Fibroblast Transformation by Fps/Fes Tyrosine Kinases Requires Ras, Rac, and Cdc42 and Induces Extracellular Signal-regulated and c-Jun N-terminal Kinase Activation*

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The small GTP-binding proteins Ras, Rac, and Cdc42 link protein-tyrosine kinases with mitogen-activated protein kinase (MAPK) signaling cascades. Ras controls the activation of extracellular signal-regulated kinases (ERKs) (JNKS). In this study, we investigated whether small G protein/MAPK cascades contribute to signal transduction by transforming variants of c-Fes, a nonreceptor tyrosine kinase implicated in cytokine signaling and myeloid differentiation. First, we investigated the effects of dominant-negative small G proteins on Rat-2 fibroblast transformation by a retroviral homolog of c-Fes (v-Fps) and by c-Fes activated via N-terminal addition of the v-Src myristylation signal (Myr-Fes). We observed that dominant-negative Ras, Rac, and Cdc42 inhibited v-Fps- and Myr-Fes-induced growth of Rat-2 cells in soft agar, indicating that activation of these small GTP-binding proteins is required for fibroblast transformation by Fps/Fes tyrosine kinases. To determine whether MAPK pathways are activated downstream of these small G proteins, we measured ERK and JNK activity in the v-Fps- and Myr-Fes-transformed Rat-2 cells. Both ERK and JNK activities were elevated in the transformed cells, suggesting that these pathways are involved in cellular transformation. Dominant-negative mutants of Ras (but not Rac or Cdc42) specifically inhibited ERK activation by v-Fps and My-Fes, demonstrating that ERK activation occurs exclusively downstream of Ras. All three dominant-negative small G proteins inhibited JNK activation by v-Fps and Myr-Fes, indicating that JNK activation by these tyrosine kinases requires both Ras and Rho family GTPases. These data demonstrate that multiple small G protein/MAPK cascades are involved in downstream signal transduction by Fps/Fes tyrosine kinases.

Members of the Ras superfamily of small GTP-binding proteins regulate diverse cellular functions, including cell proliferation, differentiation, and organization of the actin cytoskeleton (1–4). One key function of these small GTP-binding proteins is to link upstream protein-tyrosine kinases with mitogen-activated protein kinase (MAPK)1 pathways. Perhaps the best characterized MAPK pathway involves Raf/MEK/ERK activation downstream of Ras (5–7). Raf binds to the GTP-bound, active form of Ras at the plasma membrane and becomes activated. Active Raf phosphorylates and activates the dual specificity MAPK kinases MEK1 and MEK2 which in turn activate the MAPKs ERK1 and ERK2. Activated ERKs can then translocate to the nucleus and phosphorylate a number of transcription factors. In this way, the Ras/ERK pathway provides a critical link between tyrosine kinase signal transduction and transcriptional events in the nucleus.

In addition to the Ras/ERK cascade, recent work has revealed parallel small G protein/MAPK pathways to the nucleus. Small GTPases of the Rho family including Rac and Cdc42 (but not Rho itself) are linked to a kinase cascade leading to the activation of the c-Jun N-terminal kinase (JNK), an ERK-related serine kinase that specifically phosphorylates and activates the c-Jun transcription factor (8–10). This cascade is also linked to a related kinase known as p38. Both p38 and JNK are activated in response to cytokines as well as cellular stress. Rac and Cdc42 are connected to JNK via intermediate MEKK (analogous to Raf) and SEK (analogous to MEK) activities. The Rac/Cdc42/JNK cascade also includes additional kinases upstream of MEKK, such as p21-activated kinase 65, which may represent the direct targets of active Rac and Cdc42 (7, 8). Thus in addition to their roles in the regulation of cytoskeletal architecture (3, 4, 11), Rac and Cdc42 can control transcriptional events in the nucleus as well.

Activation of small GTPase/MAPK pathways is a common feature of both normal and oncogenic protein-tyrosine kinase signal transduction (12). The biochemical connection between tyrosine kinases and the activation of small GTPases downstream often involves recruitment and activation of guanine nucleotide exchange factors. In the case of Ras, autophosphorylation of activated tyrosine kinases can create binding sites for the SH2 domains of adaptor proteins such as Shc and Grb-2, which serve as intermediates in the recruitment of the SOS guanine nucleotide exchange factor to the plasma membrane, focal adhesions, or other sites of tyrosine kinase signaling (13, 14). The translocation of SOS to these sites allows for Ras activation and subsequent stimulation of the MAPK pathway downstream. In the case of Rho family GTPases, tyrosine phosphorylation of the guanine nucleotide exchange factor may be essential for activation. For example, recent studies have shown that tyrosine phosphorylation stimulates the exchange

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1 The abbreviations used are: MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MBP, myelin basic protein; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; MEK, MAPK/ERK kinase; MEK5, MEK kinase.
activity of Vav, leading to activation of the JNK pathway (15, 16). In addition, products of the phosphatidylinositol 3'-kinase pathway have recently been shown to regulate the exchange activity of Vav and SOS toward Rho family GTPases (17, 18).

The Fps/Fes family of cytoplasmic protein-tyrosine kinases are expressed primarily in myeloid hematopoietic cells and the vascular endothelium (19–23). Human c-Fes has been linked to multiple cytokine receptors (24–28) and may play a direct role in myeloid differentiation and angiogenesis (22, 29, 30). However, the critical downstream signaling pathways regulating the biological activities of Fes have not been fully characterized. Previous studies have identified Bcr, Ras GAP, and Shc as substrates for c-Fes and its transforming viral homolog, v-Fps (31–35). All of these proteins have been linked to the regulation of the Ras and Rho families of small GTPases. For example, Bcr has a C-terminal GAP domain for Rac and Cdc42 (36) as well as a central guanine nucleotide exchange region with homology to Dbl (37, 38). Previous work from our laboratory has shown that tyrosine phosphorylation of Bcr in v-Fps-transformed fibroblasts induces its association with Grb-2/SOS, suggesting that Bcr may couple Fps/Fes tyrosine kinases to both the Ras and Rho signaling pathways downstream (34). In this study, we provide evidence that small G proteins of both the Ras and Rho families are required for Fps/Fes-induced fibroblast transformation and that small G protein activation is coupled to the ERK and JNK pathways downstream. These data provide strong evidence that activation of multiple small G protein/MAPK cascades is critical to Fps/Fes signal transduction.

**MATERIALS AND METHODS**

**Construction of Plasmid Vectors**—The full-length cDNAs for N17Ras, V12N17Rac, and N17Cdc42 containing a C-terminal Myc epitope tag were the generous gift of Dr. Alan Hall, MRC, London (39). These cDNAs were subcloned into the retrovector pSRαMSVtkneo (40) and into the mammalian expression vector pCDNA3 (Invitrogen). Addition of the N-terminal myristylation signal sequence of v-Src to the N-terminal region of c-Fes to create the transforming oncoprotein Myr-Fes was accomplished using a polymerase chain reaction-based approach and will be described in detail elsewhere. Construction of a full-length human c-fes cDNA with a C-terminal FLAG epitope tag is described elsewhere (42). A similar polymerase chain reaction-based procedure was used to add the FLAG coding sequence to the C-terminal region of v-Fps and Myr-Fes. The coding sequence of the v-src oncogene was amplified by polymerase chain reaction from a Rous sarcoma virus template obtained from the ATCC and subcloned into the pSRαMSVtkneo and pCDNA3 expression vectors described above.

**Production of Recombinant Retroviruses and Stably Transfected Rat-2 Cells**—Retroviral stocks were prepared by transient transfection of 293T cells (43) with the pSRαMSVtkneo constructs together with an ecotropic packaging vector (40). Briefly, 293T cells were grown to 50–80% confluence in 10-cm² dishes and transfected using a calcium phosphate method as described previously (42). Culture medium containing the recombinant retroviruses was harvested from 2 to 5 days post-transfection. Viral supernatants were pooled and stored in frozen aliquots at −80 °C. Negative control retroviruses were prepared using the parent vector alone.

Rat-2 cells were infected with recombinant retroviruses in the presence of polybrene as described (44). G418 was added to a final concentration of 0.8 mg/ml 48 h later, and the cells were grown under G418 selection for 2–4 weeks. The expression of N17Ras, V12N17Rac, and N17Cdc42 was confirmed by immunoprecipitation followed by immunoblotting with the anti-Myc monoclonal antibody, 9E10 (39). Stable expression of c-Fes was confirmed by immunoprecipitation and immunoblotting with the anti-FLAG monoclonal antibody, M2 (Kodak Scientific Imaging Systems).

**Transformation Assay**—Rat-2 cells (5 × 10⁴) stably expressing N17Ras, V12N17Rac, N17Cdc42, or the neo marker alone were plated in 6-well dishes. Cells were infected with v-Src, v-Fps, or Myr-Fes retroviruses 24 h later as described above and incubated for 2 additional days. The cells were then trypanosed, and 1 × 10⁶ cells were replated in triplicate in Dulbecco’s modified Eagle’s medium containing 0.3% agar and 20% fetal calf serum. Transformed colonies were counted after 2 weeks.

To establish Rat-2 cell lines stably expressing each of the tyrosine kinase products, single transformed colonies were isolated from the soft agar plates and grown in Dulbecco’s modified Eagle’s medium with 5% fetal calf serum. The expression of v-Src was confirmed by immunoprecipitation and immunoblotting with an anti-Src antibody (N-16, Santa Cruz Biotechnology); Myr-Fes and v-Fps expression were confirmed with the M2 anti-FLAG antibody.

**In Vitro JNK Assay**—Rat-2 or 293T cells were incubated overnight in serum-free medium prior to harvesting. Clarified cell lysates were prepared as described previously (35). For some experiments, a solid phase JNK assay was employed (45). Cell lysates (75 μg of protein) were incubated with 2 μg of an immobilized GST-Jun fusion protein (Jun amino acids 1–72) in a final volume of 300 μl of HBb buffer (20 mM HEPES, pH 7.5, 20 mM β-glycerophosphate, 10 mM MgCl₂, 40 μM ATP, 1 mM dithiothreitol, and 50 μM NaVO₃) containing 5 μCi of [γ-³²P]ATP (3000 Ci/mmol, NEN Life Science Products) for 20 min at 30 °C. Phosphorylated proteins were separated by SDS-PAGE and visualized by storage phosphorimaging (Molecular Dynamics). Alternatively, JNK activity was assessed by immunoblot with the JNK antibody to verify equivalent levels of the JNK protein in each reaction.

**In Vitro ERK Assay**—Clarified cell lysates (75 μg) were incubated with MAPK lysis buffer (40 mM Tris-HCl, pH 7.4, 120 mM NaCl, 5 mM EDTA, 2 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 0.5% Triton X-100, 10 mM NaF, 2 mM Na₃VO₃, 10 mM sodium pyrophosphate, 10 μg/ml leupeptin, and 5 μg/ml aprotinin) and immunoprecipitated with ERK1 and ERK2 antibodies (0.4 μg each; Santa Cruz Biotechnology). Immune complexes were precipitated with protein G-Sepharose, washed with HBb buffer, and divided into two aliquots. One aliquot was tested for ERK activity using the in vitro kinase assay conditions described above for the solid phase assay. The second aliquot was immunoblotted with the ERK1 antibody to verify equivalent amounts of ERK protein in each immunoprecipitate.

**RESULTS**

**Dominant-Negative Mutants of the Small GTP-binding Proteins Ras, Rac, and Cdc42 Block Rat-2 Transformation by Fps/Fes Tyrosine Kinases**—In this study, we used fibroblast transformation as a model system to study small G protein/MAPK signaling by v-Fps and Myr-Fes, two activated forms of the Fps/Fes tyrosine kinase family. v-Fps is a potent transforming oncogene, we inhibited the function of dominant-negative mutants in which the Ser residue at position 17 was changed to Asn (N17 mutants). These mutants bind preferentially to GDP and act as dominant inhibitors of endogenous small G protein function presumably by blocking their access to exchange factors (39, 48, 49). Populations of Rat-2 fibroblasts stably expressing each of these mutants were infected with v-Fps and Myr-Fes retroviruses and assayed for soft agar colony formation as a measure of transformation. As a control, we also

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² J. A. Rogers, N. A. Dunham, and T. E. Smithgall, manuscript in preparation.
experiments and are shown as the mean observed using the vector control cells. Data are from five independent Myr-Fes (and Myr-Fes was detected in the vector control cells (polyclonal antibody. The heavy chain of the Src antibody is also visible proteins. v-Src was detected by the same procedure with an anti-Src noprecipitation followed by immunoblotting with the monoclonal anti-cells expressing dominant-negative (mutations of Ras, Rac, and Cdc42 or the populations of Rat-2 fibroblasts stably expressing dominant-negative transformation of the v-Src (Fig. 1A). To determine whether the ERK pathway is essential roles in transformation signaling downstream from oncogenic Fps/Fes tyrosine kinases as well as v-Src.

Activation of ERK by v-Fps and Myr-Fes—Activation of many tyrosine kinase families is linked to stimulation of ERK activity (see Introduction). To determine whether the ERK pathway is activated by Fps/Fes kinases, we measured ERK activity in v-Fps- and Myr-Fes-transformed Rat-2 cell lines derived from single transformed soft-agar colonies. We also examined ERK activation in a Rat-2 cell line stably expressing c-Fes and in control G418-resistant Rat-2 cells. Endogenous ERK1 and ERK2 were immunoprecipitated from clarified cell lysates and an in vitro kinase assay was conducted with MBP and \( \gamma^{32}\text{P}]\text{ATP} \) as co-substrates. As shown in Fig. 4, ERK activity was increased 6–7-fold in the v-Fps- and Myr-Fes-transformed cells. In contrast, stable expression of c-Fes, which is nontransfoming, resulted in only a 2-fold increase in ERK activity. These results suggest that transformation by activated forms of Fps/Fes kinases may require sustained ERK activation. In contrast, no activation of ERK was observed in the v-Src transformants, suggesting that maintenance of the v-Src-transformed phenotype does not require constitutive MAPK activation.

Fig. 1. Dominant-negative mutants of Ras, Rac, and Cdc42 block Rat-2 transformation by v-Src, v-Fps, and Myr-Fes. A, populations of Rat-2 fibroblasts stably expressing dominant-negative mutants of Ras, Rac, Cdc42 or the neo selection marker (vector) were infected with v-Src, v-Fps, or Myr-Fes retroviruses as described under "Materials and Methods." Transformation was determined using a soft agar colony assay. The number of transformed colonies produced as a result of infection with the v-Src (solid bars), v-Fps (stippled bars), or Myr-Fes (open bars) retroviruses is expressed as the percent of colonies observed using the vector control cells. Data are from five independent experiments and are shown as the mean ± S.E. B, expression of v-Fps and Myr-Fes was detected in the vector control cells (vector) and in the cells expressing dominant-negative (dn) Ras, Rac, and Cdc42 by immunoprecipitation followed by immunoblotting with the monoclonal antibody M2, which recognizes the C-terminal FLAG epitope tag on these proteins. v-Src was detected by the same procedure with an anti-Src polyclonal antibody. The heavy chain of the Src antibody is also visible on the immunoblot (lg).

To verify that the dominant-negative small GTPases did not induce a general suppression of cellular proliferation, the growth rates of the Rat-2 cell populations stably expressing each of the mutants were compared with that of the control cells expressing only the drug-selection marker. As shown in Fig. 2A, Rat-2 cells stably expressing dominant-negative Ras, Rac, and Cdc42 grew at the same rate and to the same saturation density as the vector control cells. Expression of the dominant-negative small G proteins was verified by immunoprecipitation followed by immunoblotting (Fig. 2B).

As an alternative experimental approach to investigate the effect of dominant-negative small G proteins on Fps/Fes-mediated transformation, soft agar colony-forming assays were performed on Rat-2 fibroblasts following co-infection with recombinant retroviruses carrying the transforming oncogenes and the dominant-negative GTPase mutants. As shown in Fig. 3, this approach also led to strong suppression of transforming activity. These results are in good agreement with those observed using the Rat-2 cell populations stably expressing the dominant-negative mutants (Fig. 1). Taken together, these data support the conclusion that Ras, Rac, and Cdc42 play essential roles in transformation signaling downstream from oncogenic Fps/Fes tyrosine kinases as well as v-Src.
The surprising result that transformation by v-Src was not associated with constitutive ERK activation led us to investigate the effects of v-Src and Fps/Fes kinases on this pathway under transient expression conditions. For these experiments, we employed the human embryonic kidney cell line, 293T (43), and the in vitro ERK assay described above. As shown in Fig. 4, both v-Src and v-Fps stimulated ERK activity in this system. However, both c-Fes and Myr-Fes were unable to stimulate ERK activity in 293T cells. This lack of activation may reflect the tight regulation of both c-Fes and Myr-Fes tyrosine kinase activity in this cell line, despite the high level of protein expression (42). A nearly identical pattern of ERK activation was also observed in Rat-2 cells transiently expressing v-Fps, Myr-Fes, c-Fes, and v-Src, validating the use of 293T cells as a model system for the investigation of small G protein/MAPK signaling following transient expression of these tyrosine kinases (data not shown).

Activation of JNK by v-Fps and Myr-Fes—Recent studies have shown that JNK is activated downstream of Rac and Cdc42 (see Introduction). To determine whether the JNK pathway is activated by v-Fps and Myr-Fes, JNK activity was measured in the v-Src-, v-Fps-, and Myr-Fes-transformed Rat-2 cells. Endogenous JNK was precipitated from cell lysates with an immobilized GST-Jun fusion protein and kinase activity of substrate-bound JNK was assayed by addition of [γ-32P]ATP. As shown in Fig. 5, JNK activity was elevated in the v-Src-, v-Fps-, and Myr-Fes-transformed Rat-2 cells, suggesting that constitutive JNK activation may contribute to transformation by these oncogenes. In contrast, no increase in JNK activity was observed in nontransformed Rat-2 cells stably expressing c-Fes. Thus, JNK activation correlates with the transforming activity of these tyrosine kinase oncogenes.

Endogenous JNK activity was also measured in 293T cells transiently expressing v-Src, v-Fps, Myr-Fes, and c-Fes. As shown in Fig. 5, expression of v-Src and v-Fps readily stimulated JNK activity in 293T cells. In contrast to Rat-2 cells, JNK activity was only modestly increased by Myr-Fes in 293T cells. Similar differences were observed for Myr-Fes-induced activation of ERK in Rat-2 versus 293T cells (Fig. 4), and are consistent with the hypothesis that cells responding with activation of...
these pathways are selected for during transformation. c-Fes also slightly activated JNK over background in 293T cells, suggesting that JNK may be more sensitive than ERK to the low level of Fes tyrosine kinase activity resulting from the overexpression of Fes in this cell line (42).

**Dominant-Negative Ras Selectively Blocks ERK Activation by v-Fps and Myr-Fes**—Given that ERKs are often activated downstream of Ras, we tested whether dominant-negative Ras selectively blocks ERK activation in response to transforming signals from v-Fps and Myr-Fes. Populations of Rat-2 cells stably expressing dominant-negative mutants of Ras, Rac, and Cdc42 (as well as vector control cells) were infected with v-Fps and Myr-Fes retroviruses. One week later, endogenous ERK activity was immunoprecipitated from cell lysates and analyzed by in vitro kinase assay using MBP as a substrate. As shown in Fig. 6, dominant-negative Ras dramatically inhibited ERK activation by both v-Fps and Myr-Fes. In contrast, dominant-negative Rac and Cdc42 showed no effect on ERK activation. These results clearly indicate that constitutive ERK activation in Myr-Fes and v-Fps transformed cells occurs downstream of Ras.

The effects of dominant-negative Ras, Rac, and Cdc42 on ERK activation by v-Src and v-Fps were also examined in 293T cells. Myr-Fes was not tested because it does not activate ERK in this cell line (Fig. 4). v-Src and v-Fps were transiently expressed either alone or together with the dominant-negative small G proteins, and endogenous ERK activity was analyzed by in vitro kinase assay. As shown in Fig. 7, dominant-negative Ras inhibited ERK activation by both v-Src and v-Fps in 293T cells, while the other mutant GTPases were without effect. Expression of the mutant small G proteins and the tyrosine kinase oncogenes was verified by immunoblotting (data not shown). Similarly, co-infection of Rat-2 cells with recombinant retroviruses carrying the transforming tyrosine kinase oncogenes and dominant-negative Ras also blocked ERK activation under transient expression conditions (data not shown). These results indicate that transient ERK activation by v-Src and v-Fps is also regulated by Ras.

**DISCUSSION**

In this study, we investigated the role of small GTPases of the Ras and Rho families and their cognate MAPK pathways in signal transduction by transforming variants of Fps/Fes tyro-
Data presented here suggest that activation of the Rac/Cdc42/JNK pathway contributes to fibroblast transformation by v-src, v-fps, and other tyrosine kinase oncogenes. Consistent with this hypothesis are the previous findings that Bcr-Abl, the oncogenic protein-tyrosine kinase associated with chronic myelogenous leukemia (54), preferentially induces JNK activation in fibroblasts and hematopoietic cells transformed by this oncprotein (55). JNK-mediated activation of the c-Jun transcription factor may be an important aspect of transformation by both tyrosine kinase oncogenes as well as Ras. For example, fibroblast transformation by Bcr-Abl is blocked by dominant-negative mutants of c-Jun (55), and Ras is unable to transform fibroblasts in which Jun has been inactivated by gene targeting (56). However, in addition to the activation of JNK, Rac and Cdc42 also regulate the organization of the actin cytoskeleton which correlates with its ability to induce cellular transformation (57). Another alternative pathway downstream of Rho family GTPases may involve JNK-independent activation of nuclear factor-kB (58). Therefore, dominant-negative mutants of Rac and Cdc42 may inhibit transformation by Fps/Fes, v-Src, and other tyrosine kinases by blocking multiple intracellular signaling events in addition to interfering with JNK activation.

Comparison of small G protein/MAPK signaling by v-src and fps/fes oncogenes revealed some important differences. While both v-Src and v-Fps both potently activated ERK in the 293T cell transient expression system in a Ras-dependent manner (Figs. 4 and 7), constitutive ERK activation was not observed in the v-Fps-transformed Rat-2 cells (Fig. 4). This finding suggests that although ERK activation may be an essential early event in v-Src-mediated transformation, maintenance of the transformed phenotype does not require continuous activation of this kinase pathway. Our results agree with other recent work showing that rodent fibroblasts transformed by v-Src as well as mutationally activated c-Ha-Ras do not demonstrate constitutive ERK activation (59). Unlike v-Src, transformation by both v-Fps and Myr-Fes does correlate with constitutive ERK activation, suggesting that this pathway is important to the maintenance of transformation by Fps/Fes tyrosine kinases. In contrast to the ERK pathway, constitutive activation of JNK was observed with v-src and both fps/fes oncogenes, suggesting that JNK activation may play a general role in tyrosine kinase-mediated transformation.

A second major difference between v-Src- and Fps/Fes-mediated transformation may involve the signaling pathway leading from the tyrosine kinase to JNK activation downstream. While dominant-negative Ras, Rac, and Cdc42 all inhibited JNK activation by both v-Fps and Myr-Fes, only dominant-negative Rac and Cdc42 blocked JNK activation by v-Src; N17 Ras was without effect (Fig. 8). This finding suggests that the major pathway from v-src to Rho family GTPases does not require Ras. One possible mechanism linking v-Src with Rho family small G protein activation may involve direct phosphorylation of a guanine-nucleotide exchange factor. A precedent for this idea is provided by the recent observation that Vav guanine nucleotide exchange activity toward Rac and Cdc42 requires tyrosine phosphorylation by the Src family tyrosine kinase, Lck (15, 16). Although Vav is restricted in its expression to hematopoietic cells (60), a related protein may exist in other cell types that is a direct target for v-Src.

Although our results show a strong correlation of Ras/ERK signaling and Fps/Fes-mediated fibroblast transformation, activation of this pathway in other cell types may contribute to Fps/Fes-mediated differentiation. Several previous studies have implicated Fps/Fes kinases in the regulation of hematopoietic differentiation. For example, expression of v-Fps is suf-
ficient to induce differentiation of chicken myeloid stem cells into macrophages in the absence of colony-stimulating factor-1 (61). Similarly, introduction of c-Fes into the leukemia cell line K-562 induces growth suppression and terminal myeloid differentiation (29). Interestingly, recent work with K-562 cells shows that activation of the ERK pathway may be sufficient to induce terminal differentiation as well. In one study, constitutively active MEK mutants were shown to be sufficient to promote megakaryocytic differentiation in this cell line (62). These results suggest that MEK may be an important downstream effector for Fes-induced differentiation in a hematopoietic cell context.

Other work points to the Ras/Raf/ERK pathway as a general regulator of hematopoietic differentiation. Induction of monocytic differentiation of human myeloid leukemia cells has been correlated with activation of Raf-1 and ERK activities (63). Furthermore, introduction of a constitutively activated mutant of Ras induced monocytic differentiation of the human monocytic cell line, U-937 (64), while dominant-negative Ras was shown to block phorbol-ester-induced differentiation of HL-60 cells into macrophages (65). These data strongly implicate the Ras/Raf/ERK pathway as a general pathway for Fes-induced differentiation in a hematopoietic cell line, U-937 (64), while dominant-negative Ras was sufficient to induce differentiation of chicken myeloid stem cells into macrophages (61). Similarly, introduction of c-Fes into the leukemia cell line K-562 induces growth suppression and terminal myeloid differentiation (29). Interestingly, recent work with K-562 cells shows that activation of the ERK pathway may be sufficient to induce terminal differentiation as well. In one study, constitutively active MEK mutants were shown to be sufficient to promote megakaryocytic differentiation in this cell line (62). These results suggest that MEK may be an important downstream effector for Fes-induced differentiation in a hematopoietic cell context.

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