The nucleotide sequences of chloroplast 4.5 S rRNAs from four species of plants, celery *(Apium graveoleus)*, barley *(Hordeum vulgare)*, Chinese chive *(Allium tuberosum)* and dayflower *(Commelina communis)*

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Four chloroplast 4.5 S rRNAs were isolated from the respective plant leaves by a simple method. The complete nucleotide sequences were determined using rapid gel sequencing techniques. The sequences are highly conserved among chloroplast 4.5 S rRNAs. The 4.5 S rRNAs of celery, Chinese chive and dayflower are 103 nucleotides long and that of barley 95 nucleotides long. The 7-nucleotide sequence from position 28–34 is absent in monocotyledon barley 4.5 S rRNA but is present in monocotyledon Chinese chive and dayflower.

Chloroplast *RNA* *RNA sequencing* *Sequence homology* *Evolution* *Phylogenesis* *(Plant)*

1. INTRODUCTION

Chloroplast 4.5 S rRNA is found not only in higher plants [1–12], but also lower plants [13]. Since the reports by Dyer and Bowman [1] and Whitfield et al. [3], 4.5 S rRNAs from a number of plants have been sequenced [6–13]. The nucleotide sequences of 4.5 S rRNAs are strongly conserved among themselves and homologous with the 3′-terminal region of prokaryotic 23 S rRNAs [12,17]. The structural homology suggests that there is some functional homology between 4.5 S rRNA and the 3′-terminal region of prokaryotic 23 S rRNA. The appearance of 4.5 S rRNA is interesting in plant molecular evolution and phylogenesis.

We have determined the nucleotide sequences of chloroplast 4.5 S rRNAs of celery, barley, Chinese chive and dayflower and compared the sequence homology.

2. MATERIALS AND METHODS

2.1. *Materials*

Plants were obtained from the local commune or grown in our laboratory. [γ-^{32}P]ATP (5000 Ci/mmol) was purchased from the Radiochemical Centre (Amersham, England) or the Chinese Nuclear Research Centre, Beijing. Polynucleotide kinase, RNA ligase and RNase T_1 were purchased from the Biochemical Factory of the Institute of Biophysics, Academia Sinica, Beijing. RNase U_2, RNase Phy M and RNase *B. cereus* were obtained from Pharmacia P-L Biochemicals. [5′-^{32}P]pCp was prepared from Cp and [γ-^{32}P]ATP by polynucleotide kinase reaction.

2.2. *Purification of 4.5 S rRNA*

Chloroplast 4.5 S rRNAs were prepared from young leaves primarily according to Wildeman and Nazar [6] with modifications. 500 g leaves were mixed with 500 ml of 0.3% SDS, 50 mM sodium acetate (pH 5.0), 0.14 M NaCl and 500 ml phenol saturated with distilled water and containing

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0.08% hydroxyquinoline. The mixture was homogenized in a blender at maximum speed (20000 rpm) for 2 min. The homogenate was stirred at 50°C for 15 min and centrifuged at 6000 × *g* at 4°C for 15 min to remove the debris. The aqueous phase was made 2 M with LiCl and stirred at room temperature for 20 min, then centrifuged to remove DNA, high-M_r RNA and part of the proteins. 2.5 vols ethanol were added and the preparation kept at −20°C overnight. The RNA precipitate was collected by centrifugation, washed with 70% cold ethanol and lyophilized. 4.5 S rRNA was purified using 8% polyacrylamide/7 M urea gel electrophoresis.

2.3. *5′- or 3′-end labeling*

The 5′- or 3′-end of purified 4.5 S rRNA was labeled as in [14]. The 5′-end was labeled with polynucleotide kinase and [γ-^{32}P]ATP without prior dephosphorylation and the 3′-end with RNA ligase and [5′-^{32}P]pCp. The end-labeled RNA was purified as in [6].

2.4. *RNA sequence analysis*

The nucleotide sequences were determined using the enzymatic method of Donis-Keller et al. [15] and the chemical method of Peattie [16]. Both 5′- and 3′-end-labeled RNA were used in enzymatic sequencing. 3′-end-labeled RNA was used in chemical sequencing. The 3′-terminal residue was identified by PEI-cellulose thin-layer chromatography after complete digestion of the end-labeled RNA with RNase T_2.

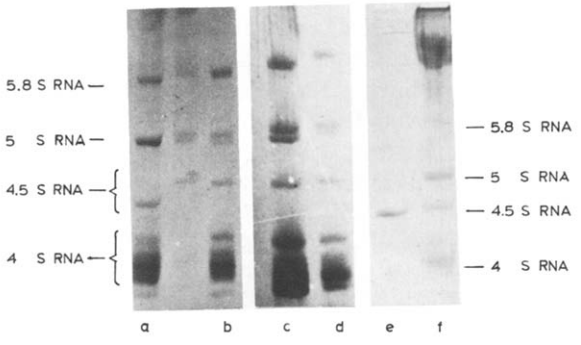

Fig.1. Separation of low-M_r RNAs from various plant leaves on 8% polyacrylamide gel. Bands were stained in toluidine blue. (a) Barley, (b,c) Chinese chive, (d) dayflower, (e) purified 4.5 S rRNA of celery, (f) celery.

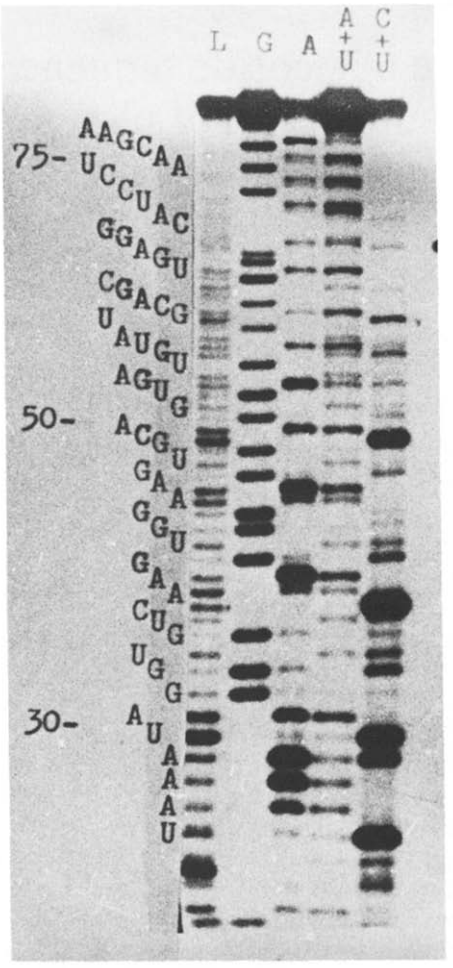

Fig.2. Autoradiograph of sequencing gel with 5′-end-labeled chloroplast 4.5 S rRNA of barley. The partial digests were obtained by incubation with RNase T_1 (G), RNase U_2 (A), RNase Phy M (A + U), RNase *B. cereus* (C + U), and alkali (L). Electrophoresis was carried out on a 20% polyacrylamide gel for 8 h at 1500 V. Numerals indicate residue numbers relative to the 5′-end.

3. RESULTS AND DISCUSSION

The 4 chloroplast 4.5 S rRNAs were purified from the 4 plant species by a simple method, hot-phenol extraction.

The nucleotide sequences were determined as described by Peattie with 3′-end labeled 4.5 S rRNA and according to Donis-Keller with both 3′- and 5′-end labeled 4.5 S rRNA. The 4 nucleotide sequences from different plants have been deter-

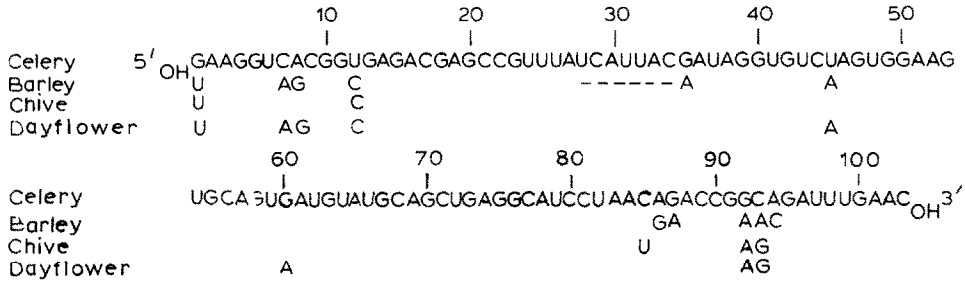

Fig.3. Alignment of chloroplast 4.5 S rRNA sequence of celery, barley, Chinese chive and dayflower. Nucleotides identical to those found in celery chloroplast 4.5 S rRNA sequence are not shown. Bars denote gaps introduced to maximize homology.

mined. All are 103 nucleotides long, except for barley 4.5 S rRNA which is 95 nucleotides in length (fig.3). The sequence of at least 90 nucleotides from the 3′-end was unambiguously determined by both enzymatic and chemical methods with [3′-^{32}P]RNA. The sequences from the 5′-end to about position 80 were determined enzymatically using [5′-^{32}P]RNA. There were about 50–80 nucleotide sequences overlapping from different end determinations. The 3′-terminal residue as pyrimidine C could not be read out from the autoradiograph with 3′-end-labeled RNA because the last fragment was GAAC^{32}pCp in the lane of RNase *B. cereus* in the autoradiograph mapping (not shown).

From the determined nucleotide sequences of the chloroplast 4.5 S rRNAs it was proved that the sequences of 4.5 S rRNAs were strongly conserved among themselves (fig.3). For example, the highest homology between celery and Chinese chive, Chinese chive and dayflower is up to 95%. However, there is an additional phenomenon shown in fig.3, namely a 7-nucleotide sequence deletion from position 28 to 34 of barley 4.5 S rRNA. The sequence homology between barley and the others in fig.3 is only about 80% when the homology is estimated with the inclusion of the 7-nucleotide fragment. It is present in the 4.5 S rRNAs of dicotyledon tobacco [7], spinach [9] and tomato [12]. It is also present in the 4.5 S rRNAs of typical monocotyledon Chinese chive and dayflower. However, this 7-nucleotide fragment is absent in monocotyledon barley, wheat [6] and maize [8], all of which are classed as monocotyledon grasses. The absence of the 7-nucleotide fragment in the sequence of 4.5 S rRNA may be a feature of grasses. Therefore, we consider that the deletion of this fragment must have occurred after the evolutionary separation of monocotyledon grasses. It might play a particular structural or functional role in one group of ribosomes.

The nucleotide sequences of the four 4.5 S rRNAs were compared with that of the 3′-terminal region of *E. coli* 23 S rRNA, essentially according to the alignment of Edwards and Kössel [17], resulting in a homology of about 60%. This suggests that the gene of chloroplast 4.5 S rRNA might arise from the 3′-terminal region of the gene of prokaryotic 23 S rRNA. This lends support to the hypothesis of endogenesis.

The four 4.5 S rRNAs that we sequenced all have a free 5′-terminal hydroxyl. The property of having an unphosphorylated 5′-end is in accord with the general features of chloroplast 4.5 S rRNAs [1–12]. This suggests that 4.5 S rRNAs might differ in the post-transcription process from other RNAs in chloroplasts.

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