The accuracy of poly(U) translation by different eukaryotic tRNAs

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In this work we present comparative data on rates of phenylalanine and leucine incorporation into the poly(U) dependent product of cell-free translation by different eukaryotic tRNAs at high Mg^{2+} concentration. The frequency of translation errors has been found to depend upon the value of the $tRNA^{Phe}$:$tRNA^{Leu}$ ratio and the peculiarities of isoacceptor $tRNAs^{Leu}$ of different origin.

Translation accuracy *Poly(U) misreading* *Isoacceptor $tRNAs^{Leu}$* *Aminoacyl-tRNA competition*

1. INTRODUCTION

It is well known that in cell-free systems the stimulation of amino acid incorporation by poly(U) is not restricted to phenylalanine. The accuracy of translation depends upon the temperature, Mg^{2+} concentration [1], aminoglycoside antibiotics [2,3], organic solvents [4], mutational alterations of the ribosomal proteins [5], the presence of 'translation factors' and the rate of elongation [6–8]. The importance of the ratio of individual tRNAs in their total pool for the misreading may be assumed from theoretical premises but has not been studied. We here present comparative data on the rates of phenylalanine and leucine incorporation into the poly(U) dependent product of cell-free translation by different eukaryotic tRNAs at high Mg^{2+} concentrations. The frequency of translation errors has been found to depend upon the value of the $tRNA^{Phe}$:$tRNA^{Leu}$ ratio and the leucine isoacceptor tRNA composition of total tRNAs of different origin.

2. MATERIALS AND METHODS

The total tRNAs from differentiated and undifferentiated mammary gland, rabbit liver, brewer's yeast and highly purified $tRNA^{Leu}$ and $tRNA^{Phe}$ were obtained as in [9,10]. The wheat germ cell-free system of protein synthesis was obtained as in [11] with subsequent gel chromatography of the S-30 fraction of homogenate on Sephadex G-100 [12]. The system was completely dependent on exogenous tRNA and messenger polynucleotide. The reaction mixture (0.05 ml) consisted of 25 mM Hepes (pH 7.6), 75 mM KCl, 12 mM $Mg(CH_3COO)_2$, 1 A_{260} unit of S-30, 25 μg poly(U), 25 μg total tRNA, 1 mM ATP, 0.4 mM GTP, 10 mM phosphocreatine, 1 μg phosphocreatine kinase, 2 mM dithiothreitol, 0.06 mM [^{14}C]leucine (240 mCi/mmol) and [^{12}C]phenylalanine or [^{14}C]phenylalanine (120 mCi/mmol) and [^{12}C]leucine. All samples were preincubated for 10 min at 37°C before the addition of poly(U) and the amount of aminoacyl-tRNA was determined. The incubation time for poly(U) translation was 1–10 min. During this time the rate of incorporation of [^{14}C]leucine and [^{14}C]phenylalanine into polypeptides did not change and the amount of aminoacyl-tRNA remained constant due to the presence of endogenous aminoacyl-tRNA synthetases in the system (fig.1). Control samples did not contain poly(U) or tRNA, which were added after trichloracetic acid precipitation of polypeptides [14]. The radioactivity of the acid insoluble product of poly(U) translation was estimated as in [12].

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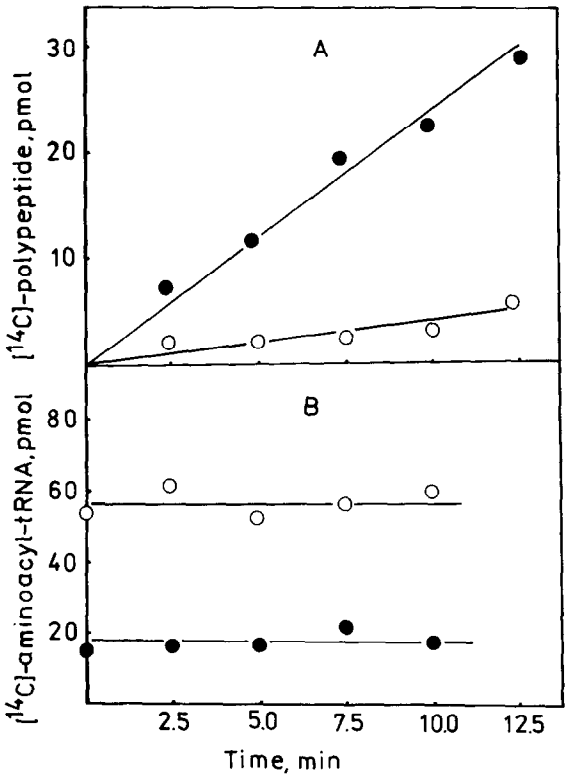

Fig.1. (A) Kinetics of [^{14}C]leucine (○) and [^{14}C]phenylalanine (●) incorporation into the trichloroacetic acid insoluble product of poly(U) translation. (B) Amount of [^{14}C]Leu-tRNA (○) and [^{14}C]Phe-tRNA (●) in the system during polypeptide synthesis; tRNA from differentiated mammary gland.

3. RESULTS AND DISCUSSION

The initial rates (V_o) of [^{14}C]leucine and [^{14}C]phenylalanine incorporation into the trichloracetic acid insoluble product of poly(U) translation by different eukaryotic tRNAs were compared (table 1). The results were calculated from the linear parts of the kinetic curves and recounted for 1 pmol of aminoacyl-tRNA in the system. The accuracy index was measured as

$$\frac{V_o^{Phe}}{[\text{Phe-tRNA}]} \Big/ \frac{V_o^{Leu}}{[\text{Leu-tRNA}]}$$

where V_o^{Phe} and V_o^{Leu} are the initial rates of Phe-peptide and Leu-peptide synthesis, respectively. It is seen that the minimum accuracy for poly(U) translation was found for tRNA from differen-

Table 1

The accuracy of poly(U) translation by eukaryotic tRNAs of different origin

Source of tRNA	Experiment no.	$[V_o^{Phe}]$/[Phe-tRNA] (*A*) (min^{-1})	$[V_o^{Leu}]$/[Leu-tRNA] (*B*) (min^{-1})	Accuracy index (*A*/*B*)	M ± σ
Differentiated mammary gland	1	0.145	0.019	7.6	
	2	0.470	0.038	12.3	
	3	0.306	0.064	4.8	
	4	0.862	0.082	10.5	8.8 ± 3.3
Undifferentiated mammary gland	1	0.204	0.018	11.3	
	2	0.558	0.027	20.7	
	3	0.397	0.054	7.3	
	4	0.948	0.061	15.5	13.7 ± 5.7
Brewer's yeast	1	0.251	0.013	19.3	
	2	0.785	0.023	34.1	
	3	0.649	0.031	20.9	
	4	0.923	0.039	23.7	24.5 ± 6.6
Rabbit liver	1	0.256	0.007	36.6	
	2	0.452	0.015	30.1	
	3	0.768	0.027	28.4	
	4	0.597	0.039	15.3	27.6 ± 8.9

tiated mammary gland. Comparison of maximum levels of leucine misincorporation into poly(U) translation product by different tRNAs has given the same result [14]. The difference in the accuracy of poly(U) translation by the tRNAs studied may be connected with different values of the $tRNA^{Phe}$:$tRNA^{Leu}$ ratio and (or) the peculiarities of isoacceptor $tRNAs^{Leu}$ in their total pool. The $tRNA^{Phe}$:$tRNA^{Leu}$ ratio for tRNA from differentiated and undifferentiated mammary gland, rabbit liver and brewer's yeast was found to be 0.26, 0.53, 0.51 and 0.96, respectively [14]. To verify this suggestion the accuracy indexes of poly(U) translation were compared for different values of the Phe-tRNA:Leu-tRNA ratio in the system. The samples contained 15 μg of total yeast tRNA purified from $tRNA^{Phe}$ by BD-chromatography [10] and 0.1–2.5 μg $tRNA^{Phe}$. The increase of the $tRNA^{Phe}$:$tRNA^{Leu}$ ratio increases the accuracy,

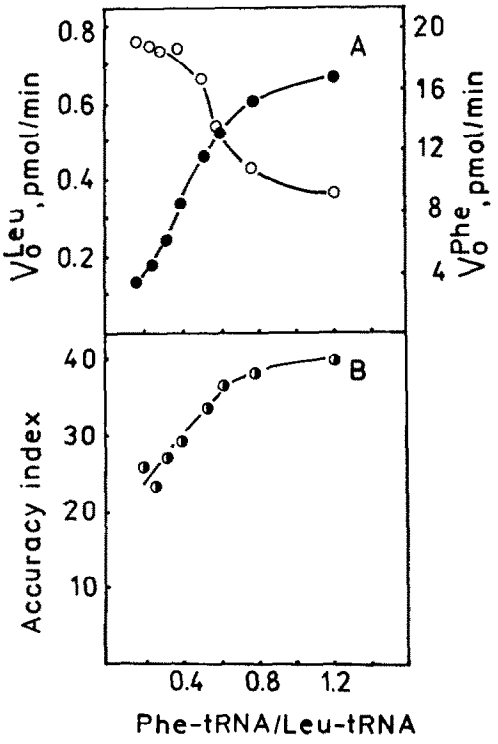

Fig.2. (A) Dependence of the initial rates of [^{14}C]leucine (○) and [^{14}C]phenylalanine (●) incorporation into the trichloroacetic acid insoluble product of poly(U) translation on the Phe-tRNA:Leu-tRNA ratio. (B) Dependence of the accuracy index on the Phe-tRNA:Leu-tRNA ratio.

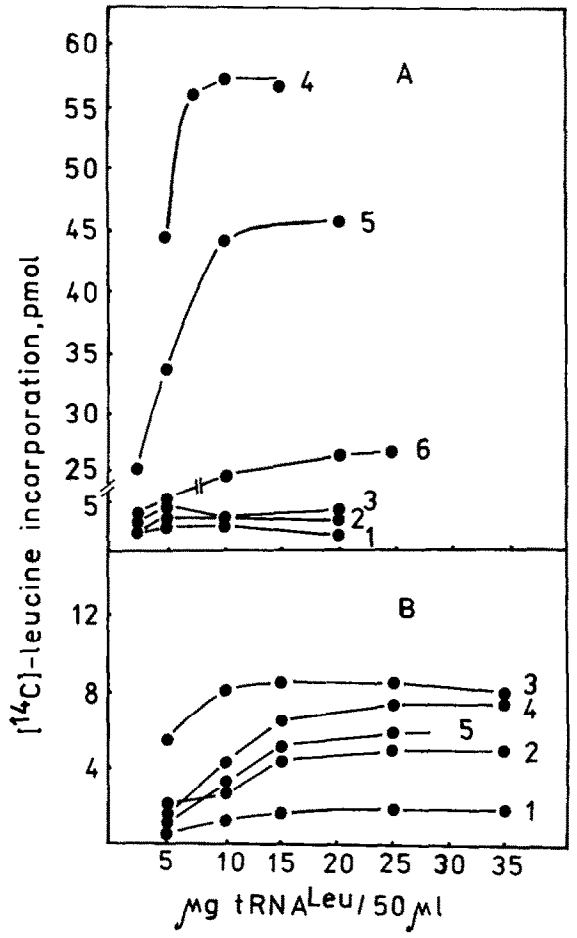

Fig.3. Leucine incorporation into the trichloroacetic acid insoluble product of poly(U) translation by different isoacceptor $tRNAs^{Leu}$ from differentiated mammary gland (A) and brewer's yeast (B). Translation was performed for 60 min. 1–6, numbers of $tRNAs^{Leu}$ according to RPC-5 chromatography.

but to a definite level (fig.2). We have shown that such a change in accuracy is due to Phe-tRNA and Leu-tRNA competition in the translation step rather than during aminoacylation or interaction with elongation factors.

The abilities of isoacceptor $tRNAs^{Leu}$ from yeast and differentiated mammary gland to incorporate leucine into poly(U) translation product are compared and the results presented in fig.3. Isoacceptor $tRNAs^{Leu}$ essentially differ in their ability to misread poly(U). This is in agreement with theoretical premises and literature data [15]. The minor $tRNAs^{Leu}$ fractions are characterized by a

high level of miscoding, especially $tRNA^{Leu}_{4,5}$ from differentiated mammary gland. It is of great interest that the maximum rate of poly(U) misreading was observed for $tRNA^{Leu}_4$, which appears in mammary gland epithelium during differentiation. The synthesis of polyleucine on poly(U) seems to occur in a cell-free system which contains highly purified $tRNAS^{Leu}$ only. The possibility of the process occurring in principle has been demonstrated [16]. The difference in the rates of leucine incorporation into poly(U) translation product by isoacceptor $tRNAS^{Leu}$ may be connected with their different ability to initiate polypeptide synthesis on ribosomes. To verify this assumption we have compared poly(U) misreading by mammary gland $tRNA^{Leu}_2$ and $tRNA^{Leu}_4$ in the presence of a small amount of $tRNA^{Phe}$ and the same result has been obtained. Thus the lower accuracy of poly(U) translation by total tRNA from differentiated mammary gland in comparison with other eukaryotic tRNAs may be explained by the low value of the $tRNA^{Phe}$:$tRNA^{Leu}$ ratio and the presence of special isoacceptor $tRNAS^{Leu}$. The primary structure, coding response of isoacceptor $tRNAS^{Leu}$ from differentiated mammary gland and their ability to misread the UUU-codon both in UUU-dependent binding to ribosomes and in poly(U) translation are the aims of further study.

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