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# cAMP-dependent activation of protein synthesis correlates with dephosphorylation of elongation factor 2

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The addition of 5 mM cAMP to a cell-free translation system from rabbit reticulocytes increases the rate of protein synthesis 3–5-fold. Lower concentrations of cAMP (0.005, 0.05 and 0.5 mM) have no effect on translation in this system. cAMP at all the concentrations tested stimulates the phosphorylation of the same pattern of polypeptides, while 5 mM cAMP additionally stimulates dephosphorylation of the 95 kDa polypeptide identified as elongation factor 2 (EF-2). Testing of the preparations of EF-2 with a different content of the phosphorylated form in poly(U)-directed poly(Phe) synthesis reveals that the EF-2 activity correlates with the fraction of non-phosphorylated EF-2. Thus cAMP-dependent activation of protein synthesis seems to be due to dephosphorylation of EF-2.

Protein phosphorylation; Elongation factor 2; Protein synthesis regulation; cyclic AMP-dependent activation; (Rabbit reticulocyte)

## 1. INTRODUCTION

It is well known that cAMP at 1 mM or more drastically stimulates cell-free protein synthesis [1–4]. This fact is usually attributed to the cAMP-dependent inactivation of the haemin-controlled repressor (HCR) followed by dephosphorylation of the initiation factor 2 $\alpha$-subunit (eIF-2$\alpha$) and stimulation of the initiation process [2–4]. On the other hand, Malkin and Lipmann [1] reported that cAMP at greater than 1 mM increases polyphenylalanine synthesis on poly(U) up to 5-fold, indicating that cAMP could activate the elongation stage of protein synthesis.

The aim of our study was to reveal changes in the pattern of protein phosphorylation in a working cell-free translation system from rabbit reticulocytes under cAMP-dependent stimulation. We found that dephosphorylation of just one protein, identified as EF-2, correlates with the activation of protein synthesis. Moreover, experiments with pure EF-2 revealed that phosphorylated EF-2 is significantly less active than the native form.

We suggest that the dephosphorylation of EF-2 causes the stimulation of protein synthesis by cAMP.

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## 2. MATERIALS AND METHODS

Pure preparations of EF-2 and eIF-2 from rabbit reticulocytes obtained by chromatography on RNA-Sepharose, hydroxyapatite, Mono Q and Mono S were a gift from Dr E.K. Davydova. Phosphorylated and non-phosphorylated EF-2 were separated by chromatography on a Mono Q column (Pharmacia) as in [5]. To prepare the cell-free translation system, rabbit reticulocytes obtained by the method of Adamson et al. [6] were lysed by an equal volume of 0.5 mM $MgCl_2$ solution containing 2 mM dithiothreitol and 40 $\mu$M haemin. Cellular debris was removed by centrifugation. Creatine kinase (Boehringer) was then added to the supernatant and the lysate placed in liquid nitrogen.

The mixture for cell-free translation (30 $\mu$l) contained 22 $\mu$l reticulocyte lysate, 20 mM Hepes (pH 7.6), 150 mM KOAc, 1.2 mM $MgCl_2$, 10 mM creatine phosphate, 1 mM ATP, 0.2 mM GTP, 0.5 mM spermidine, 1.0 mM dithiothreitol, 0.6 $\mu$Ci (40 $\mu$M) [$^{14}$C]Leu (Czechoslovakia), the 19 other amino acids (40 $\mu$M each) and cAMP in concentrations as indicated in section 3. Radioactivity incorporated into the protein was determined as in [7].

Protein phosphorylation in the cell-free translation system was studied in the mixture described above, which contained, in addition, 10 $\mu$Ci [$\gamma$-$^{32}$P]ATP (> 1000 Ci/mmol). 2.5-$\mu$l aliquots

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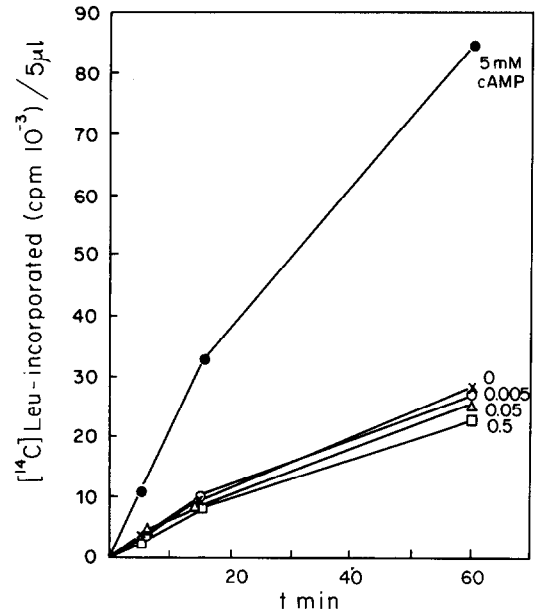


Fig.1. Influence of different cAMP concentrations on the rate of protein synthesis in a cell-free translation system from rabbit reticulocytes. Translation was carried out in the absence of cAMP (×) and in the presence of cAMP at 0.005 mM (○), 0.05 mM (□), 0.5 mM (△) and 5 mM (●).

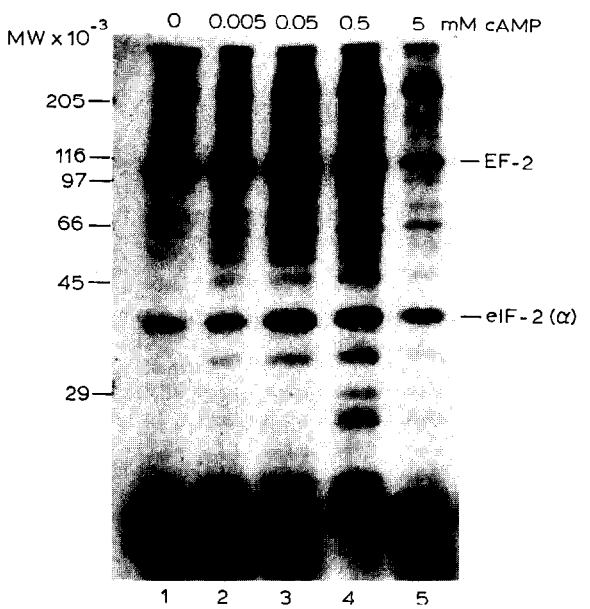


Fig.2. Influence of different cAMP concentrations on protein phosphorylation in the cell-free translation system from rabbit reticulocytes. The cell-free system was incubated with [$\gamma$-$^{32}$P]ATP in the absence of cAMP (1), in the presence of cAMP at 0.005 mM (2), 0.05 mM (3), 0.5 mM (4) and 5 mM (5). Radioautograph after SDS gel electrophoresis is presented.

were taken and subjected to electrophoresis with SDS [8] or two-dimensional separation according to O'Farrell [9]. Coomassie G-250 stained and dried gels were exposed with RM-V film (Tasma, USSR) at −70°C for 24 h.

Poly(U)-directed poly(Phe) synthesis was carried out as described [5].

## 3. RESULTS

The addition of 5 mM cAMP to the cell-free translation system from rabbit reticulocytes results in several-fold activation of [$^{14}$C]leucine incorporation into the protein. A lower concentration of cAMP has no effect on translation (fig.1). This result is in accordance with previous data [1,3,4].

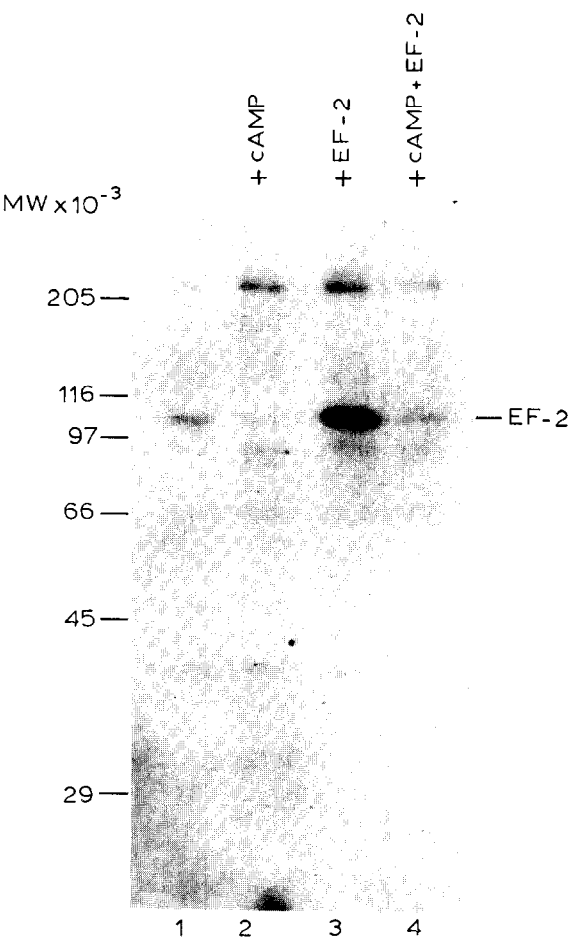


Fig.3. Protein phosphorylation in the cell-free translation system upon addition of EF-2 and cAMP. (1) No additions; (2,4) cAMP up to 5 mM added; (3,4) 4 µg EF-2 added. Radioautograph after SDS gel electrophoresis is presented.

A parallel incubation of the working cell-free translation system with [$\gamma$-$^{32}$P]ATP reveals the incorporation of $^{32}$P into a number of polypeptides (fig.2, lane 1). cAMP stimulates additional incorporation of $^{32}$P into some polypeptides (fig.2, lanes 2–5). Phosphorylation of these polypeptides is observed at all cAMP concentrations tested. However, upon addition of 5 mM cAMP, i.e. when protein synthesis is stimulated, a dramatic decrease of $^{32}$P incorporation into just one polypeptide (95 kDa) is observed. It has been shown previously that the major phosphorylated

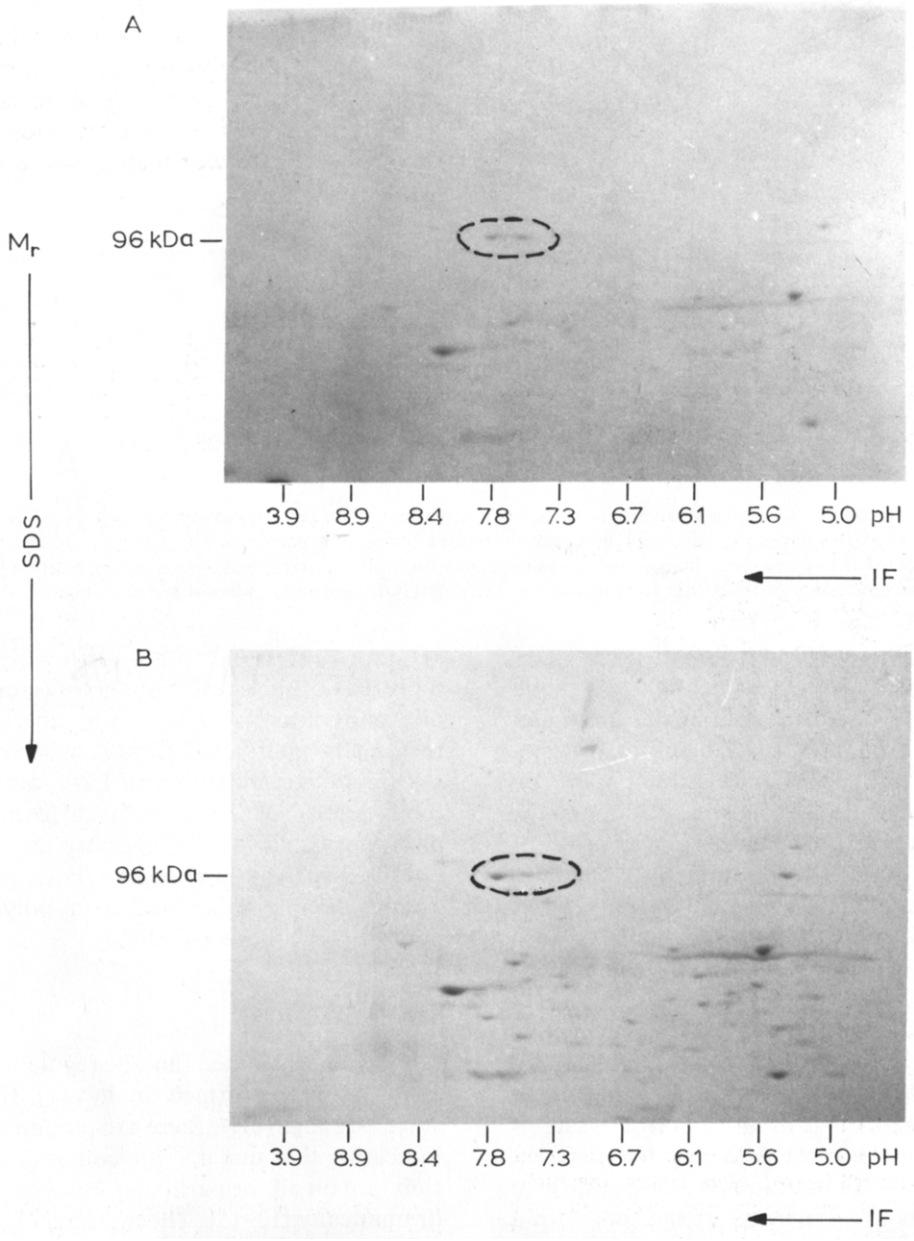


Fig.4. O'Farrell two-dimensional analysis of proteins of the cell-free translation system. The cell-free system was preincubated with [$\gamma$-$^{32}$P]ATP for 15 min at 32°C in the absence of cAMP (A) and in the presence of 5 mM cAMP (B). Staining with Coomassie.

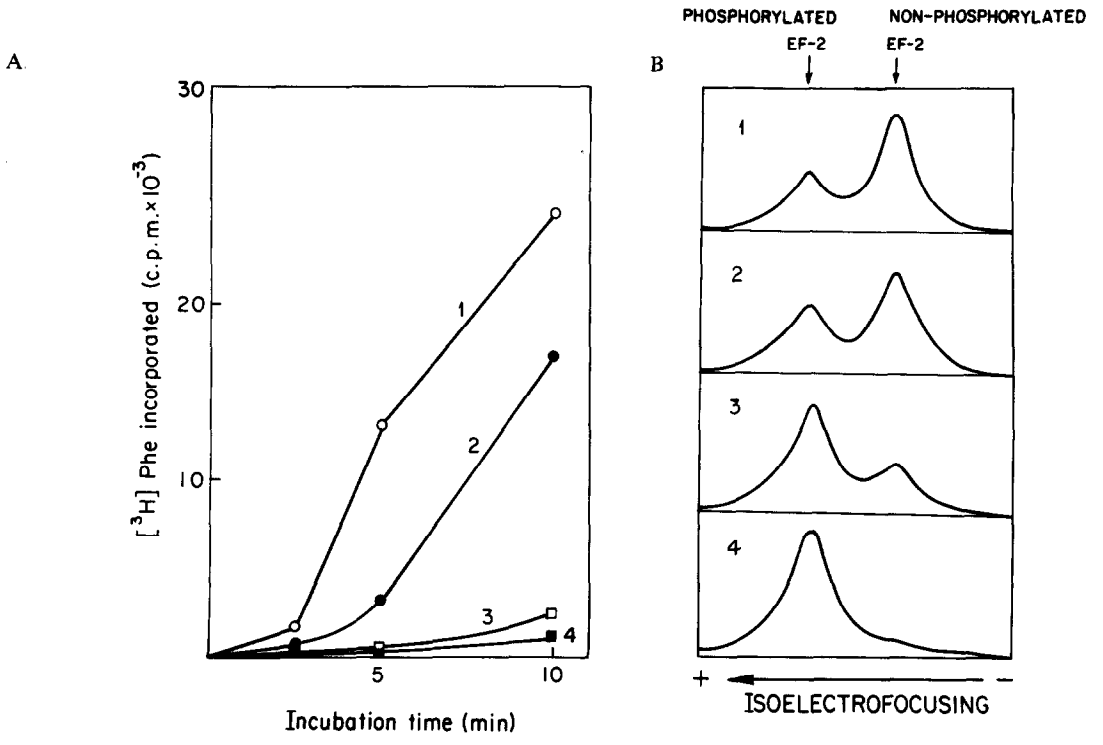


Fig.5. (A) Kinetics of polyphenylalanine synthesis in the poly(U)-dependent translation system upon addition of EF-2 preparations with different ratios of the phosphorylated and non-phosphorylated forms (see panel B). (B) The result of gel scanning after isoelectrofocusing of EF-2 preparations obtained by chromatography on Mono Q and used in experiments described in panel panel A. The conditions of isoelectric focusing and of the poly(U)-dependent translation are described in [5].

protein in the ribosome-free extract of rabbit reticulocytes is EF-2, which has a similar molecular mass, nearly 95 kDa [10]. Therefore, we investigated the influence of 5 mM cAMP on phosphorylation of EF-2 exogenously added to the lysate. As shown in fig.3 (lanes 1,3), addition of pure EF-2 to the lysate markedly stimulates phosphorylation of the 95 kDa polypeptide, while the addition of cAMP almost completely suppresses this phosphorylation of exogenous EF-2 (fig.3, lane 4).

A final identification of the 95 kDa polypeptide as EF-2 was made using the O'Farrell two-dimensional separation technique [9]. It is evident from fig.4A that EF-2 in the control lysate is represented by two spots with slightly different isoelectric points. The addition of 5 mM cAMP to the lysate results in an increase of the spot with a more basic isoelectric point (fig.4B). Radioautographs of these gels reveal that the spot with a more basic isoelectric point corresponds to non-phosphorylated EF-2 while the other corresponds to the phosphorylated form (not shown).

Direct determination of EF-2 activity in the preparations of EF-2 with different ratios of phosphorylated and non-phosphorylated forms reveals that the non-phosphorylated form of EF-2 is considerably more active in poly(U)-directed poly(Phe) synthesis (see fig.5).

## 4. DISCUSSION

While it is assumed that the regulation of protein synthesis is performed mainly at the initiation stage (see e.g. [11]), there are a number of reports indicating that under some conditions the elongation rate on all, or particular mRNAs, may change dramatically [12–15]. The molecular mechanism of such regulation is unclear, but covalent modifications of the elongation factors may be involved. It

has been shown that elongation factor EF-1 can be phosphorylated [16], and EF-2 can be ADP-ribosylated by a cellular ADP-ribosyl transferase [17,18]. In the latter case inhibition of the elongation process was observed [17].

The phosphorylation of EF-2 by a $Ca^{2+}$/calmodulin-dependent protein kinase (EF-2 kinase) has been reported recently [10,19]. The EF-2 kinase seems to be identical to the previously described $Ca^{2+}$/calmodulin-dependent protein kinase III [20]. This kinase was found to be activated in vivo, after treatment with growth factors and hormones [21] and may play a crucial role in cell metabolism.

The results of this work show (see also [5]) that the phosphorylated form of EF-2 is considerably less active than the native one in poly(U)-directed translation, and the cAMP-dependent activation of protein synthesis in rabbit reticulocyte lysate is most likely explained by the influence of cAMP on the degree of EF-2 phosphorylation.

It is noteworthy that treatment of PC-12 cells with nerve growth factor or agents increasing intracellular cAMP levels cause specific dephosphorylation of a 100 kDa protein [22,23]. This protein seems to be identical to EF-2 according to different criteria, including sequence analysis (Guroff, G. and Hama, Y.; Palfrey, H.C. and Nairn, A.C., personal communications). Thus, the influence of cAMP on the rate of protein synthesis through dephosphorylation of EF-2, observed in rabbit reticulocyte lysates, appears to be a model of the in vivo situation.

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