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Isolation and Sequencing of a cDNA Clone Homologous to the v-*sis* Oncogene from Human Endothelial Cells

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A clone containing the 3′ end of the mRNA for the human c-*sis* gene (homologous to the B chain of platelet-derived growth factor) was isolated from a cDNA library derived from human umbilical vein endothelial cells and then sequenced. The analysis of possible translation products in all three reading frames indicated that the A chain of platelet-derived growth factor was not coded for within the 3′ end of the c-*sis* mRNA. The 3′ end of the mRNA for c-*sis* is contained in or adjacent to exon 6.

The platelet-derived growth factor (PDGF) is the most potent mitogenic polypeptide in human serum for cells of mesenchymal origin (38, 41). Purified PDGF consists of two heterodimeric proteins of equal mitogenic activity which differ only in carbohydrate content. Reduced PDGF consists of a larger A chain (~17 kilodaltons) and a smaller B chain (~14 kilodaltons) (14, 15). (In previously published work based upon our original separations of the heterodimeric polypeptide chains of PDGF, we assigned the larger chain as the B chain and the smaller chain as the A chain [12, 14, 15, 45]. The individual chains have been variably called A, B, I, and II, based upon individual laboratories' nomenclature. To arrive at a common nomenclature for the chains of PDGF, we have used the B chain to indicate the smaller, heterodimeric chain of PDGF which has >90% homology with the transforming protein of the simian sarcoma virus [SSV] [45] consistent with the chain nomenclature we used in collaborative works identifying the c-*sis* gene structure as identical with the small chain of PDGF [27].) Amino acid sequence analysis of the B chain established a remarkable (>90%) homology with the deduced amino acid sequence of the transforming gene product ($p28^{v\text{-}sis}$) of SSV. This homology provided evidence that the transforming activity of SSV is initiated and maintained through PDGF-like mitogenic activity (18, 45). The human cellular proto-oncogene (c-*sis*) analog of v-*sis* encodes the B chain of PDGF (8, 27, 28), whereas the genetic locus and functional role of the related A chain of PDGF remains to be identified.

A 4.2-kilobase (kb) mRNA has been identified in cells expressing the c-*sis* gene product (3, 5, 19, 20, 22, 26, 34, 44, 47). An additional 2.7-kb transcript has also been identified in some of these cell lines (3, 19, 20, 22, 26, 44, 47). The expression of the c-*sis* mRNA appears to be regulated by other growth factors, as demonstrated by the high levels of c-*sis* transcripts found in endothelial cells stimulated by the endothelial cell growth factor (26).

The presumed protein products of the v-*sis* and c-*sis* genes have mitogenic, antigenic, and receptor binding properties similar to those of purified PDGF (4, 5, 13, 17, 20–22, 24–26, 31, 32, 34, 37, 39, 42). Several SSV-transformed cells also secrete the transforming gene product. Growth of this type of SSV-transformed cells with anti-PDGF antibody (25) results in a marked reduction of [*methyl*-^{3}H]thymidine incorporation, which suggests that the secreted v-*sis* gene product interacts with PDGF cell surface receptors to stimulate the autocrine synthesis of DNA and presumably cell growth.

The genomic organization of human cellular DNA has been studied by cloning regions homologous to v-*sis* (8–10, 27, 29). The gene is ~14 kb long and contains six exons. Four of these exons contain sequences homologous to the B chain of PDGF. The nucleic acid sequences of v-*sis* and c-*sis* are over 90% conserved. The nucleotide sequence of regions homologous with v-*sis* was determined from a cDNA clone obtained from a library derived from a human T-cell lymphotropic virus-transformed cell line (HUT 102) and from genomic clones (8, 27–29). We report the isolation and sequencing of a clone from a cDNA library constructed from mRNA from cultured normal human umbilical vein endothelial (HUVE) cells. The clone consists of a region homologous to the 3′ end of v-*sis* and an additional 3′ sequence. No sequences encoding the A chain (non-*sis* homologous) were observed in any of the three reading frames isolated.

By using the methods of Buell et al. (7), cDNA was synthesized from poly(A)$^+$ mRNA (1) isolated from monolayer cultures of HUVE cells as previously described (26). *Eco*RI linkers were ligated to the cDNA, and the cDNA was then ligated to *Eco*RI-digested lambda gt11 (49). Five different clones were isolated that hybridized to the *Bam*HI fragment that contained exons 5 and 6 of c-*sis* (11). To further characterize the clones, they were checked for hybridization to the *Pst*I fragment containing v-*sis* (16). Only one, pTP (the largest of the five clones), hybridized to v-*sis* and was subcloned into pUC19 (33).

The restriction map and sequencing strategy of the c-*sis* region of pTP are shown in Fig. 1. The cDNA was 3′ of the region homologous to v-*sis* and the open reading frame coding for the B chain of PDGF. The insert began in exon 6 at nucleotide pair 849 (as referenced from spliced c-*sis*) (28) and continued for ~1,380 base pairs (bp) to the putative polyadenylation site. The sequencing strategy for pTP using the partial chemical degradation method (30) is shown (Fig. 1B). DNA fragments were obtained by digesting with either *Bam*HI, *Eco*RI, *Bst*EII, or *Sst*I. Labeling of the fragments was accomplished either at 5′ protruding ends by

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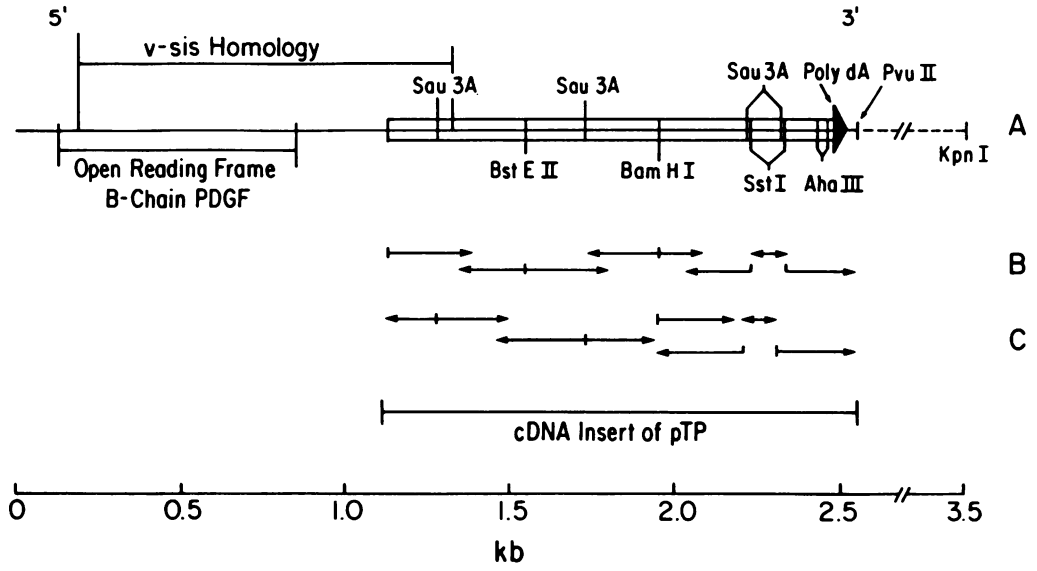

FIG. 1. Restriction map and sequencing strategy for pTP. (A) Position of the c-*sis* insert in pTP relative to the region homologous to v-*sis* and relative to the open reading frame coding for the B chain of PDGF. ▭, cDNA insert in pTP; -----, lambda gt11 sequences; ——, previously sequenced regions of c-*sis* (9). (B) Maxam-Gilbert (30) sequencing strategy. Arrows, Distance sequenced; vertical lines, restriction sites that were labeled with ^{32}P. The 90-bp *Sst*I fragment was labeled on both ends with ^{32}P, strand-separated, and sequenced. (C) Dideoxy chain termination sequencing strategy (40). Arrows, Distance sequenced: vertical lines, *Sau*3A site closest to the hybridization site of the 17-mer (New England Biolabs, Inc., Beverly, Mass.) used for priming the synthesis. The 102-bp *Sau*3A fragment was sequenced in both directions.

treatment with calf intestinal alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) followed by phosphorylation with [γ-^{32}P]ATP (New England Nuclear Corp., Boston, Mass.) and polynucleotide kinase (Boehringer Mannheim) or at 3' protruding ends with [α-^{32}P]dATP (Amersham Corp., Arlington Heights, Ill.) and terminal transferase (Pharmacia, Inc., Piscataway, N.J.). After digestion with a second enzyme and electrophoresis on polyacryl-

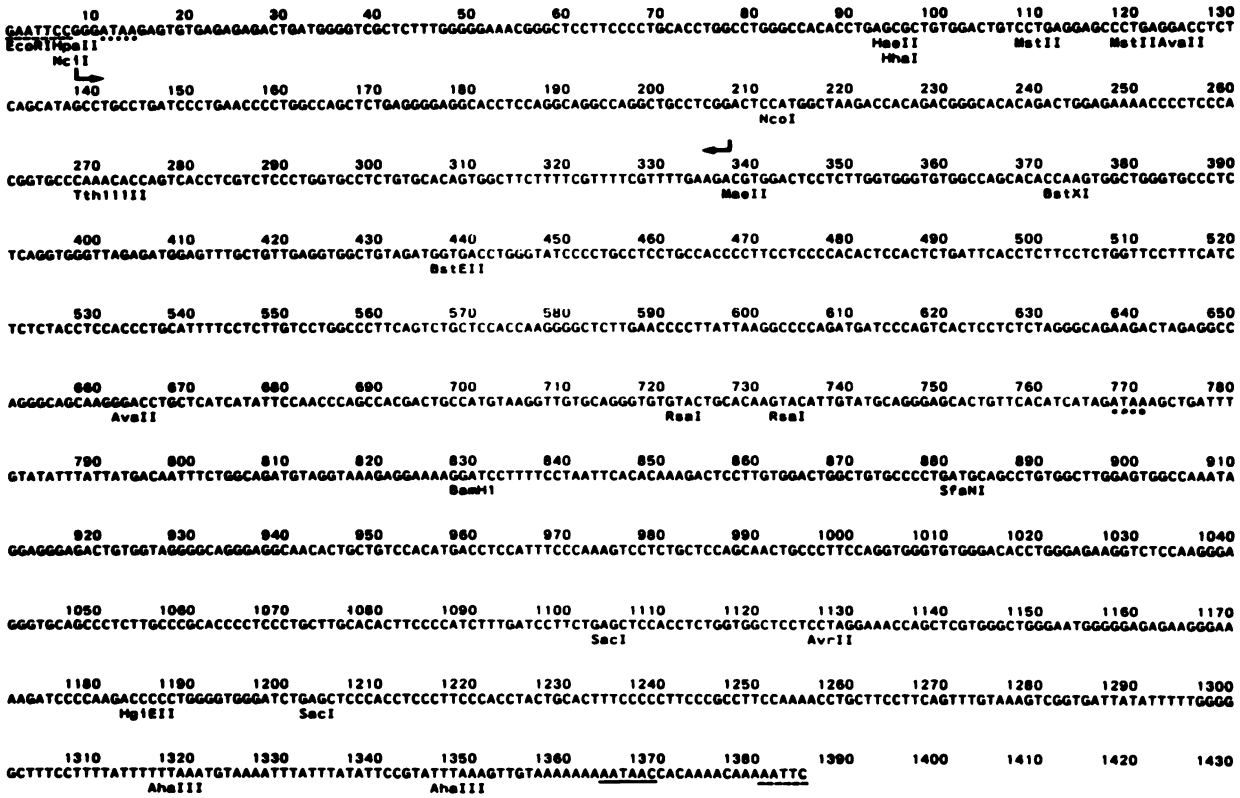

FIG. 2. Nucleotide sequence of c-*sis* cDNA clone pTP. ------, *Eco*RI linkers; ——, putative polyadenylation signal; ·····, potential minor polyadenylation sites. The region homologous to that reported for pSM1 (9) is in brackets.

amide gels, the fragments were isolated by electroelution and sequenced (New England Nuclear sequencing kit NEK-010).

The strategy devised for M13 sequencing (40) is shown in Fig. 1C. The *Eco*RI-*Kpn*I fragment containing the c-*sis* region was isolated from pTP, digested with *Sau*3A, and subcloned into bacteriophage M13 WB2348 (a modification of WB2344 [2] obtained from W. Barnes, Department of Biological Chemistry, Washington University) previously digested with *Bgl*II or *Bgl*II and *Eco*RI. Sequencing of these fragments was done with a dideoxy sequencing kit (Pharmacia), [α-^{35}S]dATP (New England Nuclear) and the modifications suggested by Biggin et al. (6).

The sequence of the cDNA insert of pTP is shown in Fig. 2. The sequence agrees with that previously published (28, 29) at 196 of 198 bp; at nucleotide pair 59, a C is present in this clone and in the viral v-*sis* sequence, whereas a T was reported in a human genomic c-*sis* clone, λL33 (28), and at nucleotide pair 161, a G is present in this clone and in the v-*sis* sequences, whereas an A was reported in λL33. Most mRNAs contain the sequence AATAAA preceding the polyadenylation site (35). This sequence is not present in c-*sis*. However, the sequence AATAAC is present 9 bp 5′ of the putative polyadenylation site. The substitution of a C for the fifth A (AATAAC) is a rare event (48), but it has been observed in soybean Bowman-Birk protease inhibitor (23). Ratner et al. (36) recently completed the sequencing of pSM-1, a cDNA clone isolated from transformed HUT 102 cells. Although there were a number of differences (two additional Cs before the polyadenylation cleavage site, a 5-bp insert at nucleotide pair 190, and numerous single base changes) between this clone and pSM-1, the same putative polyadenylation signal that is found in c-*sis*, AATAAC, was found 14 bp from the polyadenylation site of pSM-1. A portion of this sequence, ATAA, was identical to that found 5′ of the polyadenylation sites of the three short forms of mouse dihydrofolate reductase mRNA (43). The sequence ATAA is found at two other locations in c-*sis*, nucleotide pairs 11 to 14 and 769 to 772 (Fig. 2). The occasional use of these sites, in addition to the putative polyadenylation signal, nucleotide pairs 1,370 to 1,374, would yield transcripts 3.4 and 2.8 kb long in some cells. c-*sis* mRNA species of approximately these lengths have been reported in some c-*sis*-producing cells (3, 19, 20, 22, 26, 34, 44, 47). Computer-generated analyses of all three reading frames showed a large open reading frame (bp 930 to 1,373; 16.2 kilodaltons) that could code for a peptide the size of the A chain of PDGF. However, the amino acid sequence was different from that of the A chain of PDGF (18, 45). Also, no portion of the 3′ untranslated region of c-*sis* could code for a portion of the A chain of PDGF.

If the mRNA for c-*sis* is 3.5 kb, as suggested by Collins et al. (10), there is no room in the 5′ end of the mRNA to code for the A chain of PDGF. If the mRNA coding for c-*sis* is 4.2 kb, as suggested by Jaye et al. (26), Eva et al. (19), and Ratner et al. (36), the combination of the sequence described by Collins et al. (10) and our sequence would account for ~3,400 bp and would leave 800 bp. This could encode a protein the size of the A chain of PDGF. The conclusive localization of the sequences coding for the A chain of PDGF in relation to that of the B chain will require the isolation of the cDNA encoding the A chain and its use as a probe in chromosome studies and in genomic DNA mapping studies.

Human genomic DNA was cleaved with restriction endonucleases and separated with agarose electrophoresis and analyzed by Southern blotting analysis with either the *Pst*I fragment containing v-*sis* (16) or the *Eco*RI-*Pvu*II fragment containing c-*sis* as the probe (data not shown). The c-*sis* probe hybridized to fragments >23 kb long in the *Eco*RI- and *Hin*dIII-digested DNA in agreement with those reported for hybridization with v-*sis*. The c-*sis* probe hybridized to a 2.3-kb *Pvu*II fragment, which indicated that the 3′ end of the gene for c-*sis* was contained in a single *Pvu*II fragment. The v-*sis* probe hybridized to 9.4- and 2.3-kb fragments in the *Bam*HI digest, whereas the c-*sis* probe hybridized to *Bam*HI fragments 2.3 and 1 kb long. Combining this mapping data with the positioning of a 1-kb *Bam*HI fragment 3′ to exon 6 as reported by Chiu et al. (8) for a genomic clone of human c-*sis* suggests that c-*sis* sequences extend into the 1-kb *Bam*HI fragment 3′ to the 3′ end of the v-*sis* homologous region.

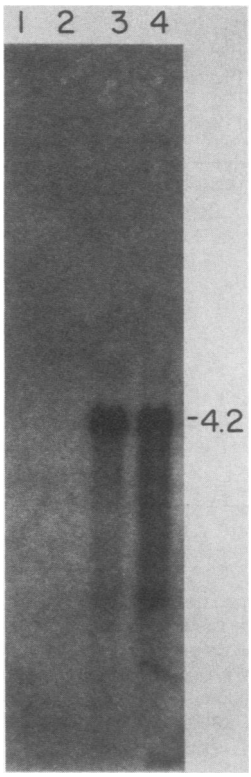

FIG. 3. Northern blot analysis of HUVE, A172, and RPM cell mRNA and tRNA with the insert of pTP as a probe. A total of 10 μg each of tRNA (lane 1), RPM cells (lane 2), HUVE cells (lane 3), and A172 cells (lane 4) was analyzed. mRNA was electrophoresed on a 1% agarose gel containing formaldehyde. Transfer and analysis was performed as previously reported (26). The position of the 4.2-kb mRNA is indicated.

mRNA was isolated from HUVE, A172 (19), and rat promegakaryoblast (RPM) (46) cells, electrophoresed in an agarose-formaldehyde gel, and blotted as described by Jaye et al. (26). Only one band at 4.2 kb hybridized to the nick-translated *Eco*RI-*Pvu*II fragment of pTP in the lane containing HUVE mRNA. The lane containing A172 mRNA contained smaller RNA bands that also hybridized to c-*sis*. In the control lanes containing RPM mRNA and tRNA, no hybridization was observed (Fig. 3). mRNA for c-*sis* has been reported to be 4.2 kb long (19); hybridization to a fragment of this size thus provided additional evidence that this clone is derived from mRNA coding for c-*sis*. While this manuscript was in preparation, Collins et al. (10) presented

cDNA cloning of a *sis* homologous region from endothelial cells. The c-*sis* insert pTP presented here begins at residue 2,037 and proceeds substantially 3′ of the cDNA clone of Collins et al. and contains the signal for the cleavage and polyadenylation of the molecule.

Earlier work demonstrating that the expression of the c-*sis* gene is enhanced when endothelial cells in culture are exposed to endothelial cell growth factor (26) suggests important roles for the c-*sis* gene product in regulating vessel wall growth. PDGF is a potent mitogen for fibroblasts and smooth muscle cells but also is a potent chemotactic protein for neutrophils, monocytes, smooth muscle cells, and fibroblasts. Each of these activities which are mediated in vitro by PDGF or c-*sis* gene product is highly important in the inflammatory response and subsequent repair of injured blood vessels and also appears to be important in the abnormal proliferative response characteristic of atherosclerosis in humans.

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