DIFFERENCE IN RNA-BINDING ABILITY BETWEEN EUKARYOTIC AND PROKARYOTIC ELONGATION FACTORS OF TRANSLATION

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1. Introduction

It has been shown that the total fraction of RNA-binding proteins of the eukaryotic cytoplasm [1,2] contains translation factor activities including those of the two elongation factors, EF-1 and EF-2 [3,4]. The hypothesis was proposed that the ability of binding with RNA is an additional evolutionary acquisition of many eukaryotic proteins which serve protein biosynthesis and other RNA-involving processes; this property of the proteins could ensure their concentration on RNA and at least partial compartmentation due to formation of mRNA · protein complexes in the large eukaryotic cell (*Omnia mea mecum porto*) [5]. If this is true, then the corresponding prokaryotic proteins, being analogous in functions, must not possess the affinity to RNA.

Here this prediction has been checked in direct experiments. Pure elongation factors isolated from rabbit reticulocytes and *Escherichia coli* have been assayed for RNA-binding activity. It has been shown that the eukaryotic elongation factors, both EF-1 and EF-2, possess an affinity to RNA and thus are capable of forming RNA · protein complexes. Under the same conditions the prokaryotic elongation factors, such as EF-Tu, EF-Tu·Ts and EF-G, have displayed no ability to bind with RNA. Thus, the conclusion is made that eukaryotic elongation factors (EF-1 and EF-2) are RNA-binding proteins whereas their prokaryotic analogs (EF-T and EF-G) are not.

2. Materials and methods

2.1. *Elongation factor preparations*

The eukaryotic elongation factors, EF-1 and EF-2, were isolated from rabbit reticulocytes according to the procedure detailed in [6,7]. Testing of elongation factor activity in the cell-free system containing mouse liver ribosomes [8,9] was done as in [4,10]. The saturating amounts of the elongation factors were about 2 μg for EF-1 and 0.7 μg for EF-2 per 3 pmol ribosomes in 50 μl. The preparations of each of the elongation factors were free from contamination of the other factor activity. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate [11] showed that the preparations of both the 'light' and the 'heavy' forms of EF-1 (EF-1_L and EF-1_H, respectively) contained the main component of about 50 000 daltons; in the EF-1_L preparation it comprised about 90% of the total protein mass, whereas the EF-1_H displayed also significant amounts of polypeptides of about 35 000 and 30 000 daltons (fig.1b,c). The purity of the EF-2 preparation (95 000 dalton component) was about 90% (fig.1d).

The bacterial elongation factors, EF-Tu, EF-Ts and EF-G, were prepared from *Escherichia coli* MRE 600 as in [12,13]. Testing of the activity in the *E. coli* cell-free system with poly(U) was done according to [14]. The preparations of each of the elongation factors were free from contamination of the other factor activity. The electrophoretic purity of the EF-Tu, EF-Ts and EF-G preparations was no less than 80–90% (fig.1f–i).

Protein concentration was determined from the ΔA at 228.5 nm and 234.5 nm [15] or by the amido black staining technique [16,17].

2.2. *Assay for RNA-binding activity*

The RNA-binding activity of the proteins was measured by two methods:

Fig.1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the eukaryotic and prokaryotic elongation factors and the total RNA-binding proteins. (a) Total RNA-binding protein of the rabbit reticulocyte extract; (b) $EF\text{-}1_L$; (c) $EF\text{-}1_H$; (d) EF-2, (e) total RNA-binding proteins of the *E. coli* extract; (f, g) EF-G, two different preparations; (h) EF-Tu; (i) EF-Ts.

(1) By retention of [^{14}C]RNA · protein complexes on nitrocellulose filters [1,2];
(2) By direct adsorption of the proteins on the Sepharose-coupled RNA columns [17].

In the first method a certain amount of the protein tested was introduced into the incubation mixture containing radioactive [^{14}C]RNA isolated from *E. coli* ribosomes and the mixture was passed through a nitrocellulose filter; the [^{14}C]RNA · protein complex was retained on the filter while the free [^{14}C]RNA passed through [1,2]. (It has been shown earlier that *E. coli* ribosomal RNA effectively competes with eukaryotic mRNA for RNA-binding proteins and so can be equally used to assay the RNA-binding activity [18].) The incubation mixture was prepared in the buffer consisting of 10 mM Tris–HCl, $pH_{25°C}$ 7.6, 10 mM KCl, 1 mM $MgCl_2$ and 1 mM dithiothreitol. It contained a constant amount (0.2 μg or 4000 cpm) of [^{14}C]RNA and varying amounts of the protein tested, in 100 μl total volume. Complex formation took place in less than 15 s at room temperature. The reaction mixture was diluted with the same buffer, but without dithiothreitol, and passed through a nitrocellulose filter of the Millipore type. The radioactivity retained on the filter was counted in the standard toluene–PPO–POPOP system using a Beckman LS100 liquid scintillation spectrometer.

In the second method, described [17], the protein tested was applied to the column with Sepharose-coupled *E. coli* ribosomal RNA equilibrated with the buffer consisting of 10 mM Tris–HCl, $pH_{25°C}$ 7.6, 10 mM KCl, 1 mM $MgCl_2$ and 1 mM dithiothreitol.

About 2–3 mg of the protein was taken per 50 mg RNA in the column. The column was washed with the same buffer to remove the proteins incapable of binding with RNA. Elution of RNA-binding proteins was done with the same buffer but containing 1 M KCl.

3. Results and discussion

Figure 2 shows the dependence of the [^{14}C]RNA · protein complex formation measured by the nitrocellulose filter retention technique on the amount of the tested protein added to the incubation mixture containing 0.2 μg [^{14}C]RNA. First of all it is seen that the eukaryotic elongation factors do have a significant affinity to RNA. The highest affinity of protein to RNA is observed in the case of EF-1; the titration curve is steep, thus indicating a high binding constant. EF-2 is also found to be capable of forming complexes with RNA, though its affinity to RNA seems to be lower than that of EF-1; here, binding of the same amounts of RNA requires 4-times more protein by weight than in the case of EF-1.

It should be mentioned that recently the RNA-binding activity of EF-1 using the filter technique was also shown by other authors [19]. Earlier the interaction of EF-1 with poly(U) was reported [20].

It is striking that no form of the prokaryotic elongation factors tested displays any visible RNA-binding activity: neither EF-Tu, nor EF-Tu · Ts, nor EF-G, with and without nucleotides, give any retention of radioactive RNA on the filter (fig.2).

Another method of assaying the RNA-binding activity of the proteins was used to exclude the possibility that complexes of prokaryotic elongation factors with high molecular weight RNA for some reason could be incapable of being adsorbed on nitrocellulose filters of the Millipore type. Table 1 shows the results of passing the eukaryotic and prokaryotic elongation factors through the Sepharose-coupled RNA column. In all the cases at the given ionic conditions (10 mM Tris–HCl, 10 mM KCl and 1 mM $MgCl_2$) the eukaryotic elongation factors, EF-1 and EF-2, were completely retained in the column; at 1 M KCl they were eluted. On the other hand, neither of the prokaryotic elongation factors displayed the slightest affinity to RNA in the column, even under

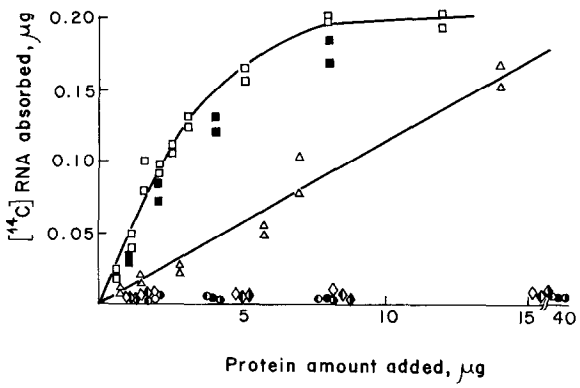

Fig.2. Amount of [^{14}C]RNA retained (in the form of RNA·protein complexes) on nitrocellulose filters versus the amount of the protein added (eukaryotic or prokaryotic elongation factors). The protein was added to the constant 0.2 μg amount of [^{14}C]RNA (4000 cpm) in 100 μl total volume. (○) EF-1_L; (■) EF-1_H; (△) EF-2; (◑) EF-Tu · GDP; (◐) EF-Tu · GTP; (●) EF-Tu · Ts; (◇) EF-G; (◑) EF-G · GDP; (◐) EF-G · GTP.

Table 1
Results of passing the eukaryotic and prokaryotic elongation factors through the Sepharose-coupled RNA column

Elongation factor	Amount of protein (μg)		
	Applied to the column	Determined in 10 mM KCl wash	Determined in 1 M KCl eluate
EF-1_L	2.5	< 0.1	2.3
EF-1_H	3.0	< 0.1	3.2
EF-2	2.1	< 0.1	1.9
EF-Tu	2.0	2.3	< 0.01
EF-G	2.0	2.1	< 0.05

the conditions used of a relatively low ionic strength (table 1).

In our special experiments the total ribosome-free supernatant of the rabbit reticulocyte lysate was passed through the Sepharose-coupled RNA column. The result was that at least 97% of the EF-1 activity and 99% of the EF-2 activity were adsorbed in the column. On the contrary, the activities of EF-Tu and EF-G were not retained at all in analogous experiments with the total ribosome-free *E. coli* supernatant, though some proteins of the bacterial extract were absorbed. Electrophoretic data confirm the absence of EF-Tu and EF-G among the proteins of the *E. coli* extract adsorbed on the Sepharose-coupled RNA column (fig.1e).

The demonstration of the RNA-binding activity of the eukaryotic elongation factors suggests that they may complex with mRNA in the cytoplasm. From this, it is likely that the elongation factors, in particular the firmly binding EF-1, are one of the constituents of free cytoplasmic informosomes and polyribosomal messenger ribonucleoproteins.

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