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Nucleotide sequence of transforming human c-*sis* cDNA clones with homology to platelet-derived growth factor

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ABSTRACT

Three c-sis cDNA clones were obtained from polyadenylated RNA of a human T-cell lymphotropic virus (HTLV) type I transformed cell line. Two clones, designated pSM-1 and pSM-2, have cDNA inserts of 2498 and 2509 base pairs (bp), respectively, excluding the sizes of the guanylate tails, and the polyadenylate tracts. These clones are shorter than the estimated size of the c-sis mRNA of 4200 bp. Both of these clones can transform NIH 3T3 cells. The third clone, designated pSM-3 has a cDNA insert of 1421 bp and lacks transforming activity. The sequence of clone pSM-1 reveals a single long open reading frame (nucleotides 118-840) encoding chain A of platelet-derived growth factor, and two segments with homology to v-sis (nucletodies 182-871 and 1021-1325). Sequence homology is noted in the 3' untranslated region to the corresponding regions of the $\beta_1$interferon (IFN), human and murine β-nerve growth factor (NGF), human interleukin 2 (IL2) genes, and tubulin pseudogenes. However, no typical AATAAA polyadenylation signal is present. An alternating $(dCdA)_n \cdot (dGdT)_n$ sequence is present in the 3' flanking cellular sequences similar to those in the corresponding position of the human proenkephalin gene, in the first intron of the γ-IFN gene, and the second intron of the β-NGF gene.

INTRODUCTION

The human cellular homologue of v-sis, the transforming gene transduced by the simian sarcoma virus, encodes one of two polypeptide chains (chain A) of PDGF (1-5). Though PDGF polypeptide chain B reveals 50% amino acid identity with that of polypeptide chain A (1,2), its genetic locus, mode of expression, and function remain to be defined. The human c-sis locus is expressed in rat pup aortic endothelial cells (6), bovine and human venous endothelial cells (7-9), developing human placenta (10) and mouse embryo tissues (11), a variety of human sarcoma (12-17) and glioblastoma cell lines (17,18), and a number of T-lymphoid cell lines transformed by HTLV-I or II (19,20). The predominant transcript is a 4.2 kb mRNA, although a 2.7 kb transcript has also been identified in at least some cell types (6,9,10,11,12,15,19). The primary translation product is a 28 kilodalton (kd) protein which undergoes dimerization and

proteolytic cleavage (15,21,22). This protein product has in many cases been shown to have mitogenic activity as well as antigenic and receptor-binding characterisitcs similar to PDGF, and may be secreted from the cell (7,8,10,13-18,20).

In order to define the role of c-sis gene expression in HTLV-I mediated transformation, we isolated a 2.6 kb cDNA clone, which was designated pSM-1 (23). Restriction enzyme map and nucleotide sequence analysis demonstrated that the entire v-sis homologous region and the open reading frame encoding PDGF polypeptide chain A are present in the 5' portion of the cDNA insert. Partial nucleotide sequences of clone pSM-1 showed no differences from that of the normal human c-sis gene (24). Transfection of pSM-1 into NIH 3T3 cells resulted in transformed foci which grew as tumors in nude or syngeneic mice (23).

We now report the complete nucleotide sequence of clone pSM-1, the corresponding portions of the normal human c-sis gene, and the 3'flanking cellular sequences. We have isolated two additional c-sis cDNA clones, designated pSM-2 and pSM-3, from an HTLV-I transformed cell line and compared them to pSM-1 by restriction enzyme mapping, by sequencing the 5' and 3' portions of their cDNA inserts, and by analyzing their transforming capability.

MATERIALS AND METHODS

Materials: Nucleotide sequencing reagents, γ-^{32}P-ATP (spec. act. >5000 Ci/mmole) and α-^{32}P-dNTPs (spec. act. >3000 Ci/mmmole) were obtained from New England Nuclear; T4 polynucleotide kinase from New England Biolabs; acryamide, bis-acrylamide, N,N,N',N'-tetramethyl-ethylenediamine, and ammonium persulfate from Biorad; α-^{32}P-dideoxy ATP (spec. act. >7000 Ci/ mmole) and terminal deoxynucleotidyl transferase from Amersham; restriction enzymes, 10x universal core buffer, urea, bromphenol blue, ethidium bromide, and Klenow fragment of DNA polymerase I from Bethesda Research Labs; and Tris (hydroxymethyl)aminomethane (Tris) and ethylene diamine tetraacetic acid (EDTA) from Sigma.
Plasmid Clones: Plasmid clones pSM-1, pSM-2, and pSM-3, and phage clone L33 were isolated as previously described (23-25). The 1.0 kb Bam HI fragment of L33 was subcloned in the Bam HI site of pBR322, and is designated B3L.
Restriction Enzyme Digests: 0.5 ug of DNA were incubated with 5 u of restriction enzyme and 1/10 volume of 10x universal core buffer for 2 hrs. Reactions were stopped by the addition of 1/10 volume of 0.5% bromphenol blue, 100 mM EDTA 10 mM Tris, pH 7.5, 50% glycerol and incuba-

tion at 60°C for 10 min. Samples were electrophoresed on 5% acrylamide, 0.17% bis-acrylamide in 0.1 M Tris-borate, 2 mM EDTA buffer on 1.5 mm thick gels of 20 cm length at 300 v for 2 hrs. The gels were stained with 10 υg/ml ethidium bromide for 30 min.

DNA Sequencing: DNA fragments were labeled at their 5'ends with T4 polynucleotide kinase and γ-^{32}P-ATP or at their 3'ends with Klenow fragment of DNA polymerase I and α-^{32}P-dNTP or terminal deoxynucleotidyl transferase and α-^{32}P-dideoxyATP and sequenced by the method of Maxam and Gilbert (26). Sequences were analyzed by the computer programs of Queen and Korn (27) and Wilbur and Lipman (28).

RESULTS AND DISCUSSION

A cDNA library was constructed with the vector pcDV1 (29) using polyadenylated RNA from an HTLV-I transformed cell line. Clone pSM-1

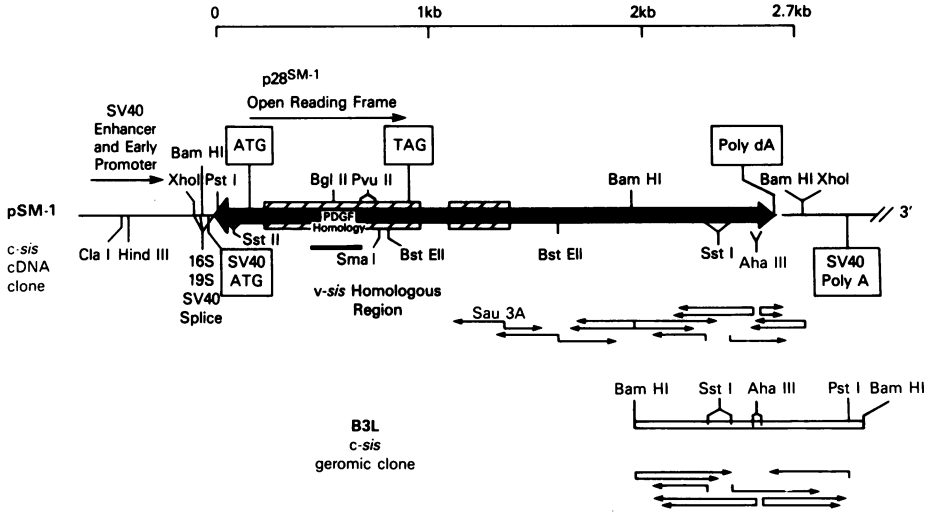

Fig. 1. Restriction maps of c-sis cDNA clone pSM-1 and genomic clone B3L. The heavy double arrowed line represents the cDNA insert in pSM-1. The polyadenylation site is indicated. The cDNA insert is flanked on the left by the SV40 enhancer and early promoter, and 16S and 19S mRNA splice donor and acceptor sites, and on the right by an SV40 polyadenylation signal. Regions homologous to v-sis are indicated by the shaded boxes. The region homologous to PDGF is shown by a single line, within the largest open reading frame, designated p28^{SM-1}. The restriction enzyme mpa of clone B3L is shown on the bottom of the figure. The scheme for newly derived sequences reported in this study are shown. The symbols |⟶ and ⟵| indicate 5' labeled fragments and |⟶ and ⟵| indicate 3' labeled fragments. The length of the arrows indicate the number of sequences obtained from each fragment.

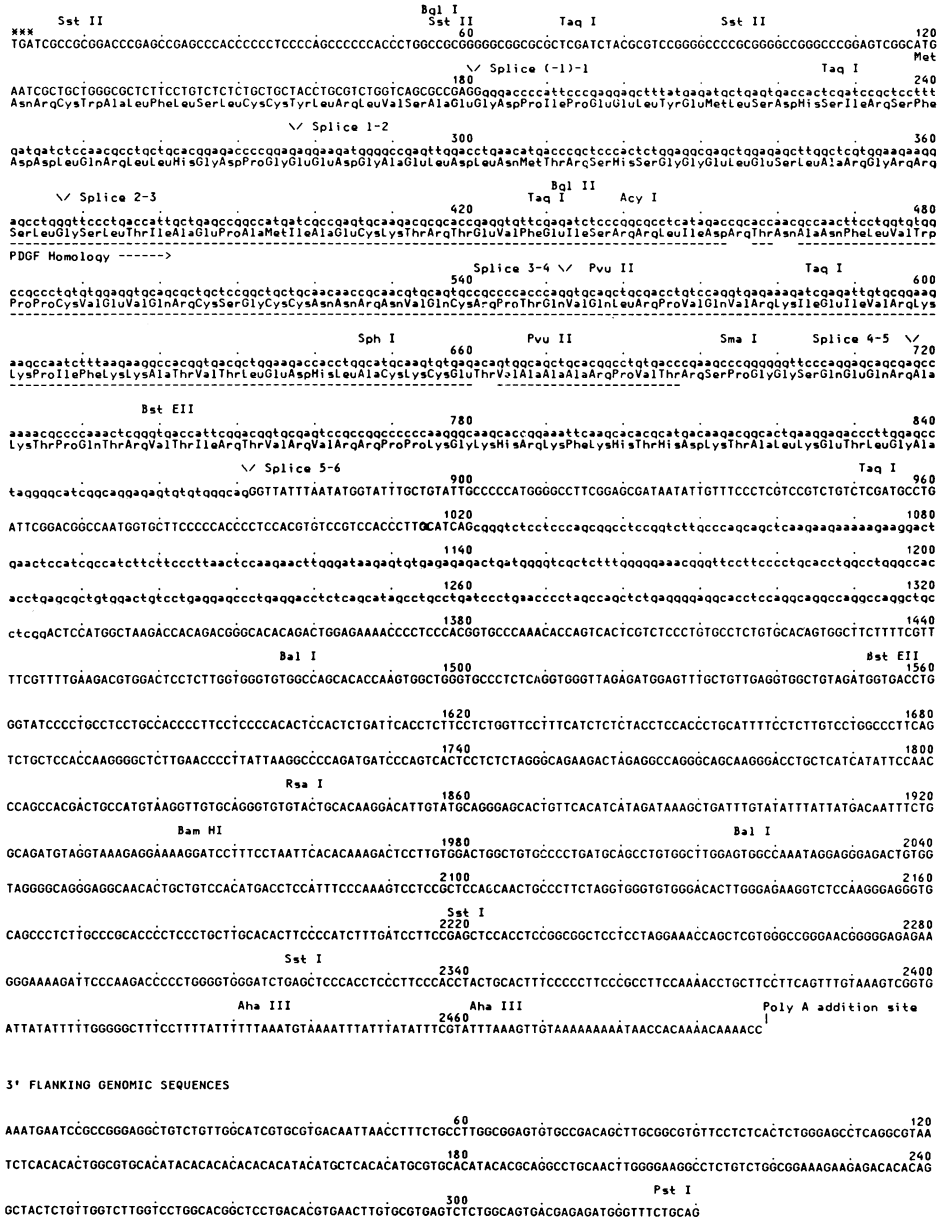

Fig. 2. Sequence of c-sis cDNA clone pSM-1 and 3' flanking genomic sequences. Lower case letters indicate those which are homologous to v-sis. RNA splice sites, the polyadenylation addition site, and restriction sites are indicated above the sequence. The predicted amino acid sequence of the largest open reading frame is shown and the amino acids identical to those determined for PDGF are underlined.

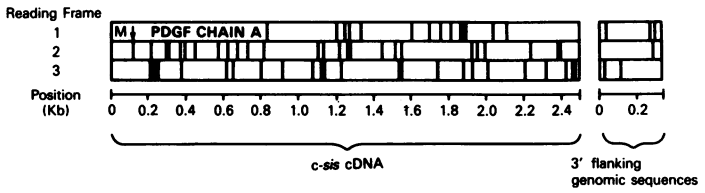

Fig. 3. Distribution of termination codons in each of the 3 reading frames of c-sis cDNA clone pSM-1 and 3'flanking genomic sequences. The position of the first ATG codon (M) is indicated within the largest open reading frame which encodes PDGF polypeptide chain A. The position in kilobases (kb) is indicated below the graph.

was isolated using a v-sis probe (23). The organization and restriction map of this clone is shown in Fig. 1. The clone includes a 2.6 kb polyadenylated cDNA insert downstream from the simian virus 40 (SV40) enhancer, early promoter, 16S and 19S mRNA splice donor and acceptor sites, and upstream from an SV40 polyadenylation signal. The restriction map of a corresponding region of the normal c-sis gene (clone B3L) is also shown.

The sequence of the cDNA insert of clone pSM-1 and a portion of clone B3L is shown in Fig. 2. The cDNA insert of pSM-1 includes 2498 bp followed by a stretch of 92 adenylate residues. The positions of RNA splice sites are indicated as determined by comparing the sequence of pSM-1 with that of the genomic c-sis sequence (3,24; Fig. 2). The exons are numbered 1-6 for those including sequences homologous to v-sis (3,24, 30) (lower case letters in Fig. 2). An upstream exon lacking homology to v-sis is designated exon -1.

A single large open reading frame is present at positions 118-840 predicting a protein product of 27,281 daltons (24) (Fig. 2 and 3). A hydrophobic leader sequence is predicted by the sequence within exon -1 (24). Predicted amino acids identical with those determined for PDGF peptides (nucleotide positions 361-687) follow a dibasic amino acid sequence which may represent a signal for proteolytic cleavage (24,31). The PDGF-homologous sequences are located predominately within exons 3 and 4. Predicted amino acid sequences within the other short open reading frames of pSM-1 (Fig. 3) reveal no similarity to those reported for PDGF polypeptide chain B.

The longest open reading frame is preceeded by an in phase termination codon at positon 1 indicating that the ATG codon at position 118 is likely

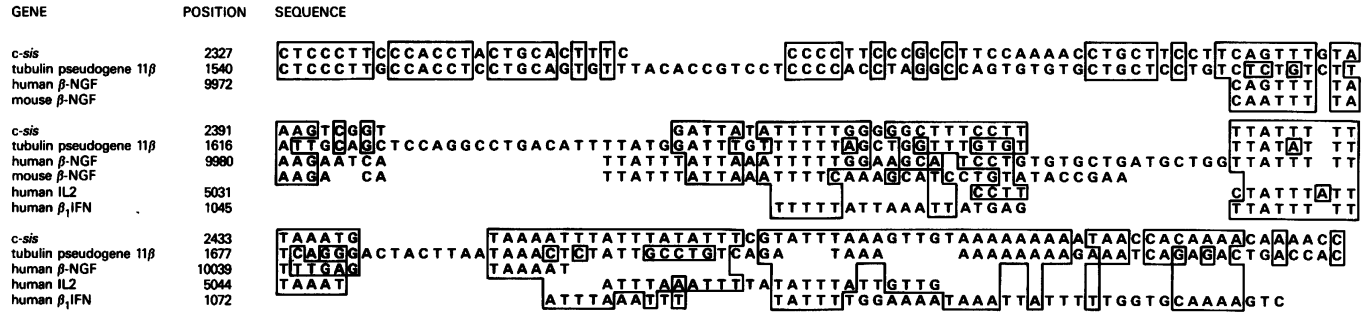

Fig. 4. Nucleotide sequence homologies in the 3'untranslated portions of mRNAs for c-sis, human tubulin pseudogene clone 11β, human IL 2, human β1-IFN, and human and mouse β-NGF. The nucleotide position of the beginning of each sequence is indicated. Identical amino acids are boxed.

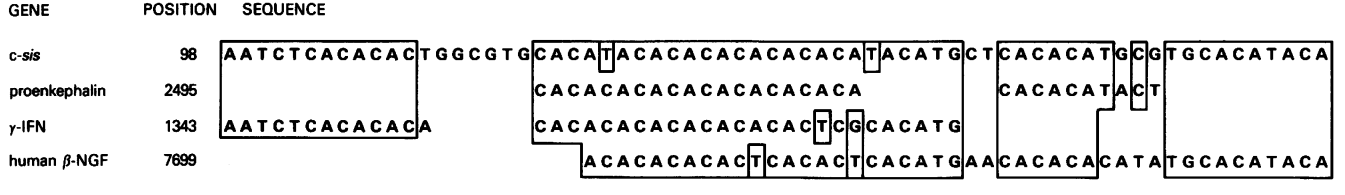

Fig. 5. Sequence homologies including $(dCdA)_n \cdot (dGdT)_n$ sequences in the 3'flanking genomic sequences of c-sis and proenkephalin, the first intron of γ-IFN, and the second intron of β-NGF. The nucleotide position of the beginning of each sequence is indicated. Identical amino acids are boxed.

to be the initiator codon (24). This finding precludes the possibility that PDGF chains A and B are synthesized from a single polyprotein precursor unless a mRNA with alternate splicing is encoded by this locus. It also indicates that the c-sis mRNA is likely to have almost 1.7 kb of 5' untranslated sequences.

The open reading frame terminates within exon 5 and is followed by a 1658 bp 3'untranslated sequence. Neither a typical AATAAA polyadenylation signal nor the less commonly utilized variants of this sequence (33-36) are present near the polyadenylation site. Furthermore, sequences homologous to those mediating the formation of histone mRNA 3' termini (37) are also absent from the c-sis gene. However, an AT-rich sequence and an oligo A stretch are present just upstream from the polyadenylation site in an analogous positon to those described for the β_1-IFN (38), β-NGF (39), and interleukin 2 (IL2) (40) genes and the tubulin pseudogenes (41) (Fig. 4). The role of these sequences in polyadenylation or mRNA stability remain to be evaluated.

The 3'flanking cellular sequence includes a $(dCdA)_n \cdot (dGdT)_n$ sequence which may be capable of forming Z-conformation DNA, and may have a role in transcriptional control (42,43). A similar region is present in an analogous region of the human proenkephalin gene (44), in the first intron of the γ-IFN gene (45), and the second intron of the β-NGF gene (39) (Fig. 5).

The v-sis homologous region (nucleotides 181-871 and 1021-1325) is interrupted by a sequence which is not homologous to v-sis (3,24). This is most likely due to the utilization of a different splice acceptor site in the human cells from which the cDNA clone was obtained compared to the woolly monkey cells from which simian sarcoma virus was isolated (24).

The sequence of the cDNA insert in pSM-1 is identical to that of the normal human c-sis gene, as determined in this study for clone B3L together with previously reported upstream sequences (3,4,5). A qualitative alteration within this region of the normal c-sis gene is thus not needed for activation of its transforming potential. This is confirmed by the demonstration of transforming activity of the normal c-sis gene (46).

Two additional c-sis cDNA clones, designated pSM-2 and pSM-3, were obtained from the HTLV-I transformed cell line. Comparative restriction enzyme digestion patterns and maps are shown in Fig. 6. The restriction map of pSM-2 is similar to that of pSM-1. The polyadenylate tail is

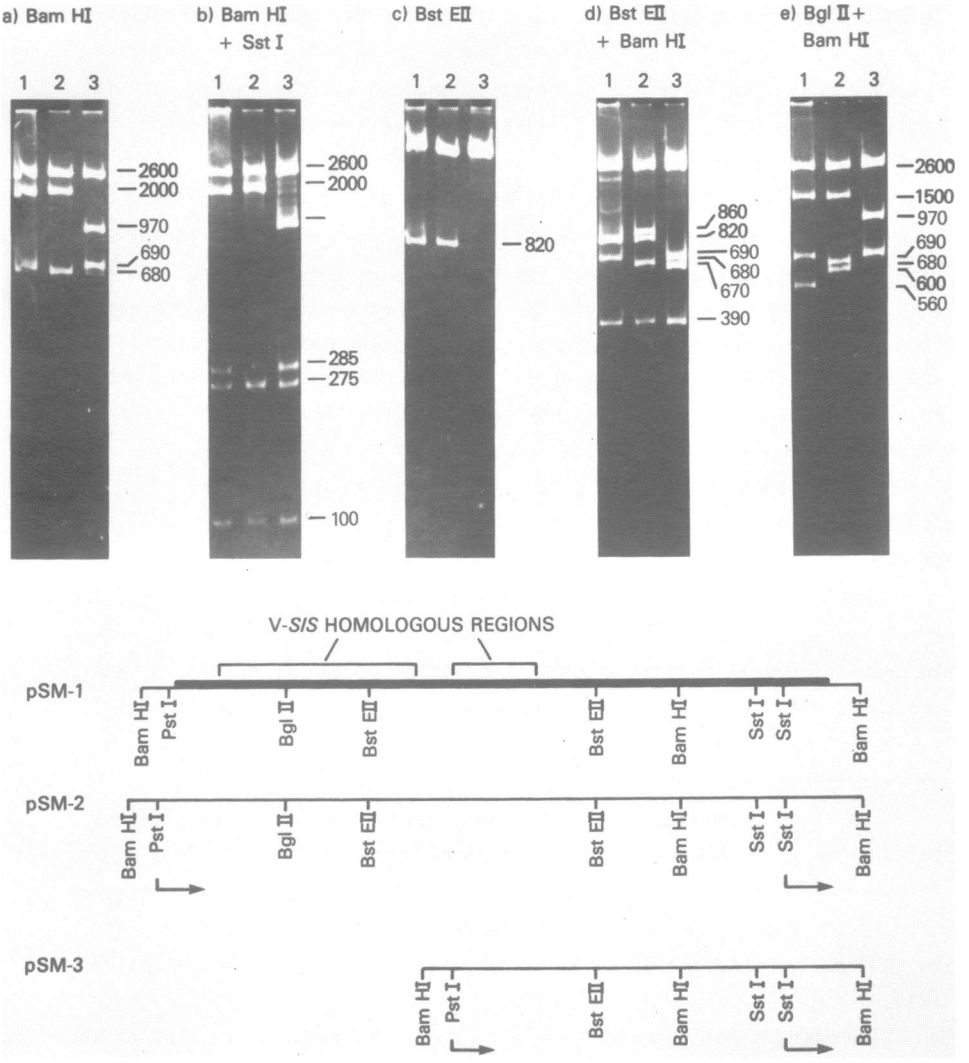

Fig. 6. Comparison of restriction maps of 3 different c-sis cDNA plasmids. Restriction enzyme digests (indicated above each figure) and polyacrylamide gel electrophoresis are described in Materials and Methods. The sizes of the DNA fragments in bp are indicated to the right of each figure. Restriction enzyme maps are indicated below the figure. The heavy line for pSM-1 shows the position of the cDNA insert. The positions of v-sis homologous regions are shown. The arrows below the maps for clones pSM-2 and pSM-3 indicate the positions of fragments which were 3'end labeled and, the length of the arrows show the number of sequences obtained.

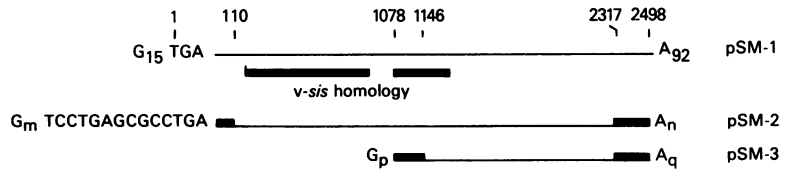

Fig. 7. Sequences of the 5' and 3' termini of c-sis cDNA plasmids pSM-2 and pSM-3. The sequences of pSM-1 are shown for comparison which begin at position 1 with TGA flanked on the 5' end by 15 guanylate residues and at the 3' end by 92 adenylate residues. The nucleotide positions for clone pSM-1 are shown at the top of the figure. The precise length of the guanylate and adenylate tails for pSM-2 and pSM-3 were not determined and are represented by G_m and G_p, and A_n and A_q, respectively. Sequences were determined at positions -11 - 110 and 2317 - 2498 for pSM-2 and 1078 1146 and 2317 - 2498 for pSM-3 based on the number of nucleotides shown in Fig. 1.

about 10 bp longer in pSM-1 compared to pSM-2, as indicated by a 690 rather than 680 bp fragment after digestion with Bam HI and a band at 285 bp rather than 275 bp (doublet) after digestion with Bam HI and Sst I, respectively. Approximately 40 additional bp are present at the 5' end of the cDNA insert in pSM-2 compared to pSM-1 as indicated by a band at 860 bp rather than 820 bp (doublet) after digestion with Bam HI and Bst EII, and a band at 600 bp rather than 560 bp after digestion with Bam HI and Bgl II, respectively.

The cDNA insert in clone pSM-3 is 1077 bp shorter than that in pSM-1. The restriction map of the 3'portion of the cDNA inserts of pSM-3 and pSM-1 are similar as indicated by the common bands at 690 bp after digestion with Bam HI, 285, 275, and 100 bp after digestion with Bam HI and Sst I, and 690 and 390 bp after digestion with Bst EII and Bam HI. The lack of difference in the positions of the internal restriction sites suggests that the same splice sites were used for processing of the precursors of the mRNAs from which all 3 clones were synthesized. This is in contrast to the alterations in the pattern of mRNA splicing demonstrated for a number of other genes including those for human c-Ki-ras (47), murine $\alpha_2$crystallin (48), human interleukin 2 receptor (49), rat fibrinogen-γ (50), and rat calcitonin (51).

The termini of the cDNA inserts in clones pSM-2 and pSM-3 were sequenced and found to be identical to the corresponding portions of pSM-1 except for the presence of an additional 11 bp at the 5' end of the cDNA insert of pSM-2 compared to that of pSM-1 (Fig. 7). The same in phase termination

codon is found in clone pSM-2 as pSM-1. Thus, the thymidylate residue at position 1 in pSM-1 is not due to a contaminant in the dGTP preparation used for tailing.

The lack of transforming activity of clone pSM-3 compared to clones pSM-1 and pSM-2 suggests that at least one region within the first 1077 bp of the cDNA insert of pSM-1 is required for transformation. A deletion of the 3' 600 bp of the pSM-1 cDNA sequence had no effect on the efficiency of transformation (our unpublished data with E. Westin). More recent studies have defined as essential regions for transformation those encoding the N-terminal hydrophobic sequence (52) which is presumably a signal sequence for insertion into or across the membrane, and the region of predicted amino acid sequence homology with PDGF (53). Further localization of the minimum transformation region in the c-sis product, and the definition of functions associated with other portions of the protein remain to be studied. Furthermore, the mechanism of transformation of 3T3 cells by this c-sis cDNA clone, whether by over-expression or deregulation of the c-sis product, loss of regulatory regions from upstream mRNA sequences, and/or interaction of the presumed homodimer product with a different set of cellular receptors, must also be clarified.

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