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Control of Protein Synthesis. Phosphorylation and Dephosphorylation of Eukaryotic Peptide Initiation Factor 2

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Differentiation, virus infection, or certain pathological conditions are examples of physiologic events that cause dramatic qualitative and quantitative changes in protein synthesis. It has been assumed and directly shown in several model systems that these changes involve a block in peptide initiation. Systems in which some of the underlying molecular mechanisms have been studied include dimethyl sulfoxide (DMSO) induced differentiation in Friend leukemia cells (FLC), iron or heme deficiency in reticulocytes or their lysates, and interferon-treated cells that are sensitive to double-stranded RNA (dsRNA). It has been shown with FLC that DMSO and other inducers of differentiation cause a rather rapid inhibition of peptide initiation that precedes the induction of hemoglobin synthesis (Bilello et al. 1979). These authors also demonstrated that inhibitors of peptide initiation, but not inhibitors of elongation, may be able to induce these cells to differentiate. Cell-free systems have been used to study the mechanism by which peptide initiation is blocked during heme deficiency and in the presence of dsRNA. It was found that the peptide initiation factor eIF-2 plays a key role in the regulation of eukaryotic protein synthesis. The activity of eIF-2 in the reaction by which Met-tRNA$_f$ is bound to 40S ribosomal subunits is controlled by phosphorylation/dephosphorylation of its smallest subunit, eIF-2α (reviewed by Kramer et al. 1980). Binding of Met-tRNA$_f$ to 40S ribosomal subunits that are dependent on eIF-2, GTP, and other initiation factor(s) is inhibited if eIF-2α has been phosphorylated by the cAMP-independent, heme-controlled reticulocyte protein kinase that is specific for this substrate (Pinphanichakarn et al. 1976). Conversely, a phosphoprotein phosphatase isolated from reticulocytes will reverse this inhibition (Grankowski et al. 1980a).

The eIF-2α protein kinase and the counteracting phosphatase thus form a pair of regulatory components in the control of eukaryotic peptide initiation. The activity of the regulatory proteins is controlled by other factors. Here we consider some aspects of the regulation of the phosphoprotein phosphatase.

A. Materials and Methods

Preparation of the following components from rabbit reticulocytes has been described in detail: eIF-2 (Odom et al. 1978), phosphatase (Grankowski et al., 1980a), and phosphatase activators (Grankowski et al. 1980b). eIF-2 was phosphorylated in its αsubunit by the heme-controlled protein kinase from reticulocytes, reisolated by chromatography on phosphocellulose, and used as substrate in the phosphatase assay as reported previously (Grankowski et al. 1980a).

B. Results and Discussion

In contrast to the highly specific protein kinase that phosphorylates eIF-2α, phosphoprotein phosphatase activity isolated from rabbit reticulocytes has been found to have a rather broad substrate range (Grankowski et al. 1980a). Proteins phosphorylated by both cAMP-independent and cAMP-dependent protein kinases are readily dephosphorylated. However, stimulation and a certain degree of substrate specificity may be imposed on phosphatase activity by specific low molecular weight, heat-stable peptides. We have purified two such peptides from reticulocytes to homogeneity (Grankowski et al. 1980b).

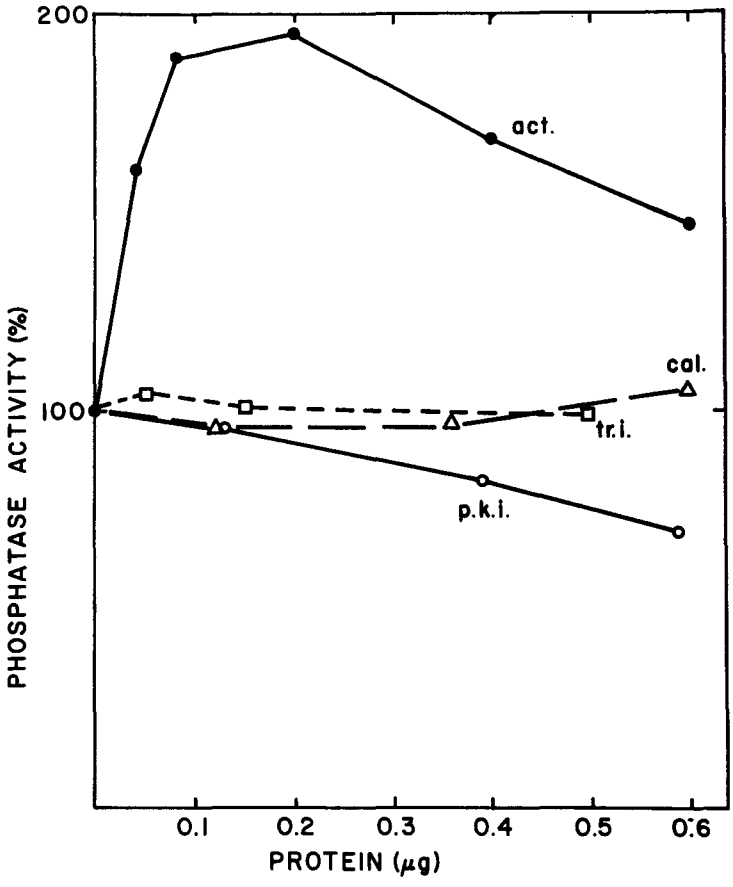

Fig. 1. Specificity of the activator that stimulates phosphatase activity for phosphorylated eIF-2α. Phosphatase (0.5 μg) was preincubated for 10 min at 37°in the absence or presence of the indicated amounts of phosphatase activator (●—●, *act.*), soybean trypsin inhibitor (□–□, *tr. i.*), calmodulin (△–△, *cal.*), or the inhibitor of cAMP-dependent protein kinases (○—○, *p.k.i.*). Then phosphatase activity was determined with [^{32}P]eIF-2α as substrate as described (Grankowski et al. 1980a)

One of them is a heat-stable, acidic, 17,400 dalton protein that specifically stimulates dephosphorylation of eIF-2α by the reticulocyte phosphatase. The results given in Fig. 1 show an increase in phosphatase activity for eIF-2α by up to 100% depending on the amount of activator added. As demonstrated in Fig. 1, no other peptide tested has been found to produce a similar effect for a few of these components (calmodulin, soybean trypsin inhibitor, and the heat-stable inhibitor of cAMP-dependent protein kinase activity, all of which are heat-stable, low molecular weight proteins with an acidic isoelectric point). This phosphatase activator does not affect dephosphorylation of phosphoproteins that have been phosphorylated by a cAMP-dependent protein kinase (Grankowski et al. 1980b). The data in Fig. 2 show that the activator increases the rate of dephosphorylation appreciably, but not the extent. The results of other experiments indicate that the activator interacts with the enzyme, not the substrate (Grankowski et al. 1980b). Thus it appears that the activator is a specific, low molecular weight peptide effector that may function in the control of phosphatase activity for eIF-2α.

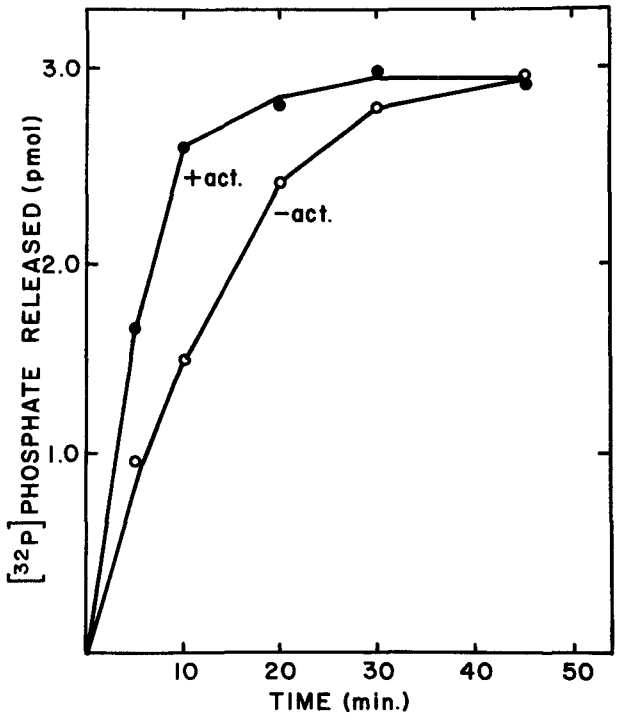

Fig. 2. Phosphatase activator increases the rate of dephosphorylation of eIF-2α. Phosphatase was preincubated in the absence or presence of the activator, then [^{32}P]eIF-2α was added and the reaction stopped at the times indicated. ●—●, *act.*, incubated in the presence of phosphatase activator

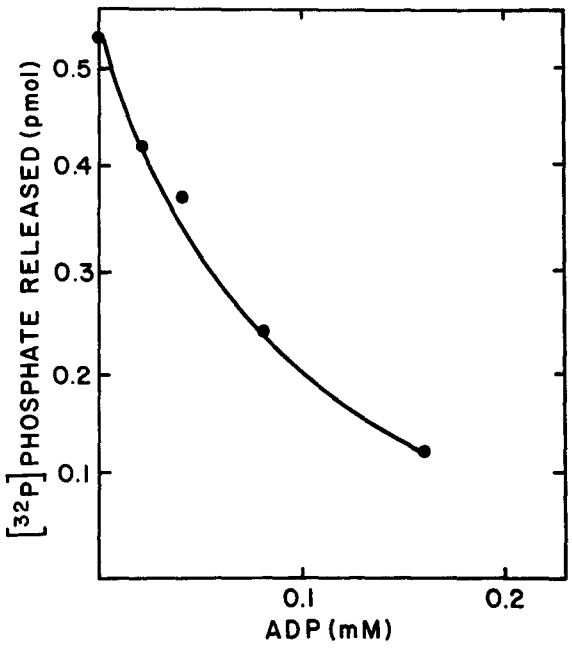

Fig. 3. Inhibition of Phosphatase Activity by ADP. ADP was included in the reaction mixture to give the concentrations shown on the abscissa. Release of [^{32}P]phosphate from eIF-2α was determined (Grankowski et al. 1980)

The results presented in Fig. 3 indicate that ADP is a potent inhibitor of eIF-2α phosphatase activity; 50% inhibition is observed with 80–100 μM. GDP has been found to be slightly less active (cf. Grankowski et al. 1980a). In vivo levels of the adenylate pool (about 2 mM) are 10-fold to 15-fold greater than the guanylate pool (Colby and Edlin 1970). The ratio of ATP to ADP appears to vary greatly and decreases under adverse nutrient conditions, thereby directly and indirectly influencing macromolecular synthetic pathways. It controls nucleoside diphosphate kinase activity affecting the conversion of GDP to GTP and thus controls protein synthesis and, especially, peptide initiation (Walton and Gill 1976). However, the direct effect of ADP on eIF-2α phosphatase activity (Fig. 3) seems to be an immediate additional way of controlling peptide initiation in situations in which the eIF-2α protein kinase is activated.

References

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