Novel Negative Regulatory Element in the Platelet-derived Growth Factor B Chain Promoter That Mediates ERK-dependent Transcriptional Repression*

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Platelet-derived growth factor (PDGF), which consists of an A and/or B chain, stimulates migration and proliferation in vascular smooth muscle cells as well as a large number of other cell types. Investigations over recent years have defined roles for several positive regulatory transcription factors in the PDGF-B promoter. However, little is known about the transcriptional mechanisms that negatively regulate this gene. Here, we used transient transfection and 5′ deletion analysis to define a specific region in the PDGF-B promoter-mediating repression in vascular smooth muscle cells. Gel retardation assays revealed this region is bound by nuclear protein(s) in a specific manner. Supershift assays excluded the direct association of Sp1, Sp3, and Egr-1. Mutation of the negative regulatory element no longer supported nucleo-protein complex formation and, when introduced into the PDGF-B promoter, rescued the promoter from repression. Promoter activity was also restored by transfection of oligonucleotide decoys bearing the repressor binding site. The MEK1/2 inhibitor, PD98059, and a dominant negative construct generating inactive ERK1 increased reporter expression driven by the PDGF-B promoter. In contrast, the MEK inhibitor had no effect on the activity of the mutant PDGF-B promoter. These effects were cell type-specific, since neither suppression of the PDGF-B promoter nor nucleoprotein complex formation was observed in vascular endothelial cells. These findings define a distinct negative regulatory element in the PDGF-B promoter that interacts with nuclear protein(s) and inhibits PDGF-B promoter-dependent gene expression in an ERK-dependent manner.

Platelet-derived growth factor (PDGF)† comprises a disulfide-linked homo- or heterodimer of an A and/or B chain. It is a potent mitogen and chemoattractant for cells of mesenchymal origin. A large number of cell types, including vascular smooth muscle cells, produce PDGF (1), suggesting that PDGF may be involved in autocrine/paracrine growth loops. Several lines of evidence implicate PDGF B-chain in vascular pathologic settings and in remodeling after mechanical injury to the artery wall. PDGF-B is associated with smooth muscle cells in human atherosclerotic plaques (2, 3) and post-angioplasty restenotic lesions (4). In rat and pig models of balloon angioplasty, PDGF-BB stimulates intimal thickening (5–7).

PDGF-A and -B chain subunits are encoded by distinct genes on chromosomes 7 (8, 9) and 22 (10, 11), respectively, whose expression is independently regulated. The minimal PDGF-B promoter comprises approximately 100 bp (12–15). In vivo footprinting demonstrated that the 5′-CACCC-3′ sequence in this minimal region is occupied in intact cells (16). A number of positive-regulatory zinc finger transcription factors have been found to interact functionally with this region of the PDGF-B promoter, including Sp1, Sp3, and Egr-1 (13, 17, 18). NF-κB interacts with the shear stress response element located further upstream (19).

Negative regulation of PDGF-B gene expression at the level of transcription is poorly understood. To date, ZNF174 is the only known transcriptional repressor with the capacity to down-regulate the activity of the PDGF-B promoter (20). Here, we used a variety of approaches to search the PDGF-B promoter for additional cis-acting silencer elements. Using gel shift, mutational, decoy, and transient transfection analysis, we have identified a novel negative regulatory element in the PDGF-B promoter that mediates repression of the promoter in a sequence-specific and MEK/ERK-dependent manner.

EXPERIMENTAL PROCEDURES

Cell Culture—Pup SMCs (WKY12–22) were cultured in Waymouth’s MB752/1 medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum, 30 μg/ml l-glutamine, 10 units/ml penicillin, and 10 μg/ml streptomycin at 37 °C and 5% CO2. Cultures were passaged every 4–5 days in 75-cm2 flasks. Cells seeded for preparation of nuclear extracts were grown to 80–90% confluence, and nuclear extraction was performed.

Transient Transfection Analysis—Pup SMCs were seeded in 100-mm tissue culture plates for 48 h before transfection. When approximately 60–70% confluent, the cells were transfected with 8 μg of the indicated promoter-reporter plasmids. Transfections were performed using FuGENE6 (Roche Molecular Biochemicals). A precipitate was formed using 3 μl of FuGENE/μg of transfected DNA, and the transfection mix was made up to 1 ml with serum-free Waymouth’s medium. After incubation at 22 °C for 10 min, the DNA/FuGENE mixture was added to cells containing 4 ml of complete Waymouth’s medium. Two days post-transfection, cell lysates were prepared for assessment of chloramphenicol acetyltransferase (CAT) activity as described (13). The concentration of protein in the cell lysates were assessed using the BCA protein assay kit and used to correct CAT reporter activity.

Oligonucleotide Synthesis, Purification, and Radiolabeling—Oligonucleotides were synthesized by Pacific Oligos. Double-stranded oligonucleotides were radiolabeled with [γ-32P]dATP (GeneWorks) using T4

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The abbreviations used are: PDGF, platelet-derived growth factor; CAT, chloramphenicol acetyl transferase; ERK, extracellular signal-regulated protein kinase; EMSA, electrophoretic mobility shift assay; SMC, smooth muscle cells; bp, base pairs; MAP, mitogen-activated protein; MEK, MAP kinase/extracellular signal-regulated kinase.
Nuclear Silencer Element in PDGF-B Promoter

Evidence for the Existence of a Negative Regulatory Element in the Proximal PDGF-B Promoter—WKY12–22 pup SMCs, which express PDGF-B mRNA (21), were transiently transfected with a series of CAT reporter constructs driven by various-sized fragments of the PDGF-B promoter (Fig. 1, upper panel). Basal CAT activity was readily detectable and not significantly different in SMCs transfected with constructs d77 and d26, bearing 92 and 153 bp of PDGF-B promoter sequence (relative to the TATA box), respectively (Fig. 1, lower panel). In contrast, construct dNco (−227), which bears an additional 74 bp of PDGF-B promoter sequence upstream (Fig. 1, upper panel) was, like construct d18 (Fig. 1, upper panel), virtually inactive (Fig. 1, lower panel). These data contrast with our previous finding that using serial 5′ deletion analysis in bovine aortic endothelial cells, where no major difference was observed in CAT activity driven by the reporter vectors (13). The inability of construct dNco to mediate basal PDGF-B promoter-dependent expression in SMCs prompted us to explore the apparent negative regulatory role of this region in the promoter.

Nuclear Protein(s) Interact with the Negative Regulatory Element in the Proximal PDGF-B Promoter—We next performed EMSA to gain insight into the mechanisms underlying the negative regulation of the PDGF-B promoter. Since the preceding findings demonstrated that the putative repressor site is located between the 5′ PDGF-B promoter end points in constructs dNco (−227) and d26 (−153), we synthesized a series of overlapping double-stranded oligonucleotides spanning this region (Table I). These 32P-labeled oligonucleotides were incubated with nuclear extracts of pup SMCs, and the adducts were resolved by nondenaturing gel electrophoresis. 32P-Oligo B A (bp −230/−201) produced a single, discrete nucleoprotein complex (Fig. 2A, arrow) that was virtually absent when the probe was substituted with 32P-Oligo B C (bp −205′/−176) and 32P-Oligo B C (bp −180′/−151) (Fig. 2A).

Competition experiments were performed to determine whether the interaction between nuclear protein and oligonucleotide was specific. A 35-fold molar excess of unlabeled Oligo B C abrogated the formation of the nucleoprotein complex (Fig. 2B, third lane). In contrast, the same fold molar excess of two unrelated oligonucleotides, Oligo A (22) and Oligo LKSSRem3 (19), had no effect (Fig. 2B).

The PDGF-B promoter sequence between the 5′ end points in d26 and dNco bears a consensus binding element for Sp1. Previously, we determined that Sp1 as well as Sp3 and Egr-1

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**TABLE I**

| Oligonucleotides | 32P-Oligo B A | 32P-Oligo B C |
|------------------|--------------|--------------|
| Wild type        | 230-gtccttgtcatctgtgctgctttatggac-201 | 205-gggacgttggtgctacccatgctttttttt-176 |
| Oligo B A        | 230-gtccttgtcatctgtgctgctttatggac-201 | 180-ccccccccccgcagctattgaagt-151 |
| Oligo B C        | 230-gtccttgtcatctgtgctgctttatggac-201 | 205-gggacgttggtgctacccatgctttttttt-176 |
| Mutant oligonucleotides | 230-gtccttgtcatctgtgctgctttatggac-201 | 230-gtccttgtcatctgtgctgctttatggac-201 |

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**FIG. 1.** Serial 5′ deletion analysis of the PDGF-B promoter in pup SMCs. **Upper panel**, schematic representation of PDGF-B promoter CAT constructs. The length of the PDGF-B promoter in each construct is indicated by the number of base pairs upstream of the TATA box. **Lower panel**, transient transfection analysis. Pup SMCs were transfected with the indicated PDGF-B promoter-CAT constructs, and CAT activity in the lysates was determined after 24 h. CAT activity was normalized to the concentration of protein in the cell lysates. The data are representative of two independent determinations. Error bars represent S.E. of the mean.
interact with a second site (bp -30' - 13') located downstream in the promoter, which positively regulates basal PDGF-B expression (18). Supershift analysis was performed with $^{32}$P-Oligo B$_A$ using antibodies with specificity directed to these nuclear factors. However, the nucleoprotein complex was unaltered by the presence of either antibody (Fig. 3A).

Novel Silencer Element in PDGF-B Promoter

Fig. 2. Specific interaction of nuclear protein(s) with the negative regulative element in the PDGF-B promoter. A, EMSA was performed using pup SMC nuclear extracts (NE) and several overlapping double-stranded $^{32}$P-labeled oligonucleotides spanning the region between the 5' PDGF-B promoter end points in constructs dNco and d26. Binding reactions and nondenaturing gel electrophoresis were performed as described under “Experimental Procedures.” The nucleoprotein complex is indicated by an arrow. Oligo B$_A$ (5'gtccatggtcactgtgctga-3') sense strand, Oligo B$_B$ (5'ggaggtgggtgatgttagtttttttt-3') sense strand, Oligo B$_C$ (5'ggaggtgggtgatgttagtttttttt-3') sense strand are shown. B, oligonucleotide competition experiments. Nuclear extracts from pup SMCs were incubated in the absence or presence of a 35-fold molar excess of unlabeled Oligo B$_A$, Oligo A, or LKSSREm3 for 10 min before the addition of the $^{32}$P-labeled probe. Binding reactions and nondenaturing gel electrophoresis were performed as described under “Experimental Procedures.” Where indicated, 1 mg of the appropriate antibody was incubated with the binding mixture for 10 min before the addition of the $^{32}$P-labeled probe. Where indicated, 1 mg of the appropriate antibody (Sp1, Sp3, or Smad1) was incubated with the binding mixture for 10 min before the addition of the $^{32}$P-labeled probe. NE denotes nuclear extract. The data are representative of two independent determinations.

Fig. 3. Nuclear Sp1, Egr-1, or Sp3 do not interact with the negative regulatory element in the PDGF-B promoter. A, EMSA was performed with $^{32}$P-Oligo B$_A$ and pup SMC nuclear extracts under the conditions indicated under “Experimental Procedures.” Where indicated, 1 mg of the appropriate antibody was incubated with the binding mixture for 10 min before the addition of the $^{32}$P-labeled probe. B, EMSA was performed with pup SMC nuclear extracts and $^{32}$P-Oligo B$_A$ or $^{32}$P-Oligo A using conditions suited for Sp1, Egr-1, and Sp3 (22). Where indicated, 1 mg of the appropriate antibody (Sp1, Sp3, or Smad1) was incubated with the binding mixture for 10 min before the addition of the $^{32}$P-labeled probe. NE denotes nuclear extract. The data are representative of two independent determinations.

using antibodies with specificity directed to these nuclear factors. However, the nucleoprotein complex was unaltered by the presence of either antibody (Fig. 3A). To confirm the lack of a direct physical association of Sp1 with $^{32}$P-Oligo B$_A$, we performed EMSA and supershift analysis using binding conditions suited to Sp1 (22). These conditions did not support the formation of a nucleoprotein complex involving $^{32}$P-Oligo B$_A$ and Sp1 (Fig. 3B). In contrast, multiple nucleoprotein complexes were observed using a second oligonucleotide, $^{32}$P-Oligo A (Fig. 3B), as described previously (18, 22), thus demonstrating the pres-
Serial mutational binding analysis defines the site in the PDGF-B negative regulatory element crucial for interaction with nuclear protein(s). Pup SMC nuclear extracts were incubated with $^{32}$P-Oligo BAm1 (5'-gtcaacgtgaactgctaggggggac-3') sense strand, $^{32}$P-Oligo BAm2 (5'-gtcaacgtgaactgctaggggggac-3') sense strand, or $^{32}$P-Oligo BAm3 (5'-gtcaacgtgaactgctaggggggac-3') sense strand under the conditions indicated under "Experimental Procedures." Nucleoprotein complexes were resolved by nondenaturing electrophoresis and visualized by autoradiography. NE denotes nuclear extract. The data are representative of two independent determinations.

Mutation of the −227/−221 element in construct d18 rescues the PDGF-B promoter from repression. Pup SMCs were transiently transfected with constructs d26, d18, or md18Am1. After 24 h, the cells were lysed, and CAT activity was normalized to the concentration of protein. The data are representative of two independent determinations. Error bars represent S.E. of the mean.

Oligonucleotide decoys targeting the negative regulatory element rescues the PDGF-B promoter from repression. A, transient transfection analysis. Pup SMCs were transfected with construct d18, either alone, or together with 100 nM or 1 μM Oligo B_A or Oligo B_Am1, as indicated in the figure. CAT activity in the cell lysates was determined after 24 h and normalized to the concentration of protein. B, Oligo B_A and Oligo B_Am1 localize in the nucleus upon transfection. Nuclear and cytoplasmic extracts of pup SMCs transfected with $^{32}$P-labeled Oligo B_A or Oligo B_Am1 (500 fmol, $1 \times 10^6$ cpm) were prepared at various times. Radioactivity associated with each of these fractions was assessed using a β-scintillation counter. The data are representative of two independent determinations. Error bars represent S.E. of the mean.

Mutation of the −227/−221 Site in d18 Rescues CAT Activity—To determine the functional consequence of the −227/−221 mutation in the PDGF-B promoter, this sequence was introduced into construct d18 before transient transfection analysis in pup SMCs. CAT activity in SMCs transfected with construct d26 was used, consistent with earlier findings (Fig. 1, lower panel). Construct md18Am1, bearing the −227/−221 mutation, was basally active and produced CAT activity only faintly observed using $^{32}$P-Oligo B_Am2 (Fig. 5). In contrast, Oligo B_Am1 was unable to affect the appearance of this nucleoprotein complex (Fig. 4). These findings demonstrate the requirement of bp −227/−221 (5’-CATGGTCACA-3’) in the repressed region of the PDGF-B promoter for optimal nucleoprotein complex formation.

Mutation of the −227/−221 Site in d18 Rescues CAT Activity—To determine the functional consequence of the −227/−221 mutation in the PDGF-B promoter, this sequence was introduced into construct d18 before transient transfection analysis in pup SMCs. CAT activity in SMCs transfected with construct d26 was used, consistent with earlier findings (Fig. 1, lower panel). Construct md18Am1, bearing the −227/−221 mutation, was basally active and produced CAT activity only faintly observed using $^{32}$P-Oligo B_Am2 (Fig. 5). In contrast, Oligo B_Am1 was unable to affect the appearance of this nucleoprotein complex (Fig. 4). These findings demonstrate the requirement of bp −227/−221 (5’-CATGGTCACA-3’) in the repressed region of the PDGF-B promoter for optimal nucleoprotein complex formation.

Mutation of the −227/−221 Site in d18 Rescues CAT Activity—To determine the functional consequence of the −227/−221 mutation in the PDGF-B promoter, this sequence was introduced into construct d18 before transient transfection analysis in pup SMCs. CAT activity in SMCs transfected with construct d26 was used, consistent with earlier findings (Fig. 1, lower panel). Construct md18Am1, bearing the −227/−221 mutation, was basally active and produced CAT activity only faintly observed using $^{32}$P-Oligo B_Am2 (Fig. 5). In contrast, Oligo B_Am1 was unable to affect the appearance of this nucleoprotein complex (Fig. 4). These findings demonstrate the requirement of bp −227/−221 (5’-CATGGTCACA-3’) in the repressed region of the PDGF-B promoter for optimal nucleoprotein complex formation.

Mutation of the −227/−221 Site in d18 Rescues CAT Activity—To determine the functional consequence of the −227/−221 mutation in the PDGF-B promoter, this sequence was introduced into construct d18 before transient transfection analysis in pup SMCs. CAT activity in SMCs transfected with construct d26 was used, consistent with earlier findings (Fig. 1, lower panel). Construct md18Am1, bearing the −227/−221 mutation, was basally active and produced CAT activity only faintly observed using $^{32}$P-Oligo B_Am2 (Fig. 5). In contrast, Oligo B_Am1 was unable to affect the appearance of this nucleoprotein complex (Fig. 4). These findings demonstrate the requirement of bp −227/−221 (5’-CATGGTCACA-3’) in the repressed region of the PDGF-B promoter for optimal nucleoprotein complex formation.

Mutation of the −227/−221 Site in d18 Rescues CAT Activity—To determine the functional consequence of the −227/−221 mutation in the PDGF-B promoter, this sequence was introduced into construct d18 before transient transfection analysis in pup SMCs. CAT activity in SMCs transfected with construct d26 was used, consistent with earlier findings (Fig. 1, lower panel). Construct md18Am1, bearing the −227/−221 mutation, was basally active and produced CAT activity only faintly observed using $^{32}$P-Oligo B_Am2 (Fig. 5). In contrast, Oligo B_Am1 was unable to affect the appearance of this nucleoprotein complex (Fig. 4). These findings demonstrate the requirement of bp −227/−221 (5’-CATGGTCACA-3’) in the repressed region of the PDGF-B promoter for optimal nucleoprotein complex formation.
at levels even higher than construct d26 (Fig. 5). These data provide functional evidence for the existence of a negative regulatory element at position 227/221 in the PDGF-B promoter.

**In Vivo Competition of Construct d18 Using Decoys Rescues CAT Activity**—An oligonucleotide decoy strategy was used to confirm our observations using the mutant PDGF-B promoter. Pup SMCs were cotransfected with construct d18 plasmid and either unlabeled Oligo BA, which binds to the repressor in a specific manner (Figs. 2, A and B, and 4), or Oligo BAm1, which is unable to bind to the nuclear protein (Fig. 4). Transfection experiments revealed that transfection of Oligo B_A increased CAT activity generated by construct d18 (Fig. 6A), whereas Oligo B_Am1 failed to influence reporter activity (Fig. 6A). These findings demonstrate the sequence-specific nature of transcriptional repression at the 227−221 site in the PDGF-B promoter. The wild-type oligonucleotide serves as a decoy that competes with the authentic site in the promoter for interaction with the repressor.

To ensure that both oligonucleotides localized to the nucleus of these cells independently of differences in sequence, we traced 32P-labeled Oligo B_A or Oligo B_Am1 after transfection over time in both cytoplasmic and nuclear fractions. Both oligonucleotides demonstrated a similar spatial and temporal pattern of cellular localization, entering the cytoplasm before localizing in the nucleus shortly thereafter (Fig. 6B). After 12 h, the majority of each oligonucleotide had localized in the nucleus (Fig. 6B). Therefore, whereas both oligonucleotides reached the nuclear compartment with similar kinetics, only Oligo B_A, which bound the repressor (Fig. 3A), rescued the PDGF-B promoter from transcriptional repression.

**The Repressor Is Cell-specific**—Previous examination of the PDGF-B promoter by our group in vascular endothelial cells revealed no difference in CAT activity driven by a series of PDGF-B promoter-reporter constructs bearing or not bearing the negative regulatory element (23). Indeed, in contrast to findings in pup SMCs, no significant difference was observed in

**FIG. 7. Repression of PDGF-B promoter via the negative regulatory element is cell-specific.** A, Pup SMC and bovine aortic endothelial cells (BAEC) cells were transiently transfected with 8 µg of d18 or md18Am1. CAT activity after 24 h was normalized to the concentration of protein in the cell lysate. Error bars represent S.E. of the mean. B, comparative EMSA using pup SMC and bovine aortic endothelial cells nuclear extracts and 32P-Oligo B_A. Binding reactions and nondenaturing gel electrophoresis were performed as described under “Experimental Procedures.” C, EMSA confirms the integrity of pup SMC and bovine aortic endothelial cells nuclear extracts. Extracts of both cell types were incubated with 32P-Oligo A under conditions previously described for this probe (22). Nucleoprotein complexes were visualized by autoradiography. The data are representative of two independent determinations.

**FIG. 8. PDGF-B repressor is ERK-dependent.** A, Pup SMCs transiently transfected with 8 µg of construct d18 or md18Am1 were exposed to 30 µM MEK inhibitor PD98059 for 24 h. B, cotransfection of pup SMCs with d18 and 10 µg of either pcDNA3 or pcDNA3 dominant negative ERK1. After 24 h, CAT activity in the lysates was normalized to the concentration of protein. The data are representative of two independent determinations. Error bars represent S.E. of the mean.
activity generated by construct d18 or md18Am1 (Fig. 7A). Given the present data, we hypothesized that the lack of repression via this site in endothelial cells may be the consequence of low expression or complete absence of the repressor in this cell type.

We assessed whether 32P-Oligo B3 could support nucleoprotein complex formation with endothelial nuclear extracts in EMSA. Pup SMC extracts, as expected, formed the nucleoprotein complex formation with endothelial nuclear extracts (Fig. 7B). However, this complex was completely absent when endothelial nuclear extracts were used (Fig. 7B). To ensure that these findings were not merely the consequence of unequal protein loading or degradation of the endothelial extract, we performed EMSA with each extract using 32P-Oligo A (Fig. 7C). This produced an identical electrophoretic pattern of shifted complexes in both cell types, entirely consistent with previous observations (18, 22). These data indicate the cell-specific nature of –227/–221-directed repression and the capacity of this element to interact with nuclear protein(s).

Repression of the PDGF-B Promoter Is Mitogen-activated Protein Kinase-dependent—Previous studies have determined that certain transcriptional repressors are regulated by MAP kinases. For example, the repressor activity of the Ets family member, GETS-1, is abrogated by overexpression of Ras/MAP kinase or by mutation of Ser-405 MAP kinase phosphorylation site in GETS-1 (24). Similarly, MAP kinase phosphorylation of repressor protein, Bcl-6, leads to its rapid degradation via the ubiquitin/proteasome pathway (25). ERK1/2 is basally active in pup SMCs (data not shown). We investigated whether repression of the PDGF-B promoter via the –227/–221 site was dependent upon ERK. PD98059, an inhibitor of MEK1/2, the upstream activator of ERK, was incubated with SMCs transfected with construct d18. The flavone rescued reporter activity driven by the PDGF-B promoter (Fig. 8A), as previously observed using the wild-type oligonucleotide decoy (Fig. 6A). In contrast, PD98059 had no effect on higher basal expression generated by construct md18Am1 (Fig. 8A). To demonstrate the involvement of ERK in PDGF-B transcription, we overexpressed a dominant-negative mutant form of ERK1 in transient transfection setting with construct d18. CAT activity generated by construct d18 increased upon cotransfection with dominant negative ERK1 (Fig. 8B). Thus, unlike the paradigm of GETS-1 (24) and Bcl-6 (25), repression of the PDGF-B promoter conferred by the –227/–221 element requires active Raf/MEK/ERK (Fig. 8B).

In this paper, we have identified a novel negative regulatory element in the PDGF-B promoter, located 227/222 bp upstream of the TATA box, that binds nuclear proteins in a specific fashion and inhibits PDGF-B promoter-dependent gene expression in a Raf/MEK/ERK-dependent manner. Gel retardation and supershift assays excluded the direct association of Sp1, Sp3, and Egr-1 with this element. Repression of the PDGF-B promoter was rescued by (i) mutation of the –227/–221 site that ablates nucleoprotein complex formation, (ii) cotransfection with double-stranded oligonucleotide decoys, and (iii) pharmacological and dominant negative inhibitors of the Raf/MEK/ERK pathway. Comparative binding and transient transfection studies in endothelial cells revealed that repression of the PDGF-B promoter via this element is cell type-specific. These findings thus define a key negative regulatory element in the PDGF-B promoter.

The –227/–221 repressor element in the PDGF-B promoter does not fit the consensus motif for any known transcription factor. Competition studies by EMSA in vitro and decoy experiments in vivo illustrate the specific nature of this element.

That mutation ablates nucleoprotein complex formation and rescues the promoter from inactivity suggests that the repressor may function by active rather than passive means. For example, active transcriptional repressors that directly contact DNA include the human Kruppel-related factor YY1 (26), the Wilms’ tumor gene product WTI1 (27), and the human bZIP protein E4BP4 (28). The repressor may also inhibit PDGF-B expression by inhibiting the activity of positive transcriptional regulators such as Sp1, Sp3, and Egr-1, whose sites are located downstream in the proximal region of the promoter. This may involve protein-protein interactions while bound to the promoter in a three-dimensional setting. The MAP kinase dependence of repression via this site demonstrates that phosphorylation is a key event in the regulation of PDGF-B transcription. ERK may directly phosphorylate the repressor in the nucleus and/or modulate its stability or cellular localization. Isolation and characterization of this cell-restricted repressor should provide important insights into the molecular mechanisms negatively regulating PDGF-B transcription in vascular cells.

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