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Identification of Proteins Encoded by the L1 and L2 Open Reading Frames of Human Papillomavirus 1a

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The human papillomavirus 1 (HPV-1) virion is composed of two virally encoded proteins: a 57,000-molecular-weight polypeptide (57K polypeptide), which is the product of the L1 open reading frame (ORF), and a 78K polypeptide, which is derived from the L2 ORF. The 57K (L1) product, which represents the major structural component, appears to be disulfide cross-linked in virus particles. The 78K (L2) protein is a minor component of the virion and does not appear to be disulfide linked either to the L1 gene product or to itself. Analysis of virus particles banding at different buoyant densities revealed differences in the L2 content of heavy-full and light-full virions. Antiserum prepared against a bacterially expressed fragment of the L1 ORF was found by immunofluorescence to cross-react with HPV-2 and bovine papillomavirus 1 virions in wart sections. No cross-reactivity was observed with antisera prepared against either the N- or C-terminal halves of the L2-encoded protein. Similarly, antisera prepared against purified virus particles (disrupted and nondisrupted) reacted only with an expressed fragment of the L1 ORF and not with either L2-encoded polypeptides or proteins derived from the E1, E2, E4, E6, or E7 ORFs. This indicates that the L1 protein contains the papillomavirus common antigens.

Papillomaviruses produce benign epithelial tumors in animals and humans (29). On the basis of their physical properties, they have been classified as members of the papovavirus group (22, 23), which includes simian virus 40 (SV40) and polyomavirus. These viruses are naked icosahedral capsids containing double-stranded circular DNA.

The genomic organization of the papillomaviruses however is distinct from that of SV40 or polyomavirus (5), whose genomes can be functionally divided into early and late regions. The early regions of these viruses are expressed before the onset of viral DNA replication, and the late genes are expressed after viral DNA replication has commenced.

For the papillomaviruses, the lack of an in vitro culture system has meant that the same functional division cannot be made. The putative papillomavirus early region corresponds to a 69% fragment of the bovine papillomavirus 1 (BPV-1) genome, which is sufficient to transform mouse cells in vitro (19). The remainder of the genome, which is not required for in vitro transformation, is thought to encode the papillomavirus structural proteins and contains two large open reading frames (ORFs), L1 and L2. By indirect evidence obtained from amino acid analysis, the major capsid protein purified from BPV-1 viral particles has been assigned to the L1 ORF (21). More conclusively, antisera prepared against biosynthetically produced L1 and L2 proteins of BPV-1 (prepared in *Escherichia coli*) have been shown to prevent BPV-1 from transforming mouse C127 cells in vitro (34), and in an enzyme-linked immunosorbent assay, antisera prepared against disrupted BPV-1 virions were shown to react with the biosynthetic BPV-1 L1 protein (34). This suggests that the L1 ORF encodes the major papillomavirus structural component and that the L2 ORF encodes an as yet unidentified minor virion protein. This conclusion is supported by the observation that RNA species mapping to the late region can only be detected in productively infected papillomas (8, 24, 33). These late mRNAs, however, frequently have 5′ ends which map in the early region, suggesting that certain early genes may represent exons of the late mRNAs. In particular, the human papillomavirus 1a (HPV-1a) E4 gene has been shown to be expressed at high levels in the upper layers of productively infected papillomas (4, 7).

To identify and characterize the virally encoded proteins present in HPV particles, we prepared antisera against HPV-1a gene fragments expressed in bacteria. We used these antisera to identify the products of the L1 and L2 genes of HPV-1a and to determine if these proteins contain large segments derived from certain parts of the HPV-1 early region.

MATERIALS AND METHODS

Construction of fusion proteins and preparation of antisera. The construction of plasmids expressing HPV-1 DNA fragments, the preparation of fusion proteins, and the raising of antisera were performed as described earlier (7). Antiserum to HPV-1 virions was the gift of T. S. Burnett (Ph.D. thesis, University of Birmingham, Birmingham, United Kingdom, 1983). Cross-reactive antipapillomavirus antiserum was obtained commercially (Dakopatts).

Typing of warts and purification of virions. DNA was extracted from small slivers of individual wart biopsies after overnight digestion at 37°C in 100 mM NaCl–1 mM EDTA–0.5% sodium dodecyl sulfate (SDS)–5 mM Tris hydrochloride (pH 8.0) containing 500 μg of proteinase K per ml. Purified DNA was analyzed by Southern blotting (20) by using as probes cloned HPV-1a, HPV-2, HPV-4, or BPV-1 DNA which had been nick translated (35) with [α-^{32}P]dCTP (Amersham International).

Individual typed warts were minced as finely as possible by using a scalpel blade and then suspended in 3 ml of phosphate-buffered saline (PBS) and ground in a chilled mortar containing a few grains of carborundum powder (80 grit; BDH Chemicals). After a smooth suspension was

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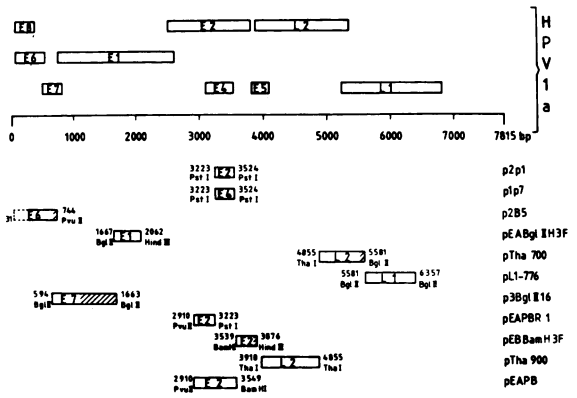

FIG. 1. Location of HPV-1 DNA fragments expressed to high levels in *E. coli*. The positions of the major ORFs of HPV-1a are illustrated at the top, and specific restriction fragments which were cloned into the pEX series of bacterial vectors are indicated beneath. The names of constructs containing each fragment are shown on the right. Sizes are given in base pairs.

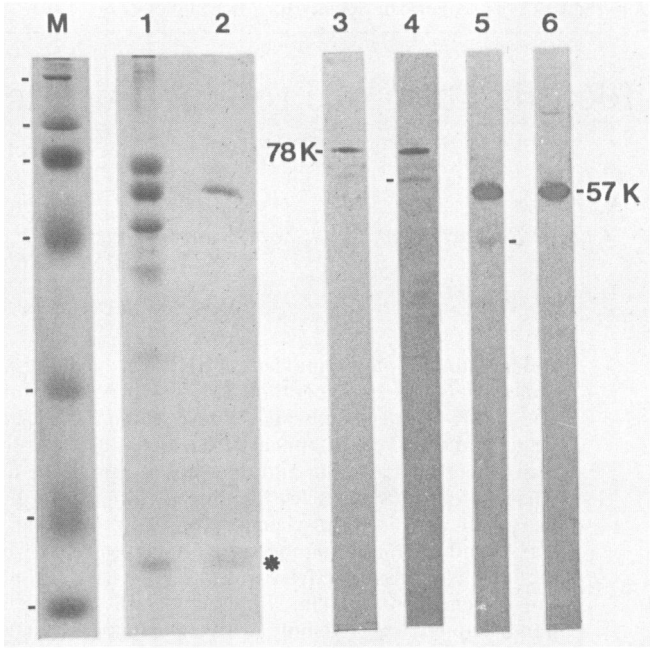

FIG. 2. SDS-polyacrylamide gel (stained with Coomassie blue) of total protein obtained from an HPV-1-induced wart (lane 1) or from HPV-1 virions purified from the same papilloma (lane 2). The 57K major structural protein is clearly visible (lane 2), with no indication of contaminating keratins.The low-molecular-weight polypeptide (*) in lanes 1 and 2 reacted with anti-p1p7 (E4) antiserum (data not shown). Lanes 3, 4, 5, and 6 are Western blots of HPV-1 virion proteins after SDS-polyacrylamide gel electrophoresis. Blotting was carried out to anti-pTha 900 (L2; lane 3), anti-pTha 700 (L2; lane 4), anti-pL1-776 (L1; lane 5), and anti-SDS-disrupted HPV-1 virion (lane 6) antisera. The positions of the 78K (and 65K) L2 proteins and the 57K (and 43K) L1 polypeptides are indicated. Molecular weight markers (200,000, 97,400, 68,000, 43,000, 25,700, 18,400, and 14,300) are shown in lane M.

obtained, the cell debris was removed by centrifugation at 12,000 × *g* for 10 min. Viral particles were then pelleted from the supernatant by centrifugation at 125,000 × *g* for 60 min and then resuspended in 14 ml of a solution containing 1.3 g of CsCl per ml. After centrifugation at 125,000 × *g* for 20 h, virus bands were removed by puncturing the bottom of the tube and collecting 0.5-ml fractions. To obtain highly purified virions, virus-containing fractions were subjected to a second round of purification by cesium chloride density centrifugation, and virus particles were collected as before. The densities of the individual fractions were determined by using an Abbe 60 refractometer.

Analysis of total wart proteins and purified virions. Individual typed warts were minced and solubilized in 7 M urea–15 mM β-mercaptoethanol–50 mM Tris hydrochloride (pH 8.0) by homogenization (Polytron) and sonication, and the extracts were analyzed by SDS-polyacrylamide gel electrophoresis (17) or by alkaline urea gel electrophoresis (14). Virus particles were prepared for SDS-polyacrylamide gel electrophoresis by boiling in 2% SDS–50 mM β-mercaptoethanol–50 mM Tris hydrochloride (pH 6.8)–10% glycerol–0.01% bromophenol blue for 5 min. Western blotting (immunoblotting) was carried out as previously described (39), except that filters were blocked in PBS (pH 7.5) containing 0.5% gelatin. Antibody binding was detected by using a commercially available peroxidase-linked streptavidin-biotin system (Amersham International).

Indirect immunofluorescence staining was carried out on Formalin-acetic acid-methanol (1:1:8)-fixed paraffin sections by using rat antiserum as the first antibody followed by fluorescein isothiocyanate-labeled goat anti-rat immunoglobulin G as the second antibody. Immunoperoxidase staining was performed by using a commercially available streptavidin-biotin system (Amersham International) and was followed by development in PBS containing 0.5 mg of diaminobenzidine and 0.02% H_2O_2. Sections were counterstained with fast green.

Electron microscopy of purified virions. After dialysis against PBS (pH 7.2), purified virus particles were negatively stained with 2% sodium phosphotungstate solution (pH 7.2) and were viewed under a Phillips 301 transmission electron microscope.

RESULTS

Construction and identification of HPV-1a expression plasmids. Specific restriction fragments from the HPV-1a genome were cloned into the pEX series of bacterial expression vectors (38) as described previously (7) and illustrated in Fig. 1. With the exception of the L1 construct, all the recombinants expressed fusion proteins of the expected size with no appreciable degradation. The pEX/HPV L1 plasmid produced multiple fusion protein bands after analysis by SDS-polyacrylamide gel electrophoresis and staining with Coomassie blue, suggesting that in this case, the fusion protein may be partially degraded. High-titer antisera were successfully raised against all the fusion proteins as previously described (7).

Identification of the HPV-1 L1 gene product. By Western blotting, antiserum prepared against the L1 (pL1-776) fusion protein detected the major 57,000-molecular-weight HPV-1 structural protein (57K protein) consistently in extracts of warts induced by HPV-1 and in purified HPV-1 virions (Fig. 2, lane 5). Two additional lower-molecular-weight polypeptides of 50,000 and 43,000 were occasionally also detected. These species were very variable in amount, were always present at lower levels than the major 57K L1 gene product was, and may represent degradation products of the larger protein.

Antiserum prepared against purified HPV-1 virions produced a staining pattern identical to that observed by using

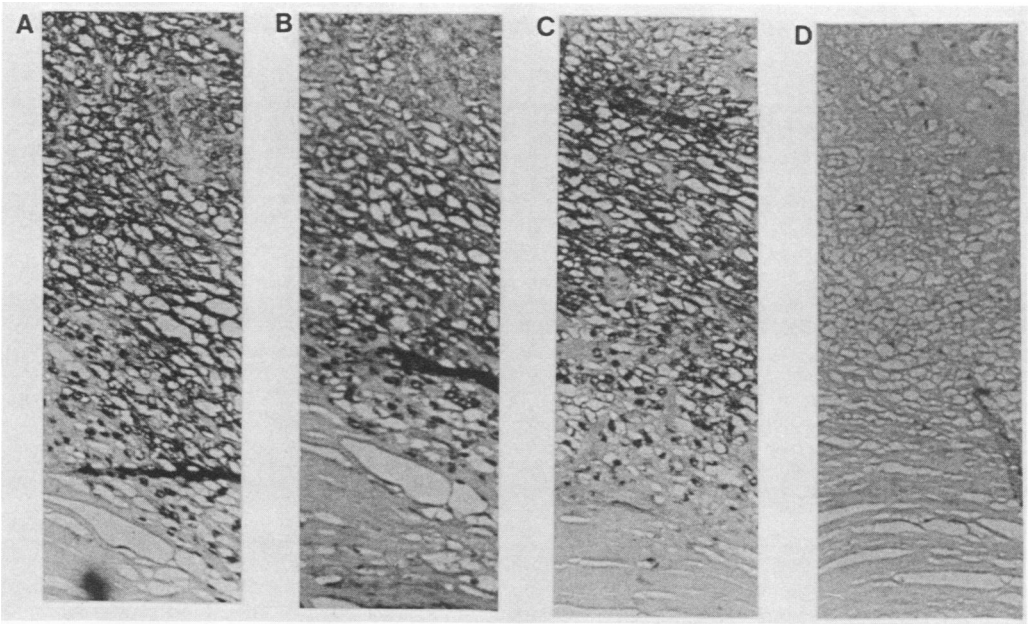

FIG. 3. Immunoperoxidase staining of adjacent frozen sections from an HPV-1-induced papilloma by using anti-pL1-776 (L1) antiserum (A), anti-pTha 900 (L2) antiserum (B), anti-HPV-1 virion antiserum (C), or normal rat preimmune serum (D). Peroxidase labeling was developed with diaminobenzidine, and sections were counterstained with fast green. Magnification, 100×.

anti-L1 (pL1-776) antiserum (Fig. 2, lane 6). No proteins were detected in extracts of HPV-2- or HPV-4-induced warts or in extracts of BPV-1-induced fibropapillomas (data not shown). To ensure that the lack of cross-reactivity of anti-L1 (pL1-776) antiserum by Western blotting was not due to the lower number of viral particles in non-HPV-1 warts (2), BPV-1 virions were purified from an individual BPV-1-induced fibropapilloma by two rounds of purification on cesium chloride density gradients. Although antiserum raised against disrupted BPV-1 virions reacted strongly with purified BPV particles (see Fig. 6B, lane 2), no reactivity was observed by using the anti-L1 (pL1-776) antiserum (see Fig. 6B, lane 3).

By immunoperoxidase staining of tissue sections, the anti-L1 (pL1-776) antiserum produced strong nuclear staining in cells of the granular layer in HPV-1-induced warts (Fig. 3A). The pattern of staining was indistinguishable from that obtained by using an HPV-1 type-specific antiserum (Fig. 3C). No staining was observed below the granular layer, and staining was not observed in the cytoplasm, except in the stratum corneum after degeneration of the nuclear membrane. Although no cross-reactivity was observed by Western blotting, the anti-L1 (pL1-776) antiserum produced typical viral staining on sections of warts induced by HPV-2 and BPV-1 (Fig. 4). The cross-reactivity of this antiserum was expected by comparison of the predicted L1 protein sequences from papillomaviruses which have been DNA sequenced (3, 6, 11, 12, 36, 37).

Identification of the HPV-1 L2 gene product. Antisera prepared against both the N-terminal (pTha 900) and C-terminal (pTha 700) regions of the HPV-1a L2 protein were both found to detect a 78K protein specifically in extracts of HPV-1-induced papillomas (data not shown) and in purified HPV-1 virions (Fig. 2, lanes 3 and 4). A minor 65K polypeptide was occasionally detected and may represent a degradation product of the larger protein. These antibodies failed to detect any proteins in extracts of warts induced by HPV-2, HPV-4, or BPV-1 and showed no reactivity against purified BPV-1 virions. No reactivity was observed against the 57K major capsid protein identified by the anti-L1 (pL1-776) antiserum. Except for the N-terminal 50 amino acids, the L2 proteins are only poorly conserved between papillomaviruses of different types (5).

Anti-L2 (pTha 900 and pTha 700) antisera showed typical virion staining on sections of HPV-1-induced papillomas (Fig. 3B). No cross-reactivity was observed in sections of either HPV-2- or HPV-4-induced warts or in BPV-induced fibropapillomas.

Distribution of proteins in heavy-full and light-full virions. HPV-1 virus particles were prepared from an individual HPV-1-induced wart following two cycles of purification by cesium chloride density gradient centrifugation. After dialysis, fractions from the gradient were analyzed by gel electrophoresis and Western blotting to anti-L1 (pL1-776), anti-L2 (pTha 900 and pTha 700), or anti-E4 (p1p7) antisera. Full virus particles (confirmed by electron microscopy) were found to band at buoyant densities between 1.345 and 1.36 g/ml, and two peaks were apparent after blotting of the fractions to anti-L1 (pL1-776) antiserum (Fig. 5iii). The 78K L2 gene product was primarily associated with heavy-full virus particles and was present at lower levels in light-full virions (Fig. 5i).

Surprisingly, HPV-1 E4 gene products could also be detected at buoyant densities between 1.345 and 1.365 g/ml (Fig. 5ii), although the significance of this is not clear. By alkaline urea gel electrophoresis, differences were observed between the migration patterns of E4 polypeptides which sedimented at different buoyant densities. This may represent alterations in the charge of the E4-encoded proteins or partial proteolytic degradation.

Disulfide cross-linking of the L1 protein. The L1 proteins of different papillomaviruses are highly conserved and contain conserved cysteine residues at six positions (amino acid residues 166, 179, 189, 233, 385, and 435 on the HPV-1 L1 protein). To determine the role of disulfide cross-linking in virus assembly, purified HPV-1 virions were analyzed before

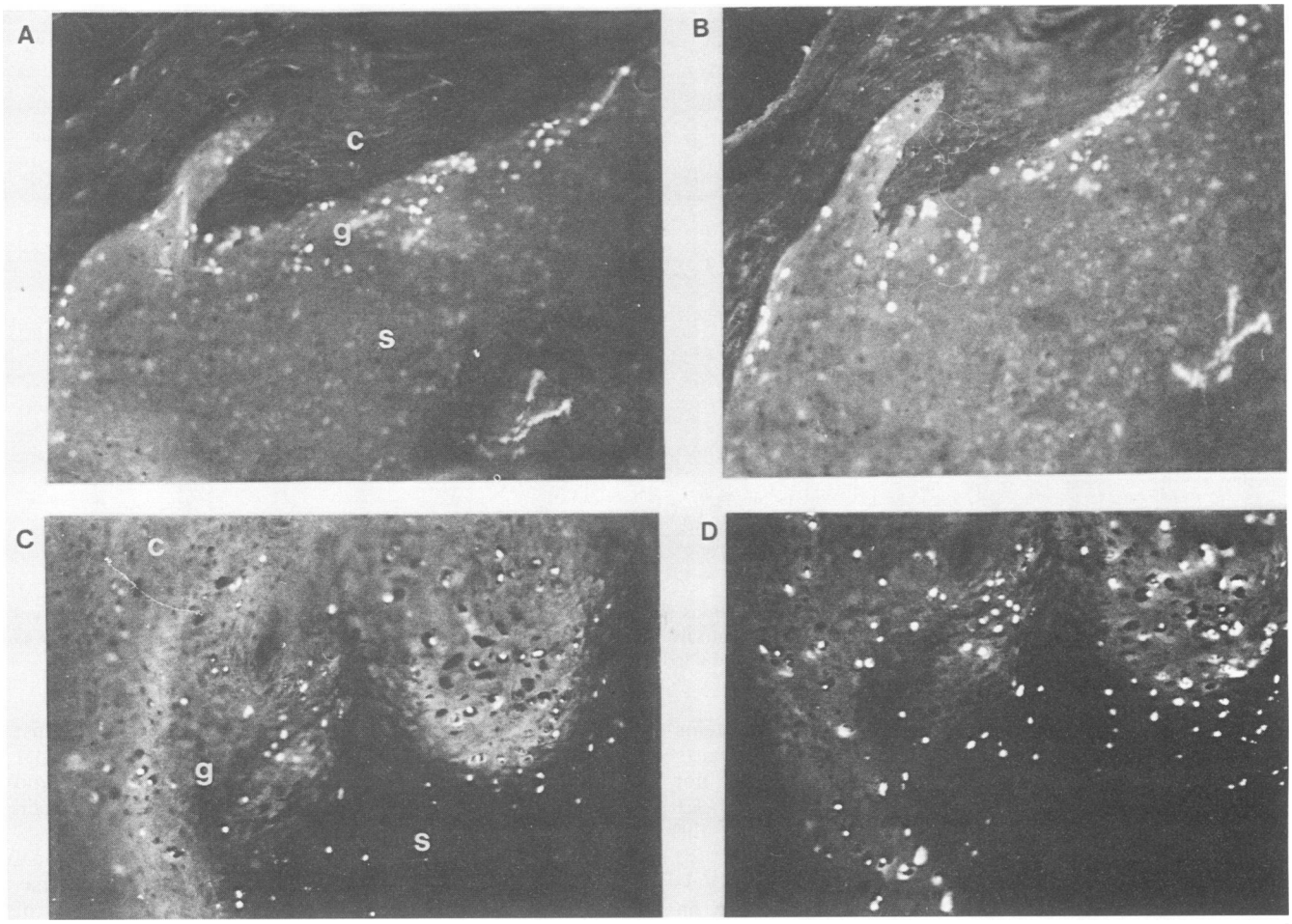

FIG. 4. Cross-reactivity of anti-pL1-776 (L1) antiserum by immunofluorescence. Formalin-acetic acid-methanol-fixed paraffin sections of a BPV-2-induced bovine papilloma (A and B) or a HPV-2-induced human wart (C and D) were stained with either anti-pL1-776 (L1) antiserum (A and C) or cross-reactive antiserum prepared against SDS-disrupted BPV virions (B and D). Magnification, 110×. The spinous (s), granular (g), and cornified (c) layers are indicated.

and after reduction with β-mercaptoethanol. The L2 antibodies (pTha 700 and pTha 900) detected a single 78K polypeptide in both unreduced and reduced virion extracts (Fig. 6A, lanes 3 and 4), while the L1-encoded protein was found to migrate as two high-molecular-weight species (>200,000) when not reduced but as a single 57K product after treatment with β-mercaptoethanol (Fig. 6A, lanes 1 and 2). The L1 protein was thus able to form disulfide cross-linked oligomers but does not appear to be attached to the L2 gene product in this way. Disulfide cross-linkage is not involved in the association of the L2 gene product with HPV-1 virions.

Analysis of proteins detected by antivirion antiserum. Antiserum prepared against SDS-disrupted HPV-1 virions was screened against the panel of HPV-1a fusion proteins, which contained expressed regions from the L1, L2 (N terminus and C terminus), E4, E2 (N terminus, middle, and C terminus), E1, E6, and E7 ORFs. Antivirion antiserum reacted only with the fusion proteins encoded by plasmid pL1-776 and did not react with proteins derived from the HPV-1a early region or with the two L2 fusion proteins (Fig. 6C). Similarly, against purified HPV-1 virions, antiserum raised against disrupted virus was found to react only with the 57K L1-encoded protein and not with the 78K product of the L2 ORF. These results suggest that the major cross-reactive papillomavirus antigen is located on the L1 protein rather than on L2.

Analysis of HPV-1 virions for polypeptides encoded by other HPV-1 ORFs. Antisera raised against expressed fragments from the HPV-1 E1, E6, E2 (N terminus, middle, and C terminus), E4, and E7 ORFs (Fig. 1) showed no reactivity against either the L1-encoded 57K polypeptide or the L2-encoded 78K protein. This suggests that the 57K (L1) and 78K (L2) proteins do not contain significant regions derived from parts of the HPV-1a genome which are expressed in the early-region fusion proteins described above. Antisera prepared against the products of the E6, E2, and E1 ORFs failed to detect any proteins in either extracts of HPV-1-induced warts or in purified HPV-1 virions.

DISCUSSION

Previous analyses of papillomavirus structural proteins have indicated the presence of up to 15 polypeptides in purified HPV-1 virions (9, 13, 26, 27, 30, 32). The major HPV-1 structural protein, however, has a molecular weight of 57,000 and corresponds to the previously identified VP1 (9) or protein 2 (13). We showed that this protein was the

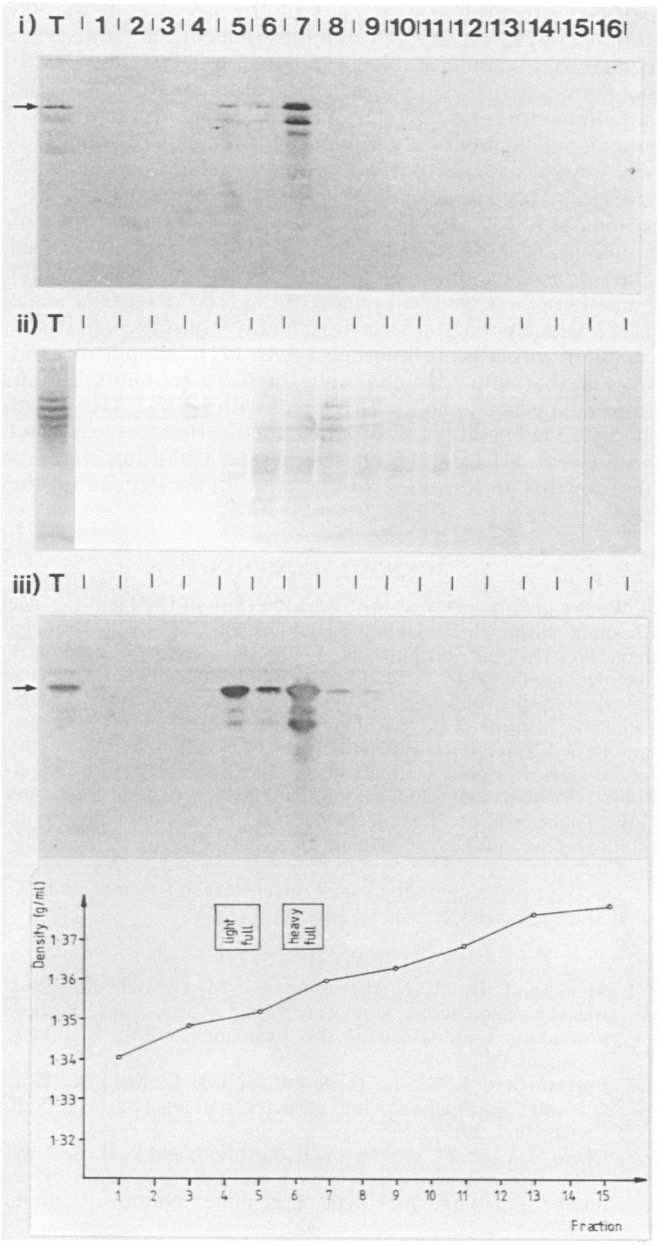

FIG. 5. Analysis of heavy-full and light-full virions after cesium chloride density gradient centrifugation. (i) Western blot (after SDS-polyacrylamide gel electrophoresis) to anti-pTha 900 (L2) antiserum of fractions (1 to 16) obtained after density gradient centrifugation of purified HPV-1 virions. A crude-protein extract of the HPV-1-induced wart from which the virions were purified was run in lane T. The 78K L2-encoded polypeptide is indicated by an arrow. The minor 65K L2-encoded protein is apparent in lanes 5, 6, and 7. (ii) Western blot (after alkaline urea gel electrophoresis) to anti-p1p7 (E4) antiserum. Lanes T and 1 to 16 are as described above. (iii) Western blot (after SDS-polyacrylamide gel electrophoresis) to anti-pL1-776 (L1) antiserum. Lanes T and 1 to 16 are as described above. The 57K L1-encoded protein is indicated by an arrow. The 50K and 43K L1 products are visible in lanes 5, 6, and 7. The density (grams per milliliter) of each gradient fraction is indicated in the graph at the bottom.

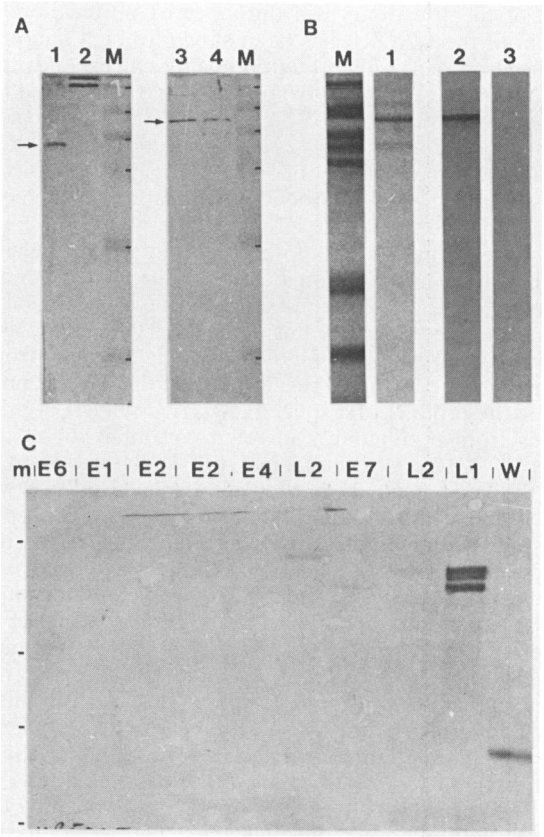

FIG. 6. (A) Disulfide cross-linking of L1- but not L2-encoded proteins. Western blots of purified HPV-1 virion proteins after SDS-polyacrylamide gel electrophoresis. The virus particles were disrupted in sample buffer with (lanes 1 and 3) or without β-mercaptoethanol (lanes 2 and 4) prior to being applied to the gel. Blotting was carried out by using anti-pL1-776 antiserum (lanes 1 and 2) or anti-pTha 900 (L2) antiserum (lanes 3 and 4). Molecular weight markers (200,000, 97,400, 68,000, 43,000, 25,700, and 18,400) are shown in lanes M. (B) Cross-reactivity of anti-pL1-776 antiserum. Lanes 1, 2, and 3 contain extracts of purified BPV-1 virions after SDS-polyacrylamide gel electrophoresis and staining with Coomassie blue (lane 1) or after Western blotting to either anti-BPV-1 virion antiserum (lane 2) or anti-pL1-776 antiserum (lane 3). Molecular weight markers (116,000, 66,000, 45,000, 36,000, 29,000, 24,000, 20,000, and 14,200) are illustrated in lane M. (C) Detection of L1 fusion protein by using anti-HPV-1 virion antiserum. Western blot of bacterial extracts containing expressed fragments from the E6, E1, E7, (N and C termini), E4, L2 (N terminus), E7, L2 (C terminus), and L1 ORFs. Lane W contains an extract from an HPV-1-induced papilloma. Blotting was carried out by using antiserum prepared against SDS-disrupted HPV-1 virions. Specific staining of the L1 fusion protein (pL1-776) and the 57K major HPV-1 structural protein is seen in lanes L1 and W. The faint bands in lanes L2 (N terminus), E7, and L2 (C terminus) were also observed with preimmune serum. The positions of molecular weight markers (200,000, 97,400, 68,000, and 43,000) are indicated in lane M.

primary product of the L1 ORF, confirming the assumptions drawn from earlier studies (21, 34). The two smaller L1-encoded proteins of molecular weights 50,000 and 43,000, which were infrequently present at low levels, may be equivalent to the previously identified proteins 3 and 4 (13) or to VP2 and 3 (9). As these protein species were frequently absent, it seems likely that they may represent breakdown products of the larger 57K L1 gene product. The prevalence of these components in previous studies (9, 13, 30) may be a

result of the use of trypsin during virus purification. The product of the L2 ORF has been shown to be a minor 78K structural protein. This protein probably corresponds to the 76K (27) or 86 K (13) polypeptide previously reported to be a minor component of HPV-1 virions. The additional 65K L2-encoded protein (Fig. 2, lanes 3 and 4) which was detected in a small number of HPV-1 virus preparations may represent a degradation product of the larger 78K species.

The observed size of the L1-encoded protein (57,000) is in close agreement with its predicted molecular weight of 57,550 (assuming initiation begins at the first methionine residue [16]). The size of the L2 gene product, however, was larger than expected. The unspliced L2 ORF has the ability to encode a polypeptide of molecular weight 55,243 (from its first methionine residue), suggesting that the 78K L2 protein may be the product of a spliced mRNA. Analysis of mRNA species from productively infected cottontail rabbit papillomas (24, 33) has shown that two mRNA classes exist which span the late region. In particular, a spliced mRNA which fuses sequences from the E7, E2 (C terminus), L2, and L1 ORFs has been identified (24), and it is likely that a similar mRNA occurs in warts induced by HPV-1. From our results, however, it is unlikely that the 78K L2 gene product contains large regions derived from either the E2 or E7 ORFs, as antisera prepared against the products of these genes did not react with the 78K protein. It must be borne in mind, however, that such negative results do not confirm this. By similar reasoning, the 78K L2 polypeptide is unlikely to represent a straightforward L1-L2 fusion, although the possibility that L2 is spliced either to the extreme 5′ end or to the 3′ end of L1 cannot be ruled out. It is interesting that a minor structural protein of 65,000 molecular weight has been found in BPV particles and that a 78K polypeptide has not been detected (10). It seems reasonable to assume that this protein represents the product of the BPV L2 ORF.

The ability of HPV-1 virions to band at different buoyant densities has previously been observed by other workers (28, 30), and it has been shown that differences exist between the proteins present in heavy-full and light-full virions (30). In the present study, the L2 protein was found to be primarily associated with heavy-full virions. Although E4-encoded proteins were also found within the gradient, even after two cycles of purification, it is not known if they are associated with virus particles. It is interesting, however, that several workers have indicated differences between the low-molecular-weight polypeptides present in HPV-1 virions and those extracted from other papillomavirus types (13, 17). The possibility exists that such differences may be due to the presence of E4 16K and 17K proteins in HPV-1 preparations.

The ability of the L1 gene product, but not the L2 protein, to disulfide cross-link bears similarities to the organization of structural proteins in SV40 and polyomavirus. In these viruses, the VP1 (L1) protein subunits are covalently cross-linked to each other by disulfide bridges (40). Analysis of the L1 protein sequences from a number of papillomavirus types (3, 6, 11, 12, 36, 37) shows that cysteine residues are highly conserved in position. The minor VP2 and VP3 proteins of both SV40 and polyomavirus are not involved in such linkages, and like the HPV-1 L2 protein, can easily be dissociated from virus particles by treatment with SDS (40). This suggests that a common mechanism may exist for the formation of icosahedral virus particles by both the papillomaviruses and other members of the papovavirus group.

On tissue sections, all our late antisera (anti-L1 and anti-L2) produced similar staining to that observed with antiserum prepared against SDS-disrupted HPV-1 virions. This is consistent with previous studies indicating that viral particles are produced in cells of the upper spinous and granular layer (1, 25). The finding that anti-L1 antiserum could cross-react with other papillomavirus types by immunofluorescence is supported by the observation that the papillomavirus L1-encoded protein is highly conserved among papillomavirus types (15). Our results are consistent with the cross-reactivity observed by other workers of antiserum prepared against SDS-disrupted papillomavirus virions (15, 18, 31). This cross-reactivity is the result of antibodies directed against the L1 protein and not L2, as antivirion antiserum reacted only with a fusion protein containing the expressed region of the HPV-1 L1 ORF. The L2-encoded polypeptide is less highly conserved between papillomaviruses of different types (11), supporting our finding that anti-L2 antiserum (both N terminus and C terminus) was unable to cross-react with HPV-2, HPV-4, or BPV-1. The inability of our anti-L1 antiserum to cross-react with purified BPV-1 virions by Western blotting suggests that for this antiserum, cross-reactivity may depend on the tertiary structure of the L1 polypeptide.

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LITERATURE CITED

1. **Almeida, J. D., A. F. Howatson, and M. G. Williams.** 1962. Electron microscope study of human warts; sites of virus production and nature of the inclusion bodies. J. Invest. Dermatol. **38**:337–345.
2. **Barrera-Oro, J. G., K. O. Smith, and J. L. Melnick.** 1962. Quantitation of papova virus particles in human warts. J. Natl. Cancer Inst. **29**:583–595.
3. **Chen, E. Y., P. M. Howley, A. D. Levinson, and P. H. Seeburg.** 1982. The primary structure and genetic organisation of the bovine papillomavirus type I genome. Nature (London) **299**:529–534.
4. **Croissant, O., F. Breitburd, and G. Orth.** 1985. Specificity of cytopathic effect of cutaneous human papillomaviruses. Clin. Dermatol. **3**:43–55.
5. **Danos, O., I. Giri, F. Thierry, and M. Yaniv.** 1984. Papillomavirus genomes: sequences and consequences. J. Invest. Dermatol. **83**(Suppl.):7–11.
6. **Danos, O., M. Katinka, and M. Yaniv.** 1982. Human papillomavirus 1a complete DNA sequence: a novel type of genome organization among papovaviridae. EMBO J. **1**:231–236.
7. **Doorbar, J., D. Campbell, R. J. A. Grand, and P. H. Gallimore.** 1986. Identification of the human papillomavirus-1a E4 gene products. EMBO J. **5**:355–362.
8. **Engel, L. W., C. A. Heilman, and P. M. Howley.** 1983. Transcriptional organization of bovine papillomavirus type 1. J. Virol. **47**:516–528.
9. **Favre, M., F. Breitburd, O. Croissant, and G. Orth.** 1975. Structural polypeptides of rabbit, bovine, and human papillomaviruses. J. Virol. **15**:1239–1247.
10. **Favre, M., F. Breitburd, O. Croissant, and G. Orth.** 1977.

Chromatin-like structures obtained after alkaline disruption of bovine and human papillomaviruses. J. Virol. **21**:1205–1209.
11. **Fuchs, P. W., T. Iftner, J. Weninger, and H. Pfister.** 1986. Epidermodysplasia verruciformis-associated human papillomavirus 8: genomic sequence and comparative analysis. J. Virol. **58**:626–634.
12. **Giri, I., O. Danos, and M. Yaniv.** 1985. Genome structure of the cotton tail rabbit (shope) papillomavirus. Proc. Natl. Acad. Sci. USA **82**:1580–1584.
13. **Gissmann, L., H. Pfister, and H. zur Hausen.** 1977. Human papillomaviruses: characterization of 4 different isolates. Virology **76**:569–580.
14. **Grand, R. J. A., S. V. Perry, and R. A. Weeks.** 1979. Troponin C-like proteins (calmodulins) from mammalian smooth muscle and other tissues. Biochem. J. **177**:521–529.
15. **Jenson, A. B., J. R. Rosenthal, C. Olson, F. Pass, W. D. Lancaster, and K. Sha.** 1980. Immunological relatedness of papillomaviruses from different species. J. Natl. Cancer Inst. **64**:495–500.
16. **Kozak, M.** 1983. Comparison of initiation of protein synthesis in procaryotes, eucaryotes, and organelles. Microbiol. Rev. **47**: 1–45.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
18. **Lancaster, W. D., and A. B. Jenson.** 1981. Evidence for papillomavirus antigens and DNA sequences in a laryngeal papilloma. Intervirology **15**:204–212.
19. **Lowy, D. R., I. Dvoretzky, R. Shober, M.-F. Law, L. Engel, and P. M. Howley.** 1980. In vitro transformation by a defined subgenomic fragment of bovine papillomavirus DNA. Nature (London) **287**:72–74.
20. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Meinke, W., and G. C. Meinke.** 1981. Isolation and characterization of the major capsid protein of bovine papillomavirus type 1. J. Gen. Virol. **52**:15–24.
22. **Melnick, J. L.** 1962. The papova virus group. Science **135**: 1128–1130.
23. **Melnick, J. L., A. C. Allison, J. S. Butel, W. Eckhart, B. E. Eddy, S. Kit, A. J. Levine, J. A. R. Miles, J. S. Pagano, L. Sachs, and V. Vonka.** 1974. Papovaviridae. Intervirology **3**:106–120.
24. **Nasseri, M., and F. O. Wettstein.** 1984. Differences exist between viral transcripts in cottontail rabbit papillomavirus-induced benign and malignant tumors as well as non-virus-producing and virus-producing tumors. J. Virol. **51**:706–712.
25. **Noyes, W. F., and R. C. Mellors.** 1957. Fluorescent antibody detection of the antigens of Shope papillomavirus in papillomas of the wild and domestic rabbit. J. Exp. Med. **106**:555–562.
26. **Orth, G., F. Breitburd, M. Favre, and O. Croissant.** 1977. Papillomaviruses: possible role in human cancers, p. 1043–1068. *In* H. H. Hiatt, J. D. Watson, and J. A. Winsten (ed.), Origins of human cancer. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Orth, G., M. Favre, and O. Croissant.** 1977. Characterization of a new type of human papillomavirus that causes skin warts. J. Virol. **24**:108–120.
28. **Pass, F., and J. V. Maizel.** 1973. Wart-associated antigens. II. Humoral immunity to viral structural proteins. J. Invest. Dermatol. **60**:307–311.
29. **Pfister, H.** 1984. Biology and biochemistry of papillomaviruses. Rev. Physiol. Biochem. Pharmacol. **99**:111–191.
30. **Pfister, H., L. Gissmann, and H. zur Hausen.** 1977. Partial characterization of the proteins of human papillomavirus (HPV) 1-3. Virology **83**:131–137.
31. **Pfister, H., and J. Meszaros.** 1980. Partial characterization of a canine oral papillomavirus. Virology **104**:243–246.
32. **Pfister, H., and H. zur Hausen.** 1978. Characterization of proteins of human papilloma viruses (HPV) and antibody response to HPV 1. Curr. Top. Microbiol. Immunol. **166**:13–19.
33. **Phelps, W. C., S. L. Leary, and A. J. Faras.** 1985. Shope papillomavirus transcription in benign and malignant rabbit tumours. Virology **146**:120–129.
34. **Pilacinski, W. F., D. L. Glassman, R. A. Krzyzek, P. L. Sadowski, and A. K. Robbins.** 1984. Cloning and expression in *E. coli* of the bovine papillomavirus L1 and L2 open reading frames. Bio/Technology **1**:356–360.
35. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**: 237.
36. **Schwarz, E., M. Durst, C. Demankowski, O. Lattermann, R. Zech, E. Wolfsperger, S. Suhai, and H. zur Hausen.** 1983. DNA sequence and genome organization of genital human papillomavirus type 6. EMBO J. **2**:2341–2348.
37. **Seedorf, K., G. Krammer, M. Durst, S. Suhai, and W. G. Rowekamp.** 1985. Human papillomavirus type 16 DNA sequence. Virology **145**:181–185.
38. **Stanley, K. K., and J. P. Luzio.** 1984. Construction of a new family of high efficiency bacterial expression vectors: identification of cDNA clones coding for human liver proteins. EMBO J. **3**:1429–1434.
39. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4356.
40. **Walter, G., and W. Deppert.** 1974. Intermolecular disulphide bonds: an important structural feature of the polyomavirus capsid. Cold Spring Harbor Symp. Quant. Biol. **39**:255–257.