p38 Mitogen-activated Protein Kinase Activation Is Required for Fibroblast Growth Factor-2-stimulated Cell Proliferation but Not Differentiation*

Pamela Maher
From the Department of Cell Biology, Scripps Research Institute, La Jolla, California 92037

Basic fibroblast growth factor (FGF-2) is a member of a family of polypeptides that have roles in a wide range of biological processes. To determine why different cell types show distinct responses to treatment with FGF-2, the array of FGF receptors present on the surface of a cell which differentiates in response to FGF-2 (PC12 cells) was compared with that present on the surface of a cell that proliferates in response to FGF-2 (Swiss 3T3 fibroblasts). Both cell types express exclusively FGFR1, suggesting that there are cell type-specific FGFR1 signaling pathways. Since mitogen-activated protein kinases function as mediators of cellular responses to a variety of stimuli, the roles of these proteins in FGF-mediated responses were examined. FGF-2 activates extracellular signal-regulated kinases with similar kinetics in both fibroblasts and PC12 cells, and a specific inhibitor of extracellular signal-regulated kinase activation blocks differentiation but has little effect on proliferation. In contrast, while p38 mitogen-activated protein kinase is activated weakly and transiently in PC12 cells treated with FGF-2, a much stronger and sustained activation of this kinase is seen in FGF-2-treated fibroblasts. Furthermore, specific inhibitors of this kinase block proliferation but have no effect on differentiation. This effect on proliferation is specific for FGF-2 since the same concentrations of inhibitors have little or no effect on proliferation induced by serum.

Basic and acidic fibroblast growth factors (FGF-2 and FGF-1)³ are the prototypes for a large family of multifunctional growth factors, which have been identified in a wide variety of tissues (for reviews see Refs. 1–4). Although FGFs were first characterized on the basis of their ability to stimulate cell proliferation, it is now known that FGFs can modulate a number of other cellular functions, including promotion or inhibition of differentiation, survival, protease synthesis and secretion, and chemotaxis. However, the pathways that mediate these cell type-specific effects of FGFs are not well understood.

The FGFs interact with two classes of FGF receptors: high affinity receptors, which bind FGFs with picomolar affinity and are thought to mediate the cellular responses to FGF; and low affinity receptors, which bind FGFs with nanomolar affinity and are characterized by the presence of heparan sulfate moieties. The family of high affinity FGF receptors contains four closely related members (for reviews, see Refs. 4–7), which all possess intrinsic tyrosine kinase activity. In addition, a number of alternately spliced mRNAs corresponding to multiple isoforms of FGF receptor-1 (FGFR1), FGF receptor-2 (FGFR2), and FGF receptor-3 (FGFR3) (8–11) have been identified. These isoforms include receptors lacking Ig-like domain I (two Ig-like domain isoform) and/or alternative sequences for the second half of Ig-like domain III (receptor isoforms a, b, and c).

In a recent study, the interaction of nine members of the FGF family with the four FGF receptors was examined using the induction of mitogenesis as an end point (12). This study demonstrated that each of the FGF receptors is capable of binding and responding to more than one type of FGF. Furthermore, both the two Ig-like domain isoform and the three Ig-like domain isoform (with Ig-like domain 1) of FGF receptors 1 and 3 showed similar responses to all of the FGFs tested, indicating that the first Ig-like domain is unlikely to play a role in regulating intracellular signaling by the receptors. In addition, cells transfected with either FGFR1b or FGFR1c showed a strong mitogenic response to treatment with FGF-2, suggesting that the third Ig-like domain is also unlikely to play a role in regulating signaling through FGFR1.

The pathways involved in FGF receptor signal transduction have not yet been fully elucidated. Since the FGF receptors are tyrosine kinases, an early event in the cellular response to receptor activation is substrate binding and tyrosine phosphorylation. Indeed, autophosphorylation of tyrosines 653 and 654 in the tyrosine kinase domain of FGFR1 is required for activation of the kinase activity of the receptor, as well as subsequent substrate phosphorylation and stimulation of proliferation or differentiation (13). However, many of the proteins known to be phosphorylated on tyrosine by other receptor tyrosine kinases do not appear to be phosphorylated in response to treatment of cells with FGFs (for examples, see Refs. 7 and 13–15) suggesting that FGF receptors have a distinct set of substrates. Furthermore, porcine aortic endothelial cells transfected with cDNAs encoding either the PDGF receptor or FGFR1 show very different patterns of protein tyrosine phosphorylation in response to PDGF or FGF-2, respectively, although both growth factors generate a mitogenic response (16). Members of the Raf-Ras-MAP kinase pathway have been implicated in FGFR signaling (17–21), based on the use of gain-of-function or dominant-negative constructs. However, since this pathway is activated in cells that show different responses to FGF treatment (22–26), it is unclear whether it plays a role in mediating the
FGF-induced Proliferation Requires p38 MAP Kinase Activity

cell type-specific effects of FGFs. Although it was suggested (27, 28) that the MAP kinase pathway can mediate distinct cellular responses depending on whether the activation of the pathway is transient or sustained, the kinetics of kinase activation with respect to distinct responses to FGF have not been examined.

Two cell lines that show distinct responses to FGF treatment (proliferation versus differentiation) have been used to examine the role of receptor-specific versus cell type-specific signaling pathways in mediating the two different outcomes. The results presented here demonstrate a previously unrecognized role for the p38 MAP kinase pathway in FGF-stimulated cell proliferation.

EXPERIMENTAL PROCEDURES

Materials—PD98059 was obtained from Biomol and solubilized in Me2SO. SB202190, SB203580, and Ros18220 were obtained from Calbiochem and solubilized in Me2SO. U0126 was obtained from Promega and solubilized in Me2SO. Recombinant human FGF-2 was prepared as described (29). PDGF was purchased from R & D Systems.

Cell Culture—Swiss 3T3 cells were obtained from the American Type Culture Collection (Rockville, MD) and grown in Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Inc.) supplemented with 10% fetal calf serum (Hyclone), and antibiotics. To examine the effects of FGF-2 or other growth factors on the Swiss 3T3 cells, they were grown to confluence and then made quiescent by washing with serum-free DMEM, followed by incubation for 2 days in DMEM with 0.5% calf serum. PC12 cells were obtained from D. Schubert (Salk Institute) and grown in Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Inc.) supplemented with 5% horse serum (Hyclone), and antibiotics. To examine the effects of FGF-2 or other growth factors on the PC12 cells, they were grown to confluence and then made quiescent by washing with serum-free DMEM, followed by incubation for 2 days in DMEM with 0.5% calf serum.

PC12 cells were obtained from D. Schubert (Salk Institute) and maintained in DMEM supplemented with 10% fetal calf serum (Hyclone), 5% horse serum (Hyclone), and antibiotics. To examine the effects of FGF-2 on the PC12 cells, the culture medium was removed and replaced by the chemically defined N2 medium (Life Technologies, Inc.). T-47D cells were obtained from the American Type Culture Collection and grown in DMEM supplemented with 10% fetal calf serum and antibiotics.

Stimulation with Growth Factors—Quiescent Swiss 3T3 cells or PC12 cells in N2 medium were treated with the growth factors and other agents as described in the figure legends and, after the indicated time periods, the cells were solubilized in SDS-sample buffer containing 0.1 mM Na3VO4 and 1 mM PMSF, boiled for 5 min, and either analyzed by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose. Transfers were blocked for 2 h at room temperature with 5% nonfat milk in TBS, 0.1% Tween 20 and then incubated overnight at 4°C in the primary antibody diluted in 5% bovine serum albumin in TBS, 0.05% Tween 20. The primary antibodies used were: phosphospecific cAMP-responsive element-binding protein (CREB) antibody (1/1000), phosphospecific ATF-2 antibody (1/1000), phosphospecific p38 MAPK antibody (9211; 1/1000), phosphospecific MAPK antibody (9101, 1/1000) and ATF-2 antibody (1/500) from New England Biolabs; p38 MAP kinase antibody (sc-728, 1/1000) and CREB-1 antibody (sc-186; 1/1000) from Santa Cruz Biotechnology; pan ERK antibody (1/5000) from Transduction Laboratories. The transfers were rinsed with TBS, 0.05% Tween 20 and incubated for 1 h at room temperature in horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse (Bio-rad) diluent-1/5000 in 5% nonfat milk in TBS, 0.1% Tween 20. The immunoblots were developed with the Super Signal reagent (Pierce).

Measurement of the Stimulation of DNA Synthesis—Cells were grown in 96-well dishes (1 × 104 cells/well) for 2 days in DMEM containing 10% calf serum, at which time they were confluent. After washing with serum-free DMEM, they were incubated for another 2 days in DMEM with 0.5% calf serum. The rate of DNA synthesis was measured 24 h after the addition of growth factors and other agents to the cells by the addition of 0.2 μCi/well [methyl-3H]thymidine (6.7 Ci/mmol, ICN), followed by incubation for 5 h. The cultures were then processed for scintillation counting as described (30).

Receptor Cross-linking and Immunoprecipitation—[125I]-FGF-2 was prepared as described (31), using lactoperoxidase. The free iodine was removed over a heparin-Sepharose column. The specific activity was 4–6 × 106 cpm/ng. For cross-linking, cells were incubated with 106 cpm/ml [125I]-FGF-2 in DMEM containing 20 mM HEPES and 0.2% gelatin for 2 h at 4°C with shaking. Cells were rinsed twice with PBS and incubated with 0.15 mM disuccinimidyl suberate (Pierce) in PBS for 15 min at room temperature with shaking. The cross-linking was stopped by the addition of 200 mM ethanolamine and the cells solubilized in Triton X-100 buffer (1% Triton X-100, 50 mM HEPES, pH 7.5, 50 mM NaCl, 5 mM EDTA, 1 mM Na3VO4, 1 mM PMSF). FGFs were immunoprecipitated overnight at 4°C with rabbit antibodies against the C-terminal domains of the four different FGFs (Santa Cruz Biotechnology, sc-121, sc-122, sc-123, and sc-124). The immunoprecipitates were washed twice on protein A-Sepharose, washed twice with 0.1% Triton X-100 in 20 mM HEPES, pH 7.5, 150 mM NaCl; washed once with PBS; and solubilized in SDS-sample buffer. The samples were separated on 7.5% SDS-polyacrylamide gels, and the gels were dried overnight and autoradiographed.

MAPKAP Kinase-2 Assay—Cells were grown in 60-mm dishes was solubilized in 50 mM sodium Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA, 1 mM Na3VO4, 0.1% 2-mercaptoethanol, 5 mM sodium pyrophosphate, 10 mM β-glycerophosphate, 50 mM NaF, 1 mM PMSF, 1 μg/ml aprotinin, and 1 μg/ml leupeptin. MAPKAP kinase-2 in the supernatants was collected with sheep anti-rabbit MAPKAP kinase-2 (2 μg/immunoprecipitate; Upstate Biotechnology, Inc.) preabsorbed to protein G-Sepharose. The immunoprecipitates were washed once with solubilization buffer containing 500 mM NaCl, once with solubilization buffer, and once with kinase assay buffer and resuspended in 30 μl of kinase assay buffer (20 mM MOPS, pH 7.2, 25 mM β-glycerophosphate, 5 mM EGTA, 1 mM Na3VO4, and 1 mM dithiothreitol) containing 25 mM MgCl2, 150 mM ATP, 10 μCi/assay [γ-32P]ATP (ICN), and 62.5 μM MAPKAP kinase-2 substrate peptide (Upstate Biotechnology). Following incubation at 30°C for 10 min the MAPKAP kinase-2 immunoprecipitate was pelleted and the supernatants transferred to P-81 phosphocellulose paper disks. The disks were washed three times with 0.85% phosphoric acid, once with H2O, and counted in a liquid scintillation counter.

Stress-activated Protein Kinase (SAPK)/c-Jun N-Terminal Kinase (JNK) Assay—Cells were treated with FGF-2 (25 ng/ml) for 10 min and solubilized with 50 μl of 1% Triton X-100 in 50 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM Na3VO4, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM PMSF, and 1 μg/ml leupeptin. Preliminary experiments using an antibody to the activated form of SAPK/JNK indicated that this time point represented the peak of the very low level of SAPK/JNK activation induced by FGF-2 in the Swiss 3T3 cells. GST-c-Jun (1–89) bound to glutathione-Sepharose was used to "pull down" total SAPK/JNKs from the FGF-treated cell extracts, and kinase activity was assayed as described (32) in the absence or presence of 5 μM SB202190 or SB203580 except that c-Jun phospho-tyrosine was detected with an antibody specific for the phosphorylated form of the protein (New England Biolabs; 9810; 1/1000) in conjunction with SDS-PAGE and immunoblotting.

RESULTS

To identify the signaling pathways involved in different responses to FGF-2, two cell lines were chosen for study which show distinct responses to FGF-2 treatment. When treated with FGF-2, Swiss 3T3 fibroblasts undergo a mitogenic response that can be monitored by [3H]thymidine incorporation. In contrast, PC12 cells differentiate and this can be monitored visually by the extension of neurites. They do not show a mitogenic response as defined by [3H]thymidine incorporation. To determine if these distinct cellular responses are mediated at the level of the FGF receptor, the receptors present at the surface of each cell type and capable of binding FGF-2 were characterized using [125I]-FGF-2 and chemical cross-linking, followed by immunoprecipitation with antibodies specific to each of the four different FGF receptors. Fig. 1A shows that this approach is capable of detecting all four FGF receptors when they are present on a single cell type, T-47D. Fig. 1B shows the results obtained with Swiss 3T3 cells and PC12 cells and demonstrates that both cell lines express only cell surface FGF-R1.

The two FGF-R1 bands seen in the Swiss 3T3 cells correspond to the two and three Ig-like domains of the receptor (data not shown), whereas the single FGF-R1 band seen in the PC12 cells corresponds to the three Ig-like domain form of the receptor. These results suggest that the distinct cellular responses exhibited by the two cell lines following treatment with FGF-2 are mediated by different intracellular signaling pathways which are activated by the same cell surface FGF receptor.

As an initial approach to identifying the signaling pathways
A was seen in both cell lines. Similar results were obtained with Fig. 2, sustained ERK activation following addition of FGF-2, ERK activation was monitored at various time points of the kinase in conjunction with immunoblotting. As shown in Fig. 3, in vitro, sustained ERK activation was promoted long term activation of ERKs (28). To determine how the activation of p38 MAPK by FGF-2 compares with that of stress-inducing agents, cells were treated with two classical stress-inducing agents and extracts probed with the phosphospecific p38 MAPK antibody. As shown in Fig. 5, the level of p38 MAPK activation by FGF-2 in Swiss 3T3 cells was very similar to that seen with either anisomycin or sorbitol, indicating that, in Swiss 3T3 cells, FGF-2 is a strong activator of this MAPK family member.

To further compare the induction of p38 MAPK activity by FGF-2 in Swiss 3T3 cells with that in PC12 cells, the activation of MAPKAP kinase-2 by FGF-2 was examined. MAPKAP kinase-2 is a specific and direct substrate of p38 MAP kinase (45, 46). Furthermore, by using an in vitro kinase activity assay on partially purified ERKs (42) (data not shown).

To determine if ERK activity was necessary for either FGF-2 stimulated differentiation or proliferation, the specific MEK1 inhibitor, PD98059, was used (43). Treatment of PC12 cells with PD98059 brought about a significant reduction in FGF-2-induced ERK activation, which decreased to control levels at 100 nM PD98059 (Fig. 3A). This same concentration of PD98059 inhibited PC12 cell differentiation by ~90% (Fig. 3B), suggesting that ERK activation is required for the induction of differentiation by FGF-2. In contrast, the same concentrations of PD98059 had only a slight effect on ERK activation in Swiss 3T3 cells (Fig. 3A). Recently, a second MEK1 inhibitor (U0126), which has a 100-fold higher affinity for MEK1 than does PD98059, was described (44). In contrast to PD98059, U0126 effectively blocked ERK activation in Swiss 3T3 cells treated with FGF-2, PDGF, or 10% serum (Fig. 4). Although 10 μM U0126 reduced PDGF-stimulated Swiss 3T3 proliferation by ~80%, this same concentration of inhibitor had little or no effect on cell proliferation induced by either FGF-2 or serum (Fig. 3C). These results suggest that ERK activation is not required for the induction of fibroblast proliferation by FGF-2.

Since p38 MAPK was found to be activated by FGF-2 in several different cell types (45, 46), its activation by FGF-2 in both the PC12 cells and Swiss 3T3 cells was tested using an antibody specific for the dual phosphorylated, and thereby active, form of the kinase. In contrast to the results with ERKs, quite different time courses of p38 MAPK activation were seen in PC12 cells as compared with Swiss 3T3 cells (Fig. 5). Whereas in the FGF-2-treated PC12 cells, only a brief induction of p38 MAPK activation relative to control levels was observed, in Swiss 3T3 cells, FGF-2 induced a much more prolonged activation of the same enzyme (Fig. 5). As noted above, p38 MAPK activation has been associated primarily with the cellular response to stress rather than proliferation.

As mentioned above, ERKs are implicated in the promotion of both cell proliferation and differentiation. It has been suggested that the specific cellular response to ERK activation is dependent upon whether that activation is transient or sustained (27). Many of the observations supporting this hypothesis derive from studies with PC12 cells where epidermal growth factor, which failed to promote differentiation, only activated ERKs for a brief time period whereas growth factors such as nerve growth factor and FGF-2, which induce differentiation, promoted long term activation of ERKs (28). To determine if similar differences in the time course of ERK activation are seen between Swiss 3T3 cells and PC12 cells treated with FGF-2, ERK activation was monitored at various time points up to 8 h after stimulation with FGF-2 using an antibody specific for the dual phosphorylated, and therefore active, form of the kinase in conjunction with immunoblotting. As shown in Fig. 2, sustained ERK activation following addition of FGF-2 was seen in both cell lines. Similar results were obtained with an immunoprecipitation/in vitro kinase activity assay and with

![Fig. 2. Time course of ERK activation in response to FGF-2.](image)
MAPKAP kinase-2 activity as a measure of p38 MAP kinase activity, it is possible to assess the effects of several specific, reversible p38 MAP kinase inhibitors on the activity of p38 MAP kinase in FGF-2-treated cells. As shown in Fig. 7, FGF-2 induced a 20–30-fold activation of MAPKAP kinase-2 activity in Swiss 3T3 cells, whereas the same concentration of FGF-2 only brought about a 2–3-fold increase in MAPKAP kinase-2 activity in PC12 cells. These results are consistent with the data on p38 MAP kinase activation obtained using the antibody to the phosphorylated form of the enzyme (Fig. 5). Fig. 7 also shows that low concentrations of the specific p38 MAP kinase inhibitors, SB202190 and SB203580, reduced MAPKAP kinase-2 activity to near control levels in FGF-2-treated cells, indicating that these inhibitors can be used to assess the role of p38 MAP kinase in FGF-induced proliferation and differentiation. These inhibitors are highly specific for p38 kinase both in vitro and in vivo and, even at concentrations as high as 100 μM,
were found to have no effect on the activity of many other protein kinases, including other MAP kinase family members (35, 39, 47, 48). Although inhibition of SAPK/JNK activity by SB203580 at concentrations above 1 μM was reported recently (32, 49), JNK2 isoforms were much more susceptible to inhibition than JNK1 isoforms. No inhibition of SAPK/JNK activity was observed in the Swiss 3T3 cells treated with FGF-2 in the presence of either SB202190 or SB203580 (Fig. 6B). In addition, neither inhibitor had any effect on ERK activation (data not shown).

To assess the role of p38 MAPK in the cellular response to FGF-2, the effects of the two p38 MAPK inhibitors described above on both Swiss 3T3 proliferation and PC12 cell differentiation were tested. Both inhibitors were very effective at blocking Swiss 3T3 cell proliferation, as measured by [3H]thymidine incorporation, with maximal inhibition occurring at 5 μM (Fig. 8A). These results correlate well with the results for the inhibition of MAPKAP kinase-2 activation by the two compounds (Fig. 7). In contrast, 5 μM SB202190 or SB203580 had very little effect on cell proliferation induced by the addition of 10% serum to quiescent cells (Fig. 8A). Neither p38 MAPK inhibitor had any effect on PC12 differentiation induced by FGF-2 (Fig. 8B).

The requirement for p38 MAP kinase activity for FGF-2-stimulated cell proliferation suggests that one or more downstream substrates of this kinase are essential for the proliferative response. As mentioned above, one of these substrates is MAPKAP kinase-2, which is activated by FGF-2 and whose activation is blocked by the p38 MAP kinase inhibitors. MAPKAP kinase-2 phosphorylates HSP27, which, in turn, can modulate actin filament dynamics (46). However, how this effect on microfilaments could contribute to the proliferative response remains to be determined. p38 MAPK also activates a number of other proteins, including several kinases and transcription factors (45, 50–56). To determine whether any of these latter
The absence or presence of 5 T3 cells were untreated or treated with 25 ng/ml FGF-2 for 5 min in the absence or presence of 5 μM SB202190, 10 μM SB203580, 100 μM PD98059, or a combination of the drugs as indicated in the figure. Cell extracts were prepared and equal amounts of protein analyzed by SDS-PAGE and immunoblotting with antibodies specific for phosphorylated CREB and total CREB or phosphorylated ATF-2 and total ATF-2. Data from a single experiment are shown. Similar results were obtained in three independent experiments.

Fig. 9. Analysis of p38 MAPK substrate activation. Quiescent 3T3 cells were untreated or treated with 25 ng/ml FGF-2 for 5 min in the absence or presence of 5 μM SB202190, 10 μM SB203580, 100 μM PD98059, or a combination of the drugs as indicated in the figure. Cell extracts were prepared and equal amounts of protein analyzed by SDS-PAGE and immunoblotting with antibodies specific for phosphorylated CREB and total CREB or phosphorylated ATF-2 and total ATF-2. Data from a single experiment are shown. Similar results were obtained in three independent experiments.

substrates were activated by FGF-2 in a p38 MAPK-dependent manner in the Swiss 3T3 cells, a combination of inhibitors and phosphospecific antibodies was used. As shown in Fig. 9, FGF-2 stimulated the phosphorylation of CREB, ATF-1, and ATF-2 at sites essential for the activation of transcriptional activity. However, the phosphorylation of none of these transcription factors was inhibited by the p38 MAPK inhibitors (Fig. 9) alone. The phosphorylation of both CREB and ATF-1 was, however, inhibited by a combination of the p38 MAP kinase inhibitors and the MEK1 inhibitor, PD98059 (Fig. 9). Similar results were reported for nerve growth factor activation of CREB in PC12 cells (55, 57) and FGF-2 activation of CREB in SK-N-MC cells (55). In contrast, the phosphorylation of ATF-2 was only slightly inhibited by the combination of inhibitors (Fig. 9). However, since the p38 MAPK inhibitors alone have no effect on the activation of CREB, ATF-1 or ATF-2, but do block FGF-stimulated cell proliferation, it is unlikely that the activation of any of these transcription factors is required for the proliferative response to FGF-2.

The finding that a combination of inhibitors was required to inhibit CREB phosphorylation suggested that CREB phosphorylation in response to FGF-2 treatment of Swiss 3T3 cells may result from the activation of mitogen and stress-activated protein kinase-1 (MSK-1), a novel protein kinase recently shown to be activated by both ERKs and p38 kinase in a variety of different cell lines (55). To test this idea, Ro318220, a protein kinase inhibitor that can inhibit the activity of MSK-1 (55), was used. Treatment of cells with Ro318220 blocked the phosphorylation of CREB by FGF-2 (Fig. 10), as reported previously (55), but had little or no effect on the phosphorylation of ATF-2 (Fig. 10). These data indicate that, while FGF-induced phosphorylation of CREB is likely to be mediated by MSK-1, the phosphorylation of ATF-2 must be mediated by other, as yet undefined, kinases.

**Discussion**

The mechanisms whereby a single growth factor can induce very distinct responses in different types of cells is still not clear. While it is possible that growth factors which interact with multiple members of a receptor family can have distinct effects on cells depending on the specific receptor family member expressed by a given type of cell, it is likely that there are other mechanisms regulating cell type-specific responses. The results presented here demonstrate that, in the case of FGF-2, cell type-specific signaling pathways can mediate the distinct cellular responses to this growth factor. Although Swiss 3T3 cells and PC12 cells show very different responses to treatment with FGF-2, both cell lines express only FGFR1 on their cell surfaces. An earlier study found that a different clone of PC12 cells expressed FGFR3 and FGFR4 along with FGFR1 but that FGFR1 was the only receptor required for FGF-2 to induce differentiation (58), consistent with the data presented here. The failure to detect FGFR3 and FGFR4 in the PC12 cells used in the present study is not due to an inability to detect these receptors by the method used to analyze receptor expression (Fig. 1A). Thus, the absence of FGFR3 and FGFR4 from the PC12 cells used in the present study is probably due to variations among the different clones of this cell line.

Since members of the MAPK family have been implicated in both cell proliferation and differentiation, it was likely that these kinases were also involved in FGF-2 signaling. Indeed, a number of studies suggest that MAP kinases play a role in one or more cellular responses to FGF-2 (20, 24, 25, 59–61). Furthermore, one hypothesis as to how growth factors induce neuronal differentiation is based on the idea that prolonged ERK activation is required (28). However, the kinetics of FGF-2-stimulated ERK activation in PC12 cells and Swiss 3T3 cells are indistinguishable (Fig. 2), suggesting that other signaling pathways are necessary for the distinct cellular responses to this growth factor. Nevertheless, ERK activity does appear to be required for FGF-2-induced differentiation since an inhibitor of ERK activation blocks neurite outgrowth by 90%. This result is consistent with previous studies on FGF-2 signaling in PC12 cells (60, 62), but not with a recent study on FGF-2 signaling in a conditionally immortalized rat hippocampal cell line (63). However, this difference may be a reflection of the fact that these two cell lines are models for different types of neurons, which, therefore, may show distinct, cell type-specific responses to FGF-2 treatment.

In contrast, a role for ERK activity in FGF-2-induced cell proliferation could not be demonstrated. Surprisingly, another member of the MAP kinase family, p38 MAPK, was found to be specifically required for the proliferative response to FGF-2. p38 MAPK was strongly activated by FGF-2 in Swiss 3T3 cells for an extended time period, whereas only a weak activation for a much shorter time period was observed in the PC12 cells. Furthermore, two specific inhibitors of p38 MAPK blocked FGF-2-induced cell proliferation at concentrations that had no effect on either serum-stimulated proliferation or FGF-2-induced neurite outgrowth in PC12 cells. Although several previous studies reported activation of p38 MAPK following treatment with FGF-2 (45, 46), this activation was not associated with a specific cellular response. Thus, the studies described here are the first demonstration that this member of the MAPK family plays an important role in FGF-induced cell proliferation.

p38 MAPK was first identified several years ago in lipopolysaccharide-stimulated mouse macrophages (64) and was shown to be the target of a series of anti-inflammatory pyridi-
FGF-induced Proliferation Requires p38 MAP Kinase Activity

N. van der Hoeven and L. van der Hoeven

In this study, the authors investigate the role of p38 MAP kinase in the proliferative response to FGF-2 in PC12 cells. They report that p38 MAPK activation is required for FGF-induced cell proliferation and that inhibition of p38 MAPK activity using specific inhibitors leads to a decrease in cell proliferation. This work provides evidence for a critical role of p38 MAPK in the proliferative response to FGF-2 in PC12 cells.

**References**

1. Baird, A., and Bohlen, P. (1990) in *Peptide Growth Factors and their Receptors* (Sperl, M. B. and Roberts, A. B., eds) pp. 369–418, Springer-Verlag, Berlin.

2. Burgess, W. H., and Maciag, T. (1989) *FASEB J.* 3, 1651–1656.

3. Kyriakis, J. M., and Avruch, J. (1996) *Curr. Opin. Cell Biol.* 8, 353–361.

4. Schlessinger, J., and Ullrich, A. (1993) *Science* 260, 1195–1202.

5. Han, J., Jiang, Y., Li, A., Kravchenko, V., and Ulevitch, R. (1997) *EMBO J.* 16, 3372–3384.

6. Hou, J., Kan, M., McKeehan, K., McBride, G., Adams, P., and McKeehan, W. L. (1997) *Mol. Cell. Biol.* 17, 1921–1933.

7. Waskiewicz, A., Flynn, A., Proud, C., and Cooper, J. (1997) *EMBO J.* 16, 1909–1920.

8. Han, J., Jiang, Y., Li, A., Kravchenko, V., and Ulevitch, R. (1997) *Science* 273, 1651–1656.

9. Hou, J., Kan, M., McKeehan, K., McBride, G., Adams, P., and McKeehan, W. L. (1997) *Mol. Cell. Biol.* 17, 1195–1202.

10. Johnson, D. E., Chen, H., Werner, S., and Williams, L. T. (1991) *Adv. Cancer Res.* 60, 1–41.

11. Eisenman, A., Ahi, J. A., Graziani, G., Tronick, S. R., and Ron, D. (1991) *Oncogene* 6, 1195–1202.
Lax, I., and Schlessinger, J. (1997) *Cell* **89**, 693–702
62. Hadar, Y., Kouhara, H., Lax, I., and Schlessinger, J. (1998) *Mol. Cell. Biol.* **18**, 3966–3973
63. Kao, W., Abe, M., Rhee, J., Eves, E., McCarthy, S., Yan, M., Templeton, D., McMahon, M., and Rosner, M. (1996) *Mol. Cell. Biol.* **16**, 1458–1470
64. Han, J., Lee, J.-D., Bibbs, L., and Ulevitch, R. J. (1994) *Science* **265**, 808–811
65. Wang, X., Diener, K., Manthey, C., Wang, S., Rosenzweig, B., Bray, J., Delaney, J., Cole, C., Chan-Hui, P., Mantlo, N., Lichenstein, H., Zukowski, M., and Yao, Z. (1997) *J. Biol. Chem.* **272**, 23668–23674
66. Enslen, H., Raingeaud, J., and Davis, R. (1998) *J. Biol. Chem.* **273**, 1741–1748
67. Morley, S. J., and McKendrick, L. (1997) *J. Biol. Chem.* **272**, 17887–17893
68. Morley, S. J. (1996) in *Protein Phosphorylation in Cell Growth Regulation* (Clemens, M. J., ed) pp. 197–224, Harwood Academic Publishers, Amsterdam
69. Wiedlocha, A., Paines, P., Madshus, I., Sandvig, K., and Olsnes, S. (1994) *Cell* **76**, 1039–1051