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Short communication

New intron-containing human tRNA^{Leu} genes*

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Three new human nuclear tRNA^{Leu} genes have been isolated and sequenced using the PCR technique. Two of them represent genes containing a CAA anticodon and both contain introns of 22 nucleotides in length but differing in sequence. Intron-containing prolongated anticodon stems can be folded into a secondary structure similar to that of yeast pre-tRNA^{Leu}. The evolutionary conserved secondary structure suggests the same role of intron sequences in the human and yeast pre-tRNA^{Leu} maturation pathway.

Maturation of eukaryotic cytoplasmic tRNA is a complex multistage process. It includes processing of 5′ and 3′ ends of pre-tRNA transcripts, splicing in the case of intron-containing tRNAs and modifications of numerous nucleosides [1]. Depending on the organism, the number of intron containing nuclear tRNA genes varies strongly. In the case of yeast there are ten families of different isoacceptor genes, containing introns [2, 3] while in plants and vertebrates only three families of intron-containing tRNA genes are known. Among them are the genes coding for tRNA^{Tyr} (GΨA) and tRNA^{Leu} (m^{5}CAA) [4].

Several studies carried out on yeast have demonstrated that pseudouridine in the middle position of tRNA^{Tyr} and 5-methyl-cytosine in the first position of the anticodon of tRNA^{Leu} are introduced into tRNA as late as at the stage of intron-containing precursors. The constructs of these tRNA genes deprived of the introns create transcripts that do not undergo modification at the positions mentioned above either *in vitro*, nor *in vivo* [5, 6]. The observation of intron-dependent conversion of U_{35} to Ψ_{35} in yeast was later confirmed for pre-tRNA^{Tyr} from man, *Xenopus laevis, Drosophila melanogaster and Arabidopsis thaliana* [4]. However, there are no reports showing intron-dependent introduction of 5-methyl cytosine in the case of pre-tRNA^{Leu} in higher vertebrates. Since the presence of m^{5}C in the first position of the tRNA^{Leu}CAA anticodon is crucial for the proper mRNA decoding process in yeast [6], we decided to isolate human tRNA^{Leu} genes and investigate whether m^{5}C is introduced at specific steps into pre-tRNA^{Leu} during its matura-

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tion.Here we report the sequences of three new tRNA^{Leu} genes, two of which contain anticodon CAA and introns.

MATERIALS AND METHODS

DNA was isolated from human blood using the procedure described by Chomczyński & Sacchi [7]. The PCR reaction was carried out in a mixture, containing in a volume of 50 µl 10 mM Tris/HCl, pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 200 mM each of dNTP, 1.0 µM each of 5′- and 3′-tRNA^{Leu} gene primers, 5.0 µg of whole human DNA and 0.5 U PrimeZyme DNA polymerase. The first step was 5 min denaturation at 94°C, followed by 25 cycles of 1 min at 94°C,1 min at 50°C and 1 min at 72°C. The first primer (45-mer) contained the *Eco*RI restriction site and the T7 RNA polymerase promoter sequence followed by the first 20 nucleotides of the tRNA^{Leu} gene from the 5′ end (GGAATTCCTAATACGACTCACTATAGTCAGGATGGCCGAGTGGTC). The second oligonucleotide (33-mer) comprised 22 nucleotides of the tRNA^{Leu} gene from the 3′ end (GCGGATCCTGGTGTCAGAAGTGGGATTCGAACC), *Bst*NI and *Bam*HI restriction sites. The products of amplification were purified by gel electrophoresis, digested with *Eco*RI and *Bam*HI and cloned into pUC 19 vector digested with the same enzymes. Clones containing the tRNA^{Leu} gene were identified by DNA sequencing using Pharmacia Sequencing Kit. A search of the NCBI GeneBank sequence database for human tRNA^{Leu} was carried out using the BLAST program.

RESULTS AND DISCUSSION

Sequencing of cloned PCR products revealed the presence of four different tRNA^{Leu} isoacceptor genes. Two of them (anticodon CAG and UAG) do not contain introns. Search of NCBI database using the BLAST program showed that an identical tRNA^{Leu} gene with anticodon UAG has been already identified. The other one, tRNA^{Leu} containing anticodon CAG, is not listed in NCBI GenBank Database and we presume it is the first human tRNA^{Leu} gene coding for CAG tRNA isoacceptor isolated so far. Figure 1 presents the cloverleaf structure of a putative product of the sequenced tRNA^{Leu}CAG gene. Probably, its origin is nuclear because there is no resemblance between this gene and two known human mitochondrial tRNA^{Leu} genes [8]. Two other isolated tRNA^{Leu} genes contain anticodon CAA and, as expected, introns. Two introns are in the same canonical position as the other known introns in nuclear tRNA genes. Both introns are 22 nucleotide long, compared with 22–24 nucleotide long introns of the earlier isolated human tRNA CAA isoacceptor genes [9] and 32 nucleotides for a similar yeast tRNA gene [6]. Figure 2 presents the cloverleaf structures of both intron-containing tRNA^{Leu}CAA precursors. In comparison to the earlier isolated five human intron-containing tRNA^{Leu}CAA genes, the two genes presented in this paper show a different nucleotide sequence in the intronic domain. Additionally one of them (pHLIVS1) shows a new nucleotide substitution in the loop of variable arm, not observed in the other six tRNA^{Leu}CAA genes. The prolongated anticodon stems of two newly isolated tRNA^{Leu} genes can be folded into secondary structures similar to those described for five human tRNA^{Leu}CAA genes [9]. Moreover, the putative secondary structure of the yeast tRNA^{Leu}CAA prolongated anticodon stem exhibits similar features. In all cases folding shows the presence

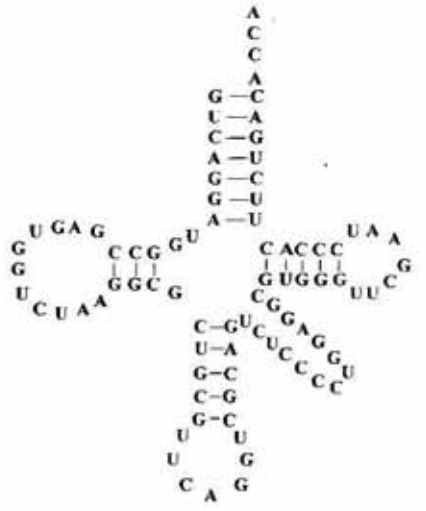

Figure 1. The cloverleaf structure of tRNA^{Leu} with CAG anticodon, based on sequencing of PCR amplified human tRNA^{Leu} gene.

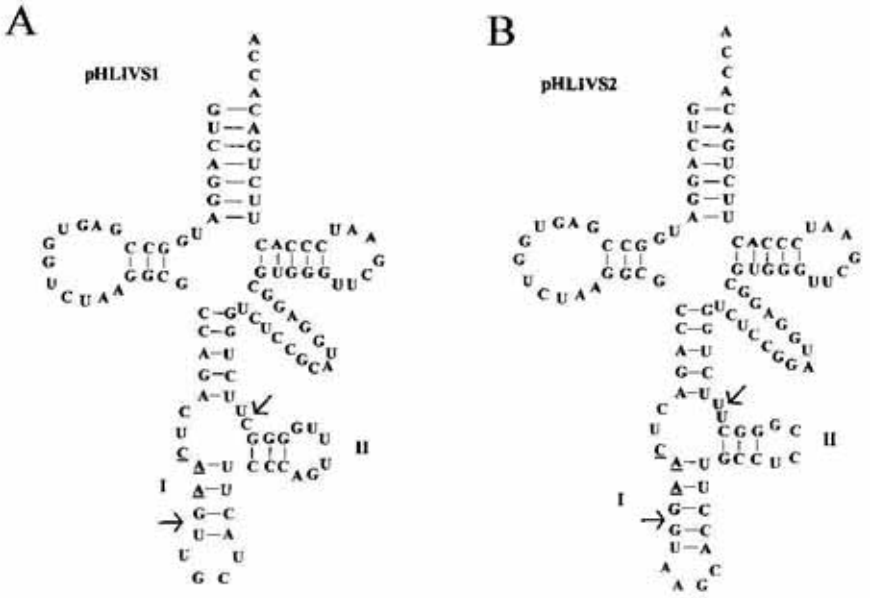

Figure 2. Cloverleaf structure of tRNA^{Leu}CAA precursors containing introns.

A, The sequence of PCR amplified pHLIVS1 gene; B, pHLIVS2 gene. Arrows point to the splicing sites, underlined letters specify anticodon sequence. I and II represent arms of the prolongated anticodon.

of two arms (depicted in Fig. 2 as I and II). The largest variation in nucleotide sequence is observed in the loop regions, while any substitution in the stem region results in the presence of a compensatory substitution restoring base-pairing. Table 1 presents a comparison of seven human intron sequences and of one sequence from yeast. Conserved regions represent stem regions of arms I and II.

ture in pre-tRNA maturation. It is important to note that the structure exists only transiently, before intron excision at the nuclear envelope. We suppose that this structure, necessary for the enzymatic introduction of m^5C into the first position of the anticodon of yeast pre-tRNA^{Leu}CAA, may play the same role in the case of human tRNA^{Leu} precursors. Introns that allow modification of specific nucleosides in tRNA could be compared

Table 1. The comparison of intron sequences of pre-tRNA^{Leu} pHLIVS1 and pHLIVS2 with already known sequences of human pre-tRNA^{Leu} (A–E) and yeast.

Bolded letters represent base paired nucleotides of the II stem in the prolongated anticodon secondary structure while underlined letters represent conserved nucleotides in the I stem that base-pair with the nucleotides of the anticodon stem that are not a part of the intronic sequence (see Fig. 2).

pHLIVS1	5'-	U -	- U	GC	UA	CU	U -	- C	CC	AG	--	--	--	UU	UG	GG	GC	
pHLIVS2	5'-	GU	AA	GC	AC	CU	U -	- G	CC	UC	C -	--	--	--	- G	GG	CU	
A	5'-	GU	AA	GC	AC	CU	U -	- G	CC	UG	C -	--	--	--	- G	GG	CU	
B	5'-	C -	--	UU	GG	CU	U -	- C	CU	CG	U -	--	--	GU	UG	AG	GA	
C	5'-	C -	--	UA	AG	CU	U -	- C	CU	CC	G -	--	- C	GG	UG	GG	GA	
D	5'-	C -	UU	AC	UG	CU	U -	- C	CU	GU	G -	--	--	UU	CG	GG	UC	
E	5'-	C -	UU	AC	UG	CU	U -	- C	CU	GU	G -	--	--	UU	CG	GG	UC	
yeast	5'-	G -	AA	AU	AU	CU	UG	AC	CG	CA	GU	UA	AC	UG	UG	GG	AA	UA

The striking similarity of the secondary structure of the prolongated anticodon stems containing introns in pre-tRNAs^{Leu} from yeast and man suggests a role for this structure to *cis*-guiding elements that define RNA editing sites and *trans*-guiding elements directing specific nucleoside modifications.

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