Rat Embryo Fibroblast Cells Expressing Human Papillomavirus 1a Genes Exhibit Altered Growth Properties and Tumorigenicity

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Human papillomavirus 1a (HPV1a) induces benign tumors (papillomas or warts) in humans under natural conditions of infection but has not been found to replicate significantly in cell culture or in experimental animals. To establish model systems to study the oncogenic properties and expression of HPV genes, we established cell lines by cotransfecting the 3Y1 rat fibroblast cell line with HPV1a DNA constructs containing an intact early gene region and the Tn5 neomycin resistance gene. Most cell lines selected for expression of the neomycin resistance gene by treatment with the antibiotic G-418 contained viral DNA in a high-molecular-weight form. The growth characteristics of several cell lines containing high copy numbers of HPV1a DNA were studied further. They were shown to differ from the parental cell line and from G-418-resistant cell lines that did not incorporate viral DNA in the following properties: (i) morphological alteration, (ii) increased cell density at confluence, (iii) growth in 0.5% serum, (iv) efficient anchorage-independent growth in soft agar, and (v) rapid formation of tumors in nude mice. Those cell lines that possessed altered growth properties and tumorigenicity were found to express abundant quantities of polyadenylated virus-specific RNA species in the cytoplasm.

The human papillomaviruses (HPVs) are scientifically interesting and medically important (for reviews, see references 8 and 13). There are a minimum of 42 different HPVs, most of them discovered within the past few years (tabulated by T. Broker and L. Chow, National Cancer Institute Workshop on Transformation Mechanisms of Papillomaviruses, February, 1986). HPVs are different from conventional DNA tumor viruses. First, they are natural causes of benign tumors and recent studies strongly implicate a role for HPVs in the etiology of human urogenital cancers (1, 4, 7, 9, 23). Second, expression of HPV genes appears to be regulated by the differentiated state of the host cell. Third, HPV DNA replicates as an episome in benign tumors, a strategy that differs from that of most well-studied DNA tumor viruses (8).

Our understanding of HPV gene functions and oncogenicity is poorly developed, mainly because cell culture systems for analyzing cell transformation and viral gene expression have not been available. In fact, it has not been established whether HPVs encode transforming genes. Regarding cell transformation, several groups have tried with limited success to isolate HPV-transformed cells by standard procedures, e.g., focus formation in cultured cells (see Discussion). We used an alternate procedure which has been used with other systems to determine whether expression of HPV1a genes can transform cells. HPV1a DNA constructs containing an intact early gene region were cotransfected with the Tn5 neomycin resistance gene into the 3Y1 rat embryo fibroblast cell line, a cell line that has proven useful for transformation by other DNA tumor viruses (10), and G-418-resistant cell lines were established (17). In this study, we showed that clonal cell lines containing high copy numbers of HPV1a DNA and expressing high levels of polyadenylated viral RNA in the cytoplasm were modified in several growth properties, exhibited efficient anchorage-independent growth, and formed tumors in nude mice. These cell lines and this approach may be useful for mapping viral

mRNA molecules and for identifying putative viral transforming genes.

MATERIALS AND METHODS

Construction of HPV1a plasmids. The HPV1-pBR322 recombinant plasmid (clone 1-40) (2), consisting of *Bam*HI-linearized HPV1a DNA (see Fig. 1A) cloned into the *Bam*HI site of pBR322, was recloned to generate two constructs, pHPV1a(17-4) and pHPV1a(13-29) (see Fig. 1B), as follows. Clone 1-40 was digested partially with *Hpa*I to yield a mixture of two linear molecules cleaved at nucleotide (nt) 1 or 6575, upstream of the early gene region. *Sal*I linkers were added, the linearized structures were recircularized with DNA ligase, and the two clones with a *Sal*I site at nt 1 or 6575 were isolated by transformation of *Escherichia coli* HB101. HPV1a DNA was excised from each clone with *Bam*HI, recircularized, cleaved with *Sal*I, and cloned into the *Sal*I site of pBR322 to yield pHPV1a(17-4) and pHPV1a(13-29) (see Fig. 1B). pHPV1a(17-4) contains the viral genome with *Sal*I linkers inserted at the *Hpa*I site at nt 1 and cloned into the *Sal*I site of pBR322. pHPV1a(13-29) contains the viral genome with *Sal*I linkers inserted at the second *Hpa*I site at nt 6575 and cloned into the *Sal*I site of pBR322. pHPV1a(17-4E) (E denotes the early region) and pHPV1a(13-29E) were constructed by deletion of a portion of the late region (nt 4529 at the *Eco*RV site in HPV1a DNA to the *Eco*RV site in pBR322) from pHPV1a(17-4) and pHPV1a(13-29), respectively, followed by addition of *Sal*I linkers to the *Eco*RV site (see Fig. 1B).

Cotransfection of the 3Y1 rat fibroblast cell line with HPV1a plasmids and pNEO5 and selection of G418-resistant clones. Rat 3Y1 cells (10) at passage 8 were grown in Dulbecco modified Eagle medium (DME) supplemented with 10% fetal bovine serum. The HPV1a inserts in each of the four HPV1a constructs described above were excised by digestion with *Sal*I followed by heat inactivation of the enzyme. We formed DNA-calcium phosphate precipitates by slowly adding 500 μ l of 2 $\times$ HBS (280 mM NaCl, 15 mM sodium phosphate, 42 mM HEPES[N-2-hydroxyethyl]piperazine-N'-2-ethanesul-

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fonic acid)] with aeration to 500 μ l of 250 mM CaCl_2 containing 25- μ g amounts of linearized HPV1a plasmid and 2 μ g of plasmid pNEO5 (11). After 20 to 30 min at room temperature, each suspension was added directly to the medium of a 100-mm (diameter) dish of 3Y1 cells at 60 to 80% confluence. After overnight incubation, the precipitates were removed and the cells were refed. Cells were trypsinized 48 h after transfection and expanded onto 100-mm dishes at a split ratio of 1 to 20. G-418 (GIBCO; 400 μ g/ml) was added 24 h later, and G-418-resistant colonies were picked starting at about 3 weeks (17).

Growth of cells in monolayer culture, anchorage-independent growth, and tumorigenicity in nude mice. The growth rate of control and transfected cell lines was determined in DME containing either 10 or 0.5% fetal bovine serum in 25-cm² flasks. We determined anchorage-independent growth by seeding 5,000 cells from each line in 4 ml of soft agar overlay (0.3% agarose in DME-5% fetal bovine serum maintained at 45 to 50°C) onto hardened 10-ml layers of 0.5% agarose in DME-5% fetal bovine serum contained in 25-cm² flasks. Flasks were incubated in a CO₂ incubator at 37°C, and colonies were scored 2 to 3 weeks later. Tumorigenicity was determined by subcutaneous injection into BALB/c athymic nude mice of 0.4-ml amounts of trypsinized, well-washed suspensions of 10⁶ cells in phosphate-buffered saline. Animals were examined twice weekly for the appearance of tumors. Representative tumors derived from each clonal cell type were excised at 14 days, and tumor cell lines were established in culture by digestion of minced tumors with 0.025% collagenase-0.25% dispase in phosphate-buffered saline.

DNA and RNA blot hybridization. Cells were harvested from monolayer cultures by scraping and separated into nuclear and cytoplasmic fractions as previously described (2, 6). High-molecular-weight DNA was isolated from washed nuclei by digestion with proteinase K plus RNase followed by phenol-chloroform extraction and ethanol precipitation. DNA blot hybridization was performed, as previously described (2), with HPV1a DNA excised from plasmid as the probe, double gel purified, and labeled by nick translation to 2×10^8 cpm/ μ g with a nick translation kit (Bethesda Research Laboratories, Inc.). RNA blot hybridization was performed, as previously described (2), with 1.4% formaldehyde-agarose gels and a nick-translated HPV1a DNA probe or a riboprobe prepared as follows. An HPV1a DNA sequence representing mostly the E7 open reading frame (ORF) (nts 595 to 967) was cloned into the polylinker of pGEM-3 (Promega Biotec). RNA antimesage (about 6×10^8 cpm/ μ g) was synthesized with *Hind*III-linearized plasmid and T7 RNA polymerase by the protocol provided by Promega Biotec.

RESULTS

Construction of HPV1a plasmids containing intact early gene regions. In previous studies from our laboratory investigating expression of the HPV1a genome, we introduced an intact HPV1a-pBR322 construct into mouse cells by cotransformation with the herpesvirus thymidine kinase gene (2). Viral DNA was incorporated in a complex integration pattern and appeared to be expressed as hybrid HPV1a-pBR322 RNA molecules, presumably because of the interruption of the viral early region at the single *Bam*HI site by pBR322 DNA (2) (see Fig. 1A for a map of the HPV1a genome). To prepare HPV1a vectors more suitable for gene transfer, we recloned the HPV1a genome to provide four different

FIG. 1. (A) Structure and organization of the HPV1a genome. (B) Structure of pHPV1a(17-4), pHPV1a(13-29), pHPV1a(17-4E), and pHPV1a(13-29E) constructs. See Materials and Methods for details of construction.

HPV1a DNA plasmids (Fig. 1B) (see Materials and Methods for details on constructions). These four constructs, pHPV1a(17-4), pHPV1a(13-29), pHPV1a(17-4E), and pHPV1a(13-29E), possess an intact early region and different

amounts of 5' upstream sequences. The putative early promoter (TATA box) is located at nt 68, and the putative poly(A) site is at nt 3984 (Fig. 1A).

Cotransfection of the rat 3Y1 fibroblast cell line with HPV1a constructs and pNEO5, selection of clonal cell lines, and identification of lines that have incorporated viral DNA. The HPV1a genomes (17-4, 13-29) and HPV1a early regions (17-4E, 13-29E) were excised from pBR322 sequences by digestion with *Sa*I. Each of the resulting digests containing a linearized HPV1a DNA construct was introduced into 3Y1 cells by cotransfection with the selective marker pNEO5, which contains the bacterial neomycin resistance gene under the transcription control of the Harvey murine sarcoma virus long terminal repeat (11). Cells expressing the neomycin resistance gene were selected by growth on G-418-containing medium (17). Clones resistant to G-418 were expanded in culture and analyzed for the presence of HPV1a DNA sequences by DNA blot hybridization. A total of 188 rat 3Y1 cell clones were derived in this manner.

Of 98 that were analyzed by DNA blot hybridization, 90 had incorporated viral DNA. No significant differences were noted in the frequency of incorporation of the four DNA constructs. About 20% of the clones contained low levels of viral DNA (about 1 to 5 copies per cell), 40% contained moderate levels (about 5 to 10 copies), and 40% contained high levels (greater than 10 copies). Figure 2 shows DNA blots of five of the cell lines that represent a focus for our studies. With most cell lines, a complex pattern of viral-DNA-containing bands was observed on DNA blots after digestion of cell DNA with *Bam*HI, a one-cut enzyme for HPV1a DNA (Fig. 2, lanes B). Viral DNA was found in a high-molecular-weight band in blots of cell DNA sheared by passage through a 26-gauge needle (Fig. 2, lanes S). Furthermore, digestion with *Xho*I, a no-cut enzyme for HPV1a, yielded similar patterns (data not shown). These results were consistent with the integration of viral DNA into cellular

FIG. 2. Viral DNA sequences detected by blot hybridization of DNA isolated from 3Y1 rat cell lines established by cotransfection with HPV1a DNA constructs and pNEO5. DNA blot hybridization was performed, as described in Materials and Methods, with nick-translated HPV1a DNA as the probe. The lane furthest to the left is a reconstruction experiment containing five copies of HPV1a DNA per cell. Lane M represents size markers containing 32 P end-labeled adenovirus 2 *Hind*III DNA fragments. B refers to *Bam*HI-digested DNA. S refers to sheared DNA.

FIG. 3. Morphological alterations at confluence of rat 3Y1 cell lines established by cotransfection with HPV1a DNA constructs and pNEO5. Panels A to J show the following cell lines: A, the parental 3Y1 cell line at passage 8; B and C, G-418-resistant clones B8.2.1 and B2.3.3, respectively, which did not incorporate significant levels of viral DNA; D to J, HPV1a DNA-containing lines B4.3.1, B4.5.6, B3.3.1, B4.4.2, B4.3.3, B5.4.2, and B2.5.2, respectively.

DNA and the absence of episomal viral DNA. However, the possibility that some HPV1a DNA sequences are present as multimeric episomes was not excluded.

Growth properties of HPV1a DNA-containing cell lines. Photomicrographs of the cells in monolayer at confluence revealed that cell lines that have incorporated HPV1a DNA often possess an altered morphology (Fig. 3). Whereas the parental 3Y1 cell line exhibited a cobblestone appearance (Fig. 3, panel A), many viral-DNA-containing cell lines displayed a spindle-shaped and disoriented appearance and appeared to be smaller than the parental cell and to grow to a higher cell density (Fig. 3, panels F to J, clones B3.3.1, B4.4.2, B4.3.3, B5.4.2, and B2.5.2, respectively). G-418-resistant cell lines that did not incorporate viral DNA were similar in morphology to parental 3Y1 cells (Fig. 3, panels B and C). Clones B4.3.1 and B4.5.6 (Fig. 3, panels D and E) appeared intermediate in morphology and less disoriented

FIG. 4. Growth curves in low-serum-containing medium of 3Y1 rat cell lines established by cotransfection with HPV1a DNA constructs and pNEO5. Cells were plated at 2×10^5 cells per 25-cm² flask in DME containing either 10 or 0.5% fetal bovine serum. Cells in individual flasks were trypsinized at intervals and counted in a hemacytometer. Control 3Y1 cell line (○); HPV1a DNA-containing clones B3.3.1 (Δ), B5.4.2 (□), and B3.5.2 (▲).

than the cells shown in panels F to J, and they grew to a lower saturation density (see below).

The rate of growth of HPV1a DNA-containing cell lines was examined in DME containing 10% fetal bovine serum. The parental 3Y1 line, the two nonviral-DNA-containing lines, and clones B4.3.1 and B4.5.6 (shown to express low levels of viral RNA) exhibited similar growth rates with a doubling time of about 14 h and reached similar saturation densities of 4×10^6 to 5×10^6 cells per 25-cm² plate (data not shown). Four of the cell lines that possessed an altered morphology (Fig. 3, clones B3.3.1, B4.4.2, B4.3.3, and B5.4.2) grew at about the same rate as the 3Y1 parent but reached a twofold higher cell density at confluence (data not shown).

The growth of three of the HPV1a DNA-containing cell lines, B5.4.2, B3.3.1, and B2.5.2, was further examined in DME containing 0.5% fetal bovine serum. All three HPV1a DNA-containing cell lines grew at almost the same rate in 0.5% serum as in 10% serum; the doubling time was 15 to 17 h in 0.5% serum as compared with 14 h in 10% serum (Fig. 4). In contrast, parental 3Y1 cells did not grow appreciably in 0.5% serum (Fig. 4).

To determine whether the morphological alteration, increased density at confluence, and growth in low serum could be confirmed by a more stringent test for cell transformation, we tested the cell lines for anchorage-independent growth in 0.3% agarose (soft agar). Cells that displayed an altered morphology and increased saturation density formed large colonies in 0.3% agarose in 2 to 3 weeks, whereas the parental 3Y1 cell line formed no colonies among 5,000 cells that were seeded (Table 1). Cell lines containing HPV1a DNA possessed cloning efficiencies ranging from 1.5 to 12% (Table 1). An adenovirus 12-transformed 3Y1 cell line had a 3.0% cloning efficiency under similar conditions (Table 1).

Cell lines were established from the soft-agar clones, and growth rates were measured. The rates of growth and terminal densities of clones isolated from soft agar were

TABLE 1. Anchorage-independent growth of 3Y1 rat embryo fibroblast lines established by cotransfection with HPV1a DNA constructs and pNEO5

Cell line	Transfected construct ^a	Cloning efficiency in soft agar (%) ^b
3Y1, passage 8		0
3Y1, passage 14		0
Ad12-5 ^c		3.1
B2.2.3 ^d	pHPV1a(13-29)	0
B8.2.1 ^d	pHPV1a(13-29E)	0
B4.3.1 ^e	pHPV1a(13-29E)	0.2
B4.5.6 ^e	pHPV1a(13-29E)	0.2
B5.4.2	pHPV1a(17-4)	1.5
B5.4.1	pHPV1a(17-4)	8.4
B3.3.1	pHPV1a(17-4E)	8.3
B3.5.2	pHPV1a(17-4E)	9.8
B2.5.2	pHPV1a(13-29)	7.9
B6.2.8	pHPV1a(13-29)	12.1
B4.3.3	pHPV1a(13-29E)	5.4
B4.4.2	pHPV1a(13-29E)	11.9
B4.4.3	pHPV1a(13-29E)	5.4

^a Constructs were cotransfected with pNEO5.

^b Colonies were scored 2 to 3 weeks after seeding of 5,000 cells per 25-cm² flask.

^c 3Y1 cell line established by transformation with adenovirus 12.

^d These cell lines did not incorporate HPV1a DNA.

^e These cell lines expressed low levels of HPV1a RNA.

indistinguishable from those of the parent HPV1a DNA-containing clones (data not shown). This result implies that no additional selective pressure was imposed by growth in soft agar.

Tumorigenicity of HPV1a DNA-containing rat cell lines in nude mice. We tested the cell lines for tumorigenicity in BALB/c athymic nude mice by injecting 10^6 cells subcutaneously. The 3Y1 cell line at passages 8, 10, and 14 did not induce tumors at 36 days after inoculation, whereas all of the HPV1a DNA-containing 3Y1 cells that grew in soft agar and appeared to be altered morphologically formed tumors quite rapidly (Table 2). Tumors grew to a diameter of 0.5 cm by 6 to 10 days after inoculation and 1 to 2 cm by 14 days.

Tumors were excised at 14 days, and cell culture lines were established. Cell lines derived from the tumors were found to retain viral DNA (data not shown), indicating that each tumor originated from the rat 3Y1 transformant used for inoculation. Tumor cell lines possessed a spindle-shaped morphology identical to that of the cell lines used for inoculation. The growth rates of tumor-derived cell lines in culture were 1.4 to 2.4 times faster than those of the

TABLE 2. Tumorigenicity of rat embryo fibroblast lines transfected with HPV1a DNA constructs

Cell line ^a	No. of animals with tumors/no. injected ^b	Time until tumor appearance (days)
3Y1, passage 10	0/6	44 (no tumors)
B5.4.2	2/2	10
B5.4.1	2/2	10
B3.3.1	2/2	10
B3.5.2	2/2	6
B2.5.2	2/2	10
B6.2.8	2/2	10
B4.4.3	2/2	10

^a Cell lines were derived by cotransfection with HPV1a DNA constructs and pNEO5.

^b BALB/c athymic nude mice were injected subcutaneously with 10^6 cells.

FIG. 5. Viral RNA sequences detected by blot hybridization of 3Y1 rat cell lines established by cotransfection with HPV1a DNA constructs and pNEO5. (A) Total cytoplasmic RNA analyzed by hybridization to nick-translated HPV1a DNA; autoradiographic exposure was for 15 to 36 h with an X-ray intensifying screen. (B) Polyadenylated cytoplasmic RNA hybridized with a ^{32}P -labeled riboprobe encoding nts 595 to 967 on the HPV1a genome; autoradiographic exposure was for 6 h with an X-ray intensifying screen.

soft-agar-derived clones or the parental HPV1a DNA-containing transformants (data not shown). The cell densities of tumor lines at confluence was about 1.4×10^7 cells per plate (data not shown), about 1.5 times those of soft-agar clones and the initial transformants and about 3 times those of the parent 3Y1 cells.

Expression of viral genes in rat cell lines containing HPV1a DNA sequences. To determine whether HPV1a genes are expressed in transfected 3Y1 cell lines, we performed RNA blot hybridization on 30- μg amounts of total cytoplasmic RNA by using nick-translated HPV1a DNA as the probe. Our results suggested that there may be a correlation between HPV1a gene expression and altered cell growth. This is illustrated in Fig. 5A. Strong hybridization signals were observed after 15 to 36 h of autoradiographic exposure with cytoplasmic RNA from the five clonal lines B5.4.2, B2.5.2, B3.3.1, B3.5.2, and B4.3.3; these lines cloned efficiently in soft agar (Table 1) and induced tumors in nude mice (Table 2). On the other hand, clones B4.3.1 and B4.5.6 synthesized very low levels of viral RNA, requiring exposures of 7 days to detect a weak signal (data not shown); these lines possessed an intermediate morphology (Fig. 3) and displayed a low cloning efficiency in soft agar (Table 1; 0.2%).

Individual viral RNA bands were difficult to resolve in the RNA blots of total cytoplasmic RNA by using the whole viral genome as the probe (Fig. 5A). This was due in part to high levels of ribosomal RNA, which impede resolution, and probably to the occurrence of multiple HPV1a mRNA species, as anticipated from the complex transcription pattern found for bovine papillomavirus 1-transformed mouse cells (5, 18, 21). However, individual HPV1a mRNA species can be visualized by analyzing polyadenylated cytoplasmic RNA with subgenomic viral RNA probes. We identified three major HPV1a-specific RNA species ranging from about 1.8 to 5 kilobases by RNA blot analysis of clones B3.3.1 and B3.5.2 by using a riboprobe mapping at nts 595-967 in HPV1a DNA (Fig. 5B).

DISCUSSION

Most HPVs are known to induce benign tumors in humans, and several HPVs have been associated with the progression of benign lesions to invasive cancer. However, efforts to detect putative HPV transforming gene activity in vitro by standard focus assays have proven difficult. Watts et al. (20) reported that transfection with chimeric plasmids containing the genome of HPV5 (associated with epidermodysplasia verruciformis patients who develop cancer) or HPV1a (cause of plantar warts) induced foci in mouse C127 cells; the transfecting plasmid was detected as an episome in the transformants. No evidence was presented for the expression of viral genes; the early gene region which, by analogy with BPV1, encodes the putative transforming genes is interrupted by pBR322 in these plasmid constructs. HPV5- but not HPV1a-transformed cells were reported to form tumors in nude mice (20). More recently, Burnett and Gallimore (3) introduced an HPV1a plasmid into a thymidine kinase-deficient rat fibroblast cell line. Viral DNA was incorporated into the high-molecular-weight cell DNA fraction, and viral RNA transcripts were detected in two of three cell lines examined; however, viral RNA was present at extremely low levels and required 14 days of autoradiographic exposure to visualize on RNA blots. No evidence was found for alteration of the growth properties of the cells; the researchers suggested that the lack of cell transformation may have been due to inefficient expression of the viral genome in these cell lines (3). Very recently, a recombinant dimer of HPV16 (22) and HPV16 DNA sequences in total DNA of a cervical tumor (19) were reported to transform mouse NIH 3T3 cells. HPV16 is considered an oncogenic virus since it is present in a high proportion of cervical tumors (4). An unusually long latency period was required for transformation by the recombinant HPV16 plasmid (22).

The fact that HPV DNAs do not readily transform cells in culture is not entirely unexpected. Tumor induction is considered a multistep process, and the functions of at least two genes or protein domains are often required for transformation of cultured cells (reviewed in reference 8). It is possible that HPV genomes provide only one of the functions needed to produce recognizable foci or to transform cultured cell lines morphologically. Furthermore, the expression of papillomavirus genes may be limited in many cell types, possibly because of regulation by host- and differentiation-specific *trans*-acting factors. Perhaps additional oncogenic functions that are provided by a second tumor virus or by a cell mutation are required for tumorigenicity or cell transformation.

In the present study, we introduced four different HPV1a DNA constructs into the 3Y1 rat embryo fibroblast cell line by cotransfection with pNEO5. Most of the G-418-resistant clonal cell lines that were generated incorporated HPV1a DNA sequences. Representative cell lines containing high copy numbers of viral DNA were found to exhibit anchorage-independent growth with a high cloning efficiency in soft agar and to form tumors rapidly in nude mice. Those cell lines that exhibited altered growth and tumorigenicity synthesized abundant levels of viral RNA which were detected as distinct polyadenylated viral RNA species in the cell cytoplasm. Our results differ from those of Burnett and Gallimore (3) in the sense that most of our HPV1a-transformed rat cell lines exhibited oncogenic properties, whereas their cell lines did not. Our transformed cells expressed high levels of HPV1a-specific RNA. However,

two of our cell lines expressed low levels of viral RNA, and these cells had minimal alterations in growth properties. These two lines resemble those of Burnett and Gallimore (3), which expressed low levels of HPV1a RNA and did not possess oncogenic properties. Therefore it appears reasonable, from our data as well as those of Burnett and Gallimore (3), that the early region of HPV1a is capable of transforming cells but that abundant expression of HPV1a genes is required to effect transformation. We do not know why our G-418-selected 3Y1 cells appeared to express HPV1a genes more readily than the thymidine kinase-deficient rat cells; perhaps different *trans*-acting factors play a role.

As discussed above, our data suggest that a correlation may exist between expression of HPV1a genetic information and transformation in the growth properties of 3Y1 rat embryo fibroblast cells. The correlation between RNA expression and transformation suggests that HPV1a may encode a classical transforming gene whose expression is required for cell transformation. Nothing is known about HPV transforming genes. Recent studies have identified two BPV1 genes, E5 and E6, whose action can independently transform C127 mouse cells (12, 14–16, 21). Alternatively, it is conceivable that HPV1a may transform cells by a mechanism that differs from that of BPV1 and the classical tumor viruses. Further studies are needed to clarify this issue. We are currently performing mapping studies on the viral RNA species expressed in several of the cell lines described in this study.

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LITERATURE CITED

1. Boshart, M., L. Gissmann, H. Ikenberg, A. Kleinheinz, W. Scheurlen, and H. zur Hausen. 1984. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. *EMBO J.* 3:1151–1157.
2. Brackmann, K. H., M. Green, W. S. M. Wold, A. Rankin, P. M. Loewenstein, M. A. Cartas, P. R. Sanders, K. Olson, G. Orth, D. Kremsdorf, and M. Favre. 1983. Introduction of cloned human papillomavirus genomes into mouse cells and expression at the RNA level. *Virology* 129:12–24.
3. Burnett, T. S., and P. H. Gallimore. 1985. Introduction of cloned human papillomavirus 1a DNA into rat fibroblasts: integration, *de novo* methylation and absence of cellular morphological transformation. *J. Gen. Virol.* 66:1063–1072.
4. Dürst, M., L. Gissmann, H. Ikenberg, and H. zur Hausen. 1983. A papillomavirus virus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc. Natl. Acad. Sci. USA* 80:3812–3815.
5. Engel, L. W., C. A. Heilman, and P. M. Howley. 1983. Transcriptional organization of bovine papillomavirus type 1. *J. Virol.* 47:516–528.
6. Favalaro, J., R. Treisman, and R. Kamen. 1980. Transcription maps of polyoma virus-specific RNA: analysis by two-dimensional nuclease S1 gel mapping. *Methods Enzymol.* 65:718–749.
7. Gissmann, L., L. Wolnik, H. Ikenberg, U. Koldovsky, H. G. Schurch, and H. zur Hausen. 1983. Human papillomavirus types 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers. *Proc. Natl. Acad. Sci. USA* 80:560–563.
8. Green, M. 1985. Transformation and oncogenesis: DNA viruses, p. 183–234. In B. N. Fields, D. M. Knipe, R. M. Chanock, J. L. Melnick, B. Roizman, and R. E. Shope (ed.), *Virology*. Raven Press, New York.
9. Green, M., K. H. Brackmann, P. R. Sanders, P. M. Loewenstein, J. H. Freil, M. Eisinger, and S. A. Switlyk. 1982. Isolation of a human papillomavirus from a patient with epidermodysplasia verruciformis: presence of related viral DNA genomes in human urogenital tumors. *Proc. Natl. Acad. Sci. USA* 79:4437–4441.
10. Kimura, G., A. Itagaki, and J. Summers. 1975. Rat cell line 3Y1 and its virogenic polyoma- and SV40-transformed derivatives. *Int. J. Cancer* 15:694–706.
11. Lusk, M., and M. R. Botchan. 1984. Characterization of the bovine papilloma virus plasmid maintenance sequences. *Cell* 36:391–401.
12. Nakabayashi, Y., S. K. Chattopadhyay, and D. R. Lowry. 1983. The transforming function of bovine papillomavirus DNA. *Proc. Natl. Acad. Sci. USA* 80:5832–5836.
13. Pfister, H. 1984. Biology and biochemistry of papillomaviruses. *Rev. Physiol. Biochem. Pharmacol.* 99:112–181.
14. Sarver, N., M. S. Rabson, Y.-C. Yang, J. C. Byrne, and P. M. Howley. 1984. Localization and analysis of bovine papillomavirus type 1 transforming functions. *J. Virol.* 52:377–388.
15. Schiller, J. T., W. C. Vass, and D. R. Lowy. 1984. Identification of a second transforming region in bovine papillomavirus DNA. *Proc. Natl. Acad. Sci. USA* 81:7880–7884.
16. Schiller, J. T., W. C. Vass, K. H. Vousden, and D. R. Lowy. 1986. E5 open reading frame of bovine papillomavirus type 1 encodes a transforming gene. *J. Virol.* 57:1–6.
17. Southern, P. J., and P. Berg. 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. *J. Mol. Appl. Genet.* 1:327–341.
18. Stenlund, A., J. Zabielski, H. Ahola, J. Moreno-lopez, and U. Pettersson. 1985. Messenger RNAs from the transforming region of bovine papilloma virus type 1. *J. Mol. Biol.* 182:541–554.
19. Tsunokawa, Y., N. Takebe, T. Kasamatsu, M. Terada, and T. Sugimura. 1986. Transforming activity of human papillomavirus type 16 DNA sequences in a cervical cancer. *Proc. Natl. Acad. Sci. USA* 83:2200–2203.
20. Watts, S. L., W. C. Phelps, R. S. Ostrow, K. R. Zachow, and A. J. Faras. 1984. Cellular transformation by human papillomavirus DNA in vitro. *Science* 225:634–636.
21. Yang, Y.-C., H. Okayama, and P. M. Howley. 1985. Bovine papillomavirus contains multiple transforming genes. *Proc. Natl. Acad. Sci. USA* 82:1030–1034.
22. Yasumoto, S., A. L. Burkhardt, J. Doniger, and J. A. DiPaolo. 1986. Human papillomavirus type 16 DNA-induced malignant transformation of NIH 3T3 cells. *J. Virol.* 57:572–577.
23. Zachow, K. R., R. S. Ostrow, M. Bender, S. Watts, T. Okagaki, F. Pass, and A. J. Faras. 1982. Detection of human papillomavirus DNA in anogenital neoplasia. *Nature (London)* 300:771–773.