

The nucleotide sequence of a mung bean (*Vigna radiata*) mitochondrial lysyl-tRNA gene

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Abstract. The nucleotide sequence of a gene coding for tRNA^{Lys} and its flanking regions from mung bean mitochondrial genome are presented and compared with known mitochondrial tRNA^{Lys} genes from other higher plant species. This tRNA sequence shows less similarity with its chloroplast counterpart. Several regulatory motifs in the 5' and the 3' flanking regions were identified. The 3' motif could be folded into a stem-loop structure. The functions of these regulatory motifs are discussed.

Keywords: tRNA^{Lys}; Mitochondria; Mung bean; *Vigna radiata*.

Abbreviations: DAPI, 4',6-Diamidino-2-phenylindole, hydrochloride.

Introduction

Mitochondrial genomes in plants are large and structurally complex, ranging in size from 200 to 2400 kb (Lonsdale, 1989); however, the plant mitochondrial genomes, unlike their animal counterparts, do not encode the whole set of essential tRNAs required for translation in mitochondria (Hanson and Folkerts, 1992). Systematic investigations of flowering plant mitochondrial tRNAs have revealed that they fall into three groups: the first group includes native tRNAs encoded by the mitochondrial genome; the second group contains chloroplast-like tRNAs with 90–100% homology to their respective chloroplast counterparts; and the final group is cytosolic-like tRNAs that are encoded by nuclear genes (Dietrich et al., 1992; Hanson and Folkerts, 1992). Not all plant species contain the same sets of tRNAs from each of the three different groups. For example, the potato mitochondrial genome encodes 15 tRNAs for the first group, but wheat mitochondrial genome encodes only 10 such tRNAs (Joyce and Gray, 1989; Marechal-Drouard et al., 1990). Hence it is important to determine the number and the origin of tRNAs encoded in the mitochondrial genome of different plant species. Here we report the sequence of a mung bean native mitochondrial tRNA^{Lys} together with its flanking regions. The sequence is also compared with tRNA^{Lys} genes of other species.

Materials and Methods

Mitochondrial DNA Extraction

Mitochondria were isolated from 4-day-old etiolated mung bean seedlings (*Vigna radiata* L. (Wilzed) cv. Tainan

No. 5) by sucrose gradient purification (Dai et al., 1991). After treatment of the purified mitochondria with DNase and protease K, mitochondrial DNA was purified by CsCl-DAPI density gradient (Chiang, 1968).

Cloning of the Mung Bean Mitochondrial tRNA^{Lys} Gene

Mung bean mitochondrial DNA was digested with *EcoRI*, and the fragments were cloned into the *EcoRI* site of the vector pBlueScript[®] II SK(+)(Stratagene). A clone of 3.7 kb, which contained the tRNA^{Lys} gene, was further subfragmented and recloned into pBlueScript[®] II SK(+). A resultant subclone containing a 2.5 kb *PstI-EcoRI* fragment was used for sequencing (Figure 1A) (Sambrook et al., 1989).

DNA Sequencing

The sequence analysis was performed as described by the manufacturer on the A.L.F.[™] DNA sequencer (Pharmacia).

Results and Discussion

We identified a mung bean mitochondrial gene for tRNA^{Lys} (trnK(UUU)) located in the internal 2.5 kb *PstI-EcoRI* fragment of a 3.7 kb *EcoRI* insert (Figure 1A). The coding sequence of 73 nt (Figure 1B) shows 100% homology with the sequences of mitochondrial tRNAs^{Lys} of 3 dicots, common sunflower (Ceci et al., 1996), *Arabidopsis* (Unsold et al., 1997), rapeseed (Handa and Nakajima, 1992), and 2 monocots, maize (Sangare et al., 1989) and wheat (Joyce and Gray, 1989). However, this coding sequence shows only about 70% similarity when compared with its counterparts in chloroplasts (Sugita et al., 1985; Hiratsuka et al., 1989). Sequence analyses, there-

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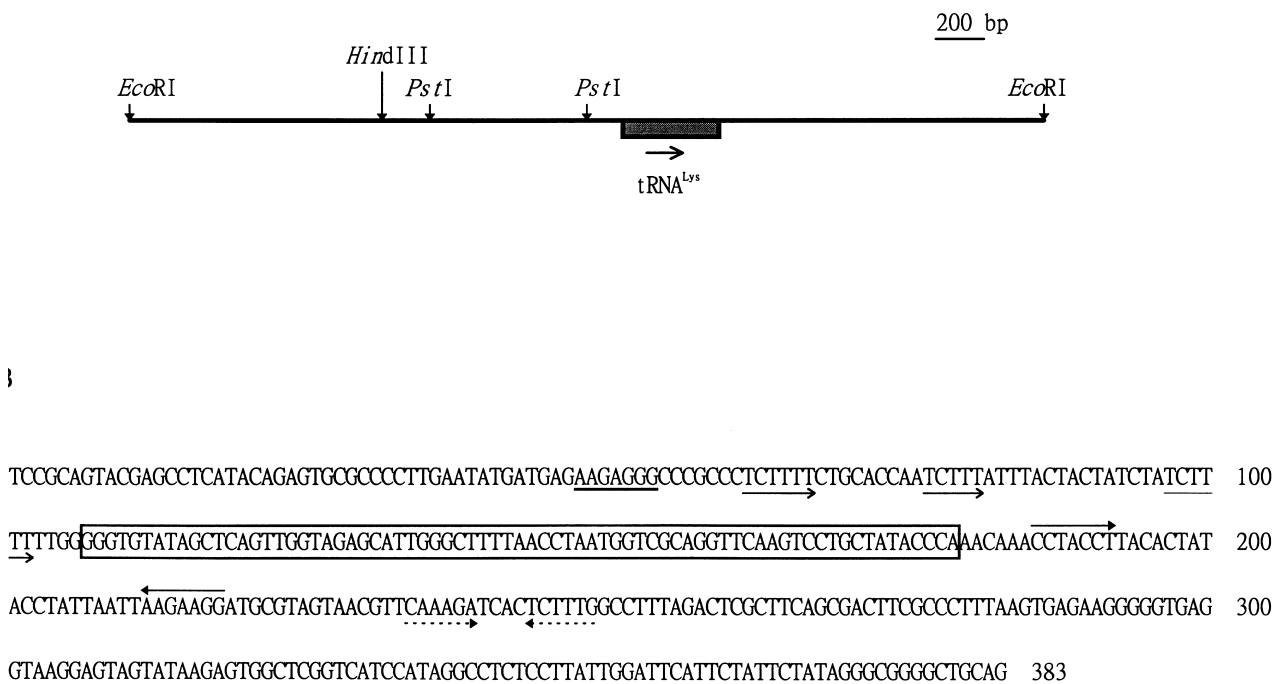


Figure 1. A, Restriction map of the 3.7 kb mung bean *EcoRI* fragment of mitochondrial DNA. The position of tRNA^{Lys} gene is indicated the arrow indicates the transcriptional direction. B, Nucleotide sequence of the mitochondrial tRNA^{Lys} gene of mung bean. The region encoding the tRNA^{Lys} is boxed. The purine-rich sequence, AAGAANRR, is underlined. Direct repeats or inverted repeats are indicated by arrows. The sequence is available from GenBank Caccession number AF013756.

fore, clearly indicate that this 73-nt fragment is a mung bean mitochondrial tRNA^{Lys} belonging to the first group of tRNA genes. The mung bean tRNA^{Lys} gene sequence could be folded into the standard cloverleaf secondary structure with no structural deviation (see the figure in Joyce and Gray, 1989). Like other plant mitochondrial tRNA genes, the mung bean mitochondrial tRNA^{Lys} does not encode the 3'-terminal $-\text{CCA}_{\text{OH}}$ sequence, which must be added post-transcriptionally (Hanic-Joyce and Gray, 1990).

Analysis of the 5' upstream DNA sequence of the mung bean tRNA^{Lys} coding region shows a purine-rich motif, which is similar to the 5'-AAGAANRR-3' sequence found between 70 bp and 130 bp upstream of the wheat tRNA coding regions (Figure 1B). Such a motif has also been identified in yeast mitochondrial promoters (Joyce et al., 1988). However, the 5'-NNRAANNNCRTA-3' promoter motif, which was found immediately upstream from the transcription initiation site of the mitochondrial protein-coding genes, could not be identified within 100 bp upstream of the mung bean tRNA^{Lys} gene, supporting the notion that different transcription machineries are used for different classes of mitochondrial genes (Muisse and Hauswirth, 1992; Rapp and Stern, 1995). Alignment of the 5' upstream sequence of the mitochondrial tRNA^{Lys} cod-

ing region of different species shows a pyrimidine-rich region which is highly conserved among both dicots and monocots (Figure 2). Several short direct repeats could also be identified in this region although it is unknown if these repeats are involved in transcription or processing of tRNA .

When we aligned the 3' downstream from the mung bean tRNA^{Lys} coding sequence with other species, we found the region is only conserved among dicot plants (Figure 3). Two pairs of possible inverted repeats are present at the 3' end of the mung bean tRNA^{Lys} coding region, which could form a stem-loop structure within the conserved region (Figures 1B and 3). Although the importance of these stem-loops is not clear, these structures have been implicated in affecting transcription termination and RNA processing of mitochondrial genes (Schuster et al., 1986; Gray et al., 1992).

In summary, we have identified a mung bean native mitochondrial tRNA^{Lys} gene. Several highly conserved regions were identified in regions upstream and downstream from the tRNA^{Lys} coding region among different plant species, both dicots and monocots. Further investigation using an in vitro transcription and processing system would be necessary to unravel the functions of these motifs.

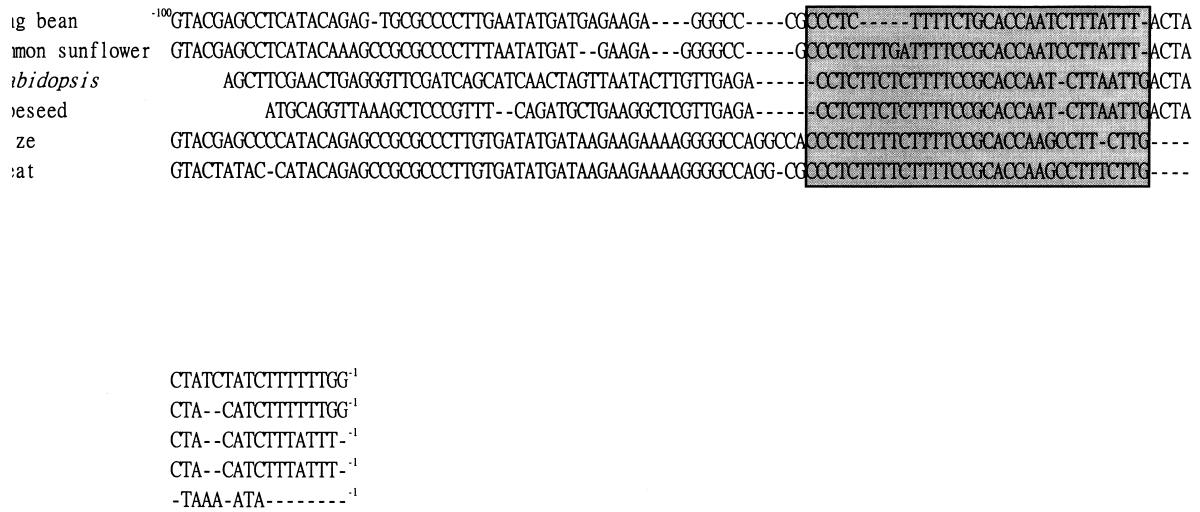


Figure 2. Alignment of the 5' upstream sequence of the mung bean mitochondrial tRNA^{Lys} gene with the mitochondrial tRNA^{Lys} genes of other species. The conserved pyrimidine-rich region is shaded. Numbers refer to nucleotides, starting with -1 at the first nucleotide just 5' from the coding region. Dashes represent gaps introduced for alignment of the respective sequence.

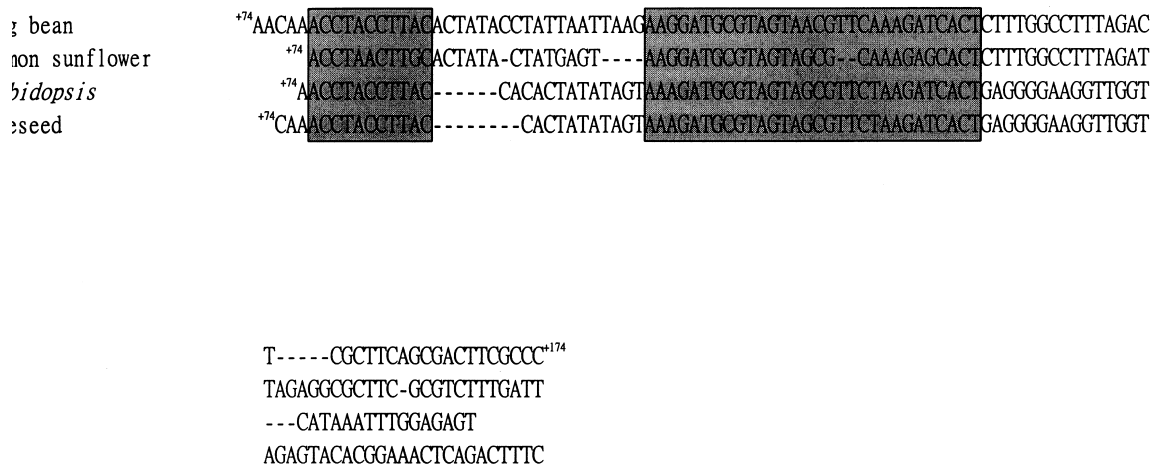


Figure 3. Alignment of the 3' downstream sequence of the mung bean mitochondrial tRNA^{Lys} gene with mitochondrial tRNA^{Lys} genes of other species. The conserved region among 3 dicots is shaded. Numbers refer to nucleotides, starting with 74 at the first nucleotide just 3' from the coding region. Dashes represent gaps introduced for alignment of the respective sequence.

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綠豆粒線體賴氨酸一轉移核糖核酸基因之序列

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在本研究中，我們將綠豆粒線體之賴氨酸一轉移核糖核酸 (lysyl-tRNA) 基因及其 5' 與 3' 上下游之 DNA 定序，並將所得到之 DNA 序列與其他已知之高等植物粒線體 lysyl-tRNA 基因相互比較。我們亦分析此基因上下游所含可能之基因調控片段。DNA 序列顯示綠豆粒線體 lysyl-tRNA 基因與位於葉綠體內之 lysyl-tRNA 基因有明顯之不同。

關鍵詞：賴氨酸一轉移核糖核酸；粒線體；綠豆。