Regulation of Stat3 Activation by MEK Kinase 1*

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Stat3 is a latent transcription factor activated by various cytokines and growth factors. Phosphorylation on Tyr-705 is a prerequisite for dimer formation, nuclear translocation, binding to its cognate DNA sequences, and regulation of the target gene transcription. Ser-727 phosphorylation of Stat3 plays an additional role in the regulation of transcription. MEK kinase 1 (MEKK1) is a mitogen-activated protein kinase (MAPK) kinase (MAPKKK) that activates the c-Jun NH2-terminal kinase signaling pathway. Here we report that MEKK1 is involved in the regulation of Stat3 activation by growth factors. Kinase-inactive MEKK1 inhibits Stat3 phosphorylation on tyrosine and serine, and its transactivation activity stimulated by epidermal growth factor and platelet-derived growth factor in different cell types. In contrast, active MEKK1 induces Stat3 tyrosine and serine phosphorylation leading to a functionally active Stat3 capable of binding DNA and enhancing transcription. Ser-727 is phosphorylated by MEKK1 in vitro, whereas Tyr-705 phosphorylation induced by MEKK1 involves Src and Janus kinases in vivo. These data demonstrate for the first time a novel role of MEKK1 to modulate tyrosine kinases that results in the activation of specific members of STAT family.

STAT1 family was first identified in the regulation of interferon-inducible gene transcription (1). These transcription factors were named Signal Transducers and Activators of Transcription, by virtue of their novel and unique dual functions as signaling molecules in the cytoplasm and as transcription factors following nuclear translocation. Concurrent to the expression of STAT family was the discovery of a group of tyrosine kinases called Janus kinases (JAKs) in interferon and cytokine signaling (2, 3). Upon cytokine stimulation, these receptor-associated kinases transphosphorylate themselves and the receptors, creating recruitment sites for binding proteins containing the Src homology 2 (SH2) domains, such as STATs. The STATs are subsequently phosphorylated by JAKs on a single tyrosine site at the COOH terminus, form homo- or heterodimers via the reciprocal interactions between SH2 domain and the phosphorylated tyrosine, and translocate into the nucleus where they bind to DNA and regulate the transcription of target genes (reviewed in Refs. 4–6).

There are seven known mammalian STAT proteins, denoted by Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, and Stat6, that are activated in various cytokine signaling. Stat3 was identified as an acute-phase response factor activated by interleukin-6 (IL-6) in mouse liver and by homology to Stat1 (7, 8). In addition to cytokines, Stat3 is also strongly activated by growth factors, such as EGF, platelet-derived growth factor (PDGF), and colony-stimulating factor-1 (8–11). Although the mechanisms of Stat3 activation by growth factors are less defined compared with cytokines, the intrinsic tyrosine kinase activity of the EGF receptor (EGF-R) and the nonreceptor tyrosine kinases, Src and JAKs, are implicated in its tyrosine phosphorylation and activation (12–15). IL-6 and EGF also induce serine phosphorylation of Stat3 on Ser-727, and this event appears to play an additional role in the regulation of Stat3-targeted gene transcription, contributing to its maximum transcriptional activity (16, 17). The MAPK family is composed of extracellular signal-regulated kinase (ERK), induced by growth factors and cytokines, JNK/stress-activated protein kinase (SAPK), and p38/HOG1 (p38), both activated by pro-inflammatory cytokines and environmental stresses (reviewed in Refs. 18 and 19). Phosphorylation at Ser-727 of Stat3 by all three MAPK family members has been reported in response to different extracellular stimuli (17, 20–24), suggesting a cross-talk between MAPK cascades and JAK-STAT pathways.

MEKK1 is a mammalian Ser/Thr protein kinase initially identified on the basis of its homology with STE11 and Byr2, the MAPKKKs that activate the phoromone-responsive MAPK cascade in yeast (25). The full-length 196-kDa murine MEKK1 is a target for proteolytic cleavage by caspases. Cleavage of MEKK1 at Asp-874 releases the 91-kDa fragment containing the COOH-terminal catalytic domain, which renders this portion constitutively active (26, 27). Expression of the constitutively active forms of MEKK1 that contain either the catalytic domain or the COOH-terminal fragment lead to the activation of JNK via phosphorylation of its upstream kinase, JNKK1/MKK4 (28). MEKK1 also has the ability to activate ERK, but the effect is less potent (29). These data suggest MEKK1 to be an upstream kinase in the MAPK cascade. In addition, MEKK1 is found to participate in the activation of the transcription factor NF-kB, which is activated by various extracellular stimuli, including inflammatory cytokines and stresses (reviewed in Ref. 30). A key step in the activation of NF-kB is the phosphorylation of its inhibitor, IκB, by IκB kinases (IKKs) (31). Transient transfection of the active MEKK1 phosphorylates and stimulates the kinase activity of IKKα and IKKβ, which results in the phosphorylation of IκB and the release of NF-kB to translocate
into the nucleus (32). This evidence suggests that the function of MEKK1 is not restricted to the MAPK cascade.

EGF stimulates activation of Stat3 by phosphorylation on tyrosine and serine. Since MEKK1 is an upstream kinase of MAPKs that are involved in serine phosphorylation of Stat3, and MEKK1 itself is also activated by EGF in various cell type (33, 34), we investigate a possible role of MEKK1 on Stat3 phosphorylation and activation. To our surprise, the dominant negative MEKK1 inhibits phosphorylation of Stat3 not only on serine but also on tyrosine induced either by EGF in COS-1 and HeLa cells or by PDGF in NIH3T3 cells. On the other hand, a constitutively activated MEKK1 induces both serine and tyrosine phosphorylation of Stat3, which is functionally active. We further showed that MEKK1 phosphorylates Stat3 Ser-727 directly in vitro and induces Stat3 tyrosine phosphorylation via Src and Jaks in vivo. We suggest that MEKK1 is involved in Stat3 activation by growth factors via modulating the Jak and Src activities and enhancing serine phosphorylation either di-

**FIG. 1.** MEKK1 is required for the EGF- and PDGF-induced Stat3 phosphorylation. A, COS-1 cells were transfected with the expression plasmid of Stat3 in the absence or presence of 5 μg of MEKK1-C (KM) expression plasmid. Cells were either left uninduced (U) or stimulated with 100 ng/ml EGF for 15 min. An aliquot of total cell lysates (30 μg) was resolved on a 10% SDS-PAGE, transferred to a PVDF membrane, followed by Western blot analyses with anti-phospho-Tyr-705 Stat3 (pY705 Stat3), anti-phospho-Ser-727 Stat3 antibody (pS727 Stat3), anti-Stat3, or anti-MEKK1 (C-22) antibodies. B, HeLa cells were transfected with vector alone or increasing amounts of MEKK1-C (KM) expression plasmid (5 μg for lane 3 or 10 μg for lane 4), and cells were either left uninduced (U) or stimulated with 100 ng/ml EGF for 5 min. Endogenous Stat3 protein was immunoprecipitated (IP) from total cell lysates with anti-Stat3 (C-20) antibody, resolved on a 10% SDS-PAGE, and analyzed by Western immunoblotting with anti-phosphotyrosine (4G10) antibody (BLOT: pTyr). The blot was stripped and re-blotted with anti-phosphoserine (BLOT: pSer), and subsequently with anti-Stat3 antibody (BLOT: Stat3). An aliquot of total cell lysates (30 μg) was also resolved and blotted with anti-MEKK1 (C-22) antibody to analyze expression of MEKK1-C (KM) protein (TC BLOT: MEKK1). C, NIH3T3 cells were transfected with vector alone (2) or 5 μg MEKK1-C (KM) (1) expression plasmid, and cells were either left uninduced (U) or stimulated with 50 ng/ml PDGF for 5 min. Immunoprecipitation and analyses of the endogenous Stat3 protein and analysis of MEKK1-C (KM) expression were performed as described in B.
Activation of Stat3 by MEKK1

FIG. 2. MEKK1-C (KM) inhibits EGF-induced Stat3 transcriptional activity. COS-1 cells were transfected with an empty vector (vec) or expression plasmids encoding Stat3 (St3), either alone or together with 5 or 10 μg of MEKK1-C (KM), in the presence of the reporter plasmid pSIE-CAT and pCMV-β-gal. Cells were either left uninduced (lanes 1–4) or stimulated with 100 ng/ml EGF for 6 h (lanes 5–8). CAT assays were normalized with equivalent β-galactosidase activity and performed as described under “Experimental Procedures.” Acetylated and non-acetylated forms of [14C]chloramphenicol were separated by thin layer chromatography, followed by autoradiography. A representative autoradiograph of three independent experiments is shown in the upper panel. The CAT activities were quantified using a Bio-Rad GS700 imaging densitometer, and the average fold of induction is illustrated in the graph in the lower panel and indicated on top of the error bars. Error bars represent the standard error of the means.

EXPERIMENTAL PROCEDURES

Construction of Plasmids—The expression plasmid of Stat3, pRc/CMV-Stat3 (8), was a gift from Dr. J. E. Darnell (The Rockefeller University). pRc/CMV-Stat3 mutants were cloned by substitutions of the phosphorylation site Tyr-705 to Phe (named Y1) or Ser-727 to Ala (named S1), performed with the QuikChange™ site-directed mutagenesis kit (Stratagene). The bacterially expressed glutathione S-transferase (GST)-Stat3 fusion protein and the point mutant GST-S1, in which Ser-727 of GST-Stat3 was replaced by Ala, were constructed as described previously (12, 22). The full-length Stat3 was also cloned in vector pXJ40-GST or pXJ40-GST-Stat3 and the expression plasmids of wild-type MEKK1-C (KM), a kinase-inactive mutant containing the catalytic domain of murine MEKK1 with a point mutation was expressed in mammalian cells. The constitutively active MEKK1-C (34) was provided by Dr. R. Janknecht (Mayo Foundation). The point mutant MEKK1-C (KM) was constructed by replacing Lys-1253 to Met (27) and was confirmed by sequencing. The expression plasmids of wild-type Src (pSGTsrcK) and the kinase-inactive Src (pSGTsrcK-) (35) were gifts from Dr. S. Courtneidge (Sugen Inc., Redwood City, CA). Wild-type Jak2 (Jak2) and the kinase-inactive Jak2 (Jak2K) (36) were obtained from Dr. O. Silvennoinen (University of Helsinki, Finland). The reporter plasmid pSIE-CAT for CAT assays was prepared by inserting three copies of the high affinity sis-inducible element (hSIE) sequence (TTCCTCCTAA) upstream of a c-fos minimal promoter followed by the CAT gene in plasmid pFOSCATΔ56 as described (22).

Antibodies, Growth Factors, and Inhibitors—Anti-phospho-Tyr-705 Stat3, anti-phospho-Ser-727 Stat3, and anti-phospho-Tyr-701 Stat3 were purchased from New England Biolabs. Monoclonal anti-Stat3, anti-EGF-R (activated), and anti-EGF-R antibodies were obtained from Transduction Laboratories. Polyclonal anti-Stat3 (C-20), anti-Stat1 (C-24), anti-MEKK1 (C-22), and anti-Jak2 (C-20 and HR-758) antibodies were purchased from Santa Cruz Biotechnology. Recombinant mouse MEKK1 enzyme, anti-phosphotyrosine (4G10), and anti-phospho-Ser-727 Stat3 were purchased from Upstate Biotechnology, Inc. Anti-phospho-Ser-707 Stat1 monoclonal antibody was obtained from Sigma, and anti-v-Src (Ab-1) antibody was acquired from Oncogene Science, Inc. EGF was purchased from Genzyme and Upstate Biotechnology, Inc., and PDGF was from Genzyme. EGF-R inhibitor, AG1478, was obtained from Calbiochem.

Immunoprecipitation/Western Blotting and Immune Complex Protein Kinase Assay—Lysis of cells, immunoprecipitation, Western blotting, and in vitro kinase assays were performed as described previously (23). The MEKK1-C was immunoprecipitated by an MEKK1 (C-22) antibody, and MEKK1 kinase assay was performed in a buffer as described by Xu et al. (37) with minor modifications. Briefly, the buffer contains 10 mM Hepes (pH 7.3), 10 mM MgCl2, 1 mM benzamidine, 1 mM dithiothreitol, 20 μM cold ATP, and 10 μM of [γ-32P]ATP, and MEKK1 phosphorylation was assayed using GST-Stat3 fusion proteins as substrates. For cold in vitro kinase assay coupled with Western immunoblotting, a similar procedure as described above was performed except by incorporating 100 μM cold ATP instead of [γ-32P]ATP in the assay. GST-Stat3 resolved in an SDS-PAGE and transferred to a PVDF membrane was subjected to Western immunoblotting with an anti-phosphotyrosine (4G10) and anti-Stat3 (C-20) antibodies. For kinase assay using recombinant MEKK1 and GST or GST-Stat3 as substrates, cells were transfected with pXJ40-GST or pXJ40-GST-Stat3, and the expressed GST and GST-Stat3 were immobilized onto glutathione-Sepharose beads overnight at 4 °C, washed twice with RIPA, and twice with phosphate-buffered saline. Kinase reaction was performed in the absence or presence of 0.2 μg of recombinant MEKK1 at 30 °C for 15 min.

RESULTS

Dominant Negative MEKK1 Inhibits Stat3 Phosphorylation and Transcriptional Activity Induced by Growth Factors—Stat3 is phosphorylated on Tyr-705 and Ser-727 upon stimulation by growth factors such as EGF and PDGF. We and others (17, 20, 22, 23) have shown that JNK and ERK can phosphorylate Stat3 on serine. Since MEKK1 is an upstream Ser/Thr kinase of JNK and ERK and is also activated by EGF in various cell types (33, 34), we investigated whether MEKK1 plays a role in Stat3 phosphorylation stimulated by growth factors.

MEKK1-C (KM), a kinase-inactive mutant containing the catalytic domain of murine MEKK1 with a point mutation was cotransfected with Stat3 into the COS-1 cells, which express very little endogenous Stat3. The phosphorylation of Stat3 in response to EGF stimulation was examined in Western blot analysis using antibodies that specifically recognize the phosphorylated forms of Stat3. As shown in Fig. 1A (lane 2, upper and 2nd panels), EGF induced both tyrosine and serine phosphorylation of Stat3. Surprisingly, the tyrosine phosphorylation of Stat3 was significantly inhibited by dominant negative mutant MEKK1-C (KM), but not by its wild-type counterpart, MEKK1-C, which contains the catalytic domain and is constitutively active (lanes 3 and 4, upper panel). The EGF-induced Ser-727 phosphorylation of Stat3 was also reduced (lanes 3 and 4, 2nd panel), whereas the expression level of Stat3 remains constant (3rd panel). To confirm this finding, we further tested the effect of MEKK1-C (KM) on endogenous Stat3 in other cell types. We found that the tyrosine phosphorylation of the endogenous Stat3 stimulated by EGF was inhibited in HeLa cells (Fig. 1B, upper panel). Serine phosphorylation of Stat3 in this cell line was also diminished (Fig. 1B, 2nd panel), although a high basal level of serine phosphorylation was observed compared with COS-1 cells. Furthermore, PDGF induces tyrosine (10) and serine phospho-
Cells were harvested 40 h after transfection. An aliquot of total cell lysates (10 μg) or presence of MEKK1-C expression plasmid (Stat3 in the absence of MEKK1-C, (KM)). MCF-7 cells were transfected with or without MEKK1-C. Total cell lysates were resolved and analyzed by Western immunoblotting using an anti-phospho-Tyr-701 Stat1 antibody (pY705 Stat1) or subsequently with anti-phospho-Ser-727 Stat3 antibody (pS727 Stat3) to analyze the ability of Stat3 phosphorylation by MEKK1-C. The blots were finally stripped and re-blotted with anti-MEKK1 (left panel, lane 4). In contrast, phosphorylation of Stat3 was further investigated by measuring its DNA binding and activation stimulated by growth factors.

**Active MEKK1 Induces Phosphorylation and Activation of Stat3**—To study further the role of MEKK1 on Stat3, we tested whether MEKK1 phosphorylates Stat3. The constitutively active murine MEKK1-C and Stat3 were cotransfected in COS-1 cells, and the phosphorylation of Stat3 was examined. Both Tyr-705 and Ser-727 of Stat3 were strongly phosphorylated by MEKK1-C in COS-1 cells (Fig. 3A, left panels). Furthermore, MEKK1-C also induced tyrosine and serine phosphorylation of the endogenous Stat3 in MCF-7, a human breast cancer cell line (Fig. 3A, right panels). In contrast, phosphorylation of transfected Stat1 on Tyr-701 in COS-1 cells was not induced by MEKK1-C or its kinase-inactive mutant, MEKK1-C (KM), although its Ser-727 phosphorylation can be induced by MEKK1-C (Fig. 3B). As a control, EGF treatment of the cells induced both Tyr-701 and Ser-727 phosphorylation of Stat1.

To demonstrate further the specificity of Stat3 phosphorylation by MEKK1, varying amounts of MEKK1-C were cotransfected with Stat3 in COS-1 cells. Stat3 Tyr-705 and Ser-727 phosphorylations can be detected with as little as 0.5 μg of the transfected MEKK1-C plasmid, and the phosphorylations were dose-dependent (Fig. 3C). Moreover, similar phosphorylations were also observed by a human MEKK1-C (not shown). These results indicate that active MEKK1 specifically induces both Ser-727 and Tyr-705 phosphorylation of Stat3.

**MEKK1-C Stimulates the DNA Binding and Transcriptional Activities of Stat3**—The functionality of the MEKK1-C-phosphorylated Stat3 was further investigated by measuring its DNA binding and transcriptional activities. The DNA binding activity was examined using EMSA with a [32P]-labeled oligonucleotide probe hSIE (Fig. 4A). The Stat3-DNA complex (SIF-A) was observed when COS-1 cells were cotransfected with Stat3 and MEKK1-C (left panel, lane 4), which could be competed out by excess unlabeled wild-type hSIE (lane 5) but not by mutant hSIE oligonucleotides M2 and M1 (lanes 6 and 7). SIF-A was confirmed by the supershift with a specific antibody against Stat3 (lane 8) but not Stat1 (lane 9).

The transcriptional activity of Stat3 was tested in the absence or presence of MEKK1-C. The results showed that MEKK1-C increased the transcriptional activity of Stat3 to 4.1-fold, compared with 6.5-fold induced by EGF (Fig. 4B, left panel).
To investigate the contribution of the Tyr-705 and Ser-727 phosphorylation to Stat3 activities induced by MEKK1-C, the mutant Stat3 genes bearing either a single mutation on Ser-727 to Ala (S1) or Tyr-705 to Phe (Y1) were transfected into COS-1 cells. The EMSA and CAT assays results (Fig. 4, A and B, right panels) showed that mutation on Tyr-705 completely abrogated the DNA binding and transcriptional activities of Stat3, whereas mutation on Ser-727 showed a strong DNA binding but reduced transcriptional activity. These results demonstrate that in accordance with growth factor and cytokine stimulation, the MEKK1-C-induced Stat3 phosphorylation results in a functionally active Stat3, capable of binding to its cognate regulatory sequence and activating transcription. Phosphorylation of Tyr-705 is absolutely required for Stat3 activities, whereas Ser-727 phosphorylation plays a dual role, by positively regulating transcriptional activity but negatively affecting its DNA binding activity as reported previously (17, 20, 23).

**Phosphorylation of Stat3 by MEKK1-C in Vitro—** MEKK1 is a Ser/Thr kinase. To investigate the possible mechanism of Stat3 phosphorylation induced by MEKK1, we tested whether Stat3 itself is a direct substrate of MEKK1 by *in vitro* kinase assay. MEKK1-C or the kinase-inactive MEKK1-C (KM) (Fig. 5A), indicating that GST-STAT3 is phosphorylated by MEKK1-C, but not by MEKK1-C (KM) (Fig. 5A), indicating that GST-STAT3 is phosphorylated by MEKK1-C, but not likely by any kinase(s) that may have associated with MEKK1-C. Immunoprecipitated MEKK1-C used in the kinase assay was shown by Western blot analysis (lower panel), and equal loading of GST-Stat3 fusion proteins was shown by staining of the blot with Amido Black (middle panel). Furthermore, a recombinant mouse MEKK1 enzyme phosphorylated GST-Stat3 fusion protein expressed in COS-1 cells but not the GST protein alone (Fig. 5B, left panel).

To examine the Tyr-705 phosphorylation of Stat3 by MEKK1-C, a similar *in vitro* kinase assay was performed in the absence of [γ-32P]ATP in the reaction. Instead, it contained 100 μM cold ATP, and the reaction mixtures were subjected to Western blot analysis using the anti-phosphotyrosine 705 Stat3 antibody. The results revealed that whereas the positive control, Src, immunoprecipitated from the Src-transfected cells, was able to phosphorylate GST-STAT3 on tyrosine, MEKK1-C failed to do so (Fig. 5D). These findings support the hypothesis that MEKK1, a Ser/Thr kinase, is capable of phosphorylating Stat3 on Ser-727 directly but on Tyr-705 but by an indirect mechanism.

**Induction of Tyr-705 Phosphorylation of Stat3 by MEKK1-C via Src and Jak2 but Not EGF-R Tyrosine Kinases—** The above results led us to investigate the possible tyrosine kinase(s) that may be involved in the MEKK1-C-induced Stat3 activation. Jak2, Src, and EGF-R tyrosine kinases known to phosphorylate Stat3 were examined using the kinase-inactive Jak2 and Src or a specific inhibitor of EGF-R, AG1478. As shown in Fig. 6A, the tyrosine phosphorylation of Stat3 was induced by the wild-type Jak2 and Src in the absence of MEKK1-C and further enhanced in the presence of MEKK1-C. However, the MEKK1-C-induced Tyr-705 phosphorylation was inhibited by the kinase-inactive Jak2 or Src. Expressions of Stat3, MEKK1-C, Jak2, and Src are
shown in lower panels. These results suggest that Jak2 and Src are required for the Stat3 Tyr-705 phosphorylation by MEKK1-C.

To test the role of EGF-R on the tyrosine phosphorylation of Stat3 by MEKK1, transfected COS-1 cells were either left untreated or treated with EGF, in the presence or absence of AG1478. As shown in Fig. 6B (upper panel), EGF-induced EGF-R activation was inhibited by AG1478 examined by using an antibody against the activated EGF-R (compare lanes 5 and 6), whereas MEKK1-C could not induce the activation of EGF-R (lane 2). Coincidentally, Tyr-705 phosphorylation of Stat3 induced by EGF-R or MEKK1-C, or wild-type Src as a positive control. MEKK1-C and Src were immunoprecipitated with their respective antibodies, and a cold in vitro kinase assay was performed as described above except in the presence of 100 μM cold ATP instead of [γ-32P]ATP, using bacterially expressed GST-Stat3 as substrate. Western immunoblotting was performed with an anti-phosphotyrosine antibody, 4G10 (pTyr Stat3, upper panel). The blot was stripped and blotted with an anti-Stat3 (C-20) antibody (lower panel).

**DISCUSSION**

The function of MEKK1 in mammalian cells has been established as a MAPKKK that activates JNKK1 (M KK4), and subsequently activates JNK, and also as an upstream kinase that phosphorylates IkB kinases leading to the activation of NF-κB by using expression of either the catalytic domain of MEKK1 or a 672-amino acid COOH-terminal fragment (38, 39). In this study, we used a similar strategy and identified the JAK-STAT pathway as another target of MEKK1. We demonstrate that MEKK1 regulates Stat3 activity via inducing a unique dual phosphorylation on both Tyr-705 and Ser-727, which leads to a functionally active Stat3. Since the Tyr-705 phosphorylation is a prerequisite for Stat3 activity, the ability of MEKK1, a Ser/Thr kinase, to stimulate tyrosine phosphorylation of Stat3 is rather remarkable. However, we consider this effect specific and unlikely to be due to the overexpression of the active MEKK1. We observed that a low amount of MEKK1-C (0.5 μg) already significantly induces Tyr-705 phosphorylation of Stat3 (Fig. 3C). In addition, full-length MEKK1 also induces tyrosine phosphorylation of Stat3, albeit weaker (data not shown). However, none of the other kinases in the MAPK cascades we have examined, including MAPKs MKK3, MKK6, MKK7, and MEK1, can induce Tyr-705 phosphorylation of Stat3 when overexpressed (data not shown). We also detected Stat5 phos-
phorylation by MEKK1-C (not shown), but not Stat1 (Fig. 3B), under the same conditions. The specificity was also supported by the observation of phosphorylation of endogenous Stat3 in MCF-7 (Fig. 3A).

Our data suggest that MEKK1 selectively induces phosphorylation and activation of certain members of STAT family. EGF stimulates Stat3 phosphorylation on Tyr-705 and Ser-727 (8, 17). The dominant negative mutant of MEKK1 blocks such phosphorylations, indicating its essential role in mediating Stat3 activation in EGF signaling. A compelling question is how MEKK1 is involved in the EGF-induced phosphorylation of Stat3.

Earlier studies have shown that all three subtypes of MAPK are involved in Stat3 serine phosphorylation. ERK and JNK phosphorylate Stat3 in response to EGF (17, 20, 22) and various stresses (23), respectively, whereas p38 regulates Stat3 serine phosphorylation in response to a combination of IL-12 and IL-2 stimulation (21). We also observed that MAPKKs, including MEK1, MKK3, MKK6, and MKK7, induce Ser-727 phosphorylation of Stat3 to various degrees (data not shown). Our results show that MEKK1 phosphorylates Stat3 on Ser-727 directly in vitro, suggesting that it may phosphorylate Stat3 in vivo. However, it may also induce Stat3 serine phosphorylation through the downstream kinases in vivo. Collectively, these data indicate an extensive cross-talk between STATs and kinases in the different levels of the MAPK cascades, presumably to maximize the Stat3 activity.

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and activation or vice versa.

The possible role of MEK1 in Stat3 activation in growth factor signaling is summarized in a simplified model (Fig. 7). Binding of growth factors to their respective receptors triggers their intrinsic tyrosine kinase activity that leads to the activation of tyrosine kinases, Src and JAKs, GTPase Rac1, and its associated MEKK1. The activated MEK1 further increases the activity of Src and JAKs and leads to an enhanced phosphorylation of Stat3. On the other hand, MEKK1 activates both the MKK4-JNK pathway and the ERK pathway, the latter by interacting with GTP-bound active Ras (42), and Raf-1, MEK1, and ERK2 in a scaffold complex (43). Therefore, Stat3 serine phosphorylation in EGF signaling may be mediated by multiple kinases in the MAPK pathways including MEKK1, MEK1, ERKs, and JNKs.

Although JNK and NF-κB reside in two different signaling pathways and have distinct downstream targets, both can be activated by inflammatory cytokines, such as tumor necrosis factor-α and interleukin-1, as well as environmental stresses (44), and therefore can be coordinately activated via MEKK1. However, we previously observed that only Ser-727, but not Tyr-705 phosphorylation, was induced by tumor necrosis factor-α and various stress treatments, suggesting that activation of MEKK1 by these stimuli does not lead to activation of Stat3. On the other hand, MEKK1 activated by EGF results in the activation of Stat3, JNK, and ERK (28, 29). Therefore, MEKK1 responds to different extracellular stimuli by specifically activating a subset of the downstream signaling molecules to exert their intrinsic tyrosine kinase activity that leads to the activation of Stat3 by MEKK1.

Acknowledgments—We are grateful to Drs. R. Janknecht for the constitutively activated MEKK1-C plasmid; J. E. Darnell for pRCCMV-Stat3; S. A. Courtneidge for wild-type pSGTsrcK plasmid; R. Tham and R. Chng for photography. Dr. V. Novotny, and T. Zhang for the critical reading of the manuscript; J. E. Darnell for pRC/CMV-MEK1, ERK2, and JNKs. Therefore, Stat3 responds to different extracellular stimuli by specifically activating a subset of the downstream signaling molecules to exert their intrinsic tyrosine kinase activity that leads to the activation of Stat3 by MEKK1. Although JNK and NF-κB reside in two different signaling pathways, both can be coordinately activated via MEKK1. However, we previously observed that only Ser-727, but not Tyr-705 phosphorylation, was induced by tumor necrosis factor-α and various stress treatments, suggesting that activation of MEKK1 by these stimuli does not lead to activation of Stat3. On the other hand, MEKK1 activated by EGF results in the activation of Stat3, JNK, and ERK (28, 29). Therefore, MEKK1 responds to different extracellular stimuli by specifically activating a subset of the downstream signaling molecules to exert their intrinsic tyrosine kinase activity that leads to the activation of Stat3 by MEKK1.
