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Regulation of translation

Regulación de la traducción

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It has been known for several years that protein synthesis in reticulocyte lysates is but briefly maintained in the absence of added hemin (1). Gross and Rabinovitz (2) showed that hemin prevents the formation of an inhibitor of chain initiation from a proinhibitor of similar molecular weight. As seen in Figure 1, the synthesis of protein in a lysate of rabbit reticulocytes comes to a sharp decline in about 10 minutes if hemin is not added to the incubation mixture. Addition of inhibitor, in the presence of hemin, has the same effect as omission of hemin (Figure 1).

The proinhibitor is present in the postribosomal supernatant of reticulocyte lysates. It can be converted to inhibitor by incubation for several hours at 34°-37° or by incubation with N-ethylmaleimide (NEM) for a few minutes at 30° (2). Upon chromatography on a Biogel P-150 column, the proinhibitor is eluted in the void column region (Figure 2, left panel). The molecular weight of both proinhibitor and inhibitor (Figure 2, right panel) was found to be about 300,000 as determined by Sephadex G-200 filtration (2). The conversion of proinhibitor elicited by incubation of postribosomal supernatants for 4 hours at 34° was markedly retarded by hemin (2).

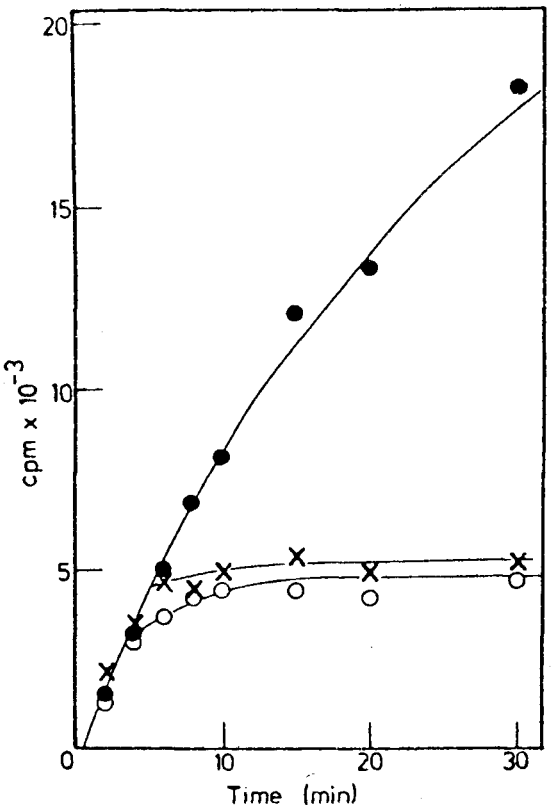
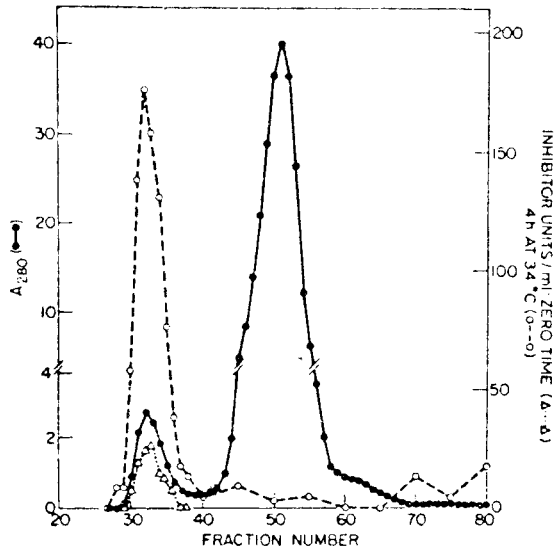


Fig. 1. Protein synthesis in the reticulocyte lysate in the absence of added hemin or in the presence of added inhibitor Samples (100 µl) were incubated at 30° in the presence or absence of hemin (30 µM) and inhibitor protein (25 µg of a partially purified preparation). Aliquots were removed at the times indicate for assay of [^{14}C] leucine incorporation into protein (×) minus hemin; (●) plus hemin; (○) plus hemin and inhibitor. From Clement *et al.* (1).

Translational Inhibitor of Reticulocyte Lysates

Recently, it has been shown that the inhibitor is a cyclic AMP-independent protein kinase that catalyzes the phosphorylation of the small (38.000 dalton) subunit of the initiation factor eIF-2 (3-6). This factor forms a ternary complex with Met-tRNA$_i$ and GTP which,



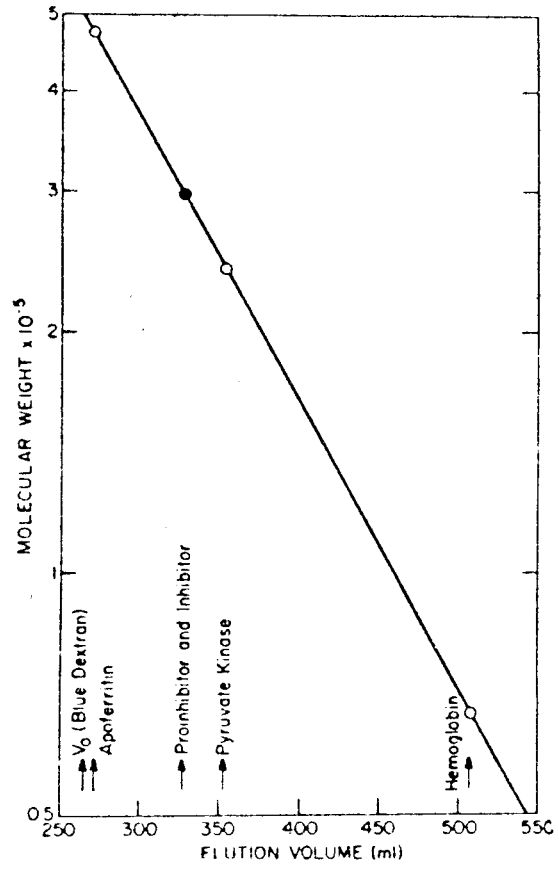


Fig. 2. **Top panel. Chromatography of proinhibitor on** Biogel P-150. Fresh supernatant was applied to a Biogel P-150 column equilibrated with a buffer containing 3.7 mM N-2-hydroxyethylpiperazine-N'-2-etha-nesulfonic acid (Hepes) in 27.5 mM KCl and the column was developed with the same buffer. The inhibitory activity of the fractions on protein synthesis in hemin-containig reticulocyte lysates was assayed before (Δ——Δ) and **after (00——0) incubation for 4 hrs at 34°. Bottom: panel** Molecular weight determination of proinhibitor and inhibitor on a Sephadex G-200 column. From Gross and Rabinovitz (2).

components of the reaction mixtures were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The gels were dried, stained, and autoradiograms were prepared. The four tracks to the left correspond to stained gels; the four tracks to the right to their autoradiograms. The fraction labelled DS2 was a DEAE-cellulose fraction having both cyclic AMP (cAMP)-dependent protein kinase (as assayed with histone as substrate) and translational inhibitor activity. On further fractiona-

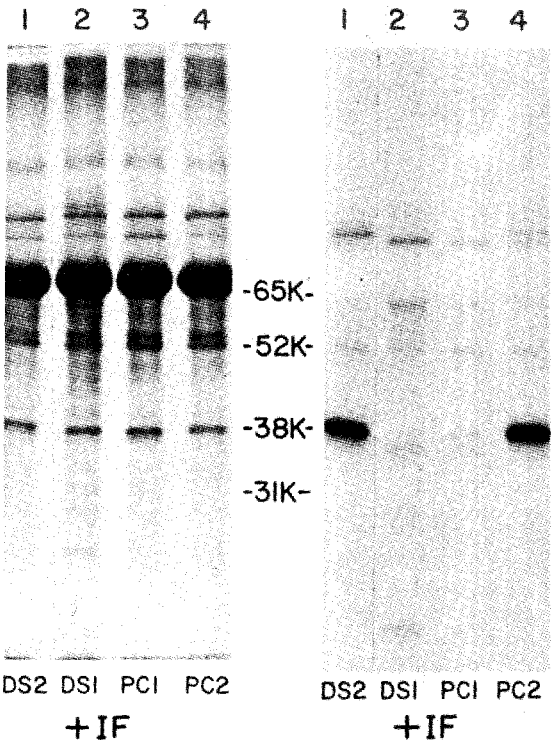


Fig. 3. Phosphorylation of small (38K) subunit of initiation factor eIF-2 by cAMP-independent protein kinase activity in preparations of translational inhibitor from the postribosomal supernatant of rabbit reticulocyte lysates. For explanations see text. From Levin *et al.* (4).

on interaction with a 40S ribosome, gives rise to a 40S initiation complex (7-9). Phosphorylation of eIF-2 renders the factor inactive in chain initiation. In Figure 3, eIF-2 (IF) was incubated with various protein fractions from rabbit reticulocyte postribosomal supernatant, in the presence of $[\gamma^{-32}P]$ATP, and the

tion by chromatography on phosphocellulose it yielded fractions PC1 and PC2. PC1 had the cAMP-dependent protein kinase activity. It did not phosphorylate eIF-2 (Figure 3) and did not have translational inhibitor activity. PC2 had virtually no activity toward typical protein kinase subsrates (e.g., histone, casein) but, as seen in Figure 3, it phosphorylated the 38.000 dalton subunit of eIF-2. This activity was cAMP-independent. PC2 also had high inhibitor activity. Essentially identical, independent data by another group are shown in Figure 4. Highly purified eIF-2 and two preparations of translational inhibitor of different degrees of purity were used. The A tracks co-

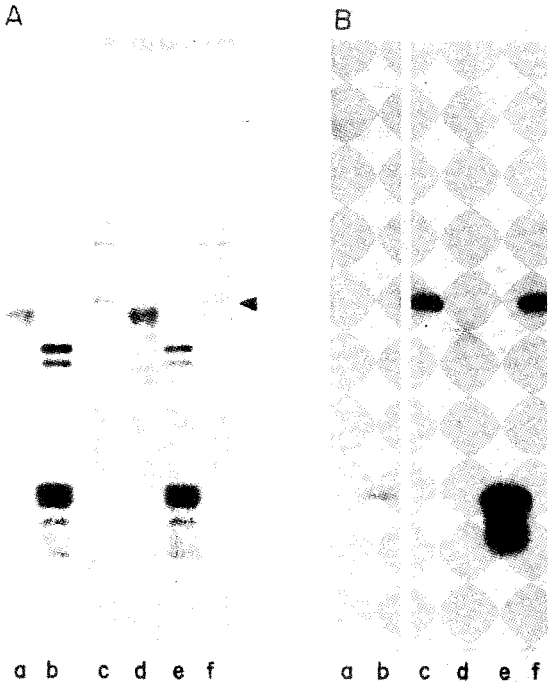


Fig. 4. Further evidence of phosphorylation of small subunit of initiation factor eIF-2 by translational inhibitor. The less pure inhibitor preparation (tracks e and f) contained a cAMP-dependent protein kinase, that catalyzed the phosphorylation of eIF-2 and had translational inhibitor activity. The purer preparation (tracks b and c) was almost free of cAMP-dependent protein kinase. See text for other details. From Kramer *et al.* (5).

rrespond to the stained gels, the B tracks to their autoradiograms. The arrow marks the position on the gel of glyceraldehyde-phosphate dehydrogenase used as molecular weight marker (subunit mol. wt., 37.000). The less pure preparation of inhibitor contained a kinase(s) that phosphorylated histone besides eIF-2 (tracks e and f), the more highly purified one phosphorylated eIF-2 but had only weak activity with histone (tracks b and c). Casein was not phosphorylated by either preparation.

Conversion of Proinhibitor to Inhibitor

The mechanism of conversion of proinhibitor (inactive eIF-2 kinase) to inhibitor (active eIF-2 kinase) was unknown. We have shown (10) that the conversion of proinhibitor to inhibitor can be promoted by cAMP-dependent protein kinase or its catalytic subunit. This observation in consistent with the view that, as in the case of phosphorylase kinase (11, 12), inactive eIF-2 kinase is activated by phosphorylation catalyzed by cAMP-dependent protein kinase. This is illustrated diagramatically in Figure 5. We have further shown (13) that hemin blocks the binding of cAMP to the regulatory subunit of cAMP-dependent protein kinase and, in this way, interferes with the

REGULATION OF POLYPEPTIDE CHAIN INITIATION BY CYCLIC AMP

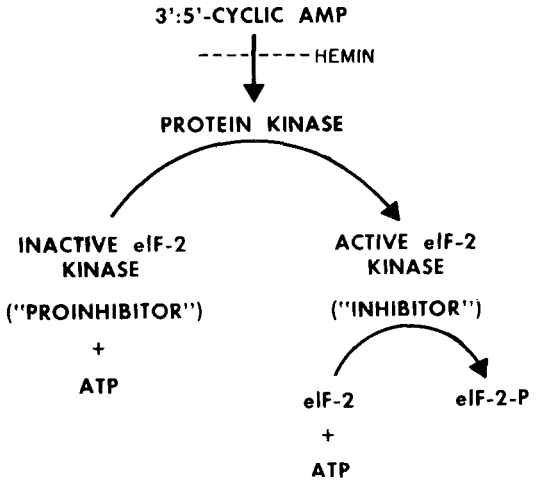


Fig. 5. Regulation of polypeptide chain initiation by cAMP. cAMP releases protein kinase catalytic subunits (see Table 1) which catalyze the phosphorylation of proinhibitor (inactive eIF-2 kinase) to inhibitor (active eIF-2 kinase) by ATP. The cAMP-independet active eIF-2 kinase catalyzes the phosphorulation of the 38K subunit of the initiation factor eIF-2 by ATP, an event which is eventually responsible for inactivation of the factor and inhibition of initiation. Hemin blocks the dissociation of the kinase by cAMP and prevents the release of the catalytic subunits.

cAMP-promoted activation of eIF-2 kinase, i.e., with the conversion of proinhibitor to inhibitor. The experimental evidence reviewed in this paper is consistent with this model.

As seen in Table 1, phosphorylase kinase and the eIF-2 kinase belong to the same group of cAMP-independent protein kinases. As regards the cAMP-dependent enzymes note their subunit structure R_2C_2 and the mode of action of cAMP. cAMP binds to the regulatory subunit (R) releasing the active catalytic subunit (C). Phosphorylase kinase was known to exist in an inactive, nonphosphorylated form and to be converted to an active form by phosphorylation catalyzed by cAMP-dependent protein kinase (11-12).

TABLE 1: PROTEIN KINASES

ATP + PROTEIN ⟶ ADP + PHOSPHOPROTEIN

cAMP-INDEPENDENT

TYPE A	TYPE B
P DONOR: ATP OR GTP	P DONOR: ATP
SUBSTRATES:	SUBSTRATES:
ACIDIC PROTEINS (CASEIN, PHOSVITIN)	PHOSPHORYLASE (PHOSPHORYLASE KINASE)
CERTAIN SUBUNITS OF SOME INITIATION FACTORS.	38K SUBUNIT OF INITIATION FACTOR eIF-2 (eIF-2 KINASE)

cAMP-DEPENDENT

$R_2C_2 + 2cAMP \longrightarrow R_2cAMP_2 + 2C$

P DONOR" ATP

SUBSTRATES:

BASIC PROTEINS (PROTAMINES, HISTONES, RIBOSOMAL PROTEINS), ENZYMES (GLYCOGEN SYNTHETASE, HORMONE-SENSITIVE LIPASE, PHOSPHORYLASE KINASE)

We found that protein synthesis in hemin-containing reticulocyte lysates is inhibited not only by the translational inhibitor, i.e., by cAMP-independent eIF-2 kinase, but also by cAMP-dependent protein kinase, in the presence of cAMP, or by its catalytic subunit. Typical results are shown in Figure 6. Panel A compares the effect of inhibitor (solid circles) and bovine heart protein kinase (squares). Panel B shows the effects of the catalytic subunit. The effect of kinase and catalytic subunit as a function of their concentration is shown on Panels C and D. Both are strong inhibitors but, per unit weight, the catalytic subunit is much more effective. Figure 7 shows stimulation by cAMP of the inhibitory effect of both bovine heart and rabbit reticulocyte protein kinases.

The above results raised the question whether eIF-2 could be phosphorylated by both cAMP-independent eIF-2 kinase and cAMP-dependent protein kinase or whether the effect of the latter enzyme was indirect. To answer this question, we used a ternary complex formation assay. We found that formation of the ternary complex eIF-2 · GTP · Met-tRNA$_i$ (Figure 8, reaction 1) is inhibited if eIF-2 is briefly preincubated with eIF-2 kinase and ATP. This is true only when partially but not highly purified

INHIBITION OF PROTEIN SYNTHESIS IN RETICULOCYTE LYSATES BY cAMP-DEPENDENT BOVINE HEART PROTEIN KINASE (BHK) AND ITS CATALYTIC SUBUNIT (C)

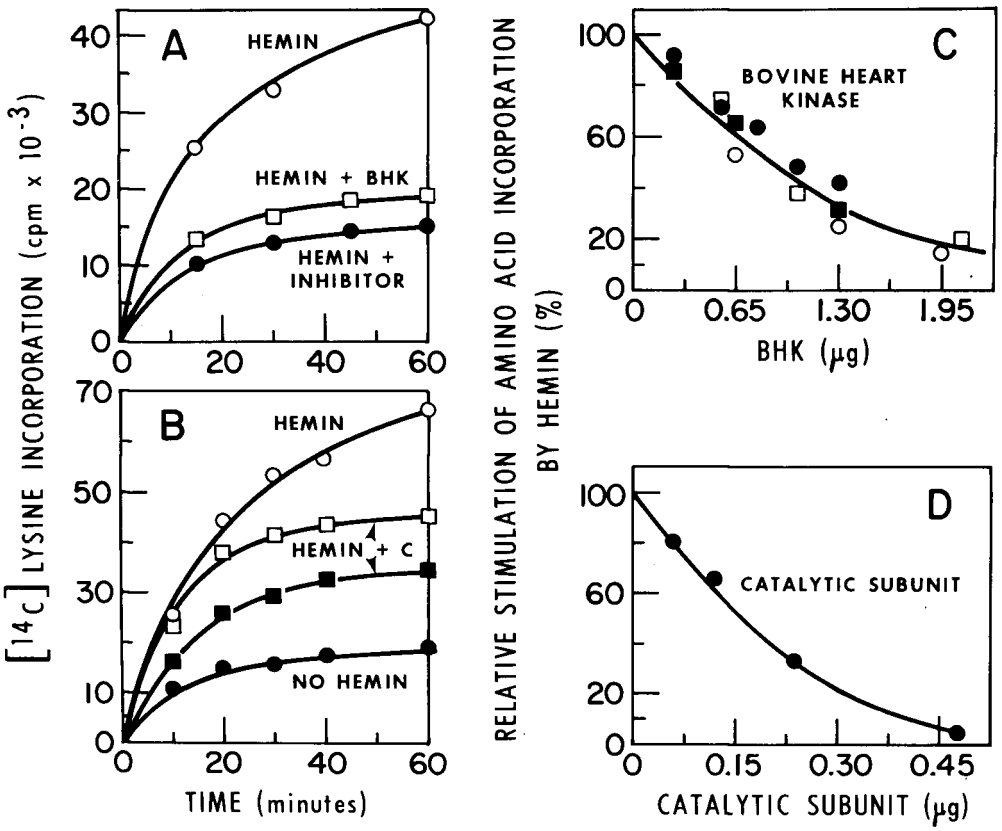


Fig. 6. Inhibition of protein synthesis in reticulocyte lysates by cAMP-dependent bovine heart protein kinase (BHK) and its catalytic subunit (C). Protein synthesis was assayed through the incorporation of [^{14}C] lysine into acid-insoluble material under standard conditions. The concentration of hemin (when present) was 34 μM, that of cAMP, 10 μM. The concentration of (partially purified) inhibitor in the lower curve of panel A was 10 μg/50 μl; that of catalytic subunit in the corresponding curves of panel B, 0.15 and 0.3 μg/50 μl. From Datta *et al.* (10).

eIF-2 is used. We interpret this to mean that eIF-2 is not inactivated by simple phosphorylation of its 38.000 dalton subunit (Figure 8, reaction 2) but by interaction of the phosphorylated factor with another protein (factor X) present as a contaminant of eIF-2 preparations (Figure 8, reaction 3). As seen in Figure 9, the inactivation is fast (Panel A) and, with short incubation times, proportional to the eIF-2 kinase (inhibitor) concentration within a limited concentration range (Panel B). The inactivation of eIF-2 is clearly a consequence of the phosphorylation of the factor for, as seen in Panel A., there is no inhibition when ATP is omitted. As further shown in Panel A., cAMP-dependent protein kinase, whether from bovine heart or rabbit reticulocytes, or the catalytic subunit, are inactive in this assay. It is clear from these results that the inhibition of translation in reticulocyte lysates by cAMP-dependent protein kinases is direct.

The most likely explanation for the inhibition of translation in lysates by cAMP-dependent protein kinase is that it acts as a kinase kinase to catalyze the conversion of proinhibitor to inhibitor by transfer of phosphate from ATP. This would be in strict analogy to the activation of phosphorylase kinase by cAMP-dependent

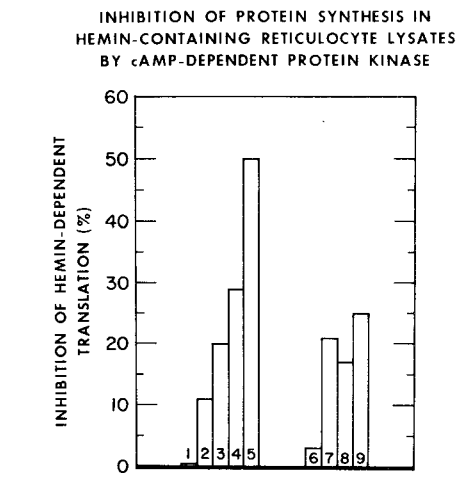


Fig. 7. Inhibition of protein synthesis in reticulocyte lysates by cAMP-dependent protein kinases. Protein synthesis assay in the presence of added hemin (34 μM) as in Fig. 6 with various additions as indicated BHK, bovine heart protein kinase. Retic PK, partially purified cAMP-dependent protein kinase from postribosomal supernatant of rabbit reticulocyte lysate. From Datta *et al.* (10).

protein kinase. To test this hypothesis, crude proinhibitor prepared from fresh lysate by chromatography on CM-Sephadex G-50, according to Gross and Rabinovitz (2), was incubated with or without catalytic subunit or cAMP-dependent protein kinase and ATP, and the reaction mixtures were assayed for inhibitor formation with the ternary complex or protein synthesis assay.

As seen in Figure 10, there was little or no inhibition of ternary complex formation with proinhibitor and ATP but there was significant inhibition when proinhibitor was incubated with catalytic subunit and ATP. The poor proportionality of the assay (inhibition in the last sample should have been 26% rather than 18%) may have been caused by the presence of protein phosphatase in the crude proinhibitor preparations. Figure 11 shows that conversion of proinhibitor to inhibitor required catalytic subunit and ATP and provides convincing evidence for the involvement of phosphorylation in this reaction. In the experiments of Figure 12, the conversion of proinhibitor to inhibitor was assayed by protein synthesis in the hemin-containing reticulocyte lysate. The experiment to

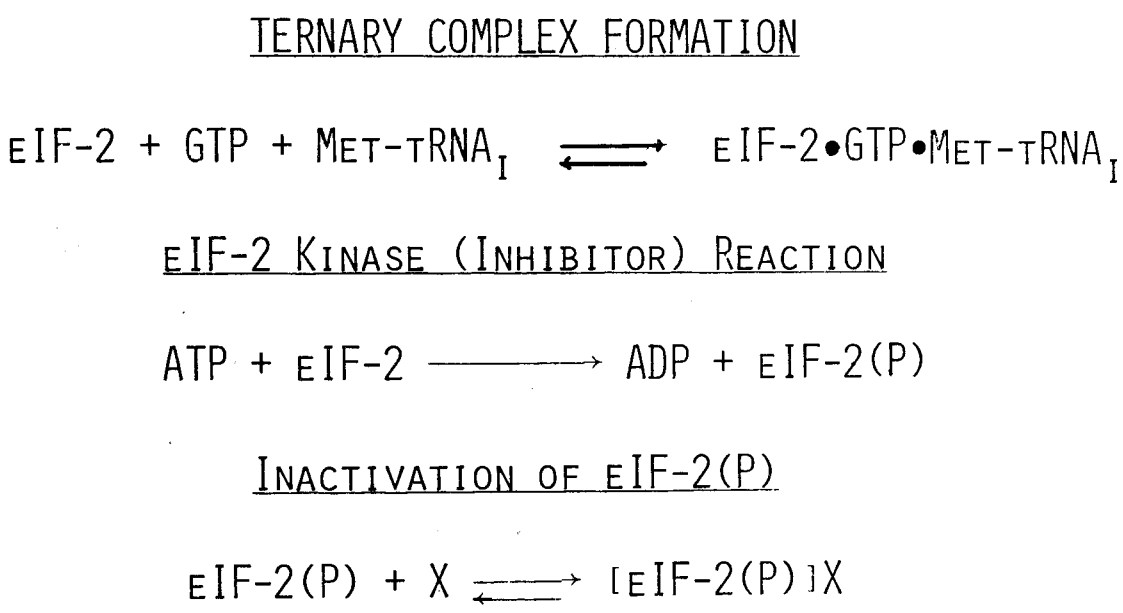


Fig. 8. Hypothetical inactivation of eIF-2 for ternary complex formation by phosphorylation and interaction with another protein factor (see text). Met-tRNA$_f$, initiator species of methionyl transfer RNA.

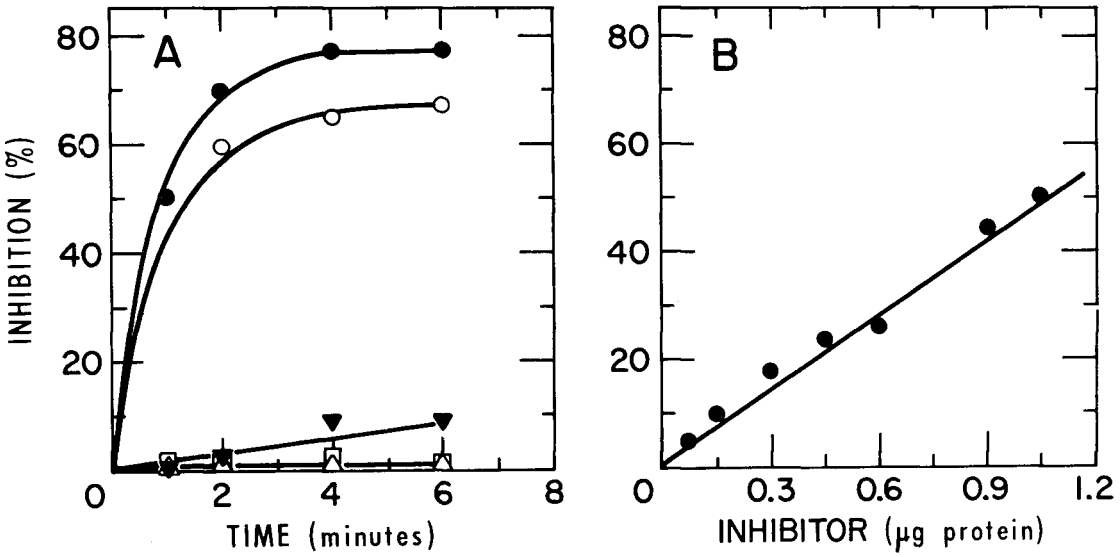


Fig. 9. Assay of inhibitor through its effect on ternary complex formation. (A) Kinetics of inhibition. Partially purified eIF-2 was incubated for various times at 30° with the indicated supplements, before GTP and [^{35}S] Met-tRNA$_f$ were added. The samples were then incubated for a further 5 min and assayed for ternary complex formation by the Millipore filtration procedure. (●) Inhibitor, 1.5 μg of protein, ATP, 0.4 mM; (0) inhibitor, 0.75 μg of protein, ATP, 0.4 mM; (▼) BHK, 0.38 μg, cAMP, 5 μM, ATP, 0.4 mM; (□) inhibitor, 1.5. μg of protein (no ATP); (△) either BHK catalytic subunit, 0.3 μg, ATP, 0.4 mM;, or cAMP-dependent rabbit reticulocyte protein kinase (partially purified), 2 μg of protein, cAMP, 5 μM, ATP 0.4 mM. (B) Inhibition of ternary complex formation as a function of the inhibitor concentration. eIF-2 was incubated with ATP, 0.4 mM, and with or without the indicated amounts of inhibitor for 1 min at 25° before assay. BHK, bovine heart protein kinase. From Datta *et al.* (10).

the left (bars 1-4) shows the effect of the addition of cAMP-dependent protein kinase from rabbit reticulocytes with and without cyclic AMP and ATP. The experiment to the right (bars 6,7) compares the effect of cAMP-dependent reticulocyte protein kinase + cAMP and bovine heart catalytic subunit.

Mode of Action of Hemin

Hemin was reported to inhibit the activity of cAMP-dependent protein kinase from rabbit reticulocytes (14). We confirmed this observation but found that hemin had no effect on the activity of the catalytic subunit. These results together with our finding that inactive eIF-2 kinase (proinhibitor) is converted to active eIF-2 kinase (inhibitor) by phosphorylation catalized by cAMP-dependent protein kinase, made in likely that hemin blocks the proinhibitor-inhibitor conversion by preventing the dissociation of cAMP-dependet protein kinase by cAMP:

$$C_2R_2 + 2\,cAMP \xrightarrow[\text{hemin}]{} R_2cAMP_2 + 2\,C$$

This hypothesis could be demonstrated directly using crude proinhibitor prepared by CM-Sephadex chromatography of fresh postribosomal supernatant from reticulocyte lysate (13). We established that this preparation, assayed with histone as substrate, also contained a very active protein kinase highly dependent on cAMP.

Typical experiments, patterned after those of Figure 10, are shown in Figure 13 (left panel). It will be seen that the conversion of proinhibitor to inhibitor, as gauged by inhibition of ternary complex formation, required either cAMP or catalytic subunit (C) besides ATP and that, whereas the conversion promoted by cAMP was markedly inhibited by hemin, that elicited by the catalytic subunit was not significantly affected by the porphyrin.

It can in fact be shown that hemin blocks the binding of cAMP to the regulatory subunit of

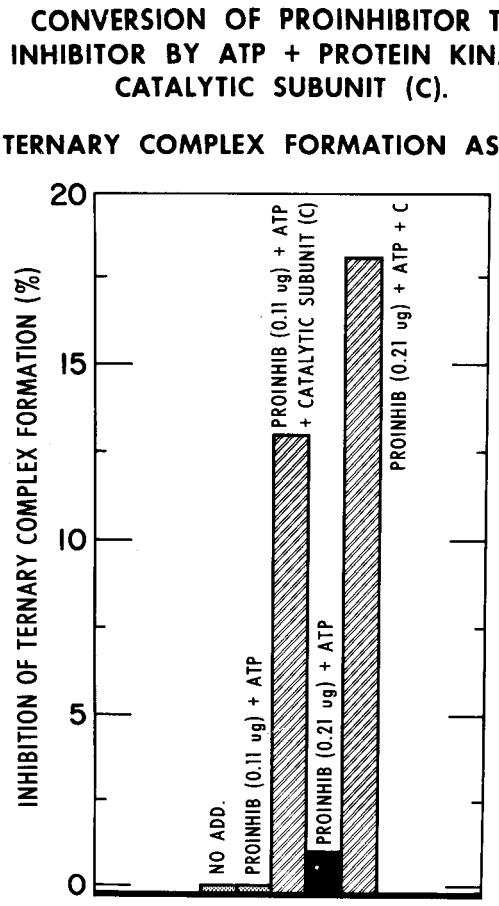


Fig. 10. Conversion of proinhibitor to inhibitor by bovine heart kinase catalytic subunit. Samples (25 μl) containing Hepes buffer, pH 7.6, 20 mM; KCl, 50 mM; Mg^{2+}, 2 mM; and ATP, 0.4 mM; with or without crude proinhibitor from postribosomal supernatant of rabbit reticulocyte lysate and/or catalytic subunit as indicated, were incubated for 8 min at 30°. Aliquots were then assayed for inhibition of ternary complex formation. From Data *et al.* (10).

cAMP-dependent protein kinase. In Figure 13 (right panel) the binding of [^{3}H]cAMP to bovine heart kinase (open circles), bovine heart kinase regulatory subunit (squares) and cAMP-dependent protein kinase from rabbit reticulocytes (solid circles), was measured in the absence and in the presence of increasing concentrations of hemin. It may be seen that, at concentrations at which it affords complete protection of translation in reticulocyte lysates, the binding of cAMP to reticulocyte protein kinase is severely curtailed by hemin. Recently we have shown (paper in preparation) that ^{3}H-labelled hemin binds specifically to the regulatory subunit of bovine heart protein kinase with an affinity which is only two orders of magnitude lower than that of cAMP. Moreover, whereas unlabelled hemin can displace bound labelled hemin as well as labelled cAMP, unlabelled cAMP can displace bound labelled cAMP but not hemin. This shows that hemin prevents the binding of cAMP to the regulatory subunit of cAMP-dependent protein kinase in a non-competitive fashion. Hemin binds to a site different from the cAMP binding site and affects the binding of cAMP in an allosteric manner. These observations explain why hemin, unlike cAMP, does not activate, i.e., does not dissociate cAMP-dependent protein kinase.

Proinhibitor-Inhibitor Pair in Other Cells

The presence of translational inhibitor in ascites tumor cells (15), liver (16), and Friend leukemia cells (17) has been reported. We have evidence for the occurrence of this translational control system in organisms evolutionarily far removed from mammals (18). Thus *Artemia salina* embryo cells have a proinhibitor-inhibitor system which, like its reticulocyte counterpart, is activated by a cAMP-dependent protein kinase. There is a similar system in wheat germ although it does not seem to be cAMP-dependent.

As shown in Figure 14, bovine heart protein kinase catalytic subunit is a potent inhibitor of translation in the *A. salina* cell-free system (solid circles). In view of our data with the reticulocyte system these results can only mean that in *A. salina* the protein kinase catalytic subunit is also mediating the transfer of phosphate from ATP to a proinhibitor to form an inhibitor of polypeptide chain initiation. This conclusion is strengthened by the fact that, as shown in Figure 14 (righ panel) the catalytic subunit does not inhibit chain elongation (open circles) or poly(U) translation (squares) which, at relatively high Mg^{2+} concentrations, does not involve initiation factors. Inhibition of translation by the catalytic subunit was also observed in wheat germ systems (Figure 15).

Preparations derived from postribosomal supernatant from dormant or developing *A. salina* embryos by CM-Sephadex chromatography were found, with use of the ternary complex formation assay, to contain proinhibitor. As assayed with histone as substrate they were also found to contain cAMP- dependent protein kinase(s). Figure 16 shows that the *A.*

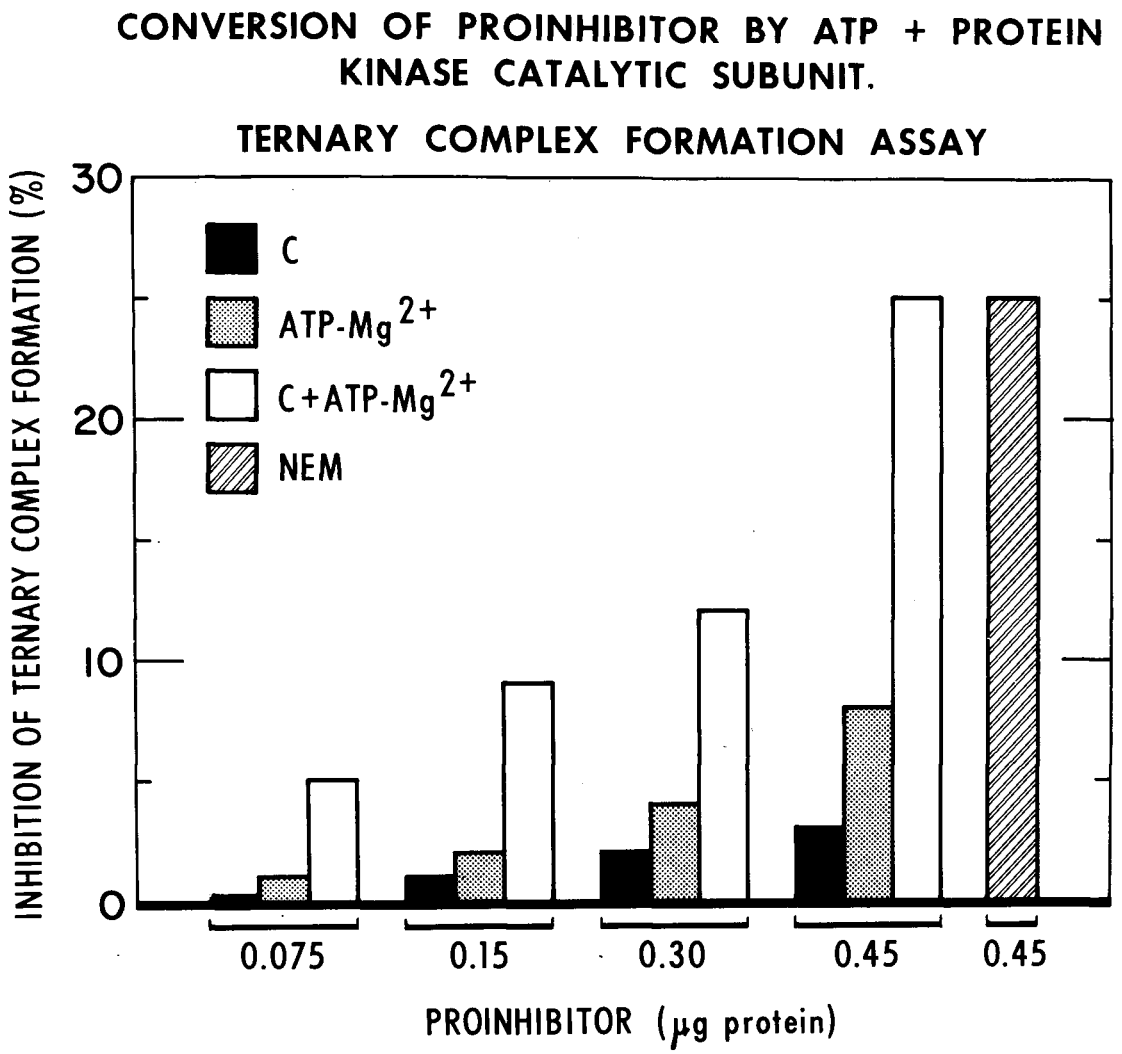


Fig. 11. Conversion of proinhibitor to inhibitor by bovine heart kinase catalytic subunit: requirement for catalytic subunit and ATP. The experimental plan was similar to that of Fig. 10 but the proinhibitor, along with any formed inhibitor, was reisolated by CM-Sephadex chromatography prior to ternary complex formation assay. In the last bar the proinhibitor content of the preparation was assayed with N-ethylmaleimide (NME) which causes the quantitative conversion of proinhibitor to inhibitor (2). Comparison of the last two bars shows that the enzymatic conversion was complete. From Datta *et al.* (10).

salina proinhibitor, like the reticulocyte proinhibitor, is converted to inhibitor by incubation with ATP and either bovine heart kinase catalytic subunit or cAMP but not by incubation with ATP alone. Moreover, as with the reticulocyte proinhibitor, hemin inhibits the conversion promoted by cAMP. It may be noted that hemoglobin is the oxygen-carrying pigment in *A. salina* (19).

CM-Sephadex chromatography of wheat germ postribosomal supernatant (18) appears to yield a mixture of proinhibitor and inhibitor. This is deduced from the observation that there is substantial inhibition of ternary complex formation on addition of ATP to the wheat germ enzyme fraction, although more inhibition is observed upon the further addition of bovine heart kinase catalytic subunit. Here, however, cAMP did not have an effect comparable to that of the catalytic subunit; in fact; it had little if any effect. On examination of the wheat germ preparation for protein kinase activity,

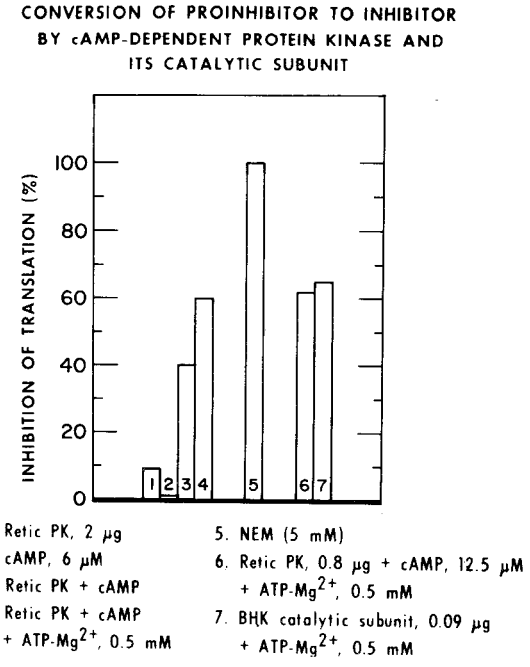


Fig. 12. Conversion of proinhibitor to inhibitor by cAMP -dependent protein kinases and catalytic subunit. Crude proinhibitor from postribosomal supernatant of rabbit reticulocyte lysates was incubated (8 min at 30°) with additions as indicated. Formation of inhibitor was assayed in the reticulocyte lysate translation system in the presence of hemin. The proinhibitor content of the preparations was determined by treatment of an aliquot with NEM (bar 5), the inhibition of translation produced by NEM (100% proinhibitor-inhibitor conversion) being taken as 100%. From Data *et al.* (10).

with histone as substrate, only a cAMP-independent (and cGMP-independet) activity was found. Evidently, further work is required to clarify the mechanism controlling the proinhibitor-inhibitor conversion in this system

Nature of factor X

Pursuing the idea that phosphorylated eIF-2, which is active in ternary and 40S initiation complex formation, is inactivated by interaction with another factor (X in Figure 8, reaction 3), we isolated a protein from ribosomal salt washes required for inhibition of ternary complex formation with purified eIF-2 upon incubation

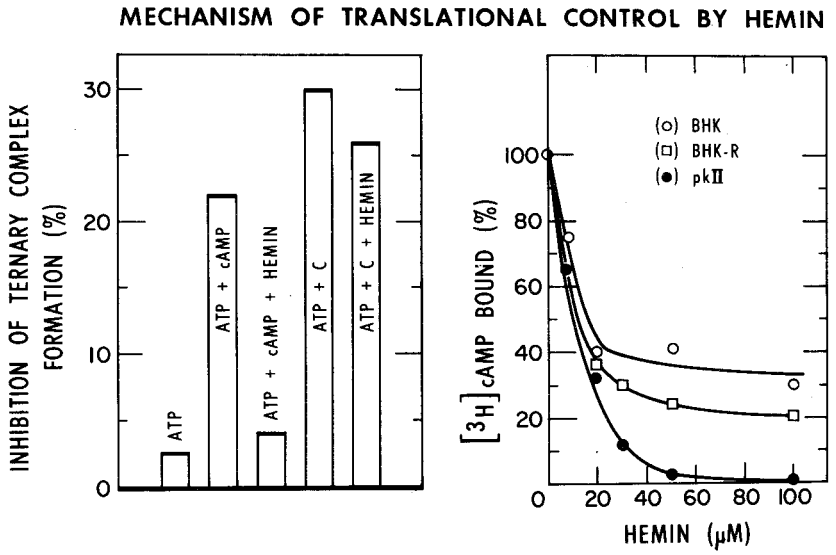


Fig. 13. Left panel. Effect of hemin on the conversion of proinhibitor to inhibitor. Conversion was promoted either by endogenous cAMP-dependent protein kinase, upon the addition of cAMP, or by bovine heart protein kinase catalytic subunit, and was measured by the ternary complex formation assay. All samples contained proinhibitor (0.5 μg of protein) and ATP (0.67mM). Bars from left to right: no further additions, cAMP (15 μM), cAMP (15 μM) and hemin (45 μM), catalytic subunit (0.4 μg), and catalytic subunit (0.04 μg) and hemin (45 μM). Right panel. Blocking by hemin of cAMP binding to the regulatory subunit of cAMP-dependent protein kinase. Binding of [^{3}H] cAMP to cAMP-binding protein (kinase holoenzyme or regulatory subunit) as a function of the hemin concentration. (0) Homogeneous bovine heart protein kinase, 1.0 μg; (□) Bovine heart protein kinase regulatory subunit, 0.4 μg; (●) cAMP-dependent rabbit reticulocyte protein kinase (partially purified), 4.8 μg. From Datta *et al.* (13).

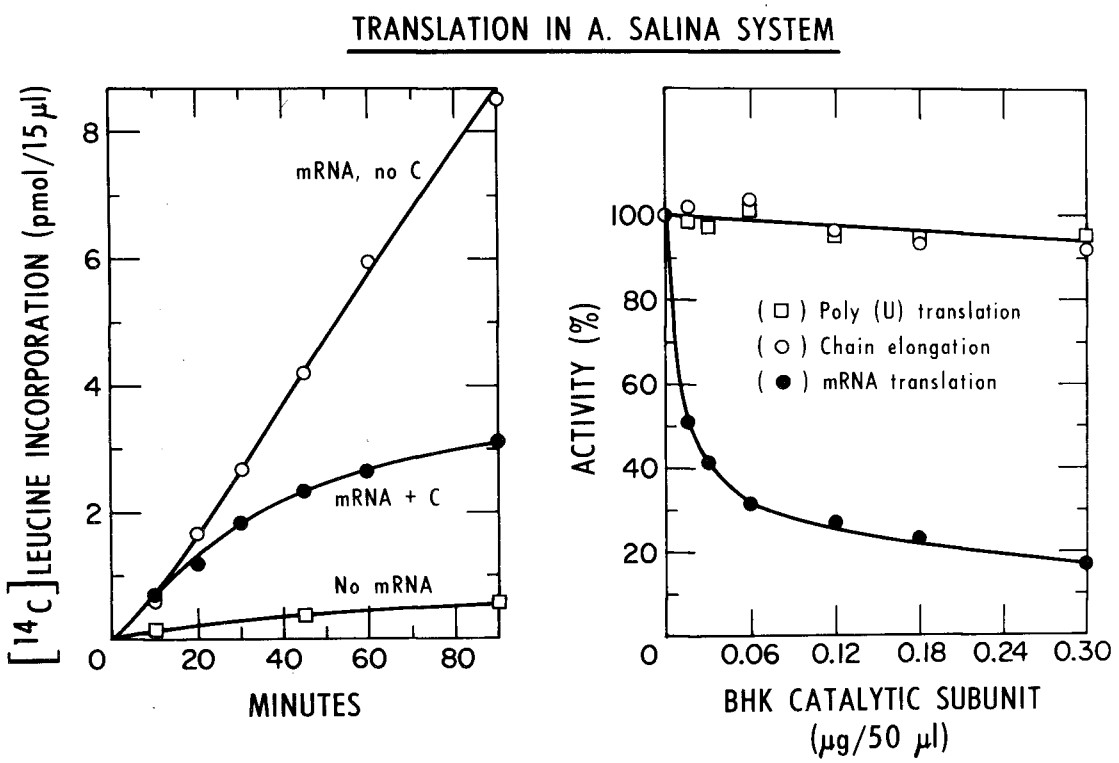


Fig. 14. Inhibition of translation in *A. salina* extracts by bovine heart protein kinase catalytic subunit. The amounts of components present/50 µl sample were: poly (A)-containing *A. salina* polysomal mRNA, 3 µg; catalytic subunit (C), 0.3 µg; poly (U), 0.8 A_{260} units. Chain elongation (edeine resistant) was assayed with polysomes from developed *A. salina* embryos. Incubation at 25°: right panel (□, ○), 45 min; (●) 60 min. From Sierra *et al.* (18).

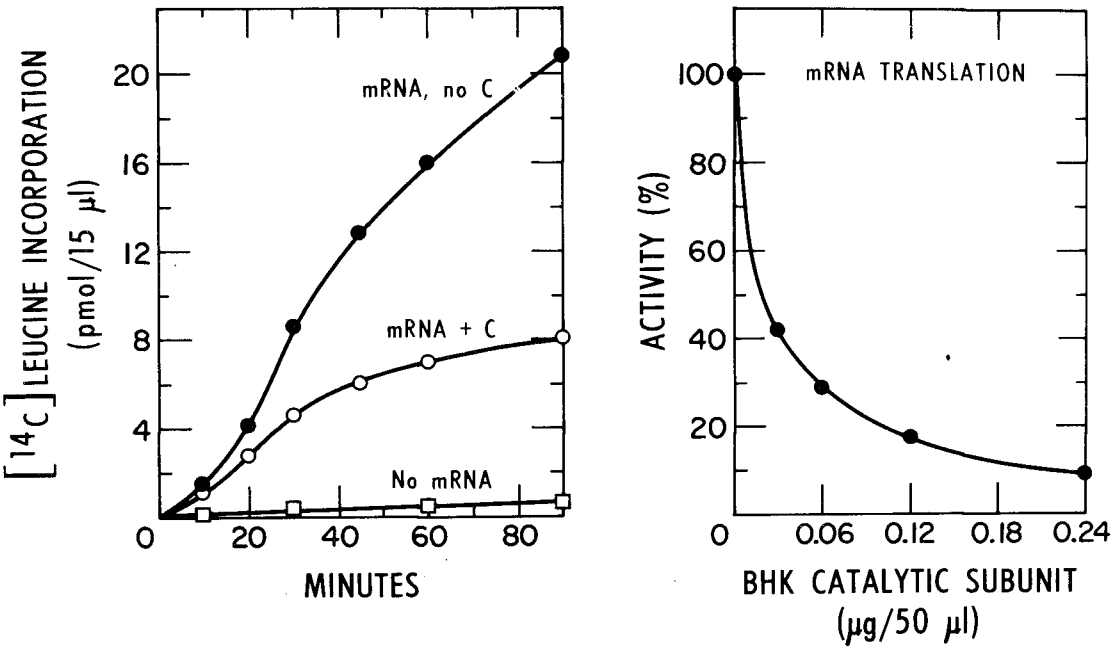


Fig. 15. Inhibition of translation in wheat germ S30 extracts by bovine heart protein kinase catalytic subunit (C). Conditions as in Fig. 14. From Sierra *et al.* (18).

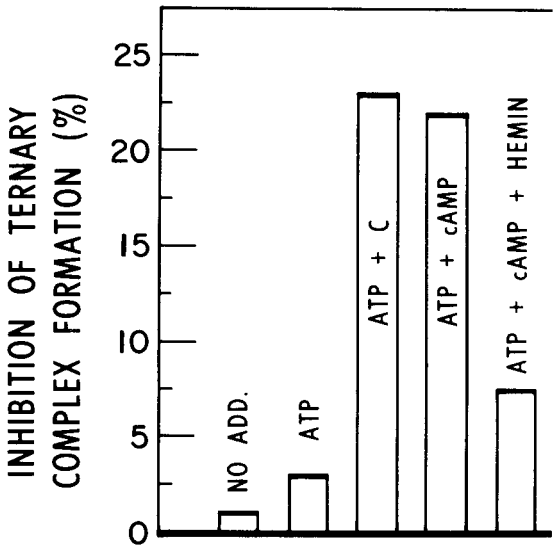


Fig. 16. Conversion of proinhibitor to inhibitor in *A. salina* extracts and the effect of hemin. Crude proinhibitor was prepared from postribosomal supernatant of developing *A. salina* embryos by chromatography on CM-Sephadex as described for reticulocyte lysate supernatants (10). The experiments were patterned after those of Fig. 13 (left panel). All samples except the one at the extreme left contained proinhibitor (0.5 μg of protein) and ATP (0.67 mM). Bars from the second from the left to right: no further additions, catalytic subunit (0.04 μg), cAMP (15 μM), and cAMP (15 μM) and hemin (45 μM). From Sierra *et al.* (18).

with eIF-2 kinase and ATP (20). Surprisingly the new factor proved to enhance the ability of unphosphorylated, but not that of phosphorylated eIF-2 to form initiation complexes, whether ternary or 40S. We refer to this factor as ESP for eIF-2 stimulating protein. At low concentrations, such as occur in reticulocyte lysates, eIF-2 is virtually unable to form an initiation complex unless activated by ESP.

It is therefore understandable that, through blocking of the ESP activation, phosphorylation of eIF-2 by eIF-2 kinase will cause pronounced inhibition of translation in reticulocyte lysates.

Conclusions and Summary

One important fact arising from our studies is that protein synthesis can be, and probably is, regulated by cAMP. It had already been observed as far back as 1960 that glucagon and cAMP inhibit protein synthesis in liver slices (21) but the significance of this finding could not be assessed at that time. More recently (22) cAMP has been shown to inhibit protein synthesis in cellfree preparations of rat liver in an ATP-dependent reaction. These observations strongly suggest the involvement of cAMP-dependent protein kinase in translational control. The inverse relationship between cell growth and cAMP levels disclosed by the work of Pastan and others (23) can now be explained in view of the identical relationship between protein synthesis and cAMP levels suggested by our work. Tomkins and collaborators (24) showed that cAMP and prostaglandin E_1 (which raises the intracellular level of cAMP) inhibited the growth of cultured mouse lymphoma S49 cells but were much less effective on a variant that had low levels of cAMP-dependet protein kinase. Thus, the inhibition by cAMP of both protein synthesis and cell growth is mediated by cAMP-dependent protein kinase. Probably inhibition of cell growth is a consequence of protein synthesis inhibition.

In summary, a system of translational control in eukaryotes consists of (a) a proinhibitor and (b) an inhibitor of polypeptide chain initiation. The inhibitor (active eIF-2 kinase) is a cAMP-independent protein kinase. It catalyzes the phosphorylation of the small subunit of the initiation factor eIF-2. Phosphorylated eIF-2 cannot interact with eIF-2 stimulating protein and become inactive in chain initiation. Our observations are consistent with the view that the proinhibitor (inactive eIF-2 kinase) is converted to the inhibitor by phosphorylation catalyzed by a cAMP-dependent protein kinase. This is in analogy to the conversion of inactive phosphorylase kinase to active phosphorylase kinase. Hemin blocks the cAMP-induced dissociation of the regulatory and catalytic subunits of cAMP-dependent protein kinase by preventing cAMP from binding to the regulatory subunit. Hemin, therefore, blocks the conversion of proinhibitor to inhibitor by inhibiting the activity of the cAMP-dependent protein kinase. The mode in which translation in reticulocyte lysates is inhibited in the absence of hemin and maintained in its presence is now understood.

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