Transforming Growth Factor-β1 Stimulates Protein Kinase A in Mesangial Cells

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Lewei Wang, Yanqing Zhu, and Kumar Sharma‡

From the Department of Medicine, Division of Nephrology, Thomas Jefferson University School of Medicine, Philadelphia, Pennsylvania 19107

We recently demonstrated that transforming growth factor-β (TGF-β) stimulates phosphorylation of the type I inositol 1,4,5-trisphosphate receptor (Sharma, K., Wang, L., Zhu, Y., Bokkala, S., and Joseph, S. (1997) J. Biol. Chem. 272, 14617–14623), possibly via protein kinase A (PKA) activation in murine mesangial cells. In the present study, we evaluated whether TGF-β stimulates PKA activation. Utilizing a specific PKA kinase assay, we found that TGF-β increases PKA activity by 3-fold within 15 min of TGF-β1 treatment, and the enhanced kinase activity was completely reversed by the inhibitory peptide for PKA (PKI; 1 μM). In mesangial cells transfected with a PKI expression vector, enhanced PKA activity could not be demonstrated with TGF-β1 treatment. TGF-β1 was also found to stimulate translocation of the α-catalytic subunit of PKA to the nucleus by Western analysis of nuclear protein as well as by confocal microscopy. TGF-β1-mediated phosphorylation of cAMP response element-binding protein protein was completely reversed by H-89 (3 μM), a specific inhibitor of PKA. Stimulation of fibronectin mRNA by TGF-β1 was also attenuated in cells overexpressing PKI. We thus conclude that TGF-β stimulates the PKA signaling pathway in mesangial cells and that PKA activation contributes to TGF-β stimulation of cAMP response element-binding protein phosphorylation and fibronectin expression.

Recent studies have demonstrated that TGF-β initially binds to its type II receptor and then forms a heteromeric complex with the type I receptor (reviewed in Ref. 6). Cross-phosphorylation of the type I receptor by the type II receptor is critical for subsequent phosphorylation of various members of the recently described Smad family of proteins. Phosphorylation of Smad2 and Smad3 has been demonstrated to play an important role in mediating the effects of TGF-β on cell proliferation (7, 8); however, their role in stimulation of matrix molecules such as fibronectin is unclear. The protein kinase A (PKA) pathway induces many effects on cells similar to TGF-β, and in particular, activation of both TGF-β and PKA stimulates fibronectin production, at least in part, by stimulating gene transcription (4). A critical step involved in the transcriptional gene regulation by the PKA pathway is the phosphorylation of the cAMP response element-binding protein (CREB). Interestingly, CREB phosphorylation by TGF-β has been demonstrated in several cell types (9–11), and the consensus CAMP response element (CRE, TGAGTCA) has been implicated in mediating the effects of TGF-β on the fibronectin promoter in rat mesangial cells (12) and on the cyclin A promoter in Chinese hamster lung fibroblasts (13). Although these findings would suggest that the PKA pathway is involved in TGF-β signaling, this possibility was considered unlikely primarily because cAMP levels were not elevated following TGF-β treatment (9, 10).

PKA is composed of two regulatory subunits and two catalytic subunits in its inactive state (14). Elevation of cAMP levels leads to dissociation of the heteromeric complex, thus allowing the free catalytic subunit to be active as a serine/threonine kinase in the cytoplasm and nucleus. Until recently, activation of PKA without a rise in cAMP levels was not thought to occur. In an elegant study by Zhong et al. (15), the α-catalytic subunit of PKA was found to be bound to Ip6B in the cytoplasm, rather than by one of its regulatory subunits; degradation of Ip6B led to activation of the α-catalytic subunit. Therefore, it is apparent that other mechanisms are operant to allow for PKA activation independently of cAMP. In support of a role for the PKA pathway being involved in TGF-β signaling, we recently demonstrated that TGF-β-induced phosphorylation of the type I inositol 1,4,5-trisphosphate receptor in mesangial cells appeared to be mediated by PKA (16). In the present study, we demonstrate that TGF-β stimulates PKA activity without elevating intracellular CAMP levels in mesangial cells and that inhibition of PKA attenuates TGF-β-induced stimulation of CREB phosphorylation and fibronectin gene expression.

EXPERIMENTAL PROCEDURES

Materials—[γ-32P]ATP was purchased from NEN Life Science Products. An enhanced chemiluminescence system was purchased from Amersham Pharmacia Biotech. TGF-β1 was purchased from R&D Systems (Minneapolis, MN). All other reagents were from Sigma unless otherwise noted.

Cell Culture—An SV40-transformed murine glomerular mesangial...
cell line, which has been previously described (17), was primarily used in these studies. These cells retain many of the differentiated characteristics of mesangial cells in primary culture. We also performed a limited series of experiments in rat glomerular mesangial cells that were conducted between passages 4 and 6 and in the mink lung epithelial cell line (14), obtained from American Type Culture Collection, Rockville, MD).

In Vitro Kinase Assay for PKA Activity—Murine mesangial cells (MMCs) were grown to confluence in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum and then grown for 24 h in serum-free DMEM. Cells were treated with agonists for various periods of time, washed with PBS, and homogenized in 10 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 0.5 mM EGTA, 10 mM β-mercaptoethanol, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride. Protein concentrations of the crude lysates were quantitated, and equal amounts of protein were added to a reaction mixture containing 40 mM Tris-HCl, pH 7.4, 20 mM MgCl2, 0.1 mg/ml bovine serum albumin, 100 μM biotinylated PKA peptide substrate (Kemptide, LRRASLG; Promega, Madison, WI), 3000 Ci/mmol [γ-32P]ATP, and 0.5 mM ATP per reaction. Experiments were performed in parallel with the addition of a PKA inhibitor peptide (TTYADFIAS-GRTGRRNAHID, 1 μM; Promega). The reaction was allowed to proceed for 5 min at 30 °C and then terminated by the addition of 2.5 M guanidine hydrochloride. Five μl of sample were spotted onto streptavidin-coated discs, washed repeatedly, dried in an oven, and resuspended in scintillation vials for radioactive counting. To visually demonstrate that the Kemptide peptide was indeed phosphorylated, the in vitro kinase reaction was also performed with fluorescently tagged Kemptide (Promega). Phosphorylation of this peptide alters the peptide’s net charge from +1 to −1, allowing the phosphorylated peptide to be separated from the unphosphorylated peptide on an agarose gel at neutral pH.

Transfection of MMCs with Protein Kinase A Inhibitor (PKI) Expression Vector—The expression vectors for PKI and mutant PKI driven by the Rous sarcoma virus promoter were obtained from Dr. Richard Maurer (Oregon Health Sciences University, Portland, OR) (18). The mutant PKI sequence codes for glycine rather than arginine at positions 20 and 21. The arginines at these sites are essential for inhibition of catalytic subunit activity. Subconfluent MMCs were transfected with 10 μg of plasmid and Superfect transfection reagent (Qiagen Inc., Chatsworth, CA) for 3 h. Cells were washed and then cultured in serum-free DMEM for an additional 16 h prior to the addition of agonists.

Western Blot Analysis—MMCs were treated with TGF-β and/or the PKA inhibitor H-89 (3 μM, 30 min) prior to the addition of TGF-β in serum-free DMEM. Cell extracts were prepared as described previously (16). Nuclear extracts were prepared according to the method of Schreiber et al. (19). Total cellular or nuclear protein was quantitated using the Bio-Rad DC protein assay, and 20 μg of protein were resolved on a 10% SDS-polyacrylamide gel and transferred to nitrocellulose membrane. Immunoblotting was performed with antibodies against the α-catalytic subunit of PKA (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-phospho-CREB antibody (Upstate Biotechnology, Inc., Lake Placid, NY), anti-NF-κB antibody, or anti-NF-κB p65 antibody (Santa Cruz Biotechnology, Inc.). Immunoreactive bands were detected using the enhanced chemiluminescence system.

Confocal Analysis—MMCs were grown in 6-well tissue culture dishes on No. 1 coverslips pretreated with poly-l-lysine (0.1 mg/ml for 5 min) with DMEM containing 10% fetal calf serum. After cells were adherent, they were then cultured in serum-free DMEM overnight and then exposed to TGF-β1 (10 ng/ml) or forskolin (10 μM) for 15 min. After exposure to agonists, cells were washed with PBS three times and fixed with 3.7% formaldehyde for 10 min at room temperature. After repeated washing, cells were permeabilized with 0.05% Triton X-100 in PBS (PBS/TX) for 10 min at room temperature, washed with PBS/TX three times, blocked with 4% normal goat serum in PBS/TX for 10 min, and then incubated with anti-α-catalytic antibody (1:200) for 30 min at 37 °C. Cells were washed with PBS/TX and blocked again with 4% normal goat serum, and secondary antibody (fluorescein-conjugated goat anti-rabbit antibody, 1:500 dilution; Rockland Inc., Gilbertsville, PA) was applied for 30 min at 37 °C. After repeated washing, cells were post-fixed with 3.7% formaldehyde for 10 min at room temperature and washed, and coverslips were placed on glass slides and mounted with SlowFade (Molecular Probes, OR). Slides were visualized with a confocal microscope (courtesy of Dr. James Keen, Thomas Jefferson University) and representative regions were photographed. Control cells, stained only with secondary antibody, showed minimal fluorescence.

Measurement of cAMP—Cyclic AMP levels were measured in MMCs after treatment with TGF-β1 (10 ng/ml, 5, 15, and 30 min) or forskolin (10 μM, 15 min) using a Biotrak cAMP enzyme immunoassay kit (Amersham Pharmacia Biotech). MMCs were trypsined and resuspended in PBS with 5% (v/v) ethanol. The cell precipitates were centrifuged, the supernatants were drawn off, and the extracts were dried in a vacuum oven. Extracts were resuspended in assay buffer, acetylated, and assayed for cAMP following the instructions supplied by the manufacturer. MMCs were assayed in the absence and presence of isobutylmethylxanthine (100 μM) 10 min prior to the addition of TGF-β1 or forskolin.

Northern Analysis—To assess whether TGF-β1-induced fibronectin mRNA expression was affected by inhibition of PKA, MMCs transiently transfected with PKI expression vector or mutant PKI were treated with TGF-β1 (10 ng/ml) for 24 h and washed with ice-cold PBS, and total RNA was isolated using acid guanidinium thiocyanate/phenol/chloroform (20). Twenty μg of total RNA were loaded onto a 1.2% agarose gel containing 2.2 M formaldehyde, electrophoresed, and transferred onto nylon membrane. The probe for murine fibronectin has been described previously (3). Hybridization and washing conditions were performed as described previously (21). To standardize for loading, membranes were stripped and reprobed with a β-actin cDNA probe (kindly provided by Dr. Pamela A. Norton). Densitometric analysis was performed as described previously (16), and mRNA levels were calculated relative to those of β-actin. RESULTS

TGF-β Stimulates PKA Activity—MMCsa were treated with TGF-β1 for various time points, and an in vitro kinase assay was performed utilizing a biotinylated substrate for PKA (Fig. 1A). The reaction mixture was spotted onto avidin-coated discs to bind only the biotinylated peptide, thus increasing the specificity of the assay. With 15 min of administration of TGF-β1, PKA activity was increased by 3-fold relative to control values and remained elevated at 30 min. The addition of a specific peptide inhibitor of the catalytic subunit of PKA (PKI) completely blocked the increased kinase activity in TGF-β1-treated samples, demonstrating that the increased kinase activity was specific for PKA (Fig. 1A). A dose-response relationship was noted, with maximal stimulation of PKA noted at a concentration of 10 ng/ml TGF-β1 (Fig. 1B). To further demonstrate that PKA activity was being stimulated by TGF-β1 or forskolin, cells were transiently transfected with an expression vector for the PKI peptide to inhibit PKA activity or a PKI peptide that was mutated in its PKA catalytic recognition site. TGF-β1 or forskolin stimulated PKA activity in cells expressing the mutant PKI peptide, but not in MMCs expressing wild-type PKI (Fig. 1C). To visually demonstrate that the peptide substrate for PKA was indeed phosphorylated by TGF-β1, a fluorescently tagged peptide substrate was employed in the in vitro kinase assay. Phosphorylation of the peptide promotes migration to the positive electrode on an agarose gel. TGF-β1 treatment for 5 and 15 min (lanes 3 and 4) stimulated migration of this peptide to the positive electrode (Fig. 1D).

To determine whether TGF-β1 stimulates kinase activity in other cell types, we evaluated nontransformed rat mesangial cells (between passages 4 and 6) and the mink lung epithelial cell line, which has commonly been found to be very sensitive to TGF-β. PKA activity was increased by ∼1.5-fold in rat mesangial cells and by ∼2-fold in mink lung epithelial cells treated with TGF-β1 (Fig. 2, A and B).

TGF-β Stimulates Translocation of α-Catalytic Subunit of PKA—Activation of PKA leads to dissociation of the catalytic subunit from its regulatory subunit and translocation to the nucleus (14). To demonstrate that PKA is activated and that the catalytic subunit is dissociated from its inhibitory regulatory subunit, MMCs were isolated from control and TGF-β1-treated MMCs, and immunoblot analysis was performed with an antibody against the α-catalytic subunit of PKA. Fig. 3 demonstrates accumulation of the catalytic subunit in the nucleus with TGF-β1 treatment. Confocal microscopy (Fig. 4) also demonstrated a redistribution of the α-catalytic subunit from a primarily perinuclear distribution in untreated MMCs to a more diffuse distribution within the nucleus.
MMCs (Fig. 4A) to a nuclear localization in TGF-β1-treated (Fig. 4B) and forskolin-treated (Fig. 4C) MMCs. As compared with control cells, TGF-β1- and forskolin-treated cells demonstrated a similar redistribution of the α-catalytic subunit diffusely into the cytoplasm.

TGF-β Does Not Increase Intracellular cAMP Levels or Affect NF-κB/IκB—Increased intracellular cAMP levels enhance binding of cAMP to the regulatory subunits of PKA and promote dissociation from the catalytic subunit. Therefore, cAMP levels were measured following TGF-β1 treatment after various time intervals. MMCs stimulated with TGF-β1 for 5, 15, or 30 min did not raise intracellular cAMP levels (Fig. 5). Forskolin treatment did cause a marked increase in cAMP levels in MMCs, as would be expected. In the presence of the phosphodiesterase inhibitor isobutylmethylxanthine, TGF-β1 also did not increase cAMP levels (data not shown).

To evaluate if TGF-β1-induced activation of PKA may involve degradation of IκB as a cAMP-independent mechanism of PKA activation (15), we analyzed protein levels of IκB after TGF-β treatment. In whole cell lysates, treatment of MMCs with TGF-β1 for 30 min did not reduce IκB (Fig. 6A). Immunoblot analysis of nuclear protein from MMCs treated with TGF-β1 for 30 min did not reveal accumulation of NF-κB (Fig. 6B). Analysis by electrophoretic mobility shift assay also did not demonstrate enhanced binding of nuclear protein to a consensus NF-κB probe with TGF-β1 treatment for 30 min (data not shown). Therefore, it is unlikely that IκB degradation is responsible for TGF-β1-induced stimulation of PKA α-catalytic subunit activation.

TGF-β1 Induces CREB Phosphorylation Is Blocked by Inhibition of PKA—To determine the functional significance of TGF-β1-induced PKA activation, we evaluated if CREB was phosphorylated in response to TGF-β1 in MMCs by performing immunoblot analysis of nuclear protein with anti-phospho-CREB antibody. Fig. 7 demonstrates enhanced CREB phosphorylation (43-kDa band) in nuclear protein isolated from TGF-β1-treated cells as compared with control cells. Pretreatment of MMCs with H-89, a relatively specific inhibitor of PKA, was sufficient to completely inhibit TGF-β1-induced CREB phosphorylation. Immunoblot analysis of the same membrane with an antibody that recognizes total CREB revealed that TGF-β1 did not lead to accumulation of CREB in the nucleus (data not shown).
whereas MMCs overexpressing wild-type PKI had a blunted
response to TGF-β1 (10 ng/ml, 15 min), and PKA activity was assessed as described for Fig. 1B. Results are expressed as means ± S.E. from three separate experiments. *, p < 0.05 compared with values for the corresponding control.

Our major conclusions are that TGF-β1 stimulates PKA in
mesangial cells and that the PKA pathway plays an important
role in TGF-β1-induced CREB phosphorylation and TGF-β1
stimulation of fibronectin gene expression. Surprisingly, we
found PKA activation by TGF-β1 in the absence of a detectable
rise in cAMP levels.

FIG. 2. TGF-β stimulates PKA activity in rat mesangial cells and mink lung epithelial cells. Rat mesangial cells (A) and mink lung epithelial cells (B) were stimulated with TGF-β1 (10 ng/ml, 15 min), and PKA activity was assessed as described for Fig. 1B. Results are expressed as means ± S.E. from three separate experiments. *, p < 0.05 compared with values for the corresponding control.

FIG. 3. Translocation of the α-catalytic subunit of PKA to the
cell nucleus with TGF-β1 stimulation of MMCs. Twenty μg of nuclear
protein were resolved by SDS-polyacrylamide gel electrophoresis,
transferred to nitrocellulose, and immunoblotted with the antibody for
the α-catalytic subunit of PKA. Shown are representative data from a
single experiment with two separate control samples (lanes 1 and 2),
TGF-β1 (10 ng/ml) treatment for 15 min (lanes 3 and 4), and TGF-β1
treatment for 30 min (lanes 5 and 6). Experiments were repeated twice
with essentially the same results. Anti-phospho-CREB antibody is raised against the
phosphoserine site of the CREB protein that shares
100% homology with the phosphoserine site of the transcription
factor ATF-1 (22); therefore, this protein may also be
demonstrated on immunoblotting. The band at 38 kDa corre-
sponds to phospho-ATF-1 and was also found to be markedly
stimulated in nuclear extracts from TGF-β1-treated cells (Fig.
7). Inhibition of PKA by H-89 also completely prevented TGF-
β1-induced phosphorylation of ATF-1. Treatment of MMCs tran-
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demonstrate increased CREB or ATF-1 phosphorylation in re-
sponse to TGF-β1 treatment (data not shown). Thus, CREB
phosphorylation by TGF-β1 appears to be largely dependent on
activation of PKA.

TGF-β1-induced Fibronectin mRNA Stimulation Is Attenu-
ated by Inhibition of PKA—Fibronectin gene stimulation is
considered to be controlled, at least in part, by CREs located in the
fibronectin promoter, and both TGF-β and PKA stimulation
may stimulate the fibronectin promoter via CRE sites (4, 12,
23). To assess if PKA may be involved in stimulation of steady-
state fibronectin mRNA levels by TGF-β, we performed Northern
analysis of MMCs transiently transfected with an expres-
sion vector for either wild-type PKI or mutant PKI (Fig. 8).
MMC overexpressing mutant PKI treated with TGF-β1 for
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are in contrast to other published reports in different cell types. Prior studies excluded the PKA pathway in TGF-β1 signaling in mink lung epithelial cells and rat mesangial cells primarily
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group reported that the α-catalytic subunit of PKA is constitu-
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function to bind the α-catalytic subunit of PKA and to regulate
its activity independently of intracellular cAMP. Therefore,
failure to observe an increase in cAMP-mediated kinase activ-
ity is not adequate evidence to exclude the involvement of the
PKA pathway in a given cellular process.

It should be noted that in two of the above-mentioned studies
(11, 13), PKA activity was measured by in vitro kinase assays
after 10–15 min of exposure to TGF-β and was found not to be
increased. The in vitro kinase assays utilized either Kemptide
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strate that binds to the phosphocellulose filter as well as other proteins that are present in the cell lysate that have basic residues (24). In addition, overall phosphatase activity in the cell lysate may also be reflected in this assay. As TGF-β has been shown to activate serine/threonine kinases (6, 25) as well as various phosphatases (26), this assay method may not be conclusive. Our studies used two separate in vitro kinase assays in which the Kemptide substrate is biotinylated or fluorescently labeled, thus assuring that phosphorylation of only the peptide substrate of interest would be measured. To enhance specificity, only the PKI-inhibitable fraction of kinase activity was taken to reflect PKA activity. In addition, we demonstrate translocation of the α-catalytic subunit to the nucleus with TGF-β treatment by immunoblot analysis as well as confocal microscopy, providing an independent means of demonstrating PKA activation.

The mechanism(s) by which TGF-β stimulates PKA activation remains unclear. Our studies failed to demonstrate that 

**FIG. 4.** Translocation of the α-catalytic subunit of PKA by confocal microscopy. MMCs treated with TGF-β1 (10 ng/ml) or forskolin (10 μM) for 15 min were fixed on coverslips, and immunofluorescence was performed with the antibody for the α-catalytic subunit of PKA. Slides were observed by confocal microscopy, and representative areas were photographed. The location of the α-catalytic subunit in the basal state is in the perinuclear location as shown in A (control). With TGF-β exposure, enhanced nuclear staining and diffuse cytoplasmic staining were observed (B). A very similar appearance occurred with exposure of MMCs to forskolin (C).

**FIG. 5.** Cyclic AMP levels are not increased with TGF-β treatment. Cyclic AMP levels were measured in MMCs after treatment with TGF-β1 (10 ng/ml; 5, 15, and 30 min) or forskolin (10 μM, 15 min) using a Biotrak enzyme immunoassay assay kit. Results are expressed as means ± S.E. from three separate experiments.

**FIG. 6.** Immunoblot analysis of IκB and NF-κB in MMCs treated with TGF-β. A, immunoblot analysis with anti-IκB antibody of the whole cell lysate (20 μg of protein) derived from control MMCs and MMCs treated with TGF-β1 (10 ng/ml) for 30 min; B, immunoblot analysis with anti-NF-κB antibody of nuclear protein (20 μg) derived from control MMCs and MMCs treated with TGF-β1 (10 ng/ml) for 30 min.

**FIG. 7.** Inhibition of PKA blocks TGF-β-induced CREB phosphorylation. Shown are the results from the immunoblot analysis with anti-phospho-CREB antibody of nuclear extract from MMCs treated with TGF-β1 (10 ng/ml, 30 min) alone or in the presence of the PKA inhibitor H-89 (3 μM, 30 min prior to TGF-β1 treatment). The upper bands correspond to phospho-CREB (P-CREB), and the lower bands correspond to phospho-ATF-1 (P-ATF). Data are representative of a single experiment. Experiments were repeated twice with essentially the same results.
IgB protein underwent degradation in whole cell lysate or that NF-κB was translocated to the nucleus with TGF-β treatment. This would suggest that TGF-β may affect degradation of another protein that associates with and inhibits the α-catalytic subunit of PKA, possibly via ankyrin repeat sites. In a study in which platelet-derived growth factor-BB was found to stimulate specific responses to TGF-β, without a concomitant increase in total cellular CAMP. These possibilities are presently being explored in our laboratory.

TGF-β-induced PKA activation likely contributes to CREB and ATF-1 phosphorylation. CREB phosphorylation by TGF-β has been demonstrated by several independent studies (9–11). In a study employing murine embryonic palatal cells (11), TGF-β-induced phosphorylation of CREB was demonstrated with an anti-phospho-CREB antibody specific for serine 133 (22). Serine 133 is not only the site of CREB phosphorylation by PKA, but also the site of CREB phosphorylation by mitogen-activated protein kinase-stimulated CREB kinase and calcium-calmodulin kinase. Evidence against the role of the latter two pathways in mediating TGF-β-induced CREB phosphorylation was provided in the above study (11). The present study demonstrates that TGF-β-induced CREB phosphorylation, employing the same anti-phospho-CREB antibody, is completely attenuated in mesangial cells when pretreated with the relatively specific inhibitor of PKA, H-89. Of note, H-89 inhibits the catalytic activity of PKA and is therefore not dependent on regulatory subunit binding of the catalytic subunit. Similar results were also found with the PKI peptide, which also binds to the free catalytic subunit of PKA. In addition, the phospho-CREB antibody also recognizes phospho-ATF-1 (22), and this nuclear transcription factor was also found to be phosphorylated by TGF-β1 treatment and reversed with PKA inhibition. The role of CREB phosphorylation in mediating gene regulation by TGF-β is unclear; however, a recent study in Drosophila demonstrated that a CREB-binding site was important in mediating decapentaplegic (the Drosophila homologue of TGF-β) stimulation of the homeotic gene Ultrabithorax (28).

TGF-β1 stimulation of the fibronectin promoter appears to require a consensus CRE (TGACGTCA) site (4, 29), and both CREB and ATF-1 derived from mesangial cells bind to this site (10). CREB phosphorylation enhances CREB binding to CRE-binding protein, thus activating gene transcription via the CRE (29). Inhibiting CREB phosphorylation would thus inhibit CREB binding to CRE-binding protein and may inhibit TGF-β-induced stimulation of fibronectin gene transcription. Our finding that inhibition of PKA in mesangial cells (by overexpressing PKI) prior to TGF-β1 treatment attenuates stimulation of fibronectin mRNA levels supports this hypothesis. The role of other transcription factors that are regulated by PKA and that bind to the CRE of the fibronectin promoter, such as ATF-1 and ATF-2, may also be relevant to TGF-β stimulation of fibronectin gene transcription. In addition, it is likely that cross-talk among the PKA pathway, the mitogen-activated protein kinase pathway, and the Smad pathway participates in mediating specific responses to TGF-β in various cell types.

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