GC Factor 2 Represses Platelet-Derived Growth Factor A-Chain Gene Transcription and Is Itself Induced by Arterial Injury

Levon M. Khachigian, Fernando S. Santiago, Louise A. Rafty, Onza L.-W. Chan, Gabrielle J. Delbridge, Alex Bobik, Tucker Collins, Alfred C. Johnson

Abstract—Platelet-derived growth factor (PDGF) is a mitogen and chemoattractant for a wide variety of cell types. The genes encoding PDGF A chain (PDGF-A) and PDGF B chain (PDGF-B) reside on separate chromosomes and are independently regulated at the level of transcription. Regulatory events underlying inducible PDGF-A expression have been the focus of much investigation. However, mechanisms that inhibit transcription of this gene are not well understood. In this study, we report the capacity of a newly cloned DNA binding factor, GC factor 2 (GCF2), to repress expression driven by the human PDGF-A promoter. Deletion and transient cotransfection analysis in vascular endothelial cells revealed that GCF2 repression is mediated by a nucleotide region located in the proximal region of the PDGF-A promoter. Electrophoretic mobility shift assays demonstrate that GCF2 binds to this region in a specific and dose-dependent manner. Interestingly, the site bound by GCF2 overlaps those for specificity protein-1 (Sp1) and early growth response factor-1 (Egr-1), zinc finger transcription factors that direct basal and inducible expression of the PDGF-A gene. Gel shift experiments revealed that GCF2 competes with these factors for interaction with the PDGF-A promoter. Overexpression of GCF2 suppressed endogenous PDGF-A expression in vascular endothelial cells and smooth muscle cells. GCF2 was induced on mechanical injury of cells in culture as well as after balloon injury of the rat carotid artery wall. Time course studies revealed the sustained induction of GCF2 after injury while PDGF-A levels sharply returned to baseline. Smooth muscle cell proliferation was inhibited by GCF2, an effect reversed by the addition of exogenous PDGF-AA. These findings demonstrate negative regulation of PDGF-A expression by GCF2. This is the first report of the induction of an endogenous transcriptional repressor in the rat vessel wall. (Circ Res. 1999;84:1258-1267.)

Key Words: GC factor 2  platelet-derived growth factor-A  DNA binding protein  transcription  injury

Platelet-derived growth factor (PDGF) comprises a family of cationic polypeptides with mitogenic and chemoattractant activity. PDGF consists of an A chain (PDGF-A) or B chain (PDGF-B) held in homodimeric or heterodimeric configuration by disulfide linkages. These isoforms (AA, BB, and AB) are glycoproteins with an approximate molecular mass of 26 to 30 kDa. The PDGF-A– and PDGF-B–chain genes reside on different chromosomes, and their expression is independently regulated. The A-chain gene resides on chromosome 7 (7pter-7q22) and contains \( \approx 24 \) kb of DNA spanning 7 exons. A single transcriptional start site is located 36 bp downstream of the TATA box. The promoter is highly rich in guanines and cytosines (G+C) and contains functional binding sites for a number of zinc finger transcription factors including specificity protein-1 (Sp1), Sp3, early growth response factor-1 (Egr-1), and the Wilms tumor repressor gene product WT-1. These sites occur in the proximal region of the promoter hypersensitive to cleavage by S1 nuclease. Sp1 is required for basal expression of PDGF-A in both vascular endothelial cells and smooth muscle cells. PDGF-A promoter-dependent expression is induced in endothelial cells exposed to a variety of agonists and pathophysiological conditions, including fluid shear stress, phorbol ester, and (acidic) fibroblast growth factor and involves displacement of Sp1 from the promoter by Egr-1. Egr-1 binds to the PDGF-A promoter, as well as the PDGF-B promoter within minutes of exposure to agonist.

In contrast to inducible PDGF transcription, mechanisms downregulating PDGF expression are not well characterized. Transcriptional repressors inhibit gene expression by one of at least two general mechanisms. First, passive repression, involves nuclear proteins competing with positive transcriptional regulators for common binding sites. These repressors may also modulate promoter activity by interacting with...
transcriptional activators themselves. Activity of the PDGF-B promoter can be repressed by the zinc finger protein ZNF174, cloned originally from a human fetal library, likely by passive means.

Second, active repression, involves the intrinsic inhibitory activity of the repressor. WT-1, an active repressor, can inhibit PDGF-A promoter activity as well as expression driven from the promoters of a large number of other genes. These include insulin-like growth factor type II, transforming growth factor-β1, colony-stimulating factor-1, and insulin-like growth factor-1 receptor. Despite the capacity of WT-1 to repress reporter expression driven by multiple growth factor promoters, the relevance of WT-1 repression to the regulation of the authentic gene has not been established.

Additionally, the limited pattern of WT-1 expression and the transient nature of growth factor induction suggests that other repressors may exist with the ability to negatively regulate growth factor gene expression.

GCF is a transcriptional repressor so termed because of its preference for G+C-rich elements in DNA. GCF is a 91-kDa protein that interacts and downregulates expression driven by the epidermal growth factor receptor (EGFR) promoter, which lacks TATA and CAAT motifs. A second member of the GCF family, GCF2, was recently isolated from an ovarian carcinoma cDNA library. GCF2 shares homology with GCF in a 309-bp region located at positions 1382-1690 within the C-rich sequence in the proximal region of the promoter. GCF2 represses the activity of the PDGF-A promoter in a specific and dose-dependent manner and competes with transcription factors directing expression of the gene for overlapping binding sites. GCF2 represses PDGF-A promoter-dependent expression and can inhibit the expression of the endogenous gene itself. In addition, we provide evidence for an inverse relationship between the repressor and the growth factor in the artery wall after mechanical injury.

**Materials and Methods**

**Cell Culture**

Bovine aortic endothelial cells were purchased from Cell Applications, Inc, and grown in DMEM (pH 7.4) containing 10% FBS, 50 μg/mL streptomycin, and 50 IU/mL penicillin at 37°C in a humidified atmosphere of 5% CO₂. Rat aortic smooth muscle cells were obtained from Cell Applications, Inc, and grown in Waymouth’s medium (pH 7.4) containing 10% FBS and antibiotics. Cells were routinely passaged with trypsin/EDTA and not used in experiments beyond passage 6.

**Transient Transfection Analysis and Chloramphenicol Acetyltransferase (CAT) Assay**

Endothelial cells in 100-mm-diameter dishes at 70% confluence were transfected with the indicated amounts of cesium chloride–purified plasmid DNA using a modification of a calcium phosphate precipitation method. After transfection, the cells were incubated overnight in an atmosphere of 3% CO₂, washed twice with PBS (pH 7.4) and incubated for an additional 24 hours at 5% CO₂. Cell lysates were assessed for CAT activity as previously described. Conditioned medium was sampled at the time the cells were harvested and assayed for human growth hormone (Bioclone) to correct for transfection efficiency. Total protein concentration in the lysates was assessed using a modified Bradford assay (Bio-Rad) and used to normalize transfection data.

**Electrophoretic Mobility Shift Assay (EMSA)**

Binding reactions for gel shift assays were performed in 20 μL of 10 mmol/L Tris-HCl, 50 mmol/L MgCl₂, 1 mmol/L EDTA, 1 mmol/L DTT, 5% glycerol, 1 mmol/L PMSF, 0.75 μg salmon...
sperm DNA, 5% sucrose, 0.75 μg poly dI-dC, 10 μg of BSA, 32P-labeled oligonucleotide probe (150 000 cpm), and 1.5, 3, or 6 μg of in vitro transcribed/translated GCF2 protein. The reaction was incubated for 35 minutes at 22°C. In competition gel shifts, 6 μg of nuclear extract was prepared as described and combined with 6 μg of GCF2 protein. As a control, an equal amount of BSA protein was substituted for GCF2. Bound complexes were separated from free probe by loading the samples onto a 5% non-denaturing polyacrylamide gel (PAGE) and electrophoresing at 200 V for 2 to 3 hours. The gels were dried and subjected to autoradiography overnight at −80°C.

Northern Blot Analysis
Endothelial cells or smooth muscle cells were grown to 70% confluency in 100-mm-diameter Petri dishes and transfected with 10 μg pcDNA3 or GCF2-pcDNA3 using FuGENE6 reagent (Boehringer Mannheim) at a FuGENE6:DNA ratio of 3:1 (vol:μg), per the manufacturer’s instructions. After 24 hours, total RNA was extracted with TRIzol reagent (Life Technologies), and 20 μg of each sample was resolved by electrophoresis on 1% formaldehyde/agarose/MOPS gels. RNA was transferred overnight onto Hybond nylon membranes before hybridization with a 32P-labeled 260-bp fragment of PDGF-A cDNA in ExpressHyb (Clontech). Vacuum-dried gels were autoradiographed for 1 week at −80°C, then stripped and reprobed with a 32P-labeled 1.1-kb fragment of GCF2 cDNA, and later with a 32P-labeled fragment of β-actin cDNA followed by reexposure.

Injury and Western Blot Analysis
Endothelial cells or smooth muscle cells were rendered growth-quiescent by incubation overnight in medium containing 1% or 0.1% FBS, respectively. The cells were injured by repeated scraping with a sterile stainless steel comb and solubilized in RIPA buffer. Extracts were resolved by 10% SDS-PAGE and transferred to nylon membranes by electrophoretic blotting. Nonspecific binding sites on the membrane were blocked with nonfat skim milk before probing with affinity-purified rabbit polyclonal antibodies to PDGF-A (Genzyme) or GCF2. This was followed by chemiluminescent detection using HRP-linked swine anti-rabbit secondary antiserum (NEN-DuPont).

Assays of Cell Proliferation
Cells were seeded into 96-well titer plates (Nunc-InterMed) at 2500 cells per well and allowed to grow for 48 hours in complete medium. The cells were transfected with 1 μg of the indicated plasmid in complete growth medium using FuGENE6 (Boehringer Mannheim) in accordance with manufacturer’s instructions. At the time of transfection, and again after 24 hours, the cells were rinsed with PBS (pH 7.4) and trypsinized, and the suspension was quantitated using an automated particle counter (Coulter Electronics).

Rat Carotid Artery Injury and Immunohistochemical Analysis
Sprague-Dawley rats (400 g) were obtained from the Biology Research Unit, Baker Medical Research Institute, Melbourne, Aus-
GCF2 Represses PDGF-A Promoter-Dependent Expression

To determine whether GCF2 can influence expression driven by the PDGF-A promoter, we transfected endothelial cells with a GCF2 expression vector driven by the cytomegaloviral (CMV) promoter (construct GCF2-pcDNA3), together with a CAT reporter construct, ΔSac, bearing 643 bp of PDGF-A promoter sequence. CAT activity was normalized for transfection efficiency after assessment of human growth hormone levels secreted into the culture medium. Overexpression of GCF2 in this cotransfection setting strongly inhibited reporter expression driven by the PDGF-A promoter (Figure 1). GCF2 repression of PDGF-A promoter-driven CAT activity was also observed in rat vascular smooth muscle cells (data not shown). In contrast, overexpression of a transcriptional activator of PDGF-A, Egr-1, with the same backbone (pcDNA3) driven by the PDGF-A promoter, we transfected endothelial cells with a 153 bp of the PDGF-B promoter,23 which also observed in rat vascular smooth muscle cells (data not shown). GCF2 in this cotransfection setting strongly inhibited reporter expression driven by the PDGF-A promoter (Figure 1). GCF2 repression of PDGF-A promoter-driven CAT activity was also observed in rat vascular smooth muscle cells (data not shown). In contrast, overexpression of a transcriptional activator of PDGF-A, Egr-1, with the same backbone (pcDNA3) stimulated CAT activity 2-fold (Figure 1).

Repression by GCF2 Is Mediated by Nucleotide Elements Located in the Proximal Region of the PDGF-A Promoter

GCF2 binds preferentially to nucleotide sequences rich in guanines and cytosines.17 The proximal region of the PDGF-A promoter is intensely G+C-rich.1 We hypothesized that GCF2 repression of the PDGF-A promoter may involve elements located in the proximal region. Transient cotransfection experiments revealed that expression driven by construct f28, which contains 71 bp of PDGF-A promoter sequence,2 was inhibited by GCF2 coexpression (Figure 2). However, when GCF2-pcDNA3 was cotransfected with construct f36, which bears 55 bp of promoter sequence,2 repression by GCF2 was no longer observed (Figure 2). These findings suggest that GCF2 repression of the PDGF-A promoter is mediated by nucleotide elements located between the 5’ promoter end points of f28 and f36. The GCF2 binding site in the epidermal growth factor receptor promoter has a core binding sequence of 5’-AGC CCC CCG CG-3’ (or 5’-CG CCG GGG GGT-3’).17 The nucleotide sequence between the f28 and f36 5’ end points bears the 5’-CGG GGG C-3’ motif (Figure 2). Separate experiments revealed that a CAT reporter construct driven by 153 bp of the PDGF-B promoter,23 which does not bear the GCF2 core binding sequence, was unaffected by cotransfection with GCF2-pcDNA3 (data not shown).

GCF2 Interacts With Proximal PDGF-A Promoter

To determine whether GCF2 binds directly to the PDGF-A promoter, we incubated in vitro transcribed/translated GCF2 with a 32P-labeled probe, Oligo A, whose sequence spans the 5’ end points of f28 and f36, and resolved the adducts by electrophoresis under nondenaturing conditions. GCF2 bound to the oligonucleotide in a dose-dependent manner as two distinct nucleoprotein complexes (Figure 3A). The upper and more intense complex represents dimeric GCF2, whereas the lower complex is the monomeric form.17 Neither complex was observed when an oligonucleotide bearing an unrelated sequence, 32P-LKmdc, was substituted for 32P-Oligo A (Figure 3A). Furthermore, neither complex formed when 32P-Oligo Am was substituted for 32P-Oligo A (Figure 3A). Oligo Am was generated by altering the 5’-CGG GGG C-3’ repeated motif in Oligo A by insertion of additional guanines (5’-CGG GGG gC-3’) thus disrupting the promoter sequence.
When wild-type $^{32}$P-Oligo A was incubated with GCF2Δ510, an in vitro transcribed/translated form of GCF2 that carries a deletion in its DNA binding domain, nucleoprotein complex formation was no longer observed (Figure 3B). Additional experiments revealed that wild-type GCF2 failed to interact with a $^{32}$P-labeled G+C-rich RNA oligomer composed of 23 bases (data not shown). Taken together, these findings demonstrate that GCF2 interacts with the proximal PDGF-A promoter in a dose-dependent and specific manner.

**Competition Between Several Zinc Finger Binding Proteins and GCF2 for Binding Sites in the Proximal PDGF-A Promoter**

The preceding findings demonstrate the capacity of GCF2 to bind to the PDGF-A promoter and inhibit promoter-dependent reporter gene expression. We next addressed the question of the mechanism underlying GCF2 repression. Previous studies by our group have shown that a number of zinc finger transcription factors interact with the GCF2 binding site and mediate basal and inducible expression of the PDGF-A gene. These nuclear factors include Sp1 and Egr-1.2 We explored the possibility that GCF2 competes with these factors for binding to the PDGF-A promoter. Endothelial extracts were incubated with $^{32}$P-Oligo A in the absence or presence of GCF2 or a corresponding amount of BSA, before resolution of $^{32}$P-labeled nucleoprotein complexes by electrophoresis. This produced the characteristic profile of $^{32}$P-labeled nucleoproteins composed of Sp1, Egr-1, Sp3, and other nuclear proteins2,3,24 (Figure 4). Inclusion of GCF2 in the binding mixture markedly attenuated the intensity of the Sp1, Egr-1, and Sp3 complexes, among other complexes (Figure 4). In contrast, these complexes were unaffected by substitution of GCF2 with an identical amount of BSA (Figure 4). Thus, GCF2 competes with these positive regulatory transcription factors for overlapping binding sites in the PDGF-A promoter.

**Mechanical Injury Stimulates PDGF-A and GCF2 Expression In Vitro**

To determine the potential pathophysiological relevance of the above findings to vascular disorders, we examined the relationship between GCF2 and PDGF-A expression in an in vitro model of cell injury. Endothelial cell monolayers were scraped repeatedly with a sterile stainless steel comb, and lysates were assessed for levels of PDGF-A. PDGF-A was weakly expressed in unmanipulated endothelial cells (Figure 5A). However, within 4 hours of injury, PDGF-A levels increased 3-fold (Figure 5A, open bars), consistent with a previous report of inducible PDGF-A expression in aortic endothelium within 4 hours of mechanical injury.25 By 8 hours, PDGF-A levels increased almost 10-fold and returned to preinjury levels by 12 hours (Figure 5A).

We next determined whether GCF2 was induced by injury in this model. GCF2 is basally expressed in resting cells, and...
levels increased 4 hours and 8 hours after injury (Figure 5B).
By 12 hours, however, GCF2 levels increased 3-fold (Figure 
5B, closed bars), at which time PDGF-A levels had returned 
to background (Figure 5A). The inverse temporal correlation 
between GCF2 and PDGF-A after peak expression of the 
growth factor in this model suggests a regulatory role for 
GCF2 in the expression of PDGF-A after injury.

Mechanical Injury of Arteries In Vivo Stimulates 
GCF2 Expression
To provide evidence for a similar relationship between these 
genes in the artery wall, levels of PDGF-A and GCF2 were 
assessed in the arterial media by immunohistochemical anal-
ysis various times after balloon catheter injury of the rat 
carotid. PDGF-A antigen was weakly, if at all, expressed in 
the unmanipulated artery wall (Figure 6A). Within 4 hours of 
injury, PDGF-A was clearly expressed in the media (Figure 
6B, arrow) as previously reported. Expression was only 
transient, because PDGF-A immunoreactivity was not de-
tected at 6 hours (Figure 6C) or after 10 days (Figure 6D), 
when a significant neointima had formed, a hallmark of this 
model.

In contrast to PDGF-A, GCF2 was basally expressed in 
normal artery wall (Figure 7A). Injury dramatically increased 
GCF2 expression in the media within 4 hours (Figure 7B). 
Unlike PDGF-A, GCF2 levels remained elevated over the 
next 18 hours (Figures 7B through 7D). Indeed, intense GCF2 
expression even 24 hours after injury (Figure 7D) contrasts 
dramatically with the rapid decline in PDGF-A levels be-
tween 4 and 6 hours (Figures 6B through 6C). Ten days after 
injury, GCF2 was still expressed at levels comparable to 
those in the uninjured vessel wall (compare Figure 7E to 7A). 
Staining for both GCF2 and PDGF-A was specific, because 
neither the secondary antibody nor components of the immu-
nohistochemical detection system produced positive staining 
in the absence of primary antibody. These findings provide a 
spatial and inverse temporal correlation between GCF2 and 
PDGF-A after peak PDGF-A expression after injury to the 
vessel wall.

We performed Western blot analysis for GCF2 protein 
with lysates of cultured smooth muscle cells prepared various 
times after mechanical injury to verify findings from immu-
nostaining of the injured artery wall (Figure 7). GCF2 protein 
was inducibly expressed within 1 hour of injury; levels 
continued to increase at 4 hours and 6 hours (Figure 8). These 
data are consistent with the temporal pattern of inducible 
GCF2 protein expression in medial smooth muscle cells of 
the injured vessel wall (Figure 7).

Overexpression of GCF2 Attenuates Endogenous 
PDGF-A Expression
The temporal relationship between GCF2 and PDGF-A in 
these models suggests that GCF2 may downregulate the 
expression of endogenous PDGF-A. Because our earlier 

Figure 7. Sustained activation of GCF2 by injury. Immunohisto-
chemical analysis was performed on 5-μm cross sections of 
balloon-injured rat carotid arteries using affinity-purified rabbit 
polyclonal antibodies raised against in vitro transcribed/trans-
lated GCF2. A, Uninjured artery. B, Four hours after injury. C, 
six hours after injury. D, Twenty-four hours after injury. E, Ten 
days after injury. F, Uninjured artery without GCF2 (primary) 

antibody but with secondary (goat anti rabbit IgG) antibody and 
components of ABC kit. Brown staining indicates specific signal. 
The blue stain is a consequence of Evans blue infusion before 
sacrifice. Sections were counterstained with hematoxylin to 
identify cell nuclei. Magnification ×200.
studies used PDGF-A promoter reporter constructs in an overexpression setting, we performed Northern blot analysis using total RNA of two different vascular cell types 24 hours after transfection with GCF2-pcDNA3 or the backbone alone. Because this approach requires high transfection efficiency, we used a lipid-based formulation that enabled transfection of virtually the entire cell population (data not shown). PDGF-A mRNA, as expected, was expressed at higher levels in smooth muscle cells than endothelial cells (Figure 9A). In both cell types, however, endogenous PDGF-A expression was completely inhibited by GCF2-pcDNA3 transfection (Figure 9A). To confirm the expression of GCF2 on transfection of this plasmid, the blot was stripped and reprobed with a 32P-labeled GCF2 cDNA fragment. Strong hybridization was observed in both cell types with an mRNA species of ~2.3 kb (Figure 9A), consistent with the size of GCF2 cDNA insert present in the vector. In contrast, β-actin mRNA levels were unaffected by GCF2 overexpression (Figure 9A). These findings demonstrate the capacity of GCF2 to suppress native PDGF-A expression in two vascular cell types. To demonstrate expression of GCF2 protein, as well as mRNA, after transfection with GCF2-pcDNA3, we prepared nuclear extracts of cells transfected with this construct as well as the backbone alone. EMSA, using these extracts together with 32P-labeled Oligo A, indicates that in vivo expressed GCF2 protein can interact with its binding site in the proximal PDGF-A promoter (Figure 9B).

Figure 8. GCF2 protein is inducibly expressed by cultured smooth muscle cells after mechanical injury. Quiescent confluent smooth muscle cells were injured by repeated scraping, and lysates were resolved by SDS-PAGE. Western immunoblot analysis was performed with GCF2 antibodies. Bands were visualized by chemiluminescent detection. Gels were stained with Coomassie blue to demonstrate equal protein loading.

Figure 9. Overexpression of GCF2 represses PDGF-A mRNA expression. A, Total RNA (20 μg) from endothelial cells (ECs) or smooth muscle cells (SMCs) transfected with 10 μg pcDNA3, GCF2-pcDNA3, or GCF2Δ428-pcDNA3 using FuGENE6 in complete growth medium. Cell numbers per well were determined immediately before (day 0) or 24 hours after (day 1) the addition of serum (6 PDGF-AA, 30 ng/mL), by quantitation of trypsinized suspensions by Coulter counter. Cell numbers were not assessed on subsequent days because of artifact introduced by contact inhibition. SFM (empty bars) indicates cells that were incubated with serum-free medium and exposed to the same amount of FuGENE6 as those transfected with DNA. Mean ± SEM of 3 experiments performed in triplicate is shown. **P < 0.0001 relative to the pcDNA3 value by Student paired t test.

Figure 10. GCF2 overexpression inhibits smooth muscle cell replication. ECs or SMCs were seeded in 96-well plates and left undisturbed for 48 hours. Cells were transfected with 1 μg pcDNA3, GCF2-pcDNA3, or GCF2Δ428-pcDNA3 using FuGENE6 in complete growth medium. Cell numbers per well were determined immediately before (day 0) or 24 hours after (day 1) the addition of serum (6 PDGF-AA, 30 ng/mL), by quantitation of trypsinized suspensions by Coulter counter. Cell numbers were not assessed on subsequent days because of artifact introduced by contact inhibition. SFM (empty bars) indicates cells that were incubated with serum-free medium and exposed to the same amount of FuGENE6 as those transfected with DNA. Mean ± SEM of 3 experiments performed in triplicate is shown. **P < 0.0001 relative to the pcDNA3 value by Student paired t test.

Overexpression of GCF2 Inhibits Smooth Muscle Cell Replication

Finally, we determined whether exogenous GCF2 had the ability to influence cell proliferation. Endothelial cells or smooth muscle cells transfected with either GCF2-pcDNA3
or pcDNA3 were resuspended by trypsinization and quantitated. Endothelial cell replication after 24 hours was unaffected by GCF2 transfection (Figure 10A). However, proliferation was inhibited in smooth muscle cells transfected with GCF2 (Figure 10B). Cell growth in this group 24 hours after transfection was 37% less than the population of cells transfected with pcDNA3 alone (Figure 10B). In contrast, proliferation of smooth muscle cells transfected with construct GCF2Δ428-pcDNA3, which lacks the GCF2 DNA binding domain, was not inhibited (Figure 10B). Interestingly, addition of PDGF-AA (30 ng/mL) rescued the cells from growth inhibition by GCF2 (Figure 10B). Morphological inspection of both cell types as well as trypan blue exclusion experiments revealed that differences in cell number were not due to cytotoxicity (data not shown). Therefore, although GCF2 suppressed endogenous PDGF-A expression in endothelial cells and smooth muscle cells, proliferation of only the latter cell type was attenuated as a consequence.

**Discussion**

The specificity and efficiency with which eukaryotic genes are transcribed depend on specific, ordered interactions between transcription factors and cis-acting nucleotide elements in promoter regions. Models of inducible gene transcription involve the formation of 3-dimensional stereospecific enhancer complex composed of transcription factors that can bend DNA and components of the basal transcriptional hancer complex composed of transcription factors that can involve the formation of 3-dimensional stereospecific enhancer complex composed of transcription factors that can bend DNA and components of the basal transcriptional machinery. Repression is less well understood but is, nonetheless, an equally important aspect of transcriptional regulation. Here, we have described the ability of a newly cloned DNA binding factor, GCF2, to repress expression driven by the human PDGF-A promoter. Briefly, deletion and transient transfection analysis revealed that GCF2 repression is mediated at a region located between the 5’ end points of PDGF-A promoter constructs f28 and f36. Gel shift experiments demonstrated that GCF2 interacts with this region of the promoter in a specific, dose-dependent manner and competes with activating transcription factors for overlapping binding sites. GCF2 is activated by mechanical injury in culture as well as in the artery wall. GCF2 can suppress endogenous PDGF-A expression in both endothelial cells and smooth muscle cells but attenuates proliferation in only the latter cell type.

The precise mechanism(s) with which GCF2 regulates PDGF-A transcription, or the transcription of other genes, is not clear. Active repressors, such as WT-1, and the human Kruppel-related factor, YY1, possess intrinsic repressing activity and the capacity to downregulate transcription through modular domains. On the other hand, passive repressors downregulate transcription by modulating the activity of positive regulatory factors. An example of a passive repressor is GCF, which may compete with transcription factors for common binding sites or interact directly with these proteins. In addition, NAB1 and NAB2 repress Egr-1-dependent transactivation by interacting with this zinc finger protein. GCF2 may also function as a passive transcriptional repressor, because it competes with Sp1 and Egr-1 for common binding sites in the PDGF-A promoter, and these transcription factors each activate PDGF-A transcription. Alternatively, or in addition, GCF2 may interact with DNA to destabilize bound factors or interact directly with factors to prevent binding or increase off rates. GCF2, like some repressors, may have the capacity to activate transcription under certain conditions. For example, whether WT-1 functions as a transcriptional repressor or activator seems to be dependent on physical interactions with p53. Although the present findings define a transcriptional regulatory role for GCF2, we cannot yet rule out the possibility that GCF2 controls gene expression by additional mechanisms, such as by regulation of mRNA stability. Detailed structural charaterization of GCF2 should provide more detailed insights into the functional properties of this transcriptional repressor.

Many genes, whose products can influence chemotactic, mitogenic, adhesive, and thrombotic events are activated in response to mechanical injury (reviewed in References 38 through 42). Several of these genes are under the transcriptional control of factors, such as c-Fos, c-Jun, c-Myc, c-Ets-1, nuclear factor-κB, and Egr-1, which are themselves activated by injury to the vessel wall. In contrast to positive regulatory factors, the present study is the first to report the inducible expression of an endogenous transcriptional repressor after mechanical injury of vascular cells in the rat model. Previously, Weir et al demonstrated downregulation of the growth arrest homeobox gene, gax, after balloon injury to the rat carotid. Recently, Aoyagi et al demonstrated p53 induction in the rabbit carotid artery wall after balloon denudation. Because GCF2 inhibits both PDGF-A promoter-dependent and endogenous PDGF-A mRNA expression, the repressor could influence the course of PDGF-A expression after injury. The capacity of GCF2 to inhibit smooth muscle cell replication without influencing endothelial cell growth is consistent with the notion of autocrine growth loop(s) involving PDGF in the former cell type. Smooth muscle cell responsiveness can be inhibited by substances such as suramin, and antisense oligonucleotides directed at PDGF-A mRNA, and α1-macroglobulin, which block the PDGF ligand–PDGF receptor circuit. GCF2 may interfere with autocrine growth involving PDGF-A at the earliest stage in the cycle by inhibiting the production of the growth factor. That GCF2 inhibition of smooth muscle cell proliferation could be rescued by addition of exogenous PDGF-AA is consistent with an autocrine role for the PDGF A chain.

Unlike smooth muscle cells, large vessel endothelial cells in culture do not express PDGF receptors. It is therefore not surprising that GCF2 could not inhibit the growth of cells lacking receptors for PDGF-A.

The promoters of many growth factor genes induced by injury, such as transforming growth factor-β, fibroblast growth factor-2, and PDGF-B, also contain G+C-rich elements that are bound by Sp1 and Egr-1. The capacity of GCF2 to compete with these and other zinc finger transcription factors indicates that negative regulation by GCF2 may not be confined to PDGF-A alone. Because Sp1 is required for COL1A1 and α2 collagen expression, and collagen accumulation in the neointima occurs when GCF2 levels have already returned to background (Figure 8), it is conceiv-
able that GCF2 may also play a regulatory role in the composition of the matrix in the neointima.

The mechanisms underlying the induction of GCF2 itself, in the context of injury or any other stimulus, are not known. Elucidation of the genomic structure of GCF2 should provide valuable insights for the regulation of this gene. Future studies should determine whether positive regulatory factors such as Sp1 and Egr-1 can influence the inducible expression of this repressor and whether GCF2 can repress its own expression.

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