Ras mediates translation initiation factor 4E-induced malignant transformation

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Translation initiation factor elf-4E binds to the eukaryotic mRNA 5' cap structure (m^7GpppN, where N is any nucleotide). elf-4E is a limiting factor in translation and plays a key role in regulation of translation. We have shown previously that overexpression of elf-4E in rodent fibroblasts results in tumorigenic transformation. elf-4E also exhibits mitogenic activity when microinjected into serum-starved NIH-3T3 cells. To understand the mechanisms by which elf-4E exerts its mitogenic property, we examined the involvement of the Ras signaling pathway in this activity. Here, we report that Ras is activated in elf-4E-overexpressing cells, as the proportion of GTP-bound Ras is increased. Overexpression of the negative effector of cellular Ras, GTPase activating protein, causes reversion of the transformed phenotype. Furthermore, we show that neutralizing antibodies to Ras, or a dominant-negative mutant of Ras, inhibit the mitogenic activity of elf-4E. We conclude that elf-4E exerts its mitogenic and oncogenic activities by the activation of Ras.

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iting amounts in the cell relative to other initiation factors (Hiremath et al. 1985; Duncan et al. 1987). Moreover, the biochemical (Joshi-Barve et al. 1990) and biological (Morley et al. 1991) activities of elf-4E depend on its state of phosphorylation on Ser-53 (Rychlik et al. 1990). Phosphorylation of elf-4E is increased in response to mitogens, growth factors, and the transforming src and ras genes, suggesting that elf-4E is an important component of various mitogenic signaling pathways (Marino et al. 1989; Morley and Traugh 1989; Kaspar et al. 1990; Frederickson et al. 1991, 1992; Rinker-Schaeffer et al. 1992).

Ras proteins play a critical role in transduction of mitogenic signals in mammalian cells. Ras exists in an active GTP-bound state and in an inactive GDP-bound state (Barbacid 1987; Bourne et al. 1990). We demonstrated previously that transformation of primary cells by elf-4E requires the cooperation of an immortalizing gene such as myc or E1A (Lazaris-Karatzas and Sonenberg 1992). Furthermore, expression of transforming tyrosine kinases, which function through a Ras pathway (Smith et al. 1986), in NIH-3T3 cells or expression of an activated Ras mutant in rat embryo fibroblasts (REF) cells leads to a significant increase in elf-4E phosphorylation (Frederickson et al. 1991; Rinker-Schaeffer et al. 1992). In addition, we reported that elf-4E phosphorylation in PC12 cells, in response to nerve growth factor (NGF), is abrogated in cells expressing a dominant-negative mutant of c-Ras (Ha-c-Ras Asn-17; Frederickson et al. 1992). Taken together, these data raise the possibility that the Ras signaling pathway mediates cellular transformation by elf-4E. In this report we test this hypothesis and show a direct link between elf-4E expression and Ras function by demonstrating that elf-4E overexpression leads to an increase in GTP–Ras complex and thus to Ras activation. In addition, overexpression of GAP (GTPase activating protein) reverts the elf-4E-induced transformed phenotype. Furthermore, we show that neutralizing anti-Ras antibodies or a dominant-negative mutant of Ras (Ha-c-Ras Asn-17) block the mitogenic activity of elf-4E.

Results

elf-4E overexpression activates Ras

One possible mechanism of elf-4E transformation is through activation of the Ras signaling transduction pathway. To address this possibility directly, we examined the effect of elf-4E overexpression on Ras activity by measuring the percentage of GTP-bound Ras relative to total Ras-bound nucleotides. Ras is biologically active when bound to GTP and inactive when bound to GDP (Barbacid 1987; Bourne et al. 1990). The GTP/GDP + GTP ratio was determined following metabolic labeling with 

\[ ^{32}P \]

orthophosphate, immunoprecipitation of Ras, and analysis of GTP and GDP by thin layer chromatography (TLC). The amount of GTP-bound Ras as a proportion of total Ras-bound nucleotides is elevated threefold in NIH-3T3/elf-4E-overexpressing cells relative to the parental cells (20% as compared with 7% GTP; Fig. 1A, cf. lanes 1 and 2 with lanes 3 and 4; Table 1). There was no difference in the level of Ras protein between parental NIH-3T3 and elf-4E-overexpressing cells, as determined by immunoblot analysis with anti-Ras antibody (Fig. 1B, cf. lane 1 with 2). These results demonstrate that overexpression of elf-4E effects Ras activation.

GAP overexpression reverts the elf-4E-transformed phenotype

To further substantiate the conclusion that Ras is directly involved in elf-4E function and cellular transformation, we wished to determine the effect of overexpression of GAP on elf-4E-induced cell transformation. GAP increases the rate at which Ras is converted from the active GTP-bound to the inactive GDP-bound state (Trahey and McCormick 1987). GAP is therefore a negative regulator of Ras. We reasoned that if transformation by elf-4E is mediated by Ras, then down-regulation of Ras activity by overexpression of GAP should revert elf-4E-induced transformation. In the experiments shown here, we used REFs that had been transformed by elf-4E and selected for G418 resistance (Lazaris-Karatzas and Sonenberg 1992). These cells were chosen over elf-4E-transformed NIH-3T3 cells, because they exhibit a more conspicuous transformed morphology than NIH-3T3, a feature that facilitated the screening for revertants. elf-4E...

![Figure 1.](image-url)
4E-transformed REFs were cotransfected with an expression vector containing the human GAP cDNA under the control of the cytomegalovirus (CMV) promoter and a vector containing the hygromycin resistance gene. To obtain control elf-4E-transformed REFs, we cotransfected the expression vector, lacking the human GAP cDNA, together with the vector containing the hygromycin resistance gene, and used pooled hygromycin-resistant cells for further experiments. Thirty hygromycin-resistant clones were further examined by immunoprecipitation of \(3\text{S}\)-labeled cell extracts. Control elf-4E-transformed REF cells and revertant clones had 5–10 times more elf-4E protein relative to the E1A-immortalized REFs [Fig. 3C, cf. lane 1 with lanes 2–6]. These results demonstrate that reversion of the transformed phenotype does not result from a reduction in elf-4E protein.

We then assayed for overexpression of hGAP. Western blot analysis was performed for three of the revertant clones (C1–C3) by use of a polyclonal human GAP antibody capable of recognizing both the human and murine GAP proteins. The steady-state amount of GAP protein in these clones is six to eight times higher than in control elf-4E-transformed REF cells (Fig. 4, cf. lanes 3–5 with lane 1) and approximately three times higher than in control elf-4E-transformed REF cells (Fig. 4, cf. lanes 3–5 with lane 2). Hygromycin-resistant clones that still displayed the transformed phenotype were also examined for GAP expression. The amount of GAP in these cells is similar to that in the transformed control cells (data not shown). Thus, there is an excellent correlation between the extent of GAP overexpression and the reversion of the transformed phenotype. It is noteworthy, however, that the level of endogenous GAP is elevated (about threefold) in elf-4E-transformed cells relative to E1A-immortalized cells [cf. lane 1 with 2]. This phenomenon has precedence, as it has been shown that transformation of NIH-3T3 cells by src or lck results in an increase in the steady-state level of GAP [Ellis et al. 1990; DeClue et al. 1991; Veillette et al. 1992].

Ras activity, as determined by the percentage of GTP-bound Ras, was also examined. Ras in control E1A-immortalized REFs is almost entirely GDP bound [5.6% GTP; Fig. 5, lanes 1, 2; Table 1]. In E1A-immortalized REFs overexpressing elf-4E and in control REF cells overexpressing elf-4E, the proportion of GTP-bound Ras, was also examined. Ras in control E1A-immortalized REFs is almost entirely GDP bound [5.6% GTP; Fig. 5, lanes 1, 2; Table 1]. In E1A-immortalized REFs overexpressing elf-4E and in control REF cells overexpressing elf-4E, the proportion of GTP-bound Ras rises threefold [15% GTP; Fig. 5, cf. lanes 1 and 2 with lanes 3–6, summarized in Table 1]. As expected, GAP overexpression in elf-4E-transformed cells results in a decrease of GTP-bound Ras to levels and have observed reversion of the transformed phenotype upon GAP overexpression [data not shown].

**Revertants arise owing to GAP overexpression**

Revertants could have arisen because of the overexpression of GAP or the loss of elf-4E overexpression. To distinguish between these two possibilities, we performed Northern blot and immunoprecipitation analyses of elf-4E RNA and protein. Northern blot analysis revealed that control elf-4E-transformed REFs and revertant clones overexpressed elf-4E mRNA to the same extent [50- to 100-fold as compared with E1A-immortalized REFs; Fig. 3A, cf. lanes 1–4 with lane 5; Fig. 3B is a longer exposure of lanes 4 and 5]. Two transcripts of 1.8 and 5.0 kb are detected. The shorter transcript arises from the use of the polyadenylation signal in the elf-4E cDNA. Readthrough of this signal and termination in the 3’ long terminal repeat (LTR) of the vector yields a 5.0-kb transcript [Lazaris-Karatzas et al. 1990]. elf-4E protein levels were analyzed by immunoprecipitation of \(3\text{S}\)-labeled cell extracts. Control elf-4E-transformed REFs and revertant clones had 5–10 times more elf-4E protein relative to the E1A-immortalized REFs [Fig. 3C, cf. lane 1 with lanes 2–6]. These results demonstrate that reversion of the transformed phenotype does not result from a reduction in elf-4E protein.

**Table 1. Proportion of GTP-bound Ras in transformed and nontransformed cell lines**

| Cell line           | GTP-bound Ras (%) | Avg.   | Response [fold stimulation] |
|---------------------|-------------------|--------|-----------------------------|
| NIH-3T3             | 6.8, 7.2, 7.0     | 7.0    | —                           |
| NIH-3T3/elf-4E      | 20.0, 21.0, 20.0  | 20.3   | 2.9                         |
| E1A                 | 5.6, 4.4, 5.2, 7.2| 5.6    | —                           |
| E1A/pMV7/elf-4E     | 15.2, 16.0, 17.6, 14.8| 15.9 | 2.8                         |
| pMV7/elf-4E         | 14.4, 14.0, 15.2, 15.2| 14.7 | 2.6                         |
| + hGAP C1           | 5.0, 5.2         |        |                             |
| pMV7/elf-4E + hGAP C2| 5.2, 5.2        | 4.7    | 0.8                         |
| pMV7/elf-4E + hGAP C3| 3.2, 4.4        |        |                             |

aValues were calculated from the following formula:

\[ \text{GTP bound} = \frac{GTP}{1.5 \times GDP + GTP} \times 100 \]

4E-transformed REFs were cotransfected with an expression vector containing the human GAP cDNA under the control of the cytomegalovirus (CMV) promoter and a vector containing the hygromycin resistance gene. To obtain control elf-4E-transformed REFs, we cotransfected the expression vector, lacking the human GAP cDNA, together with the vector containing the hygromycin resistance gene, and used pooled hygromycin-resistant cells for further experiments. Thirty hygromycin-resistant clones, from the transfection experiments with hGAP, were isolated for detailed studies. The majority of the clones (-80%) exhibited flat morphology, indicative of reversion of the transformed phenotype [Fig. 2]. All of the 30 hygromycin-resistant clones were further examined for another transformation-specific property—growth in soft agar. A comparison of the morphology and growth in soft agar of revertant cell lines with those of the parental transformed cells is shown in Figure 2. The control, hygromycin-resistant-transformed cells, pMV7/elf-4E, exhibit a refractile and spindle-shaped morphology, as compared with the flat morphology of E1A-immortalized REFs [Fig. 2, cf. A with B]. The revertant cells (clones C1–C3) are flat, translucent, grow in ordered arrays, and do not grow in soft agar [Fig. 2C–E]. All of the clones that exhibited flat morphology [24 of 30] were incapable of growing in soft agar. The rest of the clones [20%] exhibited a transformed morphology and grew in soft agar. One such transformed clone (C4) is shown in Figure 2F. The three revertant clones (C1–C3) were injected into athymic nude mice to examine their tumorigenic potential. Cells from the three clones failed to form tumors in nude mice, whereas the control elf-4E-transformed cells formed tumors after a short latency period of 10–15 days (Table 2). We have repeated these experiments with elf-4E-transformed NIH-3T3 cells,
similar to those detected in E1A-immortalized REFs [Fig. 5, cf. lanes 7–9 with lanes 1 and 2; Table 1]. Taken together, our results strongly suggest that eIF-4E transforms cells by a Ras-dependent pathway.

**Table 2. Tumorigenicity in nude mice**

| Cells             | Number of tumors/mice injected | Latency (days) |
|-------------------|--------------------------------|----------------|
| E1A               | 0/2                            |                |
| pMV7/eIF-4E       | 3/3                            | 10–15*         |
| pMV7/eIF-4E + hGAP| 0/6                            |                |

*Tumors displayed unlimited growth.

**eIF-4E phosphorylation levels in eIF-4E-transformed REFs and revertant cells are comparable**

eIF-4E activity correlates positively with its state of phosphorylation. It is possible that the revertant cells contain hypophosphorylated eIF-4E. This would explain their nontransformed phenotype, as eIF-4E[ala]-overexpressing cell lines are not transformed [Lazaris-Karatzas et al. 1990]. To test this possibility, revertant cells were metabolically labeled with ³²P and immunoprecipitated with eIF-4E antibody. Control eIF-4E-transformed REFs contain increased amounts of phosphorylated eIF-4E, relative to E1A-immortalized REFs [Fig. 6, cf. lanes 1 and 2 with lanes 3 and 4]. Revertant cell lines displayed a sim-
Ras mediates eIF-4E-induced transformation

Figure 3. Northern and immunoprecipitation analysis of eIF-4E in E1A-immortalized, eIF-4E-transformed, and hGAP revertant REFs. (A) Poly(A)+ mRNA was separated on a 1.25% formaldehyde agarose gel, blotted onto a nylon membrane, and hybridized to a eIF-4E cDNA probe as described in Materials and methods. The blot was exposed for 2 hr at -70°C on Kodak X-Omat XAR-5 film. The arrow indicates the 5-kb transcript; the arrowhead indicates the 1.6-kb transcript. (Lanes 1-3) pMV7/eIF-4E + hGAP, revertant clones C1-C3; (lane 4) control pMV7/eIF-4E-transformed REFs; (lane 5) E1A-immortalized REFs. (B) Overexposure of lanes 4 and 5 from A. The blot was exposed for 24 hr. (C) eIF-4E was immunoprecipitated from [32S]methionine-labeled cell extracts as described in Materials and methods with polyclonal rabbit antibody against a mouse eIF-4E synthetic peptide (Lazaris-Karatzas et al. 1990). Exposure was for 24 hr. The description of the lanes is as in A.

Figure 4. Immunoblot analysis of hGAP in control, transformed, and revertant cell lines. Total cell extracts (75 µg) were electrophoresed on 8% polyacrylamide gel, transferred to nylon, and blotted with anti-GAP antibody, as described in Materials and methods. The autoradiograph represents a 24-hr exposure. (Lane 1) E1A-immortalized REFs; (lane 2) control pMV7/eIF-4E-transformed REFs; (lanes 3-5) pMV7/eIF-4E + hGAP, revertant clones C1-C3.

Figure 5. hGAP overexpression decreases the Ras/GTP complex. An autoradiograph is shown of TLC of the nucleotides eluted from Ras, performed as described in Fig. 1 and Materials and methods. TLC plates were exposed for 2 days. Experiments were performed in duplicate and contained the following cell lines: (Lanes 1,2) E1A-immortalized REFs; (lanes 3, 4) E1A-immortalized REFs transformed by eIF-4E, E1A/pMV7/eIF-4E; (lanes 5,6) control pMV7/eIF-4E-transformed REFs; (lanes 7-9) pMV7/eIF-4E + hGAP, revertant clones C1-C3.

ilar increase in the amount of phosphorylated eIF-4E (Fig. 6, cf. lanes 1 and 2 with lanes 5-8). These results were further confirmed by two-dimensional gel electrophoresis (data not shown). Consequently, we conclude that the reversion of the transformed phenotype is not the result of a decrease in the phosphorylation state of eIF-4E.

Anti-Ras antibody inhibits the mitogenic activity of eIF-4E

To further support our conclusion that eIF-4E activity is mediated through Ras, we examined eIF-4E mitogenic
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Figure 6. hGAP overexpression does not affect eIF-4E phosphorylation. Cells were labeled with \( ^{32}P \) orthophosphate and lysed, and eIF-4E was immunoprecipitated as described in Materials and methods. The blot was exposed for 24 hr. Experiments were performed in duplicate and contain the following cell lines: (lanes 1,2) E1A-immortalized REFs; (lanes 3,4) control pMV7/eIF-4E-transformed REFs; (lanes 5-8) pMV7/eIF-4E + hGAP, revertant clones C1 and C2. activity in serum-starved cells. Microinjection of eIF-4E into serum-starved NIH-3T3 cells induces DNA replication (Smith et al. 1990). To examine the involvement of Ras in this activity, we used an anti-Ras monoclonal antibody, Y13-259 (Furth et al. 1982) that blocks Ras activity when microinjected into cells (Mulcahy et al. 1985). This monoclonal antibody also neutralizes the mitogenic activity of coinjected purified Ras protein (Kung et al. 1986) and inhibits the proliferation of several tumor cell lines (Stacey et al. 1987). The Y13-259 antibody is not a general suppressor of mitogenic activity, inasmuch as it does not block the mitogenic activity and transformation induced by viral Raf or Mos (Smith et al. 1986). Microinjection of recombinant eIF-4E into quiescent NIH-3T3 cells induced DNA synthesis (14-fold; Fig. 7A, Table 3), whereas the mutant eIF-4E[ala] protein caused a much smaller induction (less than threefold) in DNA synthesis (Fig. 7B; Table 3), as did injection of BSA (Table 3). Coinjection of anti-Ras neutralizing antibody Y13-259 with eIF-4E effectively repressed the mitogenic activity of eIF-4E (Fig. 7C; Table 3). In contrast, a non-neutralizing Ras antibody, Y13-238 (Furth et al. 1982), had minimal inhibitory activity (< 10%) on eIF-4E mitogenic activity (Fig. 7D; Table 3). This experiment strongly argues that functional Ras is required for eIF-4E activity and that eIF-4E shares a common mitogenic signaling pathway with Ras.

A dominant-negative mutant of Ras inhibits eIF-4E mitogenic activity

In a different approach to show that eIF-4E mitogenic activity is dependent on Ras, we used a dominant-negative mutant of Ha-c-Ras in which Ser-17 was changed to asparagine (Feig and Cooper 1988). Expression of Ha-c-Ras Asn-17 inhibits the proliferation of NIH-3T3 cells presumably by competing with c-Ras, thereby blocking normal Ras protein function (Feig and Cooper 1988). Microinjection of recombinant wild-type Ha-c-Ras into serum-starved NIH-3T3 cells caused a significant induction (18-fold) of DNA synthesis (Table 4), as reported previously (Stacey and Kung 1984). In contrast, microinjection of the Ha-c-Ras Asn-17 mutant, at the same con-
rap, 4E-mediated transformation by using three different approaches. First, we demonstrate that overexpression of eIF-4E results in Ras activation as evidenced by a three-fold increase in the ratio of GTP/Ras to GDP/Ras in a dose-dependent manner, inhibiting ~50% of activity at a concentration of 2 μg/ml but fully abrogating activity at 150 μg/ml. In a similar fashion, mutant Ha-c-Ras abrogated the induction of DNA synthesis by eIF-4E, when coinjected into serum-starved NIH-3T3 cells (Table 4, Fig. 8, cf. A and B). Microinjection of mutant Ha-c-Ras by itself had no effect on DNA synthesis (Fig. 8C).

Discussion

We present evidence for the involvement of Ras in eIF-4E-mediated transformation by using three different approaches. First, we demonstrate that overexpression of eIF-4E results in Ras activation as evidenced by a three-fold increase in the ratio of GTP/Ras to GDP/Ras in transformed relative to parental cells. This increase in Ras activity is not the result of an increase in the amount of Ras protein. Also, an increase in Ras protein is not expected to affect the equilibrium between GTP- and GDP-bound Ras (Barbacid 1987). Second, we demonstrate that GAP negatively regulates eIF-4E-transforming activity. Down-regulation of Ras by overexpression of GAP reverses the transforming phenotype caused by src [DeClue et al. 1991a; Nori et al. 1991] and CSF-1R [Bortner et al. 1991], placing these oncogenes upstream of Ras. We present similar evidence, through overexpression of GAP, that eIF-4E lies upstream of Ras. This conclusion is reinforced by experiments showing that expression of rap, or a dominant-negative mutant of ras, also results in reversion of the transformed phenotype [A. Lazaris-Karatzas and F. Lejbkowitz, unpubl.]. Third, we show that anti-Ras antibodies or a dominant-negative Ras protein block the mitogenic activity of eIF-4E when coinjected with eIF-4E into serum-starved NIH-3T3 cells. Cumulatively, these data indicate that overexpressed eIF-4E is signaling through Ras and therefore lies upstream of Ras in a common signal transduction pathway.

How does eIF-4E increase the amount of Ras/GTP leading to activation of the Ras signaling cascade and transformation? One possible model is depicted in Figure 9. eIF-4E is believed to function in the unwinding of mRNA 5’ secondary structure [Ray et al. 1985; Rozen et al. 1990]. Consequently, this factor is predicted to enhance translation of inefficient mRNAs that contain extensive secondary structure in their 5’-noncoding region [Lazaris-Karatzas et al. 1990; Fagan et al. 1991]. Because eIF-4E is limiting in the cell [Hiremath et al. 1985; Duncan et al. 1987], these mRNAs are expected to be discriminated against. Consistent with this hypothesis, recent results from our laboratory demonstrate that mRNAs containing extensive secondary structure in their 5’-untranslated region (UTR) could be translated efficiently in cells overexpressing eIF-4E [Koromilas et al., 1992]. A high proportion of mRNAs with long 5’-noncoding regions and extensive secondary structure encode proteins such as oncogene products, growth factors, and growth factor receptors, which play critical roles in cell growth, development, and differentiation. Thus, eIF-4E overexpression could engender a specific increase in the translation of mRNAs that control cell growth. Several growth factors and proto-oncogenes are translationally regulated. These include c-sis [Rao et al. 1988], lck [Marth et al. 1988], and FGF-5 [Bates et al. 1991]. An increase in translation of a growth factor, such as FGF-5 or PDGF, which after secretion will bind to its receptor and activate signal transduction pathways, is consistent with our model. Several growth factors directly or through induction of a second messenger activate Ras into a GTP-bound, signal-generating state [Gibbs et al. 1990; Satoh et al. 1990]. Thus, Ras is a major relay for several growth factor-mediated signaling pathways. Significantly, we have obtained preliminary evidence for an autocrine loop involving a growth factor in eIF-4E-over-

| Table 3. The mitogenic signal of eIF-4E is blocked by neutralizing anti-Ras monoclonal antibody |
| --- |
| Microinjected sample* | DNA synthesis [fold induction] |
| eIF-4E [3 mg/ml] | 13.7 (4.7) |
| eIF-4E [Ala-33] [2.5 mg/ml] | 2.2 (1.1) |
| BSA [2 mg/ml] | 2.8 (1.5) |
| eIF-4E [3 mg/ml] + α259 [2 mg/ml] | 1.7 (0.8) |
| eIF-4E [3 mg/ml] + α238 [2 mg/ml] | 12.9 (4.8) |

*Recombinant eIF-4E was purified as described [Smith et al. 1990] [for details, see Materials and methods]. †Data from at least three separate determinations. The values in parentheses are standard deviations of the mean [for details, see Materials and methods].

| Table 4. Ha-c-Ras Asn-17, a dominant-negative mutant of Ha-c-Ras, inhibits the mitogenic signal of eIF-4E |
| --- |
| Microinjected sample* | DNA synthesis [fold induction] |
| c-RAS (250 μg/ml) | 17.6 (5.8) |
| BSA [2 mg/ml] | 1.9 (1.2) |
| Ras Asn-17 [200 μg/ml] | 1.7 (0.8) |
| Ras Asn-17 [2 μg/ml] + c-Ras [250 μg/ml] | 12.3 (4.5) |
| Ras Asn-17 [150 μg/ml] + c-Ras [250 μg/ml] | 2.6 (1.9) |
| eIF-4E [3 mg/ml] | 14.7 (5.6) |
| eIF-4E [3 mg/ml] + Ras Asn-17 [200 μg/ml] | 1.5 (1.0) |

*Data from at least three separate determinations. The values in parentheses are standard deviations of the mean [for details, see Materials and methods].
Figure 8. The mitogenic activity of eIF-4E is inhibited by coinjection of the mutant Ha-c-Ras Asn-17. Approximately 75 cells in the area of the photomicrograph were microinjected with eIF-4E (3 mg/ml) [A]; eIF-4E (3 mg/ml) + Ha-c-Ras Asn-17 (150 μM) [B]; and Ha-c-Ras Asn-17 (200 μM) [C]. After injection, the cultures were treated as described in the legend to Figure 7.

expressing cells, by showing that conditioned media from eIF-4E-transformed cells is capable of stimulating DNA synthesis in serum-starved NIH-3T3 cells (A. Lazaris-Karatzas, unpubl.). Whether growth factor expression results directly from eIF-4E overexpression or indirectly from Ras activation remains to be determined. Ras-transformed cells have been shown to produce and secrete their own growth factors (e.g., Peles et al. 1992). Additionally, growth factors can exert their activity without secretion, as demonstrated for v-sis, which ac-

Figure 9. Schematic model for transformation by eIF-4E (for details, see Discussion).
activates Ras intracellularly [Bejcek et al. 1989]. If our model is correct, it would seem to predict that microinjection of elf-4E should activate DNA synthesis in surrounding uninjected cells, yet this is not observed in Figures 7 and 8. A likely explanation is that the amount of putative growth factor secreted from elf-4E-microinjected cells is too small to exert a discernible effect on neighboring cells.

We have shown previously that elf-4E phosphorylation in PC12 cells is mediated through Ras. Inactivation of Ras in PC12 cells, by expression of a dominant-negative mutant [Ha-c-Ras-Asn-17], prevents the NGF-mediated increase in elf-4E phosphorylation. This result suggests that elf-4E activity in these cells depends on and lies downstream of Ras [Frederickson et al. 1992]. Also, factors that stimulate protein synthesis such as mitogens and expression of tyrosine kinases, some of which has been demonstrated to activate Ras, increase elf-4E phosphorylation (for a recent review, see Frederickson and Sonenberg 1992). Furthermore, elf-4E phosphorylation is increased in CREF cells transformed with activated Ras and in v-ras-transformed Rat 1 cells [Rinker-Schaeffer et al. 1992; R.M. Frederickson, unpubl.]. A possible activity of Ras in elf-4E-overexpressing cells would therefore be to act in a possible feedback loop to enhance elf-4E activity, by increasing its phosphorylation levels. The results presented in this paper, however, demonstrate that down-regulation of Ras activity does not affect elf-4E phosphorylation, suggesting that overexpression of GAP does not down-regulate Ras sufficiently to cause a decrease in elf-4E phosphorylation. Revertant cell lines maintain high levels of elf-4E phosphorylation with reduced levels of active Ras. We suggest, therefore, that the comparatively low levels of active Ras in GAP-overexpressing cells are sufficient to maintain elf-4E phosphorylation, whereas higher levels of active Ras are required to transform cells.

In summary, we have established an important link between Ras, which plays a key role in cellular signal transduction, and elf-4E, which is a critical component of the translation machinery. The data presented here strongly support the idea that Ras and elf-4E function along the same signaling pathway and that the transforming and mitogenic activities of elf-4E are mediated through the activation of Ras. Thus, the interaction between the Ras signaling system and elf-4E should control cell proliferation. Inasmuch as translation activation is a key event in the repertoire of cellular responses to extracellular growth stimuli, further studies on the interdigitation between the Ras signaling pathway and protein synthesis should yield a better understanding of the regulation of cell growth.

Materials and methods

Cell culture

NIH-3T3 and E1A-immortalized REFs were cultured in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS, GIBCO Laboratories, Grand Island, NY). Transformed NIH-3T3/elf-4E cell line (clone P2, generated as described by Lazaris-Karatzas et al. 1990) and parental REF/E1A/pMV7/elf-4E and REF/pMV7/elf-4E cell lines (generated as described by Lazaris-Karatzas and Sonenberg 1992) were cultured in DMEM supplemented with 10% FCS and 500 μg/ml of G418 (Geneticin, GIBCO Laboratories). Revertant cell lines, REF/pMV7/elf-4E + hGAP, and their control cell line REF/pMV7/elf-4E were maintained in DMEM plus 10% FCS, 500 μg/ml of G418, and 50 μg/ml of hygromycin B (Sigma).

Overexpression of hGAP and isolation of revertants

The human GAP gene was overexpressed by use of CMV-based expression vector CDM-8 (Aruffo and Seed 1987) as follows: pUC101a, a generous gift from F. McCormick (Chiron Corporation, CA), was restricted with EcoRI, releasing a 4-kb cDNA fragment encoding the full-length human GAP protein. The fragment was blunt-ended with Klenow and subcloned into the expression vector CDM-8. The hGAP expression vector or control expression vector lacking hGAP was cotransfected at a 10:1 ratio with the plasmid pSV2Hyg, which expresses the hygromycin resistance gene under the control of the SV40 promoter. Transfections into elf-4E-transformed REFs were performed by use of the calcium phosphate-mediated method [Wigler et al. 1972]. Briefly, elf-4E-transformed REFs were plated at 5 x 10⁵ cells/100-mm dish 24 hr before transfection. Cells were cotransfected with 5 μg of recombinant CDM-8, 0.5 μg of pSV2Hyg, and carrier DNA to a final concentration of 15 μg. The precipitate was applied to the cells for 24 hr before selection with hygromycin B (50 μg/ml). Plates were refed every 2-3 days with DMEM supplemented with 10% FCS, G418, and hygromycin B. Individual colonies were isolated by use of the cloning cylinder method and expanded after 3-4 weeks.

Growth in soft agar and tumorigenicity assay

Analyses of soft agar growth and tumorigenesis in nude mice were performed as described previously [Lazaris-Karatzas et al. 1990]. Briefly, for analysis in soft agar, 2 x 10⁵ cells were seeded in 30-mm dishes with 4 ml of DMEM containing 20% FCS with the appropriate selection and 0.33% agar solution at 37°C. Cells were fed with 2 ml of DMEM plus G418 every 7 days. Growth was scored as colonies containing >10 cells, 21 days after plating.

To test for tumorigenicity, CD1 nu/nu mice were injected subcutaneously with 10⁶ cells, reseeded in 100 μl of PBS. Mice that developed tumors were killed after 21 days. Mice that did not develop tumors were observed for 90 days.

Northern blot analysis

Northern blot analysis was performed as described previously [Lazaris-Karatzas et al. 1990]. Briefly, 2 μg of poly(A)⁺ mRNA was electrophoresed, blotted, and hybridized to a 3²P-labeled, random-primed probe, containing the entire coding region of elf-4E. Filters were washed at a final stringency of 0.5 x SSC and 0.1% SDS for 60 min at 65°C.

Metabolic labeling of cells and immunoprecipitation

For steady-state metabolic labeling with [³⁵S]methionine, ~5 x 10⁶ cells were seeded in 60-mm culture dishes 24 hr before labeling. Cells were washed twice with methionine-free DMEM and labeled for 18 hr with 2 ml of methionine-free DMEM containing 0.45 μCi/ml of [³⁵S]methionine (New England Nuclear, 597.5 Ci/mmol) and supplemented with 10% dialyzed FCS in 10 mM HEPES KOH (pH 7.0). To prepare ex-
tracts, cells were washed twice with ice-cold PBS, lysed with 1 ml of RIPA buffer [150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl (pH 8.0), 20 μM sodium vanadate, 0.2 mM PMSF, 50 mM NaF, 10 mM NaPPi, 1 mM EGTA], and clarified by centrifugation for 15 min at 10,000g. The supernatant was removed and frozen at −70°C.

For metabolic labeling, cells were starved overnight in phosphate-free DMEM and labeled for 3 hr with 0.5 mCi [32P]orthophosphate [New England Nuclear, 8500 Ci/m mole]/35-mm dish in the same media.

Immunoprecipitation of [32S]methionine-labeled extracts was performed with lysates containing equal amounts of trichloroacetic acid (TCA)-precipitable counts per minute. Equal numbers of counts per minute of [32P]-labeled extracts were immunoprecipitated. The lysates were incubated overnight with a polyclonal rabbit antibody against a mouse eIF-4E synthetic peptide at 4°C with rotation and then with 60 μl of 10% suspension of protein A–Sepharose, prewashed in lysis buffer, for an additional hour. Immunocomplexes were washed eight times with 1 ml of ice-cold washing buffer [50 mM Tris-HCl, 20 mM MgCl2, 0.1% Triton X-100, 0.005% SDS, and 100 mM NaCl] and once with 10 mM Tris-HCl (pH 7.5) and 20 mM MgCl2. GTP/GDP was eluted in 20 mM Tris-HCl (pH 7.5), 0.2% SDS, 0.5 mM GDP, and 0.5 mM GTP at 68°C for 20 min, separated on polyethyleneimine (PEI–cellulose thin layer plates [Brinkmann Instruments, Canada], and developed in 0.75 M potassium phosphate [KH2PO4, pH 3.4]]. Plates were dried and exposed to film at −70°C with an intensifying screen.

Autoradiographs were quantified with Biolmager (Milligen/Biosearch, Millipore, Canada). Results are expressed as the percentage of the amount of GTP relative to total GTP + GDP and corrected for moles of phosphate per mole of guanosine, assuming uniform labeling of all phosphates.

**Microinjections**

NIH-3T3 cells (3 × 10⁴) were seeded on glass coverslips in 35-mm dishes and allowed to grow to confluence. The medium was removed and medium containing 0.5% FCS was applied for 24 hr. Coded samples were mixed together and injected into quiescent cells at the concentrations indicated. The coverslips were maintained in low-serum media for 20 hr and then pulsed with [3H]thy midine (0.5 μCi/ml) for 4 hr. Cells were washed with PBS, fixed in 3.5% gluteraldehyde/PBS, coated with photographic emulsion [NTB2], and exposed to X-ray film for 48 hr. elf-4E and monoclonal antibodies against Ras (a259 and a238) were purified as described (Smith et al. 1990; Kung et al. 1986, respectively).

Fold induction of DNA synthesis was calculated by determining the percentage of injected cells that incorporated [3H]thymidine and dividing by the percentage of uninjected cells in the vicinity of the incorporated label. Standard deviation from at least four separate experiments is shown in Tables 3 and 4.

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**References**

Aruffo, A. and B. Seed. 1987. Molecular cloning of a CD28 cDNA by a high efficiency COS cell expression system. Proc. Natl. Acad. Sci. 84: 8573–8577.
Barbacid, M. 1987. Ras genes. *Annu. Rev. Biochem.* 56: 779–827.

Bates, B., J. Hardin, X. Zhan, K. Drickamer, and M. Goldfarb. 1991. Biosynthesis of human fibroblast growth factor-5. *Mol. Cell. Biol.* 11: 1840–1845.

Beijcek, B.E., D.Y. Li, and T.F. Deuel. 1989. Transformation by v-sis occurs by an internal autoactivation mechanism. *Science* 245: 1496–1499.

Bonneau, A.M. and N. Sonenberg. 1987. Involvement of the 24kDa cap binding protein in regulation of protein synthesis in mitosis. *J. Biol. Chem.* 262: 11134–11139.

Bortner, D.M., M. Ulivi, M.F. Roussel, and M.C. Ostrowski. 1990. The carboxy terminal catalytic domain of the GTPase activating protein inhibits nuclear signal transduction and morphological transformation mediated by the CSF-1 receptor. *Genes Dev.* 6: 1777–1785.

Bourne, H.R., D.A. Sanders, and F. McCormick. 1990. The GTPase superfamily: A conserved switch for diverse cell function. *Nature* 348: 125–132.

deBenedetti, A. and R.E. Rhoads. 1990. Overexpression of eukaryotic protein synthesis initiation factor 4E in HeLa cells results in aberrant growth and morphology. *Proc. Natl. Acad. Sci.* 87: 8312–8318.

DeClue, J.E., K. Zhang, P. Redford, W.C. Vass, and D.R. Lowy. 1991a. Suppression of src transformation by overexpression of full-length GTPase-activating protein (GAP) or of the GAP C terminus. *Mol. Cell. Biol.* 11: 2819–2823.

DeClue, J.E., J.C. Stone, R.A. Blanchard, A.G. Papageorge, P. Martin, K. Zhang, and D.R. Lowy. 1991b. A ras effector domain mutant which is temperature sensitive for cellular transformation: Interactions with GTPase-activating protein and NF-1. *Mol. Cell. Biol.* 11: 3132–3138.

Duncan, R., S.C. Milburn, and J.W.B. Hershey. 1987. Regulated phosphorylation and low abundance of HeLa cell initiation factor elf-4F suggest a role in translation control. *J. Biol. Chem.* 262: 3820–3828.

Ellis, C., M. Moran, F. McCormick, and T. Pawson. 1990. Phosphorylation of GAP and GAP-associated proteins by transforming and mitogenic tyrosine kinases. *Nature* 345: 377–381.

Fagan, R., A. Lazaris-Karatzas, N. Sonenberg, and R. Rozen. 1991. Translation control of ornithine aminotransferase: Modulation by initiation factor elf-4E. *J. Biol. Chem.* 266: 16518–16521.

Feig, L.A. and G.M. Cooper. 1988. Inhibition of NIH 3T3 cell proliferation by a mutant ras protein with preferential affinity for GDP. *Mol. Cell. Biol.* 8: 3235–3243.

Frederickson, R.M. and N. Sonenberg. 1992. Signal transduction and the regulation of translation. *Sem. Cell Biol.* 3: 105–113.

Frederickson, R.M., K. Montine, and N. Sonenberg. 1991. Phosphorylation of eukaryotic translation initiation factor 4E is increased in src-transformed cell lines. *Mol. Cell. Biol.* 11: 2896–2899.

Frederickson, R.M., W.E. Mushynski, and N. Sonenberg. 1992. Phosphorylation of translation initiation factor elf-4F is induced in a ras-dependent manner during nerve growth factor mediated PC12 cell differentiation. *Mol. Cell. Biol.* 12: 1239–1247.

Furth, M.E., L.J. Davis, B. Fleurdeleys, and E.M. Scolnick. 1982. Monoclonal antibodies to the p21 products of the transforming gene of Harvey murine sarcoma virus and of the cellular ras gene family. *J. Virol.* 43: 294–304.

Gibbs, J.B., M.D. Schaber, V.M. Garsky, U.S. Vogel, E.M. Scolnick, R.A.F. Dixon, and M.S. Marshall. 1990. Structure function relationships of Ras and guanosine triphosphatase-activating protein. In *G-proteins and signal transduction* (ed. N.M. Nathanson and T.K. Harden), pp. 77–85. Rockefeller University Press, New York.

Hershey, J.W.B. 1991. Translational control in mammalian cells. *Annu. Rev. Biochem.* 60: 717–755.

Hiremath, L.S., N.R. Webb, and R.E. Rhoads. 1985. Immunological detection of the messenger RNA cap-binding protein. *J. Biol. Chem.* 260: 7843–7849.

Huang, J. and R.I. Schneider. 1991. Adenovirus inhibition of cellular protein synthesis involves inactivation of cap binding protein. *Cell* 65: 271–280.

Jaramillo, M., T.E. Dever, W.C. Merrick, and N. Sonenberg. 1991. RNA unwinding in translation: Assembly of helicase complex intermediates comprising eukaryotic initiation factors elf-4F and elf-4B. *Mol. Cell. Biol.* 11: 5992–5997.

Joshi-Barve, S., W. Rychlik, and R.E. Rhoads. 1990. Alteration of the major phosphorylation site of eukaryotic protein synthesis initiation factor 4E prevents its association with the 48S initiation complex. *J. Biol. Chem.* 265: 2979–2983.

Kasar, R.L., W. Rychlik, M.W. White, R.E. Rhoads, and D.R. Morris. 1990. Simultaneous cytoplasmic redistribution of ribosomal protein L32 mRNA and phosphorylation of eukaryotic initiation factor 4E after mitogenic stimulation of Swiss 3T3 cells. *J. Biol. Chem.* 265: 3619–3622.

Koromilas, A.E., A. Lazaris-Karatzas, and N. Sonenberg. 1992. mRNAs containing extensive secondary structure in their 5' non-coding region translate efficiently in cells overexpressing initiation factor elf-4E. *EMBO J.* (in press).

Kozak, M. 1986. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell* 44: 283–292.

Kung, H.-F., M.R. Smith, E. Bekesi, V. Manne, and D.W. Stacey. 1986. Reversal of transformed phenotype by monoclonal antibodies against Ha-ras p21 protein. *Exp. Cell Res.* 162: 363–371.

Lazaris-Karatzas, A. and N. Sonenberg. 1992. The mRNA 5' cap-binding protein, elf-4E, cooperates with v-myc or E1A in the transformation of primary rodent fibroblasts. *Mol. Cell. Biol.* 12: 1234–1238.

Lazaris-Karatzas, A., K.S. Montine, and N. Sonenberg. 1990. Malignant transformation by a eukaryotic initiation factor elf-4E is required for the transformation to NIH 3T3 cells. *Mol. Cell. Biol.* 10: 1841–1842.

Marino, M.W., L.M. Pfeffer, P.T. Guidon, and D.B. Donner. 1989. Tumor necrosis factor induces phosphorylation of a 28kDa mRNA cap-binding protein in human cervical carcinoma cells. *Proc. Natl. Acad. Sci.* 86: 8417–8421.

Martindale, J.D., R.W. Overell, K.E. Meier, E.G. Krebs, and R.M. Perlmutter. 1988. Translational activation of the lck proto-oncogene. *Nature* 332: 171–173.

Morley, S.J. and J.A. Traugh. 1989. Phorbol esters stimulate phosphorylation of eukaryotic initiation factors 3, 4B and 4F. *J. Biol. Chem.* 264: 2401–2404.

——. 1990. Differential stimulation of phosphorylation of initiation factors elf-4F, elf-4B, elf-3 and ribosomal protein S6 by insulin and phorbol esters. *J. Biol. Chem.* 265: 10611–10616.

Morley, S.J., T.E. Dever, D. Ethchison, and J.A. Traugh. 1991. Phosphorylation of elf-4F by protein kinase C or multipotential S6 kinase stimulates protein synthesis at initiation. *J. Biol. Chem.* 266: 4669–4672.

Mulcahy, L.S., M.R. Smith, and D.W. Stacey. 1985. Requirement for ras proto-oncogene function during serum-stimulated growth of NIH 3T3 cells. *Nature* 313: 241–243.

Nielsen, P.J. and H. Trachsel. 1988. The mouse protein synthet-
sis initiation factor 4A gene family includes two related functional genes which are differentially expressed. *EMBO J.* 7: 2097–2105.

Nori, M., U.S. Vogel, J.B. Gibbs, and M.J. Weber. 1991. Inhibition of v-src induced transformation by a GTPase-activating protein. *Mol. Cell. Biol.* 11: 2812–2818.

Pause, A. and N. Sonenberg. 1992. Mutational analysis of a DEAD RNA helicase: The translation initiation factor eIF-4A. *EMBO J.* 11: 2643–2654.

Peles, E., S.S. Bacus, R.A. Koski, H.S. Lu, D. Wen, S.G. Ogden, R.B. Levy, and Y. Yarden. 1992. Isolation of the Neu/HER-2 stimulatory ligand: A 44kd glycoprotein that induces differentiation of mammary tumor cells. *Cell* 69: 205–216.

Pelletier, J. and N. Sonenberg. 1985. Insertion mutagenesis to increase secondary structure within the 5' non-coding region of a eukaryotic mRNA reduces translational efficiency. *Cell* 40: 515–526.

Rao, C.D., M. Pech, K.C. Robbins, and S.A. Aaronson. 1988. The 5' untranslated sequence of the c-sis/platelet-derived growth factor 2 transcript is a potent translational inhibitor. *Mol. Cell. Biol.* 8: 284–292.

Ray, B.K., T.G. Lawson, J.C. Kramer, M.H. Cladaras, J.A. Griño, R.B. Abramson, W.C. Merrick, and R.E. Thach. 1985. ATP-dependent unwinding of messenger RNA structure by eukaryotic initiation factors. *J. Biol. Chem.* 260: 7651–7658.

Rhoads, R.E. 1988. Cap recognition and entry of mRNA into the protein synthesis initiation cycle. *Trends Biochem. Sci.* 13: 52–56.

Rinker-Schaeffer, C.W., V. Austin, S. Zimmer, and R.E. Rhoads. 1992. ras transformation of cloned rat embryo fibroblasts results in increased rates of protein synthesis and phosphorylation of eukaryotic initiation factor 4E. *J. Biol. Chem.* 267: 2593–2598.

Rozen, F., I. Edery, K. Meirovitch, T.E. Dever, W.C. Merrick, and N. Sonenberg. 1990. Bidirectional RNA helicase activity of eukaryotic translation initiation factor 4A and 4F. *Mol. Cell. Biol.* 10: 1134–1144.

Rychlik, W., M.A. Russ, and R.E. Rhoads. 1987. Phosphorylation site of eukaryotic initiation factor 4E. *J. Biol. Chem.* 262: 10434–10437.

Satoh, T., M. Endo, M. Nakafuku, S. Nakamura, and Y. Kaziro. 1990. Platelet-derived growth factor stimulates formation of active p21ras • GTP complex in Swiss mouse 3T3 cells. *Proc. Natl. Acad. Sci.* 87: 5993–5997.

Smith, M.R., S.J. DeGudicibus, and D.W. Stacey. 1986. Requirement for c-ras proteins during viral oncogene transformation. *Nature* 320: 540–543.

Smith, M.R., M. Jaramillo, Y.-L. Liu, T.E. Dever, W.C. Merrick, H.-F. Kung, and N. Sonenberg. 1990. Translation initiation factors induce DNA synthesis and transform NIH 3T3 cells. *New Biol.* 2: 648–654.

Sonnenberg, N. 1987. Regulation of translation by poliovirus. *Adv. Virus Res.* 3: 175–204.

——. 1988. Cap binding protein of eukaryotic messenger RNA: Function in initiation and control of translation. *Prog. Nucleic Acid Res. Mol. Biol.* 35: 174–207.

Stacey, D.W. and H.-F. Kung. 1984. Transformation of NIH 3T3 cells by microinjection of Ha-ras p21 protein. *Nature* 310: 508–511.

Stacey, D.W., S.J. DeGudicibus, and M.R. Smith. 1987. Cellular ras activity and tumor cell proliferation. *Exp. Cell. Res.* 171: 232–242.

Trahey, M. and F. McCormick. 1987. A cytoplasmic protein stimulates normal N-ras p21 GTPase, but does not affect oncogenic mutants. *Science* 238: 542–545.

Veillette, A., L. Caron, M. Fournel, and T. Pawson. 1992. Regulation of the enzymatic function of the lymphocyte-specific tyrosine protein kinase p56^{Lck} by the non-catalytic SH2 and SH3 domains. *Oncogene* 7: 971–980.

Wigler, M., A. Pellicer, S. Silverstein, R. Axel, G. Urlaub, and L. Chasin. 1972. DNA-mediated transfer of the adenine phosphoribosyltransferase locus into mammalian cells. *Proc. Natl. Acad. Sci.* 76: 1373–1376.
Ras mediates translation initiation factor 4E-induced malignant transformation.

A Lazaris-Karatzas, M R Smith, R M Frederickson, et al.

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