Vol. 83, No. 2, 1978
July 28, 1978

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 379-384

Phosphorylation of Translational Initiation Factor 3 (eIF-3) by Cyclic AMP-regulated Protein Kinase

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Received May 11,1978

SUMMARY

Translational initiation factor 3 (eIF-3) is phosphorylated by the cyclic AMP-regulated protein kinases from rabbit reticulocytes. eIF-3 is a large molecular weight complex which facilitates binding of the ternary complex containing met $tRNA_f$, GTP and initiation factor 2 to 40S ribosomal subunits. A single polypeptide with a molecular weight of 130,000 is modified. The phosphorylation is dependent upon the presence of cyclic AMP and is inhibited by the inhibitor protein diagnostic for cyclic AMP-regulated protein kinase. Assuming a molecular weight of 700,000 for eIF-3, one mole of phosphate is incorporated per mole of eIF-3. Thus the phosphorylation of two interacting components of the protein synthesizing system, 40S ribosomal subunits and eIF-3, is controlled by cyclic AMP.

INTRODUCTION

Eucaryotic initiation factor 3 (eIF-3), formerly IF-E3 (1) and IF-M5 (2), has been shown to be associated with native 40S ribosomal subunits from a number of sources (3-5). This factor facilitates entry of the ternary complex consisting of met-$tRNA_f$·GTP·eIF-2 onto 40S ribosomal subunits and stabilizes formation of the 40S initiation complex (1,6). In addition, Thompson et al. (5) have shown that the factor prevents reassociation of the subunits to form 80S ribosomes. eIF-3 has a reported molecular weight of 500,000-700,000, and consists of approximately 10 different proteins which range in molecular weight from 35,000 to 130,000 (2,3,7). Four of these subunits have been shown to be phosphorylated by cyclic nucleotide-independent protein kinase activities (8). In this report we demonstrate that the subunit with a molecular weight of 130,000 is modified by the cyclic AMP-regulated protein kinases.

0006-291X/78/0832-0379$01.00/0

MATERIALS AND METHODS

The type I and II cyclic AMP-regulated protein kinases were isolated by chromatography on DEAE-cellulose and phosphocellulose as previously described (8). Free catalytic subunit was obtained from the type II enzyme by further chromatography on phosphocellulose. A 10 ml column was equilibrated in Buffer A (25 mM potassium phosphate, pH 6.8; 1 mM EDTA, 10 mM 2-mercaptoethanol, 0.02% sodium azide; and 1×10^{-5} M cyclic AMP). The sample (235 mg in Buffer A) was applied to the column, and the column was washed with two column volumes of Buffer A to remove contaminating protein and undissociated protein kinase. The free catalytic subunit was eluted with 0.4 M NaCl in Buffer A and stored at 4°C.

The reaction mixtures of 0.045 ml contained: 50 mM Tris-HCl, pH 7.4; 10 mM $MgCl_2$, 0.14 mM (γ-^{32}P)ATP (100-300 cpm/pmol); 1.4×10^{-6} M cyclic AMP (where indicated) and 24 enzyme units (EU) of type II cyclic AMP-regulated protein kinase or 35 EU of catalytic subunit; and eIF-3 (14-21 µg) or 40S ribosomal subunits (26 µg). An EU is that amount of enzyme which incorporates 1 pmol of phosphate into histone per min at 30°C. Purification of eIF-3 (2) and ribosomal subunits (9) have been described elsewhere. After incubation for 30 min at 30°C the reactions were terminated and analyzed by slab gel electrophoresis and autoradiography (8) or by precipitation with trichloroacetic acid (8).

RESULTS AND DISCUSSION

Phosphorylation of eIF-3 by type I and type II cyclic AMP-regulated protein kinases was examined in the presence and absence of cyclic AMP. The type I and type II protein kinases (previously identified as II and III_H respectively; 10) were partially purified from rabbit reticulocytes by chromatography on DEAE-cellulose and phosphocellulose. After phosphorylation, the individual subunits comprising the factor were separated by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, and the modified components were identified by autoradiography. The data expressed in Figure 1 show the results of this experiment with the type II enzyme and (γ-^{32}P)ATP. When the protein kinase was examined in the absence of added substrate, no phosphorylated bands were observed either in the presence or the absence of cyclic AMP. Upon addition of eIF-3 to the reaction mixture, a protein band with a molecular weight of 130,000 was highly phosphorylated in the presence of cyclic AMP. Essentially no incorporation of phosphate was observed in the absence of cyclic AMP. Identical results were obtained with type I protein kinase.

In the same experiment, phosphate incorporation into 40S ribosomal subunits was also examined. As previously observed (9,11-13), incorporation of radioactive phosphate into a single protein in the 40S subunit was stimulated

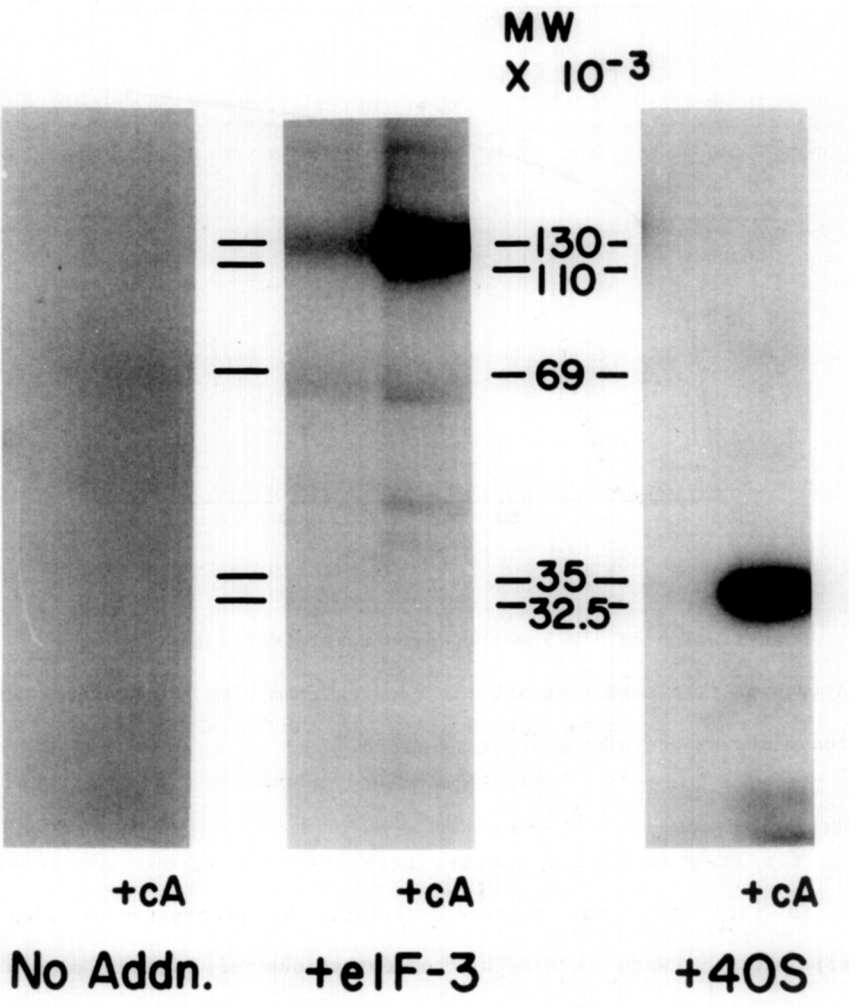

Figure 1. Phosphorylation of eIF-3 and 40S ribosomal subunits by cyclic AMP-regulated protein kinase. Left panel, protein kinase alone. Middle panel, addition of eIF-3. Right panel, addition of 40S ribosomal subunits.

by cyclic AMP (Figure 1). This protein, identified in rat liver as S-6 (12), and in rabbit reticulocytes as S-13 (9), has a molecular weight of approximately 32,500 and contains multiple phosphorylation sites. No phosphorylation was observed in the absence of the cyclic nucleotide. Thus two components in the protein-synthesizing complex, eIF-3 and 40S ribosomal subunits, were phosphorylated by the cyclic AMP-regulated protein kinases.

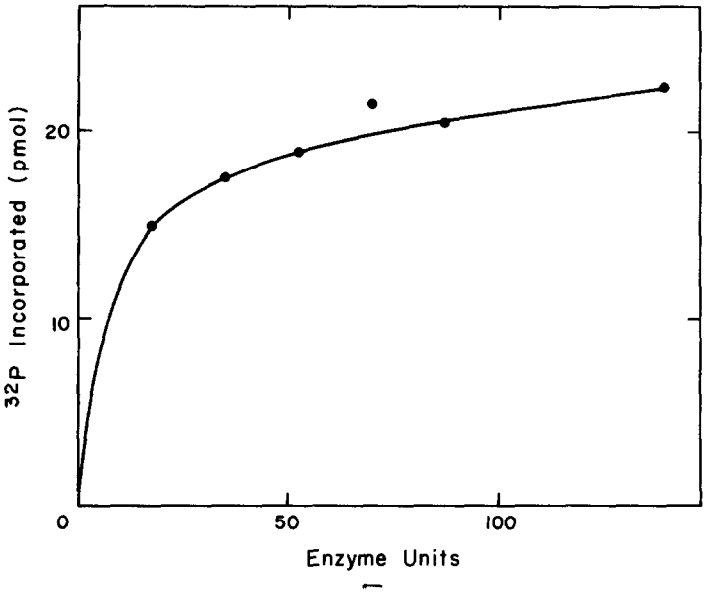

Figure 2. Quantitation of ^{32}P Incorporation into eIF-3. Increasing concentrations of free catalytic subunit ranging from 0 to 140 EU were added to reaction mixtures containing 14 µg of eIF-3.

Using the free catalytic subunit from the type II enzyme, phosphate incorporation into eIF-3 was measured directly by precipitation with trichloroacetic acid. When increasing concentrations of protein kinase were added to the reaction mixture, up to 1 mole of phosphate was incorporated per mole of eIF-3 using a molecular weight of 700,000 for the factor (Figure 2). This phosphorylation was inhibited by the addition of the heat-stable inhibitor protein specific for the cyclic AMP-regulated protein kinases (Table 1).

Attempts have been made to correlate the role of cyclic AMP and phosphorylation of 40S ribosomal subunits with translational control. Although increased phosphorylation of this protein has been observed in rat liver with glucagon (12,14) cyclic AMP (12), and under conditions of regeneration (12); in reticulocytes (11), pituitary slices (15), and hamster islet cell tumor (16) by cyclic AMP; and in tissue culture by addition of serum (17,18); no correlation has been made between this phosphorylation and specific molecular

Table I. Effects of heat-stable inhibitor protein

Additions	^{32}P Incorporated (pmol) -I	+I
eIF-3	14.2	2.2
Histone	426.9	15.1

The reaction mixture contained 18 EU of free catalytic subunit, 80 units of inhibitor (I), 38 µg eIF-3 and 300 µg histone.

events. In addition, in vitro studies examining the role of cyclic AMP-regulated phosphorylation of the 40S subunit on the partial reactions of protein synthesis have been inconclusive (19). Here we have shown that one of the initiation factors, eIF-3, is phosphorylated by the cyclic AMP-regulated protein kinases. No other initiation factor has been shown to be modified by these enzymes (20). Since 40S subunits and eIF-3 have been isolated from cell lysates as a complex (3-5), and this complex has been considered the first step in the initiation sequence, coordinate phosphorylation of these two components could potentially be a control mechanism regulating the subsequent steps in the initiation process. An examination of the role of these various phosphorylation reactions on the control of protein synthesis are currently in progress.

ACKNOWLEDGMENTS

We wish to thank Drs. William C. Merrick and Brian Safer for the purified preparations of eIF-3 used in these studies and Dr. Donal Walsh for the heat-stable inhibitor protein. This research was supported by Grant GM 21424 from the United States Public Health Service.

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