TRANSLATION FACTORS OF THE WHEAT EMBRYO EXTRACT ARE RNA-BINDING PROTEINS

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1. Introduction

A special class of proteins with a high affinity for RNA was discovered in the cytoplasmic extracts of animal cells [1–3]. Later, analogous RNA-binding proteins were found also in cytoplasmic extracts of wheat embryos [4]. The characteristic features of the RNA-binding proteins of the cytoplasm of all the eukaryotic cells studied are their existence predominantly in the form of relatively high-molecular-weight aggregates with sedimentation coefficients from 6–10 S (approx. mol. wt 100 000 – 300 000), and their ability to form stoichiometric complexes with RNA of a buoyant density in CsCl of 1.4 g/cm^3 (protein: RNA weight ratio of about 3:1). It was suggested that these cytoplasmic RNA-binding proteins are a pool involved in the formation of informosomes (messenger ribonucleoproteins) in vivo, representing free informosome-forming proteins [1,5]. The high binding constants of the proteins of this class with RNA (10^7–10^{13} M^{-1} [6]) permitted the use of the technique of their affinity adsorption on free RNA [7], or on RNA covalently bound to Sepharose [8–10], or on matrix-bound synthetic polynucleotides [10–13], for their removal from extracts and for their very effective purification from all other proteins.

Here we report data which show that protein synthesis in wheat embryo cell-free system is stopped as a result of the selective removal of the RNA-binding proteins from it. On the other hand, washed wheat embryo ribosomes are shown to be capable of synthesizing protein in the presence of aminoacyl-tRNA and natural mRNA when the total RNA-binding protein preparation is added to the system instead of a preparation of initiation and elongation factors. From this, it is concluded that eukaryotic initiation and elongation factors can be RNA-binding (informosome-forming) proteins.

2. Materials and methods

Embryos of wheat *Triticum vulgare*, 'Mironovskaya-808' variety, were used in the experiments. The embryos were prepared by a standard technique [14].

Twice-washed 80 S ribosomes from wheat embryos and RNA from tobacco mosaic virus (TMV) were isolated as in [14,15].

Total tRNA was isolated from the wheat embryo post-ribosomal supernatant by deproteinization with sodium dodecyl sulfate and phenol. Sodium dodecyl sulfate was added to final conc. 0.2% and the mixture extracted 3 times at room temperature with equal vol. phenol; the aqueous phase was adjusted to a 0.1 M concentration of potassium-acetate buffer, $pH_{25°C}$ 5.5, and tRNA was precipitated with 2.5 vol. ethanol; the tRNA was purified additionally by chromatography on a DEAE-cellulose column. Enzymic acylation of the tRNA with the complete mixture of ^{14}C-labelled amino acids was done according to a slightly modified procedure of [15]. The activity of the preparation was 1.8×10^6 cpm/mg tRNA.

The post-ribosomal supernatant freed from tRNA by a DEAE-cellulose column [16] was used as the preparation of the total initiation and elongation factors; this preparation was denoted the 'complete S100-DEAE fraction'.

RNA-binding proteins were prepared by affinity chromatography on Sepharose with covalently bound *E. coli* ribosomal RNA [10]. The post-ribosomal nucleic acid-free supernatant (complete S100-DEAE fraction) was applied to the RNA–Sepharose column equilibrated with buffer containing 10 mM Tris–HCl, $pH_{25°C}$ 7.6, 50 mM KCl, 1 mM $MgCl_2$ and 4 mM β-mercaptoethanol. The sample volume was taken such as to apply 5–9 mg total protein of the complete S100-DEAE fraction per mg RNA in the column; under these conditions the capacity of the column was sufficient to adsorb all RNA-binding activity [10]. The column was washed with the same buffer to remove proteins incapable of binding with RNA. The fraction of non-adsorbed proteins passing through the column was denoted the 'S100-DEAE deprived of RNA-binding proteins'. The fraction of 'RNA-binding proteins' was eluted from the RNA–Sepharose column with the same buffer, but containing 0.3 M KCl.

The preparations of total initiation and elongation factors (complete S100-DEAE fraction) and of RNA-binding proteins were dialysed before use for 2 h against a buffer containing 10 mM Tris–HCl, $pH_{25°C}$ 7.6, 50 mM KCl, 2 mM $MgCl_2$, 4 mM β-mercaptoethanol.

The cell-free system contained: 100 μg wheat 80 S ribosomes; 100 μg [^{14}C]aminoacyl-tRNA, 5 μg TMV RNA; 8 μg GTP; 60 μg ATP; 220 μg creatine phosphate; 16 μg creatine kinase; in vol. 70 μl. The incubation mixture was prepared in a buffer containing 40 mM Tris-HCl, $pH_{25°C}$ 7.6, 30 mM KCl, 4 mM $MgCl_2$, 3 mM dithiothreitol. In different experimental variants either 300 μg protein of the complete S100-DEAE fraction, or 200–300 μg protein of the S100-DEAE fraction deprived of RNA-binding protein, or 30–50 μg RNA-binding proteins were added to the cell-free system. Incubation was for 1 h at 25°C. The reaction was stopped by adding 3 ml 5% trichloroacetic acid. Each sample was hydrolyzed for 20 min at 90°C and cooled. The precipitate was deposited on a nitrocellulose filter and washed with 5% trichloroacetic acid; the filter was dried at 100°C and its radioactivity counted in a standard toluene–PPO–POPOP mixture.

3. Results and discussion

In preliminary experiments, we noticed that the passsing of the preparation of total initiation and elongation factors (complete nucleic acid-free S100-DEAE fraction of wheat embryos [16]) through the RNA–Sepharose column resulted in the loss of its translation factor activity. Washing of the column with standard buffers of ionic strengths about 0.1 did not elute this activity. Consequently:

(i) Either the matrix-bound RNA inactivated some of the translation factors or
(ii) At least one or some of the factors had a special affinity to RNA, i.e., behaved as RNA-binding proteins.

To test these two alternatives, we eluted the fraction of RNA-binding proteins from the RNA–Sepharose column with a high ionic strength buffer

Table 1
Translation factor activity of RNA-binding proteins

Presence of protein fractions in the cell-free system			Incorporation of [^{14}C]amino acids into trichloroacetic acid-insoluble polypeptide (cpm)	
Complete S100-DEAE fraction	S100-DEAE fraction deprived of RNA-binding proteins	Fraction of RNA-binding proteins	Exp. 1	Exp. 2
+	–	–	4400	
–	+	–	510	530
–	+	+	3380	4200
–	–	+		3350

The cell-free system contained, in addition to the protein fractions indicated: 80 S ribosomes, [^{14}C]aa-tRNA, TMV RNA, GTP, ATP and a GTP–ATP-regenerating system (see section 2)

and added it into the cell-free system with the inactive S100-DEAE fraction that passed through RNA–Sepharose. The activity of the system was significantly restored. The result is given in table 1, exp. 1. Thus, the factors were not inactivated by the RNA–Sepharose column, and the second alternative proved to be true:

> At least one or some of the translation factors were adsorbed on RNA as RNA-binding proteins.

In the next experiment we decided to test the translation factor activity of the RNA-binding protein preparation itself, without introducing either of the S100-DEAE fractions into the cell-free system. The result shown in table 1, exp. 2, was unexpected: the preparation of RNA-binding proteins ensured the active working of the system, thus substituting for the totality of the initiation and elongation factors. Consequently, RNA-binding proteins of wheat embryo cytoplasmic extract include all (or almost all) the initiation and elongation factors of the protein-synthesizing system.

On the other hand, this experiment indicates that the proteins, being initiation and elongation factors, at the same time possess yet another function, namely that of binding to RNA with a high affinity. The RNA-binding function of the eukaryotic translation factors has been recently shown in direct binding experiments, using individual factors from animal cells (T.N.V. and Domogatsky, unpublished).

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