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Nucleotide Sequences of cDNAs for Human Papillomavirus Type 18 Transcripts in HeLa Cells

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HeLa cells expressed 3.4- and 1.6-kilobase (kb) transcripts of the integrated human papillomavirus (HPV) type 18 genome. Two types of cDNA clones representing each size of HPV type 18 transcript were isolated. Sequence analysis of these two types of cDNA clones revealed that the 3.4-kb transcript contained E6, E7, the 5′ portion of E1, and human sequence and that the 1.6-kb transcript contained spliced and frameshifted E6 (E6*), E7, and human sequence. There was a common human sequence containing a poly(A) addition signal in the 3′ end portions of both transcripts, indicating that they were transcribed from the HPV genome at the same integration site with different splicing. Furthermore, the 1.6-kb transcript contained both of the two viral TATA boxes upstream of E6, strongly indicating that a cellular promoter was used for its transcription.

There have been several reports indicating the close association of human papillomavirus (HPV) types 16 and 18 with cervical cancers (4, 9, 26, 32, 36). It was also previously demonstrated that the dimer of the HPV type 16 genome or HPV type 16 DNA integrated into genomic DNA of a cervical cancer had the ability to transform NIH 3T3 cells upon transfection (31, 35). Little information is available, however, about the molecular mechanisms underlying the development of cervical cancers by HPV types 16 and 18 and about the gene products responsible for the malignant transformation by HPVs. The characterization of HPV transcripts will provide information for better understanding of the molecular mechanisms involved in the development of cervical cancers.

HeLa cells derived from a cervical cancer have been demonstrated to contain 10 to 20 copies of HPV type 18 DNA sequences and their two major transcripts of approximately 3.4 and 1.6 kilobases (kb) (27, 32). Recently, cDNA clones for HPV type 18 transcripts in HeLa cells were isolated and partially characterized (25). Information is limited, however, because these clones were not complete enough in size to represent either the 3.4- or 1.6-kb transcript.

We report here the results of analysis of HPV type 18 transcripts in HeLa cells by isolation and characterization of cDNA clones representing each size of HPV type 18 transcript.

MATERIALS AND METHODS

Cells. HeLa cells were cultured in Dulbecco modified Eagle minimum essential medium supplemented with 10% calf serum. SKG-I (17), SKG-II (13), and SKG-IIIb cells (18) were cultured cell lines established from three different cervical cancers and maintained in Ham's F12 medium with 10% heat-inactivated fetal bovine serum. SKG-I and SKG-II cells contained HPV type 18 DNA, while SKG-IIIb cells contained HPV type 16 DNA (32). The cells were cultured at 37°C under a humidified atmosphere of 5% CO_2.

Preparation of poly(A)$^+$ RNA. Semiconfluent HeLa cells were washed with phosphate-buffered saline, and total RNA was prepared by the guanidinium isothiocyanate-CsCl method (6). Poly(A)$^+$ RNA was obtained by passing total RNA through an oligo(dT)-cellulose column (1).

Construction of cDNA library and isolation and characterization of cDNA clones. A cDNA library was constructed with poly(A)$^+$ RNA from HeLa cells by the procedure of Okayama and Berg (19) and screened by colony hybridization with the HPV type 18 genome as a probe. Isolated cDNA clones were characterized by restriction enzyme digestions. Sequence analysis of cDNA clones was done by the dideoxy chain termination sequencing method (22) with M13 cloning vectors (16).

Preparation of DNA and Southern blot hybridization. High-molecular-weight DNAs were extracted by the sodium dodecyl sulfate-proteinase K-phenol-chloroform method (15) from cervical cancer-derived cell lines and human spleen tissue obtained from a patient with hepatocellular carcinoma associated with cirrhosis.

DNAs were digested with *Bam*HI, *Eco*RI, or *Hin*dIII, and 10 μg of each sample was subjected to electrophoresis on a 0.8% agarose gel. After transfer to a nitrocellulose filter by the method of Southern (28), hybridization with a nick-translated ^{32}P-labeled DNA fragment was performed in a solution of 50% formamide, 0.75 M sodium chloride, 0.075 M sodium citrate, 1× Denhardt solution (15), 0.02 M sodium phosphate (pH 6.5), 10% dextran sulfate, and 50 μg of denatured salmon sperm DNA per ml at 42°C for 20 h. The filter was washed three times with a solution of 0.3 M sodium chloride–0.03 M sodium citrate–0.02 M sodium phosphate (pH 7.0)–0.06% sodium PP_i–0.05% sodium dodecyl sulfate at 52°C. Then it was exposed to Kodak XAR-5 film with an intensifying screen at −80°C for 12 to 24 h.

Northern (RNA) blot hybridization. Poly(A)$^+$ RNA (5 μg) was electrophoresed through a 1.0% agarose gel containing 14.8% formaldehyde, transferred to a nitrocellulose filter,

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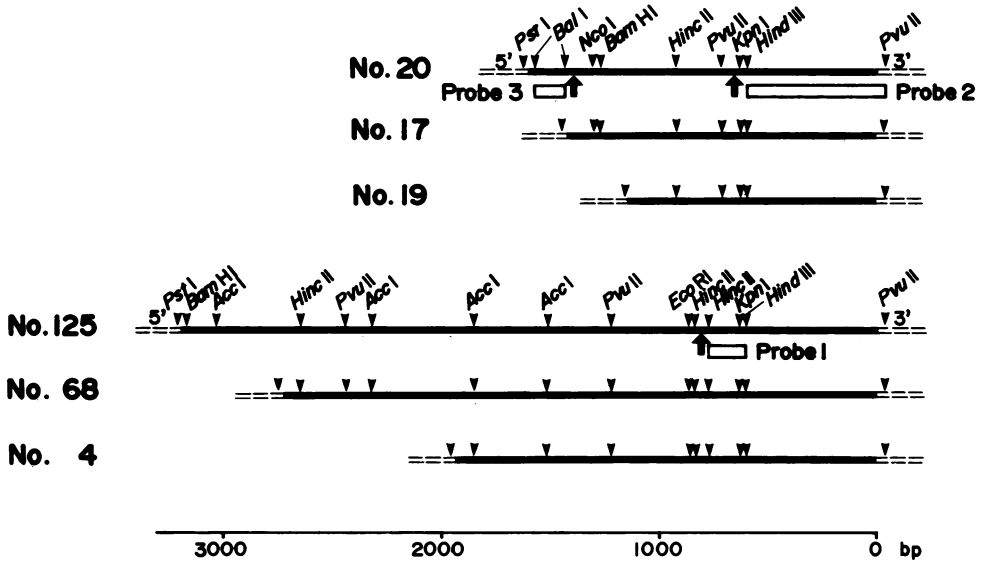

FIG. 1. Schematic presentation of the representative cDNA clones for HPV type 18 transcripts in HeLa cells. Linear maps of cDNA clones 20, 17, and 19 for the 1.6-kb transcript and clones 125, 68, and 4 for 3.4-kb transcript are shown with cleavage sites of restriction endonucleases. Solid bars and dashed bars indicate cDNAs and parts of cloning vectors, respectively. The junctions of viral and human sequences in clones 20 and 125 are shown by arrows. Fragments of human specific sequences in clone 125 (*Hin*cII-*Hin*dIII) and in clone 20 (*Hin*dIII-*Pvu*II and *Bal*I-*Bal*I) are designated probe 1 and probes 2 and 3, respectively, and are indicated by open boxes. bp, Base pairs.

and then hybridized with a probe as described above for Southern blot hybridization.

RESULTS

Isolation of cDNA clones. Fifty-two clones containing HPV type 18 DNA were isolated from a cDNA library by screening 100,000 colonies. By restriction enzyme analysis, the clones could be classified into two types: one represented a 1.6-kb HPV type 18 transcript and the other represented a 3.4-kb transcript. Some of the representative cDNA clones, numbers 20, 17, and 19 for the 1.6-kb transcript and numbers 125, 68, and 4 for the 3.4-kb transcript, are illustrated schematically in Fig. 1.

Characterization of 1.6-kb transcript. Clone 20 included 1,525 nucleotides and consisted of three portions: 225 nucleotides of human sequence in the 5′ end, 724 nucleotides of viral sequence, and 576 nucleotides of another human sequence in the 3′ end. By comparing the nucleotide sequence of clone 20 with the HPV type 18 genomic sequence which was reported recently (7), we were able to detect the two junctions of viral and human sequences in clone 20. One was located 2 bases upstream of the first TATA box in the 5′-flanking sequence of E6, corresponding to nucleotide 24 of the HPV type 18 genomic sequence numbering system (7), and the other was located in the beginning portion of E1, corresponding to nucleotide 929 (7) (Fig. 2a).

Clone 20 had two open reading frames in its HPV type 18-derived portion that coded for 57 and 105 amino acids, respectively. Using the viral genomic sequence, we found the former to be a spliced and frameshifted E6, which had been designated E6* by Schneider-Gädicke and Schwarz (25), and the latter to be the entire E7 (Fig. 3). The clone contained both of the two viral TATA boxes upstream of E6. To determine whether the human flanking sequence in the 5′ portion of clone 20 was commonly found in another cDNA clone representing the 1.6-kb transcript, we also analyzed the partial sequence of clone 17, which was 0.2 kb shorter than clone 20 but showed the same restriction enzyme map of the viral and 3′ human flanking sequences (Fig. 1). The 5′ human flanking sequence of clone 20 was not present in clone 17, and clone 17 had 23 additional nucleotides of HPV type 18 sequence upstream of the human and viral junction of clone 20 (data not shown). It also contained both of the two viral TATA boxes in the noncoding region upstream of E6. The common sequence of clones 20 and 17 is presented in Fig. 2a.

The consensus sequences of splice donor and acceptor (5) were found in the E6 region of the HPV type 18 genome (7). The sequence from nucleotide 234 to 415 of the HPV type 18 genome was spliced out, resulting in the E6* open reading frame in clone 20. There was another splice donor consensus sequence in the beginning portion of the E1 region, and at this position (nucleotide 929), the viral sequence of clone 20 probably spliced to human flanking sequence, generating the virus-human junction. There was a poly(A) addition signal, AATAAA, at the end of the 3′ human flanking sequence in clone 20.

Characterization of 3.4-kb transcript. Clone 125 included 3,135 nucleotides, and its 5′ end was just at the first ATG in the E6 open reading frame (nucleotide 105), indicating that clone 125 was nearly a full-sized cDNA for the 3.4-kb transcript. It contained three open reading frames: entire E6, entire E7, and the 5′ portion of E1 (ΔE1). There was no splicing or frameshift within the E6 and E7 open reading frames in clone 125, and they coded for 158 and 105 amino acids, respectively (Fig. 3). Using the genomic sequence of the E1 region of HPV type 18, we found that the junction of viral and human sequences in clone 125 was located next to nucleotide 2497 of the HPV type 18 genomic sequence (7), that is, 1,583 bases downstream of the first ATG in the E1 open reading frame. With the stop codon (TAA) at the beginning of the 3′ human flanking sequence, the E1 open reading frame ended just at this junction (Fig. 2b). The

FIG. 2. Nucleotide sequences and derived amino acid sequences of representative cDNA clones for the 1.6-kb transcript and the 3.4-kb transcript. The common sequence of clones 20 and 17, starting from the 5′ human-virus junction of clone 20 (a), and the entire sequence of clone 125 (b) are presented. The open reading frames E6, E6*, E7, and the 5′ portion of E1 (ΔE1) are underlined. Two TATA boxes in the noncoding region upstream of E6 and poly(A) addition signals in the 3′ end portions of both transcripts are indicated by open boxes and stippled boxes, respectively. Wavy lines indicate the tetrapeptide sequence Cys-X-X-Cys in the E6, E6*, and E7 open reading frames. The junctions of viral and human sequences in both transcripts are shown by arrowheads. Nucleotide positions 1 and 724 in panel a correspond to nucleotides 24 and 929, respectively, in the HPV type 18 genomic sequence (7), and nucleotide positions 1 and 2393 in panel b correspond to nucleotides 105 and 2497, respectively, in the HPV type 18 genomic sequence.

consensus sequences of splice donor and acceptor were not found at the junction.

Clone 125 contained 742 nucleotides of human flanking sequence with a poly(A) addition signal, AATAAA. Its 3′ part (571 nucleotides) was completely identical to that of clone 20, except that there were five more nucleotides upstream of the poly(A) addition site of clone 20. There was no open reading frame in the human flanking sequence in the 3′ portion of either clone.

In comparison with the genomic sequence of the virus, there were 10 base substitutions in the two types of HPV type 18 transcripts in HeLa cells. Three of these substitutions were in E6, two were in E7, and the remaining five were in E1. Two base substitutions resulted in amino acid changes: one from Glu to Lys at amino acid 73 in E7, and the other from Thr to Lys at amino acid 94 in E1.

The E6 open reading frame contained a fourfold repetition of the tetrapeptide sequence Cys-X-X-Cys, which was highly conserved in bovine papillomavirus type 1 and other types of HPVs, such as 1a, 6b, 11, and 16 (7, 10, 11), while the E6* open reading frame contained only one such tetrapeptide sequence. The E7 open reading frame also contained a twofold repetition of Cys-X-X-Cys (Fig. 2). Possible N-linked glycosylation sites (Asn-X-Thr/Ser) (12) were present in E6 (Asn-22), E6* (Asn-22), and E1 (Asn-39, Asn-176, Asn-177, Asn-251, Asn-335, Asn-428, and Asn-510). None of the open reading frames (E6, E6*, E7, and the 5′ portion of E1) had a leader sequence, ATP-binding site, kinase domain, GTP-binding site, or hydrophobic region characteristic of the transmembrane domain. A computer search with the GenBank nucleic acid data base and the National Biomedical Research Foundation protein data base revealed no homologous or related protein sequence for these open reading frames, except for the products of other types of papillomaviruses. The E6, E7, and 5′ portion of the E1 open reading frames of HPV type 18 at the protein level had 23, 24, and 30% homology, respectively, with those of HPV type 1a and 56, 39, and 54% homology, respectively, with those of HPV type 16. We could not find any nucleotide sequence homologous to the 5′ or 3′ human flanking sequence in clones 20 and 125 using the GenBank nucleic acid data base.

Southern blot hybridization and Northern blot hybridization with human flanking sequences. Human flanking sequences in clones 125 and 20 were subcloned and used as probes for hybridization, and they are schematically shown in Fig. 1. Southern blot hybridization analysis with the 3′ cellular flanking sequences as probes is shown in Fig. 4. They hybridized to *Bam*HI-, *Eco*RI-, or *Hin*dIII-digested human DNAs, including other cervical cancer-derived cell lines and human spleen tissue. The *Hin*cII-*Hin*dIII fragment of clone 125 (probe 1) hybridized to a single band, while hybridization with the *Hin*dIII-*Pvu*II fragment of clone 20 (probe 2) showed a definite band with some weaker bands and smears. The *Bal*I-*Bal*I fragment of clone 20 (probe 3) also hybridized to human DNAs but not to HPV type 18 DNA (data not shown). Northern blot analysis of poly(A)$^+$ RNA from HeLa cells revealed that probe 2 hybridized to both the 3.4- and 1.6-kb mRNAs, while probe 1 hybridized to only the 3.4-kb mRNA (Fig. 5). Probe 3 also hybridized to poly(A)$^+$ RNA from HeLa cells; the sizes of the two major bands were approximately 3.4 and 1.6 kb, respectively (Fig. 5). They were not expressed as mRNA, however, in any other cervical cancer-derived cell lines (SKG-I, SKG-II, or SKG-IIIb) (data not shown).

DISCUSSION

cDNA clones were isolated that represented 3.4- and 1.6-kb transcripts of the HPV type 18 genome in HeLa cells.

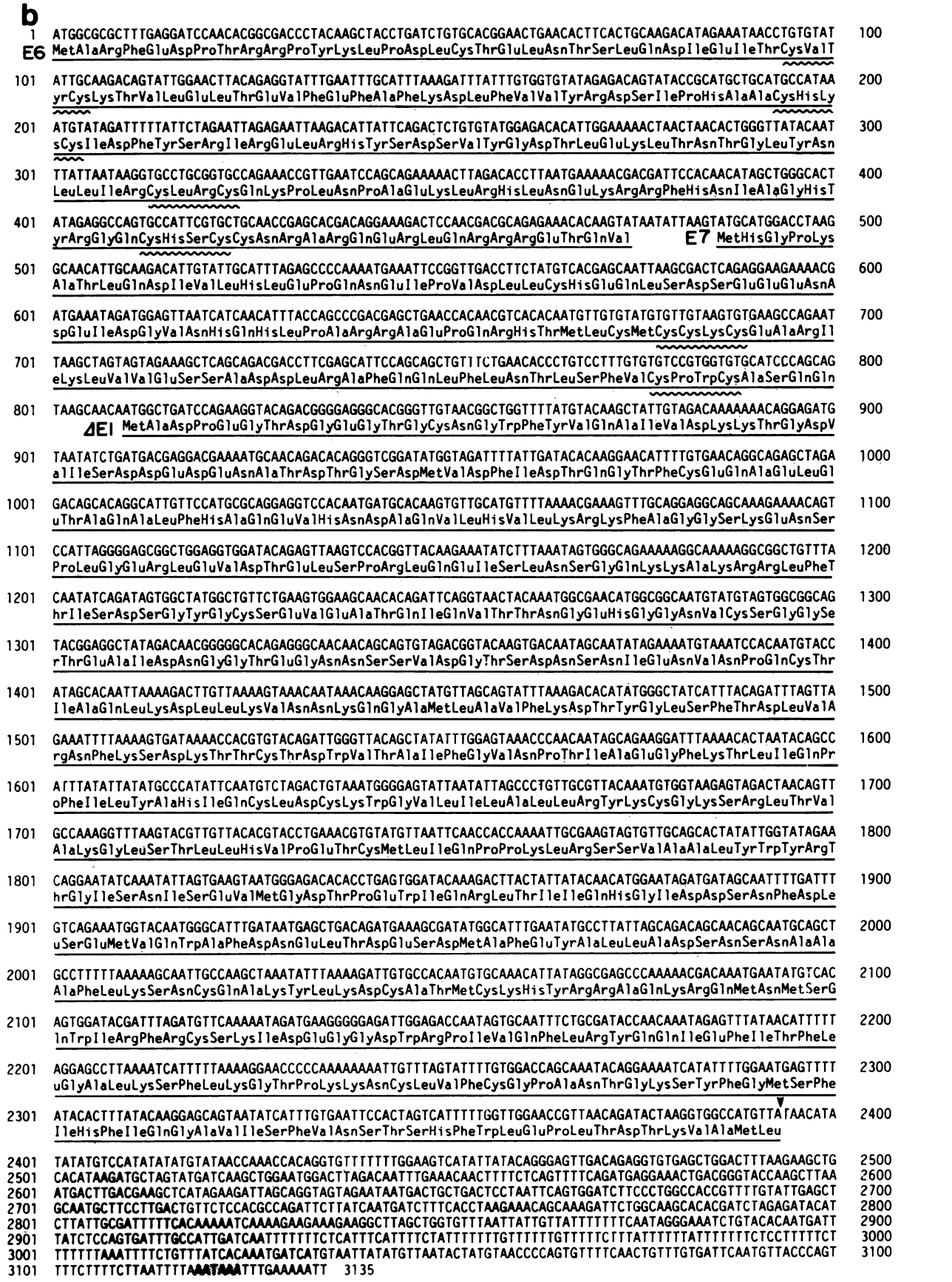

FIG. 2—*Continued.*

Sequence analysis of these two types of cDNA clones revealed that clone 20 representing the 1.6-kb transcript contained E6* and E7, whereas clone 125 representing the 3.4-kb transcript had E6, E7, and the 5′ portion of E1.

There have been several reports that the E6 and E7 regions were consistently present and that the E6 region was expressed as mRNA in cervical cancer tissues and cervical cancer-derived cell lines containing HPV type 16 or 18 (2, 14, 20, 27, 29). Furthermore, we previously demonstrated that the E6 region was present and expressed as mRNA in NIH 3T3 primary transformants induced by transfection of HPV type 16 DNA integrated into the genomic DNA of a cervical cancer (31). The E5 and E6 regions of bovine papillomavirus have transforming activity toward C127 and NIH 3T3 cells upon transfection (23, 24, 33, 34). It is likely that E6 and E7 and/or their surrounding regions are important in the development of cervical cancers. The present result showing that HPV type 18 transcripts in HeLa cells contain E6, E6*, and E7 is consistent with these previously reported results.

It was reported previously that both E6 and E6* were transcribed in SW 756 and C4-1 cells containing HPV type 18 sequences but that only cDNA clones containing E6* were isolated from HeLa cells (25). In contrast, our results reported here clearly showed that not only E6* but also genomic E6 was transcribed in HeLa cells. It was also revealed that the E6 open reading frame contained a fourfold repetition of Cys-X-X-Cys, which was highly conserved in bovine papillomavirus type 1 and other types of HPVs and was speculated to be involved in DNA binding (3, 7, 10), while only one repetition unit remained in the E6* open reading frame. It should be determined whether genomic E6 or E6* has an important role in the development of cervical cancers or in maintaining the phenotype of cervical cancers.

The present results also showed that HPV type 18 transcripts contained cellular flanking sequences. Clone 20 had two human flanking sequences in its 5′ and 3′ portions. The cell-virus and the virus-cell junctions were 2 bases upstream of the first TATA box in the 5′-flanking sequence of E6 and in the beginning portion of E1, respectively. Clone 17, another cDNA clone representing the 1.6-kb transcript, had the same restriction map as clone 20, but was 0.2 kb shorter in the 5′ end. The sequence of the 5′ portion of clone 17 was identical to that of clone 20 up to the human-virus junction. However, clone 17 contained 23 additional nucleotides of HPV type 18 sequence in place of human sequence. It remains to be determined whether clones 20 and 17 represent different forms of 1.6-kb HPV transcripts or whether the flanking human sequence in the 5′ end of clone 20 is an artifact occurring during cDNA cloning. It is, however, more likely that clones 20 and 17 represent different forms of 1.6-kb HPV mRNAs with the difference being in the 5′ end, since smaller size viral mRNA of around 1.6 kb was detected as a broad band by Northern blot hybridization with HPV type 18 genome as a probe, suggesting heterogeneity of this mRNA species. The two nucleotides in the HPV type 18 genomic sequence upstream of the 5′ human-virus junction in clone 20 are adenine and guanine, suggesting also that the junction corresponds to a splice site and is not a result of a cloning artifact. In any event, both clones 20 and 17 contain

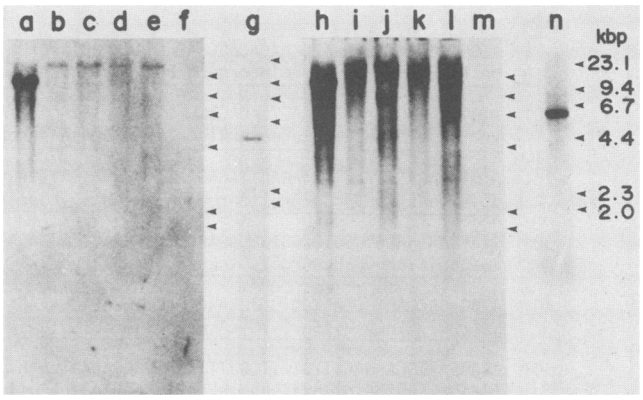

FIG. 4. Southern blot analysis of cellular DNAs from cervical cancer cell lines and human spleen tissue with the 3′-flanking sequences as probes. High-molecular-weight cellular DNAs from HeLa (lanes a and h), SKG-I (lanes b, g, i, and n), SKG-II (lanes c and j), and SKG-IIIb (lanes d and k) cells and human spleen tissue (lanes e and l) were digested with *Bam*HI (lanes a to e and h to l), *Eco*RI (lane g), or *Hin*dIII (lane n). Southern blot hybridization was done with 10-μg samples of DNAs with the following fragments of the 3′ cellular flanking sequences: probe 1 (lanes a to g) and probe 2 (lanes h to n) (probes are schematically shown in Fig. 1). The HPV type 18 genome was also subjected to hybridization with each probe (lanes f and m). kbp, Kilobase pairs.

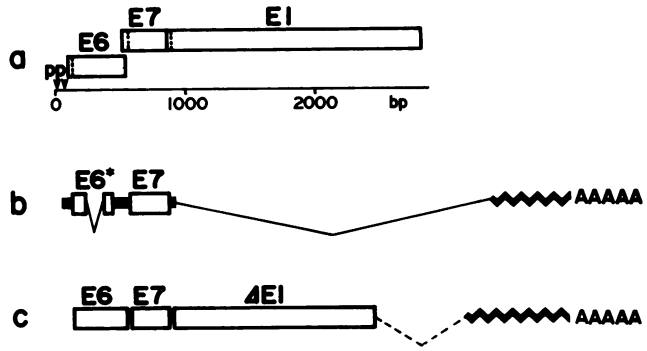

FIG. 3. Splicing pattern of HPV type 18 transcripts in HeLa cells. The 5′ portion of the early regions of the HPV type 18 genome (7) (a), 1.6-kb transcript (b), and 3.4-kb transcript (c) are schematically presented. The open reading frames E6, E6*, E7, and the 5′ portion of E1 (ΔE1) are indicated by open boxes. The first ATG in each open reading frame is shown by a dotted line in the box. Two TATA boxes (p) upstream of the E6 region are indicated by arrowheads. Zig-zag lines show flanking cellular sequences. bp, Base pairs.

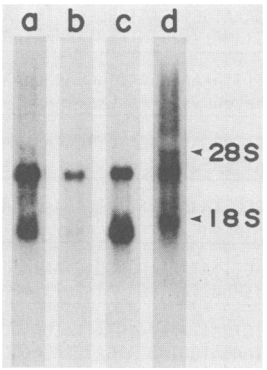

FIG. 5. Northern blot analysis of RNA for the transcripts of HPV type 18 and human flanking sequences in HeLa cells. Samples of 5 μg of poly(A)$^+$ RNA were subjected to blot hybridization with the HPV type 18 genome (lane a), probe 1 (lane b), probe 2 (lane c), and probe 3 (lane d) (schematically shown in Fig. 1).

two viral TATA boxes in the noncoding region upstream of E6. It was reported that the major HPV mRNA start site in HeLa cells was around the first ATG in the E6 open reading frame by primer extension analysis (25) and RNase protection analysis (30), and additional minor upstream start sites were also observed (25). It is difficult to determine from these experiments whether the viral or the cellular promoter is used for HPV transcription in HeLa cells. Two aspects of our results strongly suggest that a cellular promoter is utilized for HPV transcription in HeLa cells. First, the viral TATA boxes in the noncoding region upstream of E6 were included in cDNA sequences of clones 20 and 17. Second, the sizes of viral transcripts detected by Northern blot hybridization, corresponding to 1.6 and 3.4 kb, were considerably larger than those predicted if the two types of HPV transcripts had started just upstream of the first ATG of the E6 region, using viral promoters in the noncoding region upstream of E6, with the predicted sizes being less than 1.3 and 3.2 kb, respectively. In addition, Southern blot analysis showed that the genomic HPV sequence in HeLa cells lacked the 5′ end portion of the L2 region where another TATA box was located (25), further suggesting the utilization of a cellular promoter.

Since the 5′ end of clone 125 was just at the first ATG in the E6 open reading frame, we could not determine whether the 3.4-kb transcript utilizes the viral or the cellular promoter for its transcription. It is still possible, however, that the 3.4-kb transcript also contains 5′ human flanking sequence homologous to or identical with that of clone 20 and utilizes a cellular promoter, because probe 3 hybridized to mRNA with a size of approximately 3.4 kb (Fig. 5).

Clone 125 contained 742 nucleotides of human flanking sequence, and its 3′ portion (571 nucleotides) was completely identical with that of clone 20, except that there were five more nucleotides upstream of the poly(A) addition site of clone 20. It was reported that HPV type 18 DNA sequences in HeLa cells were located on chromosomes 5, 8, 9, and 22 (21). The presence of the common human sequence, together with the finding that there were consensus sequences of splice donor and acceptor at the splice site in E6* and at the 3′ virus-human junction of clone 20, indicated that both the 1.6- and 3.4-kb mRNAs were transcribed from the HPV genome at the same integration site with different splicing. Since the consensus sequences of splice donor and acceptor were not found at the virus-human junction of clone 125, it is suggested that this junction corresponds to the genomic virus-human junction and is not a result of splicing.

Although there was no open reading frame within the 3′ cellular flanking sequences of both clones, there were poly(A) addition signals in their 3′ ends, suggesting that they play a role in the stabilization of viral mRNA. The poly(A) addition site of clone 20 was 5 bases downstream from that of clone 125, although both clones utilized the same poly(A) addition signal.

The human flanking sequences hybridized to DNAs from human spleen tissue and other cervical cancer-derived cell lines containing HPV type 16 or 18 sequence. Both probes 1 and 2 were unique sequences and hybridized to a single band. Probe 2 also showed additional weaker bands and smears because it contained A+T-rich sequence and might hybridize to cellular DNAs nonspecifically. The 3′ human flanking sequences, probes 1 and 2, did not hybridize to the bands containing HPV type 16 or 18 DNA and were not expressed as mRNA in any other cervical cancer-derived cell lines. It is very unlikely that direct activation of specific cellular flanking genes occurs by insertion of HPV type 18 DNA sequences into the common integration site in cervical cancers. It was reported that one of the HPV type 18 integration sites is within 40 kb 5′ of the c-*myc* gene and that the c-*myc* mRNA levels are elevated in HeLa cells (8). HPV type 18 DNA sequences in HeLa cells were reported to be integrated into several chromosomes (21). It is yet to be determined whether the genomic sequence of HPV type 18 transcribed as mRNA in HeLa cells is located within 40 kb upstream of the c-*myc* gene.

There has been increasing evidence that more than 50% of cervical cancer tissues contain HPV type 16 or 18 sequence. There is no indication, however, of involvement of HPV type 16 and 18 DNA in the development of cervical cancers, except that the dimer of the HPV type 16 genome and HPV type 16 DNA integrated into genomic DNA of a cervical cancer could transform NIH 3T3 cells. The characterization of the HPV type 18 products based on the present sequence analysis data will certainly lead to better understanding of the molecular mechanisms involved in the development of cervical cancers.

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LITERATURE CITED

1. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA **69**:1408–1412.
2. **Baker, C. C., W. C. Phelps, B. Lindgren, M. J. Braun, M. A. Gouda, and P. M. Howley.** 1987. Structural and transcriptional analysis of human papillomavirus type 16 sequences in cervical carcinoma cell lines. J. Virol. **61**:962–971.
3. **Berg, J. M.** 1986. Potential metal-binding domains in nucleic acid binding proteins. Science **232**:485–487.
4. **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.
5. **Breathnach, R., and P. Chambon.** 1981. Organization and expression of eucaryotic split genes coding proteins. Annu. Rev. Biochem. **50**:349–383.
6. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**:5294–5299.
7. **Cole, S. T., and O. Danos.** 1987. Nucleotide sequence and comparative analysis of the human papillomavirus type 18 genome. Phylogeny of papillomaviruses and repeated structure of the E6 and E7 gene products. J. Mol. Biol. **193**:599–608.
8. **Dürst, M., C. M. Croce, L. Gissmann, E. Schwarz, and K. Huebner.** 1987. Papillomavirus sequences integrate near cellular oncogenes in some cervical carcinomas. Proc. Natl. Acad. Sci. USA **84**:1070–1074.
9. **Dürst, M., L. Gissmann, H. Ikenberg, and H. zur Hausen.** 1983. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. Proc. Natl. Acad. Sci. USA **80**:3812–3815.
10. **Giri, I., and O. Danos.** 1986. Papillomavirus genomes: from sequence data to biological properties. Trends Genet. **2**:227–232.
11. **Howley, P. M., Y. Yang, and M. S. Rabson.** 1984. The molecular biology of bovine papillomaviruses, p. 67–81. *In* P. W. J. Rigby and N. M. Wilkie (ed.), Symposium of the Society for General Microbiology. Viruses and cancer. Cambridge University Press, Cambridge.

12. **Hubbard, S. C., and R. J. Ivatt.** 1981. Synthesis and processing of asparagine-linked oligosaccharides. Annu. Rev. Biochem. **50**:555–583.
13. **Ishiwata, I., S. Nozawa, K. Kiguchi, S. Kurihara, and H. Okumura.** 1978. Establishment of human uterine cervical cancer cell line and comparative studies between normal and malignant uterine cervical cells *in vitro*. Acta Obstet. Gynaecol. Jpn. **30**:731–738.
14. **Lehn, H., P. Krieg, and G. Sauer.** 1985. Papillomavirus genomes in human cervical tumors: analysis of their transcriptional activity. Proc. Natl. Acad. Sci. USA **82**:5540–5544.
15. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–78.
17. **Nozawa, S., K. Tsukazaki, Y. Udagawa, I. Ishiwata, H. Ohta, S. Kurihara, and H. Okumura.** 1982. Human cell line (SKG-I) derived from epidermoid cancer of the cervix, p. 142–151. *In* E. S. E. Hafez and J. P. Smith (ed.), Carcinoma of the cervix: biology and diagnosis. Martinus Nijhoff Publishers, Amsterdam.
18. **Nozawa, S., Y. Udagawa, H. Ohta, S. Kurihara, and W. H. Fishman.** 1983. Newly established uterine cervical cancer cell line (SKG-III) with Regan isoenzyme, human chorionic gonadotropin β-subunit, and pregnancy-specific β_1-glycoprotein phenotypes. Cancer Res. **43**:1748–1760.
19. **Okayama, H., and P. Berg.** 1982. High-efficiency cloning of full-length cDNA. Mol. Cell. Biol. **2**:161–170.
20. **Pater, M. M., and A. Pater.** 1985. Human papillomavirus types 16 and 18 sequences in carcinoma cell lines of the cervix. Virology **145**:313–318.
21. **Popescu, N. C., J. A. DiPaolo, and S. C. Amsbaugh.** 1987. Integration sites of human papillomavirus 18 DNA sequences on HeLa cell chromosomes. Cytogenet. Cell Genet. **44**:58–62.
22. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
23. **Sarver, N., M. S. Rabson, Y. Yang, J. C. Byrne, and P. M. Howley.** 1984. Localization and analysis of bovine papillomavirus type 1 transforming functions. J. Virol. **52**:377–388.
24. **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.
25. **Schneider-Gädicke, A., and E. Schwarz.** 1986. Different human cervical carcinoma cell lines show similar transcription patterns of human papillomavirus type 18 early genes. EMBO J. **5**:2285–2292.
26. **Scholl, S. M., E. M. K. Pillers, R. E. Robinson, and P. J. Farrell.** 1985. Prevalence of human papillomavirus type 16 DNA in cervical carcinoma samples in East Anglia. Int. J. Cancer **35**:215–218.
27. **Schwarz, E., U. K. Freese, L. Gissmann, W. Mayer, B. Roggenbuck, A. Stremlau, and H. zur Hausen.** 1985. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. Nature (London) **314**:111–114.
28. **Southern, E. M.** 1975. Detection of specific DNA sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
29. **Takebe, N., Y. Tsunokawa, S. Nozawa, M. Terada, and T. Sugimura.** 1987. Conservation of E6 and E7 regions of human papillomavirus types 16 and 18 present in cervical cancers. Biochem. Biophys. Res. Commun. **143**:837–844.
30. **Thierry, F., J. M. Heard, K. Dartmann, and M. Yaniv.** 1987. Characterization of a transcriptional promoter of human papillomavirus 18 and modulation of its expression by simian virus 40 and adenovirus early antigens. J. Virol. **61**:134–142.
31. **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.
32. **Tsunokawa, Y., N. Takebe, S. Nozawa, T. Kasamatsu, L. Gissmann, H. zur Hausen, M. Terada, and T. Sugimura.** 1986. Presence of human papillomavirus type-16 and type-18 DNA sequences and their expression in cervical cancers and cell lines from Japanese patients. Int. J. Cancer **37**:499–503.
33. **Yang, Y., H. Okayama, and P. M. Howley.** 1985. Bovine papillomavirus contains multiple transforming genes. Proc. Natl. Acad. Sci. USA **82**:1030–1034.
34. **Yang, Y., B. A. Spalholz, M. S. Rabson, and P. M. Howley.** 1985. Dissociation of transforming and *trans*-activation functions for bovine papillomavirus type 1. Nature (London) **318**:575–577.
35. **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.
36. **Yoshikawa, H., T. Matsukura, E. Yamamoto, T. Kawana, M. Mizuno, and K. Yoshiike.** 1985. Occurrence of human papillomavirus types 16 and 18 DNA in cervical carcinomas from Japan: age of patients and histological type of carcinomas. Jpn. J. Cancer Res. (Gann) **76**:667–671.