PROTEIN KINASE ACTIVITY IN RNA-BINDING PROTEINS OF *AMPHIBIA* OOCYTES

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1. Introduction

Cell mRNA exists in the form of informosomes which represent complexes of mRNA with specific proteins. These RNA-binding proteins and informosomes have been found in practically all eukaryotic organisms. These proteins can play an important role in regulation of translation [1–3] and, in particular, in the processes of mRNA masking–demasking [4]. Considering that at least a part of RNA-binding proteins represents a pool for the formation of informosomes in the cell [1,5] the enzymic functions of these proteins are of interest in explaining their rôle in the processes of mRNA masking–demasking.

The presence of phosphorylated proteins within the isolated informosomes [6–8,10] and of protein kinase activity in the fraction of these particles [6,7,9] permits us to assume that phosphorylation–dephosphorylation of informosomal proteins plays an important role in the mechanism of informosome functioning. However, isolation of native pure informosomes is very difficult and the probability of protein or ribosomal contamination cannot be excluded [11]. Preparation of free informosome-forming proteins from ribosome-free extracts has been determined in [12,13].

Here, we have found protein kinase activity in the fraction of RNA-binding proteins and have characterized some of its properties which allow us to discuss a possible regulatory role of these proteins in the cells.

2. Materials and methods

RNA-binding proteins were isolated by affinity chromatography on poly(U)–Sepharose 4B from ribosome-free extracts of *Rana temporaria* ripening oocytes or from *Xenopus laevis* eggs as detailed in [13]. The RNA-binding activity in the preparations was assayed as in [15] and the protein content as in [16].

Phosphorylation of RNA-binding proteins in vitro was carried out in standard buffer: 0.01 M triethanolamine, 0.01 M KCl, 5 mM $MgCl_2$, 6 mM mercaptoethanol (pH 7.8) in the presence of [γ-^{32}P]ATP (~2000 cpm/pmol, Isotop, Tashkent). The reaction was stopped by adding trichloroacetic acid to 5% final conc. The acid-precipitated material was applied on nitrocellulose or glass filters, washed with 5% trichloroacetic acid and dried. Treatment of the cold acid-precipitated material with hot 5% trichloroacetic acid (90°C, 15 min) and 96% ethanol did not result in a decreased absorption of radioactive material on the filters, therefore these operations were not carried out further.

The degree of phosphorylation of RNA-binding proteins was expressed as the amount of radioactive phosphate (pmol) incorporated into 1 μg protein during the indicated time interval. The non-specific absorption of [γ-^{32}P]ATP at each protein concentration in the reaction mixture was taken into account. Each experimental point represents an averaged result of 3 independent measurements.

Both phosphorylated and non-phosphorylated proteins were analyzed by standard one-dimensional slab-gel electrophoresis in the presence of SDS [17] followed by autoradiography of the dried gels.

To identify *O*-[^{32}P]phosphoserine and *O*-[^{32}P]-phosphothreonine formed in the reaction, the phosphorylated proteins were partially hydrolyzed with 6 N HCl at 105–110°C under decreased pressure for 2 h [18]. Hydrolyzed samples were analyzed by high-voltage electrophoresis on Whatman 3 MM paper in 3 N acetic acid (pH 2.11) at 4900 V for 2 h. ^{32}P-containing hydrolyzates were analyzed by autoradiography.

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3. Results and discussion

RNA-binding proteins (0.05 mg) isolated from ribosome-free extracts of *Xenopus laevis* eggs by affinity chromatography on poly(U)–Sepharose were incubated with different amounts of [γ-^{32}P]ATP (from 0.25–2.5 nmol) in 200 μl standard buffer for 30 min at 22°C. The samples were then treated as in section 2; the results are presented in fig.1. A considerable incorporation of radioactive phosphate into RNA-binding proteins (~1 pmol/μg) was observed under these arbitrary conditions. In further experiments [γ-^{32}P]ATP and RNA-binding proteins were added to the reaction mixture in a ratio of $\geqslant$100 pmol ATP/1 μg protein.

The optimum temperature for incorporation of radioactive phosphate into RNA-binding proteins of *X. laevis* oocytes is ~20°C (fig.2). A decrease of the phosphorylation level when the temperature is lowered (to 0–7°C) or raised (to 30–33°C) can be explained either by inactivation of protein kinases or activation of phosphatases which are probably present in the preparation of RNA-binding proteins. The pH optimum of the reaction studied is not definitely within 7–9 pH units. Analogous results have also been obtained in a study of self-phosphorylation of RNA-binding proteins isolated from *Rana temporaria* oocytes.

High-voltage paper electrophoresis of hydrochloride hydrolyzates of proteins phosphorylated in vitro has

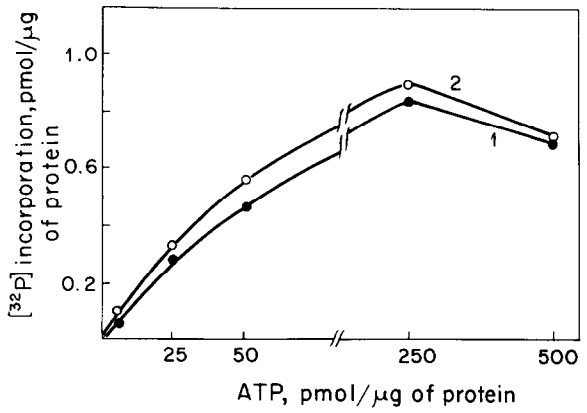

Fig.1. Dependence of radioactive phosphate incorporation into RNA-binding proteins on [γ-^{32}P]ATP concentration: (1) cold trichloroacetic acid was added to the samples to 5% and the acid-precipitated material was applied to nitrocellulose filters; (2) before applying the acid-precipitating material to the filters, it was treated with hot 5% trichloroacetic acid (90°C, 15 min) and 96% ethanol.

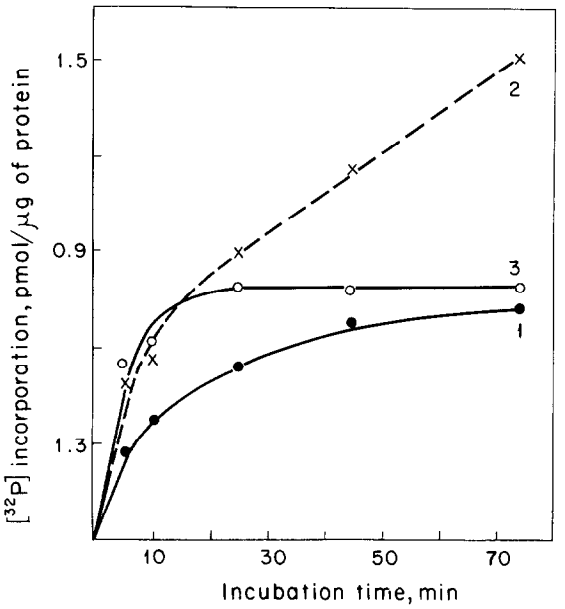

Fig.2. Dependence of the radioactive phosphate incorporation into RNA-binding proteins on incubation time at different temperatures: (1) incubation at 0–7°C; (2) incubation at 20–22°C; (3) incubation at 30–33°C.

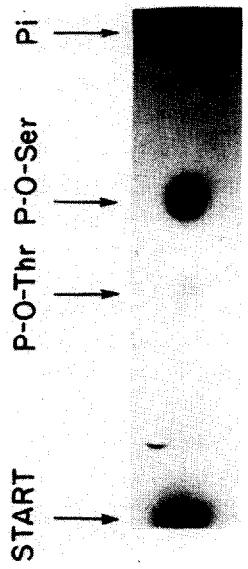

Fig.3. Autoradiographic identification of the products of hydrochloride hydrolysis of [^{32}P]RNA-binding proteins. After the end of incubation, a 3-fold volume of cooled ethanol was added to the reaction mixture. The precipitated proteins were subjected to partial hydrolysis by 6 N HCl (see section 2) and were analyzed by high-voltage paper electrophoresis. The electrophoregram with an HS-11 X-ray film (Orwo) was exposed for 24 h. Arrows indicate the positions of standards.

shown that the radioactive phosphate is found predominantly in phosphoserine residues and in small amounts in phosphothreonine residues (fig.3). As revealed by SDS–polyacrylamide gel electrophoresis not every polypeptide chain contained in the heterogeneous fraction of RNA-binding proteins (fig.4) can serve as a substrate for endogenous protein kinase.

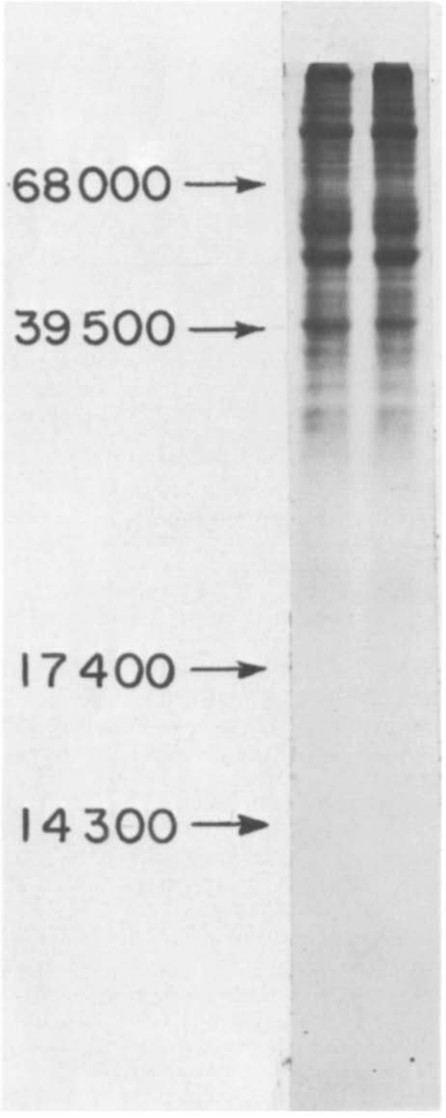

Fig.4. Electrophoresis of the preparation of RNA-binding proteins (10 μg) in a polyacrylamide gel slab in the presence of sodium dodecyl sulfate. The slab was stained with Coomassie blue R-250. Arrows indicate the positions of standards (M_r): bovine serum albumin (68 000); glyceraldehyde phosphate-dehydrogenase (39 500); TMV protein (17 400); lysozyme (14 300).

Its main substrates are polypeptide chains with $M_r \sim 75\,000$, 54 000, 33 000, 30 000, 26 000 and 17 000 (fig.5).

The addition of 5 μM cAMP to the reaction mixture does not affect the extent of protein phosphorylation. Thus, it can be considered that the fraction of RNA-binding proteins of *Amphibia* oocytes contains cAMP-independent protein kinase and endogenous substrates for phosphorylation. Their amount is limited: of the dozens of polypeptide chains contained in the fraction of RNA-binding proteins, only 6–7 polypeptides are phosphorylated by endogenous pro-

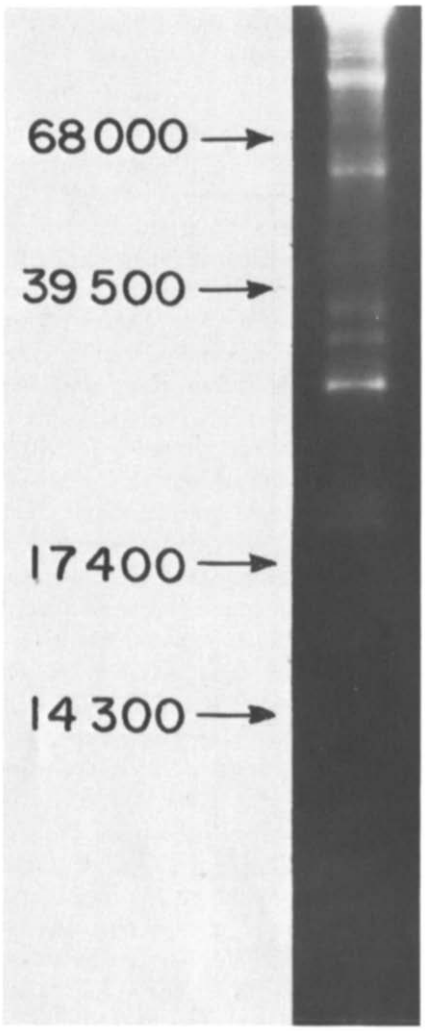

Fig.5. Electrophoresis of RNA-binding proteins phosphorylated in vitro. The figure presents the autoradiograph of the dried gel. The film was exposed for 48 h. Arrows indicate the positions of standards (see legend to fig.4).

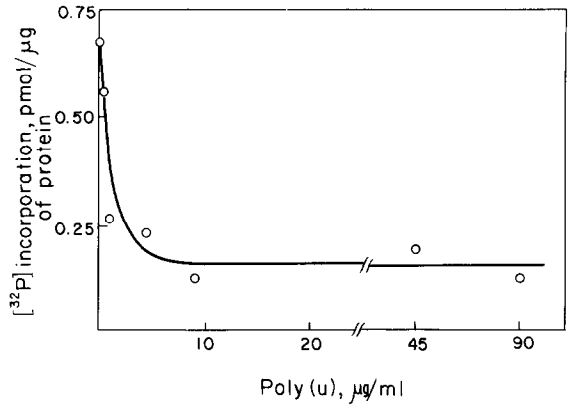

Fig.6. Dependence of self-phosphorylation of RNA-binding proteins on poly(U) concentration in the protein kinase system.

tein kinase, predominantly at the serine residues.

These results agree with [19] where endogenous protein kinase activity was detected in the fraction of RNA-binding proteins from embryonic muscle.

Fig.6 presents the data indicating that self-phosphorylation of RNA-binding proteins is suppressed by free polynucleotides. Thus, the presence of 1 μg poly(U)/ml in the reaction mixture inhibits the reaction by 70–80%. Special control experiments have shown that even the maximum poly(U) concentrations used do not affect the background of [γ-^{32}P]ATP absorption either in the presence or absence of RNA-binding proteins. An analogous inhibiting effect is noted for tRNA and poly(I):poly(C) (not shown).

Another interesting question is whether phosphorylation of RNA-binding proteins affects their ability for interaction with RNA. Experiments performed to estimate the ability of phosphorylated RNA-binding proteins to form 'minimal' (see [20]) RNP-complexes with RNA have shown that preincubation of these proteins in a standard protein kinase system containing 0.2–0.5 mM unlabeled ATP can lead to a 1.5–2-fold decrease of the ability of RNA-binding proteins to retain [^{14}C]rRNA of *Escherichia coli* on membrane nitrocellulose filters. We have also noted that during rechromatography on poly(U)–Sepharose of [^{32}P]-RNA-binding proteins phosphorylated in vitro in a standard system, some of the chains that have incorporated the radioactive phosphate can loose the ability for absorption on immobilized poly(U). On the basis of these experiments we presume that phosphorylation of RNA-binding proteins representing a pool of free informosome-forming proteins [1,5] leads to at least a partial decrease of their affinity for ribonucleic acids.

These results, as well as the observed suppression of self-phosphorylation of RNA-binding proteins by free polynucleotides indicate that the processes of mRNA masking–demasking can be regulated by phosphorylation of either the protein component of informosomes or the pool of free informosome-forming proteins.

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