Yin Yang-1 (YY1) is a multifunctional transcription factor that can repress the expression of many growth factor, hormone, and cytokine genes implicated in atherosclerosis. YY1 expression is activated in rat vascular smooth muscle cells shortly after injury. YY1 DNA binding activity paralleled elevated protein levels in the nucleus. Smooth muscle cell injury triggered the rapid extracellular release of immunoreactive fibroblast growth factor-2 (FGF-2). YY1 induction after injury was blocked by neutralizing antibodies directed against FGF-2. This growth factor increased YY1 mRNA and protein expression and stimulated YY1 binding and transcriptional activity. Overexpression of YY1 inhibited smooth muscle cell replication. Immunohistochemical analysis demonstrated YY1 staining in medial smooth muscle cells, coincident with FGF-2 expression. Proliferating cell nuclear antigen staining, in contrast, was confined mainly to the atherosclerotic intima. This is the first demonstration that YY1 is induced by either injury or FGF-2, is differentially expressed in normal and diseased human arteries, and that its overexpression inhibits vascular smooth muscle but not endothelial cell replication.

Yin Yang-1 (YY1; also called NF-E1, Δ, or UCRBP) is a GLI-Kruppel-type zinc finger nuclear factor that is able to repress, activate, and initiate transcription depending on promoter architecture and the cellular environment (1, 2). For example, YY1 activates or represses the c-myc promoter depending on the orientation of a YY1 recognition element in the promoter (3). YY1 switches between an activator or repressor of the human papillomavirus type 18 promoter depending on the integrity of a distinct element upstream in the promoter (4). YY1 competes with nuclear factor-κB for overlapping binding sites in the serum amyloid A1 promoter, inhibits promoter activity by passive means (5), and can antagonize the interaction of serum response factor to overlapping binding sites in the actin promoter (6). YY1 functionally interacts with a large number of key transcriptional regulators such as Sp1, e-Myc, adenovirus E1A, the cAMP-response element-binding protein-related factor, p300, and components of the general transcriptional apparatus including the large subunit of RNA polymerase II and transcription factor IIIB (7–11). The capacity of YY1 to bind DNA when it binds the promoter may help facilitate direct contact between regulatory proteins. YY1 can interact with histone deacetylases to repress the activity of certain promoters, including the human immunodeficiency virus, type 1 long terminal repeat (12), thereby modulating histone and chromatin structure.

The pathogenesis of common vascular disorders such as atherosclerosis and restenosis after balloon angioplasty is believed to be mediated at least in part by phenotypic changes involving smooth muscle cells of the artery wall. These cells normally adopt a “contractile” phenotype (13) in the vessel wall, but upon activation (such as mechanical injury imparted by angioplasty), these cells become “synthetic” (13) and contribute to developing lesions by migrating, proliferating, producing extracellular matrix, and elaborating and responding to myriad growth-regulatory molecules (14, 15). YY1 can repress the promoters of a wide spectrum of pro-atherogenic genes, including cytokines, hormones, and growth factors (16–21). However, whether YY1 is even expressed in the artery wall or is regulated in the adaptive response to injury is presently not known, nor is it known whether YY1 can influence the growth of smooth muscle cells or other cell types.

**Experimental Procedures**

**Cell Culture**—Primary rat aortic smooth muscle cells were obtained from Cell Applications, Inc. (San Diego, CA) and cultured in Waymouth’s medium, pH 7.4, containing 10% fetal bovine serum, 10 units/ml penicillin, and 10 μg/ml streptomycin in a humidified atmosphere of 5% CO₂ at 37 °C. The cells were rendered quiescent by incubation in Waymouth’s medium, pH 7.4, containing 0.25% fetal bovine serum for 24 h. Cells were not used beyond passage 7 in experiments. Additionally, normal medial vascular smooth muscle cells were derived and characterized from coronary arteries of patients undergoing cardiac transplantation for non-ischemic cardiomyopathy and human atherosclerotic plaque vascular smooth muscle cells from carotid endarterectomy specimens of patients with symptomatic carotid disease. Cells were cultured to passage 2–4 before isolation for nuclear extracts.

**cDNA Array Analysis**—Differential gene expression between injured and uninjured smooth muscle cells was assessed using Atlas cDNA expression arrays (CLONTECH Laboratories, Palo Alto, CA). Briefly,
total RNA (15 μg) was treated with RNase-free DNase I and then mRNA was isolated using a poly(A) Quik mRNA isolation kit (Stratagene, La Jolla, CA). CDNA was generated from equivalent amounts of mRNA, and 32P-labeled cDNAs were purified using Chromaspin-200 diethyl pyrocarbonate-water columns provided with the kit. Probes with identical copy numbers were hybridized to nylon filters and exposed to autoradiographic film under conditions specified by the manufacturer. Hybridization signals were quantitated using ImageQuant software (Amersham Pharmacia Biotech).

**RT-PCR**—Total RNA was prepared from cells that were injured (22) or exposed to FGF-2 with TRIzol in accordance with the manufacturer's instructions (Life Technologies, Inc.). RNA was reverse-transcribed to cDNA using oligo(dT) primers and Superscript (Life Technologies, Inc.). PCR was performed using Platinum Taq DNA polymerase (Life Technologies, Inc.) with the following amplification conditions: 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1 mM MgCl2, 250 μM dNTP, 0.5 μM primers, 2 μl of cDNA, and 1 unit of Platinum Taq DNA polymerase. For YY1 PCR, the same cycling conditions were used except for an annealing temperature of 58 °C and a cycle number of 25. Primer sequences for YY1 were YY1a5 (5′-GAAAACATCTGACCACTCGGCTC-3′) and YY1a3 (5′-GTCCTCTGTTGGGACCACAC-3′), whereas those of GAPDH are GAP5 (5′-ACCCAGCATCGCCACCTGAC-3′) and GAP3 (5′-TCCACCACCTGGTGCTGATAG-3′). Linearity of gene expression was established by cycle-based RT-PCR in pilot experiments for both YY1 and GAPDH.

**Western Blot Analysis**—Lysates of cells injured or exposed to FGF-2 were resolved by electrophoresis on denaturing 10% SDS-polyacrylamide gels for 2 h at 100 V. After transfer of proteins to Immobilon P nylon membranes (Millipore, Bedford, MA) and blocking nonspecific binding sites with nonfat skim milk, membranes were incubated with mouse monoclonal anti-peptide antibodies targeting YY1 (Santa Cruz Biotechnology, Santa Cruz, CA) (1:1000) prior to chemiluminescence detection (PerkinElmer Life Sciences). Cooamassie-stained gels were destained and photographed to confirm equal loading. In FGF-2 antibody preincubation experiments, growth-quiescent smooth muscle cells were incubated with neutralizing rabbit anti-human FGF-2 antibodies or rabbit pre-immune IgG (100 μg/ml) for 1.5 h prior to scraping and assessment of YY1 expression by Western blotting. The FGF-2 antibody (AB-33-NA; R & D Systems) does not cross-react with recombiant human (rh) FGF-4, rhFGF-5, rhFGF-6, rhFGF-7, recombinant murine (rm) FGF-8b, rmFGF-5c, rhFGF-9, rhFGF-10, rmFGF-15, rhFGF-17, or rhFGF-18 and has less than 5% reactivity with FGF-1 (acidic) and rhβ-ECGF, based on direct enzyme-linked immunosorbent assay by the manufacturer (R & D Systems).

**Electrophoretic Mobility Shift Analysis**—Nuclear extracts of cells injured (22) or exposed to FGF-2 were prepared as described previously (23). Binding reactions were performed using 10 μg of nuclear extract in 20 μl containing 1 μg of poly(dI-dC)-poly(dI-dC) (Sigma), 12 mM HEPES, pH 7.9, 4 mM Tris-HCl, pH 7.9, 1 mM EDTA, 1 mM dithiothreitol, 12 mM glycerol, and the 32P-labeled Oligo MVYY1 (120,000 cpm) or 32P-labeled Oligo MVYY1 (120,000 cpm) in the continuous presence of 5% fetal bovine serum. Plasmid transfection of endothelial and smooth muscle cells with FuGENE6 is extremely efficient (25). The cells were trypsinized, and suspensions were quantitated by automated Coulter counter.

**Northern Blot Analysis**—Total RNA was isolated from smooth muscle cells or endothelial cells 24 h after transfection with 10 μg of either pcPB 3′-YY1 or pcPB 5′-YY1 using FuGENE6 and probed with 32P-labeled YY1 cDNA (generated by PCR using the same primers described above) or 32P-labeled GAPDH cDNA in Northern blot analysis as described previously (22, 23).

**Immunohistochemical Detection of YY1, p53, FGF-2, and PCNA in Human Carotid Arteries**—Immunohistochemical analysis was performed with antibodies to YY1 (sc-7541, final dilution 1:200; Santa Cruz Biotechnology, Santa Cruz, CA), p53 (DO-1; Santa Cruz Biotechnology, Santa Cruz, CA) (1:50), FGF-2 (AB-33-NA; Dako), basic FGF (AB-33-NA, dilution 1:200; R & D Systems), and smooth muscle α-actin (ASM-1, dilution 1:25; Novacstra) on consecutive paraffin sections of formalin-fixed atherosclerotic carotid artery specimens obtained by endarterectomy at ST. Vincent’s Hospital, Sydney, NSW, Australia. Prior to staining, deparaffinized sections were treated with 3% hydrogen peroxide (peroxidase blocking) and boiled in citrate buffer, pH 6.0, to retrieve antigenicity. The standard avidin-biotin complex immunoperoxidase technique was used (26). After washing in Tris-buffered saline, pH 7.6, sections were incubated in the primary antibody for 60 min, followed by incubation with the appropriate secondary antibody (horse anti-mouse, Vector BA-2000 or goat anti-rabbit, Vector BA-1000) for 20 min, and finally with avidin-biotin complex (Elite Vector PK-6100) for 30 min. Immunoreactivity was visualized by treatment in 3,3'-diaminobenzidine solution for 2 min, which produced brown coloring. Sections were counterstained with Mayer’s hematoxylin. As negative control, the primary antibody was omitted, or the sections were treated with the immunoglobulin fraction of suitable non-immune serum as a substitute for the primary antibody. No positive staining was observed in any of the negative control sections (data not shown).

**RESULTS AND DISCUSSION**

**YY1 Expression and Binding Activity Is Induced in Smooth Muscle Cells in Response to Injury**—In efforts to identify new genes that are activated following mechanical injury in vascular smooth muscle cells, we reverse-transcribed mRNA isolated from injured and uninjured growth-quiescent aortic smooth muscle cells and compared the expression of specific genes by hybridization with a spatially addressable cDNA microarray. We demonstrated previously that the immediate-early gene and transcription factor early growth response factor-1 (Egr-1) is rapidly induced by injury using a well-established in vitro scraping model (24). YY1 transcript levels, like those of Egr-1, increased severalfold within 1 h of injury (Fig. 1A). In contrast, α-tubulin mRNA expression did not change in response to injury.
We next performed RT-PCR analysis using primers directed to elements within the coding region to confirm that YY1 mRNA is inducibly expressed following mechanical injury. YY1 was basally expressed in uninjured smooth muscle cells (Fig. 1B, upper panel). Injury increased YY1 expression within 2 h of injury, and levels remained elevated after 4 h. Western immunoblot analysis using monoclonal antibodies targeting YY1 produced a single protein species with a relative molecular mass of 68 kDa, which corresponds to the expected molecular mass of YY1 (Fig. 1B, lower panel) (21). YY1 protein, like YY1 mRNA (Fig. 1A and B, upper panel), was basally expressed, and levels were increased upon mechanical injury (Fig. 1B, lower panel).

To determine whether changes in YY1 mRNA and protein expression after mechanical injury correlate with the binding activity of this transcription factor, we performed electrophoretic mobility shift analysis (EMSA) using 32P-labeled Oligo MVYY1, a double-stranded oligonucleotide bearing consensus binding sites for YY1 from the upstream conserved region of the Moloney murine leukemia gene. A major nucleoprotein complex of weak intensity was detected using nuclear extracts prepared from quiescent smooth muscle cells injured and then left for various times. The gels were vacuum-dried and radioactive signals were visualized by autoradiography overnight at −80 °C. Competition analysis. Nuclear extracts prepared from cells 2 h after injury were incubated with the indicated -fold molar excesses of unlabeled Oligo MVYY1 or Oligo A for 10 min prior to the addition of 32P-Oligo MVYY1 or 32P-Oligo A. Specificity analysis. Nuclear extracts prepared from cells 2 h after injury were incubated with 2 μg of monoclonal antibodies targeting YY1 or Egr-1 for 10 min prior to the addition of the radiolabeled probe. Uninj denotes nuclear extracts prepared from uninjured cells. Radioactivity at the bottom of the gel shows free probe; the probe was run off the gel in C (left panel). The sequence of Oligo MVYY1 is 5'-TGCCTTGCAAAATGGCGTTACTGCAG-3' (sense strand), and Oligo A is 5'-GGGGGGCGGGGGCGGGGGAGGG-3' (sense strand). FF. YY1 protein is expressed in the carotid artery wall following balloon injury. Right common carotid arteries of male Sprague-Dawley rats were injured by multiple inflations of a 2F embolectomy balloon prior to sacrifice of the animals 4 h after injury. Sections (5 μm) were stained for YY1 immunoreactivity using monoclonal YY1 antibodies, rabbit ant-mouse antibodies, and chemiluminescence detection. The lowest panel shows lack of specific signal when the primary antibody (1°) is omitted.

Fig. 1. YY1 is inducibly expressed in growth-quiescent vascular smooth muscle cells following mechanical injury. A, growth-arrested smooth muscle cells were injured by scraping repeatedly with a sterile stainless steel comb or left undisturbed, and total RNA was isolated after 1 h. Reverse-transcribed 32P-labeled cDNA was hybridized to cDNA array filters (CLONTECH) prior to washing, vacuum drying, and quantitation of signal intensity by PhosphorImager analysis (ImageQuant, Molecular Dynamics). Data was normalized to levels of α-tubulin signals between injured and uninjured samples. B, inducible YY1 mRNA and protein expression at various times after injury as assessed by reverse-transcription PCR (upper panel) and Western blot (lower panel) analysis, respectively. GAPDH expression and the Coomassie-stained gel indicate unbiased loading. C, EMSA using 32P-Oligo MVYY1 or 32P-Oligo A bearing consensus binding sites for YY1 and Egr-1, respectively. Nuclear extracts were prepared from quiescent smooth muscle cells injured and then left for various times. The gels were vacuum-dried and radioactive signals were visualized by autoradiography overnight at −80 °C. D, competition analysis. Nuclear extracts prepared from cells 2 h after injury were incubated with the indicated -fold molar excesses of unlabeled Oligo MVYY1 or Oligo A for 10 min prior to the addition of 32P-Oligo MVYY1 or 32P-Oligo A. E, specificity analysis. Nuclear extracts prepared from cells 2 h after injury were incubated with 2 μg of monoclonal antibodies targeting YY1 or Egr-1 for 10 min prior to the addition of the radiolabeled probe. Uninj denotes nuclear extracts prepared from uninjured cells. Radioactivity at the bottom of the gel shows free probe; the probe was run off the gel in C (left panel). The sequence of Oligo MVYY1 is 5'-TGCCTTGCAAAATGGCGTTACTGCAG-3' (sense strand), and Oligo A is 5'-GGGGGGCGGGGGCGGGGGAGGG-3' (sense strand). F, YY1 protein is expressed in the carotid artery wall following balloon injury. Right common carotid arteries of male Sprague-Dawley rats were injured by multiple inflations of a 2F embolectomy balloon prior to sacrifice of the animals 4 h after injury. Sections (5 μm) were stained for YY1 immunoreactivity using monoclonal YY1 antibodies, rabbit ant-mouse antibodies, and chemiluminescence detection. The lowest panel shows lack of specific signal when the primary antibody (1°) is omitted.
of uninjured cells (Fig. 1C, left panel, large arrow). The intensity of this complex increased significantly within 1 h of injury, and levels remained high after 4 h (Fig. 1C, left panel). To confirm the integrity of these extracts, we performed EMSA using 32P-labeled Oligo A, whose sequence derives from the proximal PDGF-A promoter and contains overlapping consensus binding elements for Egr-1. Egr-1 binding activity increased within 1 h of injury (Fig. 1C, right panel), as demonstrated previously in vascular endothelial cells (27) and consistent with Fig. 1A.

To demonstrate the specificity of the nucleoprotein complex obtained using 32P-Oligo MVYY1, we incubated the extracts with increasing amounts of unlabeled oligonucleotide. This resulted in dose-dependent inhibition of the inducible complex and virtually complete inhibition at a 25-fold excess (Fig. 1D, arrow). In contrast, the same -fold excess of unlabeled Oligo A had no effect of the intensity of this complex. To elucidate the identity of the protein component of the inducible complex, we performed supershift analysis. Incubation of the nuclear extracts with YY1 antibodies used for Western blot analysis prior to the addition of 32P-Oligo MVYY1 eliminated the formation of the complex, instead producing a supershift (Fig. 1E). In contrast, when an identical amount of Egr-1 antibodies was used for preincubation, the complex was completely unaffected. These data provide the first demonstration that YY1 mRNA and protein are increased in vascular smooth muscle cells upon mechanical injury.

YY1 Expression Induced in Balloon-injured Rat Carotid Arteries—To determine whether the induction of YY1 by mechanical injury in vitro is also observed following injury to the intact vessel wall, we performed balloon angioplasty to the left common carotid arteries of Sprague-Dawley rats. Immunohistochemical analysis with YY1 antibodies revealed that YY1 is weakly expressed by smooth muscle cells in the medial compartment of the artery wall (Fig. 1F, top panel). The intensity of staining increased significantly in the media within 4 h of balloon injury (Fig. 1F, middle panel). The specificity of staining is evident by our inability to observe YY1 signal when primary (YY1) antibody was omitted from the protocol (Fig. 1F, bottom panel). These data demonstrate for the first time the induction of YY1 in the injured artery wall.

FGF-2 Stimulates YY1 Expression, Binding, and Transcriptional Activity—We hypothesized that the activation of YY1 following injury is regulated by endogenous factors released from the cells themselves. We focused on FGF-2, because FGF-2 mRNA and protein are basally expressed in vascular smooth cells in culture, as well as in the intact artery wall (28). We measured levels of FGF-2 in the supernatant of cultured growth-quiescent smooth muscle cells by enzyme-linked immunosorbent assay before and after scraping. Immunoreactive FGF-2 was barely detectable in the culture medium or supernatant of undisturbed smooth muscle cells (Fig. 2A). However, FGF-2 levels increased dramatically within 2 min of injury (Fig. 2A). This led us to explore the possibility that FGF-2 may regulate the expression of YY1, hitherto unreported in any cell type.

YY1 mRNA expression increased in vascular smooth muscle cells exposed to FGF-2. RT-PCR analysis revealed that YY1 transcript levels increased within 1 h of exposure to the growth factor and remained elevated even after 24 h (Fig. 2B). Western immunoblot analysis confirmed these findings of inducible YY1 expression at the level of protein (Fig. 2C).

To address the spatial distribution of inducible YY1 protein expression we performed in situ immunofluorescence analysis using YY1 antibodies as the primary antibody with secondary antibodies tagged with fluorescein isothiocyanate. YY1 immunoreactivity was preferentially detected in the nuclei of a small proportion of smooth muscle cells (Fig. 2D). After 1 or 4 h of exposure to FGF-2, a considerably greater proportion of cell nuclei showed immunofluorescence for YY1 protein. The specificity of the system was confirmed by the inability to detect immunofluorescence when the YY1 antibody was omitted (Fig. 2D).

We next used EMSA to demonstrate whether FGF-2-inducible YY1 expression protein produced increased DNA binding activity. FGF-2 increased YY1 DNA binding activity within 1 h, and levels remained elevated after 4 h (Fig. 2E, left panel), similar to our earlier observations using nuclear extracts of cells that had been injured (Fig. 1C, left panel). Unlike the rapid transient induction of Egr-1 after injury (Fig. 1C, right panel), however, Egr-1 binding activity was more sustained in cells exposed to FGF-2 (Fig. 2E, right panel).

To demonstrate that YY1 binding activity induced by FGF-2 was functionally significant, we exposed smooth muscle cells transfected with the chloramphenicol acetyltransferase-based reporter construct (E1)4TK-CAT, which contains four copies of a high affinity YY1 binding site upstream of the thymidine kinase promoter (24), to FGF-2. This construct has previously been used to gauge YY1 binding activity in an overexpression setting in fibroblasts (24). FGF-2 stimulated chloramphenicol acetyltransferase reporter expression within 24 h in a dose-dependent manner (Fig. 2F). Taken together, these findings demonstrate that FGF-2 induces YY1 mRNA and protein expression, DNA-binding activity, and can transactivate gene expression in vascular smooth muscle cells.

YY1 Induction by Injury Is Mediated by FGF-2 Release—Because injury causes the rapid release of FGF-2 from smooth muscle cells, and injury and recombinant FGF-2 each stimulate YY1 expression in this cell type, we finally determined whether the inducible expression of YY1 following injury is mediated by the local effect of endogenous FGF-2. We therefore incubated growth-quiescent smooth muscle cells with neutralizing FGF-2 antibodies prior to scraping and then assessed YY1 levels by Western immunoblot analysis. Injury increased YY1 protein levels within 2 h (Fig. 3). Interestingly, levels of the transcription factor were significantly reduced in the lysates of cells preincubated with antibodies to FGF-2 (Fig. 3). In contrast, isotype- and species-matched immunoglobulin has no appreciable effect on injury-inducible YY1 protein expression (Fig. 3). These findings demonstrate the paracrine effect of endogenous FGF-2 in the increased expression of YY1 in smooth muscle cells following mechanical injury.

Overexpression of YY1 Suppresses Cellular Proliferation—Because YY1 can repress the expression of growth factor genes, we determined whether YY1 could influence smooth muscle cell replication. Incubation of sub-confluent growth-quiescent smooth muscle cells in medium containing serum, as expected, stimulated proliferation in this population. Cells transfected with 0.5 or 1 µg of a cytomegalovirus-driven YY1 expression vector, pCB6-YY1, strongly inhibited proliferation relative to its empty vector (pCB6-) control (Fig. 4). We used Northern blot analysis to demonstrate that YY1 was indeed expressed in the smooth muscle cells following pCB6-YY1 transfection. This showed strong expression of the exogenous YY1 mRNA within 24 h of transfection (Fig. 5). These findings indicate that YY1 is a potent inhibitor of vascular smooth muscle cell proliferation. In contrast, proliferation of vascular endothelial cells was not influenced as a consequence of transfection with identical amounts of YY1 (see Fig. 4 and Fig. 5), thus indicating the cell-specific nature of inhibition by YY1. To date, YY1 has not been directly linked to cell replication in any cell type.
YY1 and FGF-2 Expression in Human Atherosclerotic Tissue—Because FGF-2 positively regulates YY1 expression, we hypothesized that YY1 and FGF-2 would colocalize in the arterial wall. Immunohistochemical staining revealed that YY1 was strongly expressed by α-actin-positive smooth muscle cells in the arterial media compared with weak expression in the intima (Fig. 6). YY1 staining in the media was distributed mosaically and was exclusively nuclear. In contrast, p53 was expressed in the intima. FGF-2 immunoreactivity was detected in the nuclear and cytoplasmic compartments of medial smooth muscle cells, consistent with previous observations in fibrous lesions (29, 30). FGF-2 immunoreactivity was coincident with YY1 expression (Fig. 6). Finally, we reasoned that because YY1 inhibits smooth muscle cell replication (Fig. 4), YY1 expression in the artery wall would inversely correlate with mitogenicity. PCNA staining was accordingly confined to smooth muscle cells and occasional macrophages in the intima, with few PCNA-expressing cells detected in the media (Fig. 6).

The mechanisms governing the activation of the YY1 gene expression are presently unknown. In smooth muscle cells, YY1 has been found to physically associate with and activate the smooth muscle cell-specific SMC22α promoter (32) but has not yet been reported to be induced by defined extracellular stimuli in this cell type. YY1 repressor activity is induced in cardiac myocytes exposed to interleukin-1 (33) and in osteosarcoma cells incubated with vitamin D (11). The hu-
The man YY1 promoter sequence cloned from a liver genomic library contains a single transcriptional initiation site located 478 base pairs upstream of the AUG translational start motif (34). Among several putative nucleotide recognition elements for known transcriptional regulators is a consensus site for cAMP-response element-binding protein/activating transcription factor and three tandem sites for Myb. cAMP-response element-binding protein can mediate transcriptional activation by FGF-2 (35). Similarly, FGF-2 can stimulate the expression of Myb factors (36), which can potentiate FGF-inducible proliferation (37). Therefore, our present demonstration of increased YY1 expression in smooth muscle cells exposed to FGF-2 may be because of the activity of these positive regulatory transcription factors. The spatial relationship established between FGF-2 and YY1 in lesions in this paper may be representative of other settings. Interestingly, FGF-2 and other members of the FGF family are expressed in the early stages of embryonic development (38), consistent with peri-implantation lethality in mice deficient in YY1 gene (39).

We observed approximately a 3-fold increase in YY1 mRNA and protein expression, as well as DNA binding and transcriptional activity with FGF-2. This magnitude induction may be sufficient to influence gene expression at local sites of FGF-2 release in the vasculature. Indeed, as little as a 2-fold induction in endogenous YY1 expression can lead to significant transcriptional repression in cardiac myocytes, which is entirely dependent on the integrity of the zinc finger structure of YY1 (33). In other contexts, minor changes in DNA binding activity can have profound effects on the transcriptional activity of dependent genes (40).

This study demonstrates that local FGF-2 release can facilitate both positive and negative influences at the level of transcription. We demonstrated previously that FGF-2 induces the expression of Egr-1, which activates the expression of many genes implicated in the initiation and progression of atherosclerosis and restenosis (22). In vascular endothelial cells, the induction of Egr-1 after injury is blocked by antibodies targeting FGF-2 (27). Thus, FGF-2 can stimulate the expression of two very different kinds of transcription factors, namely YY1 (the repressor) and Egr-1 (the activator) in the context of...
smooth muscle cell injury. It is the complex interplay of transcription factors at promoter elements that dictates gene expression and changes in cell movement, proliferation, and adhesion in the injured vessel wall.

We demonstrate here for the first time that YY1 expression and DNA binding activity increase in vascular smooth muscle cells within hours of mechanical injury. We have also shown that FGF-2 is a positive regulator of YY1 expression and moreover that endogenous FGF-2 accounts for the induction of YY1 after injury. The present study demonstrates the yin yang nature of YY1. On the one hand, YY1 expression is under the direct control of FGF-2, which stimulates smooth muscle cell growth. On the other hand, YY1 can inhibit smooth muscle cell growth. That YY1 and FGF-2 are coexpressed in growth-quiescent smooth muscle cells in human arteries suggests that YY1 may restrict pro-atherogenic gene expression and cell growth in the injured vessel wall. Because the adaptive response to arterial cell injury involves a dramatic increase in smooth muscle cell replication, the sustained activation of this enigmatic transcription factor may help restrict what otherwise may result in greater smooth muscle cell mitogenesis in early atherogenesis. Moreover, that YY1 inhibits the growth of smooth muscle cells without influencing endothelial cell proliferation suggests that strategies forcing the expression of exogenous YY1 in the injured vessel wall could be useful to inhibit intimal thickening without affecting re-endothelialization and the re-establishment of a non-thrombogenic surface.

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