Isolation and nucleotide sequence of a plant tRNA gene: petunia asparagine tRNA

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ABSTRACT

A 14.3 kb petunia genomic DNA fragment was isolated and found to contain a single tRNA gene coding for asparagine tRNA. The nucleotide sequence of the asparagine tRNA gene and its flanking regions has been determined. This gene does not contain intervening sequences nor the 3'-end CCA sequence of the mature tRNA and presents a similar overall sequence homology (70%) to both E. coli and mammalian asparagine tRNA. As in other eukaryotic tRNA genes the 5'-flanking region does not seem to contain any special sequence that could function as a regulatory element and the 3'-end is followed by a short cluster of T that may function as the transcription termination site.

INTRODUCTION

In contrast to the large amount of information that has been accumulated on the arrangement and structure of tRNA genes from a variety of eukaryotes such as yeast, Neurospora, Bombyx, Xenopus, Drosophila, rat and man (1-13), nothing is known about plant nuclear tRNA genes. To study genes for tRNA in plant cells, we have constructed a petunia genomic library and screened it with an unfractionated cytoplasmic petunia tRNA probe. Here we describe the detailed analysis of a tRNA gene-containing clone and the nucleotide sequence of a DNA fragment carrying a tRNA\_AAC(U) gene.

MATERIALS AND METHODS

Restriction enzymes were obtained from Biolabs, New England, T4 RNA ligase and T4 DNA ligase were from PL Biochemicals, \( \alpha ^{32}P \) nucleoside triphosphates and cytidine 3'-5' - \( \beta ^{32}P \), bisphosphate (pCp) were from Amersham. Petunia Genome Library. Petunia nuclear DNA was partially digested with Eco RI and \( \sim 20 \)kb DNA fragments were isolated by sedimentation through a 10-40% linear sucrose gradient as described by Maniatis et al. (14). The 20 kb petunia DNA fragments were ligated with a 2 fold molar excess of \( \lambda \)Charon 4A purified arms (12 and 19 kb) using T4 DNA ligase (14) and the recombinant DNA was packaged in \textit{vitro} into phage particles following the method of Hohn.
and Murray (15). Approximately $10^5$ independent clones were obtained. The library was amplified about $10^5$ fold by low density plating on E. coli DP50supF (14).

**Petunia tRNA clones.** The petunia genomic library was screened by the method of Benton and Davis (16) using as probe an unfractionated mixture of petunia cytoplasmic tRNAs labeled at the 3'-end with \(\overset{32P}{5'}\)-pCp. About 50 tRNA-hybridization positive clones were obtained for a petunia library of $10^5$ clones. The final isolation of tRNA gene-containing clones was carried out by plaque purification (16).

**End labeling of tRNA.** For 3' end labeling, unfractionated cytoplasmic petunia tRNA was first incubated for 120 min at 37° in 200 mM Tris-HCl pH 8.3, 200 mM KCl and 10 mM MgCl₂ to remove any 3'-end bound amino acid, then ethanol precipitated. \(\overset{32P}{5'}\)-pCp was then ligated to the tRNA using T4 RNA ligase following the procedure of England et al. (17). The \(\overset{32P}{3'}\)-tRNA preparation was further purified by electrophoresis on 10% acrylamide gel containing 7 M urea followed by elution with 500 mM NaCl, 100 mM Tris-HCl pH 7.4.

**DNA sequence analysis.** 5' and 3'-end labeling of DNA fragments and sequence analysis were performed according to the procedures of Maxam and Gilbert (18).

**In vitro transcription.** Transcription of petunia tRNA gene was carried out in Xenopus germinal vesicle extracts as described by Schmidt et al. (19). 0.5 μg plasmid DNA were transcribed with 20 μl of Xenopus germinal vesicle extracts (diluted 1:1 by volume with buffer containing 70 mM KCl, 7 mM MgCl₂, 0.1 mM EDTA, 10 mM Tris-HCl pH 7.4, 2.5 mM dithiothreitol) by incubation at 23-24°C for 90 min with \(\overset{32P}{\alpha}\)-UTP (0.01 mM, 400 Ci/mmole) and 0.1 mM each of ATP, GTP and CTP. The synthesized RNA was extracted and fractionated by two-dimensional gel electrophoresis in 10% acrylamide - 7 M urea followed by a 20% acrylamide - 7 M urea gel (20).

**RESULTS AND DISCUSSION**

**Isolation of tRNA gene-clones from a petunia genomic library**

A petunia genomic library was screened for tRNA gene-containing clones by the Benton and Davis plaque hybridization method (16), using as probe an unfractionated mixture of petunia cytoplasmic tRNAs labeled at the 3'-end with \(\overset{32P}{5'}\)-pCp. The \(32P\)-labeled tRNA probe was previously purified by electrophoresis on 10% polyacrylamide gel and the hybridization was performed in the presence of a large excess of tRNA-free petunia ribosomal RNA. Working with a collection of $10^5$ phage recombinants we have detected 50 clones carrying tRNA genes. A clone, λCh4-pN₁, containing 14.3 kb of petunia
Figure 1. Restriction endonuclease digestion of recombinant DNA from bacteriophage λCh4-pN1. DNA digests with Eco RI (A) and Hind III (B) were electrophoresed through 1% agarose slab gels and stained with ethidium bromide. In parallel with fluorescence of DNA fragments is presented an autoradiogram of a Southern blot of the same gel hybridized with 3'-32P-labeled, unfractionated petunia tRNA. An Eco RI digest of λDNA is presented in the separate lane at right.

DNA was isolated, purified and recombinant DNA analyzed by restriction with Eco RI and Hind III endonucleases. Fig. 1 shows the pattern of restriction and Southern blots obtained by transfer of the DNA fragments to nitrocellulose and hybridization with 3'-32P-labeled tRNA. The tRNA genes appear to be located within a Hind III fragment of 1.0 kb. This fragment was inserted into the Hind III site of plasmid pBR322 and the recombinant plasmid used to transform E. coli HB101. The recombinant plasmid pBR322-petunia DNA (pN1) was subsequently used for the study of petunia tRNA gene sequence and transcription.

Restriction enzyme mapping and nucleotide sequence

The subcloned 1.0 kb petunia DNA fragment was cleaved with several restriction endonucleases, thereby reducing the size of tRNA gene-containing region to a 200 bp fragment (Alu I-Bam HI). The DNA sequence presented in Fig. 2 shows that the 1.0 kb Hind III DNA fragment contains a tRNA gene coding for tRNAAsnAAC(U). Since the 200 bp fragment containing the tRNAAsn is the only fragment hybridizing with 32P-labeled tRNA, we conclude that the 14.3 kb petunia DNA insert of clone λCh4-pN1 probably contains a single tRNA gene. The 5'-flanking region (shown in Fig. 2) does not present any sequence that could be identified as a regulatory element for transcription. The 3'-flanking region contains a sequence TTTTT in the nontranscribed strand, one nucleotide from the coding region, followed by the sequence CTTTTC, 16 nucleo-
Figure 2. Restriction map of the 1.0 kb Hind III fragment of petunia DNA from AChA-pN^ and the nucleotide sequence of the tRNA-gene containing region. The non-coding strand is shown and the asparagine tRNA corresponding sequence is underlined.

The DNA sequence of the tRNA<sup>Asn</sup> gene can be arranged in a clover leaf secondary structure as shown in Fig. 3. The sequence shows that this gene does not contain an intervening DNA sequence nor the sequence corresponding to the 3'-end CCA found in mature tRNAs.

A comparison of the nucleotide sequence of petunia tRNA<sup>Asn</sup> gene with the sequences (23) of E. coli and mammalian tRNA<sup>Asn</sup> reveals a 70% homology with each. It should be observed that there is a 60% homology between tRNA<sup>Asn</sup> of prokaryote and eukaryote origin (24). There is an identity of sequences among the mammalian tRNA<sup>Asn</sup> (human, bovine, rat) (24) and a near identity of those with the sequence of a Drosophila tRNA<sup>Asn</sup> gene (9). This indicates a conservation of primary structure of cytoplasmic tRNAs during the evolution of these eukaryotes of the animal kingdom.

**In vitro transcription of tRNA<sup>Asn</sup> gene**

The RNA sequence of tRNA<sup>Asn</sup>, as that of most tRNA species in plants, is unknown and it cannot presently be determined if the tRNA<sup>Asn</sup> gene described
is expressed in the plant cell. In order to test whether petunia tRNA$^{\text{Asn}}_{\text{AAC(U)}}$ gene is recognized by RNA polymerase III and transcription factors, we have transcribed the pN$_1$ plasmid DNA in a nuclear extract of Xenopus oocytes with labeled nucleoside triphosphates and separated the products by two-dimensional acrylamide gel electrophoresis. Fig. 4 shows the synthesis of a main tRNA-size molecule (spot 5) and several minor RNA species (spots 1-4) which probably represent precursor intermediates. These minor spots indeed disappear upon an additional incubation of 2 hrs in the presence of unlabeled nucleoside triphosphates (data not shown). The \textit{in vitro} synthesized tRNA-size...
molecule (spot 5) was extracted from the gel and used as a hybridization probe with the restriction fragments of the 1 kb petunia DNA insert of pN plasmid (Fig. 2). It was found to hybridize exclusively with the 200 bp Alu I-Bam HI fragment proving unequivocally the origin of the transcript. Although the petunia tRNA<sup> asn </sup> gene transcription in a heterologous Xenopus system is not a definitive proof of its expression in the plant cell, the primary DNA sequence and secondary structure derived from it seem to contain all the characteristics required for a tRNA gene to be expressed.

This paper is the first description of the fine structure of a tRNA gene from a plant nuclear genome. The features of the structure of this plant tRNA gene indicate that the coding sequence is as close to that of other eukaryotes of animal origin as it is to that of bacteria while the flanking regions seem to be essentially the same as those found in other eukaryotes.

REFERENCES