RNA-BINDING PROTEINS OF RABBIT RETICULOCYTES CONTAIN THE TWO ELONGATION FACTORS AND SOME OF THE INITIATION FACTORS OF TRANSLATION

L. P. OVCHINNIKOV and A. S. SPIRIN
Institute of Protein Research, Academy of Sciences of the USSR, 142292 Poustchino, Moscow Region, USSR

and

B. ERNI and T. STAEHELIN
Basel Institute for Immunology, 4058 Basel, Switzerland

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1. Introduction

Cytoplasmic extracts of animal cells have been shown to contain a characteristic fraction of proteins which are capable of forming complexes with RNA [1–4]. The proteins display a high specific affinity to RNA; the binding constants have been estimated to be of the order of 10^7–10^{13} M^{-1} [5]. The protein:RNA ratio of about 3:1 and a number of physicochemical properties of the ribonucleoprotein particles formed are indistinguishable from those of natural messenger ribonucleoproteins called informosomes [2,6].

In the preceding communication [7] experiments were reported showing that the total fraction of cytoplasmic RNA-binding proteins of wheat embryos can effectively substitute for the total preparation of initiation and elongation factors in the wheat embryo cell-free translation system. This result led to the conclusion that the wheat embryo RNA-binding proteins contain most, if not all, of the initiation and elongation factors.

In the work reported here we investigated the ability of RNA-binding proteins from rabbit reticulocytes to replace individual initiation and elongation factors in a purified eukaryotic cell-free translation system. We show that preparations of RNA-binding proteins compensate completely for the absence of the elongation factors EF-1 and EF-2, as well as of the initiation factors eIF-4C and eIF-5. A partial compensation of some other initiation factors is also observed.

2. Materials and methods

RNA-binding proteins were isolated by affinity chromatography of rabbit reticulocyte ribosome-free extract on columns with *E. coli* ribosomal RNA covalently bound to Sepharose as detailed in [8]. RNA-binding proteins were adsorbed on the matrix-bound RNA at 0.01 M KCl and washed from the column by a buffer containing 1 M KCl. Two preparations of RNA-binding proteins were used in the experiments: a preparation of total RNA-binding proteins and a preparation of RNA-binding proteins with a high affinity to RNA; the latter was obtained by passing an excess of ribosome-free extract through the affinity column [8].

The functional activity of RNA-binding proteins was determined in two types of cell-free translation systems: in a poly(U)-dependent system and in a globin mRNA-dependent system. All the components of the system were isolated as in [9].

The elongation factor activities of the RNA-binding protein preparation were tested in the poly(U)-dependent polyphenylalanine-synthesizing system [9]. The incubation mixture (0.1 ml) contained:

5 pmol each of 40 S and 60 S mouse liver ribosome subunits, 15 μg poly(U), 12 μg yeast tRNA charged with [^{3}H]Phe (9 Ci/mmol) (20 pmol [^{3}H]Phe-tRNA), saturating amounts of one or both the elongation factors (2.5 μg EF-1 with a purity of about 50% and 0.2 μg 90% pure EF-2 were used for saturation) and aliquots of the RNA-binding protein preparation under investigation; incubation was in a medium consisting of 20 mM Tris–HCl (pH 7.6), 10 mM $MgCl_2$, 0.1 M KCl, 5 mM β-mercaptoethanol and 1 mM GTP for 3 min at 37°C. Trichloroacetic acid was added to 10%, the mixtures heated for 10 min at 90°C and the precipitates collected on nitrocellulose filters, washed with 5% trichloroacetic acid and dried. Radioactivity on the filters was measured in a liquid scintillation spectrometer (at 25% efficiency).

The initiation factor activities of the RNA-binding protein preparations were determined in the translation system dependent on globin mRNA described as system 'b' [9]. The system contained the following biological components per 0.1 ml: 0.14 A_{260} units 40 S and 0.36 A_{260} units 60 S mouse liver ribosome subunits, 0.05 A_{260} units globin mRNA, 0.12 A_{260} units rabbit reticulocyte tRNA, ca. 20 μg rat liver, pH 5, enzyme as a source of aminoacyl-tRNA-synthetase, 3 nmol each of the 19 unlabelled amino acids and 3 nmol [^{14}C]leucine (spec. act. 43 Ci/mol) and the elongation and initiation factors in saturating amounts; incubation was in a medium consisting of 1 mM ATP, 0.4 mM GTP, 30 mM Hepes–KOH (pH 7.3) (Hepes = *N*-2 hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid), 0.07 M KCl, 0.05 mM spermine, 10 mM β-mercaptoethanol and 2.7 mM $MgCl_2$. To saturate 0.1 ml standard assay the following amount of factors (30–90% pure) were introduced; 5 μg EF-1 (purity about 50%); 0.2 μg 90% pure EF-2; 0.1 μg eIF-1; 2 μg eIF-2; 5 μg eIF-3; 2 μg eIF-4A; 4 μg eIF-4B; 0.2 μg (eIF-4C + eIF-5). The system was incubated at 37°C for 40 min. Radioactivity of the polypeptides was determined as described for the first system (^{14}C counting efficiency was 80%).

3. Results

Figure 1 shows densitograms of Coomassie blue-stained polyacrylamide gels after electrophoresis of the two preparations of RNA-binding proteins in the presence of sodium dodecyl sulfate. The preparation consisting of all RNA-binding proteins from rabbit reticulocytes (fig.1A) contains three major polypeptide chains with molecular ratios of about 95 000, 49 000 and 36 000, and also a large number of less prominent components. The preparation of proteins

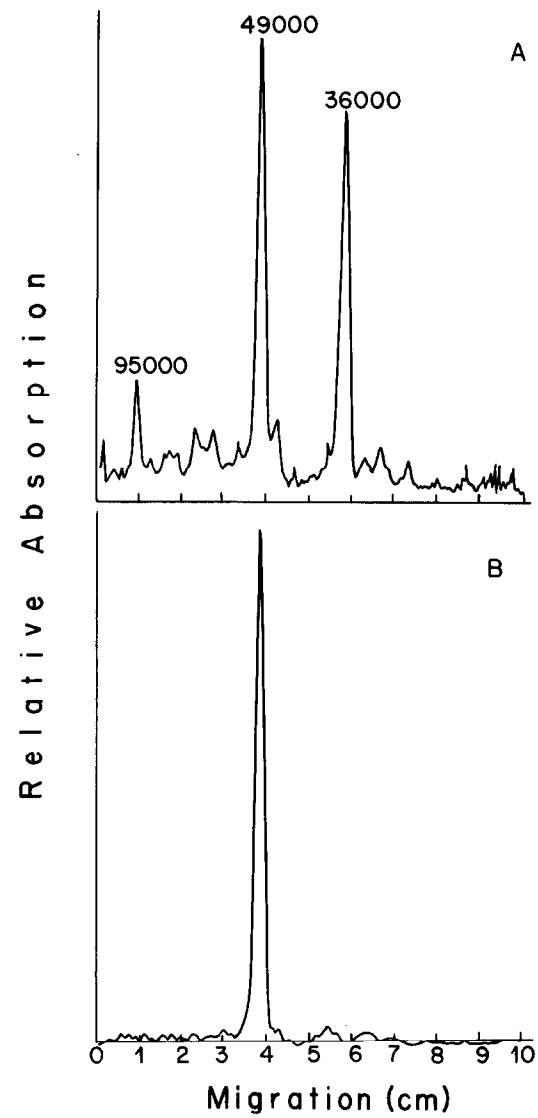

Fig.1. Results of polyacrylamide gel scanning after electrophoresis of the preparations of the total RNA-binding proteins (A) and the RNA-binding proteins with a high affinity to RNA (B). Electrophoresis was in the presence of sodium dodecyl sulfate as in [8].

with a high affinity to RNA contains one major component with a molecular ratio of about 49 000 comprising at least 70–75% total protein mass in the preparation, as well as some minor components (fig.1B). These data are in complete accord with [8].

Table 1 shows the elongation factor activities in the preparation of RNA-binding proteins with a high affinity to RNA, as determined in the system with poly(U) as template. The residual polyphenylalanine synthesis in the absence of EF-1 is about 2% and in the absence of EF-2 about 15–17% complete system. Addition of RNA-binding proteins to assays without EF-1 and EF-2 results in a recovery of the original activity, i.e., RNA-binding proteins completely substitute for EF-1 and EF-2. As little as 2 μg RNA-binding protein preparation fully compensate for the absence of EF-1 or EF-2 in the system. The addition of the RNA-binding proteins to the complete system does not give a significant stimulation.

The initiation factor activities in the preparation of RNA-binding proteins with a high affinity to RNA and in the preparation of total RNA-binding proteins were determined in the globin mRNA-dependent translation system. The results presented in tables 2 and 3 show that the RNA-binding proteins are able to compensate entirely for the absence of the (eIF-4C + eIF-5) preparation in the system. In addition, 6 μg of either preparation of RNA-binding proteins seems to substitute partially also for eIF-1 and to a lesser extent for eIF-3 and eIF-4B. On the other hand, the preparations of RNA-binding proteins contain practically no eIF-2 and eIF-4A activities.

It is noteworthy that RNA-binding proteins when added to the complete globin mRNA-directed cell-free system containing all the initiation and elongation factors in saturating amounts induce a significant (1.5-fold) increase of amino acid incorporation (table 2). This could mean either that a yet unknown stimulatory factor is present in the RNA-binding protein fraction which is limiting or absent in the translation system or that some known component(s) in the translation system was (were) partially inactivated and can be supplemented by the more active RNA-binding protein fraction.

4. Discussion

It was shown [8] that a small but quite specific fraction of cytoplasmic proteins can be purified from ribosome-free extracts of reticulocytes by affinity chromatography with immobilized RNA, such as *E. coli* RNA, or synthetic homopolyribonucleotides, such as poly(U) or poly(A). These RNA-binding proteins are the same regardless of the nature of polynucleotide used for binding. During

Table 1
Stimulation of [^{3}H]phenylalanine incorporation by the preparation of RNA-binding proteins with a high affinity to RNA in the poly(U)-dependent cell-free system

Composition of cell-free system	Incorporation of [^{3}H]phenylalanine into the acid-insoluble product (pmol)
Complete	2.02
– EF-1	0.04
– EF-1 + RNA-binding protein (2 μg)	2.23
– EF-1 + RNA-binding protein (4 μg)	2.24
– EF-1 + RNA-binding protein (6 μg)	2.36
– EF-2	0.35
– EF-2 + RNA-binding protein (2 μg)	2.13
– EF-2 + RNA-binding protein (4 μg)	2.33
– EF-2 + RNA-binding protein (6 μg)	2.52
Complete + RNA-binding protein (6 μg)	2.42

Table 2
Stimulation of [^{14}C]leucine incorporation by the preparation of RNA-binding proteins with a high affinity to RNA in the globin mRNA-dependent cell-free system

Composition of cell-free system	Incorporation of [^{14}C]leucine into the acid-insoluble product (pmol)
Complete	50.8
– eIF-1	13.0
– eIF-1 + RNA-binding protein (6 μg)	30.6
Complete	46.0
– eIF-2	4.0
– eIF-2 + RNA-binding protein (6 μg)	9.0
Complete	57.8
– eIF-3	10.6
– eIF-3 + RNA-binding protein (6 μg)	22.0
Complete	60.5
– eIF-4A	5.0
– eIF-4A + RNA-binding protein (6 μg)	6.6
Complete	47.6
– eIF-4B	12.5
– eIF-4B + RNA-binding protein (6 μg)	17.2
Complete	45.4
–(eIF-4C + eIF-5)	5.9
–(eIF-4C + eIF-5) + RNA-binding protein (6 μg)	45.9
Complete	51.2
Complete + RNA-binding protein (6 μg)	78.5

binding of excess RNA three major and many minor proteins are bound. The major components have M_r of 95 000, 49 000 and 36 000 (fig.1A). During binding of excess protein the 49 000 M_r protein displaces the other proteins and can be eluted from the RNA column at more than 70% purity (fig.1B). It is obvious that this protein has the highest affinity for RNA.

In the present study we asked whether the cytoplasmic RNA-binding proteins from rabbit reticulocytes had any functional relationship to the defined mammalian protein synthesis elongation and initiation factors. We could demonstrate in fractionated and reconstituted translation systems that both the total RNA-binding protein fraction and the high affinity RNA-binding protein fraction contain the elongation factors EF-1 and EF-2, the initiation factors eIF-4C and eIF-5 and to a lesser extent eIF-1. Quite low activities of eIF-3 and eIF-4B and no eIF-2 and eIF-4A activities were present in our RNA-binding proteins although the high affinity of eIF-2 for RNA was shown [10]. We know, however, that the reticulocyte high speed supernatant prepared according to our procedure is practically devoid of this initiation factor (B. E. and T. S., unpublished). On the other hand, eIF-4A can be purified from the high-speed supernatant by other methods but seems to have no affinity for RNA.

From electrophoretic analysis (fig.1B), the main component with a molecular ratio of 49 000 comprises no less than 70–75% high affinity RNA-binding protein mass. Two micrograms or less of this preparation tested in the poly(U) assay were equivalent to 2.5 μg of a 50% pure preparation of EF-1

Table 3
Stimulation of [^{14}C]leucine incorporation by the preparation of total RNA-binding proteins in the globin mRNA-dependent cell-free system

Composition of cell-free system	Incorporation of [^{14}C]leucine into the acid-insoluble product (pmol)
Complete	48.2
– eIF-1	15.1
– eIF-1 + RNA-binding protein (6 μg)	27.1
Complete	41.2
– eIF-2	3.2
– eIF-2 + RNA-binding protein (6 μg)	5.2
Complete	39.2
– eIF-3	9.2
– eIF-3 + RNA-binding protein (6 μg)	15.1
Complete	47.1
– eIF-4A	5.0
– eIF-4A + RNA-binding protein (6 μg)	6.1
Complete	40.8
– eIF-4B	11.5
– eIF-4B + RNA-binding protein (6 μg)	20.5
Complete	44.4
–(eIF-4C + eIF-5)	3.8
–(eIF-4C + eIF-5) + RNA-binding protein (6 μg)	28.1

(see section 2 and table 1). Therefore, the 49 000 M_r component of the RNA-binding proteins appears to represent EF-1 in accord with the molecular ratio of about 50 000 found for eukaryotic EF-1 [9]. The other factors (EF-2, $M_r \sim 90\,000$; eIF-4C, $M_r \sim 19\,000$; eIF-5, $M_r \sim 160\,000$ and eIF-1, $M_r \sim 15\,000$) present in the RNA-binding proteins are required in such minute amounts, if the preparation is active, that the absence of major components with these M_r in the RNA-binding protein fractions is not astonishing. On the other hand, the polyacrylamide gel electrophoretic band pattern, at least of the high affinity RNA-binding proteins (fig.1B), excludes the presence of significant amounts of eIF-2, eIF-3 and eIF-4B. The factor eIF-4A would in practice comigrate with EF-1.

Our results indicate that RNA affinity chromatography is a powerful method for the selective purification or enrichment of many of the factors involved in protein synthesis, in particular EF-1. This method may be even more selective and powerful than affinity chromatography with immobilized heparin [11,12]. Although the binding of these proteins to RNA may be explained in part by simple ionic interactions it is quite clear that the binding is much more selective and specific than that to an ordinary cation exchanger like phosphocellulose. It is likely that the specificity of binding to RNA of these proteins has something to do with their interaction with RNA during their functioning.

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