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TWO INTRON-CONTAINING pre-tRNAs^{Tyr} FROM *TRITICUM AESTIVUM* ARE EFFICIENTLY PROCESSED AND SPLICED IN HOMOLOGOUS CELL-FREE EXTRACT*

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Two wheat pre-tRNAs^{Tyr} containing introns and flanks are accurately and efficiently spliced in homologous wheat germ S23 extract. The initiation and termination sites upon *in vitro* transcription in HeLa cell extract have been estimated for both pre-tRNAs.

So far only a few nuclear plant tRNA genes have been sequenced and the knowledge about their expression is still sparse. The best known family of plant nuclear-encoded tRNAs is that for tyrosine tRNAs. All nuclear tRNA^{Tyr} genes, so far known, contain an intron [1]. Plant tRNA^{Tyr} genes have been isolated and characterized from *Arabidopsis thaliana* [2], *Nicotiana rustica* [3] and *Triticum aestivum* [4, 5]. In the case of *A. thaliana* it has been found that the tRNA^{Tyr} genes (containing 12 bp long introns of high homology) are arranged in 1.5 kb unit tandem arrays, each unit containing a tRNA^{Ser} gene flanked by two tRNA^{Tyr} genes [2]. Moreover,

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thirteen isolated and sequenced tRNA^{Tyr} genes were found to be efficiently transcribed in HeLa cell nuclear extract and most of them were processed and spliced, both in HeLa nuclear extract and in wheat germ extract [6]. In the case of *N. rustica*, twelve tRNA^{Tyr} genes were isolated ([3], T. Fuchs, personal communication). All of them contain introns of different sequence and length. The *N. rustica* tRNA^{Tyr} gene family shows dispersed organization in the genome (T. Fuchs, personal communication). All tobacco tRNA^{Tyr} genes isolated so far were transcribed in nuclear HeLa extract. All but one were processed and spliced in wheat germ extract (T. Fuchs, personal communication).

The organization of wheat nuclear tRNA^{Tyr} genes is dispersed, similarly as in tobacco (H. Beier, personal communication). Three members of the wheat tRNA^{Tyr} genes family have been isolated and sequenced [4, 5]. In this work the transcriptional activity of two nuclear tRNA^{Tyr} genes from *T. aestivum* var *Cheyenne* was studied in HeLa cell extract and the maturation reactions of pre-tRNAs was examined in HeLa and in wheat germ extracts.

MATERIALS AND METHODS

Plasmid pATtY3II carrying an *A. thaliana* nuclear tRNA^{Tyr} gene was a gift of H. Beier (Würzburg, F.R.G.).

HeLa nuclear extracts were prepared according to Dignam *et al.* [7]. Transcription assays and the elution of tRNA precursors from preparative gels were performed as described by Stange & Beier [8].

Cell-free wheat germ S23 extract was prepared from wheat embryos as described by Stange & Beier [8]. *In vitro* processing of tRNA^{Tyr} precursors was performed in the incubation mixture containing in a total volume of 90 μ l: 18 μ l S23 extract (30 mg protein/ml), 20 mM Tris/HCl, pH 7.4, 100 mM KOOCH₃, 6 mM Mg(OOCH₃)₂, 80 μ M spermine, 10 mM creatine phosphate, 0.4% Triton X-100, 1 mM ATP, 0.1 mM CTP and 5×10^3 c.p.m. of pre-tRNA labelled during the transcription assays in HeLa nuclear extract with α [³²P]GTP.

Primer-extension analyses were performed as described by Dingermann & Nerke [9].

RESULTS

Two intron-containing nuclear tRNA^{Tyr} genes (pTtY1 and pTtY2) located on a 5 kb *SalI* restriction fragment were cloned from an EMBL-3 genomic library of *T. aestivum* var. *Cheyenne* DNA and sequenced as described earlier [4].

The wheat tRNA^{Tyr} genes were transcribed in HeLa nuclear extract. Mature tRNA is generated *via* an intron-containing precursor with processed flanks (Fig. 1).

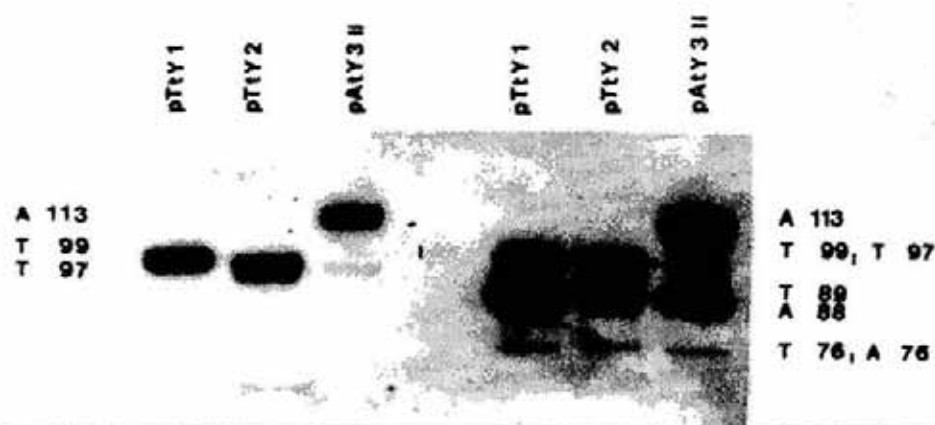


Fig. 1. *In vitro* transcription, processing and splicing of pTtY1, pTtY2 and pAtY3II in HeLa nuclear extract. The left panel represents transcription reactions in the presence of 1 mM MgCl₂. Processing and splicing reactions, in the presence of 1 mM MgCl₂, do not occur [6]. T 99, T 97 and A 113 derived from pTtY1, pTtY2 and pAtY3II, respectively, represent full intron-containing transcripts with 5' and 3' flanks. The right panel represents transcription reactions in the presence of 4 mM MgCl₂. Processing and splicing reactions are performed. T 99, T 97 and A 113 represent transcripts with 5' and 3' flanks and introns, T 89 and A 88, represent pre-tRNAs^{Tyr} containing introns with mature ends and T 76, A 76 represent mature tRNAs^{Tyr} of pTtY1, pTtY2 and pAtY3II, respectively

Transcription initiation sites were determined by primer-extension analysis [9] of pre-tRNA using a 5'-³²P-labelled oligodeoxynucleotide complementary to positions 6 to 25 of mature tRNA^{Tyr}. Major transcription initiation sites occur at positions -5 and -4 for pTtY1 and at positions -6 and -3 for pTtY2. Fingerprint analyses of the primary transcripts revealed 8 nucleotides and 5 nucleotides long trailer sequences for pre-tRNAs T 1 and T 2, respectively. In both cases transcription termination occurred at a stretch of 4 (pTtY1) and 8 (pTtY2), respectively, thymidines in accordance with the consensus termination sequence of RNA polymerase III [10]. Figure 2 shows the proposed secondary structures of pre-tRNAs^{Tyr} T 1 and T 2, which form an extended anticodon stem of 5 base pairs. The intron-anticodon interaction of plant pre-tRNAs^{Tyr} from *Nicotiana* and *Arabidopsis* includes 4 base pairs [3, 6].

The maturation of the intron-containing pre-tRNAs synthesized in HeLa nuclear extract was also studied in the homologous wheat germ S23 extract.

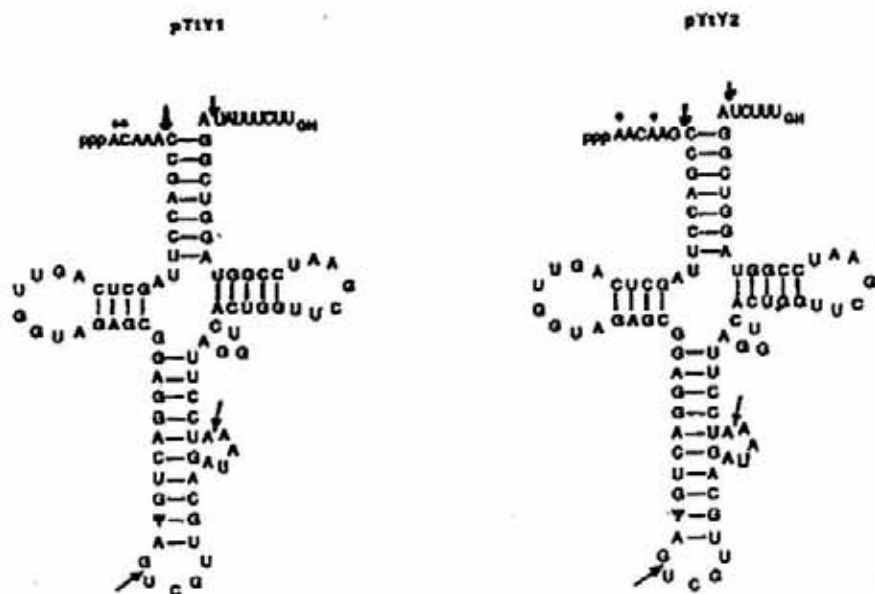


Fig. 2. Secondary structures of the pre-tRNAs^{Tyr} derived from pTtY1 and pTtY2. Major transcription initiation sites are dotted and the splice sites and sites of cleavage by 5'- and 3'- processing enzymes are marked by arrows

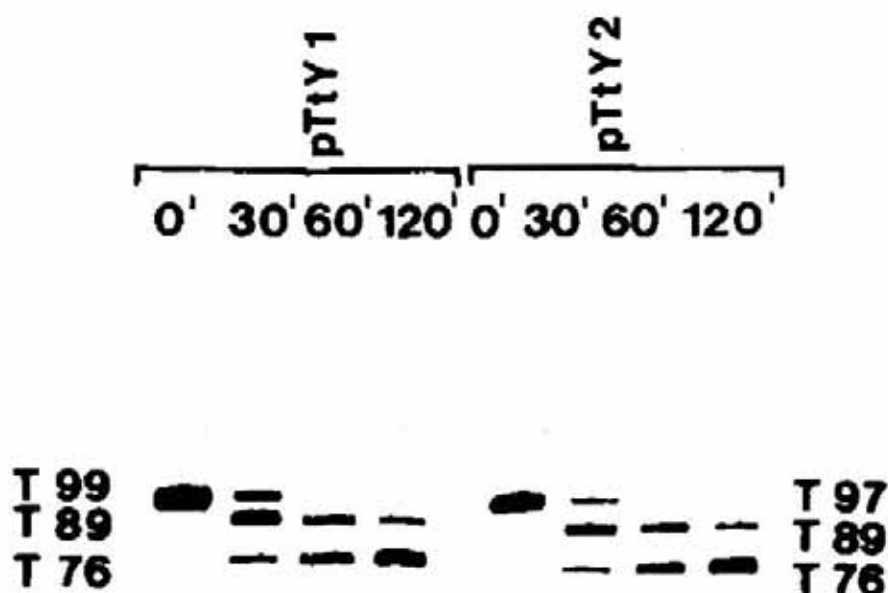


Fig. 3. *In vitro* processing and splicing of *Triticum* pre-tRNAs^{Tyr} in wheat germ S23 extract. *Triticum* tRNA^{Tyr} genes were transcribed in HeLa cell nuclear extract. The major primary transcripts (i.e. T 99 and T 97), containing flanking sequences and the intron, were recovered from a preparative gel and were used for studying their processing and splicing in wheat germ S23 extract. Aliquots were removed after 0, 30, 60 and 120 min of incubation at 30°C and analyzed on a 10% polyacrylamide/8 M urea gel. The major intermediate products are T 89, the intron-containing pre-tRNAs with mature ends. T 76 identifies mature tRNA

Both pre-tRNAs were efficiently processed and spliced to yield about 80% of mature tRNA (Fig. 3). The course of the maturation events as well as the yield of mature tRNA was the same as described for the *in vitro* maturation of *Nicotiana* and *Arabidopsis* pre-tRNAs^{Tyr} in wheat germ extract [6, 8]. The reaction products, i.e. the intron-containing precursor with mature ends T 89 and the mature tRNA T 76, were further characterized by fingerprint analysis (not shown) and revealed full accordance with the gene sequences.

DISCUSSION

The results presented here clearly show that the two wheat nuclear tRNA^{Tyr} genes are transcriptionally active in HeLa nuclear extract.

Nicotiana and *Arabidopsis* pre-tRNAs^{Tyr} obtained in HeLa extract were efficiently processed and spliced in wheat germ S23 extract [4, 6]. It is shown here that, as expected, homologous pre-tRNAs^{Tyr} from wheat underwent processing and splicing reactions in S23 wheat germ extract as efficiently as tobacco and *Arabidopsis* tyrosine tRNA precursors indicating that the two tRNA^{Tyr} genes pTtY1 and pTtY2 should be efficiently expressed *in vivo*. It is worth noting, that wheat germ splicing endonuclease does not recognize yeast, human and *Xenopus laevis* pre-tRNAs^{Tyr} as substrates and seems to be highly specific for plant intron-containing pre-tRNAs [11].

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