ERK and p38 Inhibit the Expression of 4E-BP1 Repressor of Translation through Induction of Egr-1*

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4E-BP1 plays a major role in translation by inhibiting cap-dependent translation initiation. Several reports have investigated the regulation of 4E-BP1 phosphorylation, which varies along with cell differentiation and upon various stimulations, but very little is known about the regulation of its expression. In a first part, we show that the expression of 4E-BP1 protein and transcript decreases in hematopoietic cell lines cultivated in the presence of phorbol 12-myristate 13-acetate (PMA). This decrease depends on the activation of the ERK/mitogen-activated protein kinases. 4E-BP1 expression also decreases when the p90mitogen-activated protein kinase pathway is activated by granulocyte/macrophage colony-stimulating factor but to a lesser extent than with PMA. In a second part, we examine how 4e-bp1 promoter activity is regulated. PMA and granulocyte/macrophage colony-stimulating factor induce Egr-1 expression through ERK and p38 activation, respectively. Using a dominant negative mutant of Egr, ZnEgr, we show that this transcription factor is responsible for the inhibition of 4e-bp1 promoter activity. In a third part we show that histidine decarboxylase, whose activity and expression are inversely correlated with 4E-BP1 expression, is a potential target for the translational machinery. These data (i) are the first evidence of a new role of ERK and p38 on the translational machinery and (ii) demonstrate that 4E-BP1 is a new target for Egr-1.

Control of mRNA translation plays a pivotal role in regulating gene expression under a variety of conditions in mammalian cells (1). The predominant step in translational regulation is the initiation phase, which consists of the recruitment of the 40 S ribosomal subunit to the mRNA (2). This occurs through recognition of the 5′ cap structure (m7GpppX, where X is any nucleotide) by the cap-binding protein complex eIF4F (guanylicotylic initiation factor 4F) which, in higher eukaryotes, consists of three subunits: eIF4A, eIF4E, and eIF4G. The initiation process is largely regulated through changes in the phosphorylation state of eIFs and other components involved in this process (3, 4). eIF4E activity is modulated by phosphorylation in response to mitogens, polypeptide hormones, tumor promoters, and growth factors in a mitogen-activated protein kinase (MAPK)-MAPK signal-integrating kinase (MNK) pathway-dependent manner (5). In addition to the regulation of its phosphorylation, the activity of eIF4E is tightly controlled through reversible interaction with a family of inhibitory proteins termed 4E-BP (eIF4E-binding proteins). Of the three known proteins (4E-BP1, 4E-BP2, and 4E-BP3), 4E-BP1, also named PHAS-1, is the best characterized. 4E-BP1 specifically inhibits cap-dependent translation by competing with eIF4G for binding to the cap-binding factor eIF4E and consequently preventing the formation of the eIF4F complex (6). The affinity of the 4E-BPs to eIF4E depends on their phosphorylation state. Hypophosphorylated 4E-BPs interact with high affinity with eIF4E, whereas hyperphosphorylation of 4E-BPs, elicited by stimulation of cells with hormones, cytokines, or growth factors, results in an abrogation of eIF4E-binding activity. Activation of phosphatidylinositol 3-kinase or a downstream phosphatidylinositol 3-kinase effector, Akt/protein kinase B, and FRAP/mTOR (FKBP and rapamycin-associated protein), leads to 4E-BP1 hyperphosphorylation (7–9). Six phosphorylation sites have been identified in 4E-BP1: Thr37, Thr46, Ser65, Thr70, Ser83, and Ser112 (numbering according to human 4E-BP1). FRAP/mTOR phosphorylates 4E-BP1 on Thr37, Thr46, and Thr70 (9, 12), activation of p38MSK1 (mitogen and stress kinase 1) pathway by UV light leads to phosphorylation on Thr37 and Ser65 (10), and the activation of the ERK pathway induces phosphorylation on Ser65, Thr37, Thr46, and Thr70 (11). A hierarchical phosphorylation of 4E-BP1 has been proposed: first on the Thr37 and Thr46 and then on Thr70 and Ser65 (12), showing that multiple phosphorylation events (most likely via different kinases) are required to release eIF4B from eIF-4E. Recently it has been shown that 4E-BP1 cleavage by caspase is a new step in the regulation of translation in response to insulin (13).

Signal transduction via MAPK plays a key role in a variety of cellular responses, including early embryonic development, cell death, growth factor-induced proliferation, and cell differentiation (14–21). Three main pathways have been defined in mammalian cells: the classical ERKs, the c-Jun N-terminal kinases (JNK, also known as SAPK1), and the p38 (also termed SAPK2). ERK are stimulated by growth factors and cytokines, whereas JNK and p38 are generally activated by pro-inflammatory stimuli.

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2The abbreviations used are: MAPK, mitogen-activated protein kinase; MNK, MAPK signal-integrating kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; SAPK, stress-activated protein kinase; IL, interleukin; CSF, colony-stimulating factor; GM, granulocyte/macrophage; PMA, phorbol 12-myristate 13-acetate; HDOC, histidine decarboxylase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; EGFP, enhanced green fluorescent protein; MEK, MAPK/ERK kinase; OA, okadaic acid; CHX, cycloheximide.
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interferon-

Thereby they control the expression of various genes including new insights into the regulation of HDC expression at the

We also have shown that 4E-BP1 expression is negatively tivity of the translational initiation machinery in PMA-treated post-transcriptional level (47, 48). Thus we examined the ac-

IL-4, IL-6, and IL-13 expression is regulated at the transcrip-

characterized by an induction of IL-4, IL-6, and IL-13 expres-

the growth factor GM-CSF spontaneously expresses the mRNA is modified. We derived from the pluripotent UT7 cell line a

is down-regulated during thymocyte maturation (46), but noth-

18860 primer, 5/H11032/GACTACAGCACGACCCCCG-3

were separated on agarose formaldehyde gels and blotted onto nylon membranes (Hybond™-N; Amersham Biosciences). The membrane

4e-bp1, and antisense primer, 5/H11032/GCCCGATGCTGATGAGTCCT-3

Total RNA were extracted by a modified Northern Blot Analysis

–35). Hybridized filters were washed under high stringency conditions (0.1 × SSC, 70 °C), analyzed by autoradiography, and quantified using QuantityOne. Equal loading of RNA was confirmed by stripping and reprobing the blots with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe or staining of the ribosomal RNA with ethidium bromide.

RESULTS

4E-BP1 Expression Decreases in Response to PMA—Phosphorylation of 4E-BP1 occurs in response to mitogens or growth factors by activation of the phosphatidylinositol 3-kinase

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literature that show that the Thr 37, Thr 46, and Thr 70 are phosphorylated by the FRAP/mTOR. p38 and ERK can phosphorylate several sites of 4E-BP1, notably the Ser\(^{65}\) (10, 11). This regulation did not occur in our system because treatment of the cells with SB203580 or U0126 did not have any effect on the phosphorylation pattern of 4EBP1.\(^2\) Nevertheless we could not rule out changes that could have occurred at shorter times of treatment. Northern blot analysis showed that neither rapamycin nor LY294002 affected the transcript level of 4E-BP1, whereas the PMA application resulted in a loss of 4E-BP1 transcript (Fig. 1B). We also looked at the effect of PMA on 4E-BP1 in three different cell lines: 11OC1, HEL, and K562. Twenty-four hours of PMA treatment led to an accumulation of the less phosphorylated isoforms of 4E-BP1 in 11OC1 and K562 cells, whereas in the HEL cell line only the most highly phosphorylated form of 4E-BP1 was represented in the bands at time 0, which is considered to be 100%. 20 \(\mu\)g of total RNA was analyzed by Northern blotting using 4e-bp1 or 18 S cDNA probes (loading control) as indicated (C). The results are expressed as percentages from the quantification of the bands from GM-CSF, 1 day, which is considered to be 100%.

\(^{2}\) M. Rolli-Derkinderen, F. Machavoine, and M. Dy, unpublished results.

and/or MAPK signal transduction pathways and is rapamycin-sensitive (10, 11, 50). To assess to what extend PMA modifies 4E-BP1 phosphorylation and/or expression, we analyzed by Western blot the expression and phosphorylation of 4E-BP1 in UT7D1 cells treated with or without PMA for 24 h. It should be mentioned that in all experiments UT7D1 cells were placed in fresh culture medium and GM-CSF-starved for 120 min before the addition of the inhibitors. After 30 min, we added fresh GM-CSF alone or GM-CSF and PMA. 4E-BP1 migrates in 12% SDS-polyacrylamide gels as four bands (\(\alpha, \beta, \gamma, \) and \(\delta\); Fig. 1A). The \(\alpha\) band is the less phosphorylated form, and the \(\delta\) band is the highly phosphorylated isoform. According to our personal observations and in line with the literature, the \(\beta\) band represents the 4E-BP1 isoform phosphorylated on Thr\(^{37}\) and Thr\(^{46}\); the \(\gamma\) isoform is phosphorylated on Thr\(^{37}\), Thr\(^{46}\), and Thr\(^{70}\); and the \(\delta\) isoform is phosphorylated on these three threonines and on Ser\(^{65}\) (11, 12). When the cells were cultivated with GM-CSF, the four phosphorylated forms of 4E-BP1 were present. In contrast, when the cells were treated with PMA, only the \(\alpha, \beta, \) and \(\gamma\) forms of 4E-BP1 were barely detected. These results not only reflect a change in the phosphorylation of 4E-BP1 but also a decrease in 4E-BP1 protein amount (Fig. 1A). In the presence of rapamycin, an inhibitor of FRAP/mTOR, or LY294002, an inhibitor of phosphatidylinositol 3-kinase, we observed an accumulation of the \(\alpha\) less phosphorylated form of 4E-BP1 in the absence or in the presence of PMA (Fig. 1A). These results are in line with the data from literature that show that the Thr\(^{37}\), Thr\(^{46}\), and Thr\(^{70}\) are phosphorylated by the FRAP/mTOR. p38 and ERK can phosphorylate several sites of 4E-BP1, notably the Ser\(^{65}\) (10, 11).
dectable (Fig. 1C). In all three cell lines PMA decreased 4E-BP1 expression (Fig. 1C).

4e-bp1 Transcript Level Decreases, Whereas the Expression of 4e-bp2 Is Not Sensitive to PMA—Time course analysis showed that 4E-BP1 expression transiently decreased after 4 h of GM-CSF (47 ± 17%; Fig. 2, A and C) and that the δ phosphorylated form of 4E-BP1 disappeared after 4 h of GM-CSF treatment. The α less phosphorylated form was mainly detected after 24 h of GM-CSF treatment (Fig. 2A). The expression of 4E-BP1 protein decreased after 4 h of PMA and was undetectable after 24 h (32.5 ± 13% and 4 ± 1%; Fig. 2, A and C). Northern blot analysis showed that the transcript level was strictly correlated with the protein expression level, that PMA negatively regulated 4E-BP1 expression at the transcript level (Fig. 2, B and C), and that this effect lasted 48 h (Fig. 2, D and E). Because 4E-BP1 expression was also known to vary during cell differentiation, we analyzed its expression by Northern blot. The transcript of 4e-bp2 was only present at 24 h of culture in the presence or absence of PMA (Fig. 2, D and E). Taken together, these results showed that the two repressors of translation have different ways of regulating.

ERK Is Activated by PMA and p38 Is Activated by GM-CSF in UT7D1 Cell Line—PMA and GM-CSF are known to activate ERK and p38 (27, 51–53), and the balance between these two pathways may be critical in determining cell function (54). To determine the activity of ERK and p38 in our system, we analyzed their phosphorylation by Western blot using phosphospecific antibodies. As previously mentioned, UT7D1 cells were placed in fresh culture medium and GM-CSF-starved for 120 min before addition of the inhibitors. After 30 min, we added fresh GM-CSF alone or GM-CSF and PMA. Activated ERK2/p42 was detected after 30 min of PMA treatment (Fig. 3A). This activation was maximal at 2 h of treatment and decreased thereafter to become undetectable after 48 h. It should be noted that although ERK1/p44 and ERK2/p42 were found at similar levels in UT7D1 cells, the signal was always weaker for phospho-ERK1/p44 than for phospho-ERK2/p42, and the former could not be detected in all experiments (Figs. 3A and 5A). The SB203580 inhibitor of p38/SAPK2α and β had no effect on 4E-BP1 expression (Fig. 3A). The PD98059, an inhibitor of MEKs, almost completely prevented the effect of PMA on ERK phosphorylation (Fig. 3A). Phosphorylated p38 was detected after 2 h of GM-CSF treatment. We tested the activation of the p38 pathway in UT7D1 lysates by performing kinase assays using Hap25 (56). p38 activation was maximal between 4 and 24 h and decreased thereafter (Fig. 3, B and C). The total amount of p38 did not vary during our experiments. PMA did not affect p38 phosphorylation, but the application of PD98059 increased its phosphorylation at 24 h and extended it at 48 h (Fig. 3, B and C). The SB203580 completely abolished p38 pathway activation (Fig. 3C) without affecting its phosphorylation (Fig. 3B).

Inhibition of ERK Abolished the Effect of PMA on 4E-BP1 Expression, and Inhibition of p38/SAPK2α and β Increased 4E-BP1 Expression—To analyze the role of ERK or p38 in the regulation of 4E-BP1 expression, we treated UT7D1 (Fig. 4A) or HMC1 (Fig. 4B) cells with different specific inhibitors prior to the addition of GM-CSF or PMA. Northern blot analysis showed that the application of PMA for 24 h entirely abolished 4E-BP1 transcript expression (Fig. 4A). This effect was blocked by the U0126 inhibitor of MEK (71.5% ± 19%) or the bisindolylmaleimide 1 inhibitor of protein kinase C (79% ± 33%), a well known kinase that transduces signal from PMA to ERK cascade (Fig. 4A). The SB203580 inhibitor of p38/SAPK2α and β had no effect on 4E-BP1 expression in the presence of PMA, but in the presence of GM-CSF alone, the pretreatment with SB203580 increased the transcript level of 4E-BP1 (165 ± 33%; Fig. 4A). On another cell line, the HMC1 cell line that grows independently of GM-CSF, the SB203580 had no effect at all, whereas the bisindolylmaleimide 1 and the PD98059 still reversed the PMA effect on 4E-BP1 expression (110.5 ± 18% and 59 ± 18%, respectively; Fig. 4B). This showed that 4E-BP1 expression is inhibited by both PMA-activated ERK and GM-CSF-activated p38.

Hyperosmolarity-induced Activation of JNK/SAPK1 Does Not Affect 4E-BP1 Expression—We next wanted to determine the effect of different stimuli able to activate the MAPK pathways on 4e-bp1 expression. Using Western blot analysis with phosphospecific antibodies, we analyzed the phosphorylation and thereby the activation level of the three main MAPK pathways after 8 h stimuli. Whereas the NaCl barely activated the ERK, it activated the p38 and strongly activated the JNK/SAPK1. The okadaic acid (OA) inhibitor of serine/threonine phosphatases indirectly activated the ERK and strongly acti-
vated the p38 but not the JNK (Fig. 5A). As shown by Northern blot analysis, the 4E-BP1 mRNA expression was not affected by 10% fetal calf serum or NaCl but decreased when UT7D1 cells were treated with OA to the same level as when cells were treated with PMA (Fig. 5B).

Cycloheximide Treatment Abolished PMA Effect on 4E-BP1 Expression—The MAPKs are known to regulate gene expression directly by phosphorylation of transcription factors or indirectly by controlling immediate early genes, which are themselves transcription factors. To distinguish which of these two kinds of transcription factors, ERK and p38, inhibit 4e-bp1 transcription, we blocked the protein synthesis with cycloheximide (CHX). When cells were cultivated in GM-CSF, CHX had no effect on the 4E-BP1 transcript level, neither at 4 h nor at 8 h (Fig. 6). In contrast, the PMA-induced inhibition of 4E-BP1 mRNA expression was completely reversed by the presence of CHX, demonstrating the necessity of protein neosynthesis for 4e-bp1 expression. We addressed the question of 4e-bp1 mRNA stability by performing a time course analysis of 4e-bp1 mRNA expression in the presence of actinomycin D (10 μg/ml from 0 to 270 min). The decrease of its expression was the same in the presence or the absence of PMA, showing that GM-CSF or PMA did not stabilize or destabilize 4e-bp1 mRNA.2

Fig. 4. PMA-activated ERK and GM-CSF-activated p38 inhibit 4E-BP1 expression. GM-CSF-starved (2 h) UT7D1 cells (A) or HMC1 (B) cells were cultivated in the presence or absence of PMA for 18 h. Where shown, the cells were preincubated (30 min) with bisindolylmaleimide (Bis; 3 μM), PD98059 (20 μM), U0126 (10 μM), or SB203580 (10 μM) prior to the addition of GM-CSF (2 ng/ml) or GM-CSF and PMA (10 nM). 20 μg of total RNA was analyzed by Northern blotting using 4e-bp1 cDNA probe as indicated. Equal loading of RNA was controlled by ethidium bromide staining of the ribosomal RNA (28 and 18 S). The columns represent the means of quantification of two to four independent Northern blot analyses of 4e-bp1 mRNA expression ± S.D. The results are expressed as percentages of the quantification of the bands from GM-CSF or 0, which are considered as 100% for A and B, respectively. The columns labeled with asterisks show significant differences (p < 0.01) compared with the same treatment without inhibitor.

Fig. 5. Indirect activation of ERK and p38 by OA mimics the effect of PMA, whereas hyperosmolarity activation of JNK does not. GM-CSF-starved (2 h) UT7D1 cells were cultivated in the presence or absence of fetal calf serum (10%), NaCl (0.25 M), OA (40 nM), or PMA for 24 h. Samples of cell lysates were subjected to SDS-PAGE followed by Western blotting using either anti-phospho-p44/-p42, anti-phospho-p54/p46, anti-phospho-p38, or anti-actin antibodies (loading control) as indicated (A). 20 μg of total RNA was analyzed by Northern blotting using 4e-bp1 or GAPDH cDNA probes (loading control) as indicated (B).

Fig. 6. Cycloheximide treatment abolishes the PMA effect on 4E-BP1 expression, and egr-1 expression is induced by PMA. GM-CSF-starved (2 h) UT7D1 cells were cultivated in the presence or absence of PMA for different times as indicated. Where shown, the cells were preincubated (30 min) with cycloheximide (10 μg/ml). 20 μg of total RNA was analyzed by Northern blotting using 4e-bp1, egr-1, or GAPDH cDNA probes (loading control) as indicated.
EGR-1 Expression Is Induced by PMA or GM-CSF in an ERK- or p38-dependent Manner, Respectively.—Because Egr-1 expression is known to be under the control of MAPK activities under different conditions (55, 56), we analyzed its expression in UT7D1 cells. Northern blot analysis of egr-1 expression showed an induction of its transcript by PMA (Fig. 6). CHX pretreatment increased this effect (Fig. 6), suggesting that in UT7D1 cells Egr-1 exerts a negative feedback on its expression as was demonstrated (57). Western and Northern blot analysis showed that GM-CSF induced the expressions of Egr-1 protein and transcript, which were maximal at 1 h and decreased thereafter to reach the control level at 24 h (Fig. 7, A and B). The addition of PMA prolonged the Egr-1 induction until 24 h. Pretreatment of the UT7D1 cells with U0126 or SB203580 abolished egr-1 induction by PMA or GM-CSF, respectively (Fig. 7C). This demonstrated that PMA-activated ERK or GM-CSF activated p38 induce Egr-1 expression.

4e-bp1 Promoter Activity.—To examine whether Egr-1 is involved in ERK and/or p38 inhibition of 4e-bp1 transcription, we have cloned 1020 bp upstream of the ATG of 4e-bp1 human gene sequence as described under “Experimental Procedures.” This sequence contains some potential Egr response element, Elk1, Sp1, AP4, or NFkB regulatory elements (Fig. 8A). We have fused them to the luciferase gene to measure whether this potential promoter responds to ERK and p38 activities and whether this activity depends on Egr-1. HeLa cells were transfected, and luciferase assays were performed as described under “Experimental Procedures.” 4e-bp1 promoter activity was almost entirely abolished when the cells were stimulated with PMA, and the U0126 inhibitor reversed this effect, whereas the SB203580 did not (Fig. 8B). These inhibitors applied together did not have more effect than the U0126 alone. The OA diminished 4e-bp1 promoter activity too, and this effect was partially reversed by U0126 and significantly by SB203580. The two MAPK inhibitors had an additional effect (Fig. 8B). Coexpression of the ZnEgr dominant negative mutant of Egr did not change the pGL2B nor the pGL2C activities, showing that the effect observed is specific to the ZnEgr.

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**FIG. 7.** Egr-1 expression is induced by GM-CSF-activated p38 or PMA-activated ERK. GM-CSF-starved (2 h) UT7D1 cells were cultivated in the presence or absence of PMA for different times as indicated. Samples of cell lysates were subjected to SDS-PAGE followed by Western blotting using anti-egr-1 antibody (loading control) as indicated (A). 20 μg of total RNA was analyzed by Northern blotting using egr-1 or GAPDH cDNA probes (loading control) as indicated (B). In C cells are GM-CSF starved for 2 h and pretreated with U0126 (10 μM) or SB203580 (10 μM) before 1 h of treatment with GM-CSF alone or GM-CSF and PMA. 20 μg of total RNA was analyzed by Northern blotting as described in B.

**FIG. 8.** Egr-1 is responsible for PMA- or OA-induced inhibition of 4e-bp1 promoter activity. Egr response element (shaded), Elk1 (underlined), Sp1 (boxed), AP4 (underlined), or NFkB (underlined) potential regulatory elements were found in the first 1020 bp of the sequence of the 5′ upstream region of the human 4e-bp1 gene (A). This promoter fused to the luciferase gene (bp1-p) was transfected (as described under “Experimental Procedures”) in HeLa cells and after 24 h stimulated with OA or PMA for additional 12 h. Where indicated the cells were preincubated with U0126 (10 μM) or SB203580 (10 μM) inhibitors. The dominant negative mutant ZnEgr was cotransfected as indicated, and the basal luciferase expression was estimated by transfection of the pGL2B vector. Luciferase activity was measured as described under “Experimental Procedures” and normalized by measuring the fluorescence of EGFP expressed by the pIRES-EGFP vector, which was always cotransfected. The data are the means ± S.D. of three independent experiments performed in duplicate (B). The columns labeled with asterisks show significant differences (p < 0.01) compared with the same treatment without the different inhibitors or without the ZnEgr.
Fig. 9. Histamine production and HDC activity are inhibited by U0126 and SB203580 inhibitors. GM-CSF-starved (2 h) UT7D1 cells were cultivated in the presence or absence of PMA for 24 h (open bars and B) or 48 h (black bars). Where shown, the cells were preincubated (30 min) with U0126 (U) or SB203580 (SB) prior to the addition of GM-CSF (2 ng/ml; left panel) or GM-CSF and PMA (10 nM; right panel). Intracellular histamine concentration and HDC activity were measured as described under “Experimental Procedures.” The data are the means ± S.D. of two to five independent experiments. The columns labeled with asterisks show significant differences (p < 0.01) compared with the same treatment without inhibitor (A). 20 μg of total RNA was analyzed by Northern blotting using hdc or GAPDH cDNA probes (loading control) as indicated (B).

Both the histamine production and the HDC activity when cells were cultivated in GM-CSF (Fig. 9A). When UT7D1 cells were stimulated with PMA, the histamine production and the HDC activity were significantly inhibited in the presence of U0126 (Fig. 9A). This effect seemed to be amplified when U0126 and SB203580 inhibitors were both present in the culture medium (Fig. 9A). Northern blot analysis showed that the hdc mRNAs expression did not change in the presence of PMA nor in the presence of U0126 or SB203580 inhibitors (Fig. 9B). Taken together these data suggest that the post-transcriptional regulation of HDC could be done at the translational level, depending on 4E-BP1.

**DISCUSSION**

In UT7D1 cells, PMA quickly and transiently activates the ERK through a protein kinase C-dependent pathway but does not affect p38, which is activated by the GM-CSF. Activation of one of these two pathways represses 4E-BP1 transcription but at different levels. In contrast the JNK pathway, activated by hyperosmolarity, does not affect 4E-BP1 expression. This work is the first evidence for a role of the ERK and p38 on translational machinery member expression. As determined by our CHX experiments, inhibition of 4E-BP1 expression by the MAPK depends on protein neosynthesis. ERK and p38 have common targets that could play a role in this regulation: the immediate early genes egr-1, c-jun, and c-fos, for example. Egr-1 can be the activator of transcription as well as the inhibitor of transcription and, in UT7D1 cells, we have shown that Egr-1 expression is strictly inversely correlated with 4E-BP1 expression. On the 1020-bp sequence of 4e-bp1 promoter some putative Egr response element, AP4, SRF, SP1, Elk-1, or NFκB-binding sites can be identified. Performing reporter assays in HeLa cells with the ZnEgr dominant negative mutant, we have shown that 4e-bp1 is a new gene, the expression of which is inhibited by Egr transcription factors.

As far as we know, the only repressor effect of Egr-1 was described on egr-1 gene itself to realize a negative feedback (57), and how Egr-1 is inhibitor rather than activator of transcription is not fully understood. It was first shown that ZnEgr inhibits Egr-1-dependent transcription by binding to the Egr response element of a promoter, but a recent work has demonstrated that ZnEgr disrupts the formation of a Egr-1/c-Jun complex (58). In our system c-Jun expression is induced after 4 h of PMA treatment of UT7D1.2 We cannot exclude the possibility that a Egr-1/c-Jun complex is formed on 4e-bp1 promoter to inhibit its activity, but at that time Egr-1/c-Jun has been shown to activate the transcription of the MAO-B gene (59). A detailed study of 4e-bp1 promoter regulation must be done to identify the mechanism by which retinoic acid or Me3SO represses 4E-BP1 expression. Nevertheless Egr-1 has a critical role in a variety of processes that include proliferation, apoptosis, and cell differentiation (and thereby oncogenesis) neuronal plasticity and ischemia. The new link that our work makes between Egr-1 and 4E-BP1 allows us to reconsider the role that 4E-BP1 could play in this process by being regulated through Egr-1. The induction of Egr-1 expression by PMA has been implicated in the megakaryocytic differentiation process of K562 cell line (60). According to our data, we can suppose that 4E-BP1 could be responsible for one or more of the characteristic changes that appear, like variations of cell morphology, adhesive properties, endomitosis, and expression of markers associated with megakaryocytes.

This work is the first evidence for a role of the ERK and p38 on translational machinery member expression, but a lot of works have been done demonstrating the role of the MAPK in the regulation of phosphorylation of eukaryotic initiation factors. MAPK regulate the eIF4E phosphorylation state through
MNK1 and MNK2, which can integrate signals emanating from both types of MAPK pathway in response to mitogens, polypeptide hormones, tumor promoters, and growth factors (61–64). This phosphorylation was first correlated with an increase rate in protein synthesis, and on the other hand, dephosphorylation coincides with a reduction of protein synthesis at metaphase, upon heat shock, and during adenovirus infection. However, a correlation between eIF4E phosphorylation and the overall translation rate is not observed in every situation (5), and the effects of phosphorylation on eIF4E are not completely understood. Whereas Mininch et al. (66) have described an increased affinity of phosphorylated eIF4E for the cap, Schepker et al. (65) have shown that phosphorylation of eIF4E on Ser209 diminishes its ability to bind capped mRNA. A recent work of Knauf et al. (68) demonstrates that the phosphorylation of eIF4E by MNK1 and MNK2 inhibits the cap-dependent translation. Two recent studies have shown that ERK and p38, through p90/RSK1, can influence the translational phosphorylation of eIF4E by MNK1 and MNK2 inhibits the cap-dependent translation. Two recent studies have shown that ERK and p38, through p90/RSK1, can influence the translational phosphorylation of eIF4E by MNK1 and MNK2 inhibits the cap-dependent translation. Two recent studies have shown that ERK and p38, through p90/RSK1, can influence the translational phosphorylation of eIF4E by MNK1 and MNK2 inhibits the cap-dependent translation. Two recent studies have shown that ERK and p38, through p90/RSK1, can influence the translational phosphorylation of eIF4E by MNK1 and MNK2 inhibits the cap-dependent translation. Two recent studies have shown that ERK and p38, through p90/RSK1, can influence the translational phosphorylation of eIF4E by MNK1 and MNK2 inhibits the cap-dependent translation. Two recent studies have shown that ERK and p38, through p90/RSK1, can influence the translational phosphorylation of eIF4E by MNK1 and MNK2 inhibits the cap-dependent translation. Two recent studies have shown that ERK and p38, through p90/RSK1, can influence the translational phosphorylation of eIF4E by MNK1 and MNK2 inhibits the cap-dependent translation. Two recent studies have shown that ERK and p38, through p90/RSK1, can influence the translational phosphorylation of eIF4E by MNK1 and MNK2 inhibits the cap-dependent translation. Two recent studies have shown that ERK and p38, through p90/RSK1, can influence the translational phosphorylation of eIF4E by MNK1 and MNK2 inhibits the cap-dependent translation. Two recent studies have shown that ERK and p38, through p90/RSK1, can influence the translational phosphorylation of eIF4E by MNK1 and MNK2 inhibits the cap-dependent translation. Two recent studies have shown that ERK and p38, through p90/RSK1, can influence the translational phosphorylation of eIF4E by MNK1 and MNK2 inhibits the cap-dependent translation. Two recent studies have shown that ERK and p38, through p90/RSK1, can influence the translational phosphorylation of eIF4E by MNK1 and MNK2 inhibits the cap-dependent translation.
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