The Phosphorylation of Eukaryotic Initiation Factor eIF4E in Response to Phorbol Esters, Cell Stresses, and Cytokines Is Mediated by Distinct MAP Kinase Pathways*

Initiation factor eIF4E binds to the 5′-cap of eukaryotic mRNAs and plays a key role in the mechanism and regulation of translation. It may be regulated through its own phosphorylation and through inhibitory binding proteins (4E-BPs), which modulate its availability for initiation complex assembly. eIF4E phosphorylation is enhanced by phorbol esters. We show, using specific inhibitors, that this involves both the p38 mitogen-activated protein (MAP) kinase and Erk signaling pathways. Cell stresses such as arsenite and anisomycin and the cytokines tumor necrosis factor-α and interleukin-1β also cause increased phosphorylation of eIF4E, which is abolished by the specific p38 MAP kinase inhibitor, SB203580. These changes in eIF4E phosphorylation parallel the activity of the eIF4E kinase, Mnk1. However, other stresses such as heat shock, sorbitol, and H2O2, which also stimulate p38 MAP kinase and increase Mnk1 activity, do not increase phosphorylation of eIF4E. The latter stresses increase the binding of eIF4E to 4E-BP1, and we show that this blocks the phosphorylation of eIF4E by Mnk1 in vitro, which may explain the absence of an increase in eIF4E phosphorylation under these conditions.

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§ These authors contributed equally to the work.

¶ Present address: Laboratoire de Biophysique, Musée National d’Histoire Naturelle, 43 rue Cuvier, 75231 Paris Cedex 05, France.

** Present address: Faculty of Medicine, Dept. of Medical Biochemistry, Section on Molecular Carcinogenesis, P.O. Box 9503, 2300 RA Leiden, The Netherlands.

 §§ To whom correspondence should be addressed. Present address: Dept. of Anatomy & Physiology, Medical Sciences Inst., University of Dundee, Dundee, DD1 4HN, UK. Tel.: 44-1382-344919; Fax: 44-1382-322424; E-mail: CGProud@bd.dundee.ac.uk

Xuemín Wang‡, Andrea Flynn‡§§, Andrew J. Waskiewicz§§, Benjamin L. J. Webb‡§, Robert G. Vries‡,**, Ian A. Baines‡††, Jonathan A. Cooper, and Christopher G. Proud‡§§

From the ‡Department of Biosciences, University of Kent at Canterbury, Canterbury, CT2 7NJ, United Kingdom and the ¶Fred Hutchinson Cancer Research Centre, Seattle, Washington 98109

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Phosphorylation of eIF4E in vivo is less clear. We have recently shown that insulin-induced eIF4E phosphorylation requires the MAP kinase signaling pathway (also termed the Erk, extracellular signal-regulated kinase pathway, the term used here) (7). However, eIF4E is not a substrate for the Erks, and we have shown that it is phosphorylated instead at Ser209 by a novel Erk-activated protein kinase, MAP kinase signal-integrating kinase-1 (Mnk1) (8, 9). Mnk1 is also phosphorylated and activated by an additional enzyme related to Erk, p38 MAP kinase, which lies on a distinct signaling pathway activated by cell stresses and cytokines (8–10).

Here we show that the increased phosphorylation of eIF4E brought about by the phorbol ester tetradecanoylphorbol 13-acetate (TPA), which activates members of the protein kinase C (PKC) family, requires the Erk and p38 MAP kinase pathways. Furthermore, we show that eIF4E phosphorylation is enhanced by agents, such as certain stresses and cytokines, that activate p38 MAP kinase and that this is blocked by a specific inhibitor of this enzyme. Our data support the identity of Mnk1 as a physiologically important eIF4E kinase. Certain stresses that activate p38 MAP kinase do not increase eIF4E phosphorylation. This is likely to be due to the increased association of eIF4E with 4E-BP1 that these conditions bring about, because 4E-BP1 inhibits phosphorylation of eIF4E by Mnk1.

MATERIALS AND METHODS

Chemicals and Biochemicals—Unless otherwise stated, chemicals were obtained as described previously (7, 11). Anti-(P)Erk was from New England BioLabs. Anti-human eIF4E was raised against a synthetic peptide corresponding to the carboxyl-terminal region of the human eIF4E protein (amino acids 215–225) and affinity-purified by the manufacturer.

The abbreviations used are: 4E-BP, eIF4E-binding protein; MAP, mitogen-activated protein kinase; TPA, tetradecanoylphorbol 13-acetate; PKC, protein kinase C; CHO, Chinese hamster ovary; HUVEC, human umbilical vein endothelial cell; MAPKAP-K, MAP kinase-activated protein kinase; GST, glutathione S-transferase; TNFα, tumor necrosis factor-α; IL-1β, interleukin-1β; JNK, c-jun N-terminal kinase.
Regulation of Initiation Factor 4E Phosphorylation

that the phorbol ester-induced phosphorylation of eIF4E is not, as has previously been suggested (1), directly mediated by PKC. A more likely route by which TPA increases eIF4E phosphorylation is through activation of the Erk/p38 MAP kinase cascades, via PKC, leading to the activation of Mnk1, which itself directly phosphorylates eIF4E (8). This is consistent with

RESULTS AND DISCUSSION

TPA-induced Phosphorylation of eIF4E Requires the Erk and p38 MAP Kinase Pathways—In several cell types, phorbol esters that activate PKC enhance eIF4E phosphorylation (11, 17–19). Exposure of 293 cells to TPA increased the level of eIF4E phosphorylation from almost zero to about 30% (Fig. 1A). To test whether, as for insulin (7), this effect required the Erk pathway, we used the compound PD098059, a specific inhibitor of MEK activation (20). As expected, PD098059 reduced the ability of TPA to enhance eIF4E phosphorylation (Fig. 1A) and also completely inhibited the activation of Erk by TPA in 293 cells (Fig. 1B, lanes 1–4). However, the inhibition of eIF4E phosphorylation was incomplete indicating that other signaling pathways were involved. We therefore tested the effect of a specific inhibitor of p38 MAP kinase SB203580 (21) on eIF4E phosphorylation. It has been shown not to interfere with stress-, cytokine-, or growth factor-induced activation of other signaling pathways such as Erk, JNK, or p70 S6 kinase (21–24). SB203580 partially blocked TPA-induced eIF4E phosphorylation, and its use together with PD098059 completely abolished TPA-induced eIF4E phosphorylation (Fig. 1A). As shown in Fig. 1B (lanes 5–8), TPA activates MAPKAP-K2, which is activated by p38 MAP kinase (10), in 293 cells. These data suggest that TPA acts through both Erk and p38 MAP kinase to increase eIF4E phosphorylation in 293 cells. Waskiewicz et al. (8) previously showed that Mnk1 could be activated in vitro by either Erk or p38 MAP kinase. To assess Mnk1 activity in 293 cells subjected to these treatments, we transfected 293 cells with a vector encoding wild-type Mnk1 fused to GST (8) and subjected the transfected cells to treatment with TPA in the absence or the presence of kinase inhibitors. Cells were extracted, and Mnk1-GST was isolated and assayed. SB203580 or PD098059 only partially prevented the activation of Mnk1 by TPA, but use of both completely abolished it (Fig. 1C). The changes in Mnk1 activity parallel the alterations in eIF4E phosphorylation observed under these conditions, entirely consistent with a key role for Mnk1 in mediating eIF4E phosphorylation (8).

TPA also increases eIF4E phosphorylation in CHO.K1 cells, which involves the so-called conventional isoforms of PKC (11). Analysis of the roles of signaling pathways in the phosphorylation of eIF4E in these cells is more complex than for 293 cells due to the high basal level of eIF4E phosphorylation (Fig. 1D). This was partially decreased either by PD098059 or SB203580, although both were required to completely suppress it (Fig. 1D). Each compound alone also decreased the level of eIF4E phosphorylation in TPA-treated CHO.K1 cells, but both were again required to abolish eIF4E phosphorylation completely (Fig. 1D). Taken together, the data reinforce the conclusion that both the Erk and p38 MAP kinase cascades are involved in mediating changes in eIF4E phosphorylation. Our data imply that the phorbol ester-induced phosphorylation of eIF4E is not, as has previously been suggested (1), directly mediated by PKC. A more likely route by which TPA increases eIF4E phosphorylation is through activation of the Erk/p38 MAP kinase cascades, via PKC, leading to the activation of Mnk1, which itself directly phosphorylates eIF4E (8). This is consistent with
Arsenite Induces the Phosphorylation of eIF4E, Which Is Blocked by SB203580—The above data prompted us to ask whether other treatments that activate p38 MAP kinase affect eIF4E phosphorylation. Arsenite potently activates the p38 MAP kinase pathway in 293 cells (Fig. 2A) and also markedly increased eIF4E phosphorylation (Fig. 2B, lanes 1–5). The p38 MAP kinase inhibitor SB203580 (21) blocked both this and the arsenite-induced activation of MAPKAP-K2 (Fig. 2A). Arsenite did not activate Erk in 293 cells (data not shown), and the MEK inhibitor PD098059 did not affect arsenite-induced eIF4E phosphorylation (Fig. 2B). Thus, arsenite-induced eIF4E phosphorylation appears to be mediated by the p38 MAP kinase pathway (8). Changes in Mnk1 activity again paralleled those in eIF4E phosphorylation (Fig. 2C). The ability of arsenite to increase eIF4E phosphorylation is not restricted to 293 cells because the same effect was also observed in CHO.K1 cells (Fig. 2B, lanes 6–9), and again, SB203580 blocked both arsenite-induced eIF4E phosphorylation and p38 MAP kinase activation (Fig. 2B and data not shown). Anisomycin activates Mnk1 in 293 cells, and this activation was blocked by SB203580 (Ref. 8 and Fig. 2C). Anisomycin increased eIF4E phosphorylation in 293 cells (to about 80%, data not shown), and this effect was also completely blocked by SB203580. Anisomycin also increases eIF4E phosphorylation in NIH 3T3 cells, and this increase is blocked by SB203580 (25).

The finding that arsenite stimulates eIF4E phosphorylation is surprising given that arsenite potently inhibits protein synthesis (26), whereas eIF4E phosphorylation is normally associated with its activation. It is likely that arsenite inhibits other steps in translation, and, indeed, we have shown that it increases phosphorylation of the α-subunit of eIF2, which is well known to lead to inhibition of peptide chain initiation (27).

Effects of Other Stresses on eIF4E Phosphorylation—Other stresses such as hyperosmolarity (sorbitol) and oxidative stress (hydrogen peroxide) also activate p38 MAP kinase, MAPKAP-K2 (Fig. 2A) and Mnk1 (Fig. 2C). However, unlike arsenite, they did not increase eIF4E phosphorylation (Fig. 2D, lanes 3–5). In 293 cells, where the basal eIF4E phosphorylation is low, no change was seen (Fig. 2D). In CHO.K1 cells, where the basal eIF4E phosphorylation is significant, they led to a fall in eIF4E phosphorylation (Fig. 2D). Heat shock did not appreciably activate p38 MAP kinase in 293 cells but did in CHO.K1 cells (16), and this activation is blocked by SB203580. Despite this, heat shock actually caused a decrease in eIF4E phosphorylation (Fig. 2D).

Why do some stresses increase eIF4E phosphorylation, whereas others cause a decrease, even though they also activate Mnk1? To try to explain this apparent paradox, we analyzed the association of eIF4E with its regulator 4E-BP1; we have previously shown that in CHO.K1 cells, most stresses increase binding of 4E-BP1 to eIF4E (16). This effect is also seen 293 cells (Fig. 3A). The exception here (as in CHO cells) is arsenite, which does not cause increased binding of 4E-BP1 to eIF4E. This is probably because it can activate the rapamycin-sensitive signaling pathway (28), which leads to the phosphor- ylation of 4E-BP1 and its dissociation from eIF4E (4). These findings raised the possibility that the association of 4E-BP1 with eIF4E might impair phosphorylation of the latter by Mnk1. To test this, we examined the effect of 4E-BP1 on the ability of Mnk1 to phosphorylate eIF4E in vitro. The data (Fig. 3B) clearly show that 4E-BP1 substantially inhibits the phosphorylation of eIF4E by Mnk1. The highest amount of 4E-BP1 used represents saturation of the eIF4E with 4E-BP1 as indicated by the fact that addition of further 4E-BP1 resulted in (i) it not being retained on m7GTP-Sepharose, i.e. not being associated with eIF4E, and (ii) phosphorylation of the excess 4E-BP1 by the Erk present in the activated Mnk1, with only free 4E-BP1 (and not the 4E-BP1/eIF4E complex) being a substrate for Erk (12) (data not shown). SB203580 had no effect on the association of eIF4E with 4E-BP1, either under stress or control conditions (16). 2 4E-BP1 did not affect the phosphorylation of another substrate, the cAMP-response element binding protein, by Mnk1 (data not shown). This suggests that inhibition of eIF4E phosphorylation by 4E-BP1 reflects the inability of Mnk1 to phosphorylate eIF4E in the eIF4E/4E-BP1 complex

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Regulation of Initiation Factor 4E Phosphorylation

The inhibition of Mnk1-catalyzed eIF4E phosphorylation by 4E-BP1 provides an explanation for the differing effects of stresses on eIF4E phosphorylation, and, in particular, for the ability of heat shock to reduce eIF4E phosphorylation (reviewed in Ref. 29). Some studies have shown that rapamycin reduces the level of eIF4E phosphorylation (25, 30). Our data suggest that this may be due to increased association of 4E-BP1 with 4E-BP1 caused by rapamycin (due to dephosphorylation of 4E-BP1 (4)) and resulting inhibition of Mnk1-catalyzed eIF4E phosphorylation. 4E-BP1 also blocks the phosphorylation of eIF4E by PKC (31). The binding site for 4E-BP1 in eIF4E has recently been identified (32). Because it is some distance from Ser209 in the three-dimensional structure of the protein, it seems unlikely that 4E-BP1 actually occludes the phosphorylation site. The effect of 4E-BP1 may instead reflect interference with the interaction between these kinases and other regions of the eIF4E protein required for kinase/substrate binding.

The increased binding of 4E-BP1 to eIF4E was accompanied by a decrease in the binding of eIF4G to eIF4E (Fig. 3C), as expected from the mutually competitive nature of their interactions (33). Consistent with its lack of effect on 4E-BP1 binding, arsenite also had no effect on the association of eIF4E with eIF4G.

Regulation of eIF4E Phosphorylation by Cytokines That Activate p38 MAP Kinase—It was important to ascertain whether treatment of cells with physiological activators of p38 MAP kinase, such as cytokines, also altered the phosphorylation of eIF4E. Tumor necrosis factor-α (TNFα) is a physiological regulator of endothelial cell function (34), and in HUVECs it markedly activates the p38 MAP kinase pathway without any apparent effect on Erk activity (data not shown and Ref. 34). We therefore studied its effect on the phosphorylation of eIF4E. TNFα increased the phosphorylation of eIF4E (Fig. 4A), and this increase was prevented by SB203580, which blocked activation of the p38 MAP kinase pathway and hence of MAPKAP-K2. These data show for the first time that cytokines increase phosphorylation of eIF4E through the p38 MAP kinase pathway. In CHO.K1 cells, another cytokine, interleukin-1β (IL-1β) activates p38 MAP kinase, although less markedly than TNFα does in HUVECs (data not shown). IL-1β (5 ng/ml) increased the phosphorylation of eIF4E in CHO.K1 cells (Fig. 4B). This effect, like that of TNFα in HUVECs, was prevented by SB203580 (data not shown).

Conclusions—The ability of activators of p38 MAP kinase to increase eIF4E phosphorylation was seen in three different cell types, human embryonic kidney (293) cells, CHO cells, and HUVECs and in response to stresses and cytokines. In all cases, SB203580 blocked the phosphorylation of eIF4E. The data for TNFα in HUVECs are of particular note given that the TNFα-stimulated induction of the cell adhesion molecule V-CAM is mediated by the p38 MAP kinase pathway (34) and involves post-transcriptional effects that might be related to changes in eIF4E phosphorylation. Both the stress stimuli (arsenite and anisomycin) and TNFα also activate the JNK pathway. However, the ability of SB203580 (which does not affect JNK activity in the cells used here) to block eIF4E phosphorylation indicates that the JNK pathway is not involved in modulating eIF4E phosphorylation.

We have previously shown that insulin-induced phosphorylation of eIF4E requires the Erk pathway (7). Taken together, our findings show that eIF4E phosphorylation can be mediated by two distinct signaling pathways, the Erk and p38 MAP kinase pathways, depending on the stimulus used, consistent with the established regulatory properties of the eIF4E kinase Mnk1, which is a target for activation by both (8, 9). Changes in eIF4E phosphorylation largely mirror alterations in Mnk1 activity, consistent with a physiological role for this kinase in eIF4E phosphorylation. The only exceptions are stress conditions that increase binding of 4E-BP1 to eIF4E. In almost all cases, such conditions activate Mnk1 but decrease eIF4E phosphorylation.
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