**α-Helix 2 in the Amino-terminal Mad Homology 1 Domain Is Responsible for Specific DNA Binding of Smad3**

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Smads, signal transducers of the transforming growth factor-β (TGF-β) superfamily proteins, directly bind to DNA and regulate transcription of target genes. Smad3 binds to CAGA box, whereas Smad1 and Smad5 preferentially bind to GC-rich sequences. The β-hairpin loop in the amino-terminal Mad homology 1 (MH1) domain is the direct DNA-binding site of Smad3; however, the amino acid sequences of the β-hairpin loop of Smad3 and Smad1/5 are identical, suggesting that other regions may be responsible for the differential DNA binding of Smad3 and Smad1/5. To identify regions other than the β-hairpin loop responsible for specific DNA binding of Smad3, we generated chimeras containing various regions of Smad3 and Smad1. Luciferase assays using a TGF-β-responsive reporter (CAGA)₃-MLP-Luc and gel-mobility shift assays using 3xCAGA as a probe revealed that α-helix 2 (H2) in the amino-terminal part of the MH1 domain plays an important role in specific DNA binding and transcriptional activation of Smad3. Luciferase assays using natural TGF-β-responsive reporters also revealed the functional importance of H2 in the Smad3 MH1 domain in direct DNA binding. Smad3 thus binds to DNA directly through the β-hairpin loop, and H2 supports specific DNA binding of Smad3.

Members of the transforming growth factor-β (TGF-β) superfamily, including TGF-β, activins, bone morphogenetic proteins (BMPs), and anti-Müllerian hormone (AMH), exhibit a wide variety of biological effects on diverse types of cells (1, 2). The TGF-β superfamily proteins bind to two different types of serine/threonine kinase receptors, termed type I and type II, and transmit intracellular signals through Smad proteins (3). Receptor-regulated Smads (R-Smads) are directly activated by type I serine/threonine kinase receptors, form complexes with common-partner Smads (Co-Smads), and translocate into the nucleus where they regulate transcription of target genes. The third class of Smads, inhibitory Smads (I-Smads), interfere with the signaling activity of R-Smads and Co-Smads in TGF-β superfamily signaling.

R-Smads are further classified into two subtypes, i.e. those activated by the activin and TGF-β signaling pathways (referred to as AR-Smads in this report) and those activated by the BMP signaling pathways (BR-Smads) (4). In mammals, Smad2 and Smad3 serve as AR-Smads, whereas Smad1, Smad5, and Smad8 are BR-Smads. Members of the BMP family, including BMP-2, BMP-4, BMP-6, BMP-7, and growth/differentiation factor-5 activate BR-Smads. In addition, AMH binds to a complex composed of AMH type II receptor and BMP type IB receptor and activates BR-Smads (5). A recent study revealed that BMP-3 does not induce bone formation in vivo and that it binds to activin type II and type IB receptors and transmits activin-specific signals (6). Thus, members of the TGF-β superfamily might be classified based on their abilities to activate either AR-Smads or BR-Smads.

In the nucleus, R-Smads regulate transcription of target genes through direct binding to DNA, interaction with other DNA-binding proteins, and recruitment of transcription co-activators or co-repressors (4, 7). Affinities for DNA binding of Smads are relatively low, and Smads may regulate transcription of certain target genes mainly through indirect DNA binding via other transcription factors, e.g. FAST/FoxH family proteins (8). However, direct DNA binding of Smads is essential for transcriptional regulation of certain genes. Smad3 and Smad4 have been shown to bind to CAGA boxes and related sequences (9–11), and CAGA boxes are present in promoters of various TGF-β-responsive genes, including PAI-1, junB, and Smad7 (9–12). In contrast, Smad1, Smad5, Drosophila Mad, and Medea have been shown to bind to GC-rich sequences (GCCGnCGC) (13–15), which are found in the promoter regions of BMP-responsive genes including mouse Smad6, Drosophila vestigial, and Ultrathorax genes (16, 17).

Smads have conserved motifs in the amino- and carboxy-terminal regions, termed Mad homology 1 (MH1) and MH2 domains (3). MH2 domains are important for interaction with serine/threonine kinase receptors, formation of Smad oligomers, interaction with various DNA-binding proteins, and transcriptional activation. MH1 domains, on the other hand, contain nuclear localization signals (18, 19) and are responsible for direct DNA binding. In addition, MH1 was shown to interact with certain DNA-binding proteins (4). Analysis of the three-dimensional structure of the MH1 domain of Smad3 revealed that the β-hairpin loop protrudes from the core struc-
ture of MH1 and that it is responsible for DNA binding of Smad3 (20). Smad2 cannot directly bind to DNA, because a 30-amino acid insert region (loop 2) located in the amino-terminal part of the MH1 domain interferes with the DNA binding of Smad2 (21, 22). Importantly, the amino acid sequences of β-hairpin loops are identical in all R-Smads, and that of Smad4 (Co-Smad in mammals) is also highly conserved compared with those of R-Smads. However, Smad1 and Smad5 bind to GC-rich sequences, which are different from CAGA box, and this differential DNA binding ability may, at least in part, be responsible for differences in the biological activities induced by activins/TGF-βs and BMPs.

To determine the reasons for the differential DNA binding abilities of Smad3 and Smad1/5, we generated chimeric molecules composed of different parts of Smad3 and Smad1. The present findings obtained by transcriptional response assays and gel-mobility shift assays indicate that α-helix 2 (H2) located in the amino-terminal part of MH1 plays an important role in specific DNA binding and transcriptional activation activity of Smad3.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—The original constructions of FLAG-pcDNA3, constitutively active forms of human BMP type IA and TGF-β type I receptors (BMPR-IA(QD) and TβR-I(TD), respectively), Smad1, Smad3, Smad4, and FAST1/FoxH3 cDNAs were previously described (14, 21). Smad1 and Smad3 chimeras were prepared by a polymerase chain reaction-based approach. To obtain efficient levels of protein expression, some constructs were subcloned into another expression vector, pcDEF3 (23). All of the polymerase chain reaction products were sequenced.

**Luciferase Assay**—(CAGA)₉-MLP-Luc (9), 3GC2-Lux (16), p800-Luc (24), AR3-Luc (25), and Tlx2-Lux (26) were used as reporter plasmids. R mutant Mv1Lu cells were transfected with appropriate combinations of plasmids, and luciferase activities were determined. C. transcriptional activation of (CAGA)₉-MLP-Luc by the 1-3-3 chimera in the presence and absence of TβR-I(TD). D. transcriptional activation activity of the 3-1-1 chimera on 3GC2-Lux reporter in the presence and absence of BMPR-IA(QD).

**RESULTS**

**MH1 Domain of Smad3 Is Responsible for Transcriptional Activation of a TGF-β-responsive Reporter**—We first prepared two Smad1/3 chimeras, i.e. 3-1-1 containing the MH1 domain of Smad3 and the linker and MH2 of Smad1, and 1-3-3 containing the MH1 of Smad1 and the linker and MH2 of Smad3 (Fig. 1A). In agreement with previous reports that MH2 domain specifically interacts with type I serine/threonine kinase receptors (28), the 1-3-3 chimera containing the Smad3 MH2 domain was phosphorylated by a constitutively active form of TGF-β type I receptor, TβR-I(TD), whereas the 3-1-1 chimera containing the Smad1 MH2 domain was phosphorylated by a constitutively active form of BMP type IA receptor, BMPR-IA(QD) (data not shown).
We next investigated transcriptional activation activity of these chimeras using (CAGA)$_9$-MLP-Luc, which is known to be specifically activated by AR-Smads (9). The 3-1-1 chimera but not wild-type Smad1 activated transcription of (CAGA)$_9$-MLP-Luc in response to BMPR-IA(QD) (Fig. 1B). As shown in Fig. 1C, TβR-I(TD) strongly induced transcription of (CAGA)$_9$-MLP-Luc, most likely through activation of endogenous Smad2 and Smad3. Smad3 induced further increase in the transcription of (CAGA)$_9$-MLP-Luc in the presence of TβR-I(TD). In contrast, the 1-3-3 chimera did not significantly induce additional increase in the transcription of (CAGA)$_9$-MLP-Luc in the presence of TβR-I(TD). These results indicate that the MH1 domain of Smad3 is responsible for specific transcriptional activation of (CAGA)$_9$-MLP-Luc.

We also used 3GC2-Lux, which contains the GC-rich sequence of the mouse Smad6 promoter, and is specifically activated by AR-Smads (16). The 3-1-1 chimera but not wild-type Smad1 activated transcription of (CAGA)$_9$-MLP-Luc in response to BMPR-IA(QD) (Fig. 1B). As shown in Fig. 1C, TβR-I(TD) strongly induced transcription of (CAGA)$_9$-MLP-Luc, most likely through activation of endogenous Smad2 and Smad3. Smad3 induced further increase in the transcription of (CAGA)$_9$-MLP-Luc in the presence of TβR-I(TD). These results indicate that the MH1 domain of Smad3 is responsible for specific transcriptional activation of (CAGA)$_9$-MLP-Luc.

We therefore focused on the transcriptional activity of Smad1/3 chimeras using (CAGA)$_9$-MLP-Luc in the present study. The Region Located at the Amino-terminal Part of MH1 Is Important for Transcriptional Activation—We next examined whether the amino-terminal or carboxyl-terminal half of MH1 domain is important for specific transcriptional activation of (CAGA)$_9$-MLP-Luc by Smad3. We divided the MH1 domains into the amino-terminal (N region) and carboxyl-terminal (C region) halves (C region) and constructed chimeras as shown in Fig. 2A. The N region of Smad3 and Smad1 are from Met-1 to Arg-69, and the C regions are from Ser-70 to Val-140. B, transcriptional activation of (CAGA)$_9$-MLP-Luc by Smad1/3 chimeras containing different N and C regions of Smad3 or Smad1. R mutant Mv1Lu cells were transfectected with appropriate combinations of plasmids, and luciferase activities were determined. C, chimeras containing the linker and MH2 domains of Smad3 were also examined in the presence and absence of TβR-I(TD).

**Fig. 2. Role of the N region of Smad3 in activation of (CAGA)$_9$-MLP-Luc.** A, the MH1 domain was divided into the N and C regions, and chimeras containing different parts of Smad3 and Smad1 were constructed. The N regions of Smad3 and Smad1 are from Met-1 to Arg-69, and the C regions are from Ser-70 to Val-140. B, transcriptional activation of (CAGA)$_9$-MLP-Luc by Smad1/3 chimeras containing different N and C regions of Smad3 or Smad1. R mutant Mv1Lu cells were transfectected with appropriate combinations of plasmids, and luciferase activities were determined. C, chimeras containing the linker and MH2 domains of Smad3 were also examined in the presence and absence of TβR-I(TD).

**Fig. 3. Direct binding of the Smad1/3 chimeras to 3xCAGA.** EMSA was performed using 3xCAGA as a probe. Whole cell extracts prepared from COS7 cells transfected with indicated plasmids were analyzed by EMSA. The chimeras containing the linker and MH2 domains of Smad1 (A) or Smad3 (B) were transfected with or without Smad4 and BMPR-IA(QD) (A) or TβR-I(TD) (B), respectively.

We next investigated transcriptional activation activity of these chimeras using (CAGA)$_9$-MLP-Luc, which is known to be specifically activated by AR-Smads (9). The 3-1-1 chimera but not wild-type Smad1 activated transcription of (CAGA)$_9$-MLP-Luc in response to BMPR-IA(QD) (Fig. 1B). As shown in Fig. 1C, TβR-I(TD) strongly induced transcription of (CAGA)$_9$-MLP-Luc, most likely through activation of endogenous Smad2 and Smad3. Smad3 induced further increase in the transcription of (CAGA)$_9$-MLP-Luc in the presence of TβR-I(TD). In contrast, the 1-3-3 chimera failed to induce transcription of (CAGA)$_9$-MLP-Luc, suggesting that the N region of Smad3 MH1 domain plays an important role in activation of (CAGA)$_9$-MLP-Luc. The C region may also be important for transcriptional activation because
the 3-1-1 chimera was more potent than the 31-1-1 chimera; however, the C region may require the N region for efficient transcriptional activation.

We also examined the transcriptional activity of chimeras 13-3-3 and 31-3-3, containing the linker and MH2 domain of Smad3 (Fig. 2C). TβR-I(TD) strongly induced transcription of (CAGA)₉-MLP-Luc, and Smad3 further induced transcriptional activation as shown in Fig. 1C. The 31-3-3 chimera containing the N region of the Smad3 MH1 domain also increased the transcriptional activity induced by TβR-I(TD), although it was less potent than wild-type Smad3. In contrast, the 13-3-3 chimera containing the N region of the Smad1 MH1 domain failed to induce additional increase in the transcription of (CAGA)₉-MLP-Luc.

Activation of the TGF-β-responsive Reporter Correlates with Direct DNA Binding of Smads to CAGA Boxes—We next investigated the DNA binding abilities of Smad1/3 chimeras to 3xCAGA by EMSA. The 3-1-1 and 31-1-1 chimeras bound to 3xCAGA upon stimulation by BMPR-IA(QD); this binding was more prominent in the presence than in the absence of Smad4 (Fig. 3A). In contrast, wild-type Smad1 and the 13-1-1 chimera only weakly bound or did not bind to the probe. Similarly, the wild-type Smad3 and the 31-3-3 chimera bound to DNA in the presence of TβR-I(TD), and this binding was enhanced in the presence of Smad4 (Fig. 3B). Two shifted bands were detected in the presence of Smad4 as previously reported (27). In contrast, neither the 1-3-3 nor the 13-3-3 chimera bound to DNA in either the absence or presence of TβR-I(TD) and Smad4. These results are in good agreement with those of luciferase assays using (CAGA)₉-MLP-Luc (Figs. 1 and 2) suggesting that transcriptional activation of the (CAGA)₉-MLP-Luc reporter is induced by direct binding of Smad chimeras to CAGA boxes.

Functional Roles of the Amino-terminal Part of the N Region—Because the N region of Smad3 is important for direct DNA binding of Smad3, the N region was further investigated using (CAGA)₉-MLP-Luc. The N region contains three α-helices termed H1, H2, and H3 (20). We divided the N region into two parts, i.e. the Na region containing the most amino-terminal part including H1 and H2, and the Nb region containing the remaining part of the N region including H3, and prepared chimeras as shown in Fig. 4A. In the presence and absence of BMPR-IA(QD), the 313-1-1 chimera was as potent as the 3-1-1 chimera in activation of (CAGA)₉-MLP-Luc (Fig. 4B). In addition, the 311-1-1 chimera significantly induced transcription of (CAGA)₉-MLP-Luc in the absence and presence of TβR-I(TD) (Fig. 4C). On the other hand, the 311-1-1 chimera failed to activate transcription of (CAGA)₉-MLP-Luc.

DNA binding of the Smad1/3 chimeras was also studied by EMSA using 3xCAGA as a probe. In agreement with the results obtained by luciferase assay, the chimeras containing the Na region of Smad3, i.e. 31-1-1 and 311-1-1, bound to the probe

![FIG. 4. The Na region is more important than the Nb region for transcriptional activation of (CAGA)₉-MLP-Luc.](http://www.jbc.org/)

A, structures of the chimeras containing the Na and Nb regions from Smad3 or Smad1. Na of Smad3 is from Met-1 to Leu-42, and Nb of Smad3 is from Lys-43 to Arg-69. Na of Smad1 is from Met-1 to Leu-41, and Nb is from Lys-42 to Arg-69. B, the chimeras were examined for activation of transcription using (CAGA)₉-MLP-Luc in the presence of BMPR-IA(QD). C, the chimeras containing the linker and MH2 domain of Smad3 were examined using (CAGA)₉-MLP-Luc in the presence of BMPR-IA(QD).
upon BMPR-IA(QD) stimulation in the presence of Smad4 (Fig. 5A). When the chimeras containing the linker and MH2 domain of Smad3 were investigated, those containing the Na region of Smad3, i.e. 313-3-3 and 31-3-3, were found to bind to DNA upon stimulation by TβR-I(TD) both in the presence and absence of Smad4 (Fig. 5B). These findings revealed that the Na region is more important than the Nb region for direct DNA binding of Smad3.

H2 Is Important for the Specific DNA Binding of Smad3—We further explored which parts of the Na region are most important for the specific transcriptional activation and DNA binding of Smad3. The Na region includes H1 and H2, which are conserved between Smad3 and Smad1, while the remaining parts of the Na region are less conserved (Fig. 6A). We generated mutants of Smad1 in which amino acid residues in the Na region were replaced by corresponding amino acid residues of Smad3. These mutants were termed Smad1[I], Smad1[II], Smad1[III], Smad1[H2C], and Smad1[H2KS]. Similarly, Smad3 mutants, i.e. Smad3[I], Smad3[II], Smad3[III], Smad3[IV], Smad3[H2A], and Smad3[H2DA], in which amino acid residues in the Na region were replaced by those of Smad1, were prepared (Fig. 6A). Luciferase assays using these chimeras revealed that Smad1[I], Smad1[II], and Smad1[H2C] were inactive, similar to wild-type Smad1, whereas Smad1[III] and Smad1[H2KS] acquired the ability to activate (CAGA)_9-MLP-Luc, similar to the 311-1-1 chimera (Fig. 6B). We also found that Smad3[II], Smad3[III], and Smad3[H2A] were as potent as wild-type Smad3, whereas Smad3[H2DA], Smad3[III], and the 133-3-3 chimera were less potent than wild-type Smad3 in transcriptional activation of (CAGA)_9-MLP-Luc (Fig. 6C). Smad1[IV] and Smad3[IV] were functionally similar to Smad1[III] and Smad3[III], respectively, in transcriptional activation of (CAGA)_9-MLP-Luc (data not shown).

Analyses by EMSA using 3xCAGA also showed that the 311-1-1 chimera, Smad1[III], and Smad1[IV] bound to DNA upon stimulation by BMPR-IA(QD) in the presence of Smad4, whereas the 13-1-1 and 31-1-1 chimeras and Smad1[II] bound only weakly to the DNA (Fig. 7A). The 313-3-3 and 31-3-3 chimeras and Smad3[II] bound to the probe in the presence and absence of Smad4 upon TβR-I(TD) stimulation, but the 133-3-3 chimera and Smad3[III] and Smad3[IV] did not (Fig. 7B). We further studied Smad constructs with mutations in H2. Smad1[H2KS] acquired the ability to bind to DNA, similar to the 311-1-1 chimera and Smad1[III], whereas Smad1[H2C] bound only very weakly to the probe (Fig. 7C). Furthermore, the DNA binding ability of Smad3[H2DA] but not that of Smad3[H2A] was greatly reduced, similar to that of Smad3[III] (Fig. 7D). These findings suggest that the amino acids Lys-36...
and Ser-37 in H2 are most important for specific DNA binding and transcriptional activation of Smad3.

**Effects of Chimeras on Natural TGF-β-responsive Reporters**—Because (CAGA)$_9$-MLP-Luc is an artificial promoter-reporter construct containing nine tandemly repeated CAGA boxes, we investigated the transcriptional activities of Smad1/3 chimeras and mutants using natural TGF-β-responsive reporters. p800-Luc contains the TGF-β-responsive element of the PAI-1 promoter (24). As shown in Fig. 8A, the chimeras containing the Na region of Smad3, i.e. 313-1-1, and 311-1-1, significantly induced transcription of p800-Luc in the presence of BMPR-IA(QD). Smad1[III] and Smad1[IV] also induced transcription of p800-Luc in the presence of BMPR-IA(QD), although they were less potent than the 313-1-1 and 311-1-1 chimeras. In contrast, neither the chimeras containing the Smad1 Na region nor the Smad1[II] mutant significantly induced transcription of p800-Luc in the presence of BMPR-IA(QD), Smad1[III] and Smad1[IV] also induced transcription of p800-Luc in the presence of BMPR-IA(QD), although they were less potent than the 313-1-1 and 311-1-1 chimeras. In contrast, neither the chimeras containing the Smad1 Na region nor the Smad1[II] mutant significantly induced transcription of the reporter. When the chimeras or mutants containing the linker and MH2 domains of Smad3 were examined, those containing the Smad3 Na region and the Smad3[III] mutant were found to induce transcription upon TβR-I(TD) stimulation. In contrast, the chimeras containing the Smad1 Na region and the Smad3[III] mutant were less active than Smad3 (Fig. 8B). Thus these results were in agreement with those obtained using (CAGA)$_9$-MLP-Luc.

We also examined the activity of Smad3 chimera using AR3-Luc, which contains the promoter region of Xenopus Mix.2 gene. The DNA binding of FAST/FoxH family proteins is required for transcriptional activation of AR3-Luc (25). Fig. 8C shows that FAST1/FoxH3 induced transcription of AR3-Luc in the presence of TβR-I(TD), which was further enhanced by Smad2. In contrast, Smad3 did not significantly induce transcription of AR3-Luc, probably because the direct binding of Smad3 to DNA altered the configuration of Smad/FAST1/DNA complexes (21, 29). Similar to Smad2, the chimera 133-3-3 enhanced transcription of AR3-Luc in the presence of FAST1/FoxH3 and TβR-I(TD), suggesting that this chimera did not bind to DNA and functioned in a fashion similar to Smad2.

The effects of Smad chimeras were examined using a BMP-responsive reporter, Tlx2-Lux (26), and using P19 embryonal carcinoma cells. Smads induce transcriptional activation of Tlx2-Lux through a GC-rich sequence.2 Smad1 significantly induced transcriptional activation in both the presence and absence of BMPR-IA(QD) (Fig. 8D). The 131-1-1 chimera induced transcription of Tlx2-Lux, although it was less potent than wild-type Smad1. In contrast, other chimeras, including 13-1-1 and 133-1-1, were not able to activate transcription of 2 K. Kusanagi, M. Kawabata, H. K. Mishima, and K. Miyazono, unpublished data.
Tlx2-Lux, suggesting that the Tlx2 gene is activated by a mechanism different from that for genes containing the Smad3-binding elements.

**DISCUSSION**

Smads have conserved MH1 and MH2 domains in their amino- and carboxyl-terminal portions, respectively, which are linked by linker regions. The MH2 domain plays important roles in interaction with type I receptors, hetero-oligomerization of Smads, binding to various transcription factors, and transcriptional regulation by recruitment of transcriptional co-activators or co-repressors (3, 7). The function of the linker region has not been fully elucidated, but recent studies have revealed that Smurf1 and Smurf2, which are members of the HECT family of E3 ubiquitin ligases, bind to a PY motif in the linker region of Smads and induce ubiquitin-dependent degradation of R-Smads and I-Smads (30–34). MH1 domains are conserved in R-Smads and Co-Smads, but the amino acid sequences of I-Smads are divergent from those of other Smads. The MH1 domain has multiple functions; MH1 physically interacts with the MH2 domain, and MH1 and MH2 repress each other’s function (35). The MH1 domain was also shown to directly bind to DNA and to interact with certain DNA-binding proteins, including c-Jun and vitamin D receptor (4). Recently, the MH1 domain of R-Smads but not of Co-Smads has been shown to contain a nuclear localization signal, which plays an important role in the nuclear transport of R-Smads upon receptor activation (18, 19).

The question arises how R-Smads exhibit different biological activities in response to TGF-β/activins and BMPs. R-Smads can interact with different transcription factors, e.g., Smad3 binds to FAST/FoxH proteins, c-Jun/c-Fos, TFE3, and vitamin D receptor, whereas Smad1 preferentially binds to OAZ and Hoxc8 (4). In addition to their differential interaction with DNA-binding proteins, Smad3 and Smad1 bind to distinct DNA sequences, resulting in exhibition of specific biological functions of TGF-β/activins and BMPs. The β-hairpin loop in the MH1 domain of Smad3 is the direct DNA-binding site (20). An important question is why Smad3 and Smad1 bind to different DNA sequences even though their β-hairpin loop amino acid sequences are identical. The present study revealed that H2 in the MH1 domain plays an...
important role in specific DNA binding of Smad3. Consistent with this, analysis of the three-dimensional structure of Smad3 MH1 domain revealed that H2 is located near the β-hairpin loop in solution (20).

Smad2 and Smad3 are AR-Smads activated by TGF-β and activin type I receptors. Although they are structurally highly similar to each other, analysis using mouse embryonic fibroblasts derived from Smad2- or Smad3-deficient mice revealed that they have specific roles in TGF-β signaling (36). Smad2 does not bind to DNA because the loop 2 (Fig. 9) interferes with the DNA binding of Smad2 (21, 22). Interestingly, transgenic analysis using the GAL4/UAS system revealed that a Drosophila homologue of Smad2/3 (dSmad2) is functionally more similar to human Smad2 than to human Smad3 (37). Although dSmad2 does not contain the loop 2, it poses functional similarities to human Smad2 by the ubiquitin-proteasome pathway (43, 44). More recently, it has been shown that protein kinase C induces phosphorylation of Ser-37 of Smad3, resulting in loss of DNA binding ability of this molecule (45). These findings suggest that the DNA binding ability of Smad3 may be physiologically regulated by protein modification and pathologically altered by mutations of proteins.

In conclusion, we have demonstrated the importance of H2 of the Smad3 MH1 domain in direct binding of Smad3 to specific DNA sequences. It will be important to determine how BR-Smads and Co-Smad recognize specific DNA sequences and how they contact DNA as R-Smad/Co-Smad complexes.

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