The nucleotide sequence of 4.5S ribosomal RNA from tobacco chloroplasts

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ABSTRACT

The nucleotide sequence of tobacco chloroplast 4.5S ribosomal RNA has been determined to be:


The 4.5S RNA is 103 nucleotides long and its 5'-terminus is not phosphorylated.

INTRODUCTION

Chloroplast ribosomes in higher plants are 70S in size and contain 23S, 16S, 5S and 4.5S RNAs (1). The 4.5S RNA has recently been found in the large subunit of the chloroplast from a number of higher plants (2-7). In tobacco chloroplasts, the 4.5S RNA is present in approximately equimolar amount as the chloroplast 5S RNA and is coded for by the chloroplast DNA between the 23S and 5S rRNA genes (6). We present here the nucleotide sequence of the 4.5S RNA from tobacco chloroplasts.

MATERIALS AND METHODS

Tobacco chloroplast 4.5S RNA was prepared as described (6, 8). The 5' end was labeled using polynucleotide kinase and [γ^{32}P]ATP as described previously (9). The 3' end was labeled using RNA ligase (10) and [5'^{32}P]pCp as described (11). RNA sequencing was done according to the published procedures (11-14).

RESULTS

The 5' end of tobacco chloroplast 4.5S RNA could be labeled with ^{32}P using polynucleotide kinase and [γ^{32}P]ATP without prior
Fig. 1. Sequence gel of [3'\textsuperscript{32}P]4.5S RNA digested by chemical method. The inset show a part of sequence gel of [5'\textsuperscript{32}P]4.5S RNA digested with alkali and RNases.
Fig. 2. A possible secondary structure of tobacco chloroplast 4.5S RNA.

dehphosphorylation. This indicates that the 4.5S RNA is not phosphorylated at its 5' end. The [5'32P]4.5S RNA was partially digested with base-specific RNases and alkali (12-13). The digests were analyzed by electrophoresis on 20% polyacrylamide gels in 7 M urea. By this procedure, up to 30 nucleotides from the 5' end could be read off, although C and U could not always be discriminated. In order to distinguish between the pyrimidines, the partial digests of the [5'32P]4.5S RNA with alkali were resolved by the two-dimentional gel electrophoresis (14). In combination with these results, we could unambiguously determine the sequence from the 5' end to residue 30. The [3'32P]-4.5S RNA was sequenced by the methods of chemical and enzymatic partial digestions (11-13). The sequence of residues 20-102 could be read off from the autoradiograms shown in Fig. 1. The 5' and 3' terminal residues were identified to be G and C, respectively, by PEI cellulose thin-layer chromatography after complete digestion of the [5'32P]RNA with nuclease P1 and of the [3'32P]RNA with RNase T2. The total nucleotide sequence of the 4.5S RNA is shown in the ABSTRACT and in Fig. 2. The 4.5S RNA contains 103 nucleotides and its 5' end is not phosphorylated.
DISCUSSION

Previously, we have cloned and sequenced the 4.5S RNA gene from tobacco chloroplasts (15, 16). The 4.5S RNA sequence obtained here agrees with the DNA sequence except for residues 69-70 (G-A in RNA while A-G in DNA). We, therefore, put residues 69-70 in parenthesis. The DNA sequence in this region should be true because a cleavage site for restriction enzyme Pvu II (C-A-G-C-T-G) exists in the 4.5S RNA gene (15) and corresponds to residues 68-73 in the 4.5S RNA. The discrepancy may be due to some post-transcriptional modification of residue 69 and/or 70, because the irregular ladder of the alkaline digest appeared at these residues and the spacer between residue 69 and 70 was unusually narrow (Fig. 1). Bowman and Dyer have recently reported the fingerprint analysis of tobacco chloroplast 4.5S RNA (4). All of the 20 identified oligonucleotides produced by RNase T1 digestion fit in well with our sequence. An oligonucleotide corresponding to residues 68-70 is, however, missing in their oligonucleotide catalog. This also suggests the unusualness in this region in the 4.5S RNA. Attempts to detect unusual nucleotide(s) are in progress.

A possible secondary structure for the 4.5S RNA can be constructed as shown in Fig. 2. This structure as well as the sequence show that the 4.5S RNA is not related to any known tRNA, 5S rRNA and 5.8S rRNA. The 4.5S RNA is, therefore, not a derivative or a variant of the known RNA species but is a unique component of chloroplast ribosomes in higher plants. It seems general feature in chloroplast 4.5S RNAs to have unphosphorylated 5' ends (2, 4). This fact suggests that chloroplast 4.5S RNA molecules are processed in a different way from chloroplast 23S, 16S and 5S RNAs which have phosphates at their 5' ends.

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