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Oncogenic Ras activates c-Jun via a separate pathway from the activation of extracellular signal-regulated kinases

JOHN K. WESTWICK*†, ADRIENNE D. COX‡, CHANNING J. DER‡, MELANIE H. COBB§, MASAHIKO HIBI¶, MICHAEL KARIN¶, AND DAVID A. BRENNER*

*Department of Medicine, Biochemistry and Biophysics, and ‡Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27599; ¶Department of Pharmacology, and †Program in Molecular Pathology, University of California, San Diego, La Jolla, CA 92109; and §Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX 75235-9041

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ABSTRACT **c-Jun transcriptional activity is augmented by expression of oncogenic Ras and Raf proteins. This study demonstrates a direct correlation between Ras transforming activity and c-Jun activation, supporting an important role for c-Jun in transformation by Ras. Since we observed that Ras activated c-Jun transcriptional activity by increasing phosphorylation of the c-Jun activation domain at residues Ser-63/Ser-73 and that oncogenic Ras proteins activated extracellular signal-regulated protein kinases (ERK1 and ERK2) (also known as mitogen-activated protein kinases), we evaluated the possibility that ERKs were directly responsible for c-Jun activation. Coexpression of wild-type ERKs with oncogenic Ras proteins potentiated, while kinase-defective ERKs inhibited, Ras-induced transcriptional activation from the Ras-responsive element (Ets-1/AP-1) present in the NVL-3 enhancer and the serum-response element in the c-*fos* promoter. In contrast, coexpression of either wild-type or kinase-defective ERKs inhibited Ras and Raf activation of c-Jun transcriptional activity. Thus, although activation of both ERK and c-Jun are downstream consequences of activation of the Ras signal transduction pathway, our results suggest that Ras-induced c-Jun phosphorylation and transcriptional activation are not a direct consequence of ERK1 and ERK2 activation.**

The human Ras proteins (H-, N-, and KRAS) function as critical regulators of signal transduction pathways triggered by diverse extracellular signals (1–3). The ligand-triggered autophosphorylation of receptor tyrosine kinases on tyrosine residues recruits the Ras exchange factor SOS to the plasma membrane via the GRB2 adapter protein, leading to stimulation of Ras GDP/GTP exchange and activation of Ras (4). Ras transforming potential is activated by mutations that cause either reduced intrinsic or GTPase-activating protein (GAP)-stimulated GTPase activities (at residues 12, 13, and 61) or enhanced nucleotide exchange rates (e.g., at residues 116–119 and 146) (3, 4). Activated GTP-Ras (5–7) associates with Raf (8–12). The activated Raf kinase phosphorylates and activates mitogen-activated protein (MAP) kinase/extracellular signal-regulated protein kinase (ERK) kinases, which in turn activate ERK1 and ERK2 (5–7, 13).

Genes containing certain DNA sequence motifs (e.g., Ets, AP-1, and NFκB) are preferentially activated by oncogenic Ras proteins, implicating members of the Ets, Jun, Fos, and Rel families of transcription factors as possible mediators of Ras function (6). Consistent with the involvement of these transcription factors in Ras transformation are observations that dominant-inhibitory mutants of Ets-1 and c-Jun are also potent inhibitors of oncogenic Ras transformation (13–15).

There is strong evidence that ERKs phosphorylate the transcriptional activation domain of c-Myc *in vitro* (16) and *in vivo* (16, 17), resulting in its enhanced transcriptional activity. A similar type of analysis demonstrated that the transcription factor Elk-1 (TCF-1), which participates in formation of a protein complex that binds to the serum response element of c-*fos*, is phosphorylated by ERKs, enhancing its transcriptional activity (18, 19). ERKs also phosphorylate c-Jun *in vitro*, but the specific phosphorylation sites and the physiological sequelae are unclear. Although some reports state that c-Jun phosphorylation by ERKs occurs within the c-Jun activation domain (20, 21), indicating that ERKs may be the c-Jun-activating kinase(s) (JNK), other studies have reported instead that ERKs phosphorylate the C-terminal DNA binding domain of c-Jun (22–24) so that the c-Jun-activating kinase must be distinct from ERKs.

Oncogenic Ras(61L) and v-Raf proteins activate ERKs and stimulate transcriptional activity of c-Jun by increasing phosphorylation of Ser-63 and -73 within the c-Jun activation domain (25–27). Recently, a JNK, which phosphorylates these sites, was purified (28). However, the relationship between JNK and ERKs needs to be clarified. Our results demonstrate a direct correlation between Ras transforming activity and c-Jun activation. This study further demonstrates that although mutant Ras proteins stimulate ERK activity, the signal transduction pathway for the activation of c-Jun does not directly include ERKs.

MATERIALS AND METHODS

Cell Culture. NIH 3T3 cells were grown in DME high glucose medium (GIBCO) supplemented with 10% calf serum (Colorado Serum, Denver). DNA transfections were done as described (29).

Plasmids. pGEX2T-cJun(wt), pGEX2T-cJun(Ala-63, -73), p20-Luc, Gal4-cJun, Gal4-cJun(AA), and the 5×Gal-Luc were described (28). Gal4-cJun expresses the Gal4 DNA-binding domain linked to amino acids 1–246 of human c-Jun. Gal4-cJun(AA) has Ser-63 and Ser-73 of c-Jun mutated to alanines. Gal4-ElkC contains the Gal4 DNA binding domain linked to the Elk-1 transactivation domain (18). 5×Gal-Luc contains 5 consensus Gal4 DNA binding sites (17-mers) subcloned into p20-Luc. All pZIP-Ras constructs have been described (30). p22W-Raf contains an activated form of Raf-1 (31). ERK cDNAs and mutants (32, 33) were subcloned into the expression vector pCMV5. Col3-Luc contains the collagen α1(I) promoter (34). Fos-Luc contains the human c-Fos promoter (35). RDO53-Luc contains a sequence from the murine NVL-3 long terminal repeat that confers responsiveness to both phorbol 12-myristate 13-acetate and activated Ras (36). RDO53(A1)-Luc, an AP-1 site mutated version of

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Abbreviations: MAP kinase, mitogen-activated protein kinase; ERK, extracellular signal-regulated protein kinase; GST, glutathione *S*-transferase; JNK, c-Jun-activating kinase; MBP, myelin basic protein; RRE, Ras-responsive element; GAP, GTPase-activating protein.

RDO53, and RDO53(E1)-Luc, an Ets-1 site mutated version of the same reporter, were provided by M. Ostrowski (Duke University).

Transfections and Luciferase Assays. Transient transfection assays used 2.5 μg of reporter plasmid, 250 ng of expression vector (if used), 250 ng of cotransfected Ras plasmids, and 100 ng of cotransfected ERK constructs (if used). In some experiments, 0.5 μg of pSV2Gal (Promega) was added as an internal control of transfection efficiency. Thirty hours post-transfection, cells were washed with phosphate-buffered saline and lysed in 200 μl of luciferase lysis buffer (0.1 M sodium phosphate, pH 7.8/0.5% Triton X-100/1 mM dithiothreitol).

Cell Extracts. Extracts were prepared as described (37) with the addition of the following inhibitors in all buffers: 500 mM phenylmethylsulfonyl fluoride, 2 mg of aprotinin per ml, 0.5 mg of leupeptin per ml, 0.7 mg of pepstatin per ml, 40 mg of bestatin per ml, 20 mM glycerophosphate, 50 mM Na_3VO_4, 10 mM *p*-nitrylphenyl phosphate.

***In Vitro* JNK Assays.** *In vitro* kinase assays were carried out as described (28) using a glutathione *S*-transferase (GST)–c-Jun(1–223) fusion protein as substrate.

ERK Assays. For the mobility-shift ERK assays, 5 μg of each cell extract was mixed with 2× sample buffer (38), boiled briefly, and electrophoresed through a low cross-linker 12.5% polyacrylamide gel. Proteins were transferred to Immobilon membranes (Millipore) for analysis by Western blotting with an anti-MAP kinase antibody (691 rabbit polyclonal, Santa Cruz Biotechnology) and the ECL detection system (Amersham). For the in-gel kinase assays, myelin basic protein (MBP) was used as a substrate (39) with 25 μg of each cell extract. Radioactivity was visualized by Ambis β scanning.

Cell Labeling and Immunoprecipitation. Labeling and immunoprecipitation of c-Jun were performed as described (40) with antiserum provided by A. Kraft (University of Alabama).

Two-Dimensional Phosphopeptide Mapping. Protein elution and trypsin treatment were performed as described (41). Cellulose plates were loaded with equal amounts of immunoprecipitated protein, and two-dimensional phosphopeptide mapping of c-Jun was performed as described (40, 42).

RESULTS

Oncogenic Ha-Ras Induces Hyperphosphorylation of the c-Jun Transactivation Domain by Stimulating c-Jun Kinase Activity. Previous studies have demonstrated that oncogenic Ha-Ras(61L) stimulates phosphorylation of the c-Jun activation domain, resulting in increased c-Jun transcriptional activity (25–27). To determine whether other forms of oncogenic Ras have similar activity, we examined c-Jun phosphorylation in NIH 3T3 cells and NIH 3T3 cells stably transfected with Ha-Ras(12R). Ha-Ras(12R) expression resulted in hyperphosphorylation of c-Jun, as demonstrated by a slower mobility form of Jun in [^{35}S]methionine-labeled cells and by increased $^{32}P_i$ incorporation (Fig. 1*A*). Synthesis of total c-Jun protein in these cells was increased by transient cotransfection of a c-Jun expression vector and was elevated only slightly by the presence of Ha-Ras(12R) (Fig. 1*A*). Two-dimensional phosphopeptide maps demonstrated that Ha-Ras(12R) transformation caused increased phosphorylation of Ser-63 and Ser-73 of c-Jun as indicated by the increased intensity of the x and y phosphopeptides (25, 26), while no effect on the C-terminal sites was detected (Fig. 1*B*). Radioactivity quantitation of the x site normalized for the C-terminal sites demonstrated an 18-fold increase for the Ha-ras(12R) cells [x/(a + b + c) = 1.27] compared to the control NIH 3T3 cells [x/(a + b + c) = 0.07]. Therefore, Ha-Ras(12R) transformation leads to hyperphosphorylation of c-Jun in a manner similar to that of the previously described effect of Ha-Ras(61L).

We wished to determine whether Ras-induced phosphorylation of c-Jun resulted from increased JNK activity or decreased phosphatase activity. To assay for JNK activity, we used a previously described solid-state assay (28). Immobilized GST–c-Jun protein was incubated with nuclear extracts prepared from NIH 3T3 cells that expressed different mutant Ras proteins. Whereas no increase in JNK activity was observed in cells expressing the unprocessed Ha-Ras(61L/186S) mutant protein, increased JNK activity was detected in cells expressing oncogenic Ha-Ras(61L), -(12R), or -(61P) mutant proteins (Fig. 2). As controls, a recombinant GST protein without the c-Jun activation domain was used as a substrate without any resulting phosphorylation (Fig. 2*A*). A recombinant GST–Jun fusion protein in which the Ser-63 and Ser-73 amino acids were mutated to alanines was phosphorylated to a lower extent than wild-type GST–c-Jun (Fig. 2).

There Is a Direct Correlation Between Transformation Efficiency and c-Jun Transcriptional Activity. To determine the functional significance of increased c-Jun phosphorylation in response to a variety of Ras proteins, we used a cotransfection assay to measure the transcriptional activity of the c-Jun activation domain. In this assay, NIH 3T3 cells were transiently transfected with a Ras expression vector, a chimeric expression vector containing the Gal4 DNA binding domain fused with the c-Jun activation domain (Gal4–Jun), and a

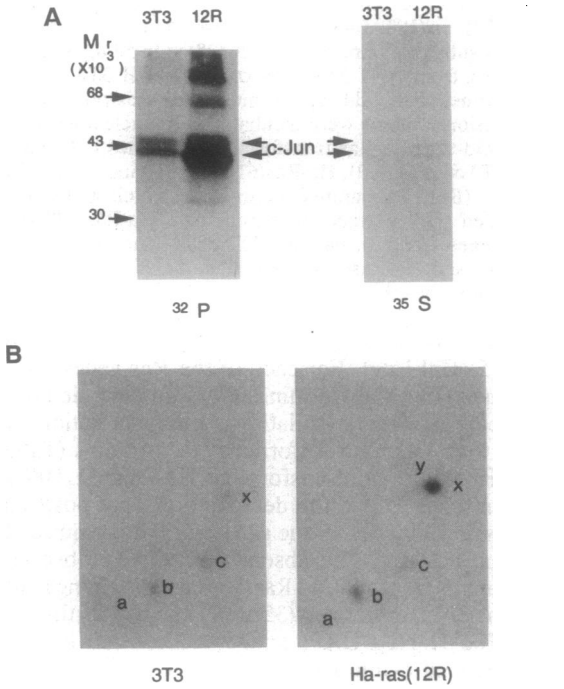

FIG. 1. The c-Jun activation domain is hyperphosphorylated in Ha-Ras(12R) transformed cells. Normal NIH 3T3 cells (3T3) or Ha-Ras(12R) transformed NIH 3T3 cells (12R) were transfected with a c-Jun expression vector and labeled with ^{32}P (*Left*) or [^{35}S]methionine (*Right*). Cell lysates were immunoprecipitated with c-Jun-specific antiserum. (*A*) Proteins were separated by SDS/PAGE, followed by blotting to nitrocellulose and exposure to film for 3 hr. Position of the c-Jun protein is indicated. The slower electrophoretic mobility forms result from phosphorylation. (*B*) ^{32}P-labeled c-Jun proteins from *A* were treated with trypsin and eluted from the nitrocellulose. Equal amounts of trypsin-treated protein were subjected to thin-layer electrophoresis in the horizontal dimension (cathode at the right) and chromatography in the vertical dimension. Origin is on the lower left of each map. Plates were exposed for 18 hr at −85°C with an intensifying screen. Peptides x (containing phosphoserine-73) and y (containing phosphoserine-63) and a, b, c (phosphopeptides adjacent to the DNA-binding domain) are indicated.

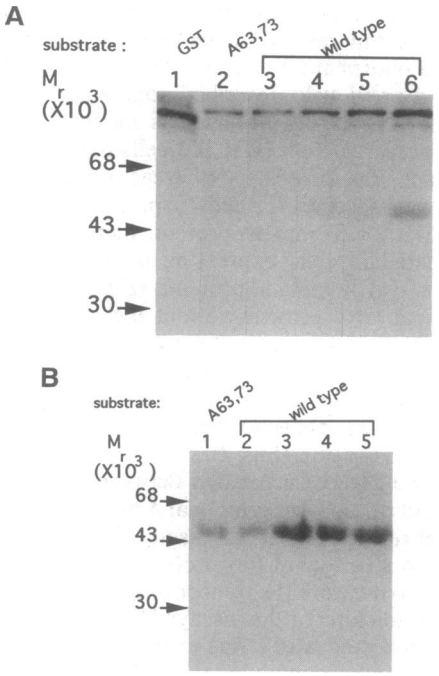

FIG. 2. Activation of a JNK in Ras-transformed cells. Ten nanograms of recombinant GST protein, GST–Jun (wild-type c-Jun, aa 1–223), or GST–Jun(A63,73) (Ser-63 and -73 mutated to Ala) was used as substrate to measure JNK (28) with 50 μg of nuclear extract prepared from NIH 3T3 cells or from NIH 3T3 cell lines stably transformed with wild-type or mutant Ha-Ras proteins. (*A*) GST or GST fusion proteins were incubated with nuclear extracts prepared from wild-type Ha-Ras-transformed cells (lanes 1, 2, and 4), parental NIH 3T3 cells (lane 3), Ha-Ras(61L/186S) (lane 5), and Ha-Ras(61L) (lane 6). (*B*) In a separate experiment, the indicated substrates were incubated with extracts prepared from Ha-Ras(12R)-transformed cells (lanes 1 and 3), parental 3T3 cells (lane 2), Ha-Ras(61P) cells (lane 4), and Ha-Ras(61L) cells (lane 5). Single band in each lane represents phosphorylated GST–Jun as in *A*.

luciferase reporter gene containing five Gal4 DNA binding sites (5×Gal-Luc). For each of the *Ras* genes (N-, Ki-, and Ha-*Ras*), the transforming Ras proteins demonstrated a markedly greater stimulation of the activation domain of c-Jun than the nontransforming Ras proteins (Table 1). The inability of the nontransforming Ha-Ras(61L/186S) mutant protein to activate c-Jun demonstrates that posttranslational processing and membrane association are required for transforming activity. The absence of activity observed with a nontransforming mutant Ras protein containing a mutation in the Ras effector domain (35A/61L) indicates that interaction with Raf-1, NF1-GAP, and/or p120 GAP is required for Ras-induced c-Jun activation. For each Ras protein, there was a direct correlation between stimulating c-Jun transcriptional activity and transforming activity, as measured by focus formation in NIH 3T3 cells ($R^2 = 0.874$; $P < 0.0002$). Thus, the activation of JNK, which is responsible for stimulation of c-Jun activity (28), is a potent predictor of Ras transforming potential.

Oncogenic Ras Proteins Stimulate ERK Activity. Next, we examined whether ERKs are activated in parallel with JNK. Like JNK, ERKs were activated in transformed, but not in untransformed, NIH 3T3 cells (Fig. 3). Western blotting of extracts prepared from cells transformed by the oncogenic Ras proteins Ha-Ras(61L), Ha-Ras(12R), and Ha-Ras(61P) demonstrated the presence of the phosphorylated active forms of ERKs (Fig. 3*A*), whereas parental NIH 3T3 cells or those expressing the nontransforming effector domain mutant Ha-Ras(35A/61L) contained only the nonphosphorylated inactive forms. To confirm that the mobility shift seen by Western

Table 1. Biochemical and biological activities of Ras mutants

Ras mutant and substitution(s)*	Relative FFU† [Ha-ras(61L) = 1.0]	c-Jun activation‡ (no Ras protein = 1.0)§
None	0.00	1.0
Ha-Ras		
Normal	0.00	1.41 ± 0.04
12R	0.80	5.31 ± 1.15
13V	0.92	3.31 ± 0.44
59T	0.90	3.37 ± 0.77
61L	1.00	5.58 ± 1.11
61P	0.09	1.85 ± 0.51
61W	0.46	3.45 ± 0.98
35W/61L	0.00	0.54 ± 0.22
35A/61L	0.00	0.99 ± 0.65
61L/186S	0.00	0.96 ± 0.30
116H	0.14	1.69 ± 1.08
117E	0.83	3.77 ± 0.85
119E	0.01	1.03 ± 0.09
N-Ras		
Normal	0.00	1.95 ± 0.78
12D	1.15	5.44 ± 1.88
Ki-Ras 4B		
Normal	0.00	0.81 ± 0.20
12V	0.71	5.26 ± 1.42

*Amino acid substitution(s) in normal Ras protein.
†Focus-forming units: values are averages of at least three experiments performed in quadruplicate.
‡Expressed as -fold induction of the 5×Gal-Luc reporter gene (2.5 μg) by the SVGal-Jun expression vector (250 ng) plus the indicated Ras mutant (250 ng) in transient transfection assays. Values are means ± SEM and are from at least three experiments performed in duplicate and normalized for transfection efficiency.
§Basal stimulation of 5×Gal-Luc by SVGal-Jun in the absence of Ras coexpression averaged 54-fold over several experiments.

blotting correlated with activation of ERK kinase activity, in-gel kinase assays were performed. Extracts of transformed, but not nontransformed, NIH 3T3 cells contained ERK kinase activity capable of phosphorylating a MBP substrate incorporated into the gel (Fig. 3*B*). Thus, only oncogenic forms of Ha-Ras constitutively activate ERKs. The absence of activity with the Ha-Ras(35A/61L) mutant indicates that an intact effector domain is required for Ras signaling through ERKs. These results prompted our assessment of whether transforming *Ras* genes activate c-Jun through ERKs.

Ras Stimulates ERK and c-Jun Transcriptional Activity by Distinct Pathways. To address the relationship between ERK and c-Jun activation, we investigated whether expression of exogenous wild-type or kinase-deficient ERKs influenced c-Jun activation. If so, we anticipated that exogenous ex-

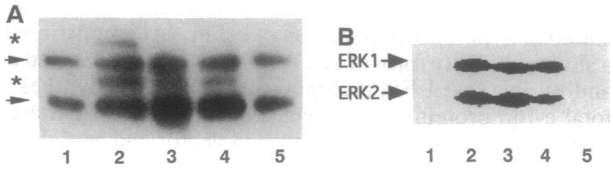

FIG. 3. Constitutive activation of ERKs in Ras-transformed cells. Extracts prepared from NIH 3T3 cells stably transformed by mutant Ha-Ras proteins were Western blotted and probed for inactive and active forms of ERKs (*A*) or used as a source of ERK activity in an in-gel MBP kinase assay (*B*). (*A*) Arrows, inactive, unphosphorylated forms of ERK1 and ERK2; asterisks, active phosphorylated forms of ERK1 and ERK2. Shown are extracts prepared from parental NIH 3T3 cells (lane 1) or from NIH 3T3 cells expressing oncogenic Ha-Ras mutants 61L (lane 2), 12R (lane 3), 61P (lane 4), or an effector domain mutant 35A/16L (lane 5). (*B*) Twenty-five micrograms of cell extracts from parental NIH 3T3 cells (lane 1) or from cells expressing Ha-Ras(61L) (lane 2), Ha-Ras(12R) (lane 3), Ha-Ras(61P) (lane 4), or Ha-Ras(wt) (lane 5).

pression of wild-type ERKs might enhance c-Jun activation, whereas the kinase-deficient mutants K71R-ERK1 and K52R-ERK2 (33) might function as dominant negative forms of ERKs to cause inhibition of Ras-induced c-Jun activation. In the absence of activated Ras, both wild-type and mutant ERK expression vectors inhibited basal c-Jun transcriptional activity as assayed with the 5×Gal4-Luc reporter gene and Gal4–Jun expression vector (Fig. 4*A*). Unexpectedly, coexpression of either wild-type ERK1 or ERK2 expression vector in the presence of activated Ras resulted in marked inhibition of c-Jun transcriptional activity when compared to its stimulation by Ras alone (Fig. 4*A*). Coexpression of the mutant ERK1 or ERK2 expression vector resulted in a greater inhibition of c-Jun transcription activity (Fig. 4*A*). Similarly, both wild-type and mutant ERK1 also inhibited c-Jun activation by oncogenic Raf (Fig. 4*B*) (43). These results suggested that activated ERKs are not directly involved in c-Jun or JNK activation.

An alternative explanation for the inhibitory activities of both wild-type and mutant ERKs is that overexpression of either form caused an inhibition of ERK function or that their overexpression was growth inhibitory. In the first control experiment, the reporter gene consisted of the collagen α1(I)

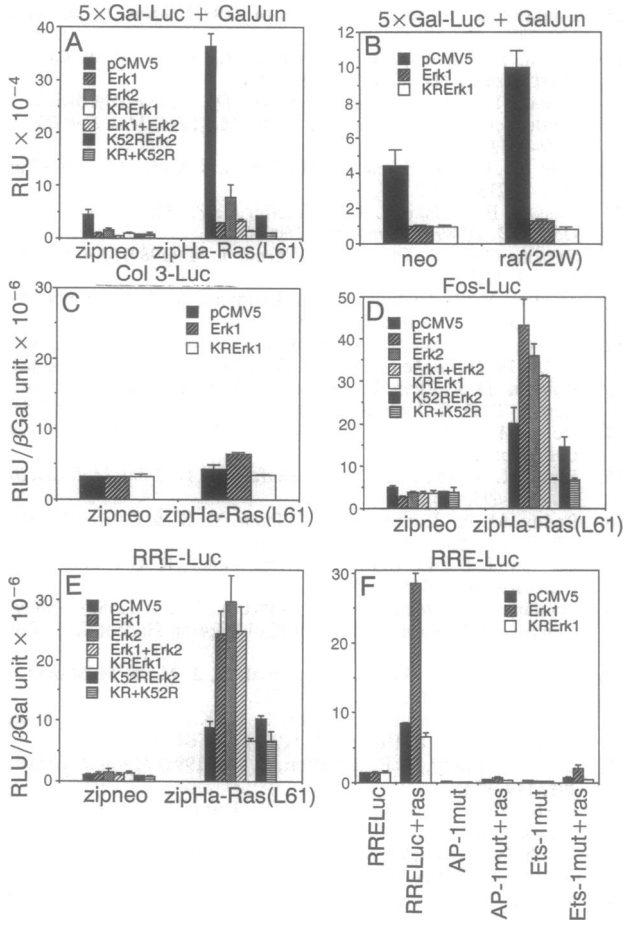

FIG. 4. Activation of reporter genes by cotransfection of wild-type or mutant ERK expression vectors. NIH 3T3 cells were transfected with 2.5 μg of the indicated reporter plasmids plus 250 ng of a vector expressing no Ras protein (pZIPneo) or 250 ng of Ha-Ras(61L) expression vector. In addition, 100 ng of empty vector (pCMV5) or expression vector for wild-type ERK1, ERK2, or both, or kinase-deficient ERK1 (KRERK1, with a mutation at residue 71), kinase-deficient ERK2 (K52RERK2), or both, was transfected per plate. Results in *B–E* were normalized for β-galactosidase (βGal) activity to allow direct comparison of the promoter/enhancer regions. RLU, relative luciferase units.

promoter driving the luciferase gene (Col3-Luc), which is not responsive to activated *Ras* genes (44). In the absence or presence of activated *Ras*, neither the wild-type nor the kinase-deficient ERK expression vector had a significant effect on Col3-Luc (Fig. 4*C*).

The c-*fos* promoter contains a serum response element, on which factors including the serum response factor and Elk-1 combine to form a ternary complex. Elk-1 is a substrate for ERKs and phosphorylation of Elk-1 enhances its transcriptional activity (18). As expected, wild-type ERK expression vectors enhanced the activation of Fos-Luc by activated Ras, while the mutant ERKs inhibited activation by Ras (Fig. 4*D*). Furthermore, wild-type but not kinase inactive ERK1 enhanced Ras activation of a Gal–ElkC chimeric transcription factor (18) using the same reporter and expression vector concentrations as in Fig. 4*A* (data not shown).

Finally, we evaluated the relationship between ERKs and Ras signal transduction by using the murine NVL-3 long terminal repeat Ras-responsive element (RRE), consisting of overlapping AP-1 and Ets-1 binding motifs (36). In the absence of a Ras expression vector, both the wild-type and kinase-inactive ERK expression vectors had a minimal effect on transcriptional activity from the RRE Luc reporter (Fig. 4 *E* and *F*). As expected, cotransfection with Ha-Ras(61L) stimulated RRE Luc activity. This activity was further enhanced by cotransfection of the wild-type ERK1 or ERK2 expression vector. On the other hand, cotransfection of the ERK1 kinase-deficient mutant vector had a slight inhibitory effect on reporter gene activity, while the kinase-inactive ERK2 vector had no effect. A 2-base mutation in the AP-1 motif (AP-1mut) or in the Ets-1 motif (Ets-1mut) of the RRE attenuated basal expression and stimulation by both Ras and ERK proteins (Fig. 4*F*). These results suggest that ERKs are critical intermediates between Ras and activation of composite AP-1/Ets-1 binding sites.

DISCUSSION

Although Ras proteins have long been implicated as critical intermediates in signal transduction pathways that dictate cell growth and differentiation (1, 3), only recently have the essential components of the Ras signaling pathway, and their functional associations, been elucidated (2–5, 8–12, 45, 46). However, the details of the pathway beyond ERKs, and the identity of the downstream substrates for ERK phosphorylation, which are important for Ras-induced cellular transformation, remain unresolved (6). Since c-Jun phosphorylation and activation have been observed in Ras-transformed cells (25, 26), and since c-Jun cooperates with Ras in oncogenic transformation (47), c-Jun represents a strong candidate for an essential component of the Ras signal transduction pathway. Therefore, we investigated the relationship between Ras transforming activity and the activation of ERKs and c-Jun.

The transcriptional activity of c-Jun is regulated by its phosphorylation status. Phosphorylation of the N-terminal transactivation domain at Ser-63 and Ser-73 results in increased transcriptional activity (25–27). In contrast, phosphorylation near the DNA binding domain results in decreased DNA binding and, consequently, decreased transcriptional activity (23, 40). The previous observations that both activated Ha-Ras(61L) and v-Raf trigger hyperphosphorylation of Ser-63 and -73 implicated a role for c-Jun activation via Ser-63/73 phosphorylation in Ras-induced transformation. In this study, we assayed c-Jun activation by a series of Ras mutant proteins that vary widely in their transforming potential, and observed a strong direct correlation between Ras transforming potential and c-Jun activation. This further implicates hyperphosphorylation of c-Jun at Ser-63 and -73 as critical events required for Ras-induced transformation.

We observed that kinase-deficient ERKs (32, 33, 48) inhibited Ras-induced stimulation of the c-*fos* promoter and the

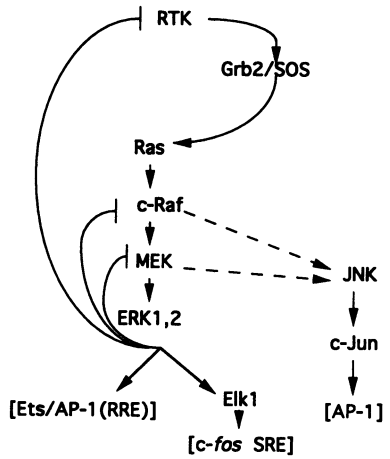

FIG. 5. Schematic representation of a Ras signal transduction pathway. RTK, receptor tyrosine kinase; SRE, serum response element.

RRE. Experiments with higher concentrations of the kinase-deficient ERK plasmids produced greater inhibition (data not shown). Thus, ERKs appear to be essential intermediates in signaling pathways that control certain Ras-responsive genes.

When we examined the role of ERKs in regulating c-Jun transcriptional activity, we observed strikingly different results. In these experiments, both the wild-type and, to a greater extent, the kinase-deficient ERKs inhibited the signal transduction pathway from Ras or Raf to c-Jun activation. This inhibition of Ras-induced c-Jun activation by both wild-type and mutant ERKs was observed over a wide range of concentrations and times (data not shown). The use of the chimeric Gal–Jun transcription factor enabled us to assess the activation of c-Jun independently of other AP-1 transcription factors, such as c-Fos, which is activated by ERKs. Therefore, enhanced ERK expression did not augment the activation of c-Jun by Ras or Raf. The simplest interpretation of these data is that the activation of c-Jun by Ras does not directly involve the ERKs but that there are common elements in both pathways extending downstream to Raf, while the role of MAP kinase/ERK kinases (MEK) is unknown (Fig. 5). At least one common element may be absorbed by overexpression of wild-type and kinase-deficient ERKs, thereby blocking the signal transduction pathway leading to c-Jun activation. Thus, the final effect of activated Ras on gene transcription represents the net interaction of sequential and parallel kinase cascades.

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