MESSENGER RNA TRANSLATION IN A tRNA DEPENDENT CELL-FREE SYSTEM: ROLE OF tRNA FROM DIFFERENT SOURCES

P. GERLINGER, M. A. LE MEUR and J. P. EBEL

Institut d'Embryologie, Faculté de Médecine et Institut de Biologie Moléculaire et Cellulaire, Laboratoire de Biochimie, Strasbourg, France

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1. Introduction

The liver and the oviduct of the laying hen are the target organs of estrogens, and synthesize the egg yolk and the egg white proteins respectively. By reverse phase chromatography we have demonstrated quantitative differences in the relative proportions of isoaccepting tRNA species purified from these two organs [1]. Although the functional adaptation of tRNA to the kind of protein synthesized in the cell is well known [2,3], it has not been demonstrated that the relative proportion of these tRNAs inside the cell has an effect on the rate of specific protein synthesis. With Krebs-II ascites cells we have developed a tRNA-dependent cell-free system and studied the translation of oviduct and reticulocyte messenger RNA in the presence of different tRNA preparations. We conclude that these messenger RNAs are translated more efficiently in the presence of tRNA from the corresponding tissues than with heterologous tRNA preparations.

2. Methods

The cell-free protein synthesizing system was prepared from Krebs-II ascites cells as described [4]. This system is claimed to be tRNA-dependent [5–7] but this is not confirmed by others [8]. In our laboratory, the system is tRNA-dependent only after filtration of the preincubated 30 000 *g* supernatant (S30) through a Sephadex G100 column which excludes the ribosomes and removes all the tRNAs present.

The polysomal RNAs from oviduct or reticulocytes were prepared according to procedure IV of Palmiter [9].

Routine assays contained in 50 μl: 30 mM Tris–HCl pH 7.5, 96 mM KCl, 2.4 mM magnesium acetate, 1 mM DTT, 1 mM ATP, 0.1 mM GTP, 0.6 mM CTP, 5 mM creatine phosphate, 0.16 mg/ml creatine kinase, 19 unlabelled amino acids (0.04 mM each), 0.1 μCi of L-[^{14}C]-phenylalanine (250 mCi/mM, 0.15 A_{260} of tRNA isolated from hen liver or oviduct [1] or from rabbit reticulocytes [10], 0.1–0.15 A_{260} of Krebs lysate, 0.2 A_{280} of tRNA free KCl wash fraction from rabbit reticulocytes polysomes [10] and 0.125 A_{260} of polysomal RNA as messenger. Incubation was at 37°C for 60 min. The determination of the radioactivity incorporated into proteins was done by the method of Mans and Novelli [11] and the filters were counted in the presence of 5 ml of toluene omnifluor.

Cell-free products were analysed on 7.5% SDS polyacrylamide gels [12] either after TCA precipitation or immunoprecipitation of the ovalbumin [13].

3. Results

The system which is described here is totally dependent on added tRNA and KCl wash fraction. A little endogenous messenger activity remains, but exogenous messenger RNAs are very efficiently translated (Table 1).

Address for correspondence: Dr P. Gerlinger, Institut d'Embryologie, Faculté de Médecine, 11, rue Humann, 67085 Strasbourg, France

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Table 1
L-[^{14}C]-phenylalanine incorporation in the cell-free system

	cpm
Complete system	14 000
minus tRNA	300
minus messenger RNA	2 500
minus tRNA and messenger RNA	150
minus KCl wash fraction	175

The reactions were performed as described in methods with 0.125 A_{260} of oviduct polysomal RNA. Blanks of 600 cpm for zero time control are subtracted.

We have studied the incorporation of L-[^{14}C]-phenylalanine in the presence of oviduct or reticulocyte polysomal RNA as a function of the amount of tRNA from different sources present in the system. Fig.1 shows that, even with a large excess of tRNA, the messenger RNAs from oviduct are translated better in the presence of tRNA from the same source than with hen liver or rabbit reticulocyte tRNA. A similar result is found with reticulocyte messenger RNA (not shown). As the magnesium curve of incorporation in this system is very sharp, we checked the total incorporation into egg proteins, and the specific incorporation into ovalbumin or globin with an excess of tRNA from the three sources, as a function of magnesium concentration. Fig.2 clearly indicates that at the optimal mag-

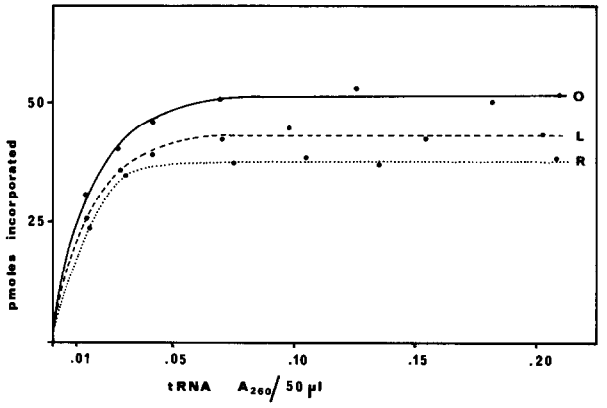

Fig.1. L-[^{14}C]-phenylalanine incorporation into proteins directed by oviduct polysomal RNA expressed in pmoles per 50 µl reaction mixture, versus the amount of tRNA from oviduct (O), liver (L) or reticulocytes (R) present in the cell free system.

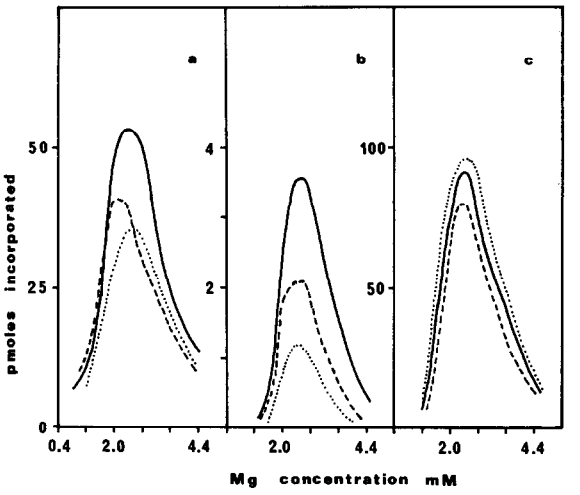

Fig.2. Magnesium curves obtained with the tRNAs from oviduct (———), liver (– – – –), or reticulocytes (. . . . .). (a) Total incorporation in the system, in the presence of 0.125 A_{260} of oviduct RNA. (b) Ovalbumin immunoprecipitation from 50 µL reaction mixture as in (a). (c) Total incorporation in the system, in the presence of reticulocyte RNA.

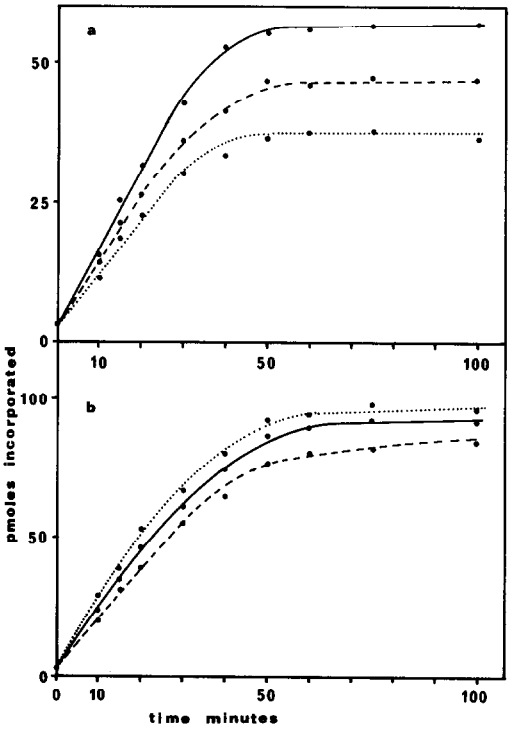

Fig.3. Kinetics of oviduct (a) and reticulocytes (b) polysomal RNA translation in the presence of an excess of tRNA from oviduct (O), liver (L) or reticulocytes (R).

nesium concentration, the oviduct messenger RNAs and particularly the ovalbumin messenger RNA are better translated in the presence of oviduct tRNA. On the other hand, globin synthesis is enhanced by reticulocyte tRNA. Hen liver tRNA gives intermediate results in both cases.

The kinetics of amino acid incorporation into egg white proteins and globin are shown in figs.3a and 3b respectively. The highest rate of egg white protein synthesis is obtained with the oviduct tRNA while the heterologous tRNAs give lower rates. Although we obtained slight differences in the case of globin synthesis, the result is highly reproducible.

The specificity of the synthesis has been examined by SDS polyacrylamide gel electrophoresis of the cell-free synthesized product (fig.4).

4. Discussion

The use of a tRNA dependent cell-free system, derived from Krebs-II ascites cells, enabled us to demonstrate that messenger RNAs are better translated with homologous tRNAs than with heterologous ones. A similar result for globin synthesis was mentioned in a tRNA dependent cell-free system from reticulocytes [10]. We think that the differences we have observed are not due to the method of purification of the tRNAs. Their purity has been demonstrated by their homogeneity on polyacrylamide gel electrophoresis and by their ability to be acylated at 93–95% with 18 amino acids.

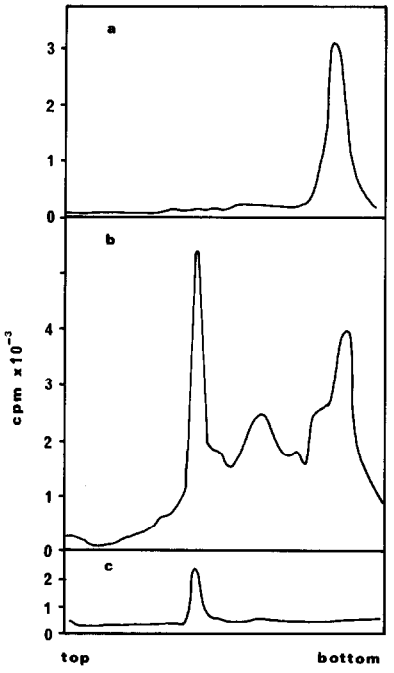

Fig.4. SDS polyacrylamide gel electrophoresis after RNase treatment and TCA precipitation of the incubated cell-free system in the presence of reticulocytes RNA (a), oviduct RNA (b), gel electrophoresis after ovalbumin immunoprecipitation (c).

Since the KCl wash fraction from reticulocytes polysomes is the only source of aminoacyl-tRNA synthetases, these enzymes are not involved in the differences which are described. Moreover, we have shown that the seryl-tRNA synthetase from several higher animals can acylate the corresponding tRNAs with the same kinetic constants [14].

With the same amount of polysomal RNA as messenger, we always obtained a more efficient translation of reticulocyte RNA than of oviduct RNA (fig.2 and 3). These differences may be related to the KCl wash fraction used as initiation factors, as it is known that rabbit reticulocyte initiation factors favour the translation of rabbit globin messenger [15,16].

Although the key role of tRNA in protein synthesis is well known, the regulatory role they could play by the existence of several isoaccepting species for each aminoacid is still unclear. In the experiments we present here, the messenger RNAs are well translated regardless of the tRNA preparations used. Nevertheless, the kinetics of incorporation indicate that the rate of synthesis increases when homologous tRNA is used. We have found quantitative differences in the tRNA populations of the liver and oviduct from the laying hen [1]. This repartition of the tRNAs in the cell may reflect an adaptation of the cell to the messenger RNAs which have to be translated and our results suggest that the tRNA pattern resulting from this adaptation could favour the synthesis of some specific proteins.

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