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THE INITIATOR TRANSFER RIBONUCLEIC ACID FROM YELLOW LUPIN SEEDS, CORRECTION OF NUCLEOTIDE SEQUENCE AND CRYSTALLIZATION

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Key Word Index—*Lupinus luteus*; Leguminosae; tRNA; methionine specific; initiator; nucleotide sequence; crystallization.

Abstract—Two methionine specific tRNAs from yellow lupin seeds have been purified to homogeneity. Initiator tRNA ($tRNA_i^{Met}$) but not $tRNA_m^{Met}$ was charged with *Escherichia coli* methionyl-tRNA synthetase. The nucleotide composition, T_1 and pancreatic RNase digestion fingerprints and nucleotide sequence of lupin $tRNA_i^{Met}$ showed its identity with wheat germ and bean initiator tRNAs. The differences in the primary structure of the lupin $tRNA_i^{Met}$ observed by other authors have not been confirmed. We have defined the conditions under which single crystals of lupin $tRNA_i^{Met}$ can be grown reproducibly.

INTRODUCTION

The most studied higher plant tRNA is phenyalanine-specific tRNA. The sequence of the $tRNA^{Phe}$ isolated from wheat germ [1], pea [2], yellow lupin seeds [3] and barley [4] has been elucidated and shown to be conserved among higher plants [5–7]. On the basis of published data, we had proposed that the structures of plant tRNAs and aminoacyl-tRNA synthetases are all highly conserved [7]. Therefore it was of interest to see if other tRNAs, initiator tRNA in particular, which has a well defined function, do obey the same rules. Recently the complete primary structure of wheat germ and bean initiator tRNAs has been determined [8, 9]. These tRNAs are missing the TΨCG sequence which is common to other eukaryotic cytoplasmic initiator tRNAs and contain a Watson–Crick base pair in the first position of the amino acid arm. Our preliminary results showed that the lupin $tRNA_i^{Met}$ primary sequence is identical with other plant initiator tRNAs [7]. Recently we have started crystallization experiments with initiator tRNA from lupin seeds. It differs in 21 positions from yeast initiator tRNA, the crystal structure of which has already been solved [10]. Apparently these differences have no effect on amino acid charging activity by methionyl-tRNA synthetase. The crystal structure of plant initiator tRNA when compared with yeast tRNA, could therefore identify the structural elements which are common and critical for enzyme recognition.

RESULTS AND DISCUSSION

The crystal structure of only a few tRNAs have been reported [10], and there is no structural data which can explain the functional difference between initiator tRNA and elongator tRNA as in the case of $tRNA^{Phe}$ and $tRNA^{Asp}$. We decided to look at the primary and tertiary structure of initiator $tRNA_i^{Met}$ from plants. We chose lupin $tRNA^{Met}$ for this study, and because there has been some contradiction as to its primary structure, we re-examined the structure of initiator tRNA of yellow lupin seeds.

Two major lupin methionine-accepting tRNAs were separated on benzoylated DEAE-cellulose. Both were further purified to homogeneity by DEAE-Sephadex A-50 and Sepharose 4B column chromatography (Fig. 1). [11]. Additionally, a small BD-cellulose column was used to concentrate and desalt the tRNA after the Sepharose 4B column. The specific activities of the two tRNAs were higher than 1500 pmol per A_{260}. To establish which methionine tRNA is the initiator one, both were charged with crude methionyl-tRNA synthetase from *Escherichia coli*. It is well known that the bacterial synthetase can charge only the eukaryotic initiator tRNA [9, 12, 13]. Only plant $tRNA_1^{Met}$ in contrast to $tRNA_2^{Met}$, is charged with bacterial enzyme (Fig. 2). Similar results have been obtained for wheat germ methionine tRNAs [12 and unpublished work]. Therefore we suggest that lupin $tRNA_{i(1)}^{Met}$ represents the initiator tRNA species.

Preliminary results obtained from nucleotide composition analysis, DEAE-cellulose column chromatography of T_1 digests and high-voltage electrophoresis of T_1 and pancreatic RNases fingerprints of both lupin and wheat germ initiator tRNAs (data not shown) strongly suggested identity of their primary structures. This was finally confirmed by sequencing of the entire molecule of $tRNA_i^{Met}$ from lupin seeds (Fig. 3). Adenosyl-2'-O-adenosine which has been found recently in position 64 of yeast $tRNA_i^{Met}$ (G. Keith, personal communication) was not present in lupin $tRNA_i^{Met}$. The nucleotide sequence of the lupin $tRNA_i^{Met}$ fragment (nucleotides 18–29) is shown in Fig. 4. Homochromatography of this fragment revealed that the sequence reads GGAAGCGUm^{2}GGUG but not GGAAGCGCm$_2^2$GGUG as was proposed by other authors (see compilation of tRNA [6]). Mobility shifts caused by C-25 are almost opposite to that of U and indeed in this case cannot be misinterpreted. Additionally, we have not found a m$_2^2$G nucleotide in our

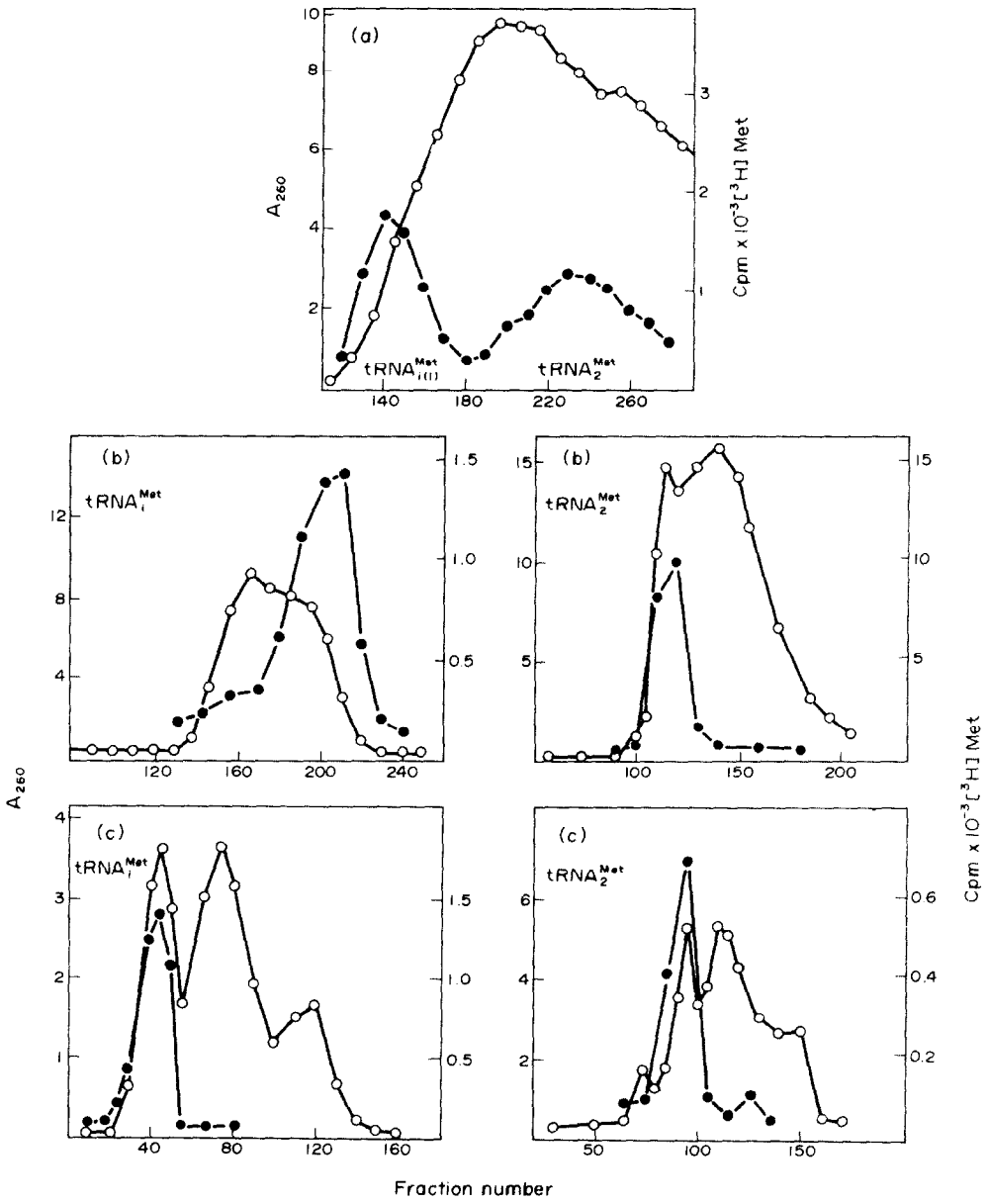

Fig. 1A. Benzoylated DEAE cellulose column chromatography of crude yellow lupin seed tRNA. The column (3.7 × 80 cm) was equilibrated with 10 l of 0.01 M sodium acetate buffer, pH 4.5, containing 0.01 M $MgCl_2$ and 0.35 M NaCl. 60 000 A_{260} of crude tRNA were applied to the column. Elution was carried out with 10 l of linear gradient 0.35–1.0 M NaCl in the same buffer. The fractions (each 20 ml) were collected at a flow rate of 1 ml/min. 10 μl aliquots were assayed for methionine acceptor activity. ○—○, A_{260}; ●—●, methionine acceptor activity. B. Purification of $tRNA^{Met}$ on DEAE-Sephadex A-50 column (1.5 × 100 cm). After equilibration with 0.05 M Tris–HCl, pH 7.5, containing 0.008 M $MgCl_2$ and 0.4 M NaCl, 5000 A_{260} of $tRNA^{Met}$ fraction from BD-cellulose were applied to the column and eluted with a 3 l linear gradient containing 0.05 M Tris–HCl, pH 7.5, 0.016 M $MgCl_2$ and 0.475 M NaCl. 5 ml fractions were collected at a flow rate of 0.5 ml/min. 10 μl aliquots were assayed. ○—○ and ●—●, as Fig. 1A, C. Chromatography of $tRNA^{Met}$ on Sepharose 4B column in a reverse gradient of ammonium sulphate. The column (1.5 × 30 cm) was equilibrated at room temperature with 0.01 M sodium acetate, pH 4.5, containing 0.01 M $MgCl_2$ and 2 M ammonium sulphate. 1000 A_{260} of purified $tRNA^{Met}$ were applied to the column. tRNA was eluted with a linear gradient of 980 ml of 2 M and 1000 ml of 1 M ammonium sulphate in the same buffer. 5.5 ml fractions were collected at a flow rate of 22 ml/hr. 5 μl aliquots were assayed. ○—○ and ●—●, as Fig. 1A.

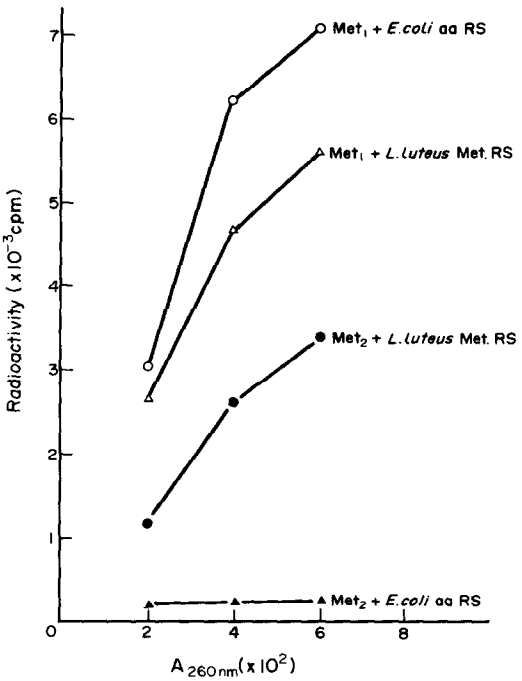

Fig. 2. The acceptor activity of the two lupin $tRNA_s^{Met}$ in the reaction with cognate and non-cognate *E. coli* methionyl-tRNA synthetases. Initiator $tRNA^{Met}$ (Met_1) is methionylated by either lupin (△—△) or *E. coli* (○—○) enzyme. Elongator $tRNA^{Met}$ (Met_2) interacts with cognate enzyme (●—●—●) but not with *E. coli* enzyme (▲—▲). The assay conditions were identical to that in ref. [14]. Concentration of *E. coli* and lupin methionyl-tRNA synthetase were 0.3 and 0.4 mg/ml respectively. Concentration of tRNA is expressed in A_{260} units. One A_{260} unit is amount of material which dissolved in 1 ml of buffer or water gives an absorbance of 1 in a 1 cm cell.

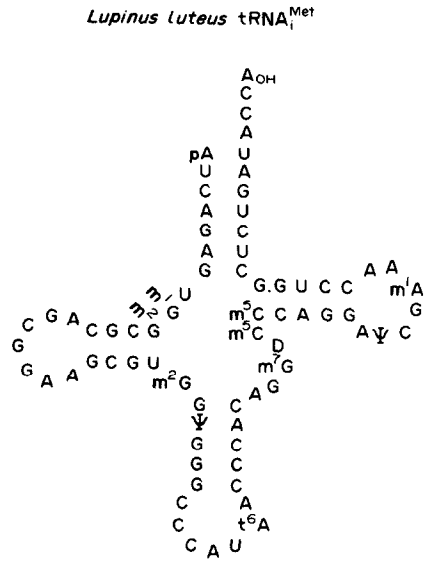

Fig. 3. Primary structure of lupin initiator tRNA. .G is 2′-*O*-methylated guanosine.

nucleotide analysis (data not shown). On the basis of these data, we conclude that the primary structure of lupin initiator tRNA is identical to wheat germ [8] and bean [9] $tRNA_i^{Met}$. Therefore, the methionine initiator tRNA is a conserved structure among higher plants, as we suggested earlier [7]. The sequence of lupin initiator tRNA shows interesting properties common to other eukaryotic initiator tRNAs. Thus, like mammalian initiator tRNA it contains seven base pairs in the amino acid stem, three G-C base pairs in the anticodon stem followed by 3 C in the anticodon loop and has A instead of T in the common TΨCG sequence (Fig. 3). However, lower plant initiator tRNA of green alga showed only 87% homology with higher plant cytoplasmic initiator tRNA [15]. Pure methionine initiator tRNA was crystallized under conditions similar to those used for yeast $tRNA_i^{Met}$ [10]. Large, well shaped single crystals (Fig. 5) grow as hexagonal bipyramids from single nucleation sites after seven to nine days, reaching the final size 0.5–1.0 × 0.4 mm after three to four weeks. Morphologically they are very similar to the crystals of $tRNA_i^{Met}$ from yeast [10]. Detailed crystallographic analysis of the lupin $tRNA_i^{Met}$ crystals grown at room temperature is now in progress.

EXPERIMENTAL

Crude tRNA from lupin seeds was prepared as described previously [16]. Unfractionated tRNA from wheat germ was obtained by the same method. Methionine specific tRNA was purified on benzoylated-DEAE cellulose (Schwarz-Mann), DEAE-Sephadex A-50 and Sepharose 4B (both from Pharmacia). *Escherichia coli* methionyl-tRNA synthetase was prepared according to a procedure published earlier [17]. Methionyl-tRNA synthetase from lupin seeds and aminoacylation conditions were the same as described previously [14]. Enzymes for digestion of tRNA, post-labelling with [γ-^{32}P]ATP and T4 kinase, fingerprinting techniques and sequence analysis were the same as described previously [18, 19]. Digestion of tRNA to mononucleotides was done according to Rogg *et al.* [20]. 2D-TLC was performed with *n*-BuOH–H_2O (1st dimension) and *i*-butyric acid–NH_3–H_2O (2nd dimension) [21]. Crystallization was done according to A. Joachimiak *et al.* [22]. Prior to crystallization pure $tRNA_i^{Met}$ was renatured in the presence of $MgCl_2$ and concentrated in a Centricon PM10 (Amicon). Crystals were grown at room temp. by vapour diffusion using the 'hanging drop' method [22]. 15–30 μl droplets were equilibrated against a 1 ml reservoir. Optimally the droplets contained tRNA at 2.6 mg/ml, $MgCl_2$ and spermine, Na cacodylate buffer, pH 6.4, and 20% $(NH_4)_2SO_4$. The reservoir was composed of 55% $(NH_4)_2SO_4$ and cacodylate buffer, pH 6.4.

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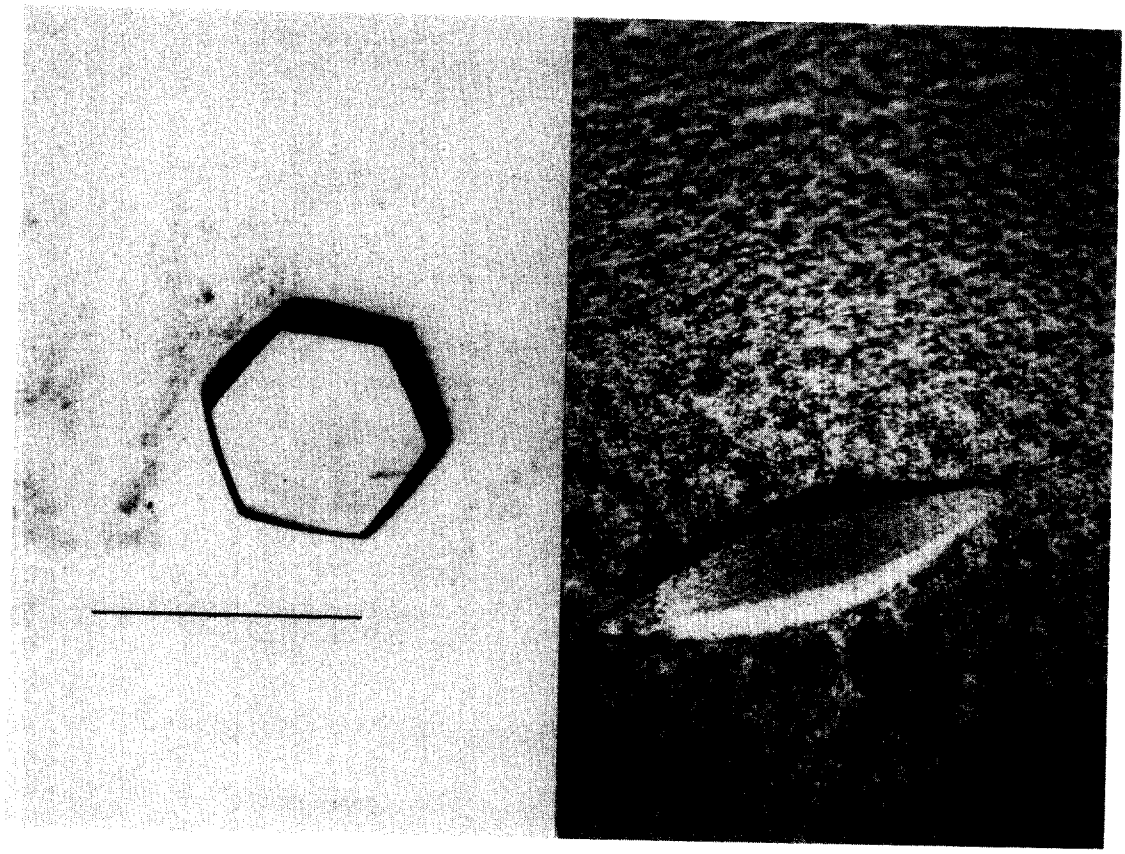

Fig. 5. Crystals of *L. luteus* initiator $tRNA^{Met}$ obtained as described in Experimental. Bar is 0.5 mm.

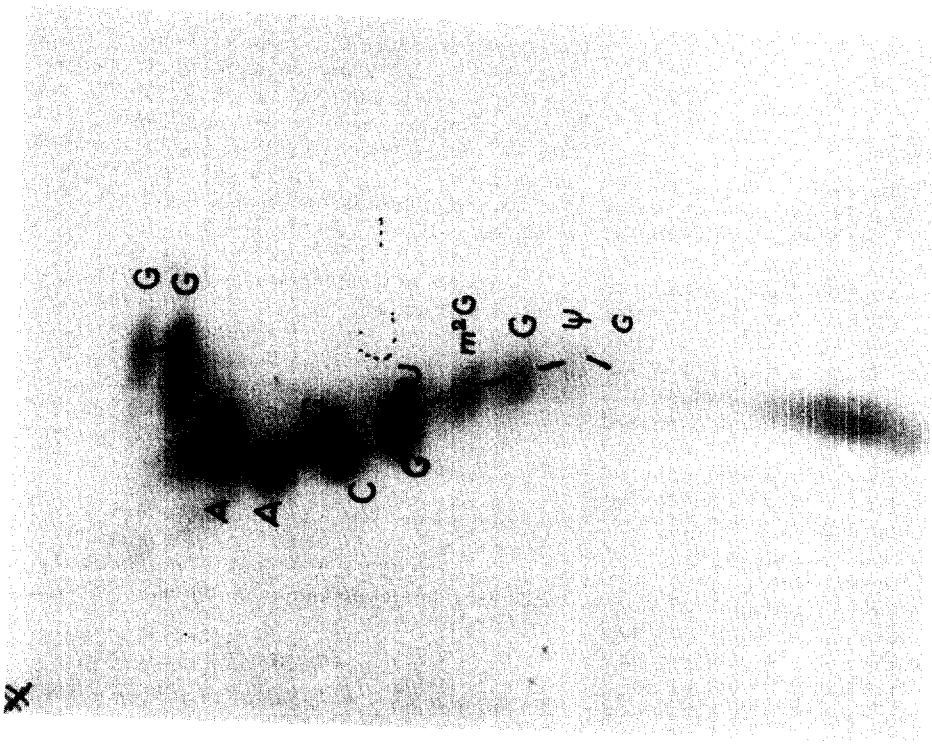

Fig. 4. Sequence analysis of lupin $tRNA_i^{Met}$ fragments on DEAE cellulose plates. The hydrolysate (5 min at 80° in formamide) was labelled with [γ-^{32}P] ATP and T4 kinase and separated in the first dimension on a 15% polyacrylamide gel at pH 3.5 and in the second on a 12% and an 18% gel, pH 8.3. Bands were eluted, hydrolysed with water and separated by homochromatography. Analysis was carried out as in ref. [19].

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