difference in the length or position of the poly(A) tail. Immunodeterminants of ACTH, endorphin, and γ-MSH have been detected in Leydig cells (*2, 16*). These determinants are derived from the midportion, carboxyl-terminal, and amino-terminal POMC domains, respectively. Hence the most likely potential splice sites within the coding region would be large nucleotide regions on either side of the γ-MSH determinant in the amino-terminal region. A deletion in the 5′ noncoding region seems unlikely since this region is small and is required for ribosomal binding. Experiments on the shortened POMC-like mRNA in testis might suggest the nature of the shortened POMC-like mRNA observed in specific regions of the brain. Finally, the relatively high concentration of POMC mRNA in testis should allow the role of potential physiological regulators of testis POMC mRNA to be assessed.

JOHN E. PINTAR*
Departments of Medicine and
Anatomy, Mount Sinai School of Medicine, New York 10029
BETH S. SCHACHTER
Department of Obstetrics, Gynecology, and Reproductive Science, Mount Sinai School of Medicine
ANDREA B. HERMAN
Department of Medicine, Mount Sinai School of Medicine
SALLY DURGERIAN
Department of Obstetrics, Gynecology, and Reproductive Science, Mount Sinai School of Medicine
DOROTHY T. KRIEGER
Department of Medicine, Mount Sinai School of Medicine

References and Notes

1. G. E. Feurle, U. Weber, V. Helmstaedter, *Biochem. Biophys. Res. Commun.* **95**, 1656 (1980); D. T. Krieger, A. Liotta, M. J. Brownstein, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 648 (1977); L.-I. Larsson, *ibid.* **78**, 2990 (1981); A. S. Liotta, D. Gildersleeve, M. J. Brownstein, D. T. Krieger, *ibid.* **76**, 1448 (1979); A. S. Liotta and D. T. Krieger, *Endocrinology* **106**, 1504 (1980); A. S. Liotta, C. Loudes, J. F. McKelvy, D. T. Krieger, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1880 (1980); E. S. Orwoll and J. W. Kendal, *Endocrinology* **107**, 438 (1980).
2. B. A. Sharp and A. E. Pekary, *J. Clin. Endocrinol. Metab.* **52**, 586 (1981); ———, N. V. Meyer, J. M. Hershman, *Biochem. Biophys. Res. Commun.* **95**, 618 (1980); S. D. Tsong *et al.*, *Endocrinology* **110**, 2204 (1982).
3. A. N. Margioris, A. S. Liotta, H. Vaudry, C. W. Bardin, D. T. Krieger, *Endocrinology* **113**, 663 (1983).
4. A. S. Liotta, H. Yamaguchi, D. T. Krieger, *J. Neurosci.* **1**, 585 (1981); S. Zakarian and D. Smyth, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5972 (1979).
5. C. J. Evans, R. Lorenz, E. Weber, J. D. Barchas, *Biochem. Biophys. Res. Commun.* **106**, 910 (1982); T. L. O'Donohue *et al.*, *Peptides* **2**, 93 (1981); E. Weber, C. J. Evans, J. D. Barchas, *Biochem. Biophys. Res. Commun.* **103**, 982 (1981); S. Zakarian and D. G. Smyth, *Nature (London)* **296**, 250 (1982).
6. O. Civelli, N. Birnberg, E. Herbert, *J. Biol. Chem.* **257**, 6783 (1982); M. J. Q. Evinger, J. L. Roberts, C. L. C. Chen, B. S. Schachter, in *Gene Expression in Brain*, C. Zomzely-Neurath and W. A. Walker, Eds. (Wiley, New York, in press).
7. C. E. Gee, C. L. C. Chen, J. L. Roberts, R. Thompson, S. J. Watson, *Nature (London)* **306**, 374 (1983).
8. J. M. Chirgwin, A. E. Przbyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979).
9. H. Aviv and P. Leder, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1408 (1975).
10. E. Potter, A. K. Nicolaisen, E. S. Ong, R. M. Evans, M. G. Rosenfeld, *ibid.* **78**, 6662 (1981).
11. P. S. Thomas, *ibid.* **77**, 5201 (1980).
12. C. L. C. Chen, F. T. Dionne, J. L. Roberts, *ibid.* **80**, 2211 (1982).
13. M. Brahic and A. T. Haase, *ibid.* **76**, 6125 (1979); C. Gee and J. L. Roberts, *DNA* **2**, 157 (1983).
14. E. Gezang-Ginsberg and D. Wolgemuth, personal communication.
15. J. Eberwine and J. L. Roberts, *DNA* **2**, 1 (1983).
16. C. Shaha, A. S. Liotta, D. T. Krieger, C. W. Bardin, *Endocrinology*, in press.
17. P. W. J. Rigby, M. Dieckman, C. Rhodes, P. J. Berg, *J. Mol. Biol.* **113**, 237 (1977).
18. We thank G. A. Stover for photographic assistance; J. Roberts, C. Gee, and C. Wilcox for discussions of in situ hybridization techniques; B. Shivers, R. Harlan, and D. Pfaff for providing adult rat brain tissue; and C. L. C. Chen and J. L. Roberts for the POMC cDNA insert I-13. Supported by HD-18592 (J.P.), HD-18110 (B.S.S.), NB-02893-15 (D.T.K.), a Hirschl career scientist award (J.P.), and the Lita Annenberg Hazen Charitable Trust.
* To whom correspondence should be addressed at the Department of Anatomy and Cell Biology, Columbia College of Physicians and Surgeons, New York 10032.

30 January 1984; accepted 30 April 1984

Cellular Transformation by Human Papillomavirus DNA in Vitro

Abstract. *Molecularly cloned DNA's of human papillomaviruses HPV-5 and HPV-1 induced morphological transformation of mouse C127 cells in culture. Single-cell clones of cells transformed by papillomavirus contained multiple persistent episomal copies of the transfected DNA species and were analyzed for growth characteristics indicating malignant potential.*

Papillomaviruses have been identified as the etiological agents of many types of benign warts in both humans and numerous animal species (*1, 2*). Furthermore, some of these viruses have been associated with malignant lesions in their natural host, particularly bovine papillomavirus (BPV) (*2, 3*), cottontail rabbit papillomavirus (CRPV) (*4*), *Mastomys natalensis* papillomavirus (*5*), and human papillomaviruses (HPV). Recent reports have shown HPV-5 DNA in primary and metastatic squamous cell carcinomas of patients with epidermodysplasia verruciformis (*6*) and HPV DNA of several types in genital neoplasias (*7*). Papillomaviruses have not been successfully propagated in culture; however, recent results have shown that papillomavirus genomes can persist in and transform cultured cells (*8–12*). The genome of BPV has been shown to induce morphological transformation of mouse C127 and NIH 3T3 cells (*11*), to replicate

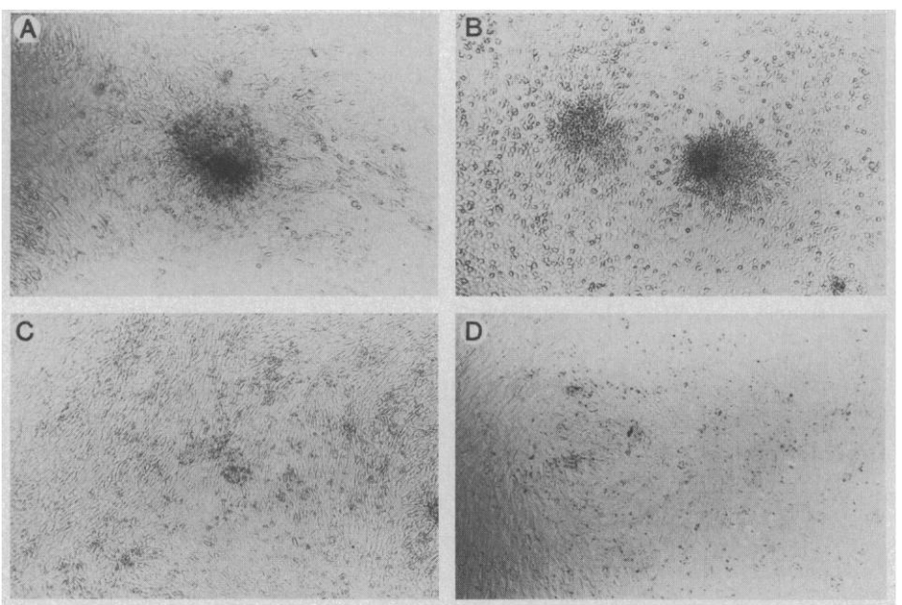

Fig. 1. Transformation of mouse C127 cells with HPV DNA. (A) Focus formation after transfection with pBR322HPV-5 DNA. (B) Focus formation after transfection with pBR322HPV-1 DNA. (C) Transformed clone C112-3A-10-2 isolated from a focus induced by transfection with pBR322HPV-5 DNA. (D) Transformed clone C112-4A-1-3 isolated from a focus induced by transfection with pBR322HPV-1 DNA.

episomally in these transformed cells (*10*), and to be transcribed into virus-specific messenger RNA species comparable to those found in cattle warts (*13*). Cultured human keratinocytes infected with HPV-1 from plantar warts have been shown to replicate HPV-1 DNA as an episome (*8*). However, the successful morphological transformation of cell cultures with human papillomaviruses has not been demonstrated previously.

We have shown that C127 and NIH 3T3 cells may be morphologically transformed by infection with CRPV or by transfection with CRPV DNA molecularly cloned in plasmid pBR322 (*12*). With the same transfection protocol we obtained foci of C127 cells transformed by cloned HPV-1 and HPV-5 DNA's (Fig. 1, A and B). Foci of transformation, which became visible after 2 weeks of incubation on monolayers treated with cloned DNA's of CRPV, HPV-1, HPV-5, and BPV-1 precipitated with calcium phosphate, were indistinguishable with respect to the species or type of transfecting DNA. Intact recombinant pBR322HPV plasmids or HPV DNA's excised from pBR322 by restriction endonuclease cleavage with the insertion-site enzyme were able to induce transformed foci at efficiencies of 100 to 400 foci per microgram of transfecting viral DNA over the range of 0.2 to 2 μg of viral DNA per assay. In contrast to some earlier reports (*11, 14*), we observed only a slight decrease in transformation efficiency with intact HPV recombinant–pBR322 DNA containing the "poison" sequence (*15*) relative to the efficiency obtained with excised HPV DNA or pMLHPV-5 DNA with the poison sequence deleted (Table 1).

Table 1. Efficiency of transformation with HPV DNA's. Low passage C127 cells originally derived from an RIII mouse mammary tumor (*17*) were transfected as described (*12*) with approximately 1 μg of cloned vector PV DNA amplified in *Escherichia coli* strain HB101 and precipitated by calcium phosphate. Foci of morphological transformation visible to the unaided eye appeared about 2 weeks after transfection. NI, not included.

Cloned DNA	Cloning site	C127 cells transformed (foci per microgram of transfecting viral DNA)		
		Experiment		
		1	2	3
pBR322HPV-1	Bam HI	120	138	132
pBR322HPV-5	Bam HI	188	152	134
pMLHPV-5	Bam HI	NI	NI	248
pBR322CRPV	Eco RI	163	162	128
pBR322BPV-1	Hind III	137	125	80
pBR322		0	0	0

Single-cell clones of C127 cells transformed by different cloned HPV DNA's were obtained by end-point dilution of focus-forming cells (Fig. 1, C and D). Total cellular DNA was isolated from these clones and analyzed for virus-specific DNA by Southern blot hybridization. DNA from two clones of C127 cells transfected with pBR322HPV-1 DNA contained multiple copies of episomal virus-specific DNA that migrated along with plasmid DNA and were identified as pBR322HPV-1 DNA by restriction endonuclease cleavage (Fig. 2A). Cellular DNA's cleaved with Bam HI, the cloning site enzyme, showed 2.6-megadalton pBR322 DNA bands and 5.0-megadalton HPV-1 DNA bands by Southern blot hybridization. Multiple clones of C127 cells transfected with pBR322HPV-5 DNA showed virus-specific DNA of forms 1 and 2 migrating with plasmid DNA (Fig. 2B). Characterization of these clones by restriction endonuclease digestion identified the virus-specific episomal DNA as unrearranged pBR322HPV-5. Focus C112-3A-8 was propagated from a few focus-forming cells, and three subclones (8-1, 8-3, and 8-5) were isolated from it approximately 5 months after they were transfected. All these subclones showed equivalent amounts of persistent episomal virus-specific DNA. Thus, single-cell clones of C127 cells transformed by pBR322HPV-1 or pBR322HPV-5 DNA's contained multiple episomes of the transfecting

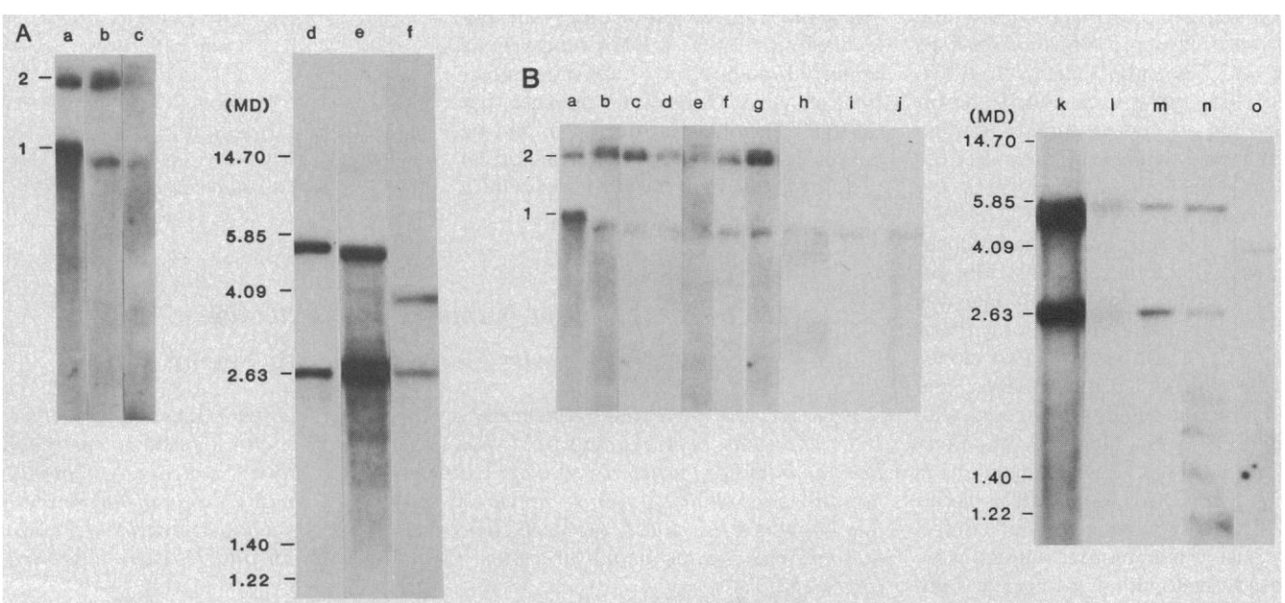

Fig. 2. Viral DNA in HPV-transfected cells. (A) Total cellular DNA from single-cell clones of C127 cells transformed by transfection with pBR322HPV-1 DNA was subjected to agarose gel electrophoresis and Southern blot hybridization to nick-translated [^{32}P]-labeled pBR322HPV-1 DNA. (Lane a) pBR322HPV-1 plasmid DNA. (Lanes b to f) Cellular DNA from clones C112-4A-1-3 (lanes b and d) and C112-4A-2-2 (lanes c, e, and f). DNA's in lanes d and e were digested with Bam HI, those in lane f with Hind III. (B) Southern blots of total cellular DNA's from pBR322HPV-5–transfected C127 cell clones were hybridized to nick-translated [^{32}P]-labeled pBR322HPV-5 DNA. (Lane a) pBR322HPV-5 plasmid DNA; (lane b) focus C112-3A-8 DNA; (lane c) clone C112-3A-8-5 DNA; (lane d) clone C112-3A-8-1 DNA; (lanes e, k, and o) clone C112-3A-8-3 DNA; (lane f) clone C112-3A-10-2 DNA; (lanes g and m) clone C112-3A-1-1 DNA; (lanes h and n) clone C622-3A-4-1 DNA; (lane i) clone C622-3A-5-4 DNA; and (lanes j and l) clone C622-3A-5-12 DNA. DNA's in lanes k through n were digested with Bam HI and in lane o with Eco RI. Molecular weight markers of Hind III–digested phage λ DNA are indicated. MD, megadalton.

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.

SUSAN L. WATTS
WILLIAM C. PHELPS
RONALD S. OSTROW
KAREN R. ZACHOW
ANTHONY J. FARAS

Department of Microbiology, University of Minnesota, Minneapolis 55455

References and Notes

1. P. H. Howley, *Arch. Pathol. Lab. Med.* **106**, 429 (1982).
2. W. D. Lancaster and C. Olson, *Microbiol. Rev.* **46**, 191 (1982).
3. W. F. H. Jarrett *et al.*, *Cold Spring Harbor Conf. Cell Prolif.* **7**, 215 (1980).
4. P. Rous and J. W. Beard, *J. Exp. Med.* **62**, 523 (1935).
5. H. Muller and L. Gissmann, *J. Gen. Virol.* **41**, 315 (1978).
6. R. S. Ostrow *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1634 (1982); D. Kremsdorf, S. Jablonska, M. Favre, G. Orth, *J. Virol.* **43**, 436 (1982).
7. M. Green *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4437 (1982); K. R. Zachow *et al.*, *Nature (London)* **300**, 771 (1982); L. Gissmann *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 560 (1983); M. Durst, L. Gissmann, H. Ikenberg, H. zur Hausen, *ibid.*, p. 3812.
8. R. F. LaPorta and L. B. Taichman, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3393 (1982).
9. M. H. Moar, M. S. Campo, H. Laird, W. F. H. Jarrett, *Nature (London)* **293**, 749 (1981).
10. M-F. Law, D. R. Lowy, I. Dvoretzky, P. M. Howley, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2727 (1981).
11. D. R. Lowy *et al.*, *Nature (London)* **287**, 72 (1980).
12. S. L. Watts, R. S. Ostrow, W. C. Phelps, J. T. Prince, A. J. Faras, *Virology* **125**, 127 (1983).
13. C. A. Heilman, L. Engel, D. R. Lowy, P. M. Howley, *ibid.* **119**, 22 (1982).
14. N. Sarver, J. C. Byrne, P. M. Howley, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7447 (1982).
15. M. Lusky and M. Botchan, *Nature (London)* **293**, 79 (1981).
16. O. Danos, M. Katinka, M. Yaniv, *EMBO J.* **1**, 231 (1982); E. Y. Chen, P. M. Howley, A. D. Levinson, P. H. Seeburg, *Nature (London)* **299**, 529 (1982).
17. I. Dvoretzky, R. Shober, S. K. Chattopadhyay, D. R. Lowy, *Virology* **103**, 369 (1980).
18. Supported by NIH grant CA 25462 and National Research Service award CA 06653 (S.L.W.).

3 February 1984; accepted 8 June 1984

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*