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The E6-E7 Region of Human Papillomavirus Type 18 Is Sufficient for Transformation of NIH 3T3 and Rat-1 Cells

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Plasmids containing the E6 and E7 open reading frames of human papillomavirus type 18 expressed from an autologous transcriptional control region were sufficient for transformation of NIH 3T3 and Rat-1 cells. Transformation by these sequences did not always involve morphological alterations even though anchorage-independent growth occurred at a high frequency. In these cells, the efficiency of transformation by the E6 and E7 construct was equivalent to or, in most cases, better than that observed with the entire viral genome. These data indicate that a major human papillomavirus type 18 transforming function is localized in either the E6 or E7 gene products or both.

Papillomaviruses are small DNA viruses which are the etiological agents of a number of proliferative lesions in many animals. Although many of these lesions are benign, those induced by specific types of papillomaviruses show a high risk of malignant conversion. Human papillomavirus (HPV) sequences are present in up to 90% of cervical carcinoma biopsies, with the majority of these tumors containing either HPV type 16 (HPV-16) or HPV-18 DNA (5, 8, 14, 15, 30). This strongly suggests a causative role for these viruses in the development of malignancy. Since the annual incidence of new cases of cervical cancer is estimated by the World Health Organization to be in excess of 450,000 (6), examination of the transforming properties of these human DNA tumor viruses is of significant interest.

The transforming properties of several animal papillomaviruses have been well studied in vivo and in cultured rodent cells. In vitro transformation by bovine papillomavirus type 1 (BPV-1) is manifested by loss of contact inhibition, anchorage-independent growth, and tumorigenicity in nude mice (9, 12, 16). Two transforming genes have been identified in BPV-1 which have differential effects in various cells (18, 19, 27, 28). The E5 open reading frame (ORF) transforms both NIH 3T3 and C127 cells, while the E6 ORF, when placed under the control of a heterologous viral promoter, can transform C127 cells but transforms NIH 3T3 cells only weakly. In addition, the product of the E6 ORF may act synergistically with E5, since deletion of this region can influence the frequency of transformation (16, 17, 27). In contrast to knowledge of BPV-1-mediated transformation, the facts about which ORFs of HPVs are involved in transformation are unclear. We now report that expression of the E6 and E7 ORFs of HPV-18, under control of the autologous HPV-18 promoter, is sufficient for transformation of rodent fibroblasts.

The HPV-18 genome has been cloned (5) and sequenced (7). By analogy to BPV-1, the HPV genome may be functionally divided into early and late regions which encode five and two ORFs, respectively (Fig. 1). The early region contains ORFs similar in size and location to those in BPV-1 and should encode functions for viral replication, transcriptional control, and transformation, while the late region encodes virion structural proteins (6). These ORFs are all located on one strand of the double-stranded genome, and a single noncoding region (also called long control region or upstream regulatory region) between the early and late regions contains sequences involved in control of replication and transcription (6). BPV-1 is the best-characterized papillomavirus, and its transcription is complex, with multiple promoters located in the noncoding region and within the E2 ORF (2). In addition, BPV-1 E2 ORF encodes both a transactivator and a repressor of early-gene transcription (11, 25). It is likely that some aspects of HPV-18 transcription and its regulation are similar to those of BPV-1.

In general, HPV DNA persists as an episome in benign lesions but is frequently integrated into the chromosomes of malignant tumors (5, 14). Integration appears to be random with respect to host sequences, but the E1 or E2 regions are often the viral sites of integration (3, 21). As a consequence of this integration, the principal viral sequences colinear with the viral transcriptional control region are the 5′-proximal sequences, and the majority of transcripts found in cervical tumor lines are those derived from the E6 and E7 regions (3, 20, 21, 23). These data suggest that expression of the 5′-proximal region of the HPV-16 or HPV-18 genome may be involved in either initiation or maintenance of the malignant phenotype.

We have localized the transforming regions of HPV-18 by introducing plasmids containing genomic and subgenomic portions of viral DNA into two rodent fibroblast cell lines commonly used in transformation assays. Both NIH 3T3 and Rat-1 cells are contact inhibited and are sensitive to transformation by a variety of cellular and viral oncogenes. The constructs used in the present study are shown in Fig. 1. The original HPV-18 plasmid (obtained from H. zur Hausen) consisted of an *Eco*RI insert into pBR322 (5). Since this insert interrupted the early region, we reconstructed an intact early region by first constructing a plasmid, p18PE (Fig. 1), by inserting a *Pst*I-*Eco*RI fragment of HPV-18 into pML. An *Eco*RI fragment containing the entire HPV-18 genome was then recloned into p18PE, thereby creating a permuted HPV-18 designated p18-1.5. In both p18PE and p18-1.5 constructs, the early regions of viral genomes are contiguous with the noncoding region which contains a transcriptional enhancer element (26; D. Gius, personal communication) and transcriptional start signals (20, 21). p18-1.5 encodes all viral early genes, while p18PE encodes

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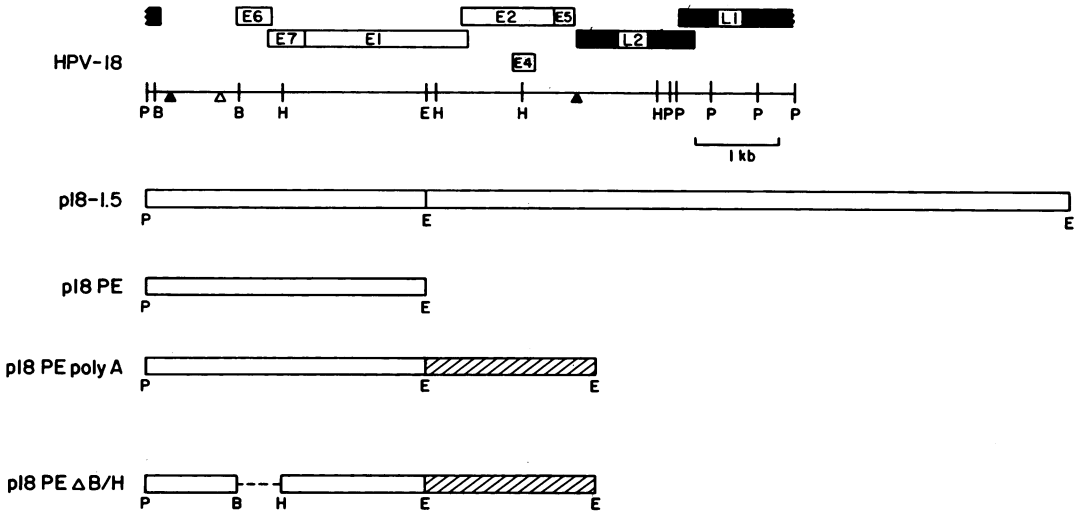

FIG. 1. Upper: Genomic organization of HPV and partial restriction map of HPV-18 (7). Early (▭) and late (▬) ORFs; restriction sites for *Bam*HI (B), *Hin*cII (H), *Pst*I (P), and *Eco*RI (E); and potential polyadenylation sequences (▲) and transcriptional start sequences (△) (7) are shown. Lower: HPV-18 constructs used in the present study. HPV sequences (▭) are shown, but plasmid sequences are not. p18-1.5 reconstructs the early region from the original *Eco*RI insert (5) and has 1.5 times the entire HPV-18 genome. p18PE contains a 3.4-kb *Pst*I-*Eco*RI fragment of HPV-18 inserted into pML, while p18PEpolyA has a 2-kb *Eco*RI insert from pSV2cat that contains the simian virus 40 late polyadenylation signal (▨). p18PEΔB/H contains a 537-base-pair deletion (– – –) from the *Bam*HI to *Hin*cII sites in the E6 and E7 ORFs and the same polyadenylation signal as p18PEpolyA.

the intact E6 and E7 regions and the N-terminal portion of E1. As the latter plasmid probably lacks polyadenylation signals, the simian virus 40 late polyadenylation signal from pSV2cat (10) was inserted at the *Eco*RI site to generate p18PEpolyA. This construct was used in transformation experiments and is analogous to the state of HPV sequences observed in cervical tumors in which the 3′ ends of E6-E7 transcripts consist of cellular sequences presumably containing polyadenylation signals (20, 21). A deletion in p18PEpolyA was made by insertion of *Bgl*II linkers after partial digestion with *Bam*HI and complete cutting at the unique *Hin*cII site to generate p18PEΔB/H. This deletion affects only E6 and E7 and leaves only five amino acids at the N terminus of E6 intact, and all other E6 and E7 sequences are deleted or out of frame.

In initial experiments with NIH 3T3 cells, focus formation in the absence of selective pressure occurred 5 to 6 weeks after transfection of either p18-1.5 or p18PEpolyA (data not shown). These foci were similar to spontaneous foci in time of appearance, and so this assay was abandoned. All subsequent experiments were therefore conducted by cotransfecting p18PEpolyA, p18PEΔB/H, or p18-1.5 DNA (30 μg) with a plasmid (3 μg) carrying a selectable marker, and individual selected colonies were analyzed. The 10:1 ratio of the two plasmids ensured that virtually every selected colony also expressed the HPV construct. The selectable marker for NIH 3T3 experiments was the neomycin resistance gene carried on pRSVneo, and selection was in 500 μg of G418 (GIBCO Laboratories, Grand Island, N.Y.) per ml. For Rat-1 experiments, either pRSVneo or pSV2gpt was cotransfected, and selection media consisted of (per milliliter) 400 μg of G418 or 15 μg of mycophenolic acid, 160 μg of xanthine, and 10 μg of hypoxanthine, respectively. Plasmids were introduced by calcium phosphate coprecipitation into NIH 3T3 or Rat-1 cells 1 day after 10^6 cells were seeded per 100-mm^2 dish. Two days after transfection, cells were trypsinized and seeded onto fresh plates at a 1:10 (NIH 3T3) or 1:3 (Rat-1) dilution, and selective media were applied. After 2 weeks, representative colonies were picked by using cloning cylinders and expanded. pEJras, containing the oncogenic c-Ha-*ras* allele (obtained from R. Pozzatti), and pUC9 were positive and negative controls, respectively, for transformation. All cultures were maintained in 10% fetal bovine serum (FBS) in Dulbecco modified Eagle medium (both obtained from GIBCO) unless otherwise indicated.

The morphologies of representative NIH 3T3 and Rat-1 lines are shown in Fig. 2 and 3 (panels a to c), respectively. In NIH 3T3 cells (Fig. 2), minimal morphological differences were observed between the control (panel a) and either HPV-18 construct (panels b and c). p18PEpolyA-containing clones were consistently more aberrant in appearance than p18-1.5 transfectants. Nonetheless, these morphological changes were not as dramatic as those seen following pEJras transfection (not shown). In contrast to NIH 3T3 cells, Rat-1 cells transfected with p18PEpolyA (Fig. 3c) appeared distinctly transformed morphologically, with characteristic refractile and disorganized cells. On the other hand, Rat-1 cells containing p18-1.5 were less refractile and were similar to controls (cf. Fig. 3b and a). Rat-1 cells thus appeared more sensitive to morphological transformation by the subgenomic HPV-18 sequences than NIH 3T3 cells were.

Representative lines of NIH 3T3 and Rat-1 transfectants were tested for anchorage-independent growth by plating 3 × 10^4 cells per 60-mm^2 dish in medium containing 0.3% agarose. Plates were scored after 2 weeks. The results for NIH 3T3 and Rat-1 experiments are summarized in Tables 1 and 2, respectively. Representative photographs of lines growing in soft agar are shown in Fig. 2 and 3 (panels d to f) for NIH 3T3 and Rat-1 cells, respectively. In both kinds of cells, pEJras was positive in 100% of the lines. Spontaneous transformation, as assayed by transfection of pUC9 DNA, was similar in the two cell types, with one of five NIH 3T3 lines and one of nine Rat-1 lines showing growth. Less than 4% of cells from each of the four pUC9 NIH 3T3 transfectants were able to grow in soft agar (data not shown). In NIH 3T3 cells, most (six of eight) of the p18-1.5 clones grew in

soft agar, with 13 to 40% of cells from each of the positive lines forming colonies. The efficiency of Rat-1 transformation by the p18-1.5 construct was high (13 of 14), but in both types of cells, growth in soft agar was slower than it was after pEJras transfection. Like the full-length HPV-18 construct, the subgenomic construct p18PEpolyA transformed both NIH 3T3 and Rat-1 cells at a high frequency. Approximately 80 and 100% of transfected NIH 3T3 and Rat-1 cell lines, respectively, were capable of anchorage-independent growth. Although the number of lines transformed by the subgenomic construct was virtually identical to that of the genomic construct, a consistent difference in the size and percentage of soft agar colonies was observed. All p18PEpolyA transformants grew two to three times larger than p18-1.5 transformants (compare panels e and f of Fig. 2 and 3). In addition, up to 90% of cells from p18PEpolyA transformants were able to form colonies, while the maximum value observed in p18-1.5 transformants was only 40% (data not shown). Clearly, deletion of E6 and E7 sequences from the subgenomic construct abolished transformation, since six of seven lines derived after transfection of Rat-1 cells with p18PEΔB/H failed to form colonies in 0.3% agarose (Table 2). This value is consistent with that observed after transfection of pUC9, the negative control.

Serum growth factor independence was assessed in NIH 3T3 transfectants by plating 0.5×10^6 cells in 0.5% FBS (Table 1) and counting cells 2 weeks later. Of eight lines that had been transfected with pEJras or p18-1.5 and were positive for growth in soft agar, six were also growth factor independent. One spontaneous pUC9 transformant (pUC9:3) was both anchorage and growth factor independent. Interestingly, all seven p18PEpolyA lines tested, of which only five were anchorage independent, were also able to grow in 0.5% FBS. After 2 weeks of growth in 0.5% FBS, the cell densities of p18PEpolyA transformants ranged from 2 to 3 times those of controls and averaged 1.5 times those of p18-1.5 transformants (data not shown).

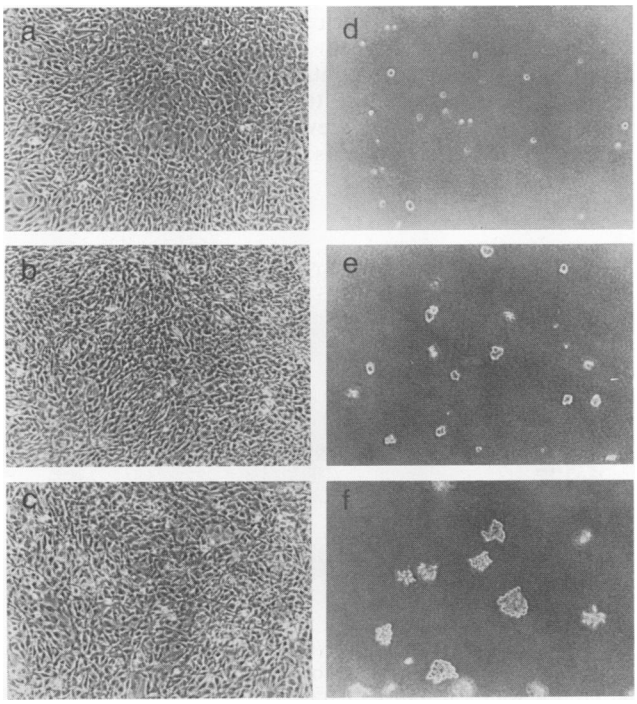

FIG. 2. Morphology and anchorage-independent growth of NIH 3T3 transfectants. Representative G418-resistant NIH 3T3 lines in media (panels a to c) or in media containing 0.3% agarose (panels d to f). a and d, pUC9; b and e, p18-1.5; c and f, p18PEpolyA. Phase-contrast, ×100.

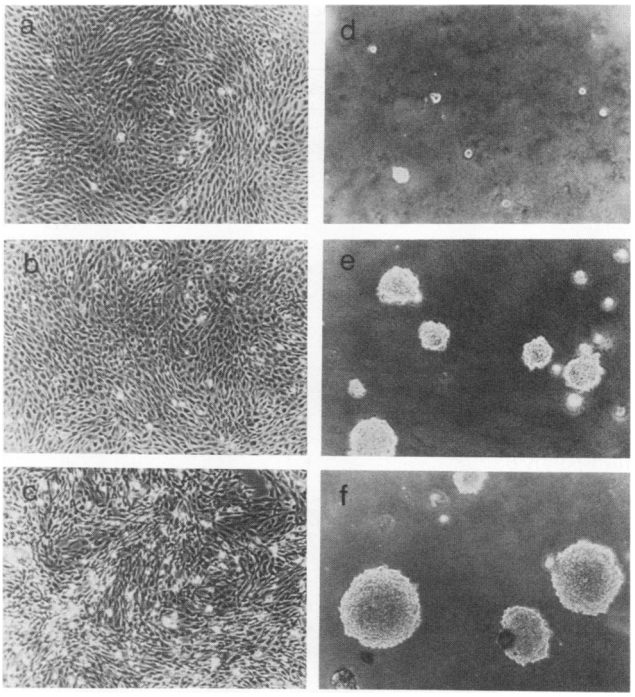

FIG. 3. Morphology and anchorage-independent growth of Rat-1 transfectants. Representative mycophenolic acid-resistant Rat-1 lines are shown. Panels are as described in Fig. 2.

The state of transfected DNA was examined in transformed cell lines by Southern analysis. DNA was isolated from 10^7 cells from lines positive for anchorage-independent growth. Cell lysates were digested with proteinase K and RNase and phenol extracted, and DNA was precipitated with ethanol. DNA (10 μg) was digested to completion with *Bam*HI, electrophoresed on 1% agarose gels, and transferred to nitrocellulose. Hybridization to a 6.4-kilobase (kb) *Pst*I fragment of p18-1.5 (Fig. 1) labeled with ^{32}P by the random primer method (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was at 42°C and was followed by high-stringency washes at 65°C. Exposures were for 2 days at −70°C with intensifier screens. Representative results are shown in Fig. 4, in which NIH 3T3 lines transformed by p18-1.5 (lanes a to c) or p18PEpolyA (lanes d to f) are shown. Lanes g and h contain *Bam*HI-digested p18PEpolyA and represent 5 and 10 copies, respectively, per genome equivalent. All transformed lines had detectable HPV DNA ranging from approximately 2 to 20 copies. HPV-18 sequences in these transformants did not undergo significant rearrangement, nor were they interrupted, as all lanes gave the expected bands. In addition, there was no evidence for episomal persistence, since undigested DNA had no detectable low-molecular-weight hybridization (data not shown).

Total cellular RNA from 10^7 cells of several anchorage-independent lines was isolated by the guanidium isothiocyanate-CsCl method (13) and analyzed by Northern blots (Fig. 5). Of the transformants listed in Table 1, the lines chosen for RNA analysis were p18-1.5:4, p18PEpolyA:3, and p18PEpolyA:4 because they had similar amounts of integrated HPV-18 sequences. In addition, two p18PE lines

TABLE 1. Growth properties of NIH 3T3 transfectants

Plasmid	Growth in:	
	0.3% Agarose[a]	0.5% Serum[b]
p18-1.5:1	−	−
p18-1.5:2	+	+
p18-1.5:3	+	−
p18-1.5:4	+	+
p18-1.5:5	+	ND[c]
p18-1.5:6	−	ND
p18-1.5:7	+	+
p18-1.5:8	+	ND
p18PEpolyA:1	−	+
p18PEpolyA:2	+	ND
p18PEpolyA:3	+	+
p18PEpolyA:4	+	+
p18PEpolyA:5	+	+
p18PEpolyA:6	+	+
p18PEpolyA:7	−	+
p18PEpolyA:8	+	+
p18PEpolyA:9	+	ND
pEJras:1	+	+
pEJras:2	+	+
pEJras:3	+	+
pEJras:4	+	−
pUC9:1	−	−
pUC9:2	−	−
pUC9:3	+	+
pUC9:4	−	−
pUC9:5	−	−

[a] Individual colonies derived from two independent cotransfections with pRSVneo were selected in 500 μg of G418 per ml and expanded, and 3×10^4 cells of each line were plated in medium containing 0.3% agarose. Plates were scored 2 weeks later and considered positive if more than 10% of the cells formed colonies.

[b] Cells (0.5×10^6) from individual clones were plated and grown in 0.5% FBS for 2 weeks. Plates were then trypsinized, cells were counted with a hemacytometer, and plates were scored positive if cell numbers were twice those of controls.

[c] ND, Not determined.

TABLE 2. Anchorage-independent growth of Rat-1 transfectants

Plasmid	Selection[a]	No. of expts	No. positive/ no. tested[b]
p18-1.5	*neo*	1	4/5
	gpt	2	9/9
p18PEpolyA	*neo*	1	5/5
	gpt	3	13/13
p18PEΔB/H	*gpt*	1	1/7
pEJras	*gpt*	1	5/5
pUC9	*neo*	1	0/3
	gpt	3	1/6

[a] *neo* selection consisted of cotransfections of HPV-18 plasmids with pRSVneo and plating of transfected cells in medium containing 400 μg of G418 (GIBCO) per ml. *gpt* selection was after pSV2gpt cotransfection of HPV-18 and plating in medium containing the following: 15 μg of mycophenolic acid, 160 μg of xanthine, and 10 μg of hypoxanthine per ml.

[b] Individual drug-resistant lines were analyzed as described in Table 1, footnote *a*.

with the same copy number were analyzed for comparison. Ten micrograms of each RNA preparation was electrophoresed in 1% agarose–formaldehyde gels and transferred to nitrocellulose paper. A 6.4-kb *Pst*I fragment of HPV-18, identical to that used for DNA blots (see above), was hybridized overnight at 45°C. The blot was washed at high stringency at 50°C, and the film was exposed for 1 week at −70°C with intensifier screens (Fig. 5a). To verify the integrity and quantity of RNA on each lane, the blot was stripped and reprobed with an actin probe (obtained from S. Taneka). Hybridization, washes, and exposure were as described for the HPV probe, except that a lower-stringency wash was used and exposure was overnight (Fig. 5b). While actin messages were identical in all cell lines, the subgenomic transformants (lanes 2, 3, 4, and 5) expressed considerably more HPV-18 RNA than did the genomic transformant (lane 1). If HPV-18 initiates transcription within the 3′ part of the noncoding region (Fig. 1), the expected size of an unspliced transcript from p18PEpolyA would be roughly 4 kb. Consistent with this, a major RNA species was observed at approximately 3.5 kb (lanes 4 and 5). Less abundant RNAs that were heterogeneous in size were also observed in both p18PEpolyA lines. However, these were not detected in the p18PE lines (lanes 2 and 3). Overall, transcription in the limited number of lines examined was greater in subgenomic than in genomic transformants. This difference may explain the differential growth of these cells in soft agar. Whether there is preferential expression or stabilization of transcripts from the subgenomic constructs is currently under investigation.

Some NIH 3T3 lines that were positive for growth in soft agar were also tested for their ability to form tumors in nude mice. These lines contained integrated HPV-18 plasmids and expressed HPV-18 RNA. Either 2×10^6 or 10×10^6 cells from individual anchorage-independent lines were injected subcutaneously into multiple sites of one to three male *nu/nu* mice. The animals were sacrificed 3 to 4 weeks later and examined for tumors at the site of injection. Two p18-1.5 transformants of NIH 3T3 cells (p18-1.5:4 and p18-1.5:8), two p18PEpolyA transformants (p18PEpolyA:4 and p18PEpolyA:9), and a single p18PE transformant formed tumors in the mice. Consistent with the results of soft agar growth, the incidence of tumors formed by genomic or subgenomic HPV-18 constructs was identical. However, a significant difference in growth of the tumors was observed, as all subgenomic transformants grew to sizes two to three times those of p18-1.5 transformants.

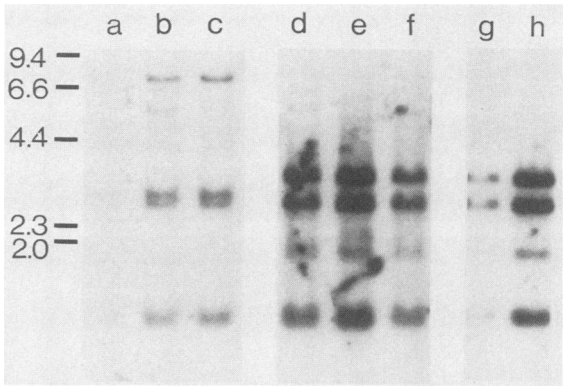

FIG. 4. Southern blot of NIH 3T3 transformants. *Bam*HI digests of genomic DNA were electrophoresed, blotted to nitrocellulose, and probed with a 6.4-kb *Pst*I fragment of p18-1.5 (Fig. 1). Size markers (in kilobases) of *Hin*dIII-digested lambda DNA are shown to the left. Lanes: a to c, cell lines p18-1.5:1 to p18-1.5:3; d to f, cell lines p18PEpolyA:1 to p18PEpolyA:3; g and h, p18PEpolyA plasmid digested with *Bam*HI at 5 (lane g) and 10 (lane h) copies per genome equivalent. (Cell lines are tabulated in Table 1.)

Our results demonstrate that two immortalized rodent fibroblast lines can be transformed by the HPV-18 E6-E7 region expressed from the autologous HPV-18 promoter-enhancer. Both types of cells were transformed to serum growth factor and anchorage independence and were able to induce tumors in nude mice. Distinct morphological alterations were not observed after transfection of the entire viral genome, even though transformation frequency was high. Only the p18PEpolyA construct exhibited distinct alterations in morphology, and this occurred in Rat-1 but not in NIH 3T3 cells. A head-to-tail dimer of the full-length HPV-16 genome, another genital HPV, has previously been shown to induce morphologically transformed foci in NIH 3T3 cells (29). These foci, however, did not appear until 4 to 6 weeks posttransfection. In our experiments with HPV-18 sequences, focus formation was concurrent with the appearance of spontaneous foci and so was not used as an assay system. Transformation of rodent fibroblasts by either HPV-16 or HPV-18 is therefore considerably different from that by pEJras, where dramatic morphological changes occurred and transformants rapidly outgrew monolayers. We have previously reported that, like pEJras, HPV-18 sequences require a second oncogene to transform primary fibroblast cultures (L. A. Laimins, M. A. Bedell, K. H. Jones, and J. A. Long, Cancer Cells, in press). However, it is not known whether the viral transforming genes act in a manner similar to that of pEJras. In view of the differences in growth properties noted above, it is likely that a different mechanism is involved.

Viral transcription in cervical carcinoma cell lines occurs primarily from the E6-E7 region of the integrated HPV genome and utilizes cellular sequences for transcription termination (3, 20, 23). The construct p18PEpolyA used in the present investigation was designed to mimic this state and contains the entire transcriptional control region, intact E6 and E7 ORFs, a truncated E1 ORF, and a heterologous polyadenylation signal. The transforming activity of this construct is most likely due to expression of E6 or E7 or both, since deletion of these sequences abolishes transforming ability. The E1 ORF is therefore probably not involved. In addition, viral transcripts in cervical tumor lines preferentially splice out E1 sequences (20). These data therefore provide strong evidence for the role of either or both of the E6 or E7 ORFs in transformation. It is of interest that p18PEpolyA was consistently more effective in all assays for transformation than the entire viral genome was. It is possible that expression of E6 and E7 in the subgenomic construct is altered from that of the full-length construct. In cervical tumors, where integration of the virus often disrupts the E1 or E2 ORFs (3, 21), viral expression may also be different from that of the intact viral genome. Cellular, rather than viral, polyadenylation sequences are used in the integrated state (21, 23). In addition, if HPV-18 E2 encodes a transcriptional activator or repressor or both, as in BPV-1, its absence or disruption in cervical tumors may effect the pattern of viral transcription. Differential regulation of integrated versus episomal forms of HPV may therefore be a critical feature in the development of malignant lesions.

The E6 and E7 regions of HPV-18 have been sequenced (7, 20, 22), and the deduced amino acid sequences are roughly 50 to 60% homologous to those of ORFs of other papillomaviruses that have been associated with genital lesions (7). Although the entire E7 ORF was found in cDNAs of cervical carcinoma lines, the E6 ORFs of both HPV-16 and HPV-18 are present as unspliced or spliced forms designated E6* (20, 23). The predicted amino acid sequences

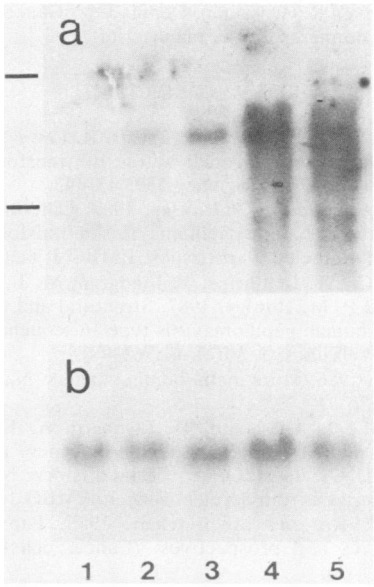

FIG. 5. Northern analysis of HPV-18 expression in NIH 3T3 transformants. Total cellular RNA (10 μg per lane) was electrophoresed in formaldehyde-agarose gels, blotted to nitrocellulose, and probed with a 6.4-kb *PstI* fragment of p18-1.5 (Fig. 1). This probe was then stripped off, and the blot was rehybridized to a human actin probe. (a) Hybridization to the HPV-18 probe; (b) hybridization to the human actin probe. The positions of 18S and 28S rRNA are indicated (—). Lanes: 1, cell line p18-1.5:4; 2 and 3, two p18PE cell lines; 4 and 5, cell lines p18PEpolyA:4 and p18PEpolyA:5. (Except for p18PE lines, cell lines are from those in Table 1.)

of all three ORFs contain conserved motifs of Cys-X-X-Cys, which are suggestive of nucleic acid-binding proteins (4); however, very little is known of the biochemical properties or viral function of these ORFs. The E6 region of BPV is sufficient for transformation in C127 cells but only when expressed from a strong heterologous promoter-enhancer (18, 28). Recently, this ORF has been shown to encode a protein present in both the cell nucleus and cellular membranes (1). In contrast to BPV-1 E6 gene product, BPV-1 E7 gene product does not seem to be involved in transformation but is required for high-copy-number maintenance of episomal forms (6). In cervical carcinoma cell lines containing either HPV-16 or HPV-18, the E7 gene product is the most abundant viral protein (22–24) and is located in the cytoplasm (24). Our data demonstrate that the HPV-18 E6 and E7 regions, when expressed from their own transcriptional control region, are capable of transforming both NIH 3T3 and Rat-1 cells. The lack of significant difference in transformation frequency between p18PEpolyA, which lacks the E5 ORF, and p18-1.5, which contains E5, suggests that the product of HPV-18 E5 is not essential for transformation of rodent cell lines. This possibility is in contrast to the situation with BPV-1 transformation, for which the E5 ORF is the major transforming gene of NIH 3T3 cells (18, 19). Whether HPV-18 E5 possesses a transforming function in other cells is unknown.

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