Interplay of Sp1 and Egr-1 in the Proximal Platelet-derived Growth Factor A-Chain Promoter in Cultured Vascular Endothelial Cells*

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The platelet-derived growth factor (PDGF) A-chain has been implicated in the initiation and progression of vascular occlusive lesions. The elements in the human PDGF-A promoter that mediate increased expression of the gene in vascular endothelial cells have not been identified. A potent inducer of PDGF-A expression in endothelial cells is phorbol 12-myristate 13-acetate (PMA). 5'-Deletion and transfection analysis revealed that a G+C-rich region in the proximal PDGF-A promoter is required for PMA-inducible gene expression. This region bears overlapping consensus recognition sequences for Sp1 and Egr-1. PMA induces Egr-1 mRNA expression within 1 h, whereas PDGF-A transcript levels increase after 2-4 h. Constitute levels of Sp1 are not altered over 24 h. A specific nucleoprotein complex is formed when an oligonucleotide bearing the G+C-rich element is incubated with nuclear extracts from PMA-treated cells. The temporal appearance of this complex is consistent with the transient increase in Egr-1 transcripts. Antibodies to Egr-1 completely supershift the PMA-induced complex. Interestingly, increased nuclear levels of Egr-1 attenuate the ability of Sp1 to interact with the oligonucleotide, implicating competition between Egr-1 and Sp1 for the G+C-rich element. Binding studies with recombinant proteins demonstrate that Egr-1 can displace Sp1 from this region. Insertion of the G+C-rich element into a hybrid promoter-reporter construct confers PMA inducibility on the construct. Mutations that abolish Egr-1 binding also abrogate expression induced by PMA or overexpressed Egr-1. These findings demonstrate that PMA-induced Egr-1 displaces Sp1 from the G+C-rich element and activates expression driven by the PDGF-A proximal promoter in endothelial cells. The Sp1/Egr-1 displacement mechanism may be an important regulatory circuit in the control of inducible gene expression in vascular endothelial cells.

The platelet-derived growth factor (PDGF)1 is a potent mitogen that has been implicated to play a role in a diversity of normal and pathological settings (for reviews see Heldin (1992), Khachigian and Chesterman (1993), and Ross (1993)). PDGF occurs as a homodimer or heterodimer of A- and B-chains covalently linked by disulfide bonds and has a relative molecular mass of approximately 28 kDa. The biological response to PDGF is mediated by high affinity cell-surface receptors with split tyrosine kinase domains. The α-receptor is bound by both polypeptide chains of PDGF, whereas the β-subunit is bound with high affinity only by the B-chain (Heldin et al., 1988). PDGF A-chain mRNA has been detected in a variety of cells of human origin in culture (Betsholtz et al., 1986; Raines et al., 1990) and undergoes alternative splicing of exons 2 (Sanchez et al., 1991) and 6 (Collins et al., 1987; Tong et al., 1987). Three transcript species can be generated from the human PDGF A-chain gene (Bonhthon et al., 1988), which are derived from the same transcriptional start site (Takimoto et al., 1991) and arise from the utilization of alternative poly(A) sites in exon 7 (Bonhthon et al., 1988). Somatic cell hybrid chromosome segregation analysis (Betsholtz et al., 1986) and in situ hybridization using endothelial cell-derived cDNA (Bonhthon et al., 1988) or non-isotopic techniques and genomic clones (Bonhthon et al., 1992) assigned the gene to chromosome 7 (7p21-p22). Isolation of genomic clones encoding the human PDGF A-chain gene allowed characterization of its structural organization and facilitated subsequent investigation of the molecular mechanisms controlling transcription of the gene (Bonhthon et al., 1988; Rorsman et al., 1988; Takimoto et al., 1991). The human PDGF A-chain gene spans approximately 24 kilobases of genomic DNA and has a single transcriptional start site located 36 bp downstream of the TATA box (Bonhthon et al., 1988). The minimal promoter region, sufficient for optimal promoter activity, consists of approximately 150 bp in human epithelial carcinoma (HeLa) cells (Lin et al., 1992) and 120 bp in African green monkey renal epithelial (BSC-1) cells (Kaetzl et al., 1994). This region, rich in G+C content, is hypersensitive to deavage by S1 nuclease (Wang et al., 1992a) and contains potential overlapping recognition elements for the zinc-finger transcription factor, Sp1 (Dyanin and Tjian, 1983; Kadonaga et al., 1987), the immediate-early growth response gene product, Egr-1 (Krox-24, zif268) (Rauscher et al., 1990), and the Wilms' tumor suppressor gene product, WT-1 (Rauscher et al., 1990). Although this G+C-rich sequence in the PDGF-A promoter is bound by nuclear proteins from cultured BSC-1 cells (Kaetzl et al., 1994), and Egr-1 and WT-1 overexpression can modulate promoter activity (Gashler et al., 1992; Wang et al., 1992b), it is unclear whether Sp1, Egr-1, or WT-1 actually play a physiologic role in the transcriptional regulation of PDGF-A. Several upstream promoter regions have also been implicated in the mobility shift assay; bp, base pair(s); DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride.

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1 The abbreviations used are: PDGF, platelet-derived growth factor; Egr-1, early growth response gene product; WT, Wilms' tumor; PMA, phorbol 12-myristate 13-acetate; CAT, chloramphenicol acetyltransferase; BAEC, bovine aortic endothelial cells; EMSA, electrophoretic

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induced expression of the gene. First, a novel element located between bp -102 and -82 interacts with nuclear protein(s) (PDGF-A-BP-1) in human mesangial cells exposed to phorbol 12-myristate 13-acetate (PMA) (Bhandari et al., 1995). Second, a consensus serum response element between bp -477 and -468 mediates PDGF-A-induced reporter gene expression in human foreskin fibroblasts (Lin et al., 1992). The PDGF-A promoter is also subject to negative transcriptional regulation. Overexpression of WT-1 represses PDGF-A promoter-dependent reporter gene expression in murine fibroblasts and human embryonic kidney cells (Gashler et al., 1992; Wang et al., 1992b). Additionally, 5'-deletion analysis in BSC-1 cells defined two negative regulatory regions located between bp -1800 and -1029 and bp -1029 and -880 (Kaetzl et al., 1994). The identity of the endogenous nuclear transcription factors that actually interact with any of the regulatory regions in the PDGF-A promoter has not been determined.

In this report, PMA was used as a model agonist to define specific nuclear transcription factors that interact with functional nucleotide elements in the proximal PDGF-A promoter in cultured vascular endothelial cells. Using 5'-deletion and transient transfection analysis, we demonstrate the requirement for the region between bp -71 and -55 for PMA-inducible expression. This region bears overlapping Egr-1 and Sp1 binding elements. Northern blot analysis reveals that PMA induces Egr-1 expression with a time course earlier than PDGF-A, whereas Sp1 transcript levels are unchanged. Gel shift and supershift studies demonstrate that both PMA-induced and recombinant Egr-1 bind to the G+C-rich element by displacing Sp1. Mutations that abolish the ability of Egr-1 to interact with the proximal PDGF-A promoter also abrogate expression induced either by PMA or overexpressed Egr-1. Thus, PMA-induced expression of the PDGF A-chain in endothelial cells is mediated, at least in part, by the rapid and transient induction of Egr-1 and involves an interchage of factors occupying the G+C-rich element in the proximal promoter. This displacement mechanism may be a common theme in the induced expression of the PDGF A-chain gene by multiple signals in a variety of biological settings.

EXPERIMENTAL PROCEDURES

Oligonucleotide Synthesis, Purification, and Radiolabeling—Oligonucleotides were synthesized using a 392 DNA synthesizer (Applied Biosystems) and purified by gel electrophoresis. Oligonucleotides were end labeled with [γ-32P]ATP (DuPont NEN) using T4 polynucleotide kinase. Oligonucleotide probes were run at 200 V (constant voltage) for approximately 2 h and then dried under vacuum and autoradiographed overnight using Kodak X-OMAT-AR film.

In Vitro DNase I Footprint Analysis—A single end-labeled fragment spanning the proximal PDGF A-chain promoter was prepared by digesting construct pPACCATΔXho with EcoRI and HindIII, dephosphorylating the ends with calf intestinal alkaline phosphatase (New England Biolabs) and isolating the 135-bp fragment by electrophoresis on a 2% agarose gel and run at 100 V. The gels were dried and autoradiographed using Kodak X-OMAT-AR film.

Cell Culture—BAEC were grown in Dulbecco's modified Eagle's medium (Life Technologies, Inc.), pH 7.4, containing 10% calf serum. Cells were passaged every 3-4 days in 75-cm² flasks (Corning) by a rinse twice with phosphate-buffered saline, pH 7.4, and incubation with 0.05% trypsin, 0.02% EDTA in Hank's balanced salts solution and 0.02% trypsin, 0.02% EDTA in Hank's balanced salts solution. Following digestion for 1 h at 37 °C, 20 μl of DNase buffer (25 μg NaCl, 10 μl EDTA, 5% glycerol, 2 mM DTT, 0.5% Nnially P40, 0.1% Triton X-100, 5% glycerol) (Rauscher et al., 1990) was generously provided by Dr. Fr. A. Rauscher, III (Wistar Institute, PA).

Electrophoretic Mobility Shift Assay (EMSA)—Binding reactions involving nuclear extracts were carried out in a total volume of 20 μl containing 2 μl of extract, 1 μg of poly(dI-dC)-poly(dI-dC) (Sigma), 1 μg of salmon sperm DNA (Sigma), 5% sucrose, and 32P-labeled oligonucleotide probe (50,000 – 100,000 cpm). Bound complexes were separated from the free probe by non-denaturing polyacrylamide gel electrophoresis using 1 X TBE running buffer. The gels were run at 200 V (constant voltage) for approximately 2 h and then dried under vacuum and autoradiographed overnight using Kodak X-OMAT-AR film.
overnight at −80 °C.

Total RNA Preparation and Northern Blot Analysis—Total RNA was prepared using TRIzol® reagent in accordance with the manufacturer’s instructions (Life Technologies, Inc.). RNA samples (15 μg) were separated on 1% formaldehyde/agarose gels, transferred to a Hybond nylon membrane (Amersham), then hybridized with cDNA that had been labeled with [α-32P]dCTP (DuPont NEN). The blot was washed with 0.5 × SSC at 65 °C and exposed at −80 °C.

RESULTS

PMA Induces the Transient Expression of PDGF-A in Vascular Endothelial Cells—To determine the temporal pattern with which the PDGF-A gene is expressed in endothelial cells exposed to PMA, bovine aortic endothelial cells were incubated with 100 ng/ml PMA for various times, and steady-state mRNA levels were assessed by Northern blot analysis. The major 2.8-kilobase PDGF-A chain transcript was virtually undetectable in untreated cells (Fig. 1). However, significant hybridization was detected following 2-4 h of exposure to PMA. PDGF-A chain transcript levels remained elevated for 16 h before returning to control levels by 24 h (Fig. 1).

Identification of Regions in the PDGF-A Promoter Required for Optimal Basal Activity and PMA-induced Gene Expression

Fig. 1. Northern blot analysis of vascular endothelial cells exposed to PMA. RNA was isolated either from unstimulated BAEC or cells that were incubated with 100 ng/ml PMA in normal growth medium for 0, 1, 2, 4, 6, 9, 12, 16, 20, or 24 h at 37 °C. Samples were normalized for total RNA (15 μg) prior to electrophoresis and transfer to a nylon membrane. The blots were hybridized with specific 32P-labeled cDNA probes, and binding was assessed by autoradiography as described under “Experimental Procedures.”
in Endothelial Cells—To define the minimal promoter region that mediates basal expression in cultured endothelial cells, BAEC were transiently transfected with reporter constructs bearing nested 5′-deletions of the PDGF-A promoter fused to CAT cDNA. Cells that were transfected with constructs pACCATΔXho, e33, and f28 (bearing 262, 110, and 71 bp of promoter sequence relative to the transcriptional start site, respectively) expressed significant levels of the reporter (Fig. 2), whereas construct f36 (−55 bp) was inactive (Fig. 2). Maximum reporter activity was obtained using construct e33 (Fig. 2). These data indicate that the minimal PDGF-A promoter region required for optimal gene expression in cultured endothelial cells consists of approximately 110 bp.

PMA-induced CAT activity was detected in cells that were transfected with constructs pACCATΔXho, e33, and f28 (Fig. 2). Construct f36, however, was unable to mediate PMA-inducible gene expression (Fig. 2). The sequence between the 5′-deletion end points of f28 and f36 corresponds to a G+C-rich element that contains overlapping consensus nucleotide recognition sequences for the zinc-finger transcription factors Sp1 and Egr-1 (Fig. 3A). Cotransfection studies indicate that constructs e33 and f28 respond to overexpressed Sp1 and Egr-1, whereas f36 does not.2 Thus, basal and PMA-induced expression mediated by the PDGF-A promoter in endothelial cells requires the proximal G+C-rich element and may involve the participation of Sp1 and/or Egr-1. The relative increase in reporter activity in response to PMA is consistent with -fold increases in CAT activity reported elsewhere (Angel et al., 1988; Fazio et al., 1991; John et al., 1995).

PMA Rapidly Induces Egr-1 Expression, but Not Sp1, in Vascular Endothelial Cells—PMA has been reported to induce the expression of Egr-1 in a variety of cell types (Esposito et al., 1994; Kanazashi et al., 1994; Larsen et al., 1994). However, the

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2 L.M. Khachigian, A.J. Williams, and T. Collins, unpublished observations.
expression of Egr-1 by endothelial cells has not yet been reported. Northern blot analysis was performed to determine whether PMA could modulate the expression of Egr-1 and/or Sp1. PMA dramatically induced steady-state Egr-1 transcript levels in BAEC within 1 h of exposure (Fig. 1). Egr-1 mRNA levels remained elevated for 16 h and returned to control levels by 24 h (Fig. 1). In contrast, Sp1 is constitutively expressed and unaffected by exposure to PMA over 24 h (Fig. 1). These findings indicate that the induction of Egr-1 transcript expression by PMA precedes that of the PDGF A-chain. Western blot analysis revealed that PMA also increased PDGF A-chain and Egr-1 expression at the level of protein, whereas constitutive levels of Sp1 were unaffected.2

An Oligonucleotide Bearing the G+C-rich Sequence in the Proximal PDGF-A Promoter Interacts with Endothelial Nuclear Proteins—To determine whether PMA could induce an interaction between proteins from endothelial nuclei and the proximal PDGF-A promoter, EMSA was performed using a 32P-labeled double-stranded oligonucleotide bearing the G+C-rich sequence (Oligo A, Fig. 3A). Using nuclear extracts from unstimulated BAEC, two prominent nucleoprotein complexes (A1 and A5) and two minor complexes with variable intensity (A2 and A4) were formed. All of these complexes were specifically competed by the presence of 50–100-fold molar excess of (A1 and A5) and two minor complexes with variable intensity by PMA over 24 h (Fig. 1). These findings indicate that the induction of Egr-1 transcript expression by PMA precedes that of the PDGF A-chain. Western blot analysis revealed that PMA also increased PDGF A-chain and Egr-1 expression at the level of protein, whereas constitutive levels of Sp1 were unaffected.2

Interaction of Egr-1 with the G+C-rich Element in the Proximal PDGF-A Promoter in Endothelial Cells Exposed to PMA—A single PMA-induced nucleoprotein complex was observed when 32P-labeled oligonucleotide A was incubated with nuclear extracts from BAEC exposed to 100 ng/ml of PMA for 1 h (A3, Fig. 3B). Complex A3 was specifically competed by 50-fold molar excess of the oligonucleotide (Fig. 3B). Complexes A1, A2, A4, and A5 were unaffected by the presence of PMA (Fig. 3B). To determine the temporal pattern of PMA-induced DNA binding activity, 32P-labeled oligonucleotide A was incubated with nuclear extracts from endothelial cells exposed to PMA for various times. The dramatic induction of complex A3 was sustained over 4 h, and levels remained elevated after 12 h before returning to control levels by 24 h (Fig. 3C). Interestingly, the time course with which complex A3 appears is remarkably similar to the pattern of Egr-1 mRNA expression induced by PMA (Fig. 1).

Polyclonal antipeptide antibodies were used to determine the identity of the nuclear protein(s) involved in the PMA-induced complex. A complete supershift of complex A3 was obtained using antibodies directed toward Egr-1 (Fig. 3D). Thus, Egr-1 interacts with the PDGF-A G+C-rich element in cultured endothelial cells within 1 h of exposure to PMA. AP-2 and WT-1 are zinc-finger transcription factors that interact with the G+C-rich nucleotide sequences, 5′-(C/T)(C/G)(C/G)(C/ A)(G/C)(G/C)(G/C)-3′ (Imagawa et al., 1987; Williams et al., 1989) and 5′-CGTGGAGT-3′ (Nakagama et al., 1995), respectively. Antibodies to AP-2, WT-1, or to PEA-3, however, failed to produce a supershift (Fig. 3D).

Displacement of Sp1 by Egr-1 in Endothelial Cells Exposed to PMA—The constitutive expression of Sp1 in endothelial cells (Fig. 1) suggests that this transcription factor occupies the G+C-rich element in the absence of PMA. Supershift analysis was used to determine whether nuclear Sp1 does indeed occupy this element in cultured BAEC. In unstimulated cells, polyclonal antipeptide antibodies directed toward Sp1 partially supershifted complex A1 (Fig. 4). Upon exposure to PMA, however, Egr-1 bound to the promoter fragment, and the Sp1 supershift was no longer apparent (Fig. 4). Antibodies to Ets-1 or Ets-2 had no effect (Fig. 4). These data indicate that PMA can affect the nature of the transcription factors interacting with this region of the promoter. Egr-1 interacts with the G+C-rich element in the PDGF-A promoter by displacing Sp1 in endothelial cells exposed to PMA. The inability of the Sp1 antibody to completely supershift complex A1 may be due to one or a combination of several possibilities: first, partial recognition of bovine Sp1 using the anti-human Sp1 antibody; second, comigration of a nucleoprotein complex unrelated to Sp1; and third, incomplete recognition due to the phosphorylation state of Sp1 (reviewed in Imagawa et al. [1987]). Partial supershifts involving the same Sp1 antibody used in this study have been reported elsewhere (Kramer et al., 1994; Minowa et al., 1994; Jensen et al., 1994).

Recombinant Sp1 and Egr-1 Interact with the G+C-rich Element in the Proximal PDGF-A Promoter in a Dose-dependent and Stable Manner—Recombinant proteins were used to further document the interaction of Sp1 and Egr-1 with the G+C-rich element in the proximal PDGF-A promoter. Both Sp1 (Fig. 5A) and Egr-1 (Fig. 5B) bound to 32P-labeled oligonucleotide A in a dose-dependent and specific manner. The appearance of higher order complexes at higher concentrations of Sp1 or Egr-1 (Figs. 5, A and B) indicates that more than one molecule, either Sp1 or Egr-1, may bind simultaneously to the oligonucleotide. Inspection of the nucleotide sequence in oligonucleotide A (Fig. 3A) indicates the presence of more than one putative binding site for each protein. Higher order complexes could also result
from direct protein–protein interactions (Pascal and Tjian, 1991). In vitro DNase I footprinting provided further evidence for the interaction of Egr-1 with the G+C-rich element in the proximal PDGF-A promoter. Egr-1 protected this region from partial digestion in a dose-dependent manner (Fig. 5C). Sequences protected by Egr-1 beyond the actual binding site (Rauscher et al., 1990) may reflect weak binding of Egr-1 to adjacent G+C-rich sequences or conformational changes induced by bound Egr-1.

To provide an indication of the stability of the nucleoprotein complexes involving Sp1 and Egr-1 over time, EMSA was carried out using the running gel technique (Franzoso et al., 1993). Recombinant Sp1 was incubated with $^{32}$P-oligonucleotide A for 30 min at 22°C and applied to a non-denaturing polyacrylamide gel following various times. The Sp1-$^{32}$P-oligonucleotide A complex was apparent even after 150 min at this temperature (Fig. 5D). Addition of 1000-fold molar excess of unlabeled oligonucleotide A displaced Sp1 from $^{32}$P-oligonucleotide A within 30 min (Fig. 5D). When recombinant Egr-1 was used instead of Sp1, the nucleoprotein complex was similarly apparent after 150 min (Fig. 5E). Unlike Sp1, however, addition of excess unlabeled oligonucleotide A resulted only in partial displacement of the prebound Egr-1 (Fig. 5E). These results indicate that both Sp1 and Egr-1 bind to the PDGF-A oligonucleotide in a stable manner. The apparent off-rate of Egr-1, however, is slower than that of Sp1.

Displacement of Prebound Recombinant Sp1 by Egr-1—The preceding results indicate that PMA-induced Egr-1 interacts with the G+C-rich element in the PDGF-A core promoter and that levels of Sp1 were unaffected in endothelial cells exposed to PMA. We hypothesized that Egr-1, induced by PMA, could displace Sp1 from this element in the promoter. Using recom-
binant proteins and the running gel technique, the effect of adding a 40-fold molar excess of Egr-1 (relative to Sp1) to a solution containing prebound Sp1-32P-oligonucleotide A was the displacement of Sp1 within 30 min (Fig. 6A). Moreover, when the 32P-labeled footprint probe was allowed to bind to a fixed amount of Sp1 for 10 min and was subsequently challenged with increasing concentrations of Egr-1, Sp1 was displaced in a dose-dependent manner (Fig. 6B). Thus, Egr-1 is able to displace Sp1 from the G+C-rich element in the proximal PDGF-A promoter.

Disruption of the G+C-rich Sequence Abrogates Egr-1 Binding and Abolishes Expression Inducible by PMA or Overexpressed Egr-1—To determine whether the interaction of Egr-1 with the G+C-rich element is necessary for inducible expression driven by the PDGF-A promoter, the oligonucleotide was mutated by the insertion of 3 guanines into the core sequence. When the mutant oligonucleotide, Oligo Am, was 32P-radiolabeled and used in EMSA, Egr-1 could no longer interact with the promoter sequence (Fig. 7A). Native and mutant A-chain promoter sequences were introduced into a heterologous promoter-reporter construct and transiently transfected into BAEC. Cells transfected with the wild-type construct expressed 5-6-fold greater CAT activity when exposed to PMA for 24 h (Fig. 7B). In contrast, CAT activity did not increase in cells transfected with the mutant construct (Fig. 7B). Similarly, expression driven by the wild-type sequence increased by 5-fold when BAEC were cotransfected with CMV.Egr-1 (Fig. 7C). However, the mutant failed to respond to overexpressed Egr-1 (Fig. 7C). These findings demonstrate the requirement of an intact Egr-1 site in the proximal PDGF-A promoter sequence for expression inducible either by PMA or overexpressed Egr-1.

**DISCUSSION**

The present report indicates that the induction of PDGF-A-chain expression in endothelial cells by PMA involves the interplay of zinc-finger transcription factors with elements in the core promoter. A schematic representation of these events appears in Fig. 8. Several lines of evidence support this model. First, 5′-deletion analysis of the PDGF-A promoter indicates that Sp1 elements are required for basal expression in endothelial cells (Fig. 2), consistent with findings in other cell types (Kaetzel et al., 1994; Lin et al., 1992). The actual number of Sp1 sites required for basal activity is not clear. However, single point mutations in individual putative Sp1 sites did not reduce PDGF-A promoter activity, suggesting that not all Sp1 sites are required for basal expression. Second, recombinant Sp1 interacts with the G+C-rich element in a specific (Fig. 5A), dose-dependent (Fig. 5A) (Kim et al., 1989), and stable manner (Fig. 5D). Third, EMSA, using nuclear extracts from unstimulated cells, indicates that the G+C-rich element in the proximal PDGF-A promoter is occupied by Sp1 (Fig. 4). Finally, the constitutive expression of Sp1 transcript (Fig. 1) and protein is not affected by PMA. Sp1, which was first identified in HeLa cells by virtue of its ability to activate the SV40 early promoter (Dynan and Tjian, 1983), mediates the basal expression of the structurally similar PDGF-B gene in cultured endothelial cells (Khachigian et al., 1994).

Upon exposure of endothelial cells to PMA, Egr-1 is induced and binds to the G+C-rich element in the proximal promoter by displacing Sp1. Several findings support this part of the model (Fig. 8). First, EMSA and in vitro DNAse I footprinting studies indicate that like Sp1, recombinant Egr-1 interacts with the G+C-rich element in a specific (Fig. 5B), dose-dependent (Fig. 5, B and C), and stable (Fig. 5E) manner. The number of functional Egr-1 sites required for induced PDGF-A expression is not clear. Second, Egr-1 mRNA is dramatically induced in endothelial cells within minutes of exposure to PMA (Fig. 1). Third, the time course of induction of Egr-1 mRNA is virtually identical to appearance of the nucleoprotein complex A3 (Fig. 3C). Fourth, supershift analysis indicates that PMA-induced Egr-1 attenuates the ability of Sp1 to interact with the promoter (Fig. 4). Finally, a molar excess of Egr-1 can displace prebound Sp1 from this region (Fig. 6, A and B). It is unlikely that Egr-1 and Sp1 interact simultaneously with the G+C-rich element in the proximal PDGF-A promoter. The presence of both Egr-1 and Sp1 in the same EMSA reaction does not result in a super complex (Fig. 6, A and B), and antibodies to Egr-1 or Sp1 do not supershift a single band from endothelial cell nuclear extracts (Fig. 4). Collectively, these findings indicate that PMA-induced Egr-1 stimulates gene expression driven by the PDGF-A promoter in endothelial cells by displacement of Sp1 from the G+C-rich element.

The molecular mechanism(s) responsible for the return of PDGF-A transcript mRNA to basal levels following induction by PMA are unclear. It is tempting to speculate, however, that one or more of the following transcriptional events may be involved. First, the transient increase in Egr-1 transcript (Fig. 1), protein, and DNA binding activity (Fig. 3B) suggests that Sp1 may reoccupy the G+C-rich element when the stoichiometric ratio of Sp1 to Egr-1 returns to preregulated levels, greatly favoring Sp1. Second, Egr-1 could be actively displaced by a third transcription factor such as WT-1. Recombinant WT-1 has been shown previously to interact with the same G+C-rich element in the proximal PDGF-A promoter (Gashler et al., 1992; Wang et al., 1992b). We did not detect a nucleoprotein complex involving endothelial WT-1 by supershift analysis; however, a WT-1-like transcription factor may be involved in the post-induction transcriptional repression of PDGF-A. Finally, other regulatory proteins could displace Egr-1 from the G+C-rich element via direct protein-protein interactions. For example, the activation of creatine kinase gene expression by WT-1 is repressed by physical association with p53 (Maheswaran et al., 1993). Interestingly, an active transcriptional repression mechanism has also been suggested in the down-regulation of PDGF-A gene expression after serum stimulation (Takimoto and Kuramoto, 1995).

The regulatory events presented in this manuscript indicate that the complex process of transcriptional activation can in
volve the interchange of transcription factors associating with distinct promoter elements (Tjian and Maniatis, 1994). Interestingly, overlapping putative nucleotide recognition elements for Egr-1 and Sp1 appear in the promoters of human tissue factor (Mackman et al., 1989), transforming growth factor-β1 (Kim et al., 1989; Dey et al., 1994), colony-stimulating factor-1 (Harrington et al., 1993), tumor necrosis factor (Kramer et al., 1994), murine thrombospordin-1 (Shingu and Bornstein, 1994), acetylcholinesterase (Mutero et al., 1995), and Egr-1 in the regulation of certain of these genes, actual cotransfection studies have implied a functional role for Sp1 and Egr-1 in the regulation of certain of these genes, actual competition between these factors has not been demonstrated. Accordingly, the exchange of Sp1 and Egr-1 at overlapping motifs may be an important common event in the control of inducible gene expression.

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REFERENCES

Angel, P., Hattori, K., Smeal, T., and Karin, M. (1988) Cell 55, 875–885

Betzshottz, C., Johansson, A., Heldin, C.-H., Wastermark, B., Lind, P., Urdea, M. S., Eddy, R., Shoves, T. B., Philpott, K., Melior, A. L., Knott, T. J., and Scott, J. (1988) Nature 338, 695–699

Bhandari, B., Wenzel, U. O., Marra, F., and Abboud, H. E. (1995) J. Biol. Chem. 270, 5541–5548

Bonthron, D. T., Morton, C. C., Orkin, S. H., and Collins, T. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1492-1496

Bonthron, D., Collins, T., Grzeschik, K.-H., van Roy, N., and Speelman, F. (1992) Genomics 13, 257–263

Cao, X., Mahendran, R., Guy, G. R., and Tan, Y. H. (1993) J. Biol. Chem. 268, 16949–16957

Collins, T., Bonthron, D. T., and Orkin, S. H. (1987) Nature 328, 621–624

Dey, B. R., Sukhatme, V. P., Roberts, A. B., Sporn, M. B., Rauscher, F. J., III, and Kim, S. J. (1994) Mol. Endocrinol. 8, 595–602

Dyson, W. N., and Tjian, R. (1983) Cell 35, 79–87

Esposito, F., Agozi, V., Morrone, G., Morra, F., Cuomo, C., Russo, T., Venuta, S., and Cinirino, F. (1994) Biochim. J. 301, 649 – 653

Fazio, M. J., O’Leary, J., Kahari, V. M., Chen, Y. Q., Salitta, B., and Uitto, J. (1991) J. Invest. Dermatol. 97, 281–285

Franzoso, G., Bours, V., Azarenko, V., Park, S., Tomita-Yamaguchi, M., Kanno, T., Brown, K., and Siebenlist, U. (1993) EMBO J. 12, 3893–3901

Gashler, A. L., Bonthron, D. T., Madden, S. L., Rauscher, F. J., III, Collins, T., and Sukhatme, V. P. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10984–10988

Harrington, M. A., Konicek, B., Song, A., Xia, X., Fredericks, W. J., and Rauscher, F. J., III. (1993) J. Biol. Chem. 268, 21271–21275

Heldin, C.-H. (1992) EMBO J. 11, 4251–4259

Heldin, C.-H., Backstrom, G., Ostman, A., Hammacher, A., Ronnstrand, L., Rubin, K., Nister, M., and Westermark, B. (1988) EMBO J. 7, 1387–1393

Imagawa, M., Chiu, R., and Karin, M. (1987) Cell 51, 251–260

Jensen, D. E., Rich, C. B., Terzstra, A. J., Farmer, S. R., and Foster, J. A. (1995) J. Biol. Chem. 270, 6535–6563

Johnson, S., Reeves, R. B., Lin, J.-X., Child, R., Leiden, J. M., Thompson, C. B., and Leonard, W. J. (1995) Mol. Cell. Biol. 15, 1798–1796

Kadowaki, T., Carner, K. R., Masliah, F. R., and Tijan, R. (1987) Cell 51, 1079–1090

Kaelzel, D. M., Maul, R. S., Lui, B., Bonthron, D. T., Fenstermaker, R. A., and Coyne, D. W. (1994) Biochem. J. 301, 321–327

Kanazashi, S., Hata, D., Ishigami, T., Jung, E. Y., Shintaku, N., Sumimoto, S., Helke, T., Katamura, K., and Mayumi, M. (1994) Mol. Immunol. 31, 21–30

Khachigian, L. M., and Chesterman, C. N. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5100–5104

Minowa, T., Minowa, M. T., and Mouradian, M. M. (1994) J. Biol. Chem. 269, 11656–11662

Mutero, A., Camp, S., and Taylor, P. (1995) J. Biol. Chem. 270, 1866–1872

Nakagama, H. G., Heinrich, J., Pelletier, J., and Housman, D. (1995) Mol. Cell. Biol. 15, 1489–1498

Pascal, E., and Tjian, R. (1991) Genes & Dev. 5, 1646–1656

Raines, R., Bowen-Pope, D. F., and Ross, R. (1990) in Handbook of Experimental Pharmacology (Sporn, M. B., and Roberts, A. B., eds) pp. 173–261, Springer-Verlag, Berlin

Rauscher, F. J., III, Morris, J. F., Tourjany, J. O. E., Cook, D. M., and Curran, T. (1990) Science 250, 1259–1262

Rorsman, F., Bywater, M., Knott, T. J., Scott, J. T., and Bonthron, D. T. (1998) Mol. Cell. Biol. 8, 511–577

Ross, R. (1993) Nature 363, 801–809

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Sanchez, A., Chesterman, C. N., and Sleight, M. J. (1991) Gene (Amst.) 98, 295–298

Shingu, T., and Bornstein, P. (1994) J. Biol. Chem. 269, 32551–32557

Takimoto, Y., and Kuramoto, A. (1993) Biochem. Biophys. Acta 1206, 176–182

Takimoto, Y., Wang, Z. Y., Kofer, K., and Deuel, T. F. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1686–1690

Tjian, R., and Maniatis, T. (1994) Cell 77, 5–8

Tong, B. D., Auer, D. E., Jaye, M., Kaplow, J. M., Ricca, G., McConathy, E., Drohan, W., and Deuel, T. F. (1987) Nature 328, 619–621

Wang, Z., Lin, X.-H., Qui, Q. Q., and Deuel, T. F. (1992a) J. Biol. Chem. 267, 17022–17031

Wang, Z.-Y., Madden, S. L., Deuel, T. F., and Rauscher, F. J., III (1992b) J. Biol. Chem. 267, 21999–22002

Williams, T., Admon, A., Luscher, B., and Tijan, R. (1989) Genes & Dev. 3, 1507–1517
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