Phosphorylation of elongation factor 1 in polyribosome fraction of rabbit reticulocytes

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A single protein, $M_r \sim 50000$, is shown to be phosphorylated during incubation of a mono- and polyribosome fraction of rabbit reticulocytes with [γ-^{32}P]ATP at a low ionic strength. This protein has been identified as the elongation factor 1α (EF-1α). The phosphorylated EF-1α, in contrast to the unmodified factor, is not detected in complexes with mono- and polyribosomes. It is suggested that the phosphorylation of EF-1α can result in its decompartmentation from polyribosomes and thus affect the rate of protein synthesis.

Elongation factor 1 *Protein phosphorylation* *Polyribosome* *Translation regulation* *Compartmentation-decompartmentation*

1. INTRODUCTION

It is known that reversible phosphorylation of cellular proteins can act as a physiological mechanism regulating their functions. The initiation factor 2 [1], ribosomal protein S6 [2] as well as several aminoacyl-tRNA synthetases [3,4] have been shown to be among the components of the eukaryotic translation machinery subjected to phosphorylation. Phosphorylation of eIF-2 leads to inhibition of protein synthesis [5]. The regulatory role of the phosphorylation of ribosomal protein S6 and that of aminoacyl-tRNA synthetases has also been discussed [2–4].

Here, we have shown that intensive phosphorylation of the elongation factor 1α (EF-1α) is observed in vitro in the fraction of mono- and polyribosomes of rabbit reticulocytes at low ionic strength. We believe that the phosphorylation of EF-1α can play a regulatory role in the cell.

2. MATERIALS AND METHODS

Reticulocytes were obtained from blood of rabbits after subcutaneous injection with phenylhydrazine according to [6]. The cells were lysed with an equal volume of 5 mM $MgCl_2$ and the lysate was centrifuged at 12000 rpm for 15 min in a JA-14 rotor of a J-21B centrifuge (Beckman) to remove cell debris and mitochondria.

EF-1 was isolated according to a slightly modified procedure of [7].

The fraction of mono- and polyribosomes was prepared by gel filtration of the mitochondria-free extract through a column with Sephacryl S-300 (Pharmacia) equilibrated with a standard buffer solution containing 10 mM Tris–HCl (pH 7.6), 1 mM $MgCl_2$. The preparation was hemoglobin-free and contained about 90% of ribosomes of the initial extract.

Protein phosphorylation was performed by incubating the samples with [γ-^{32}P]ATP 1000 Ci/mmol (Isotope, USSR) for 5 min at 37°C.

To determine the degree of phosphorylation, the protein was precipitated with 5% trichloroacetic acid, collected on GF/C (Whatman) filters and the radioactivity counted.

Protein electrophoresis was carried out in a polyacrylamide gradient gel (10–22% acrylamide) in the presence of 0.1% SDS [8].

Two-dimensional separation of proteins was done according to a modified procedure of [9]. Due to strong phosphatase activities a phosphatase

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inhibitor $ZnCl_2$ was added to the preparations to a concentration of 10 mM. Before radioautography the gels were incubated with 1 M NaOH at 60°C for 1 h.

To obtain a radioautograph of the labeled proteins the gels were dried and exposed overnight at −70°C using an RM-V film (Tasma, USSR).

Centrifugation of the preparations was carried out in an isokinetic 15–33.5% sucrose gradient made in a standard buffer at 40000 rpm for 75 min at 4°C in an SW-41 rotor.

3. RESULTS

The fraction of mono- and polyribosomes obtained by gel-filtration of the mitochondria-free extract through Sephacryl S-300 equilibrated with a standard buffer (low ionic strength) contains a considerable amount of labile associated RNA-binding proteins. Both the elongation factors 1 and 2, as well as aminoacyl-tRNA synthetases, have been found among them.

A broad range of different proteins is shown to be phosphorylated during incubation of the fraction of mono- and polyribosomes with [γ-^{32}P]ATP in the standard buffer with 150 mM KCl (fig.1A).

Incubation of this preparation with [γ-^{32}P]ATP in the standard buffer without KCl demonstrates that under these conditions the radioactive label is incorporated into only one polypeptide with a molecular mass of about 50 kDa (fig.1B, 2A)

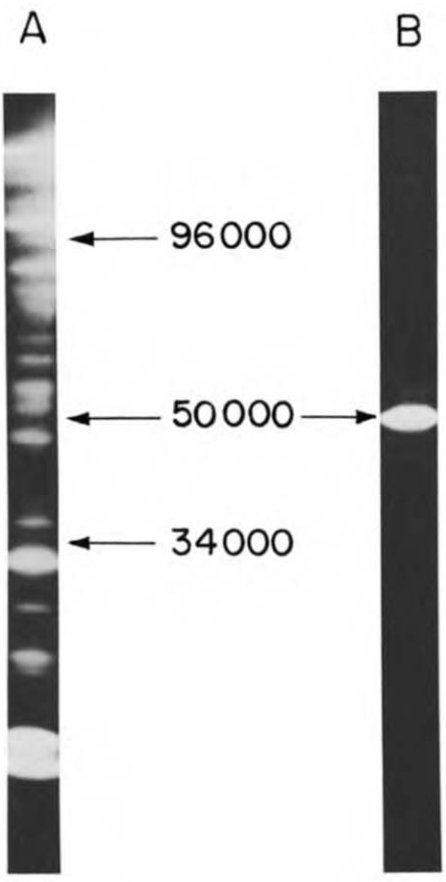

Fig.1. Radioautograph showing phosphorylation of proteins in the mono- and polyribosome fraction at 150 mM KCl (A) and under conditions of low ionic strength (B). 100-μl samples contained 0.5 A_{260} units of the polyribosome fraction and 1 μCi [γ-^{32}P]ATP.

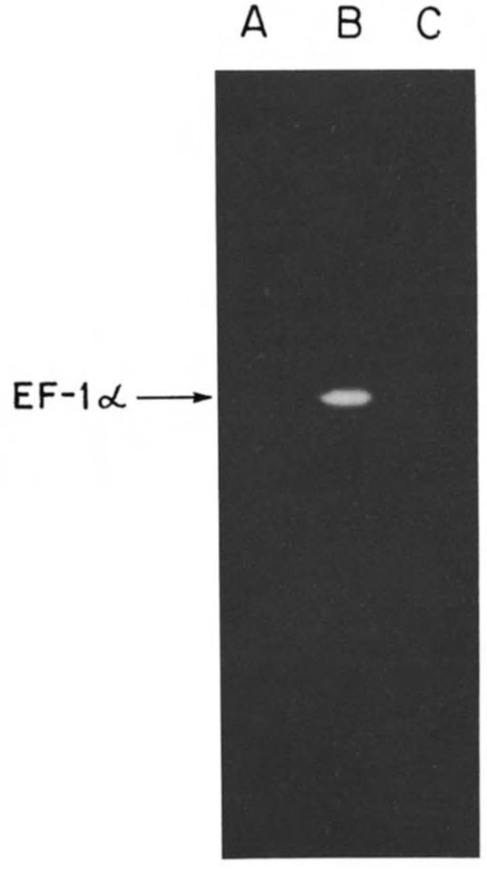

Fig.2. Radioautograph showing phosphorylation of proteins in the fraction of mono- and polyribosomes (A), in the preparation of the EF-1_H (C) and in their mixture (B) at low ionic strength. 100-μl samples contained 0.05 A_{260} units of the polyribosome fraction (A and B), 10 μg of EF-1_H (B and C) and 1 μCi [γ-^{32}P]ATP.

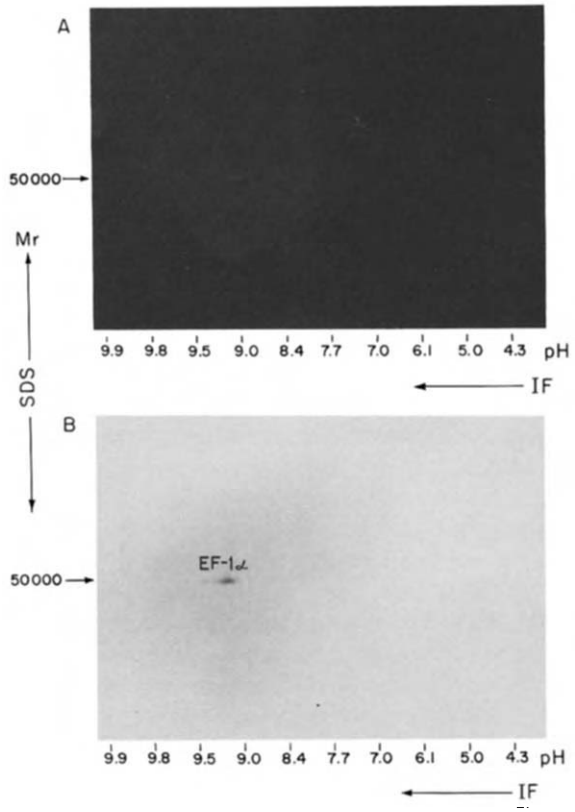

Fig.3. Two-dimensional analysis according to [7] of proteins from the mono- and polyribosome fraction incubated with [γ-^{32}P]ATP at low ionic strength (radioautograph) (A) and of the EF-1_L (stained with Coomassie) (B).

whose position in the gel corresponds to that of the α-subunit of EF-1.

Incorporation of the radioactive label into the M_r ~50000 polypeptide band is considerably increased upon the addition of a preparation of EF-1_H to the incubation mixture (fig.2B). EF-1α is practically not phosphorylated during control incubation of an EF-1_H preparation (95% purity) with [γ-^{32}P]ATP (fig.2C).

Two-dimensional analysis according to [7] corroborated the identity of the approx. 50-kDa polypeptide, phosphorylated in the mono- and polyribosome fraction, to the α-subunit of EF-1 (fig.3).

Centrifugation of the mono- and polyribosome fraction in the sucrose gradient and following incubation with [γ-^{32}P]ATP of individual fractions, has shown that the radioactive label is incorporated into the trichloroacetic acid-insoluble product in both the poly- and monoribosomes (fig.4A). Electrophoresis and radioautography demonstrate that the label in all the individual fractions of the gradient is present in the same approx. 50-kDa polypeptide which has been identified as EF-1α.

When an excess of exogenous high molecular mass RNA is added to the mono- and polyribosome fraction before centrifugation, the incorporated radioactive label is found in the post-ribosomal zone where exogenous RNA is distributed (fig.4B). This result correlates with the observation on the displacement of EF-1 from polyribosomes by an excess exogenous RNA [10].

Sucrose gradient centrifugation of the mono- and polyribosome fraction preincubated with [γ-^{32}P]ATP shows that all the acid-insoluble radioactive material is distributed at the top of the gradient and is absent from the zone of mono- and polyribosomes (fig.4C).

4. DISCUSSION

Several proteins of the eukaryotic translation machinery, including the elongation factors and aminoacyl-tRNA synthetases, are known to possess a non-specific affinity for high molecular mass RNAs. This affinity provides for their labile association with mono- and polyribosomes [11].

It is proposed that the affinity for RNA serves for partial compartmentation of the proteins of the translation machinery in places of their functioning in the big volume of the eukaryotic cell [12] while a change of this affinity may serve for regulation of protein synthesis at the translation (elongation) level [11]. In particular, inhibition of protein synthesis after ADP-ribosylation of EF-2 was shown to be accompanied by a loss of its non-specific affinity for RNA and its dissociation from the loose complexes with mono- and polyribosomes [13]. It has also been found that phosphorylation of several RNA-binding proteins with non-identified functions changes their affinity for RNA [14].

Here, we have shown that EF-1 can be phosphorylated at the α-subunit. It is noteworthy that the kinase phosphorylating EF-1α, just as EF-1α itself, is found in association with mono-

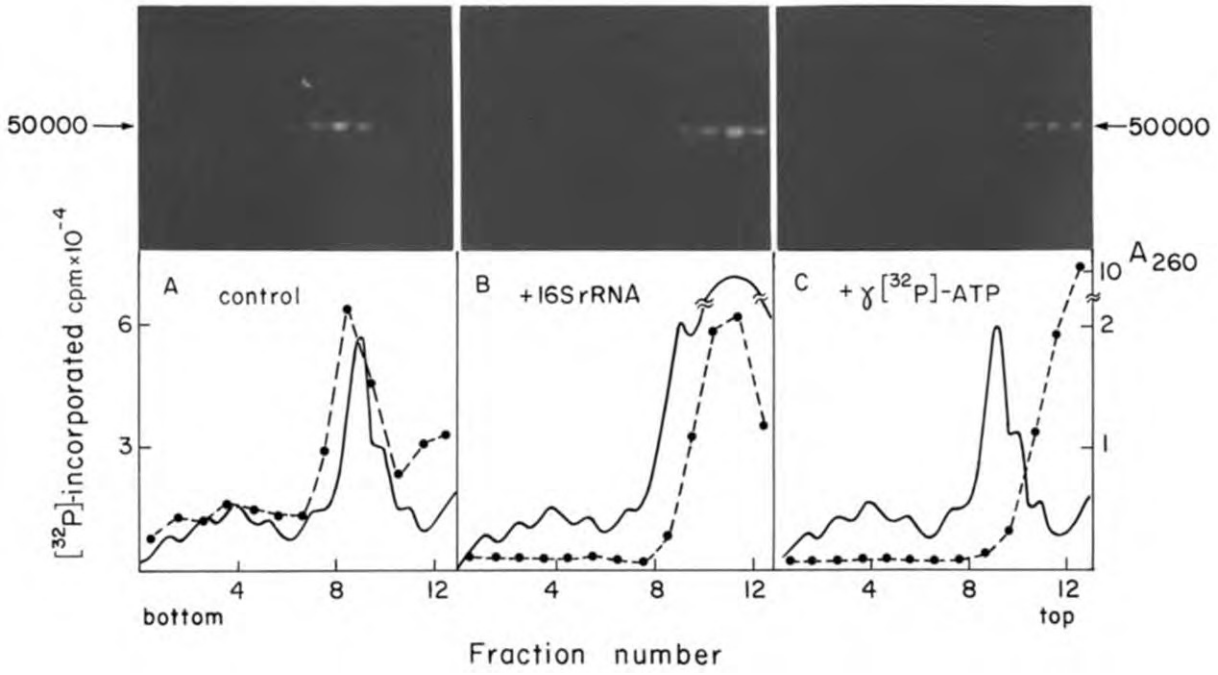

Fig.4. Sedimentation distribution of the material of the mono- and polyribosome fraction at sucrose gradient centrifugation. A, B, 1 μCi [γ-^{32}P]ATP was added to each gradient fraction after centrifugation; B, 20 A_{260} units of *Escherichia coli* 16 S ribosomal RNA were added to the polyribosome fraction before centrifugation; C, the polyribosome fraction was incubated with 13 μCi [γ-^{32}P]ATP before centrifugation. Gel electrophoresis of the labeled proteins from the corresponding gradient fractions (radioautographs) are given at the top.

and polyribosomes. Activation of the kinase in vitro results from a decrease of the ionic strength of buffer solution and its action under these conditions is strictly selective relative to the α-subunit of EF-1. The phosphorylated form of EF-1, in contrast to the unmodified EF-1, has not been found in complexes with mono- and polyribosomes. We believe that the phosphorylation of EF-1 results in its dissociation from complexes with mono- and polyribosomes, probably due to a loss of affinity for RNA.

It is not unlikely that the phosphorylation of EF-1α may affect the rate of polypeptide chain elongation and serve for regulation of the translation process in the cell.

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