An S1 nuclease-sensitive region in the first intron of human platelet-derived growth factor A-chain gene contains a negatively acting cell type-specific regulatory element

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ABSTRACT

The platelet-derived growth factor (PDGF) A-chain gene is expressed in a tissue- and developmental stage-specific manner. Here we identify an S1 nuclease sensitive region within the first intron that functions as a negative regulatory element in HeLa but not in human glioblastoma (A172) cells in transient transfection assays. A 147 bp DNA fragment that contains this element functions in a position and orientation independent manner to negatively regulate both the PDGF A-chain promoter and the heterologous herpes simplex virus thymidine kinase (TK) promoter. The cell-type specific effect of this 147 bp DNA fragment is seen when it is located downstream but not upstream of the reporter gene driven by either the PDGF A-chain or TK promoters. The negative regulatory element has been localized to a 24 bp DNA sequence within the S1 sensitive site that retains negative regulatory activity and recognizes a nuclear protein in HeLa but not in A172 cells. Furthermore, the 24 bp element functions as a cell type-specific negative element independent of its position. These results suggest that a functional silencer within the first intron exhibits a non-B-form DNA structure under superhelical stress *in vitro* and may contribute to the cell type-specific transcriptional regulation of PDGF A-chain gene *in vivo*.

INTRODUCTION

The human platelet-derived growth factor (PDGF) is an important growth factor and chemoattractant for cells of mesenchymal origin (1). PDGF is found either as a homodimer of the A- or B-chains (PDGF AA, BB) or as an AB heterodimer that elicits functional activity through the PDGF receptors (2–7). The A- and B-chains of PDGF are the products of separate but related genes that are regulated independently of each other. The gene encoding the B-chain of PDGF is a proto-oncogene, the cellular counterpart of the transforming gene (v-sis) of the simian sarcoma virus (SSV). The PDGF A-chain gene is found on a separate chromosome (10), and, in contrast to the PDGF B-chain gene, has not been demonstrated to be transforming when overexpressed in cells (11). PDGF A-chain expression has been observed in normal endothelial cells, fibroblasts, smooth muscle cells, and various transformed cell lines (10, 12–15); particularly high levels are found in glioblastoma, osteosarcoma, and rhabdomyosarcoma cell lines (10). Expression of the PDGF A-chain gene has also been found in high levels in developing mouse central nervous system (16) and in early stage xenopus embryos (17). However, little is known about the mechanisms that regulate this important gene.

Previously, we isolated, sequenced, and characterized the promoter region of the PDGF A-chain gene (18) and described *cis*-acting regulatory elements in 5' flanking sequences (19,20). However, in these analyses, *cis*-acting elements that are responsible for cell type-specific expression of the PDGF A-chain gene were not found. For this reason, we isolated and sequenced the 1431 base pair first intron and used S1 nuclease sensitivity, gel mobility shift assays, and chloramphenicol acetyltransferase (CAT) assays in transient transfections to seek potential regulatory elements in the first intron. Here we demonstrate a negatively acting regulatory element in the first intron of the PDGF A-chain gene which confers cell type-specific regulation of transcription.

MATERIALS AND METHODS

Plasmid constructions

The first intron of the PDGF A-chain gene was obtained from a 3.5 kb BamH I—BamH I genomic DNA fragment described previously (18). Small fragments encompassing the first intron were subcloned into pBluescript KS(+) (Stratagene, CA), sequenced by the dideoxy chain-termination method, inserted into downstream of CAT reporter gene in plasmid pACAT, and tested in transient transfection assays. pACAT contains the PDGF A-chain gene promoter (−250 to +386, relative to the initiation site) and the chloramphenicol acetyltransferase (CAT) reporter gene as described previously (20). Because the 147 bp Alu I—Alu I fragment of the first intron (+1517 to +1664, Fig. 1) negatively regulated PDGF A-chain promoter activity, it was selected for further analysis. In order to analyse the functional dependence of orientation, it was cloned in both orientations into the Sma I site of Bluescript and designated as pBSAlu147(+) or pBSAlu147(−). Orientation was confirmed by restriction enzyme analysis. The pBSAlu147± plasmids were digested with Sst I and Kpn I and the resultant Sst I—Kpn I fragments were cloned upstream of pACAT, and designated as AA+pACAT and AA−pACAT.

In order to study these fragments with a heterologous promoter, the pBSAlu147± plasmids were digested with Hind III and Xba I and inserted into the polylinker region upstream of pBLCAT2 in which the CAT gene was regulated by the herpes simplex virus (HSV) thymidine kinase (TK) promoter (21) and designated as AA+pBLCAT2 and AA−pBLCAT2. The same 147 bp Alu I—Alu I fragment was also cloned into a Sal I site downstream of the CAT gene of pACAT and pBLCAT2 and designated as pACAT+AA and pBLCAT2+AA, respectively.

Oligonucleotides for use in plasmid construction and gel retardation assays were synthesized with multiple restriction enzyme sites at both ends, annealed, and purified. The purified double-stranded oligonucleotides were digested with appropriate restriction enzymes and cloned into the polylinker region of the pBLCAT2 plasmid in both orientations upstream of the TK promoter or downstream of the CAT reporter gene. These plasmids were designated SHS−pBLCAT2, SHS+pBLCAT2 and pBLCAT2+SHS, respectively.

Fine mapping of S1 nuclease hypersensitive sites

Supercoiled plasmids were extracted by alkaline lysis and purified twice by ultracentrifugation through ethidium bromide—CsCl gradients, as described by Maniatis *et al.* (22). Assays for sensitivity to S1 nuclease were performed with 1.0 μg plasmid DNA in 50 μl S1 buffer (30 mM NaoAc, pH 4.5, 100 mM NaCl, 0.2 mM EDTA, and 3 mM Zn[OAC]₂) with 0.05 units of S1 for 5 minutes at 37°C. S1 nicked plasmid DNA was digested with Hind III, purified, end-labeled with either T_4 polynucleotide kinase (BRL) and $(\gamma\text{-}^{32}P)$ ATP (NEN), or with avian myeloblastosis virus (AMV) reverse transcriptase (BRL) and $[\alpha\text{-}^{32}P]$ dATP (NEN) at the 5' end or 3' end, respectively. The end-labeled plasmids were digested with BamH I, separated on agarose gels and eluted. The sites of nicking by S1 nuclease were determined after electrophoresis of end-labeled DNA fragments on 6% sequencing gels. The 3' end-labeled DNA fragments were chemically sequenced with the Maxam—Gilbert sequencing kikt (Sigma) under conditions recommended by the manufacturer and used as markers. The 5' end-labeled, S1 nicked

DNA fragment is longer than the corresponding marker ladder by three bases. Thus, the ladder band X corresponds to nicked fragment X-3.

Cell culture, nuclear extracts, gel mobility shift assays

Human glioblastoma (A172) cells express high levels of the PDGF A-chain gene whereas expression in HeLa cells is substantially lower; these cell lines were therefore used to pursue the cell type-specific regulatory elements. A172 and HeLa cells were grown in Dulbecco's Modified Eagle's Media supplemented with 10% fetal calf serum. Nuclear extracts were prepared by procedures of Dignam *et al.* (23) from 1×10^9 A172 and HeLa cells and fractionated by heparin—agarose chromatography as described by Tjian *et al.* (24). Nuclear extracts in 0.1 M KCl/HEMG (25 mM Hepes (K⁺) pH 7.5, 25 mM MgCl₂, 10% glycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol) buffer were applied to the column, washed with 0.1 M KCl/HEMG buffer until eluates contained less than 0.05 mg/ml protein, and eluted stepwise with HEMG buffer containing 0.2 M, 0.4 M, and 0.6 M KCl, respectively. Fractions were pooled, dialyzed against 0.04 M KCl HEMG, frozen, and stored at −70°C for subsequent use.

The 147 bp Alu I—Alu I DNA fragment used in gel mobility shift assays was digested from plasmid pBSAlu147+ with BamH I and Hind III. A second oligonucleotide of 24 bp from the S1 sensitive region also was synthesized based upon its homology with a negative regulatory element previously described (31). All DNA probes were end-labeled with $(\alpha\text{-}^{32}P)$dATP, $(\alpha\text{-}^{32}P)$dCTP, $(\alpha\text{-}^{32}P)$dGTP (NEN) and AMV reverse transcriptase, and purified on Sephadex G-50 spin column (Boehringer Mannheim Biochemicals). Binding assays were carried out in 25 μl sample mixtures containing 2 or 4 μg of a 0.4 M KCl heparin—agarose column eluate of nuclear extracts, 1−2 fmol of end-labeled probes, and 2 μg poly (dI:dC):poly (dI-dC) in 25 mM Hepes (pH 7.5), 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, and 5 mM MgCl₂ for 30 minutes at room temperature. For competition assays, unlabeled competitors were mixed with binding mixtures before the probes were added. Samples were fractionated on 5% or 7.5% polyacrylamide gels in 50 mM Tris HCl (pH 8.0), 0.38 mM glycine at 5−8 V/c m for 5−6 hours. The gels were dried and exposed to Kodak XAR-5 film at −70°C overnight for analysis.

DNA transfection and CAT assays

Plasmid DNA was transfected into HeLa and A172 cells by calcium phosphate co-precipitation (25). Ten μg of plasmid DNA was added to cultured cells that had been plated 24 hours previously at 1×10^6 cells per 100 mm dish. After 48 hours, extracts were prepared from transfected cells and CAT assays performed with acetyl-CoA and [^{14}C] chloramphenicol (NEN), as described by Gorman *et al.* (26). Acetylated and nonacetylated chloramphenicol was separated by thin-layer chromatography. After autoradiography, regions corresponding to acetylated and nonacetylated chloramphenicol were excised and quantitated by scintillation counting. The efficiency of transfection was normalized in cells co-transfected with SV40 early promoter/β-galactosidase plasmids (PCH110, Clontech). β-galactosidase activity in cell lysates was assayed as described (27). Each transfection experiment was performed in duplicate at least three times.

RESULTS

DNA sequence analysis of the first intron

To seek possible elements responsible for transcriptional regulation of the PDGF A-chain gene, we isolated and sequenced the first intron of the PDGF A-chain gene (Fig. 1). Analysis of this sequence indicated that a '5'-ATCCCC-3'' sequence is repeated uninterrupted four times within the first intron; this sequence is homologous to an enhancer sequence within the interferon-β gene (28). Furthermore, a poly dG:dN stretch was found which began 267 bp downstream of the 'ATCCCC' short repeat sequences. This sequence is known to form Z-DNA and also has been associated with enhancer activity (29,30). A 'TATAA' sequence was found 257 bp upstream of the second exon. However, this 'TATAA' sequence does not function as a 'TATA box' since transcripts were not found to be encoded by first intron in previous work (18). The analysis of these sequences suggests that the first intron of the PDGF A-chain gene may contain *cis*-acting regulatory elements.

The first intron of the PDGF A-chain gene contains an S1 sensitive region

We previously identified several *cis*-acting elements within the 5' flanking region of the PDGF A-chain gene that are sensitive to S1 nuclease *in vitro* (19). The approach of seeking S1 nuclease sensitive elements therefore was used to analyse the first intron; the entire span of the first intron was analyzed systematically. Discrete S1 hypersensitive sites were mapped in different regions of the first intron, including the polydG:dN region and 'AT-CCCC' repeat region as previously identified by DNA sequence analysis (Figure 1). A particularly S1 sensitive region was mapped within a 147 bp Alu I−Alu I sequence (+1517 to +1664, relative to transcriptional start site) within the first intron. To further fine map this region, the 187 bp Alu I−Alu I fragment was placed in a Bluescript plasmid and designated as pBSAlu147+. When supercoiled pBSAlu147+ was digested with S1 nuclease, labeled, and analyzed on sequencing gels (Figure 2A), an S1 sensitive region was found that spanned residues +1587 to +1656 (Fig. 2B). Fine mapping of the sites cleaved by S1 within this region indicated that the sites lay within a purine:pyrimidine rich stretch (Fig. 2B).

Cell type-specific silencer in the S1 sensitive region

In order to define a biological function of this S1 sensitive region, CAT reporter constructs were made that contained either the PDGF A-chain promoter alone, pACAT, or with the 147 bp Alu I−Alu I DNA fragment in a Sal I site located downstream of the CAT gene in pACAT (see Materials and Methods), designated as pACAT+AA (Fig. 1). pACAT and pACAT+AA were transiently expressed in human glioblastoma (A172) and HeLa cells (Fig. 3). CAT activity in extracts prepared from pACAT transfected HeLa cells was ~3-fold greater than the activity observed in extracts from pACAT+AA transfected HeLa cells, suggesting the 147 bp Alu I−Alu I DNA fragment functions to repress PDGF A-chain promoter activity (Fig. 3). However, the Alu 1−Alu 1 fragment in pACAT+AA was without effect when transfected into A172 cells. This result indicated that the negative regulatory element(s) in the Alu I−Alu I fragment fails to function in A172 cells but is functional in HeLa cells and tat this negative regulatory element(s) functions in a cell type-specific manner.
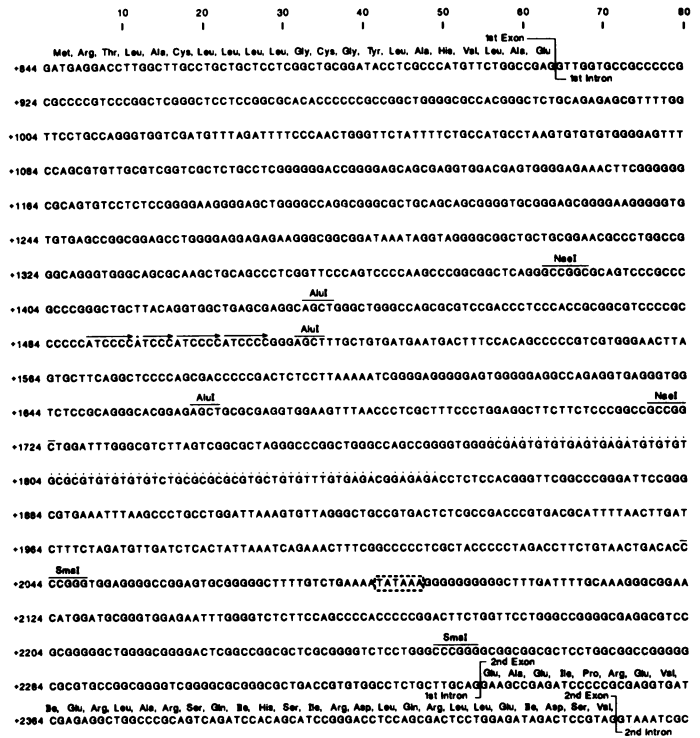

Figure 1. Nucleotide sequence of the first intron of the human PDGF A-chain gene. The 'TATAA' sequence is indicated by the dashed box. Dots overlie the poly dG:dN stretch. Splice junctions are indicated by ⌐ . Tandem repeat sequences are indicated by arrows. Several restriction enzyme recognition sites are also indicated.

To assess the orientation/position dependence of this negative effect, the 147 bp Alu I−Alu I fragment was cloned into a site immediately upstream of the PDGF A-chain promoter in pA-CAT in the 5'→3' and 3'→5' orientations, and designated as AA+pACAT and AA−pACAT, respectively. It was observed in lysates of both AA+pACAT and AA−pACAT transfected HeLa cells that CAT activity was ~10% of that in lysates of pACAT transfected cells (Fig. 3). This negative regulatory element thus is effective in both orientations and functions in a position independent manner. However, the negative regulatory effect was much stronger when the element was tested 5' to the promoter and less when tested in a position 3' to the CAT reporter gene. Interestingly, similar results were observed when these constructs were transfected into A172 cells (Fig. 3). In contrast to its failure to function as a negative element when placed downstream of the reporter gene, the Alu I−Alu I fragment in A172 cells functions also as a negative element when placed 5' of the promoter (Fig. 3). Thus, an interesting feature of these results is that the position or distance of the negatively acting element(s) relative to the transcription start site seems critical for determining the cell type-specific effect.

To determine if this negatively acting element acts similarly on a heterologous promoter, the Alu I−Alu I fragment was fused upstream of the herpes simplex virus (HSV) thymidine kinase (TK) promoter in both orientations and downstream of the TK promoter driven CAT reporter gene in the 5' to 3' orientation. These constructs were designated as AA+PBLCAT2, AA−PBLCAT2, and PBLCAT2+AA, respectively. CAT
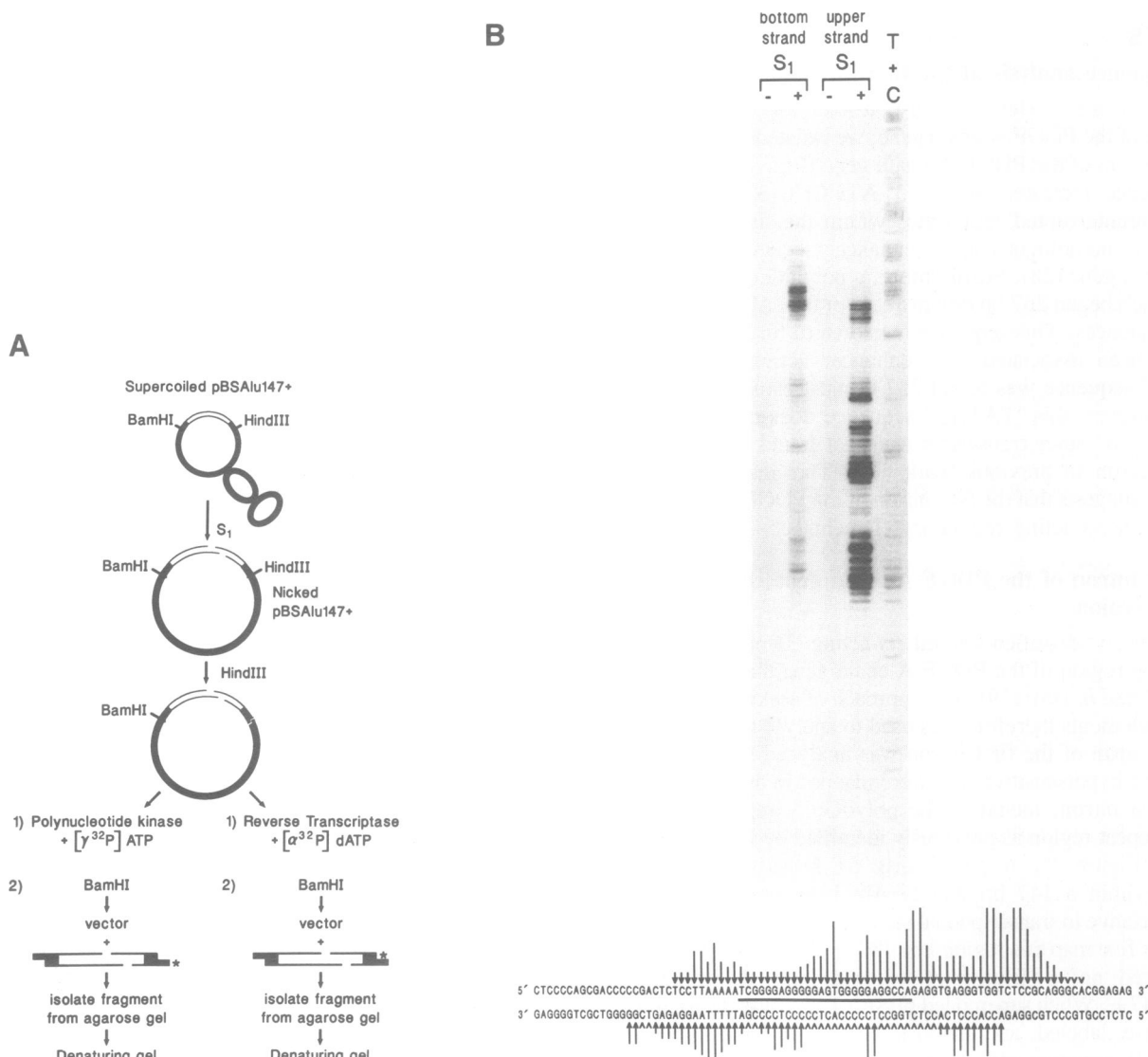
Figure 2. Mapping of S1 nuclease sensitive sites within the 147 bp Alu I—Alu I fragment of the first intron. **A.** Procedure for mapping the single stranded S1 nuclease cleavage sites within the 147 bp Alu I—Alu I DNA fragment. The plasmid used was pBSAlu147+ which contains the Alu I—Alu I fragment. **B.** Upper, Left (bottom strand). S1 nicked, 5′ end-labeled noncoding strand. Right (upper strand), S1 nicked, 3′-end-labeled coding strand. −, control, untreated with S1 nuclease. +, S1 nuclease-treated DNA. T+C: Maxam—Gilbert sequencing T+C reactions. Lower. Summary of S1 mapping experiments. Arrows above the DNA sequence represent S1 cleavages sites on the coding strand; arrows below the sequence refer to the noncoding strand. The degree of S1 sensitivity as determined by densitometric scanning is indicated by arrow length. The SHS sequence used to synthesize oligonucleotides for gel mobility shift assays is underlined.

activity was reduced to ∼20% of the levels of activity observed with the TK promoter driven CAT gene alone (Fig. 3) when each of these three constructs were transfected into HeLa cells. Consistent with the results above in which the PDGF A-chain promoter was used, this element with the TK promoter was effective as a negative regulator in A172 cells only when the Alu I—Alu I fragment was fused upstream of the promoter. It was not effective in down-regulating the TK promoter activity in A172 cells when it was cloned downstream of the CAT reporter gene (Fig. 3). Interestingly, promoter specific effects are also seen in these experiments. For example, the element in the downstream position is more potent in silencing promoter activity of the TK gene than of the PDGF A-chain in HeLa cells. It is possible that this difference may reflect a contribution of the distance from the transcription start site.

Differential binding of nuclear factors to the silencer

The mechanism underlying the cell type-specific repression of the PDGF A-chain gene promoter activity by 147 bp Alu—Alu fragment was investigated by seeking nuclear factors from HeLa and A172 cells that bind to this region. A ^{32}P end-labeled 147 bp Alu I—Alu I fragment was incubated with nuclear extracts from both cell types and gel mobility shift assays were performed. The DNA probe was retarded in gels after incubation with HeLa and A172 cell nuclear extracts; the formation of two distinct bands were observed using HeLa cell nuclear extracts (B1, B2, Fig. 4A). In contrast, the upper band (B1) was not observed when nuclear extracts from A172 cells were incubated with the Alu I—Alu I fragments whereas the lower band (B2) was consistently observed with both A172 and HeLa cell nuclear extracts (Fig.
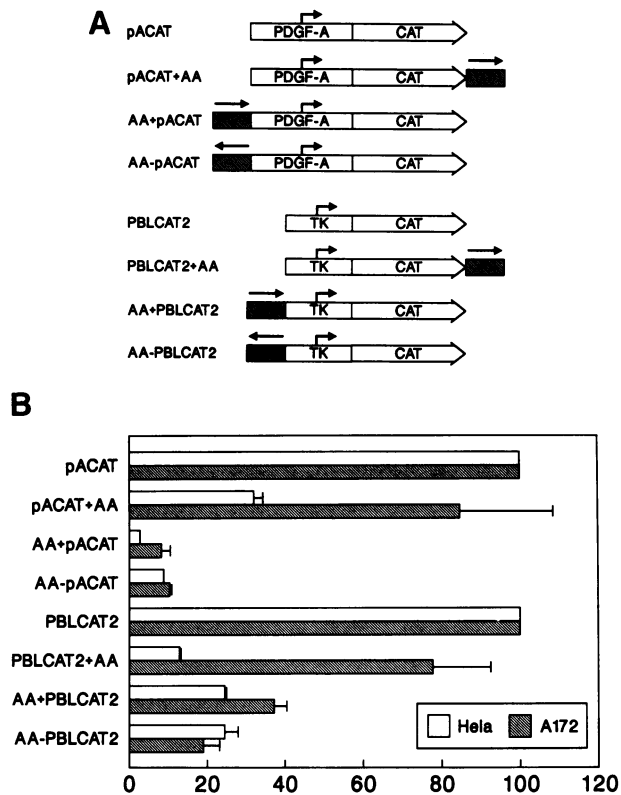
Figure 3. Inhibitory activity of the Alu I−Alu I DNA fragment. (A) CAT plasmids for transfection assays. The 147 bp Alu I−Alu I fragment was cloned in both orientations upstream and downstream of the PDGF A-chain promoter/CAT construct (pACAT) and of a thymidine kinase promoter/CAT construct PBLCAT2. (B) The constructs were transfected into the HeLa and A172 cells. CAT activities are expressed as a percentage of the activity of pACAT or pBLCAT2. pACAT: original PDGF A-chain promoter/CAT; PACAT+AA: the Alu I−Alu I fragment fused at the downstream of the CAT gene of pACAT. AA+pACAT and AA-pACAT: the Alu I−Alu I fragment cloned upstream of PACAT in 5′−3′ or 3′−5′ orientations, respectively. The pBLCAT2 constructs were designated accordingly (See Materials and Methods).

Figure 4. Gel mobility shift and competition assays with the Alu I−Alu I fragment. A. Radiolabeled fragment (2ng) was analyzed on a 5% acrylamide gel, either alone: (0) or after incubation with 1 μg or 4 μg of nuclear extract from HeLa and A172 cells. B1 and B2 indicate the two complexes formed. F indicates free probe. B. Competition analysis with Hind III digested pUC18 as nonspecific competitor. C. Competition analysis with the unlabeled probe as competitor. D. Competition analysis with the synthetic double-stranded oligonucleotide (5′-TC-GGGGAGGGGGAGTGGGGGAGGCA-3′) as competitor.

4A). These results indicated that HeLa cell nuclear extracts contain proteins capable of forming two protein−DNA complexes with the 147 bp Alu I−Alu I fragment whereas A172 cell nuclear extracts contain only one protein under conditions of assay.

Competition experiments were used to establish that the retarded bands observed result from specific protein−DNA interactions. A Hind III digest of the pUC18 plasmid was used as a nonspecific competitor and did not eliminate either of the two retarded species (Fig. 4B). The unlabeled 147 bp Alu I−Alu I fragment reduced both of the retarded bands progressively with increasing concentrations of competitor (Fig. 4C). Both bands were virtually eliminated at 50-fold molar excess of the 147 bp Alu I−Alu I fragment. A synthetic oligonucleotide which corresponded to the S1 nuclease hypersensitive sequence (designated as SHS, Fig. 2) also was used. This DNA sequence (5′-TCGGGGAGGGGGAGTGGGGGAGGCCA-3′, +1605 to +1630) was chosen because it is similar to a reported silencer sequence (5′-CCCACCTCC-3′) found in the collagen II gene promoter (31). Addition of the SHS sequence significantly reduced the intensity of the upper retarded band (B1 complex) but not the lower retarded band (B2 complex) (Fig. 4D). Since
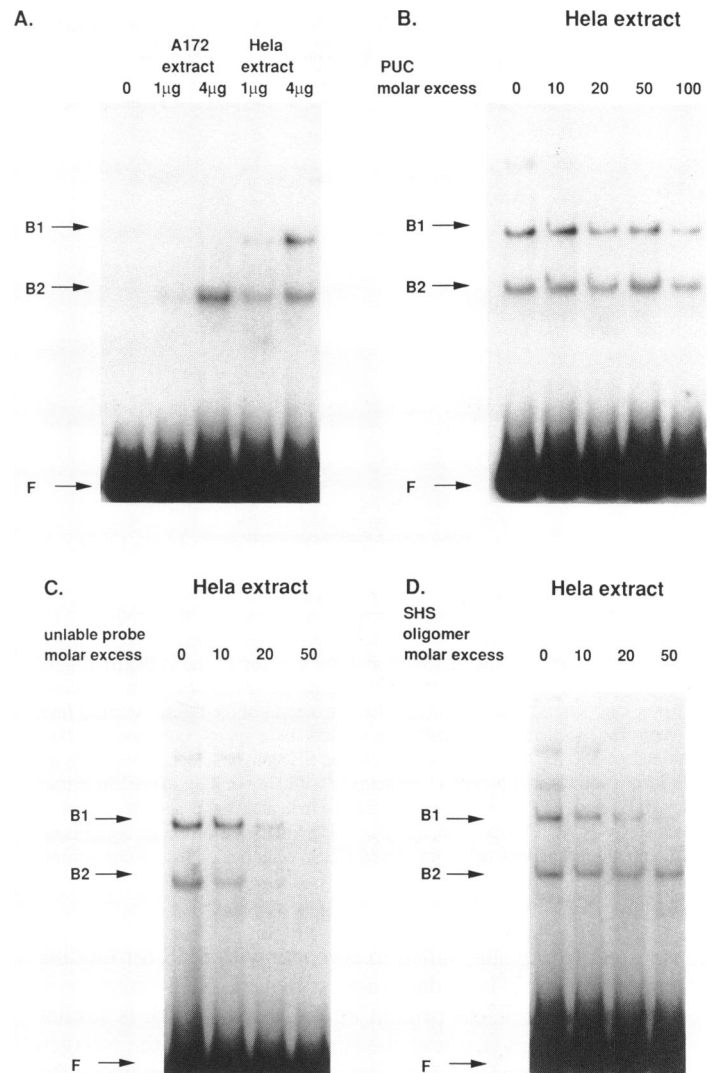
the upper complex was not observed with A172 cell nuclear extracts (Fig. 4A), this result provides presumptive evidence that the nuclear factor in this complex may be the regulatory protein that accounts for the observed cell type-specific silencer activity.

Gel mobility shift assays were then performed with the SHS oligonucleotide as probe. A discrete complex was detected with HeLa cell nuclear extracts but not with A172 nuclear extracts (Fig. 5A), even when the protein concentration of the A172 cell nuclear extracts was increased 20-fold (data not shown). However, this protein−DNA complex was effectively abolished by a 50 molar excess of unlabeled probe (Fig. 5B). Furthermore, at the same concentration, the related oligonucleotide containing the silencer sequence of collage II gene (31, Fig. 5) effectively inhibited complex formation (Fig. 5C). Oligonucleotides comprising unrelated DNA sequences, including an Sp1
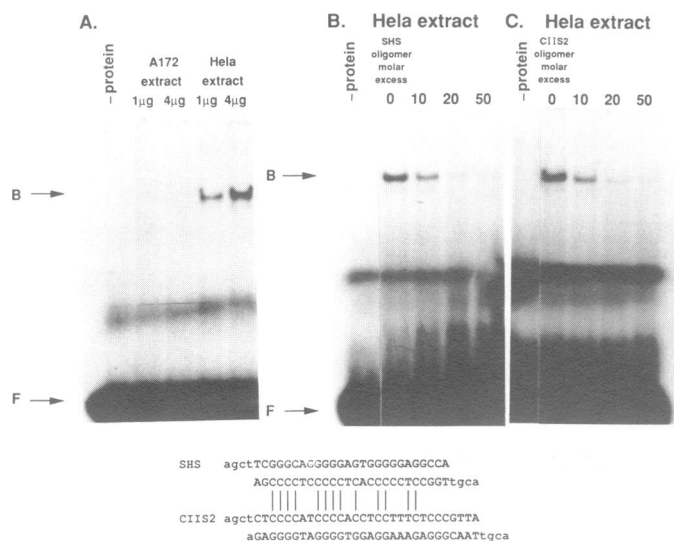
SHS   agctTCGGGCACGGGGAGTGGGGGAGGCCA
       AGCCCCTCCCCCTCACCCCCTCCGGTtgca
       |||| ||||| | || ||
CIIS2 agctCTCCCCATCCCCACCTCCTTTCTCCCGTTA
       aGAGGGGTAGGGGTGGAGGAAAGAGGGCAATtgca

Figure 5. Gel mobility shift and competition assays with the synthetic SHS oligonucleotide. Oligonucleotides representing a portion of the S1 sensitive site of PDGF A-chain first intron (SHS) and the silencer of the collagen II gene promoter (CIIS2) were synthesized with a Hind III site at both ends (indicated by lower case letters) and are illustrated at the bottom of the figure. Vertical lines between the sequences designate conserved base pairs common to both oligonucleotides. **A.** The radiolabeled synthetic SHS oligonucleotide was analysed after incubation without protein (−protein) or with 1 μg or 4 μg of nuclear extract from HeLa and A172 cells. The complex is indicated by B and free probe is indicated by F. **B.** Competition analysis using unlabeled probe as competitor. **C.** Competition analysis using unlabeled CIIS2 oligonucleotide as competitor.

consensus binding site, failed to compete with SHS for binding (data not shown). These data suggest that the SHS sequence is recognized by a nuclear protein in HeLa cells which is similar to the protein interacting with the silencer element of the collagen II gene and that the SHS sequence itself may be responsible for the cell-type specific effect observed in HeLa cells.

The SHS sequence possesses cell type-specific silencer activity

To confirm that the SHS sequence functions as a cell type-specific silencer, we cloned the 24 bp SHS double-stranded oligonucleotide (see Fig. 2B, underlined sequence) upstream of the TK promoter in both orientations and downstream of the TK driven CAT gene in the 5′→3′ orientation. These plasmids were designated as SHS+PBLCAT2, SHS−PBLCAT2, and PBLCAT2+SHS, respectively, and were tested in transient transfection assays. When the SHS 24 bp oligonucleotide was cloned upstream in both orientations or downstream in the 5′−3′ orientation, a significant loss of CAT activity was observed in lysates of the transfected HeLa cells, to levels comparable to those of the originally described silencer activity (Fig. 6). In contrast, the negative effect of the 24 bp SHS sequence was not observed in A172 cells transfected with the identical constructs. In contrast, the entire 147 bp Alu I−Alu I fragment which contains this SHS sequence actively suppressed transcription when tested in both orientations in the upstream position of the TK promoter and tested in A172 cells. These data suggest that the sequences necessary to exert the cell type-specific silencer activity in HeLa cells are present in the SHS sequence (residues from +1605 to +1630). These data also suggest that additional sequences within the 147 bp Alu I−Alu I fragment may also be required for the
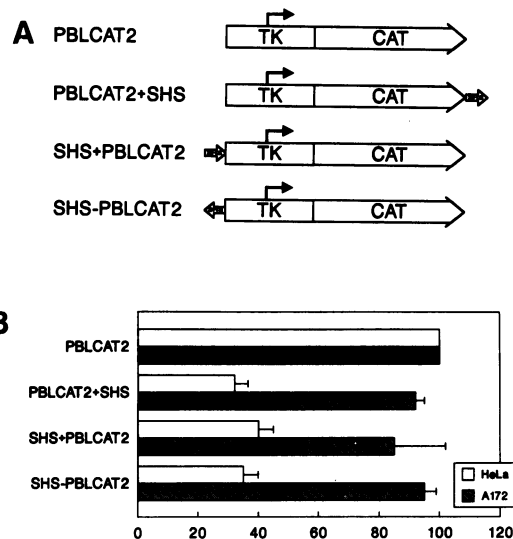

Figure 6. Cell type-specificity inhibitory effect of the SHS oligonucleotide on the thymidine kinase promoter activity. (A) CAT plasmids used in transfection assays. The synthetic SHS oligonucleotide (+1605 to +1630) was cloned in both orientations upstream or downstream of a TK promoter/CAT construct PBLCAT2. (B) The plasmids SHS+PBLCAT2, SHS−PBLCAT2, PBLCAT2+SHS, and PBLCAT2 were transfected into HeLa and A172 cells. CAT activities are expressed as a percentage of the activity of PBLCAT2.

negative influence of the 24 bp SHS sequence in A172 cells; importantly, however, the negative influence of the 147 bp Alu 1−Alu 1 sequence was essentially lost when it was placed 5′ to the promoter (Fig. 3). The sequences within the flanking regions of the SHS which are responsible for the negative function in the upstream position of reporter gene in A172 cells have not been identified.

DISCUSSION

Previously, we identified several cis-acting regulatory elements, including a serum responsive element and Sp1 binding sequences, that are located within or near homopurine-homopyrimidine stretches in the 5′-flanking sequences of the PDGF A-chain gene (19). Here we report the presence of an S1 nuclease sensitive region in the first intron of the PDGF A-chain gene that contains a negatively acting regulatory element. This combination of an S1 nuclease sensitive site and the function of the sensitive site to down regulate gene expression appears to be unique and further advances the hypothesis that non-B-form DNA conformations may be critical determinants to the transcriptional regulation (34).

The finding of a cell type-specific regulatory element in the PDGF A-chain gene is predictable, given the strict spatial and temporal patterns of expression of the PDGF A-chain gene observed during development (16). For this reason, we analysed the first intron of the PDGF A-chain gene and found that the 147 bp Alu I−Alu I sequence contains a cis-acting element that functions as a negative regulatory element for the homologous and a heterologous promoter in HeLa cells but not in A172 cells. This element thus acts in a cell-type specific manner. Consistent with the functional demonstration of cell-type specificity, we also observed that the 147 bp Alu I−Alu I sequence interacted with nuclear proteins from HeLa and A172 cells differently. HeLa

cells express a protein that recognizes this element and that is not expressed in A172 cells. The negatively regulatory element behaves as a silencer, since it functions equally with a heterologous promoter and independently of orientation and position. However, this region is unique since it fails to function as a negative element in A172 cells when positioned downstream of the promoter in its normal first intronic position but is functional when placed 5' to the promoter, suggesting that the distance of the element from the promoter region may contribute to its cell-type specific regulatory activity. The silencer region thus appears to function in a novel manner with respect to its cell type-specific function but not to its more general silencer functions.

Efforts to understand the basis of this uniquely functioning silencer region led to the identification of a 24 bp sequence (+1605 to +1630) within the 147 bp Alu I—Alu I fragment that is similar to the negative regulatory sequence (5'-CCCACCT-CCT-3') identified in the c-myc (42), lysosome (43), β-interferon (44), immunoglobulin heavy chain (45), growth hormone (46), and collagen II genes (31). This 24 bp sequence competes effectively for the HeLa cell nuclear factor in the slower moving complex (B1) in gel mobility shift assays, a complex which is absent in assays using A172 cell nuclear extracts. This 24 bp oligonucleotide functions as a negative regulator in HeLa cells but lacks the ability to negatively influence transcription in A172 cells, even when it is placed upstream of the heterologous promoter. Surprisingly, the cell type-specificity is lost when the intact Alu I—Alu I fragment which contains the 24 bp silencer is placed 5' to the promoter, suggesting that the sequences flanking the 24 bp negative regulatory element also may contain additional negatively acting element(s) which act only in the 5' flanking region of the promoter.

The regions that regulate transcription of many eukaryotic genes contain sites that are sensitive to the single-strand specific nuclease S1 when present either in active chromatin or in supercoiled DNA (32, 33). Fine mapping has demonstrated that these S1 sensitive sites are commonly located in polypurine—polypyrimidine domains, regions composed of one strand containing primarily purine residues and the complementary strand containing pyrimidine-rich residues (for review see ref. 34). It has been suggested that these polypurine—polypyrimidine stretches can adopt unusual DNA conformation(s) that endow the DNA with sensitivity to S1 (34). Polypurine—polypyrimidine stretches have been found to be essential for optimal transcription of several genes, including the human c-myc (35), epidermal growth factor receptor (36), ets-2 (37), chicken α2(I) collagen (38), mouse c-ki-ras (39), drosophila hsp26 (40), and human δ-globin genes (41).

Mechanisms of transcriptional repression *in vivo* are largely unknown (reviewed, 47,48). However, the negative transcriptional regulation of the PDGF A-chain gene by the element in the first intron appears to be mediated by DNA—protein and/or protein—protein interactions, perhaps by interacting with the transcription initiation complex through DNA binding proteins, similar to the locking model proposed by Jackson (47). It is interesting to note that Franklin *et al.* (49) showed that the first intron of the PDGF B-chain gene contains both positively and negatively acting cell type-specific regulatory elements. In both PDGF A- and B-chain genes, complex interrelationships of *cis*-acting elements within the first intron may be of critical importance in controlling the cell-type specific expression of PDGF genes.

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