



The eIF4E-binding proteins 1 and 2 are negative regulators of cell growth

Denis Rousseau¹, Anne-Claude Gingras, Arnim Pause² and Nahum Sonenberg

Department of Biochemistry and McGill Cancer Center, McGill University, Montreal, Quebec H3G1Y6, Canada

Initiation in eukaryotes is the rate limiting step of translation. The binding of the mRNA to the 40S ribosomal subunit, which is mediated by the mRNA cap structure, is a key target for control of protein synthesis. The cap binding protein, eIF4E, is the most limiting of all initiation factors and its overexpression in NIH3T3 cells causes malignant transformation. 4E-binding protein 1 (BP1) and 4E-BP2 are small proteins that bind to eIF4E and inhibit translation. Here, 4E-BPs were expressed in cells transformed by eIF4E or by *v-src* to determine the effect of 4E-BPs on cell growth and tumorigenicity. We show that 4E-BPs cause a significant reversion of the transformed phenotype. Thus, we demonstrate that the eIF4E-binding proteins act as negative regulators of cell growth. We propose that 4E-BPs are members of a class of negative regulators of cell growth acting on the translation machinery of the cell.

Keywords: malignant transformation; cap binding protein; translation inhibitors

Introduction

Negative regulators of cell growth are critical in the control of cell proliferation, differentiation and development. Many of the known negative regulators, such as p53, Rb, p27^{Kip1}, p21^{Waf/Cip1}, are key players in controlling check points in the cell cycle. Most characterized negative regulators of cell growth function as effectors of transcription (Cox *et al.*, 1995).

Regulation of translation rates plays an important role in control of cell growth (Morris, 1995; Sonenberg, 1996). An increase in translation is obligatory for entry and progression through the cell cycle, and occurs upon stimulation with a vast array of hormones and growth factors (Brooks, 1977). Conversely, protein synthesis is decreased in mitosis, or upon viral infection and heat shock (Bonneau *et al.*, 1987; Huang *et al.*, 1991; Duncan *et al.*, 1987, 1989; Lamphear *et al.*, 1990). Generally, the limiting step in translation is initiation (Mathews *et al.*, 1996). This step is regulated at several levels, one of which is mRNA binding to the ribosome. Ribosome binding is mediated by eIF4F, which binds to the cap structure that is present at the 5' end of all eukaryotic cellular (except organellar)

mRNAs (Merrick and Hershey, 1996). eIF4F is composed of three subunits: eIF4E, the cap binding protein; eIF4A, an RNA helicase and eIF4G (formerly p220), which binds both eIF4A and eIF4E (Sonenberg, 1996; Merrick and Hershey, 1996). eIF4F in combination with eIF4B is believed to unwind mRNA secondary structure, allowing for binding of ribosomes (Sonenberg, 1996).

The amount of eIF4E in the cell is limiting relative to ribosomes and other initiation factors (Duncan *et al.*, 1987; Hiremath *et al.*, 1985). Its overexpression in NIH3T3 and CHO cells causes malignant transformation (Lazaris-Karatzas *et al.*, 1990; De Benedetti *et al.*, 1994). Furthermore, eIF4E cooperates with Myc and E1A in the malignant transformation of primary embryo fibroblasts (Lazaris-Karatzas and Sonenberg, 1992a). Conversely, expression of antisense eIF4E inhibits protein synthesis and reverts the Ras-transformed phenotype (Rinker-Schaeffer *et al.*, 1993). The mechanism by which eIF4E promotes cell growth and transformation may involve the increase in translation of mRNAs encoding growth promoting factors. Many of these mRNAs possess highly structured 5' untranslated regions which inhibit mRNA binding to the ribosome (e.g. Pelletier and Sonenberg, 1985). Since eIF4F is thought to be involved in the unwinding of mRNA secondary structures, overexpression of eIF4E would promote the unwinding activity and enhance translation of mRNAs with structured 5'UTRs (Koromilas *et al.*, 1992a). At least two proteins that regulate cell growth; cyclin D1 and ornithine decarboxylase (ODC) are expressed at higher levels in NIH3T3 cells transformed by eIF4E (Rosenwald *et al.*, 1993; Shantz *et al.*, 1994; Rousseau *et al.*, 1996).

Recently two human proteins, termed 4E-BP1 and 4E-BP2 (eIF4E Binding Proteins 1 and 2), have been shown to interact with eIF4E (Pause *et al.*, 1994). 4E-BP1 (PHAS-I; Phosphorylated Heat- and Acid-Stable protein, Insulin stimulated; (Hu *et al.*, 1994)) has been initially described as a small heat-stable protein, which is phosphorylated upon insulin and growth factor treatment (Hu, *et al.*, 1994; Belsham *et al.*, 1980, 1982; Blackshear *et al.*, 1982, 1983). 4E-BP1 and 4E-BP2 share 56% identity and inhibit cap-dependent translation both *in vitro* and *in vivo* by competing with eIF4G for a common binding site for eIF4E (Pause *et al.*, 1994; Mader *et al.*, 1995; Haghighat *et al.*, 1995). Phosphorylated 4E-BP1 does not interact with eIF4E, explaining the increase in translational rates in response to insulin and growth factor stimulation (Pause *et al.*, 1994; Lin *et al.*, 1994).

Because control of translation rates is critical for proliferation, it is anticipated that overexpression of 4E-BPs would suppress cell growth. We therefore tested the hypothesis that 4E-BPs are negative regulators of cell growth and transformation.

Correspondence: N Sonenberg

Present addresses: ¹Laboratoire des protéines du cytosquelette, ²IBS J.-P. Ebel, 38027 Grenoble, FR

²CBMB, NICHD, NIH, Bethesda, Maryland 20892, USA

Received 28 March 1996; revised 16 August 1996; accepted 16 August 1996

Results and Discussion

Cell lines overexpressing 4E-BP1 and 4E-BP2

eIF4E-transformed and *v-src*-transformed cells were transfected with the expression vector pRc-CMV alone, or the vector containing the cDNAs encoding human 4E-BP1 or 4E-BP2. After selection for drug resistance and expansion, several clones or pools of clones were chosen for analysis of 4E-BPs' expression. To determine the expression of functional 4E-BPs that

are capable of interacting with eIF4E, a far-Western blotting assay with 32 P-labeled eIF4E was performed. This assay measures the capacity of 4E-BPs to bind eIF4E, and consequently the ability of 4E-BPs to inhibit eIF4E function (Pause *et al.*, 1994). In the parental, eIF4E-overexpressing cells, two small molecular weight proteins (migrating at 22 kDa and 19 kDa) interact with eIF4E (Figure 1a, lane 1). The slower migrating protein is 4E-BP1, whereas the faster is 4E-BP2 (Pause *et al.*, 1994). In different established cell lines, the magnitude of the interaction of 4E-BP1

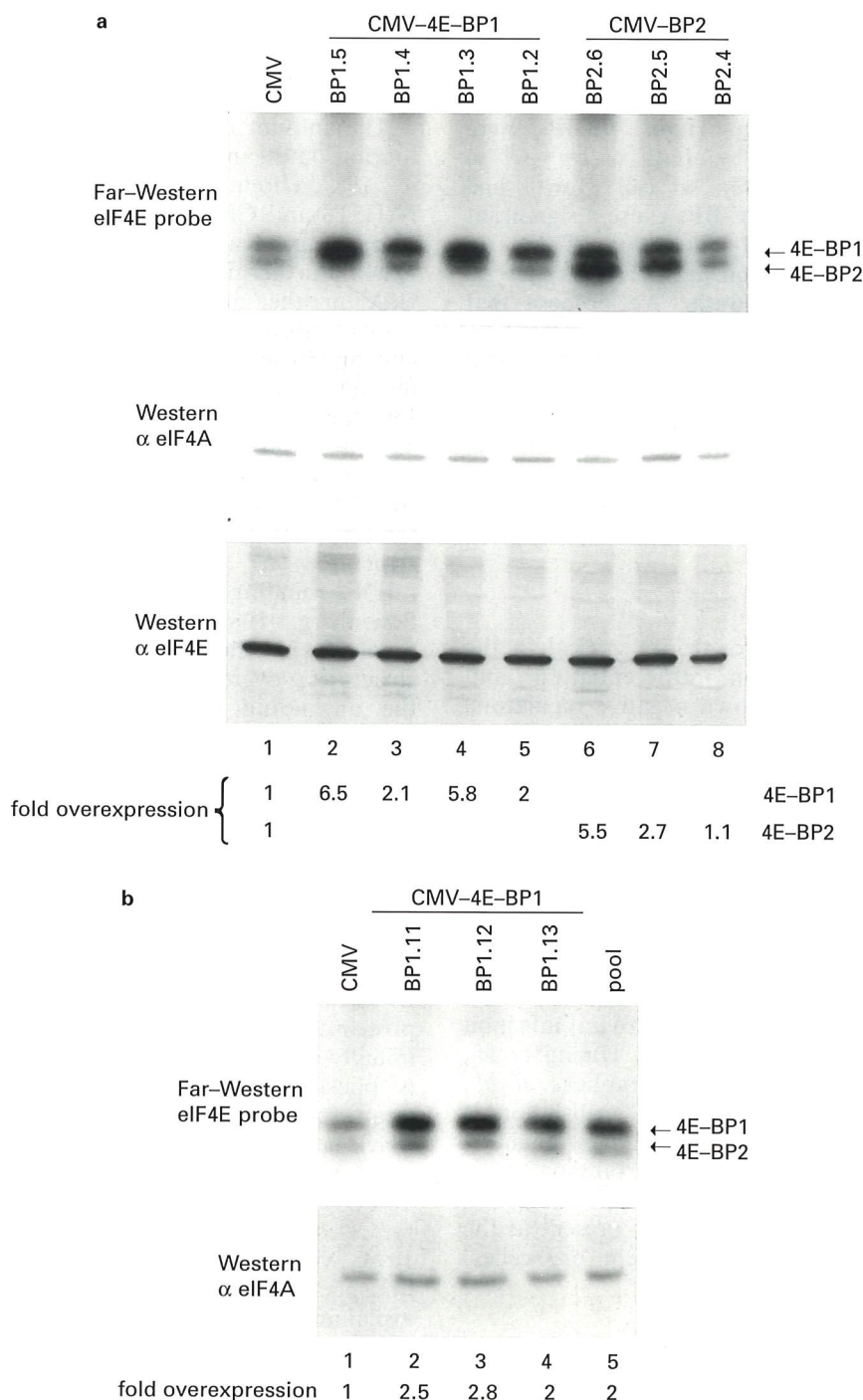


Figure 1 Overexpression of 4E-BP1 and 4E-BP2. eIF4E-transformed NIH3T3 cells (a) and *v-src*/NIH3T3 cells (b) were transfected with pRc-CMV-4E-BP1, pRc-CMV-BP2 or pRc-CMV plasmids and established cell lines were generated as described in Materials and methods. Far-Western analyses of extracts were performed using 32 P-labeled mouse recombinant HMK-eIF4E as described in Materials and methods. A monoclonal antibody against eIF4A and a polyclonal antibody against eIF4E were used in Western blotting. Quantification of the signals was as described in Materials and methods

and 4E-BP2 with eIF4E was increased to various extents relative to the parental cell line (1.1- to 6.5-fold; Figure 1a, upper panel, lanes 2–8). The same blot was reprobed with an antibody against eIF4E to show that the biological effects observed are not due to changes in eIF4E protein levels (Figure 1a, bottom panel). Anti-eIF4A antibody was used to standardize for the amount of protein loaded (Figure 1a, middle panel). In addition to the far-Western experiments, Western analyses using antibodies against 4E-BP1 and 4E-BP2 were performed to determine the extent of overexpression of 4E-BP1 and 4E-BP2 in the transfected cells. The results (data not shown) were consistent with those of the far-Western, indicating that a significant proportion of the overexpressed 4E-BPs binds to eIF4E. We note that expression of eIF4E-interactive 4E-BP1 is also increased in cells overexpressing 4E-BP2, but the explanation for this is not immediately clear.

The expression of 4E-BP1 was also analysed in *v-src*/NIH3T3 transformed cells overexpressing 4E-BP1. Total protein from established cell lines was analysed for the capacity of 4E-BPs to bind eIF4E by far-Western. 4E-BP1 was overexpressed to various degrees (2.5- to 3.7-fold), as measured by its ability to bind to eIF4E (Figure 1b, upper panel: compare lanes 2–5 to lane 1). Anti-eIF4A antibody was used to standardize for the amount of protein loaded (lower panel).

Overexpression of 4E-BP1 or 4E-BP2 partially reverts the phenotype of eIF4E transformed cells

Since eIF4E overexpression results in an increase in translation rates and cell proliferation, it is conceivable that overexpression of 4E-BP1 or 4E-BP2 would decrease translation rates and cell proliferation. Growth kinetics of pools of transfected cells were examined. Both 4E-BP1- and 4E-BP2-overexpressing cells grew significantly slower as compared to the parental cells (Figure 2a). The doubling time was 16 h for eIF4E-overexpressing cells transfected with the vector alone, 24 h for cells transfected with 4E-BP1 and 26 h for cells overexpressing 4E-BP2, as compared to 28 h for NIH3T3 cells.

Next, we determined whether cells overexpressing 4E-BP1 and 4E-BP2 display changes in the transformed phenotype. Morphologically, 4E-BPs-overexpressing cells were not different from the parental eIF4E-overexpressing cells (data not shown). A soft agar assay was performed on isolated clones and pools of transfected cells. The cloning efficiency and the size of the colonies of cells growing in soft agar were reduced for cells overexpressing 4E-BP1 or 4E-BP2 (Figure 2b). The cloning efficiency in soft agar correlated with the overexpression of the 4E-binding proteins. Significantly, the ability of cells to form colonies in soft agar was inversely proportional to the amount of 4E-BPs (Figure 2c). For example, for the cell line that overexpressed the largest amounts of 4E-BP1 (clone BP1.5; 6.5-fold overexpression) the reversion was almost complete. Pools of 4E-BP1 or 4E-BP2 clones were also tested and showed a 25 to 40% decrease in cloning efficiency (data not shown). This result is expected for an average, because of the existence of clones that do not overexpress or overexpress very little 4E-BPs.

Nude mice were used to evaluate the ability of cells overexpressing 4E-BP1 and 4E-BP2 to form tumors in animals. Overexpression of 4E-BPs resulted in a significantly slower tumor growth in nude mice as compared to the parental eIF4E-overexpressing cells, as demonstrated by longer latency periods and a slower proliferation rate (1.4- to 1.7-fold slower; Figure 2d). There was no complete reversal, however.

Overexpression of 4E-BPs partially reverts the phenotype of v-src transformed cells

Because c-Src and c-Ras regulate the phosphorylation status and consequently the activity of eIF4E, it was important to determine whether 4E-BP1 can revert the malignant phenotype of cells transformed by *v-src* or *Ha-v-ras*. Significant morphological changes in *v-src*/NIH3T3 cells were observed for the clones overexpressing 4E-BP1 or 4E-BP2. *v-src*/NIH3T3 cells transfected with the vector alone exhibited a refractile and spindle shape morphologically, characteristic of transformed cells, whereas cells transformed with 4E-BP1 and 4E-BP2 appeared flat and spread out (Figure 3a).

Overexpression of 4E-BP1 and 4E-BP2 reduced cloning efficiency in soft agar (maximum reduction of 80% and 83%, respectively, relative to control cells; Figure 3b). The extent of reduction of growth in soft agar directly correlated with the amount of eIF4E-reactive 4E-BP1 (Figure 3c). Pools of *v-src* transformed cells overexpressing 4E-BPs also showed a reduction in the ability to grow in soft agar (1.5- to 2.5-fold; data not shown). To examine the effect of 4E-BP1 overexpression on tumorigenicity, pools of clones overexpressing 4E-BPs were injected in nude mice. Tumors developed in the 4E-BPs-transfected *v-src*/NIH3T3 cells, but significantly slower than in control cell lines (1.5- to twofold slower; Figure 3d). Thus, 4E-BP1 and 4E-BP2 can also partially revert the transformed phenotype of *v-src* transformed cells. Similar results were obtained with *Ha-v-ras* transformed cells (data not shown).

Our results show that 4E-BPs inhibit cell growth and revert transformation. Our findings are consistent with earlier data showing that eIF4E acts as a proto-oncogene (Lazaris-Karatzas *et al.*, 1990; De Benedetti *et al.*, 1994) and that Ras and Src are upstream elements in a pathway that leads to eIF4E phosphorylation (Frederickson *et al.*, 1991; Rinker-Schaeffer *et al.*, 1992). These data confirm the hypothesis that the 4E-BPs are negative regulators of cell growth and proliferation. In agreement with this idea, we were not able to obtain stable cell lines of parental NIH3T3 that overexpress 4E-BPs (A-CG unpublished observations). The results obtained here are also consistent with earlier findings (Rinker-Schaeffer *et al.*, 1993) demonstrating that overexpression of an antisense RNA to eIF4E partially reverted a *v-ras* transformed phenotype.

The mechanism of the partial reversion of transformation by 4E-BPs is not known. One hypothesis is that down regulation of translation of specific mRNAs is responsible for this effect. An increase in the amount of key growth promoting proteins was postulated to be the cause of the transformation by overexpressing eIF4E (Lazaris-Karatzas *et al.*, 1992). Many of the

mRNAs encoding growth promoting factors possess long and highly structured 5'UTRs that impede translation initiation (Pelletier and Sonenberg, 1985). It was suggested that eIF4E is limiting for the translation of these mRNAs. One such mRNA encodes ornithine decarboxylase, that plays a key role in regulation of cell growth (Pegg, 1988; Auvinen *et al.*, 1992). This mRNA is a downstream target of eIF4E, and its translation is enhanced in eIF4E overexpressing cells (Shantz and Pegg, 1994; Rousseau *et al.*, 1996). Thus, inhibition of translation of mRNAs such as ODC is expected to result in reversion of the transformed phenotype. We have examined the amounts of ODC and cyclin D1 (another protein whose expression is increased in eIF4E-overexpressing cells) but have found only slight changes (A-CG, unpublished observations). It is possible therefore that translation of other mRNAs is more sensitive to the

amount of active eIF4E, and that these mRNAs play important roles in transformation. An alternative, but less likely possibility is that any non-specific decrease in translation rates would result in the reversion of transformation, as global translation rates in 4E-BPs-overexpressing cells were reduced by 1.3-fold (DR, unpublished observations).

The ability of overexpressed 4E-BPs to inhibit the function of eIF4E would argue that the 4E-BPs are maintained in the unphosphorylated form. Indeed, we found no corresponding increase in the amount of phosphorylated 4E-BP in cells overexpressing 4E-BPs relative to the parental cells (A-CG, unpublished observations). These findings suggest that the phosphorylation of 4E-BPs in cells is tightly regulated.

4E-BPs attributes are shared with other negative regulators of cell growth, many of which have been shown to act as 'tumor suppressors' and are implicated

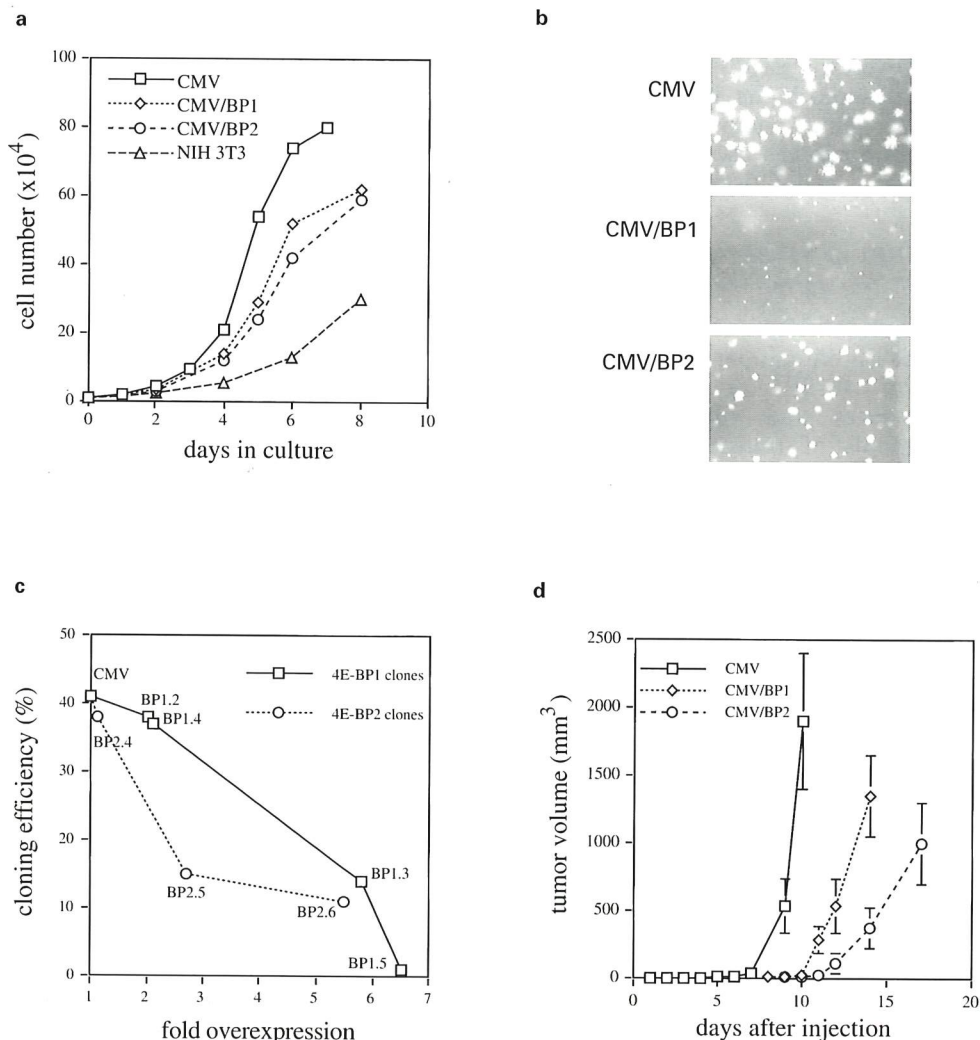


Figure 2 4E-BP1 and 4E-BP2 revert the malignant phenotype of NIH3T3 cells transformed with eIF4E. (a) Proliferation rates of 4E-BPs-overexpressing cells were analysed by measuring cell growth. Pools of cells were seeded at a concentration of $5 \times 10^3/\text{cm}^2$ and replicates were counted. Non transfected cells (parental NIH3T3) were used as a control. (b) Inhibition of anchorage-independent growth of eIF4E-transformed cells by overexpression of 4E-BPs. Stable cell lines overexpressing 4E-BP1 and 4E-BP2 were analysed for their ability to grow in soft agar, as compared to a cell line transfected with the vector alone (CMV). A representative photo is shown for 4E-BP1 (clone 1.5; c) and 4E-BP2 (clone 2.5; c). (c) Correlation between overexpression of 4E-BPs and cloning efficiency in soft agar. Cloning efficiency was determined after 10 to 14 days of growth by counting the number of foci. Fold overexpression of active 4E-BPs was derived from the far-Western analysis of Figure 1a. (d) Tumorigenicity in nude mice. 4E-BPs stably transfected cells (4E-BP1, clone 1.5 and 4E-BP2, clone 2.1) were injected subcutaneously into nude mice and tumorigenicity was assayed as described in Materials and methods

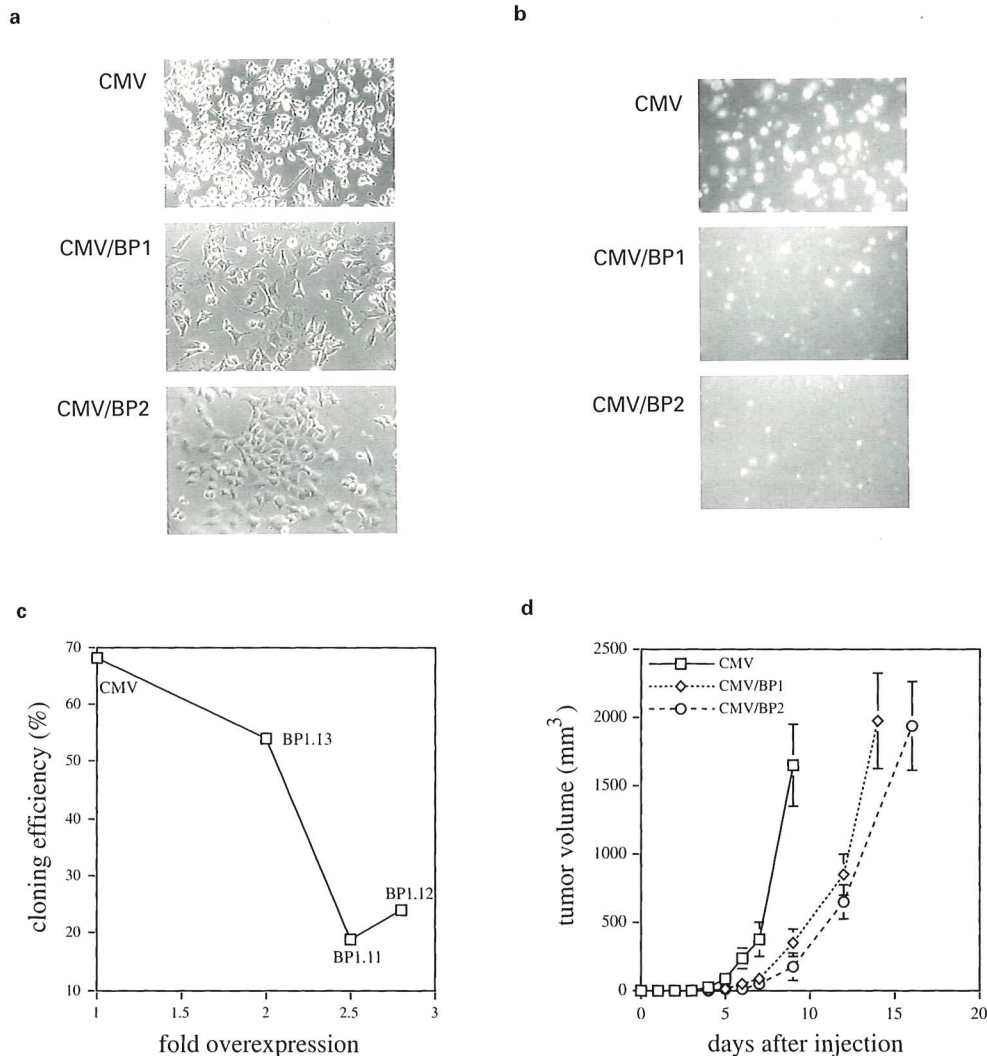


Figure 3 4E-BP1 and 4E-BP2 revert the malignant phenotype of *v-src*/NIH3T3 cells. (a) Morphological changes induced by overexpression of 4E-BPs. Cells were photographed at a magnification of 400 \times . (b) Inhibition of growth in soft agar was monitored as described in Figure 2b, using a pool of cells generated after transfection with CMV alone or cells from established cell lines BP1.12 and one BP2 cell line. (c) Correlation between the cloning efficiency of *v-src*/NIH3T3 cells overexpressing 4E-BP1 and overexpression was performed as described in Figure 2c; the far-Western data used are from Figure 1b. (d) Tumorigenicity of cells overexpressing 4E-BP1 and 4E-BP2 was monitored as described in Materials and methods

in the etiology of human tumors. We propose that 4E-BPs together with another initiation factor, eIF2 α are members of a distinct class of negative regulators of cell growth that act on the translation machinery of the cell. Expression in NIH3T3 cells of a dominant negative mutant of PKR (the kinase responsible for the phosphorylation of eIF2 α) that cannot phosphorylate eIF2 α or of a non-phosphorylatable mutant of eIF2 α causes malignant transformation (Koromilas *et al.* 1992b; Donzé *et al.*, 1995).

Materials and methods

Cell culture, transfection and cloning

NIH3T3 transformed by *v-src* (Frederickson *et al.*, 1991) were grown in DMEM, 10% FBS. Cells transformed by eIF4E (Lazaris-Karatzas *et al.*, 1990) were grown in DMEM, 10% FBS and 300 μ g/ml active G418. 4E-BP1 and 4E-BP2 cDNAs were inserted in pRc-CMVneo plasmid (Invitrogen). Since eIF4E-transformed NIH3T3 cells are already resistant to neomycin, an hygromycin

resistant vector (under control of the thymidine kinase promoter) was used for co-transfection, at a 10 times lower amount than the 4E-B vector. Transfections were performed by the calcium phosphate precipitation technique. Selection with G418 (GIBCO) and hygromycin was applied after 24 h (600 μ g active G418 for *v-src* cells and 300 μ g/ml of active G418 and 100 μ g/ml of hygromycin for eIF4E cells). After 10 to 15 days of selection, clones were isolated and expanded.

Cell extracts and protein analyses

For total cell extract, cells were rinsed twice with ice cold PBS, scraped using a rubber policeman, and lysed in 10 mM Tris-HCl, pH 7.4 buffer containing 1 mM EDTA, 1% NP40, 10 μ g/ml leupeptin, 25 μ g/ml aprotinin, 10 μ g/ml pepstatin by two cycles of freeze-thawing. After centrifugation for 20 min at 12 000 g, the supernatant was collected and added to Laemmli sample buffer for gel electrophoresis analysis. Alternatively, heat-treated extracts were prepared to enrich for 4E-BP1; subconfluent cells were rinsed twice in ice-cold PBS and lysed in 20 mM Tris-HCl pH 7.5, 400 mM KCl, 2 mM EDTA, 2 mM DTT by two cycles of freeze-thawing. Lysates were centrifuged

15 min at 10 000 g and supernatants were recovered. Protein quantification was performed in duplicate by the Bradford assay (Biorad). Samples (500 µg of protein) were boiled for 7 min at 100°C, incubated 5 min on ice and centrifuged 5 min at 10 000 g. The supernatant was recovered and protein was precipitated with 10% TCA. Pellets were washed twice with diethylether, dried and resuspended in Laemmli sample buffer. Total cell lysate (100 µg of protein) or heat-treated extracts (from equivalent volume) were resolved on SDS-15% polyacrylamide gels, transferred onto nitrocellulose membranes (0.2 µM) and processed for Western or far-Western blotting.

Immunodetection of 4E-BP1, eIF4E and eIF4A was performed by incubating the membrane in the presence of 0.5% gelatin, 0.1% Tween 20 in TBS with rabbit polyclonal antibodies against 4E-BP1 (Gingras *et al.*, 1996) and eIF4E (Frederickson *et al.*, 1991) or a mouse monoclonal antibody against eIF4A at room temperature. Antibodies were detected with [¹²⁵I]protein A (Amersham) for the rabbit antibodies or [¹²⁵I]anti mouse IgG (Amersham). Far-Western blotting was performed on total cell extracts using ³²P-labeled recombinant murine eIF4E, as described previously (Pause *et al.*, 1994).

Transformation assays

For soft agar assay, cells were trypsinized and counted. Duplicates of 10⁴ cells were suspended in soft agar medium

(0.35% agar, 20% FBS) in the presence of 100 µg/ml of active G418. Colonies composed of more than eight to ten cells were counted after 12 to 25 days. To analyse for tumorigenicity in nude mice, cells were trypsinized, rinsed in PBS and counted. Triplicates of 3 × 10⁵ cells were injected subcutaneously into mice (Charles River nude mice CD1-CD1, 4 to 6 weeks old). Tumor formation was followed every 24 h by measuring the smallest (d) and the largest (D) diameter and volume was calculated using the formula: $V = d^2 \times D \times 0.4$. Mice were sacrificed when the tumor exceeded 1–2 cm in diameter.

Acknowledgements

We thank Vinita Adkar for assistance in cell culture and Olivier Donzé for providing pRc-CMV-BPs. We thank members of the laboratory for helpful comments on the manuscript. This work was supported by a grant from the National Cancer Institute of Canada to NS; DR was supported by Institut National de la Santé et de la Recherche Médicale (CS/Amm/2943) and European Molecular Biology Organization (ALTF2-1993) fellowships. A-CG was supported by an NSERC 67 studentship and AP by a Cancer Research Society of Montreal studentship.

References

- Auvinen M, Paasinen A, Andersson LC and Hölttä E. (1992). *Nature*, **360**, 355–358.
- Belsham GJ and Denton RM. (1980). *Biochemical Society Transactions*, **8**, 382–383.
- Belsham GJ, Brownsey RW and Denton RM. (1982). *Biochem. J.*, **204**, 345–352.
- Blackshear PJ, Nemenoff RA and Avruch J. (1982). *Biochem. J.*, **204**, 817–824.
- Blackshear PJ, Nemenoff RA and Avruch J. (1983). *Biochem. J.*, **214**, 11–19.
- Bonneau AM and Sonenberg N. (1987). *J. Biol. Chem.*, **262**, 11134–11139.
- Brooks RF. (1977). *Cell*, **12**, 311–317.
- Cox LS and Lane DP. (1995). *BioEssays*, **17**, 501–508.
- De Benedetti A, Joshi B, Graff JR and Zimmer SG. (1994). *Mol. Cell. Diff.*, **2**, 347–371.
- Donzé O, Jagus R, Koromilas AE, Hershey JWB and Sonenberg N. (1995). *EMBO J.*, **14**, 3828–3834.
- Duncan R, Milburn SC and Hershey JWB. (1987). *J. Biol. Chem.*, **262**, 380–388.
- Duncan RF and Hershey JWB. (1989). *J. Cell Biol.*, **109**, 1467–1481.
- Frederickson RM, Montine KS and Sonenberg N. (1991). *Mol. Cell. Biol.*, **11**, 2896–2900.
- Gingras A-C, Svitkin Y, Belsham GJ, Pause A and Sonenberg N. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 5578–5583.
- Haghighat A, Mader S, Pause A and Sonenberg N. (1995). *EMBO J.*, **14**, 5701–5709.
- Hiremath LS, Webb NR and Rhoads RE. (1985). *J. Biol. Chem.*, **260**, 7843–7849.
- Hu C, Pang S, Kong X, Velleca M and Lawrence JC Jr. (1994). *Proc. Natl. Acad. Sci. USA*, **91**, 3730–3734.
- Huang JT and Schneider RJ. (1991). *Cell*, **65**, 271–280.
- Koromilas AE, Lazaris-Karatzas A and Sonenberg N. (1992a). *EMBO J.*, **11**, 4153–4158.
- Koromilas AE, Roy S, Barber GN, Katze MG and Sonenberg N. (1992b). *Science*, **257**, 1685–1689.
- Lamphear BJ and Panniers R. (1990). *J. Biol. Chem.*, **265**, 5333–5336.
- Lazaris-Karatzas A, Montine KS and Sonenberg N. (1990). *Nature*, **345**, 544–547.
- Lazaris-Karatzas A and Sonenberg N. (1992a). *Mol. Cell. Biol.*, **12**, 1234–1238.
- Lazaris-Karatzas A, Smith MR, Frederickson RM, Jaramillo ML, Liu YL, Kung HF and Sonenberg N. (1992b). *Genes Dev.*, **6**, 1631–1642.
- Lin TA, Kong X, Haystead TA, Pause A, Belsham GJ, Sonenberg N and Lawrence JC Jr. (1994). *Science*, **266**, 653–656.
- Mader S, Lee H, Pause A and Sonenberg N. (1995). *Mol. Cell. Biol.*, **15**, 4990–4997.
- Mathews MB, Sonenberg N and Hershey JWB. (1996). *Translational control*, Cold Spring Harbor Laboratory Press, Hershey JWB, Mathews MB, Sonenberg N (eds), pp 1–29.
- Merrick WC and Hershey JWB. (1996). *Translational control*, Cold Spring Harbor Laboratory Press, Hershey JWB, Mathews MB, Sonenberg N (eds), pp 31–69.
- Morris D. (1995). *Prog. Nuc. Acid Res. & Mol. Biol.*, **51**, 339–363.
- Pause A, Belsham GJ, Gingras A-C, Donzé O, Lin TA, Lawrence JC Jr. and Sonenberg N. (1994). *Nature*, **371**, 762–767.
- Pegg AE. (1988). *Cancer Research*, **48**, 759–774.
- Pelletier J and Sonenberg N. (1985). *Cell*, **40**, 515–526.
- Rinker-Schaeffer CW, Austin V, Zimmer S and Rhoads RE. (1992). *J. Biol. Chem.*, **267**, 10659–10664.
- Rinker-Schaeffer CW, Graff JR, De Benedetti A, Zimmer SG and Rhoads RE. (1993). *Int. J. Cancer*, **55**, 841–847.
- Rosenwald IB, Lazaris-Karatzas A, Sonenberg N and Schmidt EV. (1993). *Mol. Cell. Biol.*, **13**, 7358–7363.
- Rousseau D, Kaspar R, Rosenwald I, Gehrke L and Sonenberg N. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 1065–1070.
- Shantz LM and Pegg AE. (1994). *Cancer Research*, **54**, 2313–2316.
- Sonenberg N. (1996). *Translational control*, Cold Spring Harbor Laboratory Press, Hershey JWB, Mathews MB, Sonenberg N (eds), pp 245–269.