Ras-dependent Activation of c-Jun N-terminal Kinase/Stress-activated Protein Kinase in Response to Interleukin-3 Stimulation in Hematopoietic BaF3 Cells*

(Received for publication, October 1, 1996, and in revised form, November 15, 1996)

Koji Terada, Yoshito Kaziro, and Takaya Satoh‡
From the Faculty of Bioand Biotechnology, Tokyo Institute of Technology, Yokohama 226, Japan

Activation of the c-Jun N-terminal kinase (JNK)/stress-activated protein kinase pathway in response to stimulation of the interleukin (IL)-3 or granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor was examined in mouse hematopoietic BaF3-derived cell lines (BaF3-N6 and -V2 cells). Significant increase in the activity of JNK1 was observed within 30 min following IL-3 or GM-CSF stimulation at physiological concentrations. Dominant-negative Ras(S17N), which is conditionally expressed in the presence of isopropyl-1-thio-β-D-galactoside in BaF3-N6 cells, prevented the IL-3 stimulation of JNK1, whereas anisomycin-induced JNK1 activation was unaffected. Furthermore, a deletion mutant of the common β subunit for IL-3 and GM-CSF receptors that consists of only the membrane-proximal region, including box 1 and box 2 motifs, was incapable of facilitating JNK1 activity as well as Ras activation. These results provide evidence that Ras is required for IL-3-stimulated JNK1 activation. We also examined if constitutively active Ras(G12V) alone could stimulate JNK1 activity by using the inducible expression system. Isopropyl-1-thio-β-D-galactoside induction of Ras(G12V) in the BaF3-V2 cell line caused no significant increase in JNK1 activity, which could be activated by IL-3 or anisomycin. On the contrary, the extracellular signal-regulated kinase/mitogen-activated protein kinase pathway was fully activated following Ras(G12V) induction. Together with these results, it seems likely that the Ras protein is indispensable for the IL-3 stimulation of JNK1 although Ras activation by itself is insufficient for JNK1 activation.

Ras family GTP-binding proteins play a pivotal role in regulating proliferation of fibroblast cells, particularly in tyrosine kinase receptor-mediated signaling pathways (1, 2). A serine/threonine kinase cascade comprised of Raf family kinases, mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK), and ERK/MAPKs, functions downstream of Ras, and this cascade is essential for Ras-mediated growth promotion in fibroblast cells (3, 4). Activation of the ERK/MAPK pathway ultimately results in transcriptional activation of a serum response element-containing promoter, for example, the c-fos promoter, through phosphorylation of Elk-1 (5).

Stimulation with various cytokines, including interleukin (IL)-2, IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF), triggers Ras activation as evidenced by accumulation of an active GTP-bound form, implying that Ras acts as a signal transducer also in hematopoietic cells (6, 7). In the IL-3 signaling system, Raf and ERK/MAPK activation as well as induction of the c-fos promoter are demonstrated to be Ras-dependent (8). In addition to the Ras pathway, several distinct signaling pathways, for instance, the Janus kinase (JAK)/signal transducers and activators of transcription-mediated pathway, are stimulated by the IL-3 receptor (9).

The c-Jun N-terminal kinase (JNK)/stress-activated protein kinase (SAPK) pathway is stimulated by various kinds of stresses, such as ultraviolet (UV) irradiation, the high-temperature shock, and hyperosmolality. Moreover, protein synthesis inhibitors (e.g. anisomycin and cyclohexamide) and inflammatory cytokines (e.g. IL-1 and tumor necrosis factor α) activate JNK/SAPK (5). More recently, JNK/SAPK activation by stimulation of G protein-coupled receptors (10, 11), GTPase-deficient mutants of certain types of Gα (12, 13), as well as overproduced Gβγ subunits (14) have been reported. JNK/SAPK is phosphorylated and subsequently activated by a specific kinase, JNKK1/SEK1/MKK4, which is also activated following phosphorylation by other protein kinases, such as MEKK1 (5), Tpl-2 (15), and MLK-3 (16). Although the precise regulatory mechanism of this kinase cascade is still obscure, involvement of Cdc42 and Rac GTP-binding proteins in regulation of the JNK/SAPK pathway has recently been demonstrated using a transfection assay (17–19). Furthermore, a significant role of a mammalian homologue of the Saccharomyces cerevisiae Ste20 protein, p21-activated kinase, which is directly activated by a GTP-bound form of Cdc42 or Rac (20), has been suggested (18, 21–23). Another Ste20 homologue, germinal center kinase, is also a candidate for an activator of the JNK/SAPK pathway (24).

In this paper, we show activation of JNK1 following IL-3 treatment of IL-3-dependent hematopoietic cell lines. Conditionally expressed dominant-negative Ras abrogated the activation. Moreover, a deletion mutant of the IL-3 receptor β subunit that is unable to stimulate the Ras pathway failed to cause JNK1 activation. On the other hand, a constitutively active mutant of Ras was unable to induce JNK1 activation by itself despite its full activity to stimulate ERK2. Thus, we conclude that Ras is required, but not sufficient, for JNK1 activation in IL-3-dependent hematopoietic cells.
IL-3-induced JNK/SAPK Activation

EXPERIMENTAL PROCEDURES

Materials—A plasmid for expression of glutathione S-transferase (GST)-c-Jun(1–223) in Escherichia coli was kindly provided by Michael Karin (University of California, San Diego, La Jolla, CA). GST-c-Jun(1–223) was purified using a glutathione column. Purified mouse IL-3 and human GM-CSF are generous gifts of Robert Kastelein and Satish Menon (DNAX Research Institute of Molecular and Cellular Biology, CA), Antibodies against ERK2 (sc-154), JAK2 (sc-278), and JNK1 (sc-474) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). An anti-phosphotyrosine antibody, 4G10 (05–321), was obtained from Upstate Biotechnology, Inc. (Lake Placid, NY).

Cell Culture—BaF3-N6 and BaF3-V2 cell lines (8) were cultured in RPMI 1640 supplemented with fetal calf serum (10%, v/v), mouse IL-3 (approximately 1 nM), G418 (1 mg/ml), and hygromycin (1 mg/ml). BaF3αβ, and BaF3αβγ cells, which were kindly provided by Atsushi Miyajima (The University of Tokyo, Tokyo, Japan), were cultured in RPMI 1640 supplemented with fetal calf serum (10%, v/v), mouse IL-3 (approximately 1 nM), and hygromycin (1 mg/ml). For starvation, cells were incubated in RPMI 1640 supplemented with bovine serum albumin (1 mg/ml) for 3 h.

ERK/MAPK or JNK/SAPK Assay—Cells (1–2 × 10^6 per point) were lysed in kinase IP buffer (20 mM Tris-HCl (pH 7.5), 0.5% (v/v) Nonidet P-40, 250 mM NaCl, 3 mM EDTA, 3 mM EGTA, 3 mM β-glycerophosphate, 0.1 mM Na3VO4, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, and the supernatant of centrifugation (15,000 × g) for 10 min at 4 °C was mixed with protein A-Sepharose CL-4B (Pharmacia Biotech Inc.) and an anti-ERK2 (sc-154, 1 μg) or an anti-JNK1 (sc-474, 0.5 μg) antibody. The mixture was incubated for 1.5 h at 4 °C with gentle mixing, and the precipitate was washed twice with kinase IP buffer, twice with kinase wash buffer (25 mM Hepes-NaOH (pH 7.6), 20 mM MgCl2, 20 mM β-glycerophosphate, 0.1 mM Na3VO4, 20 mM p-nitrophenylphosphate, 2 mM benzamidine, and 100 μM leupeptin) and then three times with kinase IP buffer (25 mM Hepes-NaOH (pH 7.6), 20 mM MgCl2, 20 mM β-glycerophosphate, 0.1 mM Na3VO4, 20 mM NaF, 20 mM p-nitrophenylphosphate, 2 mM benzamidine, and 100 μM leupeptin). The precipitated proteins were subjected to the kinase assay within kinase reaction buffer (30 μl) containing 0.25 mg/ml myelin basic protein (for ERK/MAPK) or 0.05 mg/ml GST-c-Jun(1–223) (for JNK/SAPK), and 20 μM (γ-32P) ATP (307 TBq/mmol) for 20 min at 30 °C. The proteins were separated by SDS-polyacrylamide gel electrophoresis (13.5% (w/v) (for ERK/MAPK) or 10% (w/v) (for JNK/SAPK) polyacrylamide) and the radioactivity incorporated into each substrate was quantitated by an image analyzer (BAS2000, Fuji Film, Japan).

Immunoprecipitation and Immunoblotting—Cells (10^6 per point) were dissolved in IP buffer (50 mM Hepes-NaOH (pH 7.3), 150 mM NaCl, 10% (v/v) glycerol, 1% (v/v) Nonidet P-40, 2 mM MgCl2, 1 mM EDTA, 100 mM sodium fluoride, 10 mM NaF, 20 mM β-glycerophosphate, 1 mM Na3VO4, 10 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml leupeptin), and the supernatant of centrifugation (15,000 × g) for 10 min at 4 °C was used as a cell lysate. Protein A-Sepharose CL-4B (Pharmacia Biotech Inc.) and an anti-JAK2 antibody (sc-278, 2 μg) were mixed gently with the lysate for 1.5 h at 4 °C, and the precipitate was washed twice with IP buffer and twice with a wash buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 20 mM MgCl2). Then, the precipitated proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. The membrane was stained with an anti-phosphotyrosine antibody (05–321, 2 μg/ml) and enhanced chemiluminescence detection reagents (DuPont NEN).

RESULTS

Enhancement of JNK1 Activity in Response to IL-3 Stimulation in the BaF3-N6 Hematopoietic Cell Line—As a first step to examine possible involvement of JNK/SAPK in IL-3-stimulated signal transduction pathways, we measured the activity of endogenous JNK1 in IL-3-stimulated as well as unstimulated BaF3-N6 cells. The BaF3-N6 cell line was isolated from mouse IL-3-dependent hematopoietic BaF3 cells as a stable transfectant of an inducible expression system for dominant-negative Ras(S17N) (11, 12). Serum and IL-3-stimulated BaF3-N6 cells were stimulated with various concentrations of mouse IL-3 for 40 min. Endogenous JNK1 was collected by immunoprecipitation using a specific antibody, and the activity was measured by an in vitro kinase assay using GST-c-Jun(1–223) as a substrate. As shown in Fig. 1, A and B, dose-dependent increase of JNK1 activity was observed, which paralleled the induction of cell growth as measured by a colorimetric assay using 3-(4,5-di-

methythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (8). Incubation of the cells without mouse IL-3 for 40 min exhibited no effect on JNK1 activity, indicating that the increased kinase activity was not due to nonspecific stress during the incubation (data not shown). Time course of JNK1 activation following IL-3 treatment is demonstrated in Fig. 1, C and D. An increase in JNK1 activity was measurable 20 min after stimulation. The activity reached a maximum at 40 min and remained at plateau for at least another 20 min. Increase of JNK1 activity was rather slower than the case of ERK2 (Fig. 1, E and F). The activity of immunoprecipitated ERK2 was measured by an in vitro kinase assay using myelin basic protein as a substrate, where the activation was already detectable after 5 min stimulation. The observation is well correlated with time course of

![Fig. 1. IL-3-induced activation of JNK1 and ERK2 in BaF3-N6 cells.](image-url)
IPTG (5 mM) for 16 h. During the last 3 h, the cells were cultured in medium deprived of serum and IL-3. The cells were stimulated with mouse IL-3 (50 ng/ml) or anisomycin (20 μg/ml) for the indicated periods, and the activity of immunoprecipitated JNK1 was measured. Data are shown as mean ± S.E. (n = 3). B, inhibition of IL-3-induced ERK2 activation by Ras(S17N). BaF3-N6 cells were incubated with or without IPTG (5 mM) for 16 h. During the last 3 h, the cells were cultured in medium deprived of serum and IL-3. The cells were stimulated with mouse IL-3 (50 ng/ml) or phorbol 12-myristate 13-acetate (PMA) (200 ng/ml) for the indicated periods, and the activity of immunoprecipitated ERK2 was measured. Data are shown as mean ± S.E. (n = 3).

hyperphosphorylation described previously (8). Moreover, we observed similar delayed activation of JNK1 compared with ERK2 in another BaF3-derived stable clone.2

IL-3 Receptor-mediated JNK/SAPK Activation Is Ras-dependent—Since the Ras protein seems to play an important role for regulation of JNK/SAPK activity in a variety of signal transduction pathways, including those initiated by stimulation with epidermal growth factor, nerve growth factor, and G protein-coupled receptor ligands (14, 17, 18, 25), we next assessed the role of Ras in IL-3-stimulated JNK1 activation. As described previously (8), dominant-negative Ras(S17N) was induced in BaF3-N6 cells by incubation with isoprpyl-1-thio-β-D-galactoside (IPTG) for 16 h. The IL-3 stimulation of JNK1 activity was found to be diminished considerably after induction of Ras(S17N), whereas JNK1 activation in response to a translational inhibitor, anisomycin, which has been shown to be Ras-independent in COS7 cells (14), remained unaffected (Fig. 2A). Likewise, ERK2 stimulation by IL-3 was inhibited by Ras(S17N) (Fig. 2B), which is consistent with our previous results that Ras(S17N) inhibited c-Raf-1 activation as well as ERK2 hyperphosphorylation. These results suggest that both JNK1 and ERK2 pathways are regulated by Ras. Further examination of the assumption that the JNK1 pathway is dependent on Ras was performed by using a mutant receptor for GM-CSF. It has been shown that human GM-CSF is capable of stimulating proliferation of a BaF3-derived transfectant of α and β subunits of the human GM-CSF receptor (26). β544 is a mutant of the βc subunit, which is composed of intact extracellular and transmembrane domains and a C-terminal truncated cytoplasmic domain (a membrane-proximal region including box 1 and box 2 motifs) (26). The β544 receptor is incapable of activating the Ras pathway as determined by a lack of Ras-GTP formation and impaired increase in ERK2 activity, whereas human GM-CSF can stimulate the Ras pathway to an extent similar to mouse IL-3 in BaF3/αβc cells (Ref. 27 and data not shown). In contrast, the β544 receptor is considered intact in terms of stimulation of the Jak/Signal transducers and activators of transcription pathway because human GM-CSF-induced tyrosine phosphorylation of JAK2 was detected also in BaF3/αβ544 cells. Serum and IL-3-deprived BaF3/αβc and BaF3/αβ544 cells were stimulated with human GM-CSF (10 ng/ml) or mouse IL-3 (50 ng/ml) for the indicated periods. The activity of immunoprecipitated JNK1 was measured. Data are shown as mean ± S.E. (n = 3).

FIG. 2. Inhibition of IL-3-induced JNK1 and ERK2 activation by Ras(S17N) in BaF3-N6 cells. A, inhibition of IL-3-induced JNK1 activation by Ras(S17N). BaF3-N6 cells were incubated with or without IPTG (5 mM) for 16 h. During the last 3 h, the cells were cultured in medium deprived of serum and IL-3. The cells were stimulated with mouse IL-3 (50 ng/ml) or anisomycin (20 μg/ml) for the indicated periods, and the activity of immunoprecipitated JNK1 was measured. Data are shown as mean ± S.E. (n = 3). B, inhibition of IL-3-induced ERK2 activation by Ras(S17N). BaF3-N6 cells were incubated with or without IPTG (5 mM) for 16 h. During the last 3 h, the cells were cultured in medium deprived of serum and IL-3. The cells were stimulated with mouse IL-3 (50 ng/ml) or phorbol 12-myristate 13-acetate (PMA) (200 ng/ml) for the indicated periods, and the activity of immunoprecipitated ERK2 was measured. Data are shown as mean ± S.E. (n = 3).

2 K. Tago, Y. Kaziro, and T. Satoh, unpublished results.

FIG. 3. GM-CSF-induced activation of JNK1 in BaF3/αβc, but not in BaF3/αβ544 cells. A, tyrosine phosphorylation of JAK2 in BaF3/αβc and BaF3/αβ544 cells. Serum and IL-3-deprived BaF3/αβc and BaF3/αβ544 cells were stimulated with human GM-CSF (10 ng/ml) or mouse IL-3 (50 ng/ml) for the indicated periods. The JAK2 protein was immunoprecipitated with a specific antibody (sc-278), and stained with an anti-phosphotyrosine antibody (05–321). B, activation of JNK1 in BaF3/αβc and BaF3/αβ544 cells. Serum and IL-3-deprived BaF3/αβc and BaF3/αβ544 cells were stimulated with human GM-CSF (10 ng/ml) or mouse IL-3 (50 ng/ml) for the indicated periods. The activity of immunoprecipitated JNK1 was measured. Data are shown as mean ± S.E. (n = 3).

Constitutively Active Ras(G12V) Is Insufficient to Induce JNK1 Activation—BaF3-V2 cells grow in an IL-3-dependent manner, and produce constitutively active Ras(G12V) following IPTG induction (8). The induced Ras(G12V) can stimulate c-Raf-1 kinase activity, ERK2 hyperphosphorylation, as well as transcriptional activation of the c-fos promoter (Ref. 8 and data not shown). To examine the effect of Ras(G12V) expression on the induction of JNK1 activation, we first measured JNK1 activation in response to mouse IL-3 and anisomycin. Both IL-3 and anisomycin induced JNK1 activation to a similar extent as in the case of BaF3-N6 cells (Figs. 2A and 4A). Time course of the activation also was similar to BaF3-N6 cells as illustrated in Figs. 1D and 4B. Next, the activity of ERK2 and JNK1 was quantitated in parallel, and the results are described in Fig. 4C. No JNK1 activation was observed in response to IPTG induction of Ras(G12V), while ERK2 activity was markedly

hyperphosphorylation described previously (8). Moreover, we observed similar delayed activation of JNK1 compared with ERK2 in another BaF3-derived stable clone.2

IL-3 Receptor-mediated JNK/SAPK Activation Is Ras-dependent—Since the Ras protein seems to play an important role for regulation of JNK/SAPK activity in a variety of signal transduction pathways, including those initiated by stimulation with epidermal growth factor, nerve growth factor, and G protein-coupled receptor ligands (14, 17, 18, 25), we next assessed the role of Ras in IL-3-stimulated JNK1 activation. As described previously (8), dominant-negative Ras(S17N) was induced in BaF3-N6 cells by incubation with isoprpyl-1-thio-β-D-galactoside (IPTG) for 16 h. The IL-3 stimulation of JNK1 activity was found to be diminished considerably after induction of Ras(S17N), whereas JNK1 activation in response to a translational inhibitor, anisomycin, which has been shown to be Ras-independent in COS7 cells (14), remained unaffected (Fig. 2A). Likewise, ERK2 stimulation by IL-3 was inhibited by Ras(S17N) (Fig. 2B), which is consistent with our previous results that Ras(S17N) inhibited c-Raf-1 activation as well as ERK2 hyperphosphorylation. These results suggest that both JNK1 and ERK2 pathways are regulated by Ras. Further examination of the assumption that the JNK1 pathway is dependent on Ras was performed by using a mutant receptor for GM-CSF. It has been shown that human GM-CSF is capable of stimulating proliferation of a BaF3-derived transfectant of α and β subunits of the human GM-CSF receptor (26). β544 is a mutant of the βc subunit, which is composed of intact extracel-
Ras(G12V)-induced activation of ERK2, but not of JNK1, in BaF3-V2 cells. A, IL-3 and anisomycin-induced JNK1 activation. Serum and IL-3-deprived BaF3-V2 cells were stimulated with mouse IL-3 (50 ng/ml) or anisomycin (20 μg/ml) for the indicated periods, and the activity of immunoprecipitated JNK1 was measured. Data are shown as mean ± S.E. (n = 3). B, time course of IL-3-induced JNK1 activation. Serum and IL-3-deprived BaF3-V2 cells were stimulated with mouse IL-3 (50 ng/ml) for specified periods, and the activity of immunoprecipitated JNK1 was measured. Data are shown as mean ± S.E. (n = 3). C, Ras(G12V)-induced activation of ERK2, but not of JNK1. BaF3-V2 cells were incubated with IPTG (5 mM) for specified periods. During the last 3 h, the cells were cultured in medium deprived of serum and IL-3. The cells were left unstimulated (○, △) or stimulated with mouse IL-3 (50 ng/ml) for 40 min (●, ▲), and the activity of immunoprecipitated JNK1 (○, △) and ERK2 (●, ▲) was measured. Data are shown as mean ± S.E. (n = 3).

The results suggest that Ras(G12V) by itself is insufficient for activation of JNK1, where an additional signal may be required.

**DISCUSSION**

It has been demonstrated that a variety of cytokines, including IL-2, IL-3, IL-5, GM-CSF, and erythropoietin can trigger Ras activation in hematopoietic cell lines (6, 7). Although the results suggest a significant role of Ras in cytokine-mediated intracellular signal transduction, it still remains to be solved whether Ras is implicated in growth-promoting or differentiation-inducing signaling pathways. In IL-3 signal transduction, analyses using a series of deletion mutants of the receptor β subunit have shown that the Ras pathway is responsible for prevention of apoptotic cell death (30). Moreover, conditional expression of a dominant-negative Ras mutant exhibited no inhibitory effect on IL-3-dependent proliferation of BaF3 cells (8). Likewise, the Ras pathway does not seem to be necessary for IL-2 induction of cell proliferation (31–33). On the other hand, in another hematopoietic cell line 32Dc13, Ras was required for cell growth in response to IL-3, but not for granulocyte colony-stimulating factor-induced differentiation (34). As for granulocyte colony-stimulating factor receptor-mediated signaling, the Ras pathway was found to be associated with the proliferative response in myeloid leukemia cell lines, such as NFS-60 (35). Taken together, the role of Ras in cells of the hematopoietic lineage is still controversial, and thus, further investigation of Ras-regulated signaling pathways is required for a final conclusion.

In addition to the Ras protein, members of the Cdc42/Rac/Rho GTP-binding protein family have recently been shown to be important for cellular responses, particularly for the regulation of cytoskeletal organization, in various kinds of mammalian cells (36–39). They are also implicated in T-cell polarization (40), cell cycle progression (19), cell motility (41), and morphogenesis of neuronal cells (42). Furthermore, their dominant-negative-type mutants prevent Ras-induced transformation of fibroblast cells, suggesting a significant role at a point downstream of Ras (43, 44). However, the role of Cdc42, Rac, and Rho proteins in cytokine-mediated signal transduction remains to be defined.

In this report, we provide evidence that the JNK1 pathway is activated upon IL-3 stimulation, where Ras may be implicated as a regulator. Furthermore, we have obtained data showing that dominant-negative mSos1 that is capable of interfering with IL-3-induced Ras activation could block JNK1 activation upon IL-3 treatment. Collectively, it is conceivable that a Ras-dependent JNK1 pathway exists within BaF3 cells, which may play an important role in IL-3-induced cellular responses.

Although ERK/MAPK and JNK/SAPK pathways converge at the point of transcriptional activation by the AP-1 complex (5), the precise physiological role of each pathway is still obscure. Some growth factor signals preferentially stimulate the ERK/MAPK pathway, while others stimulate only the JNK/SAPK pathway. Growth factors like epidermal growth factor as well as IL-3, as illustrated in this paper, are able to activate both. Additionally, in the case of T-cell activation signaling, for example, two apparently independent signals from the T-cell receptor and the CD28 costimulatory receptor are necessary to augment JNK/SAPK activity, whereas ERK/MAPK is fully activated in response to T-cell receptor stimulation alone (45). In PC12 pheochromocytoma cells, ERK/MAPK is responsible for nerve growth factor-induced differentiation and proliferation, whereas the JNK/SAPK pathway is modulated by a signal of nerve growth factor withdrawal, which causes apoptotic cell death (46). Taking into consideration these findings, it is possible to speculate that JNK/SAPK may be responsible for cellular responses distinct from those regulated by ERK/MAPK in cytokine receptor-mediated signal transduction.

We have shown that IL-3-induced JNK1 activation occurs in a Ras-dependent manner, which is also the case in other types of cells, including PC12, HeLa, NIH 3T3, and COS7 cells (14, 17, 18, 25, 47). However, at present, the mechanism of JNK/SAPK activation through Ras has not been fully understood. It may be possible that MEKK1, which binds directly to a GTP-bound form of Ras (48), is activated by Ras, thereby stimulating a kinase cascade comprising of JNK1/SEK1/MKK4 and JNK/SAPK (5). Ras and MEKK1-dependent JNK/SAPK activation was, in fact, observed in HeLa cells (25). Instead, Rac may be involved between Ras and the JNK/SAPK cascade because Rac is thought to act as a downstream element of Ras (43, 44), and regulate the JNK/SAPK pathway (17–19). These possibilities are currently under examination.

As shown in Fig. 4, Ras(G12V) is incapable of promoting JNK1 activity by itself in contrast to ERK2, which is strongly activated by Ras(G12V) alone in BaF3-V2 cells (see also Ref. 8). In HeLa and NIH 3T3 cells lines, an activated form of Ras is able to induce JNK/SAPK activation significantly, but less than an activated Cdc42 or Rac protein (18), whereas, in COS7 cells, v-Ras induces only a slight increase of JNK/SAPK activity (17). Another paper has reported that an activated Ras induced only a partial increase in JNK/SAPK activity, which could be further enhanced by irradiation of UV light (47). Furthermore, as
described above, stimulation of the T-cell receptor, which is sufficient to induce Ras activation in T-lymphocytes, fails to activate JNK/SAPK, suggesting the activated Ras protein is unable to stimulate JNK/SAPK by itself. Taken together with these observations, a second signal, besides a signal from Ras, may be required for full activation of JNK/SAPK, especially in hematopoietic and lymphoid cells. The elucidation of the biochemical mechanism by which Ras regulates the JNK/SAPK pathway will contribute to our understanding of the role of this pathway in growth and differentiation signaling in hematopoietic cells.

Acknowledgments—We are grateful to Atsushi Miyajima for BaF3 cell lines expressing the human GM-CSF receptor and Michael Karin for the GST-c-Jun plasmid.

REFERENCES

1. Satoh, T., Nakafuku, M., and Kaziro, Y. (1992) J. Biol. Chem. 267, 24149–24152.
2. Loy, D. R., and Willumsen, B. M. (1993) Annu. Rev. Biochem. 62, 851–891.
3. Avruch, J., Zhang, X., and Kyriakis, J. M. (1994) Trends Biochem. Sci. 19, 279–283.
4. Cobb, M. H., and Goldsmith, E. J. (1995) J. Biol. Chem. 270, 14843–14846.
5. Karin, M. (1995) J. Biol. Chem. 270, 16483–16486.
6. Satoh, T., Nakafuku, M., Miyajima, A., and Kaziro, Y. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3314–3318.
7. Duronio, V., Welham, M. J., Abraham, S., Dryden, P., and Schrader, J. W. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 1587–1591.
8. Terada, K., Kaziro, Y., and Satoh, T. (1995) J. Biol. Chem. 270, 27880–27886.
9. Irie, J. N. (1995) Nature 377, 591–594.
10. Coso, O. A., Chiarlelli, M., Kalinec, G., Kyriakis, J. M., Woodgett, J., and Gutkind, J. S. (1995) J. Biol. Chem. 270, 5620–5624.
11. Zohn, J. E., Yu, H., Li, X., Cox, A. D., and Earg, H. S. (1995) Mol. Cell. Biol. 15, 6160–6168.
12. Prasad, M. V. V. S., Dormott, J. M., Heasley, L. E., Johnson, G. L., and Dhanasekaran, N. (1995) J. Biol. Chem. 270, 18655–18659.
13. Heasley, L. E., Storey, B., Fanger, G. R., Butterfield, L., Zamarrilla, J., Blumberg, D., and Maue, R. A. (1996) Mol. Cell. Biol. 16, 648–656.
14. Coso, O. A., Teramoto, H., Simonds, W. F., and Gutkind, J. S. (1996) J. Biol. Chem. 271, 3963–3966.
15. Salmeron, A., Ahmad, T. B., Carlile, G. W., Pappin, D., Narsimhan, R. P., and Ley, S. C. (1996) EMBO J. 15, 817–826.
16. Rana, A., Gallo, K., Godowski, P., Hirai, S., Ohno, S., Zon, L., Kyriakis, J. M., and Avruch, J. (1996) J. Biol. Chem. 271, 19025–19028.
17. Coso, O. A., Chiarlelli, M., Yu, J., Teramoto, H., Crespo, P., Xu, N., Miki, T., and Gutkind, J. S. (1995) Cell 81, 1137–1146.
18. Minden, A., Lin, A., Charet, F., Ahn, A., and Karin, M. (1995) Cell 81, 1147–1157.
19. Olson, M. F., Ashworth, A., and Hall, A. (1995) Science 269, 1270–1272.
20. Manser, E., Leung, T., Salehuddin, H., Zhao, Z., and Lim, L. (1994) Nature 370, 40–46.
21. Polverino, A., Frost, J., Yang, P., Hutchison, M., Neiman, A. M., Cobb, M. H., and Marcus, S. (1995) J. Biol. Chem. 270, 26067–26070.
22. Bagrodia, S., Derijard, B., Davis, R. J., and Cerione, R. A. (1995) J. Biol. Chem. 270, 27995–27998.
23. Frost, J. A., Xu, S., Hutchison, M. R., Marcus, S., and Cobb, M. H. (1996) Mol. Cell. Biol. 16, 3707–3712.
24. Pomm, C. M., Kehrl, J. H., Sanchez, I., Katz, P., Avruch, J., Zon, L. I., Woodgett, J. R., Force, T., and Kyriakis, J. M. (1995) Nature 377, 750–754.
25. Minden, A., Lin, A., McMahan, M., Lange-Carter, C., Derijard, B., Davis, R. J., Johnson, G. L., and Karin, M. (1994) Science 266, 1719–1723.
26. Sakamaki, K., Miyajima, I., Kitamura, T., and Miyajima, A. (1992) EMBO J. 11, 3541–3549.
27. Sato, N., Sakamaki, K., Terada, N., Akai, K., and Miyajima, A. (1993) EMBO J. 12, 4181–4189.
28. Quelle, J. F., Sato, N., Wilthuus, B. A., Inhorn, R. C., Eder, M., Miyajima, A., Griffin, J. D., and Ihle, J. N. (1994) Mol. Cell. Biol. 14, 4335–4341.
29. Mui, A. L., Wakao, H., O’Farrell, A., Harada, N., and Miyajima, A. (1995) EMBO J. 14, 1166–1175.
30. Kinoshita, T., Yokota, T., Arai, K., and Miyajima, A. (1995) EMBO J. 14, 266–275.
31. Satoh, T., Minami, Y., Kono, T., Yamada, K., Kawahara, A., Taniguchi, T., and Kaziro, Y. (1992) J. Biol. Chem. 267, 25423–25427.
32. Evans, G. A., Goldsmith, M. A., Johnston, J. A., Xu, W., Weiler, S. R., Erwin, R., Howard, O. M. Z., Abraham, R. T., O’Shea, J. J., Greene, W. C., and Farrar, W. L. (1995) J. Biol. Chem. 270, 28858–28863.
33. Miyazaki, T., Liu, Z., Kawahara, A., Minami, Y., Yamada, K., Tsujimoto, Y., Barsoumian, E. L., Perlmutter, R. M., and Taniguchi, T. (1995) Cell 81, 223–231.
34. Ohkita, K., Ernst, T. J., and Griffin, J. D. (1994) J. Biol. Chem. 269, 24602–24607.
35. Bashey, A., Healy, L., and Marshall, C. J. (1994) Blood 83, 949–957.
36. Vojtek, A. B., and Cooper, J. A. (1995) Cell 82, 527–529.
37. Chant, J., and Stowers, L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 11108–11122.
38. Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J., and Greenberg, M. E. (1995) Science 270, 829–834.
39. Russell, M., Lange-Carter, C. A., and Johnson, G. L. (1995) J. Biol. Chem. 270, 6362–6366.