CODON RECOGNITION PATTERN OF *PHASEOLUS VULGARIS* CYTOPLASMIC AND CHLOROPLASTIC tRNAs

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1. Introduction

Chloroplast-specific and mitochondria-specific tRNAs have been characterized in *Phaseolus vulgaris* [1–4] and in other plants (for a review see [5]). Bean chloroplast-specific tRNAs are coded for by chloroplast DNA [6] and are specifically recognized by the chloroplastic or bacterial aminoacyl-tRNA synthetase [2–4], but there is up to now no obvious explanation for the existence (or conservation) of organellar tRNAs different from their cytoplasmic counterparts. In order to see whether chloroplast-specific tRNAs can translate codewords different from those recognized by their cytoplasmic counterparts, the codon recognition pattern of cytoplasmic and chloroplast-specific $tRNAs^{Phe}$, $tRNAs^{Lys}$ and $tRNAs^{Leu}$ has been studied.

2. Materials and methods

Transfer RNAs were extracted from green leaves as already described [1], and fractionated either by reverse-phase chromatography (RPC-5) to separate the isoaccepting $tRNAs^{Leu}$ [2] and $tRNAs^{Lys}$ [4], or by BD-cellulose chromatography to separate the isoaccepting $tRNAs^{Phe}$ [7]. The iso-acceptors were detected by measuring the corresponding amino acid accepting activity in the fractions and isolated either by precipitation with ethanol or by lyophilization.

Aminoacylation of the tRNAs using high specific activity [^{3}H]phenylalanine, lysine or leucine (about 30 Ci/mmol), was performed as previously described [1] using either cytoplasmic enzymes (prepared from bean hypocotyls) to charge the cytoplasmic isoacceptors, or *Escherichia coli* enzymes to charge chloroplast-specific isoacceptors. The [^{3}H]aminoacyl-tRNAs were dialyzed, lyophilized and kept at −20°C before use.

E.coli ribosomes were prepared according to Kan et al. [8] from *E. coli* MRE 600.

Trinucleoside diphosphates (*Yp*Z*p*N) were synthetized from dinucleoside monophosphates (*Yp*Z) and nucleoside 5′-diphosphates (NDP) in the presence of polynucleotide phosphorylase, according to Hatfield [9]. A typical reaction mixture contained in a final volume of 100 µl: *Yp*Z (Pharma-Waldhof) 18 absorbance units, NDP (Pharma-Waldhof) 18 absorbance units polynucleotide phosphorylase (Boehringer) 120 µg, EDTA 0.04 µmol, Tris–HCl, pH 8.9, 20 µmol, $MgCl_2$ 1 µmol, NaCl 40 µmol. After 72 h incubation at 37°C, 50 µg phosphomonoesterase (Worthington) was added to transform the excess nucleoside 5′-diphosphate into nucleoside. To separate the trinucleoside diphosphate (*Yp*Z*p*N) from the excess of *Yp*Z and from the other oligonucleotides formed during the reaction (*Yp*Z*p*NpN, *Yp*Z*p*NpNpN, etc . . .) the mixture was chromatographed on Whatman 3 MM paper with H_2O/*n*-propanol/NH_3 (35:55:10 v/v/v) for 24 h. The spot corresponding to *Yp*Z*p*N was detected by ultraviolet absorption, eluted, and its composition was checked after hydrolysis by piperidine (yielding *Y*p, *Z*p and N) and by snake venom phosphodiesterase (yielding *Y*, p*Z* and pN), upon bi-dimensional thin-layer chromatography on cellulose plates using the following solvents: First dimension, NH_4OH (25% NH_3)/*n*-propanol/H_2O (30:60:10 v/v/v). Second dimension: HCl (37%)/isopropanol/H_2O (17.6:68:14.4 v/v/v).

ApApA was obtained by action of micrococcal

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nuclease on poly(A) according to Alexander et al. [10]. ApApG was a gift from J. Weissenbach. Poly(U) was purchased from Sigma, and poly(A) from Boehringer.

The binding of [^{3}H]aminoacyl-tRNAs to triplets (or polynucleotides) with 1–1.5 A_{260} unit of ribosomes was performed as described by Nirenberg and Leder [11], for 10 min (in the case of [^{3}H]leucyl- and lysyl-tRNAs) or 20 min (in the case of phenylalanyl-tRNAs) at 25°C. In the case of [^{3}H]leucyl-tRNA binding, 0.36 μg uncharged tRNA devoid of $tRNA^{Leu}$ was added after 10 min and the incubation continued for 3 min at 25°C. In all cases 3 ml cold buffer (0.1 M Tris–acetate, pH 7.2, 0.02 M magnesium acetate, 0.05 M KCl) was added at the end of the incubation period and the mixture was rapidly filtered on a Sartorius SM 114 membrane (pore size 0.45 μM). The tube was rinsed three times with 3 ml of the same buffer which were then poured on the filter. The filter was dried and counted.

3. Results and discussion

The results of the experiments where the binding to ribosomes of cytoplasmic and chloroplastic $tRNAs^{Phe}$ was studied, in the presence of UUU, UUC and poly(U), are summarized in table 1. The two chloroplast-specific and the cytoplasmic $tRNA^{Phe}$ all respond well to poly(U). The binding observed in the presence of UUC is less important and is even smaller in the presence of UUU. But there is no clear-cut difference in codon recognition between the three tRNAs.

The results obtained with cytoplasmic and chloroplastic $tRNA^{Lys}$ can be seen on table 2. Chloroplast-specific $tRNA^{Lys}$ and cytoplasmic $tRNA^{Lys}_2$ bind well in the presence of poly(A) and AAA, much less in the presence of AAG. Conversely the binding of cytoplasmic $tRNA^{Lys}_1$ is higher in the presence of AAG than in the presence of AAA or poly(A). Ribosome binding studies have been performed on black-eyed peas $tRNAs^{Lys}$ which can be fractionated into two fractions: one recognizes AAG, the other recognizes AAA and AAG [12]. It should be pointed out that in the case of bean $tRNAs^{Lys}$, fractionation by reverse-phase chromatography also yields two peaks, one containing chloroplast-specific $tRNA^{Lys}$, the other containing the two cytoplasmic $tRNA^{Lys}$ which can only be distinguished using an *E. coli* enzyme [4].

The results of the binding experiments performed with cytoplasmic and chloroplastic $tRNAs^{Leu}$ are summarized in table 3. The three chloroplast-specific

Table 1
Binding of cytoplasmic and chloroplastic $tRNAs^{Phe}$ to ribosomes in the presence of the two phenylalanine code-words and of poly(U)

Tri- or poly-nucleotide		Chloroplast $tRNAs^{Phe}$				Cytoplasm $tRNA^{Phe}$	
		1		2			
	Input [^{3}H]phenylalanyl-tRNA (pmol)	5.628		5.749		5.761	
[^{3}H]Phenylalanyl-tRNA bound in pmoles and % binding above background (in parentheses)							
Background		0.289		0.295		0.305	
0.5 A_{260} unit UUU		0.432	(50)	0.481	(63)	0.456	(50)
0.5 A_{260} unit UUC		0.490	(70)	0.676	(130)	0.603	(98)
0.2 A_{260} unit poly(U)		3.776	(1200)	3.513	(1090)	3.512	(1050)

Table 2
Binding of cytoplasmic and chloroplastic tRNAs^{Lys} to ribosomes in the presence of the two lysine codewords and of poly A

Tri- or poly-nucleotide	Chloroplast tRNA^{Lys}	Cytoplasm tRNA^{Lys}	
		1	2
Input [^{3}H]lysyl-tRNA (pmol)	1.850	1.280	2.892
[^{3}H]Lysyl-tRNA bound in pmoles and % binding above background (in parentheses)			
Background	0.626	0.563	1.598
0.25 A_{260} unit AAA	1.033 (65)	0.750 (33)	2.433 (52)
0.25 A_{260} unit AAG	0.756 (20)	0.838 (49)	1.754 (10)
0.2 A_{260} unit poly(A)	1.678 (168)	0.736 (30)	2.694 (69)

Table 3
Binding of cytoplasmic and chloroplastic tRNAs^{Leu} to ribosomes in the presence of the six leucine code-words

Trinucleotide	Chloroplast tRNAs^{Leu}			Cytoplasm tRNAs^{Leu}	
	1	2	3	1	2
Input [^{3}H]leucyl-tRNA (pmoles)	5.181	3.636	3.818	1.090	1.454
[^{3}H]Leucyl-tRNA bound in pmoles and % binding above background (in parentheses)					
Background	2.282	0.725	0.385	0.189	0.350
0.35 A_{260} unit UUA	2.407 (6)	0.689 (−5)	0.436 (13)	0.170 (−10)	0.354 (1)
0.35 A_{260} unit UUG	2.923 (28)	1.103 (52)	0.650 (70)	0.380 (101)	0.345 (−1)
0.35 A_{260} unit CUU	2.382 (4)	0.706 (−3)	0.305 (−11)	0.165 (−13)	0.389 (11)
0.35 A_{260} unit CUC	2.289 (0)	0.726 (0)	0.386 (0)	0.157 (−17)	0.356 (2)
0.35 A_{260} unit CUA	1.896 (−16)	0.741 (3)	0.379 (−2)	0.166 (−12)	0.346 (−2)
0.35 A_{260} unit CUG	2.132 (−6)	0.755 (4)	0.434 (13)	0.166 (−12)	0.405 (16)

tRNAs^{Leu} all respond well to UUG, but not to any of the five other leucine code-words. The fact that the three chloroplast-specific tRNAs^{Leu} all recognize the same codon is in agreement with the fact that they seem coded for by the same gene(s), as shown by the absence of additivity and the existence of competition in the hybridization experiments [6].

The first cytoplasmic tRNA^{Leu} recognizes well UUG while the second cytoplasmic tRNA^{Leu} binds in the presence of CUU and CUG. The values above background are quite low, especially in response to the triplets starting with CU, but this is a general phenomenon observed in the binding of prokaryotic and eukaryotic leucyl-tRNAs with these codons [8,9,13,14]. The binding, although weak, observed with the second cytoplasmic tRNA^{Leu} in the presence of both CUU and CUG triplets, prompted us to investigate whether this peak, isolated by RPC-5 chromatography [2], could contain two isoaccepting tRNA^{Leu} species, one recognizing CUU, the other CUG. In our laboratory, A. Steinmetz had already observed that the second cytoplasmic tRNA^{Leu} peak obtained by RPC-5 chromatography could be resolved into two distinct peaks upon RPC-6 chromatography; the result of such a fractionation is shown on fig.1. When these two fractions, shown by hatched areas on fig.1 and called cytoplasmic tRNA$^{Leu}_{2a}$ and tRNA$^{Leu}_{2b}$, respectively, were tested for codon recognition, the following results were obtained (table 4). Cytoplasmic tRNA$^{Leu}_{2a}$ (which was almost devoid of tRNA$^{Leu}_{2b}$) recognized CUU, while cytoplasmic

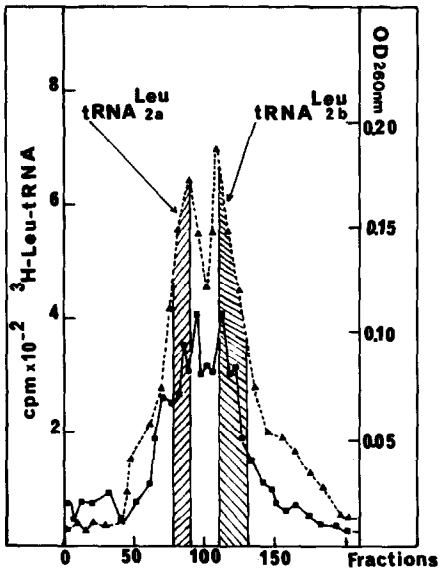

Fig.1. Reverse-phase chromatography RPC-6 [16] of the fractions corresponding to cytoplasmic tRNA$^{Leu}_2$ after RPC-5 chromatography of bean leaf tRNA. Column size: 60 X 0.6 cm. 300 μg tRNA was loaded onto the column. Elution with a 2 X 100 ml gradient of NaCl 0.29–0.3 M in Tris–HCl 0.05 M, pH 7.4, $MgCl_2$ 0.01 M. Fractions of 0.75 ml were collected. (■——■) $A_{260\,nm}$; (▲——▲) [^{3}H]leucine accepting activity.

tRNA$^{Leu}_{2b}$ responded to CUG (but as the fractions tested were still contaminated by appreciable amounts of tRNA$^{Leu}_{2a}$, it also responded to CUU).

Table 4
Binding of cytoplasmic tRNA$^{Leu}_{2a}$ and tRNA$^{Leu}_{2b}$ to ribosomes in the presence of CUU and CUG

Trinucleotide	tRNA$^{Leu}_{2a}$	tRNA$^{Leu}_{2b}$
Input [^{3}H]leucyl-tRNA (pmoles)	0.707	0.667
[^{3}H]Leucyl-tRNA bound in pmoles and % binding above background (in parentheses)		
Background	0.045	0.068
0.35 A_{260} unit CUU	0.063 (37)	0.078 (15)
0.35 A_{260} unit CUG	0.049 (8)	0.082 (21)

These results show that, in some cases at least, chloroplast-specific tRNAs recognize code-words different from those recognized by their cytoplasmic counterparts. Similar results have been obtained in the case of mitochondria-specific tRNAs [14,15]. Organelle-specific tRNAs could therefore translate codons, present in organellar mRNAs, which would not be recognized by cytoplasmic tRNAs. Whether this actually occurs in the organelles and whether it plays a role in the control of protein biosynthesis in the organelles, remains to be demonstrated. But the existence (or evolutionary conservation) of organelle-specificity cannot be explained only by their codon-specificity, as in the case of methionine for instance, coded by one triplet only. Chloroplast-specific tRNAs different from the cytoplasmic species have also been characterized [3].

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