Interdependent SMAD and JNK Signaling in Transforming Growth Factor-β-mediated Transcription*

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SMAD and JNK cascades are essential components of the transforming growth factor-β (TGF-β) signaling machinery and are implicated in common transcriptional responses. However, the relationship of these pathways to one another downstream of the TGF-β receptor complex is unknown. We show that JNK is rapidly activated by TGF-β in a SMAD-independent manner, and phosphorylates Smad3 outside its -SSXS motif. Smad3 phosphorylation by JNK facilitates both its activation by the TGF-β receptor complex and its nuclear accumulation. JNK regulates SMAD- and TGF-β-mediated transcriptional responses, yet JNK activators only partially stimulate transcriptional responses characteristic of TGF-β without coincident SMAD pathway activation. These results suggest an interdependent relationship between the JNK and SMAD pathways in TGF-β-mediated transcription.

Transforming growth factor-β (TGF-β)1 is the prototype for a conserved family of secreted polypeptides that includes bone morphogenetic proteins, activins, and several more distantly related factors (1). Through its effects on cell growth, differentiation, motility, adhesion, and apoptosis, TGF-β is an influential regulator of cell fate determination and tissue morphogenesis. TGF-β signals through a heteromeric receptor complex assembled in response to ligand binding and composed of type I (TβRI) and type II (TβRII) serine/threonine kinase receptors. Receptor activation mobilizes a complex network of signal transducers to produce pleiotropic responses. Essential elements of the post-receptor signaling machinery for TGF-β are the pathway-restricted SMADs Smad2 and Smad3 and the common mediator Smad4. In response to TGF-β, Smad2 and Smad3 are phosphorylated at their C termini by activated TβRI. These phosphorylated SMADs form stable complexes with Smad4 and translocate to the nucleus to act as transcriptional regulators. This provides a direct conduit between the activated receptor complex and target promoters in the nucleus. Underscoring their importance in TGF-β signaling, both growth and transcriptional responses to TGF-β are abated by SMAD pathway disruption, and SMAD mutations are frequently observed in human tumors (2, 3).

Emerging evidence indicates that to regulate the expression of target genes, SMADs cooperate with transcription factors previously implicated in TGF-β signaling (3–12). Among these factors is AP-1, a heterodimer of c-Fos and c-Jun family members that binds specific sequences in target promoters (13). Stimulation of AP-1-dependent transcription can be achieved by phosphorylation of the c-Jun transactivation domain by c-Jun N-terminal kinase (JNK), a member of the mitogen-activated protein kinase (MAPK) family (13–15). MAPKs have also been implicated in SMAD pathway regulation (16, 17). SMAD- and AP-1-binding elements are juxtaposed in the TGF-β-responsive regions of plasminogen activator inhibitor-1 (PAI-1) promoter, and both have been positively implicated in PAI-1 induction by TGF-β (11, 12, 18). Furthermore, a direct, TGF-β-inducible interaction between Smad3 and c-Jun has been recently described (4). Given the involvement of SMAD and AP-1 function in TGF-β signaling and the regulation of both pathways by MAPKs, we examined the relationship between the SMAD and JNK signaling pathways in TGF-β-mediated transcription.

We demonstrate that JNK activation in response to TGF-β is bimodal. Rapid, SMAD-independent JNK activation by TGF-β is followed by sustained, SMAD-dependent JNK activity. Both rapid and sustained JNK activation induced by TGF-β require the function of a Rho family GTPase. We also show that Smad3 is phosphorylated by JNK both in vitro and in vivo. JNK-mediated phosphorylation of Smad3 facilitates both its phosphorylation by TβRI and its subsequent nuclear accumulation. Additionally, the JNK pathway regulates both TGF-β- and SMAD-mediated transcriptional responses; yet only modestly activates these responses in a SMAD-deficient background. These data suggest an interdependent relationship between the JNK and SMAD signaling pathways in TGF-β-mediated PAI-1 induction beyond that suggested by the juxtaposition of their response elements in the PAI-1 promoter.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructs and Cell Culture—**Mv1Lu cells were obtained from the American Type Culture Collection. R1B cells, TGF-β receptor expression plasmids, and the 3TP-Lux reporter were generously provided by Dr. Joan Massagué. MDA-MB-468 cells were provided by Dr. Carlos Arteag. All cells were propagated in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS. RhoA and Rac1 expression plasmids were the gift of Dr. J. S. Gutkind. JNK expression plasmids were the gift of Dr. Roger Davis, and Dr. X.-F. Wang provided Smad3 expression plasmids and the 4XSBS reporter. Dr. J.-M. Gautier generously provided the CAGA12 reporter plasmid. The cDNA for C3 exotransferase from Clostridium botulinum was subcloned in pCMV5 for expression in mammalian cells.

**Immunoprecipitation / Kinase Assays—**Endogenous or transiently ex-
pressed kinases were isolated from extracts of Mv1Lu or MDA-MB-468 cells using anti-JNK (C-17; Santa Cruz Biotechnology), anti-ERK (C-16; Santa Cruz Biotechnology), or anti-FLAG (M2; Sigma) antibodies. Extracts were prepared following transfection, infection, and treatment as noted in the figure legends using lysis buffer (25 mM HEPES, 300 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 20 mM β-glycero- phosphate, 20 mM p-nitrophospholyl phosphate, 2 mM sodium pyrophosphate, 1 mM Na₂VO₄, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml pepstatin, and 10% glycerol, pH 7.5). Immune complexes were collected with protein G-Sepharose and washed four times with cold 1× lysis buffer followed by kinase assay buffer (50 mM HEPES, 10 mM MgCl₂, 1 mM dithiothreitol, 10 μg/ml p-nitrophospholyl phosphate, and 10 μg/ml β-glycero-phosphate, pH 7.5). Pellets were resuspended in 40 μl of kinase assay buffer supplemented with 10 μM ATP, 2.5 μg of GST-ATF2, His₃-Smad3, or myelin basic protein; and 10 μCi of [γ-³²P]ATP (3000 Ci/mmol) per reaction. Assays were carried out at 30 °C for 30 min and then stopped with Laemmli sample buffer. Reaction mixtures were separated by SDS-PAGE, and phosphorylated proteins were visualized by autoradiography on fixed gels.

**Immunoblot Analysis**—Following separation by SDS-PAGE, JNK, ERK, FLAG-JNK wt (where wt is wild-type), HA-Smad3, and HA-Smad3ΔSA were examined in whole cell extracts by immunoblot analysis using standard methods. Antibodies were as follows: C-17, 0.5 μg/ml; C-16, 0.5 μg/ml; mAb 22C10, 0.5 μg/ml; and 12C5 (Rho Kinase Molecular Biology Cals), 0.4 μg/ml. Bands were visualized by chemiluminescent detection.

**Transcriptional Reporter Assays**—Mv1Lu and R1B or MDA-MB-468 cells were transfected by DEAE-dextran or calcium phosphate co-precipitation methods, respectively, with expression constructs and reporters as described in the figure legends. Following overnight recovery, cells were maintained in DMEM and 0.2% FBS either with or without TGF-β for 18 h. Expression luciferase was solubilized and assayed in an Analytical Luminescence Laboratory Monolight 2010 instrument according to the manufacturer’s instructions. β-Galactosidase activity from a constitutively expressed internal control was assayed with a comprehensive time course using immunoprecipitation/kinase assays with GST-ATF2 and myelin basic protein as substrates, respectively (Fig. 1A). We observed bimodal JNK activation in response to TGF-β. The primary peak of JNK activity was rapid and transient, peaking at 10 min, followed by a decline toward base-line values by 1 h. Rapid JNK activation by TGF-β was comparable to that achieved by NaAsO₂ and tumor necrosis factor-α (Fig. 1B), both common JNK activators. The primary peak of JNK activity was followed by a gradually rising, sustained second peak, maximal between 12 and 16 h. In contrast to JNK, ERK activation was not observed in response to TGF-β throughout the time course examined (Fig. 1A), whereas epidermal growth factor (EGF) and FBS potently activated ERK toward myelin basic protein (Fig. 1B). These data indicate selectivity for TGF-β toward MAPK signaling pathways and are consistent with a role for JNK signaling in rapid transactivation of the PAI-1 gene.

JNK activation and TGF-β signaling involve the Rho family of monomeric GTPases (19, 24–26). To determine the role of Rho family proteins in both primary and secondary JNK activation elicited by TGF-β, FLAG-tagged JNK was transiently expressed in Mv1Lu cells with or without dominant-negative mutants of RhoA or Rac1 (RhoAN19 and Rac1N17, respectively) or C3 exo-transferase. C3 exo-transferase specifically inhibits Rho family proteins through ADP-ribosylation of a uniquely conserved asparagine residue (27). Following TGF-β treatment for either 10 min or 12 h, the activity of transiently expressed FLAG-JNK1 was determined in cell extracts by in vitro kinase assays (Fig. 2, A and B). We observed inhibition of JNK activity by RhoAN19 and less so by Rac1N17 after 10 min of TGF-β treatment. After 12 h of TGF-β exposure, Rac1N17 inhibited JNK activity with greater efficacy than RhoAN19. At both 10 min and 12 h following TGF-β treatment, C3 exo-transferase significantly diminished FLAG-JNK activity. These results suggest that both RhoA and Rac1 contribute to JNK regulation by TGF-β in Mv1Lu cells.

The 3TP-Lux transcriptional reporter contains TGF-β-responsive elements of the PAI-1 and collagenase promoters and is widely used to assess TGF-β signaling requirements (18, 28, 29). To further explore the role of Rho protein-dependent JNK activity in TGF-β signaling, we tested the influence of Rho and JNK mutants on 3TP-Lux reporter activation (Fig. 2C). Afifi et al. (19) have shown varying levels of 3TP-Lux transactivation by RhoA, Rac1, and Cdc42 in HepG2 cells. Similarly, we observed that constitutively active mutants of RhoA and Rac1 (RhoAQ63 and Rac1Q63) potentiated 3TP-Lux transactivation by TGF-β. Conversely, dominant-negative derivatives of RhoA and Rac1 antagonized 3TP-Lux activation by TGF-β. An inactive mutant of JNK (FLAG-JNKΔPP) similarly inhibited TGF-β-mediated 3TP-Lux activity. In contrast to previous results in HepG2 cells (19), we observed only modest activation of 3TP-Lux by constitutively active Rho proteins in Mv1Lu cells in the absence of TGF-β. This increase was blocked by coexpressed FLAG-JNKΔPP, suggesting that Rho protein-dependent 3TP-Lux signaling was transmitted via the JNK pathway. Given the bimodal pattern of JNK activation by TGF-β and the modest transactivation of 3TP-Lux by upstream activators of JNK in the absence of TGF-β, we hypothesized that the primary activation of JNK by TGF-β was independent of the SMAD signaling pathway. To test the SMAD dependence of
TGF-β-mediated JNK activation, we employed recombinant adenoviruses expressing TGF-β signaling components or β-galactosidase as a control (30) (Fig. 3A). In extracts prepared from both uninfected (Ø) and β-galactosidase-expressing adenovirus (Ad: βgal)-infected Mv1Lu cells, the bimodal activation of JNK by TGF-β was maintained. Primary JNK activation in response

FIG. 1. TGF-β elicits bimodal JNK activation with rapid, transient, and delayed sustained peaks of activity. A, endogenous JNK or ERK was immunoprecipitated from Mv1Lu whole cell extracts following TGF-β (10 ng/ml) treatment for the times indicated. GST-ATF2 or myelin basic protein (MBP) was used as a substrate for in vitro kinase assays (immunoprecipitation (IP)/JNK or ERK assay). Equal JNK and ERK expression was monitored by Western blotting (WB). Equal loading of reaction products was confirmed by Coomassie Blue staining (CBB). Normal rabbit serum (N) was employed in negative control precipitations. B, Mv1Lu cells were exposed for 15 min to either 100 µM NaAsO2 or 25 ng/ml tumor necrosis factor-α as a JNK agonist or 40 ng/ml EGF or 10% FBS as an ERK agonist. The activities of JNK and ERK were examined as described for A.

FIG. 2. TGF-β activation of JNK is dependent upon Rho family GTPases and potentiates transcriptional responses. A and B, Mv1Lu cells were transiently transfected with FLAG (FL)-JNK1 either alone or in combination with inactive RhoA or Rac1 mutants or a C3 exotransferase expression plasmid (C3 exo) as shown. Following recovery, cells were maintained in DMEM containing 0.2% FBS for 24 h and then treated with 10 ng/ml TGF-β for 10 min or 12 h as shown. Transiently expressed JNK1 was immunoprecipitated with anti-FLAG monoclonal antibody M2, followed by in vitro JNK assay as described for Fig. 1. C, Mv1Lu cells were transiently transfected with expression plasmids encoding constitutively active (RhoQL and Rac1QL) or dominant-negative (RhoN19 and Rac1N17) RhoA and Rac1 or an inactivated mutant of JNK (FLAG-JNKAPF) as indicated, together with the 3TP-Lux reporter and an internal control β-galactosidase expression plasmid. After treatment with or without 1 ng/ml TGF-β, luciferase and β-galactosidase activities were measured. Results, normalized to the internal β-galactosidase control, reflect the means ± S.D. from a representative experiment. IP, immunoprecipitation; WB, Western blot.

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to TGF-β was retained in cells infected with a adenovirus expressing Smad3Δ-FLAG (Ad: Smad3Δ: FL; a C-terminally truncated form of Smad3 with dominant-negative behavior), but the secondary peak was not observed. As anticipated, infection with an adenovirus expressing kinase-negative TβRII (Ad: DNIIR-FLAG) abolished primary JNK activation while dramatically limiting secondary JNK activity. These data suggest that primary JNK activation by TGF-β does not require the SMAD signaling pathway, but that the secondary peak is SMAD-dependent.

To confirm this hypothesis, we examined JNK activation in response to TGF-β in the breast carcinoma cell line MDA-MB-468 (Fig. 3B), which harbors a homozygous deletion of the Smad4 gene, yet retains both TβRI and TβRII expression (31, 32). These cells are refractory to TGF-β in both growth and transcriptional response assays, but both responses to TGF-β are restored by Smad4 expression (Ref. 32 and data not shown). As predicted, primary JNK activation was observed following TGF-β treatment of either uninfected or β-galactosidase-expressing MDA-MB-468 cells. However, in neither circumstance was secondary JNK activity observed. Restoration of Smad4 expression through the use of a recombinant adenovirus (Ad: Smad4: FL) partially restored secondary JNK activity. In concert, these results suggest that the SMAD signaling pathway is dispensable for primary JNK activation by TGF-β, but is required for the secondary response. They also indicate that JNK activation by TGF-β in MDA-MB-468 cells is insufficient to induce growth arrest.

In light of the SMAD independence of primary JNK activation by TGF-β, we used two strategies to elicit SMAD-independent transcriptional responses by constitutively active Rho family proteins (Fig. 3C). In the first, we examined 3TP-Lux activation in MDA-MB-468 cells. In the second, we examined transactivation of a reporter (4XSBS) driven by the TGF-β-responsive region of the collagenase promoter, modified to contain intact AP-1 sites, but mutant SMAD-binding sites (12). These experiments were done in R1B cells to eliminate the potential contribution of autocrine TGF-β signaling to reporter activity. In both circumstances, reporter stimulation induced by constitutively active Rho family proteins was comparable to their induction of 3TP-Lux in Mv1Lu cells in the absence of TGF-β (Fig. 2C). These data reinforce the SMAD independence of JNK activation in TGF-β signaling and suggest that JNK activity elicited by Rho family GTPases is insufficient to pro-
duce a robust transcriptional response characteristic of TGF-β.

The pathway-restricted SMADs show multiple consensus phosphoacceptor sites for MAPK family members. The linker regions of bone morphogenetic protein-responsive Smad1, Smad5, and Smad8 each contain a cluster of sites for ERK (P\(\times\)S/T)P (33), and ERK-mediated phosphorylation of these sites in Smad1 can inhibit bone morphogenetic protein signaling (16). In contrast, Smad3 is dominated by putative, non-ERK MAPK sites (XX(S/T)P) (33). To address potential Smad3 phosphorylation by MAPKs, we performed immunoprecipitation/kinase assays as described above using His6-Smad3 (Fig. 4A) as the substrate. Following TGF-β treatment, immunoprecipitated JNK phosphorylated His\(_6\)-Smad3 in vitro. Tumor necrosis factor-α and NaAsO\(_2\) (data not shown) also stimulated JNK phosphorylation of these sites in Smad3. As predicted by the data in Fig. 1A, ERK failed to phosphorylate His\(_6\)-Smad3 following TGF-β treatment (Fig. 4A). In contrast, ERK activated by either EGF or FBS modestly phosphorylated His\(_6\)-Smad3 in vitro, consistent with recently described Smad3 phosphorylation by ERK in vitro following EGF stimulation (17). Collectively, these data indicate that Smad3 can serve as a direct substrate for JNK in vitro following TGF-β treatment and suggest that TGF-β-induced changes in Smad3 phosphorylation do not reflect ERK activity.

Because primary JNK activation by TGF-β was SMAD-independent and Smad3 could be phosphorylated by JNK in vitro, we hypothesized that JNK, in addition to regulating AP-1 function, might also influence the SMAD signaling pathway directly. To address this hypothesis, we examined phosphorylation of transiently expressed, HA-tagged Smad3 in Mv1Lu cells in response to TGF-β. In the basal state, HA-Smad3 is a phosphoprotein that is further phosphorylated in response to TGF-β (Fig. 4B). When coexpressed with FLAG-JNK\(^{APF}\), both basal and TGF-β-stimulated phosphorylation of HA-Smad3

![Diagram](image-url)
were significantly diminished. Basal phosphorylation of HA-Smad3 was potentiated by FLAG-JNKwt expression and further increased by treatment with TGF-β for 13 min as indicated. Immunoprecipitated proteins were resolved by SDS-PAGE and visualized by autoradiography. HA-Smad3SSA expression was examined in parallel transfections by Western blotting (WB) with 12CA5. B, nuclear localization of HA-Smad3SSA in the absence and presence of TGF-β in the context of JNKwt or JNKAPF expression was examined in Mv1Lu cells following transient expression and immunofluorescence immunohistochemistry as described for Fig. 4C. C, Mv1Lu cells were transiently transfected with the indicated SMAD and JNK expression constructs along with CAGAβ-galactosidase reporters as described for Fig. 4D. SMAD-dependent CAGAβ transactivation was scored in the context of coexpressed JNKwt or JNKAPF in the absence of TGF-β. D, shown is a proposed model for interdependent transcriptional regulation by SMAD and JNK pathways in response to TGF-β. Rapid, transient, Rho-GTPase-dependent, SMAD-independent JNK activation by TGF-β provides permissive phosphorylation of Smad3 and full activation by activated TβRI. Coincident activation of SMAD and JNK/AP-1 pathways is needed for full transcriptional activation in response to TGF-β.

FIG. 5. JNK-mediated phosphorylation of Smad3 outside the -SSXS motif enhances Smad3 nuclear translocation and potentiates transcriptional activation independent of Smad3 phosphorylation by TβRI. A, HA-Smad3SSA was transiently expressed in Mv1Lu cells as shown. [32P]Orthophosphate labeling of HA-Smad3SSA was performed as described for Fig. 4B in either the absence or presence of 2 nm TGF-β for 13 min as indicated. Immunoprecipitated proteins were resolved by SDS-PAGE and visualized by autoradiography. HA-Smad3SSA expression was examined in parallel transfections by Western blotting (WB) with 12CA5. A, nuclear localization of HA-Smad3SSA in the absence and presence of TGF-β in the context of JNKwt or JNKAPF expression was examined in Mv1Lu cells following transient expression and immunofluorescence immunohistochemistry as described for Fig. 4C. B, nuclear localization of HA-Smad3SSA in the absence and presence of TGF-β in the context of JNKwt or JNKAPF expression was examined in Mv1Lu cells following transient expression and immunofluorescence immunohistochemistry as described for Fig. 4C. C, Mv1Lu cells were transiently transfected with the indicated SMAD and JNK expression constructs along with CAGAβ-galactosidase reporters as described for Fig. 4D. SMAD-dependent CAGAβ transactivation was scored in the context of coexpressed JNKwt or JNKAPF in the absence of TGF-β. D, shown is a proposed model for interdependent transcriptional regulation by SMAD and JNK pathways in response to TGF-β. Rapid, transient, Rho-GTPase-dependent, SMAD-independent JNK activation by TGF-β provides permissive phosphorylation of Smad3 and full activation by activated TβRI. Coincident activation of SMAD and JNK/AP-1 pathways is needed for full transcriptional activation in response to TGF-β.

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translocation, we examined the effect of JNK on Smad3 3SA phosphorylation and activation by Tβ motif. suggesting that the positive influence of JNK activity on HA-Smad3 3SA is a phosphoprotein in the basal state, and in its transcriptional partner, AP-1, at the promoter. However, the cytoplasm. Likewise, cytoplasmic staining was observed in Smad3 3SA mutant retains moderate transactivating ability occurs at sites distinct from those directly modified by acti-
tion of mutant Smad3 lacking the -SSX motif (21). MEKK1-mediated Smad2 phosphorylation was sufficient to stimulate nuclear localization of Smad2 in the absence of TGF-β (21). Moreover, unlike TGF-β, the MAPK activators EGF and hepatocyte growth factor induce transient Smad2 phosphorylation (22). We examined the influence of JNK on the in vivo phosphorylation and function of mutant Smad3 lacking the -SSXS motif (HA-Smad3 3SSA). HA-Smad3 3SSA is a phosphoprotein in the basal state, and in response to TGF-β treatment, we noted an increase in phosphorylation (Fig. 5A). A similar observation has been made by Liu et al. (36). A level of HA-Smad3 3SSA phosphorylation comparable to that seen with TGF-β treatment was observed in the basal state when coexpressed with FLAG-JNK wt and was further increased by TGF-β treatment. As observed for HA-Smad3, both basal and TGF-
βFLAG-JNK wt -induced HA-Smad3 3SSA phosphorylation were abolished by C3 exotransferase expression.

To gain additional insights into the significance of Smad3 phosphorylation by JNK outside the -SSXS motif, we also examined nuclear localization of HA-Smad3 3SSA in the context of FLAG-JNK wt or FLAG-JNK APF expression (Fig. 5B). In either the absence or presence of TGF-β, HA-Smad3 3SSA remained in the cytoplasm. Likewise, cytoplasmic staining was observed in both circumstances when FLAG-JNK APF was coexpressed. However, TGF-β treatment induced moderate nuclear accumulation of HA-Smad3 3SSA in cells coexpressing FLAG-JNK wt. HA-Smad3 3SSA accumulation in the nucleus was not observed in FLAG-JNK wt-expressing cells in the absence of TGF-β. These results suggest that JNK-dependent phosphorylation of Smad3 occurs at sites distinct from those directly modified by activated TpR1 and may facilitate nuclear translocation of Smad3 in response to TGF-β.

Phosphorylation of Smad3 in the -SSXS motif significantly increases SMAD-dependent transcriptional responses, yet the Smad3 3SSA mutant retains moderate transactivating ability when coexpressed with Smad4 (Fig. 5C). Given the observed influence of JNK on Smad3 3SSA phosphorylation and nuclear translocation, we examined the effect of JNK on Smad3 3SSA transactivation of the CAGA 12 reporter (Fig. 5C). When coexpressed with Smad4-FLAG, HA-Smad3 3SSA activated the CAGA 12 reporter ~15-fold (compared with 100-fold for HA-Smad3); see Fig. 4D) in the absence of TGF-β. FLAG-JNK wt potentiated and FLAG-JNK APF attenuated reporter activity, suggesting that the positive influence of JNK activity on Smad3-mediated transcription was exerted outside the -SSXS motif.

DISCUSSION

Collectively, these data are consistent with the model depicted in Fig. 5D, wherein JNK, rapidly activated by TGF-β in a Rho-dependent, SMAD-independent manner, can both activate AP-1 function and phosphorylate Smad3. Although the proposed model depicts a single pool of JNK fulfilling both roles, it is possible that TGF-β activates separate JNK populations to regulate cytoplasmic and nuclear targets. The ability of JNK to phosphorylate Smad3 3SSA and of JNK APF to inhibit Smad3 phosphorylation in response to TGF-β suggests that Smad3 phosphorylation by JNK precedes and is permissive for TpR1-dependent modification of the -SSXS motif. Following phosphorylation and activation by TpR1, Smad3 enters the nucleus in a complex with Smad4, permitting cooperation with AP-1 at the target promoter. This model predicts that robust transcription of the PAI-1 gene by TGF-β involves activation and reciprocal reinforcement between the JNK and SMAD pathways. The JNK pathway would reinforce SMAD signaling by both permissive phosphorylation of Smad3 and activation of its transcriptional partner, AP-1, at the promoter. However, without coincident Smad3 activation by TpR1, JNK would elicit only partial promoter stimulation through AP-1, as observed in transient assays. As such, the SMAD pathway would reinforce JNK signaling by augmenting AP-1 activity induced through JNK activation alone.

An activation-permissive intermediate could confer a “stimulus sampling” function to Smad3 in PAI-1 induction. In such a model, phosphorylation of Smad3 by JNK could allow Smad3 to “scan” for the source of JNK activation. JNK activation arising from the activated TGF-β receptor complex would allow Smad3 to be fully activated through phosphorylation of its -SSXS motif. Optimal TGF-β-induced PAI expression would then be achieved through activation of the SMAD signaling pathway following coincident JNK and TpR1 activation. Stimulation of PAI-1 expression by activators of either SMADs or JNK alone would be restricted.

Such an arrangement may also have implications for the effects of TGF-β on tumor formation and progression. TGF-β can act as either a tumor suppressor or a tumor promoter, depending upon the characteristics of the transformed cell and upon the host response. In tumor cells that have become refractory to TGF-β-mediated growth inhibition, the activation of preserved signaling pathways by TGF-β, in cooperation with other oncogenes, could profoundly influence tumor behavior.

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