Transforming Activity of E5a Protein of Human Papillomavirus Type 6 in NIH 3T3 and C127 Cells

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Human papillomavirus type 6 (HPV-6) is the etiologic agent of genital warts and recurrent respiratory papillomatosis. We are investigating the mechanism by which this virus stimulates cell proliferation during infection. In this paper, we report that the E5a gene of HPV-6c, an independent isolate of HPV-11, is capable of transforming NIH 3T3 cells. The E5a open reading frame (ORF) was expressed under the control of the mouse metallothionein promoter in the expression vector pMt.neo.1, which also contains the gene for G418 resistance. Transfected cells were selected for G418 resistance and analyzed for a transformed phenotype. The transformed NIH 3T3 cells overgrew a confluent monolayer, had an accelerated generation time, and were anchorage independent. In contrast, E5a did not induce foci in C127 cells, but C127 cells expressing E5a did form small colonies in suspension. The presence of the 12-kilodalton E5a gene product in the transformed NIH 3T3 cells was shown by immunoprecipitation and was localized predominantly to nuclei by an immunoperoxidase assay. A mutation in the E5a ORF was engineered to terminate translation. This mutant was defective for transformation, demonstrating that translation of the E5a ORF is required for biological activity. This is the first demonstration of a transforming oncogene in HPV-6, and the differential activity of E5a in these two cell lines should facilitate future investigations on the mechanism of transformation.

Human papillomaviruses (HPV) are naturally occurring DNA tumor viruses that induce epithelial cell proliferation during a productive infection. In the United States, an estimated one million cases of genital warts occur annually among sexually active individuals (9). The majority of these lesions are a result of HPV type 6 (HPV-6) infection (for a review, see reference 49). HPV-6 also causes juvenile-onset and adult-onset respiratory tract papillomatosis (20, 33). Respiratory papillomata are noninvasive proliferative lesions of the respiratory epithelium occurring as a solitary lesion of minimal clinical consequence or as recurring disease with life-threatening potential. The incidence of recurrent respiratory papillomatosis has been estimated at 1,500 cases, with more than 50% of the cases occurring in children less than 3 years of age (48).

HPV-6 is generally associated with benign proliferative lesions that infrequently progress to cancer. In contrast, some other HPVs, including types 16 and 18, are associated with malignant lesions (for a review, see reference 49). In genital tract cancers and carcinoma-derived cell lines, the E6 and E7 open reading frames (ORFs) are usually intact and transcriptionally active (for a review, see reference 54), and transforming activity in vitro by HPV-16 and -18 has been associated with the E6 and E7 ORFs (3, 25, 26, 39, 47, 51). HPV-6 has not been reported as being active in transformation assays (for a review, see references 24 and 29), but an isolate from an invasive squamous carcinoma of the vulva was reported as being capable of morphologically transforming mouse fibroblasts in culture (27). In HPV-6-induced papillomata, the predominant viral transcript contains 150 nucleotides from the E7 and E1 ORFs spliced with sequences from the 3' ends of the E2 and E4 ORFs as well as the E5a and E5b ORFs (13, 34, 52). Therefore, this transcript encodes an E1-E4 fusion protein, which has been detected in cutaneous warts (5, 16-18), as well as E5a and E5b proteins,

which we have detected in respiratory papillomata induced by HPV-6 (12).

We are investigating the role of HPV-6 gene products in the viral life cycle by examining their biological and biochemical activities. To explore the role of E5a in stimulating cell proliferation, we tested its transforming activity in culture. The predicted amino acid (aa) sequence of HPV-6 E5a protein shares structural similarities with that of the E5 protein of bovine papillomavirus type 1 (BPV-1), which has been shown to transform cells in culture (15, 46). Our results reported here identify the product of the E5a ORF of HPV-6 as a nuclear oncoprotein.

MATERIALS AND METHODS

Plasmid construction. The expression vector pMtE5a-b.neo was constructed by digesting pSLa1 (S.-L. Chen and P. Mounts, unpublished data) with *Hind*III and transferring the 0.9-kilobase (kb) fragment to the 6.7-kb plasmid pMt.neo.1 (37), thus placing the E5a and E5b ORFs under the control of the mouse metallothionein promoter (Fig. 1). The recombinant plasmid pMtE5a-b.neo also carries resistance to the aminoglycoside antibiotic G418 under the control of the herpesvirus thymidine kinase promoter. The plasmid pMtE5a.neo, with only the E5a ORF, was constructed by digesting pMtE5a-b.neo with *Hpa*I and circularizing the largest fragment by using T4 DNA ligase (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) to remove two fragments, one of which contains the E5b ORF.

Construction of frameshift mutation. A mutation in the E5a ORF was engineered to terminate translation. The plasmid pSLE5a-1 (Fig. 2) was constructed by digesting pMtE5a.neo with *Eco*RI and *Hpa*I and transferring the 0.5-kb fragment containing the E5a ORF into the 2.9-kb pBluescript KS+ vector (Stratagene, La Jolla, Calif.). For mutagenesis, 1 µg of pSLE5a-1 DNA was digested with *Pst*I and four 3'-unpaired nucleotides were removed with the Klenow fragment of DNA polymerase I (Bethesda Research Laborato-

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FIG. 1. Structure of the recombinant plasmid pMtE5a-b.neo. At the top of the figure is shown the arrangement of the ORFs of HPV-6c. As described in Materials and Methods, a fragment containing the E5a (solid black region) and E5b (hatched region) ORFs was cloned in the expression vector pMt.neo.1 under the control of the metallothionein (MT) promoter (stippled region). This vector (37) also contains the simian virus 40 (SV40) small t-antigen splice site and polyadenylation signals, G418 resistance gene, and bacterial sequences for the origin of replication (ori) and ampicillin resistance (Ap^R). The positions of relevant restriction enzyme sites are indicated by arrows.

ries) before ligation to circles by T4 DNA ligase. Transformed bacteria were selected for ampicillin resistance, and DNA from rapid plasmid preparations was screened for resistance to digestion by *Pst*I. The DNA was sequenced by the dideoxynucleotide method (42) as modified for double-stranded plasmids (11), using [35 S]dATP (4) to characterize the mutation. The mutated 0.5-kb *Hind*III-*Xba*I fragment was transferred into pMt.neo.1 to create pMtE5aM1.neo.

Cell culture and DNA transfection. NIH 3T3 cells and C127 cells were maintained as monolayer cultures in RPMI 1640 medium and modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.), respectively, with 10% fetal bovine serum (Hyclone, Logan, Utah). Approximately 5×10^4 cells were transfected in 60-mm dishes with $5 \mu\text{g}$ of plasmid DNA (10) purified by cesium chloride density gradient centrifugation (36) and split 1:4 after 24 h. At 24 h after replating, the cells were selected for resistance to G418 (200 $\mu\text{g}/\text{ml}$; GIBCO). After 3 to 4 weeks, the numbers of colonies were counted and scored as being transformed if they grew as foci of dense, multilayered cells. Selection in G418 was done in the absence of elevated levels of ZnSO_4 because this combination was toxic to the cells. However, the metallothionein promoter has a basal activity in the absence of Zn^{2+} , and a low level of expression would be expected.

Growth curve and anchorage independence assay. The doubling times and saturation densities were determined

from growth curves as described previously (40). Cells were plated in 35-mm dishes, and the number of cells was counted daily. The doubling time was calculated during log-phase growth, and cells reached saturation density after 9 days. For colony formation in suspension, 60-mm dishes were prepared with a bottom layer of 0.5% agarose (Bethesda Research Laboratories) in modified Eagle medium containing 10% fetal bovine serum (MEM-10). Duplicate dishes were seeded with 8×10^3 cells suspended in 0.25% agarose in MEM-10. Plates were fed twice weekly and scored for colony formation after 3 to 4 weeks.

Southern hybridization. Total cellular DNA was extracted from transformed cells (32). DNA samples (10 μg) were digested with *Nru*I or *Bam*HI, size fractionated by 1% agarose gel electrophoresis, and transferred to nitrocellulose (32). For ^{32}P labeling by nick translation, the E5a and E5b sequences were separated from the vector by digestion of pMtE5a-b.neo with *Hind*III and recovery after agarose gel electrophoresis. Hybridization and washing were as described previously (32). Filters were exposed with intensification.

Immunoprecipitation assays. For immunoprecipitation (38), subconfluent cultures of N.E5a-b and NIH 3T3 cells in 10-cm dishes were incubated with methionine-free medium (GIBCO) for 1 h and were labeled for 4 h with [^{35}S] methionine (100 $\mu\text{Ci}/\text{ml}$, 3,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) in methionine-free medium supplemented with 2% fetal bovine serum. Trichloroacetic acid-precipitable counts were determined, and equal counts were incubated on ice with preimmune serum for 1 h on an orbital rotator. Pansorbin (Calbiochem, La Jolla, Calif.) was added for 1 h and centrifuged, and the supernatants were harvested for incubation with anti-E5a serum for 1 h. Antiserum to the HPV-6c E5a-tryptophan E fusion protein was described previously (12). Pansorbin was added for 1 h, and immunocomplexes were collected by centrifugation for 10 min. Pellets were washed three times with RIPA buffer (10 mM Tris hydrochloride [pH 7.4], 0.15 M NaCl, 1% Nonidet P-40, 1% deoxycholate, 0.1% sodium dodecyl sulfate [SDS]), boiled for 5 min in 1 $\times$ sample buffer (100 mM Tris [pH 6.8], 2% SDS, 40% glycerol, 0.004% bromophenol blue), and electrophoresed on a 15% polyacrylamide gel containing SDS (28). The gel was dried and exposed for autoradiography.

Immunoperoxidase assays. For immunoperoxidase assays, N.E5a-b cells in chamber slides were rinsed with phosphate-buffered saline for 10 s and fixed in cold absolute ethanol for 2 min. The avidin-biotin modification was performed essentially as described previously (33) with the anti-E5a serum diluted 1:100. Methylene blue was the counterstain.

RESULTS

E5a expression vectors. To investigate the transforming activity of E5a, we selected the eucaryotic expression vector pMt.neo.1 (37) for two reasons. First, transfected cells containing the recombinant plasmid can be selected for G418 resistance and examined for phenotypic changes. Second, expression of the HPV-6 sequences is regulated by the mouse metallothionein promoter, and elevated levels of expression can be obtained by induction with heavy metals, such as zinc. A fragment of the HPV-6 genome containing the E5a and E5b ORFs was transferred to this vector to generate pMtE5a-b.neo, whose structure is shown in Fig. 1. HPV-6c is an independent isolate of HPV-11 with one

FIG. 2. Structure of the recombinant plasmid pSLE5a-1. As described in Materials and Methods, a fragment containing the E5a ORF (box region) was cloned in pBluescript vector KS+. This vector contains bacterial sequences for the origin of replication (*ori*) and ampicillin resistance (*amp^R*). The positions of relevant restriction enzyme sites are indicated in the pSLE5a-1 map. The DNA and aa sequences of wild-type E5a and a frameshift termination mutant of E5a created by the deletion of four base pairs at the *PstI* site are shown beneath the map. The 91-aa sequence of E5a is shown at the bottom of the figure.

nucleotide difference in the E5a ORF that results in one aa substitution and no base substitutions in the E5b ORF (31). The viral sequences in pMtE5a-b.neo correspond to HPV-11 nucleotides 3624 to 4562 (14). The plasmid pMtE5a.neo was derived from pMtE5a-b.neo as described in Materials and Methods and contains only the E5a ORF corresponding to HPV-11 nucleotides 3624 to 4159.

Focus-forming activity of E5a in NIH 3T3 cells. The plasmids were transfected into NIH 3T3 cells by a modified calcium phosphate precipitation method (10). The transfected cells were placed under G418 selection, and after 3 weeks, G418^r colonies were counted. Table 1 shows the results of three transfections for the two plasmids containing the HPV-6 E5a ORF (pMtE5a-b.neo and pMtE5a.neo), for the vector (pMt.neo.1), and for mock transfection without

plasmid DNA. The numbers given are the number of transformed foci obtained per number of G418^r colonies. As expected, no G418^r colonies were obtained in transfections without plasmid DNA. The G418^r colonies obtained from transfections with the vector pMt.neo.1 consisted of a monolayer of cells. In transfections with plasmids containing the HPV-6 E5a ORF, G418^r colonies were obtained at about the same efficiency as with the vector, but the colonies consisted of multilayers of cells or foci. The focus-forming activity of pMtE5a-b.neo was comparable to that of pMtE5a.neo, with average values of 46 and 54, respectively. Therefore, these results demonstrate that the E5a ORF has transforming activity when assayed in NIH 3T3 cells.

Frameshift termination mutant of E5a. A mutant was constructed to determine whether translation of the E5a ORF is required for transforming activity. A frameshift that results in premature termination was created by mutagenesis at the unique *PstI* site in the E5a ORF as described in Materials and Methods. Four base pairs were deleted at the *PstI* site, which resulted in the deletion of one aa and a frameshift that terminated translation prematurely (Fig. 2). Thus, the mutant E5aM1 encodes a 14-aa peptide of which the first 9 residues are the wild-type sequence. E5aM1 was tested for transforming activity by transferring the *HindIII*-*XbaI* fragment from pSLE5a-1 to the expression vector pMt.neo.1 and transfecting NIH 3T3 cells. The 64 G418-resistant colonies obtained in two independent transfections had a normal morphology and consisted of a monolayer of

TABLE 1. Focus-forming activity of HPV-6 E5a in NIH 3T3 cells

Plasmid transfected	No. of transformed foci/no. of G418 ^r colonies ^a		
	Expt 1	Expt 2	Expt 3
pMtE5a-b.neo	16/16	79/79	43/43
pMtE5a.neo	57/57	25/25	80/80
pMt.neo.1	0/27	0/23	0/45
Mock	0	0	0

^a Numbers indicate the number of colonies with transformed cells and the number of G418^r colonies for three independent experiments.

TABLE 2. Phenotypes of NIH 3T3 cells transformed by HPV-6 E5a

Cell line	Transfected DNA	Morphology	Doubling time (h) ^a	Saturation density (10 ⁶) ^a	Growth in suspension ^b
N.E5a-b	pMtE5a-b.neo	Transformed	20	2.0	156
N.E5a	pMtE5a.neo	Transformed	26	2.2	230
N.Neo	pMt.neo.1	Fibroblast	32	1.0	0

^a Numbers given are averages of duplicate determinations.

^b Duplicate dishes were seeded with 8×10^3 cells, and numbers are average numbers of colonies.

cells. Therefore, the termination mutant E5aM1 is defective for transformation.

Phenotype of NIH 3T3 cells transformed by E5a. The transformed phenotype of NIH 3T3 cells established from pools of G418^r colonies was examined. In addition to determining the doubling time and saturation density, we assayed the cells for the ability to grow in suspension because an accelerated generation time, anchorage-independent growth, and higher saturation densities are characteristics associated with a transformed phenotype (50). NIH 3T3 cells transfected with the plasmids containing the HPV-6 E5a ORF exhibited a transformed phenotype when compared with cells transfected with the vector (Table 2). The cells appeared morphologically transformed, had an accelerated generation time, and grew to higher saturation densities. In

addition, the E5a-transformed NIH 3T3 cells were capable of anchorage-independent growth when assayed for the ability to form colonies in soft agarose. The E5a-transformed cells formed large colonies (Fig. 3A and C), while NIH 3T3 cells containing the vector pMt.neo.1 did not grow in soft agarose (Fig. 3B).

Analysis of E5a in transformed NIH 3T3 cells. DNA was isolated from the morphologically transformed cell lines to demonstrate the presence of E5a sequences. The DNA was digested with either *Nru*I, which does not cut either pMtE5a-b.neo or pMtE5a.neo, or *Bam*HI, which cuts the plasmids once. Figure 4 shows the results of the hybridization analysis. Multiple bands of hybridization were observed, which is consistent with different integration sites in the pooled colonies. No evidence for extrachromosomal plasmid DNA was observed when DNA samples were digested with an enzyme that does not cut the plasmid DNA, such as *Nru*I (lanes a and b), or when the DNA samples were undigested.

To analyze the protein produced from the E5a ORF, the transformed NIH 3T3 cells were metabolically labeled with [³⁵S]methionine and the E5a protein was immunoprecipitated from extracts with anti-E5a serum (12). The immunoprecipitated proteins were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Figure 5 shows a band of 12 kilodaltons in the transformed N.E5a-b cells (lane b) that is not present in the parental NIH 3T3 cells (lane c). The size is in good agreement with the predicted size of the protein coded by the E5a ORF of 11.6 kilodaltons. The 12-kilodalton band was also present in the transformed

FIG. 3. Anchorage-independent growth of HPV-6 E5a-transformed cells. Transfected NIH 3T3 and C127 cells were assayed for colony formation in soft agarose as described in Materials and Methods. (A) NIH 3T3 cells transfected with pMtE5a-b.neo; (B) NIH 3T3 cells transfected with the vector pMt.neo.1; (C) NIH 3T3 cells transfected with pMtE5a.neo; (D) C127 cells transfected with pMtE5a.neo.

FIG. 4. Hybridization analysis of transfected plasmid DNA in HPV-6 E5a-transformed cell lines. DNA extracted from N.E5a-b cells is shown in lanes a and d; DNA from N.E5a cells is shown in lanes b and e; and DNA from NIH 3T3 cells is shown in lane c. DNA samples were digested with *Nru*I (lanes a, b, and c) or *Bam*HI (lanes d and e). The arrowhead indicates the relative position of linearized pMtE5a-b.neo (7.6 kb) used as a marker.

N.E5a cells. These results indicate that the E5a protein is present in the transformed cell at low levels. Modifications of the immunoprecipitation protocol and additional washings were unsuccessful in reducing the other bands immunopre-

FIG. 5. Presence of the E5a protein determined by immunoprecipitation assays. Cells were metabolically labeled and immunoprecipitations were performed as described in Materials and Methods. Proteins synthesized in vitro from brome mosaic virus RNA were used for size markers (lane a). Sizes of markers are indicated on the left. Immunoprecipitations are from extracts from N.E5a-b (lane b) and NIH 3T3 (lane c) cells. Arrow indicates position of 12-kilodalton E5a protein.

cipitated by the antiserum. The significance, if any, of the minor differences in the intensity between other bands immunoprecipitated from the transformed cells in comparison with the parental cells is unknown.

The subcellular location of the E5a protein was determined by an immunoperoxidase assay. The protein was found predominantly in nuclei (Fig. 6). Levels of E5a protein could be increased by incubation of N.E5a-b cells in 100 μ M ZnSO₄ as a result of induction of the metallothionein promoter in the vector (Fig. 6B). The induction of higher levels of E5a protein resulted in the cells growing to a higher saturation density as can be seen by comparing cell numbers in Fig. 6A and B.

Phenotype of C127 cells transfected by E5a ORF. C127 cells were transfected with the two plasmids containing the HPV-6 E5a ORF (pMtE5a-b.neo and pMtE5a.neo) and also with the vector (pMt.neo.1). Cells selected for G418 resistance maintained a confluent monolayer and did not exhibit an altered morphology. These cells were also assayed for anchorage-independent growth. No colonies were obtained with cells containing pMt.neo.1, and a very few, small colonies were obtained with cells containing E5a. The average number of colonies in duplicate dishes of C.E5a-b cells (C127 cells containing pMtE5a-b.neo) and C.E5a cells (C127 cells containing pMtE5a.neo) was eight and four, respectively. An example of one of the colonies of C.E5a is shown in Fig. 3D. The frequency of colony formation was approximately 20 times lower than for the NIH 3T3 cells. To confirm that the E5a protein was being expressed in the C127 cells, we used immunoperoxidase assays to determine the presence and subcellular location of the protein. The staining pattern was predominantly nuclear as presented above for NIH 3T3 cells.

DISCUSSION

In this paper, we report that the E5a gene of HPV-6 is capable of transforming NIH 3T3 cells. In assays used to study transforming activity, HPV-6 DNA molecularly cloned from benign lesions has not been reported to be as active as other HPVs that manifest a greater malignant potential (29, 45), but the basis for this difference has not been identified. Since we demonstrated transforming activity for HPV-6 DNA under the control of a heterologous promoter, the lack of activity in other systems may be due to inadequate gene expression. The transformed NIH 3T3 cells expressing E5a overgrew a confluent monolayer, had an accelerated generation time, and were anchorage independent. In contrast, E5a did not induce foci in C127 cells, but a few C127 cells containing E5a did form small colonies in suspension. The inability of E5a to transform C127 cells may result from the nonresponsiveness of the cells to the transforming activity of E5a or from aberrant structure or function of the E5a protein in C127 cells. The ability of the product of the BPV-1 E5 ORF to transform NIH 3T3 mouse cells (of presumed fibroblast origin) more readily than C127 mouse cells (of presumed epithelial origin) has also been observed (46). Transformation in vitro by papillomaviruses has been most extensively examined in BPV-1, which contains two separate transforming genes, E5 and E6 (for a review, see references 24 and 29). The expression of the BPV-1 E5 gene is sufficient to transform C127 and NIH 3T3 cells, but E6 does not transform NIH 3T3 cells (1, 43, 44) and the presence of both E6 and E7 enhances the transformed phenotype in C127 cells (35). Proteins encoded by several DNA tumor virus oncogenes including papillomavirus have

been shown to form a stable complex with host cell proteins (19, 53), and there is evidence to suggest that BPV-1 E5 interacts with other host cell proteins rather than by possessing its own intrinsic catalytic activity (30). Therefore, differences in the susceptibility of NIH 3T3 and C127 cells to transformation by HPV-6 E5a may involve cellular factors that may play a role in inducing or suppressing transformation. The differential activity of E5a in these two cell lines should facilitate future investigations on the mechanism of transformation. Not all papillomaviruses contain an E5 ORF, but there are similarities between HPV-6 E5a and BPV E5 ORFs (6, 12, 15, 43, 44). Both of these proteins are leucine rich: 18 of the 91 aa of HPV-6c E5a are leucine and 15 of the 44 aa of BPV-1 E5 are leucine. Both are hydrophobic polypeptides with two helix-breaking proline residues at the amino and carboxyl regions. Both proteins contain cysteine-X-cysteine residues near the carboxyl terminus. One difference between them, as indicated by the hydrophobic index, is that BPV E5 protein contains only two hydrophobic peaks and charged residues (aspartic acid, histidine, and glutamic acid) are located in the COOH terminus (46), while HPV-6 E5a protein exhibits three peaks of strong hydrophobicity (6) and also contains these three hydrophilic residues but in different positions (glutamic acid located in position 2, aspartic acid in position 37, histidine in position 81 [12]). Mutational analysis of E5 of BPV-1 suggests that the C-terminal residues are critical for its transforming activity and that dimerization occurs via intermolecular cross-linking between cysteine residues (7, 22, 23, 41). It has been proposed that the hydrophobic N-terminal residues probably serve mainly to anchor the protein in membranes, since other hydrophobic aa can be substituted without loss of biological activity (23, 41).

Comparison of the predicted aa sequences of the E5 ORFs of several HPV genomes (HPV-6, HPV-16, HPV-18, HPV-33) revealed little homology except for the three strongly hydrophobic domains, each of which is of sufficient length to traverse the cell membrane (2, 6). This conservation of hydrophobic domains may reflect a specific orientation of the E5 protein, since in BPV-1-transformed cells it has been detected mainly in the cell membrane fraction with a small amount located in the nucleus (8, 46). By microinjection into growth-arrested C127 cells, Green and Loewenstein (21) reported that a chemically synthesized E5 peptide functions in the cell nucleus. Our data have shown that the HPV-6 E5a protein is present in the nuclei of cells transformed *in vitro* and in those of biopsy specimens of naturally occurring infections.

A mutation in the E5a ORF of HPV-6 was engineered to terminate translation. The mutant E5aM1 encodes a 14-aa peptide of which the first 9 aa are the wild-type sequence and the remaining 5 aa are out of frame of the E5a aa sequence. E5aM1 was defective for transformation, demonstrating that translation of the E5a ORF is required for biological activity. Similarly, frameshift mutations in the BPV-1 E5 ORF cause a substantial reduction in the ability to induce the appearance of transformed foci of mouse C127 cells (15). Further analysis of mutations in the E5a ORF will enable us to

FIG. 6. Presence and subcellular localization of the E5a protein by immunoperoxidase assays. N.E5a-b cells were grown in RPMI 1640 medium with 10% fetal bovine serum (A) and in the presence of 100 μ M ZnSO_4 (B). Parental NIH 3T3 cells were treated with 100 μ M ZnSO_4 and reacted with anti-E5a serum (C). Magnification is approximately $\times 400$.

discern the importance of individual residues for biological and biochemical properties of the E5a protein.

Induction of cell DNA synthesis and cell proliferation is believed to be closely associated with the transforming capability of DNA tumor viruses, and it remains to be determined how the E5a gene product activates the cellular machinery for mitosis. The demonstration that E5a of HPV-6 has transforming activity in vitro and the presence of the mRNA (52) and protein (12) in naturally occurring infections suggest that E5a plays a role in epithelial cell proliferation. It may also be possible to design therapies to block the production or activity of this protein to reduce epithelial cell growth.

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