RNA-BINDING ACTIVITY OF EUKARYOTIC INITIATION FACTORS OF TRANSLATION

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1. Introduction

The proteins of wheat embryo cytoplasmic extract necessary for the ribosomes to translate natural templates can be selectively adsorbed on matrix-bound RNA [1]. That is, these proteins, being translation factors, behave as RNA-binding proteins. However, the activity of the eukaryotic elongation factors EF-1 and EF-2 as well as that of some of the initiation factors such as eIF-1, eIF-(4C+5), eIF-3 and eIF-4B was observed in preparations of RNA-binding proteins of rabbit reticulocytes [2]. The hypothesis was proposed that many of the eukaryotic proteins functioning with RNA and in RNA-dependent processes possess an RNA-binding activity and that the RNA-binding capability of such proteins is an additional evolutionary acquisition of the eukaryotic cell [3]. Indeed, experiments on eukaryotic elongation factors have revealed that both have an affinity to RNA whereas prokaryotic elongation factors do not bind with RNA [4].

Preparations of initiation factors eIF-2 [5–10], eIF-3 [11], eIF-4B [12,13] and eIF-5 [11,13] induced adsorption of different RNAs on nitrocellulose filters, suggesting an RNA-binding activity of the proteins. At the same time, dissociation of eIF-2 into subunits upon binding with RNA was claimed [14]. Also, eIF-3 was reported to selectively bind with mRNA but not to bind with ribosomal RNA [11].

Using the method of direct adsorption of RNA-binding proteins on Sepharose-bound *Escherichia coli* ribosomal RNA, we show here that:

(i) Initiation factor eIF-2 binds with RNA as a complex consisting of all three subunits;
(ii) Initiation factor eIF-3 also binds with this alien RNA, as a whole complex consisting of 9 polypeptides;
(iii) Initiation factor eIF-4A does not have an affinity to RNA.

2. Materials and methods

2.1. *Preparations*

Eukaryotic initiation factors eIF-2, eIF-3 and eIF-4A were isolated from rabbit reticulocytes by the method in [15]. Assays of initiation factor activity were done in a complete cell-free system of translation containing rabbit reticulocyte ribosomes, amino acid:tRNA ligases and tRNA from rabbit liver, globin mRNA, purified elongation factors EF-1 and EF-2, as well as initiation factors eIF-1, eIF-4B, eIF-(4C+5), as in [15]. The preparations of eIF-2, eIF-3 and eIF-4A were free from contamination by other initiation factor activities. Polyacrylamide gel electrophoresis with sodium dodecylsulfate (SDS) [16] showed that eIF-4A was a single polypeptide, whereas eIF-2 and eIF-3 were complexes consisting of 3 and 9 polypeptides, respectively (fig.1).

2.2. *Assay for RNA-binding activity*

The RNA-binding activity of the initiation factors was measured by direct adsorption of the proteins on Sepharose-coupled RNA columns [4,17]. The RNA–Sepharose was prepared by covalent coupling of *E. coli* 16 S ribosomal RNA to BrCN-activated Sepharose 4B [18] followed by glycine blocking of excess active groups. Control columns with glycine-blocked Sepharose without RNA were used in the same experimental conditions as a control of protein adsorption on carrier gel.

Initiation factors (10 μg) were applied on 100 μl columns with RNA–Sepharose in the buffer 10 mM Tris–HCl ($pH_{25°C}$ 7.6), 1 mM $MgCl_2$, 20 mM KCl, 1 mM dithiothreitol. Unadsorbed material was thoroughly washed off with the same buffer. Adsorbed proteins were eluted from the column with the buffer containing 1% SDS. The adsorbed and unadsorbed protein fractions were analyzed by SDS–polyacrylamide gel electrophoresis [17].

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2.3. *Analysis of the interaction of the initiation factors with different RNAs and synthetic polynucleotides*

Free *E. coli* 16 S RNA effectively competes with the RNA coupled to Sepharose, and elutes the adsorbed proteins from the column. This permitted to check the affinity of the initiation factors for different polynucleotides as compared with 16 S RNA [17].

The initiation factors adsorbed on 100 μl RNA–Sepharose columns were eluted with 40 μl 10 mM Tris–HCl ($pH_{25°C}$ 7.6), 1 mM $MgCl_2$, 20 mM KCl, 1 mM dithiothreitol containing 20 μg of one of the competing polynucleotides, such as *E. coli* 16 S RNA, rabbit liver tRNA, TMV RNA, poly(U) and poly(A). The mixture of AMP, CMP, GMP and UMP was also used as a control. The rest of proteins was then eluted from the column with 2 vol. buffer containing 4 M LiCl and 6 M urea. The protein concentration was determined on nitrocellulose filters by amido black staining [9].

3. Results and discussion

Figure 1 presents the results of electrophoresis of protein fractions obtained in the experiments on adsorption of the initiation factors eIF-2, eIF-3 and eIF-4A on RNA–Sepharose columns. Each initiation factor was passed through two parallel columns: the experimental one with RNA–Sepharose and the control one with RNA-free Sepharose. It is seen on the electrophoregrams that none of the initiation factors studied is adsorbed on the control column with RNA-free Sepharose (fig.1C). In our experiments eIF-4A does not bind also with the RNA–Sepharose (fig.1, panel 1), which agrees with the data on the absence of eIF-4A in preparations of rabbit reticulocyte RNA-binding proteins [2].

Factor eIF-3 firmly binds with RNA in the column and is eluted from it with the buffer with 1% SDS (fig.1, panel 3). It must be specially emphasized that eIF-3 is adsorbed on RNA–Sepharose as a complex consisting of 9 polypeptides (fig.1, panel 3, 2E). The adsorption of eIF-3 on the mRNA–Sepharose column was used also in [20,21]; however, it was noted [20,21] that it was very difficult to interpret the results as the material containing eIF-3 activity had contaminations of other cytoplasmic proteins and initiation factors.

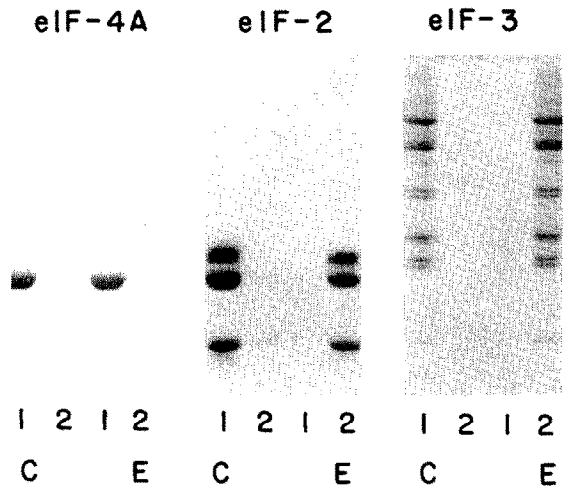

Fig.1. Determination of the RNA-binding capability of the initiation factors by their adsorption on RNA–Sepharose. Electrophoresis of protein factors not adsorbed on the column (1) and those washed off by buffer with 1% SDS (2). (C) control; (E), experiment.

Here, eIF-2 behaves analogously to eIF-3, i.e., it also binds to RNA on the column as a complex of 3 polypeptides (fig.1, panel 2, 2E). This result differs from the data in [14] where the addition of globin mRNA to eIF-2 led to its dissociation into subunits. The authors explained the result by the specific interaction of only one of the eIF-2 subunits with mRNA. However, it is quite likely that the dissociation of eIF-2 in their experiments could be induced by the migration of the complex with mRNA to the acidic region of the pH-gradient during electrofocusing.

Our results remain the same if the initiation factors are passed through RNA–Sepharose in the buffer containing 100 mM KCl instead of 20 mM KCl.

The method of adsorption of the initiation factors on RNA–Sepharose columns and their following elution with solutions containing different RNAs and synthetic polynucleotides permits the study of the specificity of the RNA–factor interaction. The results of such an experiment are given in table 1. The buffer without RNA washes off no eIF-2 or eIF-3 from RNA–Sepharose (lower line), whereas the buffer with *E. coli* 16 S rRNA elutes their significant amounts (top line) under the given conditions (see section 2.3). Substitution of the RNA by the same amount (by weight) of the nucleosidemonophosphate mixture results in no elution of the proteins. On the

Table 1
Interaction of the initiation factors with different RNAs

Competing polynucleotide (20 μg)	μg protein eluted from 16 S RNA–Sepharose column			
	eIF-2		eIF-3	
	Buffer with competitor	4 M LiCl, 6 M urea	Buffer with competitor	4 M LiCl, 6 M urea
16 S rRNA	3	3	1	0.8
TMV RNA	1	4	0.8	0.5
tRNA	2	3	1.5	0.8
poly(U)	4	2	4	0.3
poly(A)	1	4	0.3	1
AMP+GMP+CMP+UMP	<0.3	6	<0.3	1
None (buffer)	<0.3	6	<0.3	1

contrast, all other RNAs and polynucleotides tested are found to be effective (though to a different extent) as competing eluents of the initiation factors from 16 S rRNA. Consequently, the initiation factors eIF-2 and eIF-3 can bind with RNA and polynucleotides nonspecifically. No striking differences in the amount of protein eluted from RNA–Sepharose is observed with different RNAs and polynucleotides, i.e., there is no qualitative preference of binding either of eIF-2 or eIF-3 to any RNA. However, quantitatively poly(U) seems to manifest the strongest interaction with both eIF-2 and eIF-3.

These results on the capability of eIF-3 to bind with *E. coli* ribosomal RNA in a column and to be eluted from it with solutions of TMV RNA, tRNA, poly(A) and poly(U) directly contradict the conclusions on the selective binding of eIF-3 to mRNA and the absence of binding with ribosomal RNA [11]. They fully agree with the data on the adsorption of eIF-3 on a column with ribosomal RNA from rabbit reticulocyte extract [2].

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