Mutant canine oral papillomavirus L1 capsid proteins which form virus-like particles but lack native conformational epitopes

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Introduction

Papillomaviruses (PVs) induce benign epidermal and mucosal papillomas in many vertebrate species and, in humans, the development of cervical cancer is closely associated with genital mucosal infection by specific types of PVs (Lorinez et al., 1992). Approximately 500,000 people die from cervical cancer per year worldwide and in the United States it is estimated that 6 billion dollars is spent annually on the screening, diagnosis and treatment of genital papillomavirus lesions.

An effective vaccine against human papillomaviruses (HPVs) could potentially reduce the incidence of human cervical dysplasia and carcinoma by 90–95% (zur Hausen, 1989, 1991). Due to the species-specificity of these viruses, there are no animals into which HPV can be introduced to evaluate such vaccines. However, the mucosotropic, oncogenic canine oral papillomavirus (COPV) closely mimics the biology of HPVs and the capsid proteins of COPV are closely related to those of HPV-1, making this virus a unique and highly relevant animal model for the development of both veterinary and human vaccines (Bell et al., 1994).

Previous studies have shown that host protective humoral antibodies recognize native, conformational epitopes on the PV virion and it is these viral conformation-specific epitopes which are essential for generating neutralizing antibodies in the host animal (Steele & Gallimore, 1990; Ghim et al., 1991). Overexpression of the L1 protein in eukaryotic Sf9 cells using the baculovirus expression system results in the formation of virus-like particles (VLPs) which can induce high titres of neutralizing antibodies (Kirnbauer et al., 1993; Rose et al., 1993; Christensen et al., 1994; Breitburd et al., 1995). We have demonstrated that vaccination with COPV L1 completely protects beagles against mucosal infection and that circulating immunoglobulins induced by this vaccination can protect naive animals via passive transfer (Suzich et al., 1995).

Although L1 VLPs have been successfully used as a vaccine to prevent COPV mucosal infection of beagles, the purification process is expensive and requires CsCl gradients. In addition,
the purified VLPs appear to package non-specific Sf9 cellular DNA, similar to that observed for VLPs formed by the polyomavirus capsid protein expressed in Sf9 cells (Gillock et al., 1997). In order to further investigate whether it is possible to express forms of L1 which do not assemble into VLPs yet retain conformational epitopes, we constructed a series of L1 truncation mutants and evaluated their ability to form VLPs and conformational epitopes by electron microscopy and immunofluorescence microscopy, respectively.

**Methods**

**Cell culture.** COS-1 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% foetal calf serum. Sf9 cells were cultured in 1 × Grace’s Medium Supplemented (GIBCO/BRL) containing 10% foetal bovine serum and 10 µg/ml of gentamicin (GIBCO/BRL).

**Generation of polyclonal rabbit antisera against intact COPV virions and denatured COPV L1 protein.** Wild-type (wt) COPV particles were purified from experimentally induced warts (Lancaster & Olson, 1978). New Zealand White rabbits were immunized intradermally twice at 2 week intervals with 100 µg of purified intact COPV virions diluted with an equal volume of TiterMax (CytRx Corp.) as adjuvant. Animals were bled 1 week after the final boost. To prepare rabbit antiseraum against denatured COPV L1 protein, purified COPV L1 protein from E. coli (Pilacinski et al., 1984) was dissolved in 50 mM Tris–HCl, pH 8.5, containing 5% 2-mercaptoethanol and 1% SDS, boiled for 10 min, and then extensively dialysed against 50 mM Tris–HCl, pH 8.5 containing 1% 2-mercaptoethanol and 10% sucrose. A New Zealand White rabbit was immunized with 0.5 mg of dialysed protein emulsified with Freund’s complete adjuvant. The rabbit received two additional immunizations at 3 and 6 weeks following the primary immunization with 0.1 mg of dialysed protein formulated with Freund’s incomplete adjuvant. The rabbit was bled 2 weeks after the third immunization.

**Construction of transfer vectors containing the COPV L1 mutant genes.** The pYC6 expression vector, a modified pJS55 plasmid (Sparkowski et al., 1994), was generated by digesting pJS55 with BamHI and HindIII, and then ligating with a polylinker: 5′ GATCCGGCCGCACTGGGCGGCGGCACTGGGCGGCGGCGGCC 3′ 3′ CCATGGGCCGAGTCGCAGCCCTCGA 5′

The Na25, Ca26, Ca26-nls, Ca67, Ca67-nls, Ca133-nls and Ca187-nls mutants were generated by PCR from a plasmid containing the entire COPV genome (Sunberg et al., 1986). The 5′ end primers of all the mutants contain a Kozak sequence, GCCACC, upstream of the start codon and a Hin III site upstream of the Kozak sequence. The 5′ end primers of all the C-terminal mutants is 5′ GACATGGGCCGCGCAGCCCTCGA- ATGGCGCTTCTGCT 3′. The 5′ end primer of Na25 is 5′ ATATGACTGCGGCCGCGCCACCATGG 3′. The 5′ end primer of Na25 is 5′ ATATGACTGCGGCCGCGCCACCATGGGATCCA- GGAAGCTTAGACTTTGCGCTTCTTTTTCGGCGGCTGGATCCA- ATGGCGTTTGGCTTC 3′. The 3′ end primer of Na25 is 5′ CTAGGGAAGCTTAGCTTTGCGCTTCTTTTTCGGCGGCTGGATCC- TGAGGTAGG 3′. The 3′ end primer of Ca187-nls is 5′ CTAGGGAAGCTTAGCTTTGCGCTTCTTTTTCGGCGGCTGGATCC- TGAGGTAGG 3′. The 3′ end primer of Ca187-nls is 5′ CTAGGGAAGCTTAGCTTTGCGCTTCTTTTTCGGCGGCTGGATCC- TGAGGTAGG 3′.

**Construction and selection of recombinant baculoviruses.** All the COPV L1 mutants were subcloned into the NcoI–HindIII site in pBlueBac V transfer vector from pYc6. pBlueBac V is a modified pBueBac IV transfer vector from Invitrogen. The resulting plasmids were transfected with linearized AcMNPV DNA (Invitrogen) into Sf9 cells by liposome-mediated lipofection (Invitrogen). The recombinant baculoviruses were detected as blue plaques on X-Gal and purified as described by Summers & Smith (1987).

**Production and purification of VLPs.** Sf9 cells (1 × 10⁶/ml) were infected in a spinner flask with recombinant baculovirus at a m.o.i. of 25–250 p.f.u. per cell at 27 °C. Seventy-two hours after infection, the cells were harvested by centrifugation at 2000 r.p.m. for 10 min at 4 °C and resuspended in PBS. They were then homogenized by a Dounce homogenizer on ice and centrifuged at 7500 r.p.m. for 20 min at 4 °C. The pellet was resuspended in PBS and sonicated twice on ice with a Vibra Cell sonicator (Sonics & Materials Inc.) at 9 W for 30 s. The nuclear lysate was then layered over 40% (w/v) sucrose–PBS and centrifuged at 34000 r.p.m. for 2 h at 4 °C. Pelleted material was resuspended in CsCl–PBS (1.30-1.34 g/ml) and centrifuged to equilibrium at 45 000 for 20 h at 4 °C. Bands with the desired density (1.29–1.30 g/ml) were collected. PBS was added to the collected particles which were then recentrifuged at 34000 r.p.m. for 2 h. The pelleted VLPs were re-suspended in PBS.

**ELISA.** Intact COPV L1 VLPs, denatured COPV L1 proteins and intact HPV-16 L1 VLPs diluted in PBS were distributed into the wells of a 96-well plate (100 ng per well) and incubated at 4 °C overnight. The denatured COPV L1 proteins were generated by boiling the COPV L1 particles in buffer (150 mM NaCl, 25 mM -mercaptoethanol, 1% SDS and 20 mM Tris, pH 7.4) for 10 min. After washing with PBS, the plate was blocked with 1% PBSA (1 mg BSA in 100 ml of PBS) for 1 h at 37 °C. The rabbit anti-intact COPV virion antisera (1:1000) or rabbit anti-denatured COPV L1 antisera (1:1000) was then added to the wells and incubated with antigen for 1 h. After three washes, alkaline phosphatase-conjugated goat anti-rabbit IgG Fc (Accurate Chemical & Scientific Corp.) was added to the plate at 1:1000 dilution in PBS and incubated for 1 h. After three washes with PBS, phosphate substrate (Sigma) in diethanolamine buffer was added and the A405 was read by a Dynatech Micro-ELISA reader after 20 min.

**Immunofluorescence assay.** COS cells were grown on glass coverslips to 50% confluence, then transfected with 10 µg of the various pYc6 plasmid constructs by calcium phosphate transfection (Graham & van der Eb, 1973). The cells were glycerol-shocked 12 h after transfection. Forty-eight hours later, the coverslips were washed with PBS and fixed with ice-cold acetone. They were incubated for 1 h with primary antibody (Ab) diluted 1:250 (v/v) in PBS. After three washes, the coverslips were reacted with rhodamine-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories) diluted 1:50 (v/v) in PBS for 1 h. After washes with PBS, the coverslips were mounted on glass slides with Fluoromount (PanDaTa) mounting solution. Cells were viewed and photographed with a Zeiss Axioskop inverted fluorescence microscope.

**Immunoprecipitation and immunoblotting assays.** COS cells were grown on 10 cm plates to 60% confluence. Ten µg of plasmid DNA was transfected into COS cells by calcium phosphate transfection. The
cells were glycerol shocked 12 h after transfection. Forty-eight hours later, they were metabolically labelled with [35S]methionine/cysteine. The cells were solubilized with 1 ml of radioimmunoprecipitation assay (RIPA) buffer (Goldstein & Schlegel, 1990) containing 0.1 mM protease inhibitors Na-p-tosyl-l-lysine chloromethyl ketone (TLCK) (Sigma) and N-tosyl-l-phenylalanine chloromethyl ketone (TPCK) (Sigma) and 0.5 mM phenylmethylsulfonyl fluoride (Sigma). Cell extracts were incubated with 5 µl of AU1 MAb (Berkeley Antibody Co.) (Lim et al., 1990) or 5 µl of anti-denatured COPV L1 polyclonal Ab and 50 µl of a 1:1 suspension of protein A-Sepharose CL-40 beads (Pharmacia) for 1:5 h. The immune complexes were then collected by centrifugation for 15 s at 13,000 r.p.m. in a microcentrifuge, solubilized in sample buffer and analysed by 10% SDS–PAGE.

For immunoblotting assays, 5 µl of purified COPV L1 proteins (wt and mutants) was added to sample buffer and boiled for 5 min before loading on a 10% SDS–polyacrylamide gel. The proteins were then transferred onto a nitrocellulose membrane. The membrane was then immersed in buffer (150 mM NaCl, 20 mM Tris–HCl, pH 7.4 and 0.4% Tween 20) for 1 h and incubated for a further 1 h with rabbit anti-denatured COPV L1 Ab diluted 1:1000 (v/v) in the same buffer. The membrane was then washed three times with buffer and incubated for 30 min with alkaline phosphatase-conjugated goat anti-rabbit secondary Ab diluted 1:1000 (v/v). After washing, the protein bands were visually detected by incubating the nitrocellulose membrane with substrate solution (1 mg/ml of Naphthol AS-BI and 1 mg/ml of Fast Violet B (Sigma), 100 mM Tris–HCl, pH 9.5, 1 mM MgCl2) for 5 min at room temperature.

Electron microscopy. VLPs in PBS were adsorbed to carbon coated grids, stained with 2% phosphotungstic acid, pH 6.8, and examined under a JEM-100s electron microscope (JEOL) at 60 kV. All the electron micrographs were taken at 100,000 x magnification.

Results

Characterization of rabbit antisera generated against native and denatured COPV L1 protein

To evaluate the expression and native conformation of COPV L1 proteins, two polyclonal antisera were generated in rabbits as described in Methods: one against intact, native COPV virus and the other against denatured COPV L1 protein. The properties of these two antisera were analysed by ELISA. As shown in Table 1, anti-COPV antiserum has extremely strong reactivity with native COPV L1 VLPs, but very weak reactivity with either denatured COPV L1 protein or native HPV-16 L1 VLPs. Similar results were obtained when this antiserum was tested against either BPV-1, HPV-1, HPV-11 or HPV-18 VLPs (data not shown). This indicated that the antiserum was not only conformation-specific, but also type-specific for COPV L1 protein, similar to rabbit polyclonal antisera against other intact PV virions (Christensen & Kreider, 1990). In contrast, the polyclonal antiserum against denatured COPV L1 was reactive with native COPV L1 VLPs, denatured COPV L1 protein and HPV-16 L1 VLPs. It reacts with conformation-independent epitopes.

Table 1. Characterization of rabbit anti-intact COPV and rabbit anti-denatured COPV L1 antiserum by ELISA

<table>
<thead>
<tr>
<th>Absorbance</th>
<th>COPV L1 VLPs</th>
<th>Denatured COPV L1</th>
<th>HPV-16 L1 VLPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-intact COPV</td>
<td>1:155</td>
<td>0.014</td>
<td>0.037</td>
</tr>
<tr>
<td>Anti-denatured COPV L1</td>
<td>0.724</td>
<td>1.060</td>
<td>0.917</td>
</tr>
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</table>

Construction and expression of wt and mutant COPV L1 proteins

Fig. 1(A) illustrates the construction of a series of L1 truncation mutants that were evaluated for expression of type-specific epitopes by immunofluorescence microscopy (in COS and SF9 cells) and for VLP formation by electron microscopy (SF9 cells).

In several C-terminal truncation mutants, the predicted major nls of L1 (Zhou et al., 1991) was deleted. To compensate for this loss and to allow the L1 protein to translocate normally into the nucleus, an nls sequence from SV40 T antigen (Kaldener et al., 1984) was added to each of these mutants. Two mutant constructs without the T antigen nls, CA26 and CA67, were also generated for evaluating whether the nuclear location was required for proper L1 folding. Plasmid DNAs were transfected and expressed in COS cells and the L1 proteins detected by immunoprecipitation (Fig. 1, B1 and B2). AU1 MAb (Fig. 1, B1) and a rabbit polyclonal antiserum (Fig. 1, B2) were used to detect the synthesis of the wt and mutant L1 proteins. Both AU1 MAb (Lim et al., 1990) and the polyclonal rabbit antiserum (generated against denatured L1 protein) recognize linear, non-conformational L1 epitopes. The rabbit Ab was used in Fig. 1(B2) since the AU1 epitope was deleted in the CA133-nls and CA187-nls mutants.

In general, the mutant proteins migrated at their predicted molecular size. Thus, the CA67, CA133, CA187 deletion mutants showed increasing mobility on the SDS gel (Fig. 1, B2). However, the CA26 protein migrated more rapidly than the NΔ25 protein despite differing by only one amino acid in length. Protein charge or SDS-resistant folding properties of the L1 proteins may account for this aberrant observation. A cluster of positively charged amino acids located in the nls of NΔ25 is absent in CA26. No L1 protein was detected in cells transfected with pYC6 vector alone.

The first 25 amino acids of the COPV L1 N terminus are required for the expression of type-specific, conformational epitopes

To evaluate whether the COPV L1 mutants expressed in COS cells retained native conformational epitopes, immunofluorescence assays were performed with the rabbit polyclonal antiserum (generated against intact COPV virions) which
Fig. 1. (A) Expression vectors for the wt COPV L1 gene and its deletion mutants. All mutation constructs were generated by PCR from plasmid copvg/pBR322, which contains the entire COPV genome. The 5'-end and 3'-terminal primers are described in the text; the final PCR products were digested with NotI and HindIII and cloned into the corresponding sites of a modified pJS55 expression vector. Numbers of the first and last amino acids of each construct are indicated. (B) Immunoprecipitation of wt and mutant COPV L1 proteins. COS cells transfected with the expression plasmids in (A) were metabolically labelled with [35S]methionine/cysteine and cell extracts were immunoprecipitated with mouse anti-AU1 MAb (B1) or rabbit anti-denatured COPV L1 Ab (B2) as described in Methods. Immunoprecipitated proteins were resolved on a 10% SDS–polyacrylamide gel. Molecular mass markers (in kDa) are indicated on the left.

recognizes type-specific, conformation-dependent epitopes. At the same time, rabbit antiserum generated against denatured COPV L1 protein (which recognizes linear, conformation-independent epitopes) was also used to test the expression and intracellular localization of the mutant proteins in COS cells (Fig. 2).

As anticipated, wt L1 protein could be detected in the nucleus with either conformation-independent or -dependent antibodies. Cells transfected with vector alone did not react with either Ab. While N\Delta25 was clearly detectable in the nucleus with conformation-independent antibodies, it was non-reactive with conformation-dependent antibodies. Thus, N\Delta25 was synthesized and transported to the nucleus normally but did not display native conformation.

The N terminus appears to be critical for correct L1 folding

To evaluate whether the inability of N\Delta25 to display type-specific epitopes reflects the deletion of essential epitope(s) in the N terminus or whether the N terminus is essential for correct protein folding, we constructed an HPV-11–COPV L1 chimeric protein. This chimeric L1 protein contained an N terminus consisting of the first 45 amino acids of HPV-11 L1 chimeric protein. This chimera contains an N terminus consisting of the first 45 amino acids of HPV-11 L1 rather than those of COPV L1. While the chimera does not contain COPV type-specific epitope(s) in its N terminus, it does contain some conserved residues which might facilitate proper scaffolding or folding of the L1 protein. Immunofluorescence staining of Sf9 cells expressing the chimeric
Fig. 2. Immunofluorescence microscopy of COS cells transfected with wt and mutant COPV L1 constructs. COS cells were grown on glass coverslips and transfected with each of the indicated constructs. Forty-eight hours later, the cells were fixed and reacted with anti-denatured COPV L1 polyclonal Ab (which recognizes conformation-independent epitopes) or with polyclonal antiserum generated against intact COPV virions (which recognizes only conformation-dependent epitopes). Immunofluorescence microscopy was performed as described in Methods. Microphotographs of representative fields were taken at a magnification of 630 x.
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**Fig. 3.** Immunofluorescence microscopy of Sf9 cells infected with recombinant baculovirus expressing wt COPV L1 or HPV-11–COPV chimeric L1 protein. Sf9 cells were seeded onto coverslips in plastic plates and infected with the designated recombinant baculoviruses for 3 days. Immunofluorescence was done as described in the text using conformation-dependent rabbit antiserum as the primary Ab. Sf9 cells infected with HPV-11–COPV L1 chimera showed positive staining. Microphotographs were taken at a magnification of 630 x.

Protein showed that it was reactive with COPV conformation-dependent antibodies (Fig. 3), indicating that the N terminus of the L1 was required for the proper folding of the L1 protein rather than the display of a type-specific epitope.

**COPV L1 type-specific epitopes are not dependent upon the 26 C-terminal amino acids or upon nuclear localization**

To determine whether the L1 C terminus was important for L1 type-specific epitope expression, COS cells were transfected with the described C-terminal truncation mutants (Fig. 1). Some of these mutants contained an SV40 T antigen nls to compensate for the deletion of their native nls (CA26-nls, CA67-nls, CA133-nls and CA187-nls). All L1 mutant proteins were reactive with conformation-independent antibodies (Fig. 2). As anticipated, most proteins also localized to the nucleus; however, the CA26 and CA67 mutants which lacked an nls domain were expressed in both the nucleus and cytoplasm.

When the L1 C-terminal deletion proteins were reacted with conformation-dependent rabbit antiserum, only the CA26 and CA26-nls mutants were positive by immunofluorescence, indicating that the C-terminal 26 amino acids are dispensable for conformational epitopes and that deletions larger than 26 amino acids abolished reactivity with conformation-dependent antibodies. Interestingly, the CA26 mutant showed positive reactivity in the cytoplasm as well as the nucleus, demonstrating that native L1 conformation does not require protein folding to occur within the nucleoplasmic compartment.

**The abilities of L1 protein to assemble into particles and to display type-specific, conformational epitopes are dissociable**

Previous studies have already demonstrated that the papillomavirus L1 protein, when expressed in a baculovirus vector, can self-assemble into VLPs (Kirnbauer *et al.*, 1992, 1993; Rose *et al.*, 1993; Christensen *et al.*, 1994; Breitburd *et al.*, 1995). In order to determine whether VLP formation was coordinately altered by mutation-induced changes in L1 conformation, we evaluated the ability of a subset of the above L1 mutant proteins to form particles. Specifically, we chose two L1 proteins which were defective for expressing type-specific conformational epitopes (NA25 and CA67) and two which did express conformational epitopes (wt and CA26). These mutant L1 genes were subcloned into a baculovirus transfection vector and recombinant baculoviruses were made as described in Methods (Fig. 4A). The conformational epitopes of these mutant proteins in Sf9 cells were tested by immunofluorescence, with results identical to those observed in COS cells (data not shown). The Sf9-expressed proteins were then purified on CsCl density gradients and their expression was verified by immunoblotting with the rabbit antiserum which recognizes linear, conformation-independent epitopes (Fig. 4B). Surprisingly, when the structures of the NA25, CA26 and CA67 proteins were examined by electron microscopy, they all formed VLPs of uniform shape and size (Fig. 5), although the sizes of the particles formed by the three mutant L1 proteins were slightly smaller than that of the wt VLPs. However, even though the CA26 particles were smaller than wt VLPs, they expressed conformational epitopes. It appears that neither the formation nor size of particles is a valid indicator of the
expression of type-specific epitopes by a specific L1 protein. A summary of the experimental findings with the wt and mutant L1 proteins is presented in Table 2.

Discussion

In this study a series of truncation mutants of the COPV L1 protein (N-terminal and C-terminal) was generated to investigate the relationship between L1 particle formation and the expression of type-specific conformational epitopes. A rabbit anti-intact COPV virion antiserum was used to detect the existence of conformational epitopes. Although this polyclonal antiserum could potentially react with multiple L1 epitopes, the ELISA results demonstrated that it recognized only COPV L1 type-specific and conformation-specific epitopes. The property of this polyclonal Ab is similar to that of other rabbit polyclonal antisera against HPV-11, BPV-1 and CRPV virions (Christensen & Kreider, 1990).

Using the conformation-dependent antiserum, we found that the C-terminal 26 amino acids of COPV L1 were not
required to form conformational epitopes. However, deletion of an additional 43 amino acids from the C terminus abolished L1 reactivity with conformation-specific antibodies, indicating that the L1 domain from amino acid 436 to 477 (sequence between Ca67 and Ca26) was critical for conformational epitope expression (Fig. 6). A previous epitope-mapping study using synthetic peptides corresponding to the HPV-16 L1 sequence suggested that amino acids 426 to 445 of HPV-16 L1 constituted a conformation-dependent antigenic region (Heino et al., 1995). This HPV region (underlined) partially overlaps with the corresponding sequence from amino acid 436 to 477 in COPV L1, suggesting that this domain may have a role in L1 conformational epitope formation. Our study, however, cannot resolve whether this sequence represents a component of a conformational epitope or whether it regulates correct L1 protein folding. Indeed, the finding that this region is not reactive with conformation-dependent antibodies in Na25 (Fig. 3) highlights the importance of other regions of L1 for regulating its correct presentation. Interestingly, the first 45 amino acids of HPV-11 restored COPV L1 reactivity with polyclonal, conformation-dependent antiserum, suggesting not only that the L1 N terminus affects correct L1 folding but that it is not a component of type-specific epitopes. Ludmerer et al. (1996) have shown that two amino acid residues (G131 and Y132) of HPV-11 L1 protein confer type specificity to a neutralizing, conformationally dependent epitope. In our truncation study, small deletions from the N or C terminus of COPV L1 protein abolished the reactivity with the conformation-specific antiserum, despite the retention of the amino acids corresponding to the HPV-11 L1 G131/Y132 epitope.

Our experimental findings with Ca26 are consistent with a deletion mutagenesis study of the BPV-1 L1 protein which demonstrated that deletion of the C-terminal 24 amino acids of BPV-1 L1 generated a protein which could still form VLPs (Paintsil et al., 1996). However, when an additional 20 amino acids were deleted from the C terminus (amino acids 451 to 495) in the previous study, the mutant protein failed to form particles. In our study, even when 67 amino acids were deleted from the C terminus, VLP formation was observed readily. Since somewhat different conditions were used to isolate the VLPs in these two studies, it is possible that VLP stability might account for these experimental differences. We have noted that the Ca67 VLPs are less stable than those formed by Ca26 and any variations in VLP extraction and purification methods (especially buffer ionic strength) might affect the detection of VLP formation. There also appear to be significant differences in the ability of L1 mutants to form VLPs depending upon the cellular system in which they are expressed. For example, when the BPV-1 L1 protein was expressed by the baculovirus system, C-terminal deletions of 24 amino acids (Ca24; Paintsil et al., 1996) or 26 amino acids (Ca26; current study) did not interfere with the ability of L1 to form VLPs. However, when the HPV-11 L1 protein was expressed in bacteria and truncated at the C terminus by trypsinization (Li et al., 1997), it could not form stable VLPs. This could potentially reflect differences in L1 modification in eukaryotic versus prokaryotic cells; alternatively, it could reflect the multiple sites at which trypsin might be cleaving within the L1 C terminus. If trypsin were to cleave at the arginine residue which is 31 amino acids from the C terminus, it would generate an L1 protein virtually identical to Ca24 and Ca26 (which form VLPs). The failure of trypsinized HPV-11 L1 to form VLPs suggests that either there are modifications of the eukaryotic-expressed L1 proteins which alter its ability to form these higher-order structures or that trypsin cleaves at the lysine residues which are 39 and 52 amino acids from the C terminus. This could potentially generate an L1 protein which forms less stable VLPs (see above discussion). There are also significant differences between eukaryotic- and prokaryotic-expressed L1 N-terminal deletions. For example, we found that Na25 was able to efficiently form VLPs. On the other hand, when 29 amino acids were deleted from HPV-11 L1 expressed

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<th>L1 construct</th>
<th>Conformational epitopes</th>
<th>VLP formation</th>
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<tbody>
<tr>
<td>wt L1*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ca26†</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ca67‡</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Na25§</td>
<td>–</td>
<td>+</td>
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Fig. 6. Sequence alignment of the L1 C terminus of COPV, HPV-16, HPV-11 and BPV-1 L1 proteins. Homologous regions of the indicated L1 proteins are displayed. Numbers indicate the N- and C-terminal amino acids. The positions of the CΔ67 and CA26 deletions for COPV are indicated as well as the position of the CA24 deletion mutant for BPV-1. The predicted cleavage sites by trypsinization in HPV-11 L1 are indicated in bold. The underlined amino acids for HPV-16 L1 indicate the epitope which is exposed on intact but not disrupted HPV-16 L1 VLPs (Heino et al., 1995).

This study was supported by grants from the National Cancer Institute which were awarded to R.S. (R01CA57994 and R01CA53371) and a grant from the National Institute of Dental Research awarded to A.B.J. (R01DE11245). We would also like to thank JoAnn A. Suzich from MedImmune Inc. for her rabbit antiserum against denatured COPV L1 and Yu Hua Sun for her excellent technical assistance with the Sf9 cell culture work.

References


Received 13 March 1998; Accepted 14 May 1998