Table 2. Characterization of papillomavirus-transformed cells.

C127 clone*	Morphological transformation	Growth in soft agar†	Episomal viral genomes per cell‡	Tumorigenic in nude mice§
pBR322HPV-5	+	+	6	Y (3/5)
pBR322HPV-1	+	−	12	N (0/8)
CRPV	+	+	3	Y (7/7)
Control	−	−	0	N (0/10)

*The transformed clones chosen for comparison were a pBR322HPV-5–transfected clone (C112-3A-8-3), a pBR322HPV-1–transfected clone (C112-4A-2-2), and a CRPV-infected clone (C85-2E-2-1). †Growth in soft agar was scored by the number of colonies (greater than 50 cells per colony) appearing within 2 weeks after plating 103 cells per 60-mm plate. ‡Viral genomes per cell-genome equivalent was assayed by Southern blot hybridization relative to dilutions of standard plasmid DNA's hybridized at the same time with 16 pg of viral DNA per 10 μg of cellular DNA as 1 viral genome per cell-genome equivalent. §Female athymic nude mice (NIH Swiss) were inoculated subcutaneously at a single site with 2×10^6 cells in 0.2 ml of NaCl (0.85 percent). The proportion of mice showing visible tumors within 2 months after inoculation is indicated.

DNA species in persistent copy numbers varying from 2 to 30 copies per cell-genome equivalent between cell lines. No integration of viral DNA sequences was detected under conditions sensitive enough to detect 0.5 viral-genome equivalents per cell-genome equivalent.

In order to determine the extent to which our papillomavirus-transformed single-cell clones represent an appropriate model system for the study of papillomavirus gene expression in vitro, we investigated some cellular properties indicative of oncogenic transformation. Single-cell clones of C127 cells transformed by HPV-1, HPV-5, and CRPV displayed an elongated cellular morphology and a disoriented growth pattern relative to normal C127 cell cultures. Three single-cell clones representative of cells transformed by HPV-1, HPV-5, and CRPV were chosen for further analysis (Table 2). The pBR322HPV-1 transformed clone grew very poorly in soft agar and did not induce tumors in athymic nude mice within 2 months after the mice were injected with it. However, the clones transformed by pBR322HPV-5 and CRPV showed anchorage-independent growth and tumorigenicity. The differing pattern of growth in soft agar was verified for one additional clone transfected with pBR322HPV-1, two clones transfected with pBR322CRPV, and three additional clones transfected with pBR322HPV-5. The efficiency of colony formation in soft agar ranged from 15 to 30 percent for the positive clones and was less than 5 percent for control C127 cells. Nude mouse tumors were diagnosed histopathologically as undifferentiated carcinomas. Furthermore, the tumors induced by cells transformed by CRPV and HPV-5 were each shown to contain papillomavirus-specific DNA by dot blot or Southern blot hybridization or both. Phenotypic differences in the transformation characteristics of pBR322HPV-1–transformed cells relative to those transformed by pBR322HPV-5 and CRPV may be correlated with the lack of association of HPV-1 with any type of human neoplasia other than benign warts or with the Bam HI cloning site of HPV-1 in pBR322 being in that region of the genome putatively functioning in cellular transformation (*11, 16*). We have recently isolated a virus-transformed clone containing HPV-1 DNA episomes that grows well in soft agar; this result indicates that the cloning site of HPV-1 in pBR322 may have interrupted expression of the viral transforming function or functions in cells transfected with pBR322HPV-1.

Because few (if any) virus particles are produced by HPV lesions other than skin warts and because not all cell types may be susceptible to infection with intact virus, the transfection of cells with molecularly cloned HPV DNA represents a preferred method for establishing infections in vitro. Our results indicate that the HPV genome can persist in and be expressed to induce morphological transformation of certain types of cells in culture. Thus, systems for the study of HPV expression and transformation in vitro should facilitate the elucidation of the biology of papillomaviruses.

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Transforming Potential of Human c-*sis* Nucleotide Sequences Encoding Platelet-Derived Growth Factor

Abstract. *The nucleotide sequence of a transforming human* c-sis *complementary DNA shows an open reading frame 723 base pairs in length located downstream from an in-phase terminator thymine-guanine-adenine codon. Sequences within this region were identical to those previously determined for the exons of the normal human* c-sis *gene. Thus, the predicted transforming product, a protein of 27,281 daltons, may be the actual precursor for normal human platelet-derived growth factor chain A.*

The simian sarcoma virus (SSV) is a transforming, replication-defective retrovirus isolated from a fibrosarcoma of a pet woolly monkey (*1*). SSV has acquired a 1006-base-pair (bp) sequence called v-*sis*, which accounts for the transforming potential of the virus (*2*). This sequence probably originated from woolly monkey cellular DNA, but is conserved among vertebrates (*3, 4*). A similar sequence has been transduced by the Parodi-Irgens feline sarcoma virus (*5*).

The transforming gene product of v-*sis*

is a 28-kilodalton protein, $p28^{sis}$, which is probably encoded as a viral envelope–*sis* fusion product (*6*). Amino acid sequences of platelet-derived growth factor (PDGF) were found to be very similar to sequences of the v-*sis* predicted product (*7, 8*). This suggested that human c-*sis* or a related gene codes for one of the two cysteine-bonded chains of PDGF. Nucleotide sequence analysis of the six v-*sis* homologous regions within the normal human genome showed that PDGF chain A is coded by c-*sis* (*9, 10*).

In human serum, PDGF is the major mitogenic protein for mesenchymal cells (*11*), but PDGF does not cause cellular transformation when applied externally to cells, even in large amounts (*12*). Lysates of SSV-transformed cells possess mitogenic activity and immunological cross-reactivity for antibodies to PDGF (*13, 14*).

In surveys of normal and neoplastic cells, expression of the 4.2-kilobase c-*sis* messenger RNA (mRNA) has been found only in a majority of sarcoma and glioblastoma cell lines (*15*) and in the mature T-cell lymphocytic cell line HUT 102, which is productively infected with the human T-cell lymphotropic virus type I (HTLV-I) (*16*). It is interesting that *sis* expression correlates for the most part with mesenchymally derived tumors, that is, sarcomas and glioblastomas, whose normal cell counterparts are mitogenically stimulated by PDGF. This is consistent with an autostimulation model for transformation. However, lymphocytes show no mitogenic response to PDGF (*12*). Thus, HUT 102 cells do not directly fit this model. Whether *sis* expression is a contributing factor or a consequence of transformation of these cells is not known.

We previously reported cloning of a partial human c-*sis* complementary DNA (cDNA) denoted pSM-1 which transformed NIH 3T3 cells (*17*). In the study described here we determined the 5′ 1320 nucleotide sequences of the pSM-1 c-*sis* insert for comparison to sequences of the normal c-*sis* exons to better understand the structural basis of the transforming capacity of this clone (Figs. 1 and 2). Some restriction enzyme sites and the splice sites which define the exon borders in c-*sis* are shown in Fig. 2, in which areas of homology to PDGF previously reported in the predicted protein sequence are underlined (*10*).

The v-*sis* homologous region begins at position 182. The sequences upstream of the v-*sis* homologous region demonstrate the presence of at least one additional exon upstream from c-*sis* exon 1 (*18*). A potential initiator ATG codon at position

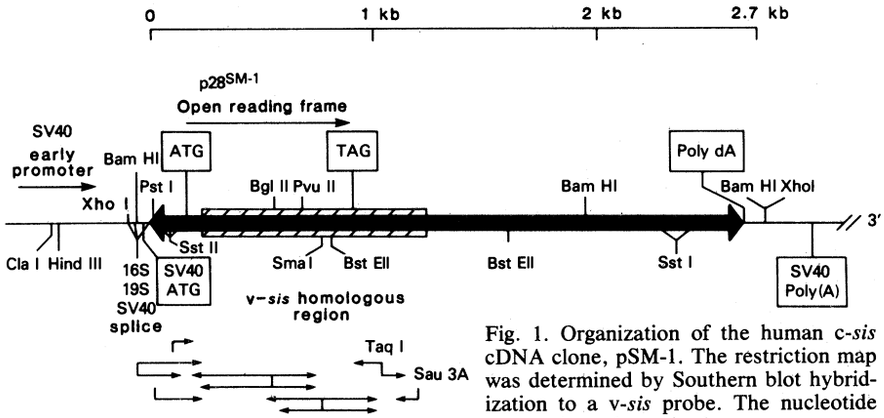


Fig. 1. Organization of the human c-*sis* cDNA clone, pSM-1. The restriction map was determined by Southern blot hybridization to a v-*sis* probe. The nucleotide sequencing scheme is shown.

118 is in phase with the previously reported open reading frame in the v-*sis* homologous region (*10*). The open reading frame extends 723 bp and terminates at the same position as in v-*sis* and the normal c-*sis* gene (*10*). The unmodified protein product designated as $p28^{SM-1}$ in Fig. 1 is predicted to be 27,281 daltons.

The predicted protein product of c-*sis* is strikingly hydrophilic (Fig. 3A). However, there is a hydrophobic region spanning the 15 amino acid residues at the NH_2-terminus; this is analogous to the signal peptide that is found for many precursor proteins and mediates translocation into the lumen of the rough endoplasmic reticulum for glycosylation and further processing (*19*). For comparison, the profile of the v-*sis* predicted product (*6, 10*) shows a similar hydrophobic region near the NH_2-terminus between amino acid residues 19 and 30 (Fig. 3B). This is encoded just downstream from the second of three possible initiator methionine codons (M) for $p28^{sis}$ located within the helper-viral envelope sequences. The presence of a hydrophobic signal peptide may be necessary for the translocation or processing of these gene products to initiate transformation since our cloned genomic c-*sis* sequences lack this signal and are not transforming even when cotransfected with Moloney murine leukemia virus long terminal repeat (LTR) sequences (*20*). There is also a significant hydrophilic to hydrophobic transition in $p28^{SM-1}$ (also conserved in $p28^{sis}$) at the arginine-arginine (R-R) amino acid residues 80–81 (nucleotides 355–360). This dibasic amino acid sequence is a potential signal of proteolytic processing (*21*). The sequence of PDGF peptide I characterized by Waterfield *et al.* (*8*) and peptide 2a determined by Antoniades and Hunkapiller (*22*) both begin at amino acid position 82, consistent with processing at this site in vivo.

A single potential *N*-glycosylation site (Asn Met Thr) is found at amino acid position 63. This site is conserved in v-*sis* as well. However, this region is presumably cleaved from processed PDGF. Therefore, any carbohydrate modification of this chain of the mature growth factor molecule probably occurs at *O*-glycosylation sites which remain to be determined.

The sequence within the v-*sis* homologous region confirms the intron-exon arrangements of c-*sis* as deduced from the v-*sis* sequence with one exception. Nucleotides 872 to 1020 in pSM-1 are not found in v-*sis* but are present in the normal human c-*sis* gene, suggesting either alternate (species-specific) mRNA splicing at the two ends of this sequence or deletion of this sequence in the woolly monkey DNA. The arrangement of exons 5 and 6 in the 1751 bp Bam HI digestion fragment subcloned from the normal c-*sis* gene is shown in Fig. 4 (*23*). Exon 5 includes 122 bp of translated sequences and 31 bp of untranslated sequences. An acceptor splice site (5′CGCTTGGCCCTGCAG 3′) is found at the Pst I site at the 5′ border of human exon 6 (solid line) (position 872 in Fig. 2). The dashed line indicates an acceptor splice site which may be conserved and utilized in the woolly monkey DNA (WM splice at position 1020 in Fig. 2). Alternatively, a deletion event is possible, since there is a partial direct repeat 5′CAG(CG)GGT 3′ at the borders of the deletion. However, the high statistical improbability of finding splice site consensus sequences at these sites suggests that the first possibility is more likely.

The 3′ terminus of v-*sis* arose by recombination within the woolly monkey sequences analogous to human exon 6 (*10*). Hybridization and restriction enzyme mapping data suggest that exon 6 probably includes the remaining sequences in the cDNA insert downstream from the v-*sis* homologous region (*24*).

The nucleotide sequence of pSM-1 and

the exons of the normal human c-*sis* gene are identical as far as we have determined; that is, from the beginning of the v-*sis* region of homology (*10*). This result and the transforming capability of pSM-1 indicate that normal human c-*sis* sequences and, possibly, normal woolly monkey sequences (within v-*sis*) can participate in the process of transformation, that is, mutations within the v-*sis* homologous region are not required for transforming potential. These results

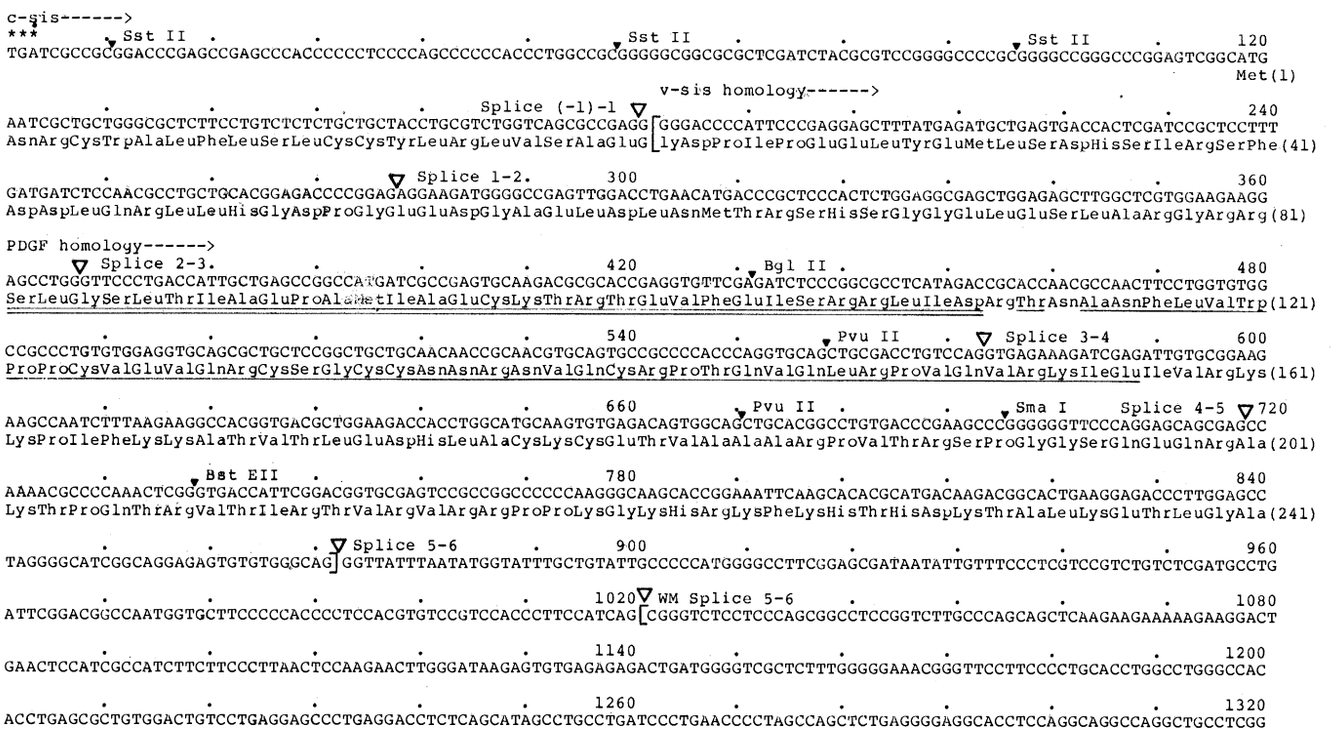
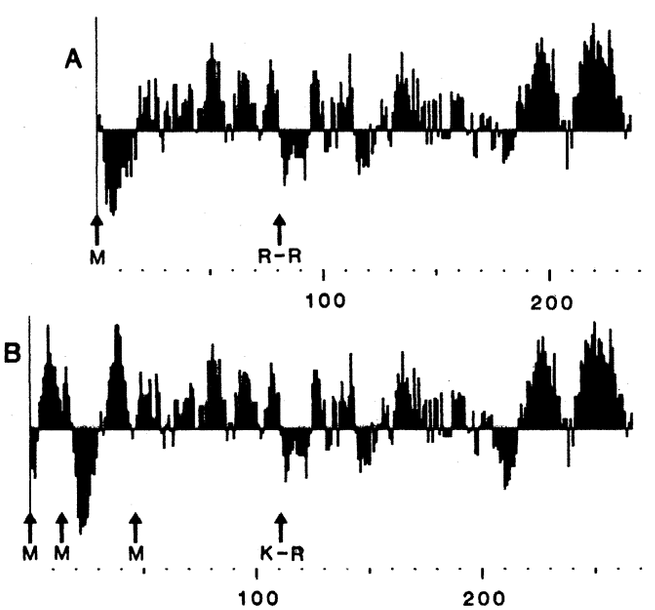


Fig. 2. The nucleotide sequence and predicted amino acid sequence of the 5′ portion of the c-*sis* cDNA clone, pSM-1, which includes the entire open reading frame within the v-*sis* homologous region. DNA fragments were end-labeled at the 5′ termini with polynucleotide kinase (*26*) and at the 3′ terminus with Klenow fragment of DNA polymerase I and α^{32}PdXTP (*27*) or with terminal transferase (*28*). Nucleotide sequencing was performed as described by Maxam and Gilbert (*26*). The brackets encase the regions of homology to v-*sis*. The doubly underlined amino acid sequence is identical to that of PDGF peptide I and the singly underlined amino acid sequences are identical to those determined for peptides III and IV (*8, 10*). The terminator codon in phase with the open reading frame of pSM-1 is marked by asterisks. Restriction sites are indicated by solid triangles and the splice sites by open triangles. Abbreviations for the nucleic acid residues are: A, adenine; C, cytosine; G, guanine; and T, thymine.



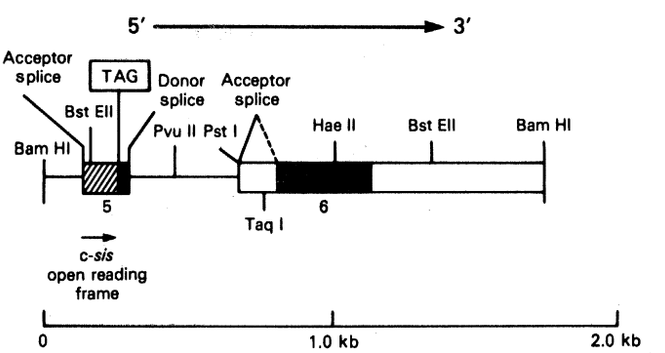


Fig. 3 (left). Hydrophilicity profile of (A) the predicted protein product of the c-*sis* cDNA clone pSM-1 and (B) of the v-*sis* product (*6, 10*). The hydrophilicity indices were determined by the methods of Kyte and Doolittle (*29*) and Hopp and Woods (*30*), which gave similar results. The profiles determined by the method of Kyte and Doolittle are shown. Deviations above the abscissae represent hydrophilic scores and below the abscissae represent hydrophobic scores. Amino acid positions are indicated beneath the graphs. Arrows show the positions of possible initiator methionine residues (M) and processing points (RR or KR) as described in the text. Fig. 4 (above right). Arrangement of c-*sis* exons 5 and 6 in clone L335 which contains a 1751-bp genomic Bam HI digestion fragment (*22*). A restriction enzyme map of the L335 insert is shown together with the 3′ terminus of the open reading frame of the v-*sis* homologous region (cross-hatched box). The solid boxes represent 3′ untranslated portions of the v-*sis* homologous region and the open boxes show regions in exon 6 which are not homologous to v-*sis*. The donor and acceptor splice sites for the human c-*sis* gene are shown by the solid lines, and the dashed line shows the possible woolly monkey acceptor splice site as deduced from comparison to the v-*sis* sequence.

also indicate a fusion of viral envelope sequences to *sis* sequences is not a sole criterion for transformation by *sis* genes.

Although pSM-1 represents an incomplete cDNA transcript, it is likely that the sequence shown here encodes the entire precursor for PDGF chain A. This is suggested by the presence of an upstream TGA stop codon at position 1 which is in phase with the open reading frame of pSM-1. Thus, the ATG at position 118 is probably the actual initiator codon for the c-*sis* protein product. Sequences of a second c-*sis* cDNA clone, pSM-2, showed that it contained 11 base pairs 5′ to the sequences of pSM-1 with the TGA stop codon at the same position as in pSM-1 (*25*). Therefore, the TGA codon was not generated as a cloning artifact. The stop codon of pSM-1 is also in phase with the SV40 initiator between the 16*S* donor and acceptor splice sites, so that no read-through SV40-*sis* product is expected.

The ability of pSM-1 to transform 3T3 cells after transfection in contrast to the inability of PDGF to transform cells when applied externally is probably due to the intracellular overproduction of PDGF chain A as a consequence of transcriptional activation by the SV40 promoter. This overproduction could result in (i) differences in the posttranslational processing transport or compartmentalization of the c-*sis* gene product, (ii) differences in the subunit structure of the pSM-1 product and PDGF, that is, a homodimer instead of a heterodimer as in PDGF, or (iii) qualitative or quantitative differences in the interaction of PDGF chain A with cellular receptors.

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Independent Developmental Programs for Two Estrogen-Regulated Genes

Abstract. *Measurement of hepatic apolipoprotein II and vitellogenin II messenger RNA during chicken embryogenesis showed that these genes acquire estrogen responsiveness at different stages of development. Sensitive solution hybridization assays with excess complementary DNA showed that apolipoprotein II transcripts were induced to 500 molecules per cell at day 9, whereas induction of vitellogenin II messenger RNA was not found until day 11. Thus, two estrogen regulated genes of common function and coordinately regulated in the adult may be on independent developmental programs.*

Embryonic chick liver is a useful system to study the acquisition of responsiveness to the steroid hormone estradiol. As in the mature hen or hormone-treated rooster, estradiol induces the hepatic synthesis of egg yolk precursor proteins in the competent chick embryo. Proteins induced by estradiol include the very low density apolipoproteins B (apo-B) and II (apo-II) and the highly phosphorylated vitellogenins (VTG) (*1*). Synthesis of apo-II is inducible by estradiol

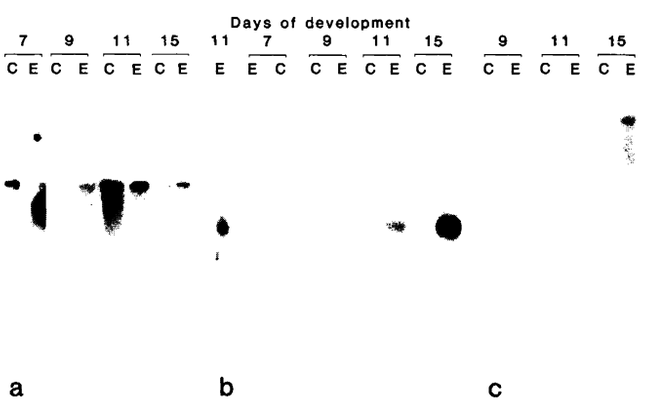

Fig. 1. Ontogeny of inducibility of apo-II and VTG-II mRNA's as assayed by Northern hybridization. For the indicated days of development, 5 μg of poly(A)$^+$-enriched RNA from the livers of estrogen-treated (E) or control (C) chick embryos were subjected to electrophoresis through 1.5 percent agarose gels containing 5 m*M* methylmercuric hydroxide. The RNA was transferred to nitrocellulose, and the blots were hybridized overnight with nick-translated recombinant plasmids (0.5×10^6 to 1.0×10^6 count/min) containing sequences complementary to chicken serum albumin mRNA (a), apo-II mRNA (b), or VTG-II mRNA (c). In each case the extent of migration corresponded to that expected for each mRNA in comparison to ribosomal RNA markers (*6*). Estrogen treatment consisted of injection of 1.25 mg of estradiol-17β in 50 μl of propylene glycol just beneath the air sac membrane 48 hours before the embryos were killed. Controls were given the vehicle alone.