Glucocorticoid receptor inhibits transforming growth factor-β signaling by directly targeting the transcriptional activation function of Smad3

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The transforming growth factor-β (TGF-β) family of cytokines and glucocorticoids regulate diverse biological processes through modulating the expression of target genes. Here we report that glucocorticoid receptor (GR) represses TGF-β transcriptional activation of the type-1 plasminogen activator inhibitor (PAI-1) gene in a ligand-dependent manner. Similarly, GR represses TGF-β activation of the TGF-β responsive sequence containing Smad3/4-binding sites. Using mammalian two-hybrid assays, we demonstrate that GR inhibits transcriptional activation by both Smad3 and Smad4 C-terminal activation domains. Finally, we show that GR interacts with Smad3 both *in vitro* and *in vivo*. These results suggest a molecular basis for the cross-regulation between glucocorticoid and TGF-β signaling pathways.

The transforming growth factor-β (TGF-β) family of cytokines and glucocorticoids regulate diverse biological processes through modulating the expression of target genes. TGF-β signaling is mediated through two types of transmembrane serine/threonine kinase receptors (1), and the highly conserved Smad family of proteins has been identified as intracellular signal transducers to relay the TGF-β signal to the nucleus. The Smad proteins can be functionally classified into three subgroups: receptor-regulated Smads, Smads1, 2, 3, 5 and 8; the common Smad, Smad4; and inhibitory Smads, Smads6 and 7 (2–5). The receptor-regulated Smads interact directly with specific TGF-β and activin receptors or bone morphogenic protein receptors and are phosphorylated at their C-terminal serines (6–8). On phosphorylation, these receptor-regulated Smads form heteromeric complexes with the common Smad, Smad4, and translocate from cytoplasm into nucleus (2–5). Smad proteins contain highly conserved N-terminal MH1 and C-terminal MH2 domains (2–5). The N-terminal domains of Smads3 and 4 have sequence-specific DNA-binding activity (9–13), whereas the C-terminal domains mediate protein–protein interactions and, together with part of the linker region, are the transcriptional activation domains (2–5, 14). Therefore, Smads are ligand-regulated transcription factors. On nuclear localization, Smad3/4 heteromeric complexes activate target gene expression by interacting with their cognate DNA-binding sites, with other sequence-specific transcription factors such as FAST1 (15, 16), FAST2 (17), AP1 (18), TFE3 (19) and 1,25-dihydroxyvitamin D_3 receptor (20), or with the CREB-binding protein (CBP)/p300 family of coactivators (21–24).

The biological effects of glucocorticoids are mediated by intracellular glucocorticoid receptor (GR), a member of the nuclear receptor superfamily (25–27). GR is a ligand-inducible transcription factor that positively and negatively modulates gene expression through diverse mechanisms. First, GR regulates target gene expression through a glucocorticoid response element (GRE)-dependent mechanism (25, 26). Depending on the nature of the GRE, GR binding can result in activation or repression of genes containing GR-binding sites (28). Alternatively, GR can also modulate the expression of genes through a GRE-independent mechanism, which is mediated in part through protein–protein interactions of GR with other sequence-specific DNA-binding factors or coactivators. GR and two groups of physiologically important transcription factors, AP1 and NF-κB, have been reported to mutually interfere with each other's activity (26, 27, 29). The cross talk between GR and AP1 on the composite response element of the proliferin gene promoter is more complex, and the outcome is determined by the composition of the AP1 subunits (30–32). GR can also cooperate with transcription factors, including octamer transcription factors Oct-1 and Oct-2, C/EBPβ, and Stat5, to activate transcription (33). The recent finding that GRE binding by GR is not required for viability (34) indicates the important role of the GRE-independent pathway in mediating the biological effects of GR.

The TGF-β and glucocorticoid signaling pathways interact both positively and negatively in regulating a variety of physiologic and pathologic processes, although the molecular mechanisms involved remain to be established. Glucocorticoids inhibit the TGF-β-induced expression of extracellular matrix proteins including fibronectin (35) and collagen (36, 37), and proteinase inhibitors such as tissue inhibitors of metalloproteinases (38). Hence, glucocorticoids and TGF-β may be important opposing physiological regulators of wound healing and fibrosis (39, 40). In addition, glucocorticoids and TGF-β antagonistically regulate bone formation (ref. 41 and refs. therein) and tight junction formation (42).

We have investigated the molecular mechanisms by which GR inhibits transcriptional activation of the type-1 plasminogen activator inhibitor (PAI-1) gene by TGF-β in human Hep3B cells. The results reported here show that GR activation by dexamethasone (Dex) inhibits the TGF-β induction of luciferase reporter constructs containing either 800 bp of PAI-1 promoter or the oligomerized 12-bp TGF-β-responsive sequence (TRS) at $-732/-721$. We further demonstrate that the inhibitory effect of GR on TGF-β signaling is mediated through both functional and physical interaction with the transcriptional activation function of Smad3. These results provide insights into the cross-regulation between GR and TGF-β signaling pathways.

Materials and Methods

Plasmids. Expression plasmids for Myc-tagged human Smad4, glutathione *S*-transferase (GST)-Smad3, and GST-Smad4 were provided by Rik Derynck (University of California, San Francisco) (7, 43). GAL4Smad4C was provided by Joan Massagué (Sloan–Kettering Memorial Institute) (14). The human GR expression plasmid pRShGRα was provided by Ronald Evans (Salk Institute). The reporter plasmids $pTRS_6$E1b-luc and

Abbreviations: TGF-β, transforming growth factor-β; GR, glucocorticoid receptor; Dex, dexamethasone; PAI-1, type-1 plasminogen activator inhibitor; TRS, TGF-β-responsive sequence; GST, glutathione *S*-transferase; GRE, glucocorticoid response element; CMV, cytomegalovirus; Smad3FL, full-length Smad3.

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pPAI(−800/+22)-luc were constructed as described (12). GST-Smad3C, containing amino acids 172–425 of Smad3, was constructed by inserting the corresponding PCR fragments into the *Bam*HI and *Eco*RI sites of pGEX-3X. GAL4Smad3C was constructed by inserting the corresponding PCR fragments into the *Bam*HI and *Kpn*I sites of pSG424 (44). The reporter plasmids pG5B-luc and pGRE$_4$E1b-luc were constructed by inserting five copies of the GAL4-binding site and four copies of a 15-bp GRE from the rat tyrosine aminotransferase gene (45) upstream of the E1bTATA box of pE1b-luc (12) respectively. The pCMVGR-Flag (cytomegalovirus, CMV) plasmid that expresses human GR with Flag-tag at its C terminus was constructed by inserting the human GR and Flag-tag into a CMV expression vector. The pCMVSmad3FL-Myc (full-length Smad3, Smad3FL) and pCMVSmad3C-Myc plasmids that express full-length and the C-terminal activation domain of human Smad3 with Myc-tag at its C terminus were constructed by inserting Smad3FL or Smad3C and Myc-tag into a CMV expression vector respectively. The plasmid for GAL4VP16 was provided by M. R. Green (St. Louis University). GR mutants GR 1–488, GR Δ 77–262, GR418–777, and GR-GAL-GR were provided by F. Lemaigre (Louvain University Medical School, Brussels) (46, 47). GR (D4X) and N454D/A458T were from A. C. B. Cato (Institute of Genetics, Karlsruhe, Germany) (48).

Transfection and Luciferase Assays. Hep3B cells were transfected with luciferase reporter constructs by using FuGENE 6 (Boehringer Mannheim). At 12 h after transfection, the cells were treated with 50 pM TGF-β and/or 100 nM Dex (Sigma) for 24 h, and luciferase activity was measured by using the Promega luciferase assay system.

Cell Extracts and Protein Purification. Whole cell extracts from transfected COS cells were prepared as described (12). GST-Smads were purified as described (12); the concentration and purity of the fusion proteins were determined by SDS/PAGE and Coomassie blue staining, with BSA as standard.

***In Vitro* Transcription and Translation.** The *in vitro* transcription and translation reactions were carried out by using the TNT-coupled reticulocyte lysate system (Promega) per the manufacturer's protocol.

***In Vitro* Protein Interaction Assays.** *In vitro*-translated proteins or whole cell extracts from transfected COS cells were incubated with the GST proteins immobilized on glutathione-agarose beads in a binding buffer containing 20 mM Tris·HCl (pH 7.9), 10% glycerol, 100 mM KCl, 5 mM $MgCl_2$, 0.5 mM EGTA, 0.5 mM EDTA, 2 mM DTT, and 0.2% IGEPAL-CA-630 with protease inhibitors. The binding mixture was incubated at room temperature for 1 h. Beads were washed four times with 300 μl binding buffer, resuspended in SDS sample buffer, and boiled for 5 min, and proteins were separated on 10% SDS/PAGE and transferred to nitrocellulose membrane. The affinity-purified GR-specific antibody was from Santa Cruz Biotechnology. Flag-GR and Myc-Smads were detected by using anti-Flag M2 monoclonal antibody (IBI; Eastman Kodak) and anti-Myc 9E10 monoclonal antibody (Santa Cruz Biotechnology), respectively, and chemiluminescence (ECL, Amersham Pharmacia Biotech).

Coimmunoprecipitation Assays. COS cells in 100-mm dishes were transfected with the Flag-GR expression vector together with Myc-Smad plasmids as indicated by using Lipofectamine (GIBCO). Cells were cultured in the presence of 100 nM Dex for 48 h before preparation of extracts (12). Cell extracts were incubated with anti-Myc or anti-Flag antibody and protein A-Sepharose for 4 h at 4°C in the binding buffer containing 75 mM KCl. The immunoprecipitates were washed four times in the binding buffer containing 50 mM KCl. The presence of GR/Smad complexes was detected by immunoprecipitation with anti-Myc 9E10 monoclonal antibody and immunoblotting by using anti-Flag M2 monoclonal antibody and chemiluminescence.

Results and Discussion

Glucocorticoids Repress TGF-β Transcriptional Activation of PAI-1. To examine the effect of glucocorticoids on TGF-β transcriptional activation of PAI-1, the TGF-β responsive Hep3B cells that express no functional endogenous GR (see Fig. 2) were cotransfected with the GR expression plasmid, pRShGRα, together with a luciferase reporter, pPAI (−800/+22)-luc containing human PAI-1 promoter sequences from −800 to +22. Subsequent treatment of the cells with TGF-β resulted in a more than 30-fold increase in luciferase activity (Fig. 1*A*). Although Dex, a synthetic glucocorticoid, caused a modest (≈2-fold) enhancement of the luciferase activity in the absence of TGF-β, Dex treatment resulted in more than 70% inhibition of TGF-β induction of this promoter (Fig. 1*A*). The Dex inhibition of pPAI (−800/+22)-luc is GR dependent because Dex showed no inhibitory effect on transcriptional activation by TGF-β in Hep3B cells in the absence of cotransfected GR (data not shown).

We have recently reported the identification of a TGF-β-responsive sequence (TRS) at −732/−721 of the human PAI-1 promoter that is capable of conferring TGF-β responsiveness to a heterologous promoter. Smad3 and Smad4 bind directly to the TRS through their conserved MH1 domains (12). Therefore, we determined whether GR could repress TRS-mediated transcriptional activation by TGF-β. Incubation of cells with TGF-β led to a more than 30-fold activation of the pTRS$_6$E1b-luc reporter, confirming previous results (12). Dex alone had no effect on this promoter, but simultaneous treatment with Dex and TGF-β caused a >90% inhibition of induction of the pTRS$_6$E1b-luc reporter by TGF-β (Fig. 1*B*). These results demonstrate that glucocorticoids repress TGF-β transcriptional activation of both pPAI (−800/+22)-luc and pTRS$_6$E1b-luc in a GR-dependent manner.

TGF-β Does Not Repress GR Induction of a GRE-Containing Promoter. Studies on the cross-regulation between GR and two groups of transcription factors, AP1 and NF-κB, have demonstrated that the inhibitory effects are mutual (26, 47–50). We therefore tested the effect of TGF-β on GR transcriptional activation of pGRE$_4$E1b-luc. Hep3B cells were transiently transfected with the pGRE$_4$E1b-luc reporter alone or together with 200 ng of pRShGRα plasmid. Cells were then incubated in the presence or absence of Dex, TGF-β, or both for 24 h before harvesting. Dex activated the pGRE$_4$E1b-luc reporter in Hep3B cells almost 1,000-fold. The effect of Dex depended completely on the exogenous expression of GR (Fig. 2), confirming that these cells do not express functional endogenous GR. TGF-β had no effect on the basal activity of the pGRE$_4$E1b-luc reporter, nor did it inhibit GR-activated transcription of the pGRE$_4$E1b-luc reporter (Fig. 2*A*). To control for problems that might arise from overexpression of transfected GR, a titration experiment was performed in which cells were cotransfected with increasing concentrations of GR from 2.5 ng to 40 ng, the latter producing an approximately half-maximal Dex response. As shown in Fig. 2*B*, GR activated the expression of pGRE$_4$E1b-luc reporter in a GR dose-dependent manner, and TGF-β showed no inhibitory effect on this transcriptional activation. Finally, overexpression of exogenous Smad3, Smad4, or both had no inhibitory effects on GR transcriptional activation of the GRE-containing reporter (data not shown). Taken together, these results suggest that the Dex repression of TGF-β is not reciprocal.

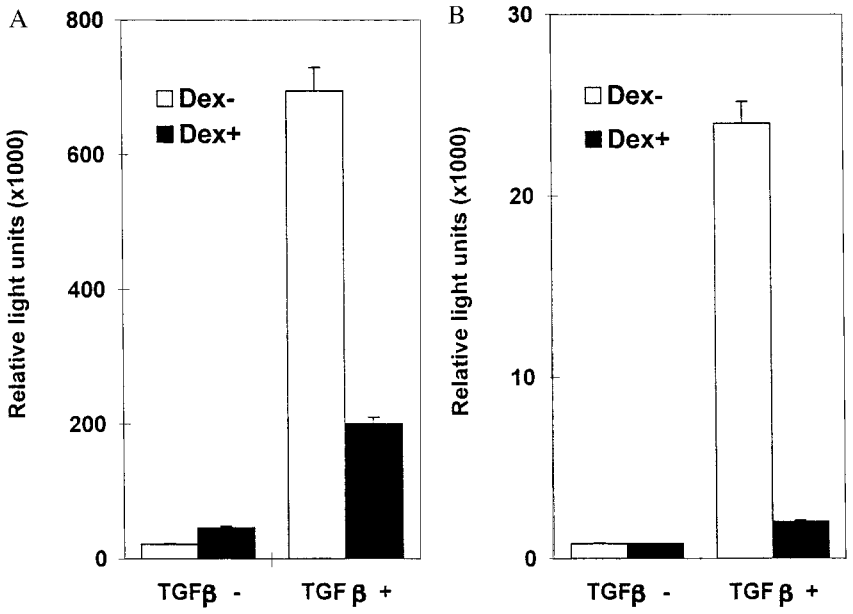

Fig. 1. Dex inhibition of the TGF-β transcriptional activation of human PAI-1. Hep3B cells cultured in 6-well plates were transiently transfected with 0.5 μg of the reporter plasmid pPAI-1(−800/+22)-luc (*A*) or pTRS$_6$E1b-luc (*B*) and 0.2 μg of human GR expression plasmid pRShGRα. Cells were cultured for 24 h in the presence or absence of 50 pM TGF-β and/or 100 nM Dex. Results are presented as the mean ± SD (n = 3) of relative luciferase activity.

GR Specifically Inhibits Smad3 and Smad4 Transcriptional Activation. The observation that GR strongly repressed TRS-mediated TGF-β transcriptional activation (Fig. 1*B*), together with our previous studies showing that Smad3 and Smad4 bind directly to TRS (12), suggested that GR might inhibit TGF-β signaling by directly targeting Smad3 and Smad4. The inhibitory effect of GR might be mediated by preventing the binding of Smad3/4 to TRS or by blocking the activation function of Smad3/4. To investigate this question, we have used the mammalian two-hybrid approach. By substituting the GAL4 DNA-binding domain for the MH1 domain of Smads 3 and 4, we hoped to avoid complications in interpretation resulting from the intramolecular interactions between MH1 and MH2 domains and their effects on the function of the activation domains. This approach should allow us to investigate directly the effect of liganded GR on the transcriptional function of Smad3 and Smad 4. Plasmids expressing fusion proteins between GAL4 DNA-binding domain and Smad3 or Smad4 activation domains were transfected into Hep3B cells together with GR and the pG5E1b-luc reporter containing GAL4-binding sites. The cells were incubated in the

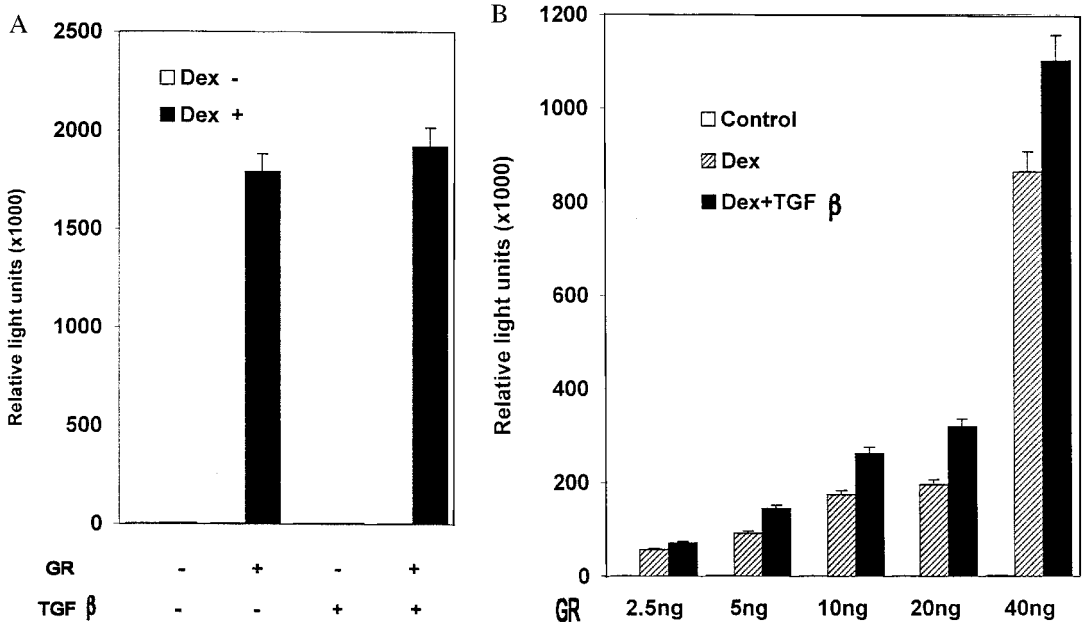

Fig. 2. GR-activated transcription is not inhibited by TGF-β. Hep3B cells were transiently transfected with 0.5 μg of the reporter plasmid pGRE$_4$E1b-luc and 0.2 μg (*A*) or 2.5–40 ng of pRShGRα, as indicated (*B*). Cells were cultured for 24 h in the presence or absence of 50 pM TGF-β and/or 100 nM Dex. Results are presented as the mean ± SD (n = 3) of relative luciferase activity.

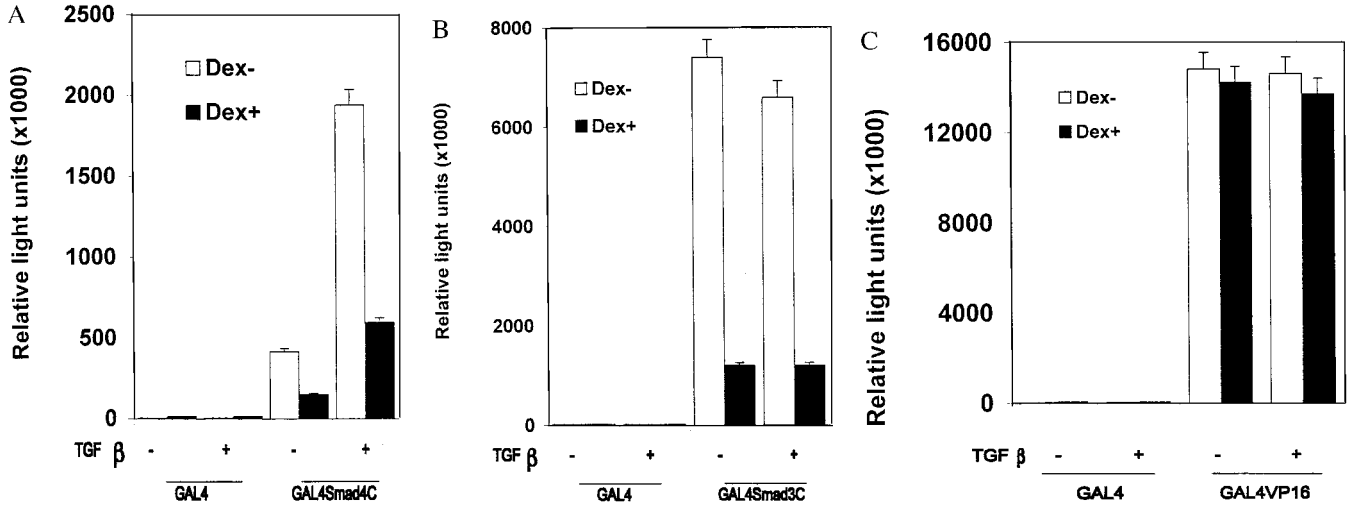

Fig. 3. Smad3/4 activation domain-activated transcription is specifically inhibited by Dex. Hep3B cells were transiently transfected with 0.5 μg of the reporter plasmid pG5B-luc, 0.5 μg of GAL4Smad4C (*A*), 10 ng of GAL4Smad3C (*B*), or 10 ng of GAL4VP16 (*C*) expression plasmids and pRShGRα. As a control, 0.5 μg of the plasmid, pSG424, which expresses the GAL4 DNA-binding domain (GAL4 (1–147) only, was transfected. Cells were cultured for 24 h in the presence or absence of 50 pM TGF-β and/or 100 nM Dex. Results are presented as the mean ± SD (n = 3) of relative luciferase activity.

presence or absence of Dex, TGF-β, or both. GAL4Smad4C (14) stimulated the expression of the reporter gene 140-fold in the absence of TGF-β and 640-fold in the presence of TGF-β. The effect of TGF-β is presumably a function of phosphorylation/activation of endogenous Smad 3 in these cells. Both TGF-β-independent and TGF-β-dependent activation by GAL4Smad4C were inhibited by approximately 70% by Dex (Fig. 3*A*). GAL4Smad3C activated the reporter gene expression >700-fold in the presence or absence of TGF-β. The observation that transactivation by overexpressed Smad3C is much stronger than that by Smad4C and that it is TGF-β independent is consistent with previous studies (21). Treatment of cells with Dex resulted in >80% inhibition of the GAL4Smad3C-activated reporter gene expression (Fig. 3*B*). To establish the specificity of the observed GR repression of Smad3 and Smad4 transcriptional activation, the effect of liganded GR on GAL4VP16 transcriptional activation was tested. As shown in Fig. 3*C*, liganded GR does not repress the equally strong transcriptional activation by GAL4VP16 under the same assay conditions, indicating the specificity of the inhibition. The results from these experiments demonstrated that GR specifically inhibits Smad3 and Smad4 activation function in a ligand-dependent manner, thus identifying the Smad3/4 activation domains as primary targets of GR action.

GR Interacts with Smad3 *in Vivo* and *in Vitro*. Results from the above studies established a functional interaction between GR and Smad3/4; therefore, we investigated their possible physical interaction. GST pull-down assays were carried out by incubating whole cell extracts prepared from COS cells expressing functional Flag-tagged GR with GST-Smad fusion proteins immobilized on glutathione-agarose beads. The bound proteins were analyzed by immunoblotting by using the M2 anti-Flag antibody. As shown in Fig. 4*A*, GSTSmad3, but not GST alone, binds a polypeptide of 94 kDa that was confirmed to be GR by immunoblotting with affinity-purified anti-GR antibody (data not shown). This experiment also showed that more GR bound the GST-Smad3C than full-length Smad3 (GST-Smad3FL). Similar results were obtained when *in vitro*-translated (IVT) GR-Flag was tested for binding to GST-Smad3C or GST-Smad3FL, indicating their direct interaction *in vitro* (Fig. 4*B*). These results suggest that GR directly interacts with Smad3, although we cannot rule out the possibility that other polypeptides in the rabbit reticulocyte lysate might mediate the interaction. The functional integrity of the Flag-tagged GR was confirmed by demonstrating its ability to both activate pGRE$_4$E1b-luc reporter and repress transcriptional activation of TRS by TGF-β (data not shown). To determine whether GR interacts with Smad3 *in vivo*, we transfected COS cells with expression plasmids for Flag-tagged GR and Myc-tagged Smads, as indicated. Coimmunoprecipitation experiments revealed the *in vivo* association between GR and Smad3C. The interaction of Smad3FL and GR is detectable, albeit to a much lesser extent than that of Smad3C (Fig. 4*C*). Full-length Smad4 showed little if any detectable interaction with GR. Because the Smads were expressed at similar levels *in vivo* (Fig. 4*C Bottom*), the observed difference in binding to GR between the Smad3C and Smad3FL could not be explained by a difference in their level of expression. This result is also in agreement with the results from *in vitro* protein interaction assays. Both IVT GR and overexpressed GR also bind more efficiently to GST-Smad3C than GST-Smad3FL. Consistent with the *in vivo* results, GST pull-down by using GST-Smad4FL failed to detect an interaction between Smad4 and GR *in vitro* under the same conditions (data not shown). Taken together, the results demonstrate that GR both functionally and physically interacts with the C-terminal activation domain of Smad3. It is likely that the functional repression of Smad4C depends on its interaction with Smad3C, which directly interacts with GR.

Repression Requires the C-Terminal Domain of GR. To begin to identify the domains of the GR required for repression, we have examined the ability of various truncation or deletion mutants of GR to repress TGF-β transcriptional activation of the pTRS$_6$E1b-luc reporter. As shown in Fig. 5, deletion of the N-terminal 417 amino acids (GR 418–777), including the AF1 activation domain, does not impair the ability of the GR to repress TGF-β-activated transcription. Similarly, an internal deletion of the AF1 domain, amino acids 77–262, is also without effect (data not shown). In contrast, deletion of the C-terminal region, including the ligand-binding domain and the AF2 activation domain (GR 1–488), completely abolished ligand-dependent repression of TGF-β transcriptional activation. Although capable of DNA binding, GR 1–488 is defective in

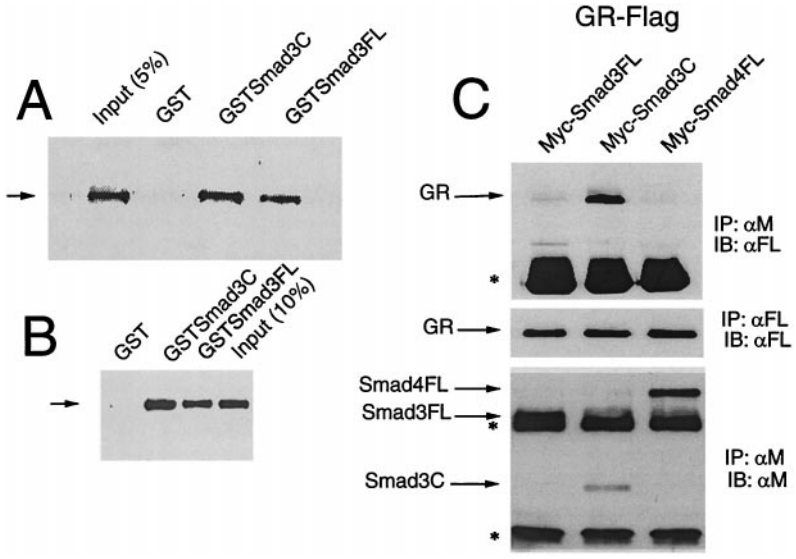

Fig. 4. Smad3 activation domain interacts with GR *in vitro* and *in vivo*. (*A*) GST pull-down assays were carried out by incubating whole cell extracts prepared from COS cells expressing functional Flag-tag GR with GST-Smad fusion proteins, including the C-terminal activation domain of Smad 3 (amino acids 172–425, GST-Smad3C) or full-length Smad3 (GST-Smad3 FL), immobilized on glutathione-agarose beads. Bound proteins were identified by immunoblotting Flag-GR, by using anti-Flag M2 monoclonal antibody and chemiluminescence. (*B*) Similar studies were performed by using *in vitro*-translated GR-Flag. *In vitro* transcription and translation reactions were carried out by using the TNT-coupled reticulocyte lysate system (Promega). (*C*) Coimmunoprecipitation analysis. COS cells were transfected with pCMVGR-Flag and Myc-tagged Smads, as indicated, by using lipofectamine, and cultured in the presence of 100 nM Dex for 48 h. Cell extracts were incubated with anti-Myc or anti-Flag antibody and protein A-Sepharose. GR/Smad complexes were detected by immunoprecipitation with anti-Myc 9E10 monoclonal antibodies and immunoblotting by using anti-Flag M2 antibodies and chemiluminescence. The arrow indicates the position of GR and the asterisk, the position of immunoglobulins. IP: immunoprecipitation; IB: immunoblotting. *Middle* shows the expression of GR-Flag; *Bottom* shows the expression of the Myc-tagged Smads.

transcriptional activation function (47). GR mutants, N454D/A458T and D4X, containing amino acid substitutions in the dimerization interface (D-loop), are defective in DNA binding and transactivation, but are able to inhibit AP1 transcriptional activation as efficiently as the wild-type GR (48). As shown in Fig. 5, GR D4X efficiently repressed TGF-β transcriptional activation, as did N454D/A458T (data not shown). These results suggest that DNA binding and dimerization of GR is not required for repression of TGF-β signaling. Similarly, replacement of the DNA-binding domain of GR with that of Gal4 also has no effect on repression (data not shown). These data suggest that the C-terminal domain of GR is required for repression, whereas the N-terminal activation domain and DNA-binding domain are not. This is in contrast to the GR repression of AP1 and NF-kB, both of which require the DNA-binding domain (refs. 48- 50 and refs. therein). Further studies will be necessary to define the exact domains required for repression.

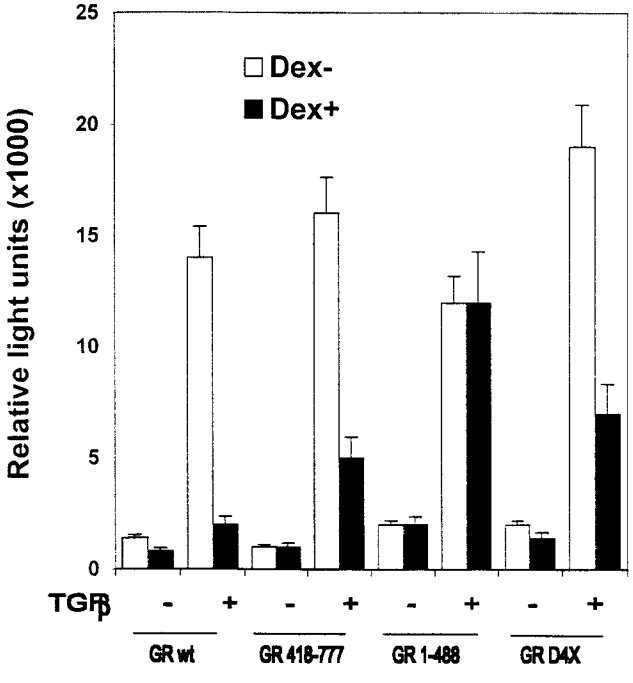

Fig. 5. The C-terminal domain of GR is required for repression of transcriptional activation by TGF-β. Hep3B cells cultured in 6-well plates were transiently transfected with 0.5 μg of the reporter plasmid pTRS6E1b-luc and 0.2 μg of expression plasmids for wild-type or mutant human GR, as indicated. Cells were cultured for 24 h in the presence or absence of 50 pM TGF-β and/or 100 nM Dex. Results are presented as the mean $\pm$ SD ($n = 3$) of relative luciferase activity.

In summary, the studies presented here demonstrate that GR inhibits transcriptional activation of human PAI-1 gene expression by TGF-β in a Dex-dependent manner. Using luciferase reporters containing a Smad3/4-binding element (TRS) or GAL4-binding sites, we further show that the target of GR in mediating the observed repression is the activation domain of Smad3. Protein–protein interaction studies show that GR interacts with the activation domain of Smad3 *in vitro* and *in vivo*. Taken together, these studies suggest that the molecular basis of the GR repression of TGF-β transcriptional activation involves direct protein–protein interactions between GR and Smad3. The repression of Smad4 transactivation is presumably secondary to this effect.

Glucocorticoid hormone receptor acts synergistically or antagonistically with a number of signaling pathways. The enhancement of 1,25-dihydroxyvitamin D_3 receptor (VDR) transactivation by Smad3 involves physical interaction of liganded VDR, Smad3, and SRC-1/TIF2 (20). The mutual inhibition between GR and AP1 or NF-κB has been attributed to their direct interaction (26, 49). The mutual inhibition between GR and AP1 has also been reported to involve competition for limiting amounts of the coactivators CBP/p300 (51) and SRC-1 (52). In

addition, GR has been reported to inhibit AP1 activation by blocking the jun N-terminal kinase pathway (53). GR can also induce the synthesis of the inhibitor protein IκB, which could play a part in GR inhibition of NF-κB transcriptional activation (54, 55). Finally, the opposing effects of GR and TGF-β on bone formation and bone matrix protein synthesis might be mediated in part by inhibiting the expression of the type I TGF-β receptor (41). Recently, it was reported that the interferon-γ/Stat pathway inhibits TGF-β signaling in U4A/Jak1 cells by inducing the expression of the inhibitory Smad, Smad7 (56). Future studies will determine whether any of these mechanisms also play some role in the glucocorticoid repression of PAI-1 gene transcription by TGF-β.

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