RhoB, Not RhoA, Represses the Transcription of the Transforming Growth Factor β Type II Receptor by a Mechanism Involving Activator Protein 1

Jalila Adnane‡, Edward Seijo§, Zhi Chen‡, Francisco Bizouarn‡, Martha Leal§, Said M. Sebti†‡, and Teresita Muñoz-Antonia§¶

From the ‡Drug Discovery Program and §Molecular Oncology Program, H. Lee Moffitt Cancer Center and Research Institute, Departments of Oncology and Biochemistry and Molecular Biology, University of South Florida, Tampa, Florida 33612

The transforming growth factor-β (TGF-β) type I (TβR-I) and type II (TβR-II) receptors are responsible for transducing TGF-β signals. We have previously shown that inhibition of farnesylation transferase activity results in an increase in TβR-II expression, leading to enhanced TGF-β binding, signaling, and inhibition of tumor cell growth, suggesting that a farnesylated protein(s) exerts a repressive effect on TβR-II expression. Likely candidates are farnesylated proteins such as Ras and RhoB, which are both farnesylated and involved in cell growth control. Neither a dominant negative Ha-Ras, constitutively activated Ha-Ras, or a pharmacological inhibitor of MEK1 affected TβR-II transcription. However, ectopic expression of RhoB, but not the closely related family member RhoA, resulted in a 5-fold decrease of TβR-II promoter activity. Furthermore, ectopic expression of RhoB, but not RhoA, in a significant decrease of TβR-II protein expression and resistance of tumor cells to TGF-β-mediated cell growth inhibition. Deletion analysis of the TβR-II promoter identified a RhoB-responsive region, and mutational analysis of this region revealed that a site for the transcription factor activator protein 1 (AP1) is critical for RhoB-mediated repression of TβR-II transcription. Electrophoretic mobility shift assays clearly showed that the binding of AP1 to its DNA-binding site is strongly inhibited by RhoB. Consequently, transcription assays using an AP1 reporter showed that AP1-mediated transcription is down-regulated by RhoB. Altogether, these results identify a mechanism by which RhoB antagonizes TGF-β action through transcriptional down-regulation of AP1 in TβR-II promoter.

Resistance to the anti-proliferative effects of transforming growth factor β (TGF-β) in a wide variety of human tumors has been associated with inactivation of either the TGF-β receptor or their substrates, the Smad proteins (1). Possible mechanisms for the inactivation of these molecules include inactivating mutations, increased degradation, and transcriptional down-regulation (2). In particular, there is a strong association between TGF-β type II receptor (TβR-II) inactivation and cancer because many tumors express low levels of TβR-II mRNA and/or protein, and this lack of TβR-II expression correlates with resistance to the anti-proliferative effects of TGF-β (3–15). Several groups have examined the genetic integrity of the TβR-II gene and found genetic alterations in both the coding and regulatory region (8, 16–23). However, there are tumors in which low levels of TβR-II mRNA could not be explained by genetic alterations in the promoter or coding region (10), which suggests that transcriptional dysregulation of TβR-II may play an important role in the escape from TGF-β growth control.

Transcriptional regulation of TβR-II likely plays an important role in modulating TGF-β responses since transformation of epithelial cells with the adenovirus E1A gene has been associated with down-regulation of TβR-II expression and TGF-β resistance (24). Recently, fusion products from the ets transcription factor gene family and the EWSRI1 gene have been shown to down-regulate transcription of TβR-II, suggesting that inactivation of TβR-II might be important in Ewing sarcoma tumorigenesis (25). Choi et al. (26) identified DNA-binding sites for ERT (ets-related transcription factor) and showed that ERT activates transcription of the TβR-II gene (26). Overexpression of ERT induced the expression of TβR-II and restored TGF-β signaling in a TGF-β-resistant breast cancer cell line (27). We have previously reported that an A to G mutation at position –364 in the TβR-II promoter in A431 tumor cells results in reduced TβR-II promoter activity (28). We identified the protein that binds to this site as the transcriptional repressor CDP/Cut (CCAAAT displacement protein) and demonstrated that overexpression of CDP/Cut decreases TβR-II transcription (28).

Recently, we have shown that inhibition of farnesylation transferase activity increases TβR-II expression, leading to enhanced TGF-β signaling and inhibition of tumor cell growth, which suggested that a farnesylated protein(s) is exerting a repressive effect on TβR-II expression (29). Potential candidates are farnesylated proteins such as the low molecular weight GTPases Ras and RhoB, which are involved in cell growth control. The Ras and Rho small GTPases serve as molecular switches that cycle between GTP-bound (active) and dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase.

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† To whom correspondence and reprint requests should be addressed:
H. Lee Moffitt Cancer Center and Research Institute, University of South Florida, 12902 Magnolia Dr., Tampa, FL 33612. For S. M. S.: Tel.: 813-979-6734; Fax: 813-979-6748; E-mail: sebti@moffitt.usf.edu. For T. M.-A.: Tel.: 813-979-3884; Fax: 813-979-3893; E-mail: antonia@moffitt.usf.edu.

‡ The abbreviations used are: TGF-β, transforming growth factor β; TβR-II, TGF-β type II receptor, AP1, activator protein 1; MTT, 3-(4,5-
GDP-bound (inactive) states. Ras proteins play an important role in cell proliferation and transformation (30), whereas Rho proteins have been shown to influence a number of cellular processes including motility, adhesion, cell polarization, metastasis, and transformation (31).

In this study, we show that RhoB antagonizes TGF-β anti-proliferative and transcriptional responses. Moreover, we show that RhoB inhibits TGF-β signaling by down-regulating TβRII transcription and protein expression. Finally, we show that API-1 is a downstream target of RhoB-mediated inhibition of TGF-β responses.

EXPERIMENTAL PROCEDURES

Cell Culture—The human pancreatic carcinoma-derived cell line, Panc-1, was grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. The normal human keratinocyte cell line, HaCaT (obtained from Dr. N. E. Fusenig, German Cancer Research Center, Germany) (32) was maintained in Dulbecco’s modified Eagle’s medium supplemented with penicillin, streptomycin, and 10% fetal bovine serum.

Chemicals—UO126, a MEK1 and MEK2 inhibitor (33), was obtained from Calbiochem. TGF-β1 was obtained from R&D Systems (Minneapolis, MN).

Plasmids—Plasmid p1233Luc contains the wild type 1233 base pairs upstream of the published 5′ end of the TβRII cDNA (34) to 50 base pairs downstream of the transcription start site fused to the luciferase reporter gene into the pGL3-Basic vector (Promega Corp., Madison, WI). TβRII promoter deletion mutants were generated by PCR amplification using specific restriction sites to facilitate directional cloning. A common 3′ oligonucleotide was used to generate the deletion mutants, 5′-ACGCGTTCAGATGTTCTGATCTACTAG-3′. After amplification, the fragments were digested with HindIII and MluI and cloned into the XhoI and MluI site of the pGL3-Basic vector. Point mutations within the transcription start site proximal region were generated by PCR using the technique of Saiki (35). The position of the different mutations in the TβRII promoter is schematically represented in Fig. 6. The sequence of the constructs was confirmed by DNA sequencing.

The pcDNA3-Ha-RasN17 vector encodes a dominant negative form of Ha-Ras (provided by Dr. Larry Feig, Tufts University School of Medicine, Boston, MA). pHa-Ras61L, which encodes a constitutively activated Ha-Ras (36), was obtained from Drs. Channing Der and Adrienne C. King (University of North Carolina). The pcDNA3-RhoA-WT vector was described before (37). The hemagglutinin-tagged RhoB construct encodes a human wild type RhoB. It was generated by inserting PCR-amplified hemagglutinin-RhoB cDNA into the pcDNA3 vector. Point mutations within the transcription start site proximal region were generated by PCR using the technique of Saiki (35). The position of the different mutations in the TβRII promoter is schematically represented in Fig. 6. The sequence of the constructs was confirmed by DNA sequencing.

RhoB, but Not RhoA or Ras, Down-regulates TβRII Gene Transcription—We have previously shown that the farnesyltransferase inhibitor, FTI-277, strongly enhances human tumor cell responsiveness to TGF-β by a mechanism involving an increase in the levels of the TβRII (29). These results suggested that a farnesylated protein(s) suppresses TβRII expression. Potential candidates are the farnesylated small GTPases RhoB and Ras, which have been shown to modulate TGF-β responses through different mechanisms (41–46). The effect of RhoB and the Ras/Raf/MEK pathway on the regulation of TβRII transcription was investigated in human keratinocytes, HaCaT, and human pancreatic carcinoma cells, Panc-1. The effect of the Ras pathways on TβRII transcription was determined by using a constitutively activated form of Ha-Ras, Ha-Ras61L, a dominant interfering mutant Ras, Ha-RasN17, and a pharmacological inhibitor of MEK1 and MEK2, UO126. Cells were transiently cotransfected with a full-length human TβRII promoter reporter, p1233Luc, and RhoB (wild type), Ha-Ras61L (constitutively activated), or Ha-RasN17 (dominant negative). Fig. 1, A and B, show that ectopic expression of RhoB repressed TβRII promoter transcriptional activity by about 3-fold in Panc-1 and HaCaT cells. In contrast, neither Ha-Ras61L or Ha-RasN17 suppressed TβRII promoter activity. The effect of Ha-RasN17 on TβRII promoter in Panc1 cells was further confirmed using the MEK inhibitor UO126. Panc1 cells were transiently transfected with p1233 and incubated with UO126 (1 μM) for 4 h before harvesting. At a concentration of 1 μM, which inhibits 100% of MEK1 and MEK2 activity (33), UO126 had no effect on TβRII promoter activity (data not shown). Fig. 1C shows that, as reported previously, RhoB and Ha-Ras61L stimulated and Ha-RasN17 suppressed SRE-mediated transcription (47–49), demonstrating that the overexpressed proteins are functional. Altogether, these results show that RhoB, but not Ras, represses TβRII transcription.

The amino acid sequence of RhoB is 86% identical to that of the closely related family member RhoA. Therefore we investigated if the down-regulation of TβRII promoter was a feature specific to RhoB or common to RhoB and RhoA. For these experiments, cells were transiently cotransfected with the full-length TβRII promoter reporter and Rhos A or RhoA expression vector (Fig. 2). Whereas RhoB-WT repressed TβRII promoter in a concentration-dependent manner, RhoA-wild type had no effect. Thus, TβRII promoter down-regulation is unlikely to be a general phenomenon of the Rho family but rather seems to be specific to RhoB.

RhoB Regulation of TβRII Expression—Protein expression in cells was visualized by immunohistochemical analysis as previously described (40).

RESULTS

Electrophoretic Mobility Shift Assay—Nuclear extracts were prepared as previously described (37). Oligonucleotides containing DNA-binding sites to API-1, Sp-1, and NF-Y, corresponding to the sites found in the TβRII gene promoter, were purchased from Invitrogen. The sequence of the probes, with the binding sites underlined, is as follows: A/GATATCTGGATCGCTCTAGGTTAATA-3′; 5′-CTGATAGCTCTTCGGGCCAGAAGA-3′; NF-Y probe: 5′-GCGTCTCTAGACTTCGACCTA-3′. The sequence of the probes, with the binding sites underlined, is as follows: A/GATATCTGGATCGCTCTAGGTTAATA-3′; 5′-CTGATAGCTCTTCGGGCCAGAAGA-3′; NF-Y probe: 5′-GCGTCTCTAGACTTCGACCTA-3′. Sense and antisense oligonucleotides with AGCT overhangs were annealed and labeled with Klenow enzyme to a specific activity of 1000 cpm/μl. Electrophoretic mobility shift assays were performed as previously described (37). For DNA binding competition, a 100-fold excess of unlabeled DNA was incubated with the protein extract for 10 min before the addition of labeled probe. Antibodies against Sp1 and API were purchased from Santa Cruz Biotechnology Inc. (San Antonio, CA). For supershift experiments, 1 μg of antibody was incubated with the reaction mixtures for 30 min at room temperature after incubation with the DNA probes.

MTT Assay—Cell growth was measured by an MTT assay as previously described (39).

Immunohistochemical Analysis—Protein expression in cells was visualized by immunohistochemical analysis as previously described (40).

RhoB Regulation of TβRII Expression—Protein expression in cells was visualized by immunohistochemical analysis as previously described (40).
Fig. 1. RhoB not Ha-Ras down-regulates TβR-II gene transcription. A, RhoB represses TβR-II promoter activity in human pancreatic cells, Panc-1. Panc-1 cells were transiently cotransfected with 6 μg of p1233Luc, a luciferase reporter carrying the full-length TβR-II promoter, and either 1 μg of RhoB (wild type), Ha-Ras61L (constitutively activated), Ha-RasN17 (dominant negative), or control vector, pcDNA3. 48 h post-transfection, samples were processed for luciferase and β-galactosidase activity, as described under "Experimental Procedures." The results are expressed as fold activation relative to the control, which was given the value of 1. B, RhoB represses TβR-II promoter activity in HaCaT cells. HaCaT cells were transiently cotransfected with p1233Luc and either RhoB or pcDNA3 control vector. The results are the averages ± S.D. of an experiment done in triplicate. C, Panc-1 cells were transiently cotransfected with a serum-responsive element luciferase reporter, pSRE-Luc, and either RhoB, Ha-Ras61L, Ha-RasN17, or pcDNA3. The results are expressed as fold activation relative to the control, which was given the value of 1. The results are representative of at least three independent experiments.

RhoB Antagonizes TGF-β-mediated Anti-proliferative and Transcriptional Responses—The inhibition of TβR-II expression by RhoB raises the question of whether RhoB-overexpressing cells become resistant to TGF-β. To test this hypothesis, we assessed the ability of TGF-β to inhibit cell proliferation in RhoB- or RhoA-overexpressing cells. Cells were incubated with different concentrations of TGF-β1 (0, 3, 10, and 30 ng/ml), and cell growth was determined by MTT assay, as previously described (39). As shown in Fig. 4A, whereas the growth of cells carrying the control vector was inhibited by 45, 50, and 50% at 3, 10, and 30 ng/ml, respectively, that of cells overexpressing RhoB was not inhibited at 3 and 10 ng/ml and inhibited by only 20% at the higher concentration of 30 ng/ml. In contrast to RhoB, RhoA-overexpressing cells were inhibited by TGF-β1 at the same level as the control cells, carrying the empty vector (Fig. 4B). Thus, RhoB overexpression interferes with TGF-β regulation of cell growth. To determine whether RhoB affects TGF-β-induced transcription, we used p3TP-Lux reporter, which carries three repeats of 12-O-tetradecanoylphorbol-13-acetate (TPA)-responsive element (TRE) and is commonly used to assess TGF-β signaling. The effect of TGF-β on p3TP-Lux reporter activity was assessed in both transiently (Fig. 4C) and stably (Fig. 4D) RhoB-transfected cells. TGF-β1 activated p3TP-Lux by 12-fold (Fig. 4C) and 2.5-fold (Fig. 4D). However, overexpression of RhoB completely inhibited TGF-β-mediated activation of p3TP-Lux. This is consistent with Engel et al. (41),
who reported similar effects in Mv1Lu cells.

**RhoB-responsive Region Is Located between −250 and −46 in TβR-II Gene Promoter**—The results described in Figs. 1 and 2 show that RhoB overexpression suppresses TβR-II transcription and protein expression. To identify the RhoB-responsive region in the TβR-II promoter, a series of progressive 5′ promoter deletion mutants was generated (Fig. 5). Panc-1 cells were transiently cotransfected with TβR-II promoter deletion constructs and RhoB expression vector. As shown in Fig. 5, the TβR-II promoter deletion constructs, p524Luc, p360Luc, and p250Luc, which lack up to 1000 base pairs from the 5′ end of TβR-II promoter, were repressed by RhoB at the same extent as with the full-length promoter (p1233Luc). However, a further deletion in the 5′ end of the promoter, which leaves 46 base pairs upstream of the transcription start site (p46Luc), abolished RhoB-mediated repression. Thus, RhoB-responsive region in TβR-II promoter is located between sequences −250 and −46, upstream of the transcription start site.

**Functional Analysis of TβR-II Gene Promoter in Response to RhoB**—The results described above identified the RhoB-responsive region in TβR-II promoter as a 250-base pair fragment upstream of the transcription start site. This region of the promoter carries DNA-binding sites for Sp1, NF-Y, and AP1 (51–53). To determine whether these DNA-binding sites are important for RhoB-mediated repression of TβR-II, mutations were introduced into this region in the p250Luc construct (Fig. 6). Panc-1 cells were transiently cotransfected with p250Luc mutants constructs and RhoB expression vector. As shown in Fig. 6, mutation of the AP1 DNA-binding site at position −185 abolished RhoB-mediated repression. Mutation of the SP1- or NF-Y-binding site at position −143 and −83, respectively, resulted in the loss of TβR-II basal activity (data not shown); thus, the effect of RhoB on these binding sites was assessed in an electrophoretic mobility shift assay.

**DNA Binding of AP1 Transcription Factor Is Inhibited by RhoB**—The functional analysis of TβR-II promoter suggested that the DNA-binding site for AP1 at position −185 is critical for RhoB-mediated repression. To further confirm the importance of this AP1 site in RhoB-mediated repression and determine whether SP1- or NF-Y-binding sites at position −143 and −83, respectively, play a role in the observed repression, electrophoretic mobility shift assays were performed (Fig. 7). Panc-1 cells were transiently transfected with RhoB expression vector, and nuclear extracts were prepared as described under...
“Experimental Procedures.” As probes, we used double-stranded oligonucleotides with sequences similar to those of TβR-II promoter containing Sp1, NF-Y, or AP1 DNA-binding site. As shown in Fig. 7A, ectopic expression of RhoB inhibits the binding of the protein complex to the AP1 probe in a dose-dependent manner (lanes 2–4). This protein-DNA binding was specific since it could be competed out by a 100-fold molar excess of unlabeled TβR-II/AP1 or consensus AP1 competitor (Fig. 7A, lanes 5 and 6) but not with a 100-fold molar excess of an unlabeled unrelated oligonucleotide (Fig. 7A, lane 7). This protein-DNA complex was supershifted by AP1 antibody (Fig. 7A, lane 8) in a specific manner, as normal IgG had no effect on the mobility of this complex (Fig. 7A, lane 9). In contrast to AP1, RhoB did not inhibit the binding of the protein complex to the Sp1-probe (Fig. 7B, lanes 2–4). This complex was supershifted by Sp1 antibody (Fig. 7B, lane 8) and competed out by a 100-fold molar excess of unlabeled TβR-II/Sp1 or consensus Sp1 competitor (Fig. 7B, lane 5 and 6). Similar to Sp1, the complex formed with the NF-Y probe (Fig. 7A and 6B, lanes 10 and 11) was not affected by RhoB overexpression. These results show the ability of RhoB to inhibit specifically AP1 DNA binding.

RhoB Down-regulates AP1 Transcriptional Activity—The results described in Figs. 6 and 7 demonstrate that the AP1 DNA-binding site, at position −185 in TβR-II, is a target for RhoB-mediated repression and suggest that RhoB may antagonize AP1-mediated transcription. To determine the effect of RhoB on AP1-mediated transcription, we used an AP1-luciferase reporter construct that carries four AP1 DNA-binding sites. For these experiments, Panc-1 cells were transiently cotransfected with AP1-Luc or, as a control, SRE-Luc and different amounts of RhoB expression vector (Fig. 8). AP1-mediated transcription was inhibited by a 2-, 3.3-, and 6.6-fold in cells transfected with RhoB at 0.2, 0.6, and 1.8 μg of RhoB expression vector, respectively (Fig. 8A). In contrast, SRE-mediated transcription was stimulated by a 2-, 3.5-, and 7-fold in a dose-dependent manner (Fig. 8B). These results show that AP1 transcriptional activity is inhibited by RhoB. The inhibition of AP1 by RhoB is a potential mechanism by which RhoB down-regulates TβR-II transcription.

DISCUSSION

Resistance to TGF-β anti-proliferative action is believed to be a major contributor to malignant progression, and evidence indicates that TGF-β receptors (TβR-I and TβR-II) and their downstream signaling molecules can act as tumor suppressors (1). One major mechanism by which tumors resist TGF-β is a decrease in TGF-β type II receptor expression. In a variety of human tumors, this decrease in TβR-II expression is due to mutations or deletions in the coding region of the TβR-II gene (8, 16–18). However, tumor types such as pancreatic carcinomas do not harbor mutations or deletions in the TβR-II gene.
but rather express very low levels of receptor (13, 54, 55). The mechanism by which this repression of TβR-II expression occurs is not known. Some studies suggest that the mechanism underlying the down-regulation of TGF-β receptors might involve either c-Ha-Ras or Ki-Ras (45, 56). However, these studies relied on 125I-TGF-β cross-linking studies and did not determine the actual expression levels of TGF-β receptors. In the present study, we provide evidence using a pharmacological inhibitor of MEK, activated Ha-Ras61L, and the dominant negative Ha-RasN17 that the Ras pathway is not involved in the regulation of TβR-II transcription in Panc-1 cells. However, Ras could still be involved in TGF-β resistance by interfering with components of the TGF-β-signaling pathways other than TβR-II. Indeed, Ras has been shown to induce TGF-β resistance by triggering Smad phosphorylation by ERK1 (extracellular signal-regulated kinase 1), resulting in the inability of the Smads to translocate to the nucleus (43, 57, 58). The lack of effect of Ras on TβR-II gene expression coupled with our previous finding showing that farnesyl transferase inhibitors enhance TβR-II expression (29) suggested that a farnesylated protein other than Ras may be responsible for the inhibition of TβR-II expression. Here we show that RhoB, a member of the Ras superfamily, suppresses TβR-II expression. Furthermore, RhoB also inhibited TGF-β-stimulated signaling and prevented TGF-β anti-proliferative effect. The fact that RhoB blocks TGF-β signaling is consistent with previous reports that show that RhoB inhibits TGF-β stimulation of p3TPLux in Mv1Lu cells (41). Interestingly, RhoA, a closely related member to RhoB, had no effect on TβR-II expression. The lack of effect of RhoA on TβR-II expression cannot be generalized to all cell types since a previous study has shown the ability of RhoA to regulate TβR-II transcription in chicken embryonic heart cells (59). It is clear, however, that the repression of TβR-II by RhoB in Panc-1 and HaCaT cells is specific because neither RhoA nor Ras affect the transcriptional activity of the TβR-II promoter.

The physiological function of RhoB appears to be different from that of other Rho family members such as, RhoA, Rac1,

**FIG. 5.** RhoB-responsive region is located between −250 and −46 in TβR-II gene promoter. *Left panel,* schematic representation of the 5’ deletion fragments of the TβR-II gene promoter. For each TβR-II deletion construct, the position from the transcription start site is indicated. *Luc,* coding region of luciferase. *Right panel,* Panc-1 cells were transiently cotransfected with full-length TβR-II (p1233Luc) or TβR-II deletion constructs (as indicated) and RhoB or pcDNA3 control vector. The results are expressed as fold induction relative to control, which was given the value of 1. The results are representative of at least three independent experiments.

**FIG. 6.** Functional analysis of TβR-II gene promoter in response to RhoB. *Left panel,* schematic representation of the wild type sequence of human TβR-II promoter and point mutations introduced into the binding sites for AP1 and Sp1 transcription factors. *Right panel,* Panc-1 cells were transiently transfected with wild type (p250Luc) and mutant (p250Sp1, p250/AP1) TβR-II promoter constructs and RhoB or pcDNA3 control vector. The results are expressed as fold induction relative to control, which was given the value of 1. The results are representative of at least three independent experiments.
and Cdc42. For example, RhoA, Rac1, and Cdc42 promote cell proliferation, transformation, and metastasis (31), whereas RhoB has been shown to antagonize malignant transformation and suppress human tumor growth in nude mice (39, 60–62).

In contrast to other Rho family members, RhoB has a short half-life, is present in low levels in growing cells, and is inducible by stress such as UV and DNA-damaging agents (63–65). The induction of RhoB during stress suggests that RhoB may play a role in cell growth control and/or DNA repair mechanisms. This is supported by the fact that overexpression of RhoB, whether in its geranylgeranylated form (RhoB-GG) or farnesylated form (RhoB-F), inhibits tumor cell growth and transformation (39).

Furthermore, RhoB and the other low molecular weight GTPases (i.e. RhoA and Rac1) also differ in their ability to modulate transcription via AP1. The results of our analysis with TβR-II promoter mutant constructs revealed that the AP1 site at position –185 from the transcription start site is critical for RhoB regulatory effect. The binding of AP1 to its DNA-binding site on the TβR-II promoter was strongly decreased by RhoB. Thus, RhoB represses TβR-II transcription by inhibiting, at least in part, the binding of AP1 to its DNA-binding site. This is consistent with our previous finding, demonstrating the ability of farnesyl transferase inhibitor, FTI-277 to enhance the binding of AP1 to DNA (29). Although RhoA and Rac1 stimulate AP1 transcriptional activity as a result of the activation of the stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK)-signaling complex (66, 67), RhoB, on the other hand, suppresses AP1-mediated transcription (this report), possibly by inhibiting the above-mentioned pathway. Our studies raise questions concerning the role of RhoB in the SAPK/JNK signaling pathway and the role of this pathway in TβR-II transcription regulation. RhoB modulation of AP1 activity provides a mechanism linking RhoB to nuclear events regulating

![Figure 7](http://www.jbc.org) DNA-binding of AP1 transcription factor is inhibited by RhoB. Panc-1 cells were transiently transfected with 8, 4, or 2 μg of RhoB expression vector or 8 μg of pcDNA3 control vector, and nuclear extracts were prepared. 32P-Labeled TβR-II/AP1, TβR-II/NF-Y, and TβR-II/Sp1 oligonucleotides were incubated with 6 μg of nuclear extract. For supershift experiments, anti-AP1 or anti-Sp1 antibodies were added to the reaction mixture for 20 min before the addition of the probe. Protein-DNA complexes were separated in a 5% PAGE gel that was subjected to autoradiography.

![Figure 8](http://www.jbc.org) RhoB down-regulates AP1 transcriptional activity. Panc-1 cells were transiently cotransfected with AP1-Luc (panel A) or SRE-Luc (panel B) reporter and 0.2, 0.6, or 1.8 μg of RhoB expression vector or 1.8 μg of pcDNA3 control vector. The results are expressed as fold activation relative to control, that was given the value of 1. The results are representative of at least three independent experiments.
gene transcription. Previous studies have shown that two transcription factors, interleukin 3, prepronendothelin-1, and c-fos, are critical for TGF-β signaling. TGF-β regulates cellular responsiveness by modulating TGFB signaling by suppressing TβR-II expression. Thus, the inhibition of TβR-II expression by RhoB may represent a negative feedback loop that regulates TGFB signal transduction, thus contributing to the maintenance of homeostasis in normal cells. Conversely, in TGF-β-resistant tumor cells, lack of TGFB signals would decrease the RhoB protein level, contributing to the dysregulation of RhoB-regulated molecules such as p21AP1 and, thus, promote tumor progression. This is consistent with our recent data demonstrating that overexpression of RhoB antagonizes malignant transformation.

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