Volume 267, number 2, 181-182 FEBS 08585 July 1990

Discussion Letter

Two phosphorylation sites on eIF-2α

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Received 8 May 1990

Protein synthesis in mammalian cells can be regulated through phosphorylation/dephosphorylation of the α subunit of initiation factor 2, eIF-2. Two specific kinases have been identified that apparently phosphorylate the same site(s). Controversy exists as to whether serine-48 is a phosphorylation site in addition to serine-51. A recent publication is discussed that, in this author's view, answers the question of the phosphorylation sites. It is suggested that phosphorylation procedes sequentially with serine-51 being the first and serine-48 the second phosphorylation site. Phosphorylation of both sites is required for inhibition of protein synthesis.

Protein synthesis regulation; Initiation factor 2; Phosphorylation site

The phosphorylation site(s) on mammalian eIF-2α, the α subunit of protein synthesis initiation factor 2, have been controversial. Phosphorylation by either of two different specific kinases leads to inhibition of protein synthesis by a common mechanism as considered in detail in [1]. The two enzymes are the heme-controlled eIF-2α kinase and the double-stranded (ds)RNA-dependent kinase. Both kinases occur in rabbit reticulocytes (references in [1]), but the latter also is found in a number of interferon-sensitive mammalian cells [2-4]. The two kinases have been reported to phosphorylate the same sites on eIF-2α [5-7]. The following discussion is based on this premise.

By direct phosphopeptide analysis and sequencing we determined [8] that serine-48 is a phosphorylation site in eIF-2α for the heme-controlled reticulocyte kinase. In collaboration with B. Kemp [9], a synthetic peptide was used that comprised amino acids 41-54 of eIF-2α containing serine-residues at positions 48 and 51. In vitro phosphorylation of this peptide resulted only in modification of serine-51. Increased phosphorylation of serine-51 relative to serine-48 was observed in eIF-2α after stimulation of the reticulocyte eIF-2α kinase by spectrin-subunits in vitro [9]. Colthurst et al. [7] found only serine-51 as a phosphorylation site in eIF-2α for either kinase.

Hershey and coworkers cloned and sequenced human eIF-2α [10] and by site-directed mutagenesis prepared mutant cDNAs encoding functionally active eIF-2α with alanine at either position 48 or 51 [11]. The in vitro expressed mutant forms were translated in the reticulocyte lysate and their phosphorylation was studied when the heme-controlled or the dsRNA-activated kinase was present. The mutant Ala-48 form was phosphorylated, the mutant Ala-51 species was not. The authors concluded serine-51 to be the only phosphorylation site for either kinase on the eIF-2α subunit.

A definite answer to the controversy regarding phosphorylation site(s) on eIF-2α may lie in a recently published paper by Davies et al. [12]. These authors studied the in vivo effects of the mutant eIF-2α forms described above on complementing deletion forms of adenovirus to support viral protein synthesis in infected human 293 cells. Adenovirus mutants defective in the synthesis of a small RNA (VAI RNA) are described and discussed in a recent review [4]. The defect allows host cells to activate the interferon-induced dsRNA-dependent eIF-2α kinase, since it has been demonstrated that adenovirus-encoded VAI RNA interacts with and prevents activation of this kinase [3]. Davies et al. [12] introduced the plasmids containing the eIF-2α mutant forms into 293 cells, then studied defective adenovirus replication and protein synthesis in these transformants. They report that a cell line carrying the eIF-2α (Ala-51) mutant that cannot be phosphorylated will relieve inhibition of viral protein synthesis. The authors are surprised by the finding that expression of the eIF-2α (Ala-48) mutant in another cell line shows the same effect. They are puzzled by this

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50 © 1990 Federation of European Biochemical Societies

finding because this mutant eIF-2α expressed in the virus-infected cells is phosphorylated by the dsRNA-dependent kinase. It should be noted that all cell lines irrespective of the level of eIF-2α synthesis during a 30-min pulse exhibited similar levels of eIF-2α protein monitored by immunoblot analysis [12].

In a previous publication [13], Kaufman et al. indicated already that expression of serine-to-alanine at either residue 48 or residue 51 could substitute for VA RNA and promote translation of plasmid-derived mRNAs in COS-1 cells. The data presented by these authors [12,13] may be interpreted in the following way. Both serine-48 and serine-51 can be phosphorylated by the eIF-2α kinases but phosphorylation proceeds in a sequential order. First serine-51 is phosphorylated (which can be accomplished even in a synthetic peptide [9]), and then subsequently serine-48 is phosphorylated. For biological effects, i.e. inhibition of protein synthesis, both sites have to be phosphorylated.

How can the data published on eIF-2α phosphorylation be reconciled with this interpretation? Our first report on the phosphorylation site for the heme-regulated eIF-2α kinase [8] identified only serine-48; however, it did not exclude serine-51. This site was not detected because not all tryptic phosphopeptides or derived chymotryptic peptides could be sequenced.

Analyses of eIF-2α from cells or lysates after different incubation conditions by isoelectric focussing have been interpreted generally to indicate one phosphorylated and one unphosphorylated form (e.g. [14, 15]). However, data presented in [16], p. 5492, may indicate more than two eIF-2α forms.

Analyses of eIF-2α isolated from reticulocyte lysates that had been incubated in the presence or absence of hemin deserve more careful consideration. Scorsone et al. [15] used monoclonal antibodies to identify eIF-2α (phosphorylated or unphosphorylated) after separating polypeptides in isoelectric focussing gels. They separate two forms, eIF-2α and eIF-2αP, from the reticulocyte lysate regardless of whether it was incubated with or without hemin or with exogenously added eIF-2α kinase. Jagus and Safer [17], on the other hand, purify eIF-2, then separate three eIF-2α forms from hemin-deficient lysates applying the same techniques. These authors also quantitate the inorganic phosphate content of eIF-2α isolated from hemin-supplemented and hemin-deficient lysates. They find 0.96–1.05 mol phosphate/mol eIF-2α in the former, and 1.25–1.30 mol phosphate/mol eIF-2α in the latter case. In vitro incubation of the same eIF-2 preparations with [γ-^{32}P]ATP and heme-controlled eIF-2α kinase leads to additional incorporation of 0.95 to 1.03 mol phosphate/mol eIF-2α into eIF-2 isolated from the former and 0.69–0.74 mol phosphate/mol eIF-2α from the latter lysates. In an earlier paper [18] it was concluded that one mole of phosphate was incorporated into eIF-2α by in vitro incubation of eIF-2 with [γ-^{32}P]ATP and the heme-controlled kinase. However, the data presented indicate more than one (up to about 1.2) mol phosphate incorporated per mol eIF-2α.

The data cited may indicate the ambiguity of the extent of eIF-2α phosphorylation both in vivo and in vitro. They do not exclude the presence of two phosphorylation sites. However, in my view, the recent data published by Davies et al. [12] unequivocally demonstrate the biological importance of both serine-48 and serine-51 as phosphorylation sites to exert translational control.

Acknowledgements: I wish to thank Drs William Picking and Boyd Hardesty for valuable discussion. Supported by a grant to B.H. from the Foundation for Research.

REFERENCES

[1] Pain, V.M. (1986) Biochem. J. 235, 625–637.
[2] Galabru, J. and Hovanessian, A. (1987) J. Biol. Chem. 262, 15538–15544.
[3] Kostura, M. and Mathews, M.B. (1989) Mol. Cell. Biol. 9, 1576–1586.
[4] Schneider, R.J. and Shenk, T. (1987) Annu. Rev. Biochem. 56, 317–332.
[5] Samuel, C.E. (1979) Proc. Natl. Acad. Sci. USA 76, 600–604.
[6] Ernst, V., Levin, D.H., Leroux, A. and London, I.M. (1980) Proc. Natl. Acad. Sci. USA 77, 1286–1290.
[7] Colthurst, D.R., Campbell, D.G. and Proud, C. (1987) Eur. J. Biochem. 166, 357–363.
[8] Wettenhall, R.E.H., Kudlicki, W., Kramer, G. and Hardesty, B. (1986) J. Biol. Chem. 261, 12444–12447.
[9] Kudlicki, W., Wettenhall, R.E.H., Kemp, B.E., Szyszka, R., Kramer, G. and Hardesty, B. (1987) FEBS Lett. 215, 16–20.
[10] Ernst, H., Duncan, R. and Hershey, J.W.B. (1987) J. Biol. Chem. 262, 1206–1212.
[11] Pathak, V.K., Schindler, D. and Hershey, J.W.B. (1988) Mol. Cell. Biol. 8, 993–995.
[12] Davies, M.V., Furtado, M., Hershey, J.W.B., Thimmappaya, B. and Kaufman, R.J. (1989) Proc. Natl. Acad. Sci. USA 86, 9163–9167.
[13] Kaufman, R.J., Davies, M.V., Pathak, V.K. and Hershey, J.W.B. (1989) Mol. Cell. Biol. 9, 946–958.
[14] Duncan, R. and Hershey, J.W.B. (1985) J. Biol. Chem. 260, 5493–5497.
[15] Scorsone, K.A., Panniers, R., Rowlands, A.G. and Henshaw, E. (1987) J. Biol. Chem. 262, 14538–14543.
[16] Duncan, R. and Hershey, J.W.B. (1985) J. Biol. Chem. 260, 5486–5492.
[17] Jagus, R. and Safer, B. (1987) J. Cyclic Nucleotide Prot. Phosph. Res. 11, 557–470.
[18] Tuazon, P.T., Merrick, W.C. and Traugh, J.A. (1980) J. Biol. Chem. 255, 10954–10958.