Molecular cloning and characterization of a nuclear gene encoding a putative subunit of tRNA splicing endonuclease from Arabidopsis thaliana

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ABSTRACT

tRNA splicing endonuclease is required to produce mature tRNAs from intron-containing tRNA precursors. To characterize the structural features of plant endonuclease, we have isolated a cDNA and a corresponding genomic DNA clone from libraries of Arabidopsis thaliana which encode a putative subunit of the endonuclease. The gene product has an apparent mass of 27 kDa and contains a homologous domain of approximately 130 amino acids at the C-terminal region commonly found in other eucaryal and archaeal counterparts. Southern hybridization analysis of Arabidopsis genomic DNA utilizing the cDNA clone as probe indicates the presence of at least two related genes.

INTRODUCTION

In plants, only nuclear genes encoding tRNA\textsuperscript{\textsuperscript{\textsubscript{Tyr}}} and tRNA\textsuperscript{\textsuperscript{\textsuperscript{\textsubscript{Met}}}} are interrupted by introns (1-4). All of these genes share similar features in the intron-containing secondary structure as well as in the centre of the tRNA mature domain (Figure 1). These properties support the notion that plant tRNA endonuclease is more specific for homologous substrates. In fact, we have previously shown that the plant enzyme cannot remove introns from other eucaryal precursors such as pre-tRNAs\textsuperscript{\textsuperscript{\textsubscript{Tyr}}} from yeast, Xenopus and human in an \textit{in vitro} splicing system from wheat germ (5,6), while pre-tRNAs transcribed from plant genes are subject to faithful splicing in the same extract (5-7). In addition, we have also demonstrated that at least for the splicing of plant pre-tRNA\textsuperscript{\textsuperscript{\textsubscript{Tyr}}}, specific nucleotides in the mature domain of tRNA are essential for the efficient intron excision (6).

Recently, the genes encoding the subunits of tRNA splicing endonuclease were successively reported from yeast and archaea (8,9). The yeast enzyme consists of four different subunits of 15, 34, 44 and 54 kDa and two of them, i.e., the 34 and 44 kDa subunits are supposed to cleave independently the 3' and 5' splice sites (8). On the other hand, the archaeal counterpart is a homodimeric or a homotetrameric enzyme in Halobacterium and Methanococcus, respectively (9,10). These proteins all have a highly conserved domain structure of about 130 amino acids, suggesting that the machinery of tRNA splicing has evolved from a common origin.

In plants, a partial sequence for an endonuclease-like protein was detected in an intron of an HMG-like gene from maize (11). However, it...
remained unclear if this sequence is in fact expressed. Here we report the molecular structure of an *Arabidopsis* gene coding for a putative subunit of the tRNA endonuclease.

RESULTS AND DISCUSSION

In order to identify an *Arabidopsis* gene coding for a subunit of tRNA splicing endonuclease, we first searched the *Arabidopsis thaliana* Database (Stanford University) utilizing the maize homologous sequence (11). A partial genomic DNA sequence of 579 bp in length (accession number B60807) displayed a significant homology to the maize sequence. This DNA fragment was PCR-amplified from *Arabidopsis* DNA with an adequate primer set. After verification of the sequence, the PCR product was used as a probe to screen a cDNA library from young seedlings of *Arabidopsis thaliana* (ecotype Landsberg). One cDNA clone was isolated which contained a 711 bp long open reading frame (ORF) and encodes a protein of 237 amino acids with a molecular mass of 27.3 kDa (Figure 2). Sequence analysis revealed 25, 29 and 48% identities with the C-terminal half (including an active domain) of the known endonuclease subunit from *Methanococcus jannaschii*, yeast and maize, respectively. We also isolated the corresponding genomic clone from *Arabidopsis*, which exhibited identical sequence in the ORF as compared to the cDNA clone. Interestingly, an intron of 259 bp was detected in the genomic clone immediately upstream of the postulated initiator codon. A 5’ race analysis of the relevant mRNA proved that a start position of its transcription is actually present in a region located upstream of the intron. Southern hybridization analysis of genomic *Arabidopsis* DNA with the cDNA clone as probe disclosed the presence of at least two copies of homologous genes in the nuclear genome. One of these genes, yielding a stronger hybridization signal, corresponds to the sequence of the identified genomic clone. The two different genes may in fact code for two distinct subunits corresponding to the 34 and 44 kDa subunits from yeast. Alternatively they may represent two copies of the same subunit. Although comparative analysis of amino acid sequence suggests that the *Arabidopsis* enzyme is closer related to the 44 than to the 34 kDa subunit, we could neither detect the presence of a transmembrane sequence nor an antiparallel coiled-coil structure characteristic for the former. To resolve these contradictory observations, *in vitro* splicing experiments with a recombinant plant endonuclease subunit protein expressed in *E. coli* are in progress.

![Restriction map of a nuclear gene for a putative subunit of tRNA endonuclease from *Arabidopsis* and its gene product (lower box). Shaded box denotes a region homologous to the endonuclease subunits reported.](https://example.com/fig2)

REFERENCES