The primary structure of maize and tobacco 5 S rRNA

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5 S ribosomal RNA structures from *Zea mays* and tobacco have been determined with two independent methods. The sequence of corn and tobacco 5 S rRNAs are identical to those of the Gramineae and the Solanaceae, respectively. There is no general structure conservation of 5 S rRNA in higher plants.

Plant 5 S rRNA *Tobacco* *Maize 5 S rRNA* *Primary structure* *RNA conservation*

1. INTRODUCTION

Considerable knowledge has been gained about the sequence of events involved in protein biosynthesis and a detailed 3-dimensional structure for the procaryotic ribosome is beginning to emerge. The role of 5 S ribosomal RNA in this process has been the subject of a great many investigations and nearly as many speculative models.

Reconstitution studies have demonstrated that 5 S rRNA is essential for the in vitro assembly of active eubacterial ribosomes [1,2]. The same is most likely true for their eucaryotic counterparts although this has not been proven. The structure of ribosomal 5 S RNA has been studied extensively and these studies have led to the proposal of a large number of structural models [3]. The relatively conserved primary and secondary structure of this small rRNA suggests an important function in protein biosynthesis and it has been proposed that the invariant CPyGAAC sequence of procaryotic 5 S rRNA interacts with the GTΨC sequence of tRNAs during peptide chain elongation. However, recently it has been shown that the invariant sequence of 5 S rRNA appears to be indispensable for accurate translation of both natural and synthetic mRNAs [4].

There is also evidence that 5 S rRNA is involved in guanosine tetra- and pentaphosphate synthesis and ribosomal subunit interaction [5].

However, there is increasing evidence that the role of 5 S rRNA is not truly universal, since mitochondrial ribosomes from *Neurospora crassa*, yeast and mammalian cells appear to lack the 5 S RNA component [6]. In any case, 5 S rRNA is becoming one of the most intensively studied macromolecules, since it is an attractive subject for studies in molecular evolution and is accessible to RNA-sequencing methods. Comparison of the primary structure of eucaryotic and procaryotic 5 S rRNA led to a universal 5-helix model [3,7]. So far, of the 213 5 S rRNA sequences already known, 10 are from different plant species [8]. We were interested to learn more about plant 5 S RNA and define to what extent they fit the general model of eucaryotic ribosomal 5 S rRNA and the degree to which the sequence is conserved. Comparative sequence analysis of small rRNAs can provide interesting insights into the evolutionary relationships between various animal and plant species.

Here, we show the nucleotide sequence of 2 new 5 S rRNAs from different plant species. We chose maize seeds as representative of the Gramineae (monocotyledons) and tobacco of the Solanaceae group in the dicotyledon family. The maize seed 5 S rRNA sequence is identical to that of wheat germ and rye [15]. This suggests that all 5 S rRNA sequences of plants belonging to the Gramineae are identical. A similar phenomenon is observed for the 5 S rRNAs from the Solanaceae.

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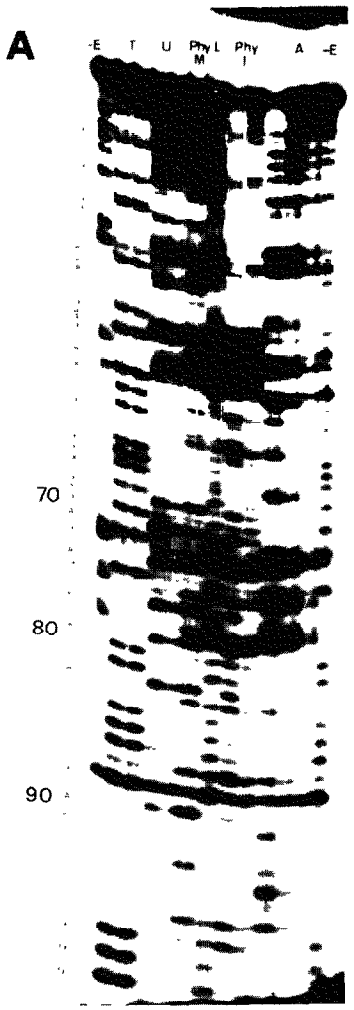
2. MATERIALS AND METHODS

Cytoplasmic 5 S rRNA from plants was isolated by following a 2-step method. First, a crude RNA preparation [9] containing tRNA, 5 S rRNA, DNA and other low-M_r RNAs was filtered through a Sephadex G-75 column (1 × 120 cm) [10]. Four times 400 A_{260} units in 0.01 M Tris-HCl, pH 7.5, buffer containing 1 M NaCl were applied to the column and eluted with the same buffer at a rate of 10 ml/h. The fraction containing 5 S rRNA was further purified by 2-dimensional gel electrophoresis on 10 and 20% polyacrylamide gels. With this approach we obtained 200 μg 5 S RNA from tobacco leaves and maize seeds. The purity of the RNA was checked on a 15% sequencing gel. Nucleotide sequence analysis by partial formamide digestion was done as described [11] for tRNA sequence analysis. RNA sequence gel analysis was performed with specific enzymes, essentially as in [12,13]. The fragments from partial enzymatic digests of ^{32}P-labeled 5 S rRNA were separated on 10 and 20% polyacrylamide gels [12,13]. To label the 5′-end, samples were dephosphorylated with calf intestinal alkaline phosphatase and then ^{32}P labeled at the 5′-end with [γ-^{32}P]ATP and T_4 kinase. To label the 3′-end, [^{32}P]pCp was enzymatically added to the 3′-end of the RNA molecule with T_4 ligase [12].

3. RESULTS

The 2 independent sequencing procedures employed, namely formamide fragment analysis and RNA sequence gels, provided enough data to determine unambiguously the complete nucleotide sequence of maize seed and tobacco cytoplasmic 5 S rRNAs (fig.1).



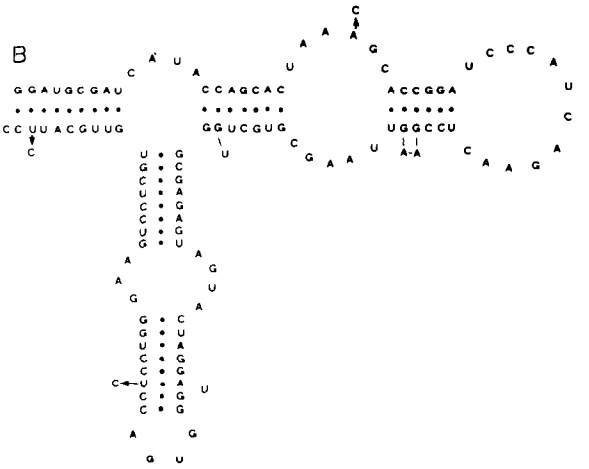


Fig.1. (A) Autoradiogram of the partial digestion products of 3′-^{32}P-labeled maize seed 5 S rRNA using specific enzymes: without endonucleases (E); T_1, incubation with RNase T_1; U_2, RNase U_2; Phy, PhyM RNase from *Physarum*; A, pancreatic RNase. Conditions were identical to those in [13]. Numbers on the left denote positions of the nucleotide in the sequence. (B). The nucleotide sequence of 5 S rRNA of maize seeds and tobacco (with arrows).

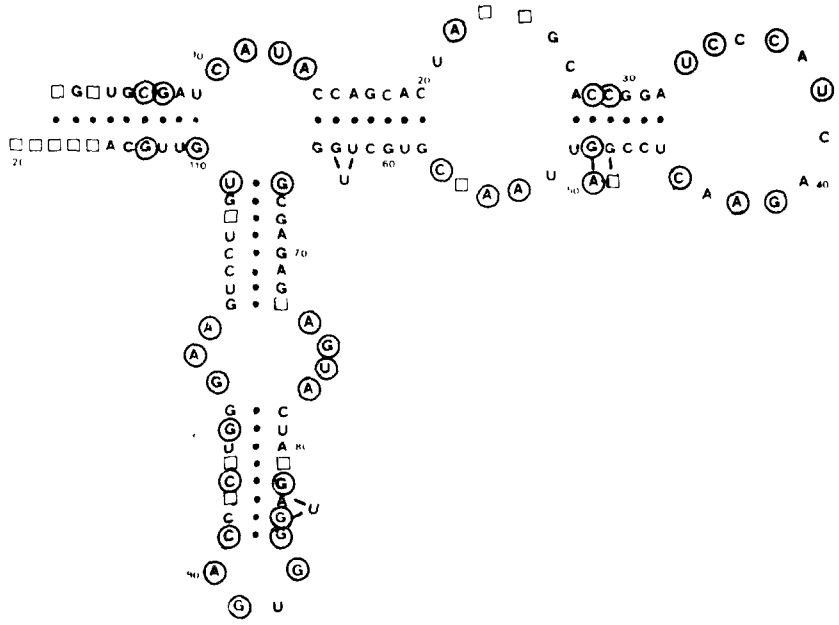


Fig.2. A possible secondary folding model of plant 5 S rRNAs. Nucleotides occupying variable positions are shown in boxes (table 1). Nucleotides in circles are common to all eucaryotic 5 S rRNAs.

The 5′-terminal nucleotide in both cases is pG. No heterogeneity was observed at the 3′-end of these RNAs. The nucleotide sequences determined fit very well the general structural model of eucaryotic 5 S rRNA [3], as seen in fig.2. The maize seed 5 S rRNA sequence is identical to those of wheat germ and rye [15] (table 1). 5 S rRNA from tobacco is different from that of maize but identical to the 5 S rRNA sequence of tomato.

4. DISCUSSION

A few years ago some of us proposed a hypothesis about the conservation of the nucleotide sequence of plant specific tRNAs [14]. The idea has also found substantial support from other findings [11]. Therefore, we wanted to learn more about the relation in other plant RNA species, namely 5 S rRNA. The question was whether 5 S rRNA follows a phylogenic relationship in plants. So far, 213 5 S rRNA sequences have been established [8,15], 10 being of higher plant origin (table 1). In the case of the Gramineae and Papilionaceae, the sequences of two 5 S rRNAs are known in each case, whereas in the other species only one sequence had been resolved. To look for structural correlations in different families we have chosen sequence analysis, maize seed and tobacco 5 S rRNAs. The primary structure of *Zea mays* 5 S rRNA (fig.1) is identical to those of wheat germ and rye 5 S rRNAs (table 1). These plants belonging to the Gramineae appear to have identical 5 S rRNA nucleotide sequences. On the other hand, the primary structure of tobacco 5 S rRNA also showed 100% homology with that of tomato 5 S rRNA. The data collected for the Gramineae and Solanaceae are in favor of the idea that plants within the same species have identical 5 S rRNA structures.

It is interesting to compare these findings with the 5 S rRNA sequences of the Papilionaceae. The broad bean [8] and dwarf bean [8] 5 S rRNA sequences differ in 3 positions, dwarf bean [8] and lupin seed [17] 5 S rRNAs differ in 1 position, but broad bean [8] and lupin seed [17] 5 S rRNAs differ in 2 positions (table 1). The lupin seed 5 S rRNA 1 and 2 sequences determined from the corresponding genes [18] showed great differences with regard to the RNA structure [17]. At present it is difficult to interpret these discrepancies. One can speculate that in the case of lupin seed one of these genes [18] represents a pseudogene as has

Table 1

Nucleotides found in the variable positions of different plant 5 S rRNA (see fig.2)

PLANT SPECIES	POSITIONS																					
	1	3	23	24	48	49	51	56	59	63	67	73	81	82	91	93	95	107	116	117	118	119	120
MONOCOTYLEDONS																							
1. Gramineae																							
rye	G	A	A	A	G	A	G	G	U	U	C	U	G	G	C	U	C	C	U	U	C	C	
wheat germ	G	A	A	A	G	A	G	G	U	U	C	U	G	G	C	U	C	C	U	U	C	C	C
zea mays	G	A	A	A	G	A	G	G	U	U	C	U	G	G	C	U	C	C	U	U	C	C	
2. Lemnaceae																							
duckweed	G	G	G	A	G	A	G	G	U	U	C	C	G	G	C	U	C	C	C	C	C	U	
DICOTYLEDONS																							
1. Papilionaceae																							
lupin seeds	A	G	A	U	G	C	G	G	U	U	C	U	G	G	C	U	C	C	C	C	U	C	
lupin seeds 1	A	G	A	U	U	C	U	G	U	U	A	U	G	C	G	U	C	C	C	C	U	C	U
lupin seeds 2	A	G	A	U	G	C	G	G	C	C	C	U	G	G	C	U	C	C	C	C	U	C	
broad bean	A	G	A	U	G	C	G	G	U	U	C	U	G	G	C	U	C	U	C	U	U	C	
dwarf bean	A	G	A	U	G	C	G	G	U	U	C	U	G	G	C	U	C	^	C	C	U	U	
2. Solanaceae																							
tobacco	G	A	A	C	G	A	G	G	U	U	C	U	G	G	C	C	C	C	U	C	C	C	
tomato	G	A	A	C	G	A	G	G	U	U	C	U	G	G	C	C	C	C	U	C	C	C	
3. Compositae																							
sunflower	G	U	A	U	G	C	G	G	U	U	C	U	G	G	C	C	C	C	A	C	C	C	C
4. Chenopodiaceae																							
spinach	G	G	A	U	G	C	G	G	U	U	C	U	G	G	C	U	C	C	C	C	C	C	U
5. Linnaceae																							
flax	G	G	A	U	G	A	G	A	U	U	C	U	A	G	C	U	U	C	C	C	C	C	C

already been suggested [8]. For the other plant species such a comparison is impossible because only one sequence of 5 S rRNA is known for each of them.

The nucleotide sequence data for tobacco and maize 5 S rRNAs presented here (fig.1) together with previous results [8,15] strongly support the hypothesis of the conservation of the 5 S rRNA nucleotide sequence of plants belonging to the same species.

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