A-G-Cp	1.2	1
P ₁₀	A-A-Up	A-A-Up	0.8	1
P ₁₁	A-A-Gp,Cp	A-A-G-Cp	0.8	1
P ₁₂	A-Gp,A-A-Cp	A-G-A-A-Cp	0.7	1
P ₁₃	Gp,Up	G-Up	3.5	3-4
P ₁₆	Gp,A-Up	G-A-Up	1.1	1
P ₁₇	A-Gp,Up	A-G-Up	1.9	2
P ₁₈	A-Gp,Gp,A-Up	(A-G,G)A-Up	1.0	1
P ₁₉	Gp ₂ ,A-Up	G-G-A-Up	1.0	1
P ₂₀	Gp ₂ ,Up	G-G-Up	1.2	1
P ₂₁	(A-Gp) ₂ ,Gp,Up	([A-G] ₂ ,G)Up	1.1	1
P ₂₂	A-A-Gp,Gp ₃ ,Up	(A-A-G,G ₃)Up	0.8	1
P ₂₃	pA-Gp,Gp,Up	pA-G-G-Up	0.9	1
P ₂₄	Gp ₂ ,Cp	G-G-Cp	0.6	1?
P ₂₅	A-Gp,Gp,Cp	(A-G,G)Cp	0.5	1?

* Expressed relative to the mean radioactivities found in the six trinucleotide spots G-A-Cp, A-G-Cp, A-A-Up, G-A-Up, G-G-Up and A-G-Up/2.

these changes can be accounted for by a single C $\leftrightarrow$ A transversion. A similar quantitation of spot T₇ reveals bimolar quantities of A-U-Gp compared with unimolar amounts in the bean and sunflower 'fingerprints'. Analysis of the 5' oligonucleotides (see below) shows the complementary sequence in broad-bean 5S rRNA is G-U-Gp, indicating a G $\leftrightarrow$ A transition.

There are several different oligonucleotide spots on the rye 'fingerprint' (Plate 2d) compared with the broad bean and all but two have been described (Table 3). Oligonucleotide T₂₄ is clearly homologous to the missing T₁₈ (C-A-C-U-A-A-U-Gp). As A-A-A-Gp is liberated after digestion with pancreatic A ribonuclease, its sequence must be C-A-C-U-A-A-A-Gp. This U $\leftrightarrow$ A transversion occurs at the same position in the molecule as the U $\leftrightarrow$ C transition described between broad bean and tomato. The oligonucleotide T₂₅ is present without the loss of a complementary oligonucleotide spot in the T₁-ribonuclease 'fingerprint' (Plate 2d), though spot P₂₀ (G-G-Up) is absent from the pancreatic A ribonuclease 'fingerprint' (not shown). The homologous sequence in broad-bean 5S rRNA is much less obvious than the others described. One possible interpretation of the results would be to assume that the sequence of T₂₅ is C-C-C-U-Gp and replaces -C-G-G-U-G- found in the other plant 5S rRNA species studied.

5'-Terminal sequence. The 5'-terminus released from 5S rRNA of the broad bean after ribonuclease T₁ digestion is pA-Gp whereas the corresponding sequence in the sunflower, tomato and rye is pGp. The pancreatic A 'fingerprints' suggest that there has been an A deletion in the sunflower (pA-G-G-Up lost and pG-G-Up gained) and an A $\leftrightarrow$ G transition in the tomato and rye (pA-G-G-Up lost and pG-G-A-Up gained). Also apparent in the latter sequence is the internal A $\leftrightarrow$ G transition which is responsible for the second sequence of A-U-Gp present in tomato and rye 5S rRNA species. The 5'-terminus of dwarf bean is the same as that of sunflower except that extra phosphate groups are present, resulting in ppGp and pppGp being produced on T₁ ribonuclease digestion.

3'-Terminal sequence. The 3'-terminal oligonucleotide detected after T₁-ribonuclease digestion differs considerably in the species examined. Pancreatic A digestion of these fragments gave similar products in the broad bean, dwarf bean and sunflower, variation occurring in the number of U residues. Two have been sequenced and are C-A-C-C-U-U_{OH} (broad bean) and C-A-C-C_{OH} (sunflower) (Table 3). The 3' terminus of dwarf-bean 5S rRNA has one more Up than that of broad bean and two more than sunflower. Its sequence is probably C-A-C-C-U-U-X_{OH}, where X_{OH} is an unidentified nucleoside but is probably U_{OH} by

Table 3. *Analysis of sequence variation in 5S rRNA from broad bean, dwarf bean, sunflower, tomato and rye*

X_{OH} refers to an unidentified nucleoside.

Fragment	Plant species	Sequence deduced from secondary digestion products	Probable homologue in the broad bean	Probable base difference to broad bean
T ₂₁	Tomato, sunflower	(A-C, C ₄ , U)Gp	A-C(C, U)C-C-U-Gp	U↔C
T ₂₂	Dwarf bean, sunflower, tomato, rye	(U ₂ , C ₃)Gp	(U, C)C-U-U-Gp	U↔C
T ₂₃	Tomato	C-A-C-U-A-A-C-Gp	C-A-C-U-A-A-U-Gp	U↔C
P ₂₆		A-A-Cp	A-A-Up	
T ₂₄	Rye	C-A-C-U-A-A-A-Gp	C-A-C-U-A-A-U-Gp	U↔A
P ₂₇		A-A-A-G-Cp	A-A-Up	
T ₄	Tomato, rye	A-A-Gp (bimolar)	C-A-Gp	C↔A
P ₂₈		G-A-A-G-Up	A-G-Up	
T ₇	Tomato, rye	A-U-Gp (bimolar)	U-Gp	G↔A
P ₂₅		pG-G-A-Up	pA-G-G-Up	
T ₂₅	Rye	(C ₃ , U)Gp	?	?
5'	Sunflower	pGp	pA-Gp	A deletion
5'		pG-G-Up	pA-G-G-Up	
5'	Dwarf bean	ppGp and pppGp	pA-Gp	A deletion
5'		ppG-G-Up and pppG-G-Up	pA-G-G-Up	
5'	Tomato, rye	pGp	pA-Gp	A↔G
5'		pG-G-A-Up	pA-G-G-Up	
3'*	Sunflower	C-A-C-C _{OH}	C-A-C-C-U _{OH}	U deletions
3'	Dwarf bean	(C ₂ , A-C, U ₂)X _{OH}	C-A-C-C-U _{OH}	pX _{OH} addition
3'	Tomato, rye	(C ₃ , A-U)X _{OH}	C-A-C-C-U _{OH}	{ C↔U U↔C

* Sequence deduced by 3'-end-group analysis in addition to digestion with pancreatic A ribonuclease.

analogy. The 3'-oligonucleotide of both rye and tomato 5S rRNA species contains A-Up instead of the A-Cp found in the other plant species examined, and three C residues. As U₂ ribonuclease digestion of this fragment in tomato gave C-Ap, in common with the broad bean (Table 3), its sequence is almost certainly C-A-U-C-C-X_{OH}. This alteration in sequence from that of the broad bean requires two C↔U transitions.

Discussion

Conservation of the sequence of 5S rRNA in eukaryotes

Our analyses of 5S rRNA clearly show that the molecule has been highly conserved during the evolution of the flowering plants. Out of a total of about 120 nucleotides, there are approximately ten sites in the molecule where sequence differences occur in those plants examined. Further, plant 5S rRNA probably has sequence homologies with the corresponding molecules of mammals and yeast. For example, the sequence -G-C-A-C-C-G- occurs in both plant and yeast 5S rRNA species, as does -G-U-U-A-A-G-C-

with the similar sequence -G-C-U-A-A-G-C- in human 5S rRNA. The large oligonucleotide T₁₅, found in all the plant 5S rRNA species studied, has the sequence A-U-A-C-C-A-U-C-A-Gp and is similar to the sequences -A-U-A-C-C-A-U-C-U-A-G- and -A-U-A-C-C-A-C-C-U-G- found in yeast and human 5S rRNA species respectively. Appreciable homology between plant, human and yeast 5S rRNA species has been confirmed by preliminary sequence analyses of oligonucleotides from broad-bean 5S rRNA after partial digestion with ribonuclease T₁ (P. I. Payne, M. J. Corry & T. A. Dyer, unpublished work).

Although highly conserved, the sequence of 5S rRNA does vary within the flowering plants, whereas within mammals it does not. Indeed, the number of differences in sequence between two flowering plants thought to be distantly related (the broad bean, a Dicotyledone, and rye, a Monocotyledone) is the same as between 5S rRNA species of mammalian and amphibian origin.

Seven of the nine sequence differences that are clearly understood are transitions. Vogel (1972) also

deduced a higher frequency of transitions compared with transversions than would be expected on a purely random basis in the DNA cistrons coding for several different proteins. Interestingly, the most common transitions were found to be C/T changes (284 out of 369). In contrast, five out of seven transitions detected in the different plant 5S rRNA species are C/U changes (equivalent to G-A in the 5S rDNA) and similarly, six of the seven transitions between *Xenopus* and human 5S rRNA sequences are due to G/A changes in the complementary DNA (Brownlee *et al.*, 1972).

Phylogenetic significance of plant 5S rRNA sequences

Despite a selection of species from different families of flowering plants, the limited number of sequence differences preclude detailed phylogenetic conclusions. However, it is probable that monocotyledonous plants are closely related to the Dicotyledones as most of the T₁-ribonuclease oligonucleotides are common to the 5S rRNA of all the species studied. The relationship between the rye and tomato 5S rRNA sequences is remarkable, and unless the sequence similarities are fortuitous, the results suggest a close phylogenetic relationship between the two. The most similar sequences of 5S rRNA, as might be expected, come from the most related plants, broad and dwarf beans, which are classified in different genera of the same family.

Secondary structure and the function of eukaryotic 5S rRNA

Our limited sequence analysis of broad-bean 5S rRNA is sufficient to show that the 5'- and 3'-ends of the molecule are complementary:

The G* residue was deduced to be G from comparative studies with tomato and rye 5S rRNA species and confirmed by partial T₁-ribonuclease digestion of the intact molecule (P. I. Payne, M. J. Corry & T. A. Dyer, unpublished work).

Tomato and rye have different 3'- and 5'-terminal sequences to the other plants studied but are also complementary:

Thus complementary 3'- and 5'-terminal sequences occur in all 5S rRNA species that have been studied (from animals, fungi, plants and bacteria) and there is

little doubt that, as in tRNA species, the two ends are hydrogen-bonded in the native state.

All other base alterations observed in plant 5S rRNA do not compensate for one another so as to preserve base-pairing. Therefore these alterations have probably occurred in the single-stranded regions of the molecule. For example, in sunflower 5S rRNA, two U residues have changed into two C residues compared with broad-bean 5S rRNA and in the corresponding molecule of the tomato there are three U/C changes and one A/C change.

The results reported here do not support the idea that a sequence of nucleotides in 5S rRNA base pair in an anti-parallel manner to the -G-T-Ψ-C-G- sequence found in tRNA species (Forget & Weissman, 1967). The complementary sequence -C/U-G-A-A-C- found in *E. coli* (Brownlee *et al.*, 1968) and mammalian 5S rRNA species (Forget & Weissman, 1967) is absent from plant 5S rRNA. Further, the homologous sequence in yeast 5S rRNA is -A-G-A-A-A- (Hindley & Page, 1972).

The present work represents part of a study designed to determine the complete nucleotide sequence of 5S rRNA species from the cytoplasm and chloroplasts of the broad bean.

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