The sequences of two nuclear genes and a pseudogene for \textit{tRNA}^{Pro} from the higher plant \textit{Phaseolus vulgaris}

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Summary

A genomic bank of nuclear DNA (nDNA) from the higher plant \textit{Phaseolus vulgaris}, constructed using the lambda EMBL-4 vector, has been screened for the presence of \textit{tRNA} genes. One of the many positive recombinants was found to hybridise several times stronger than the other positives, and has been shown to contain several \textit{tRNA} genes. We report the structure of two nuclear \textit{tRNA} genes for \textit{tRNA}^{Pro}, namely \textit{tRNA}^{Pro}(UGG) and \textit{tRNA}^{Pro}(AGG), and that of a ‘pseudogene’ for \textit{tRNA}^{Pro}. This ‘pseudogene’, despite showing 95\% homology with the other \textit{tRNA}^{Pro} species presented here, has several features which are likely to affect its transcription or its functioning as a tRNA.

Introduction

There is much information available concerning the structure, organisation and transcription of nuclear \textit{tRNA} genes in eukaryotes, but almost to the exclusion of the plant kingdom. To date there are only two reports in the literature concerning the structure of plant nuclear \textit{tRNA} genes, namely one on soybean \textit{tRNA}^{Asp}(GUC) and \textit{tRNA}^{Met}(CAU) (1), and one on petunia \textit{tRNA}^{Asn}(GUU) (2) (the latter sequence showing a 97\% homology with the tobacco chloroplast sequence, so one may wonder whether it is really a nuclear gene or perhaps a chloroplast insert in the nuclear genome). In order to obtain more information on nuclear \textit{tRNA} genes in plants, a genomic bank of nuclear DNA from the higher plant \textit{Phaseolus vulgaris}, constructed using the lambda EMBL-4 vector, was screened for the presence of \textit{tRNA} genes. Here we describe the sequences of two different genes and a ‘pseudogene’ for \textit{tRNA}^{Pro}, and their 5’ and 3’ flanking regions, and show that they appear to be typically eukaryotic in nature.

Methods

\textit{Extraction of plant nDNA(3)}

150 g of dark grown \textit{Phaseolus vulgaris} hypocotyls were ground in 50 ml of buffer A (250 mM sucrose, 50 mM EDTA, 50 mM NaCl). After filtration through gauze, and centrifugation at 600 g, the pellet was resuspended in 4 ml of buffer B (15 mM Tris-HCl, pH 8.0, 150 mM NaCl). 1 ml of 30\% SDS was added and the solution was gently mixed for 10 min at room temperature. 1 ml of 5 M sodium perchlorate was then added and gentle mixing was continued for 15 min at 4°C. Following chloroform/n-octanol extraction, the DNA was recovered by spooling after the addition of cold ethanol. The DNA was washed in 70\% ethanol and was sufficiently pure at this stage to be cloned directly.

\textit{Extraction of plant cytoplasmic tRNAs}

Total small cytoplasmic RNAs (small ribosomal RNAs and tRNAs) were extracted as described elsewhere (4).
Cloning of plant nDNA in EMBL-4

Preparation of EMBL-4 arms and the Mbo I partial digestion of \textit{P. vulgaris} nDNA were carried out as described by Schwarz-Sommer \textit{et al.} (5), except that the size selection of the plant nDNA was by agarose gel electrophoresis, the nDNA fragments being recovered by electroelution.

Screening

High density screening at 2000 phage/plate was followed by two further rounds of low density screening. The probe was total small cytoplasmic RNAs from \textit{P. vulgaris}, chemically treated as described previously (6) and labelled at the 3'-end using (\textit{ct} - 32P) ATP and tRNA nucleotidyl transferase (7), so that only tRNAs were labelled. Hybridization was at 65°C in 3 × SSC (0.45 M NaCl, 0.045 M Na citrate) for 16 h, followed by washing for 3 × 20 min at room temperature in 3 × SSC.

Subcloning in pBR 322

The positive recombinants were cleaved with EcoR I and 'shotgun' cloned into pBR 322 at the EcoR I site. tRNA gene-containing recombinants were identified as described above.

Subcloning in M13 mp8

The tRNA-containing insert was cleaved out of pBR 322 by EcoR I and the fragment was purified on low gelling agarose. The fragment was then digested either with Hinf I and filled using Klenow enzyme, or with Alu I and 'shotgun' cloned into M13 mp8 vector cut with Sma I. Sequencing was by the di-deoxy method of Sanger.

Hybrid selection of tRNAs

10 \( \mu \text{g} \) of nDNA-pBR 322 recombinant DNA was immobilized on a nitrocellulose disc in the presence of 20 × SSC, and incubated for 30 min at 65°C in 3 × SSC with the 3'-end labelled tRNA probe. After exhaustive washing in 3 × SSC, the specifically bound tRNAs were eluted by boiling in water for 5 min. The resulting solution was lyophilized and the pellet was dissolved in 10 \( \mu \text{l} \) TE (10 mM Tris-\text{HCl}, 1 mM EDTA, pH 8.0), and subjected to 2D-polyacrylamide gel electrophoresis as described elsewhere (4).

Results and discussion

The genomic bank obtained (20000 recombinants) was screened for the presence of nuclear tRNA genes using as the probe 3'-end labelled total cytoplasmic tRNAs from \textit{Phaseolus vulgaris}. After three rounds of screening, 30 positive recombinants were obtained. Initially, a phage recombinant giving a hybridization signal several times as intense as any of the other phage positives was selected for further analysis. This difference in intensity of signal suggested the presence of several tRNA genes on this cloned nDNA fragment. Southern hybridization analysis identified a 6.5 kb EcoR I fragment which alone contains the tRNA gene (s). This 6.5 kb fragment was inserted into pBR 322 at the EcoR I site by 'shotgun' cloning. Screening was by colony hybridization using the 3'-end labelled cytoplasmic tRNA probe. Preliminary mapping of this fragment revealed that tRNA genes were present on three different Hind III fragments (Fig. 1). Two fragments (1.0 kb and 4.3 kb) hybridize with relatively low intensity, whilst the third (3.5 kb) gives a signal three to four times as intense as the smallest fragment. Digestion with Taq I resulted in four hybridizing fragments, three of which are of equal intensity, the fourth being much stronger (Fig. 1). Therefore the 6.5 kb fragment probably contains six tRNA genes.

In order to assess the number of different tRNA gene species present on this fragment, the tRNAs hybridizing to this fragment were eluted from the hybrid and analyzed by 2D-polyacrylamide gel electrophoresis as described in the methods section. Two spots, of equal intensity, migrating very close to each other, were evident (Fig. 2). This would suggest that only genes for two different tRNA species, which have very similar electrophoretic mobilities, are present. A third tRNA species, which has a very different mobility, may also be present but probably only as a single copy.

The 6.5 kb fragment was subcloned, by the 'shotgun' method, into M13 mp8 vector for sequence determination by the di-deoxy sequencing technique of Sanger. Sequence analysis to date has revealed