Interaction of L2 with β-Actin Directs Intracellular Transport of Papillomavirus and Infection*

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Viruses that replicate in the nucleus, including the primary causative agent of cervical cancer, human papillomavirus type 16 (HPV16), must first cross the cytoplasm. We compared the uptake of HPV16 virus-like particles (VLPs) either with or without the minor capsid protein L2. Whereas VLPs containing only the major capsid protein L1 were diffusely distributed within the cytoplasm even 6 h post-infection, VLPs comprising both L1 and L2 exhibited a radial distribution in the cytoplasm and accumulated in the perinuclear region of BPHE-1 cells within 2 h. L2 of HPV16 or bovine papillomavirus was shown to bind to a 45-kDa cellular protein that was subsequently identified as β-actin by matrix-assisted laser desorption ionization time-of-flight analysis. A conserved domain comprising residues 25–45 of HPV16 L2 was sufficient for interaction with β-actin. HPV16 L2 residues 25–45 fused to green fluorescent protein, but not green fluorescent protein alone, colocalized with actin and caused cell retraction and disruption of the microfilament network. Finally, wild-type L2, but not L2 with residues 25–45 deleted, facilitated HPV16 pseudovirion infection. Thus, binding of β-actin by L2 residues 25–45 facilitates transport of HPV16 across the cytoplasm during infection, and blockade of this novel interaction may be useful for prophylaxis.

Passive diffusion of molecules within the cytoplasm is limited by molecular crowding and does not provide targeting to a particular subcellular domain (1). Thus, many intracellular pathogens subvert existing transport mechanisms and cytoskeletal components, both to efficiently reach their site of replication and also for the exit of their progeny (2). The cytoskeleton is highly dynamic, and its role in locomotion is regulated by a plethora of actin- and tubulin-binding proteins, kinases, and phosphatases of multiple signaling cascades. Changes in tyrosine phosphorylation of actin regulatory proteins induce the condensation of actin “comet” tails behind endosomes (3), as well as the bacteria Listeria, Shigella, and Rickettsia (4) and viruses including vaccinia, baculovirus, and SV40, for propulsion through the cytoplasm (5, 6). Other viruses employ cellular motors such as dyneins and kinesins for transport along microtubules. A single virus type can employ several intracellular transport mechanisms. Indeed, vaccinia particles are driven along microtubules by kinesin, whereupon actin tails take over propulsion (7, 8).

Compelling epidemiologic and molecular virologic studies demonstrate that infection with an oncogenic type human papillomavirus (HPV),1 typified by HPV16, is a necessary cause of cervical cancer (9). In the absence of effective screening programs, cervical cancer is a leading cause of cancer death in women (10). Furthermore, oncogenic HPV infection is also strongly associated with vulval, anal, and penile cancers, some non-melanoma skin cancers, and esophageal and salivary cancers (11). An understanding of the infectious process is critical to rational development of approaches for prevention of HPV-related cancers. Although several cellular molecules, including heparan sulfate glycosaminoglycans (12), α6 integrin (13), and CD16 (14), have been implicated as cell-surface receptors for papillomavirus, little else is known about cellular proteins that mediate cytoplasmic transport of papillomavirus and delivery of the viral genome to the nucleus.

The papillomavirus capsid comprises the major capsid protein L1 arranged as 72 pentamers, or capsomers, in a T=7d icosahedral surface lattice (15, 16) and a minor capsid protein, L2 (17), one molecule of which may be located at each vertex (18). Overexpression of L1 alone is sufficient to form empty capsids, termed virus-like particles (VLPs) (19). L1 VLPs bind to cell surfaces and compete with bovine papillomavirus type 1 (BPV1) infection in vitro (20). However, both L1 and L2 are necessary for efficient production of papillomavirus and infection (21–23). Recent studies by Kawana et al. (24, 25) suggest that residues 108–120 of L2 are displayed upon the virion exterior and bind to the cell surface, resulting in internalization. Furthermore, anti-L2 antiserum neutralizes papillomavirus without preventing virion binding to the cell surface (26). We recently demonstrated that L2 plays a critical role in infection, but is not required for interaction of papillomavirus particles with the cell surface (23). Taken together, the data suggest that L1 mediates the initial binding of virions to the cell surface, whereas L2 provides later functions critical for infection.

EXPERIMENTAL PROCEDURES

Preparation of Papillomavirus VLPs and L2—VLPs containing L1 and L2, only L1, or L1 and L2 lacking residues 25–45 were generated by infection of SF9 with recombinant baculoviruses and purified as previously described (7, 27). L2 was produced by in vitro transcription and translation of plasmid DNA as described (27). VLP preparations were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: HPV, human papillomavirus; VLP, virus-like particle; BPV, bovine papillomavirus; GST, glutathione S-transferase; GFP, green fluorescent protein; TPCK, t-1-tosylamido-2-phenylethyl chloromethyl ketone; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; PBS, phosphate-buffered saline.
ously reported (27). The HPV16 L2Δ25–45 deletion mutant was prepared by two rounds of PCR using oligonucleotides GCAGGCTGGTGACCCAGACAACTTACGTTACATTTAAG or TCCCCCGGGCTTACCGGACAAAAAGGAC and CTTTATATACGCTGACGTTGATGCTGCTGCTGGG, followed by just the outside primers. The quantity and quality of VLP preparations were analyzed by SDS-PAGE and electron microscopy, respectively. His6-tagged BPV1 L2 fusion proteins were prepared as previously described (26). For generation of glutathione S-transferase (GST)-tagged L2-green fluorescent protein (GFP) fusion proteins, L2 oligonucleotides with EcoRI and SalI overhangs were directly synthesized (HPV16 L2–13 (31), AATTCTGGACTGCTACAACCATTTATAAACAATGACACGCGGTACATGTCCACCTGACATTATACCTAAGGTTGAAGGCAAAACTATTGCT- TCAACCTTATGATTAAGTACGTTGACATGTCGTGCTGGG. and HPV16 L2–25 (31), AATTCTGGACTGCTACAACCATTTATAAACAATGACACGCGGTACATGTCCACCTGACATTATACCTAAGGTTGAAGGCAAAACTATTGCT- TCAACCTTATGATTAAGTACGTTGACATGTCGTGCTGGG, and HPV16 L2–108 (31), AATTCTGGACTGCTACAACCATTTATAAACAATGACACGCGGTACATGTCCACCTGACATTATACCTAAGGTTGAAGGCAAAACTATTGCT- TCAACCTTATGATTAAGTACGTTGACATGTCGTGCTGGG. and HPV16 L2–200 (31), AATTCTGGACTGCTACAACCATTTATAAACAATGACACGCGGTACATGTCCACCTGACATTATACCTAAGGTTGAAGGCAAAACTATTGCT- TCAACCTTATGATTAAGTACGTTGACATGTCGTGCTGGG. and HPV16 L2–299 (31), AATTCTGGACTGCTACAACCATTTATAAACAATGACACGCGGTACATGTCCACCTGACATTATACCTAAGGTTGAAGGCAAAACTATTGCT- TCAACCTTATGATTAAGTACGTTGACATGTCGTGCTGGG. and HPV16 L2–333 (31), AATTCTGGACTGCTACAACCATTTATAAACAATGACACGCGGTACATGTCCACCTGACATTATACCTAAGGTTGAAGGCAAAACTATTGCT- TCAACCTTATGATTAAGTACGTTGACATGTCGTGCTGGG. and HPV16 L2–45 deletion mutant was prepared as described for the generation of HPV16 particles. The resulting DNA was inserted into the pSFV4.2. pseudovirions were generated, and their infectivity was assayed as described previously (22). The HPV16 L2Δ25–45 deletion mutant was prepared as described for the generation of HPV16 particles. The resulting DNA was inserted into the BamHI and XmaI restriction sites of pSFV4.2.

**RESULTS**

L2 Facilitates Perinuclear Trafficking—To further define the role of L2 in the infectious process, we compared the binding, uptake, and intracellular transport of HPV16 VLPs containing both L1 and L2 (L1/L2 VLPs) with VLPs comprising only L1 (L1 VLPs) (27) in the permissive cell line BPHE-1 (29). HPV16 VLPs were incubated with BPHE-1 cells for 1 h at 4 °C and visualized by indirect immunofluorescence. As we previously demonstrated (20), VLPs comprising L1 and L2 or only L1 bound to cell surfaces with a similar pattern and degree (data not shown). Upon shifting to 37 °C for 15 min to initiate synchronized uptake, the cells were fixed, sectioned, and examined by transmission electron microscopy. No differences were noted in the cellular ultrastructure of plasma membrane-associated L1 VLPs (Fig. 1, A1 and A2) or L1/L2 VLPs (B1 and B2) or their engulfment by invagination of the plasma membrane (A2 and B2). At 30 min and 1, 2, and 6 h after shifting the BPHE-1 cells to 37 °C, the VLPs were localized (Fig. 2) by indirect immunofluorescence using the HPV16 L1-specific, conformationally dependent, neutralizing monoclonal antibody H16.V5 (30, 31). Interestingly, L1/L2 VLPs aligned along distinct radial tracks across the cytoplasm and within ~2 h at 37 °C arrived in the perinuclear region (Fig. 2, A1–A2). However, L1 VLPs were not aligned along such radial tracks. Rather, L1 VLPs remained widely distributed throughout the whole cell and showed a less clear-cut tropism toward the nucleus during this 6-h time course (Fig. 2, B1–B3). These differences in the uptake of L1/L2 and L1 VLPs suggest that L2 contributes to the transport of virions across the cytoplasm.

Because the cytoskeleton provides a framework for intracellular transport and L1/L2 VLPs exhibited a radial distribution (Fig. 2, A1 and A2) during transit to the perinuclear region, we determined the subcellular localization of cytoskeletal components during VLP uptake. Furthermore, Liu et al. (32) observed an interaction between L1 and tubulin, as well as blockade of
particle uptake by the microtubule-depolymerizing agent nocodazole. Thus, we examined the relative localization of HPV16 L1 VLPs and tubulin by immunofluorescent staining. However, limited overlap of L1 VLP and tubulin signals was noted 2 h after uptake (Fig. 2E). Cytochalasin B both disrupted the microfilament network by depolymerizing actin (Fig. 2, compare D and E) and inhibited uptake of HPV16 L1 (data not shown) and L1/L2 (Fig. 2D) VLPs, consistent with previous studies (33). Therefore, we compared the localization of actin (using rhodamine-phalloidin) and VLPs during their uptake. Upon their initial uptake, the VLPs colocalized with cortical actin at the periphery of the cell. At later time points, the radially distributed L1/L2 VLPs colocalized with actin filaments (Fig. 2A2), whereas little overlap was observed for L1 VLPs and actin (compare A and B panels). Thus, actin polymerization is critical early in VLP uptake, and the particles colocalize with actin microfilaments while traversing the cytoplasm. Although other viruses, including the structurally related SV40, induce...
His6-tagged polypeptides spanning residues 1–25, 25–333, or with GST fused to full-length HPV16 L2. The bound proteins were eluted with 50 mM Tris-HCl at pH 8.0 and analyzed by Western blotting with mouse anti-β-actin monoclonal antibody (AC-15, Sigma). E, purified GST-GFP alone or fused to HPV16 L2 fragment 25–45 or control fragment 299–333 was incubated with purified rabbit muscle actin in PBS for 1 h and then passed through a GSTrap FF column. After extensive washing, the bound proteins were eluted and visualized by SDS-PAGE and Coomassie staining.

the formation of actin comet tails (34), this phenomenon was not observed during HPV16 VLP uptake, suggesting a different mode of transport.

Residues 25–45 of L2 Bind β-Actin—BPV1 is frequently exploited in virologic studies because, unlike HPV5 (35), BPV can be readily prepared in milligram quantities, and its infectivity can be readily assayed in vitro (36). To identify cellular “targeting molecule(s)” recognized by L2, we generated in E. coli six Histagged polypeptides spanning residues 1–88, 45–173, 130–257, 216–340, 300–425, and 384–469 that together encompass the entire open reading frame of BPV1 L2 (26). These polypeptides were each incubated with [35S]methionine/cysteine-radiolabeled SiHa cell lysates and immunoprecipitated with a monoclonal antibody to GFP. After addition of Histagged BPV1 L2 fragments comprising the residues indicated, immunoprecipitation was performed with Hist-specific monoclonal antibody and protein G-Sepharose for 16 h at 4 °C with slow agitation. The immunoprecipitates were washed six times with ice-cold lysis buffer and resolved by 10% SDS-PAGE and autoradiography. B, purified GST-GFP alone (GFP) or fused to HPV16 L2 residues 1–128 (1–128) was incubated with [35S]radiolabeled and preclared SiHa cell lysates and immunoprecipitated using monoclonal antibody to GFP or isotype-matched control antibody. C, coprecipitation was performed as described for B, but using GST-GFP fused to different regions of HPV16 L2. D, HeLa cell lysate in ice-cold buffer A was clarified by centrifugation at 16,000 × g for 30 min and passed over glutathione-Sepharose precoated with GST-GFP either alone or fused to HPV16 L2 residues 25–45, 1–128, or 299–333 or with GST fused to full-length HPV16 L2. After extensive washing, the bound proteins were eluted with 50 mM Tris-HCl and 10 mM reduced glutathione (pH 8.0) and analyzed by Western blotting with mouse anti-β-actin monoclonal antibody (AC-15, Sigma). E, purified GST-GFP alone or fused to HPV16 L2 fragment 25–45 or control fragment 299–333 was incubated with purified rabbit muscle actin in PBS for 1 h and then passed through a GSTrap FF column. After extensive washing, the bound proteins were eluted and visualized by SDS-PAGE and Coomassie staining.

derived from other papillomavirus genotypes is also able bind to this 43-kDa cellular protein, we next generated in E. coli a chimera comprising GST fused in-frame to amino acids 1–128 of HPV16 L2 and GFP to form GST-HPV16 L2-(1–128)-GFP. The purified fusion protein was incubated with [35S]methionine/cysteine-radiolabeled SiHa cell lysates and immunoprecipitated using a monoclonal antibody to GFP. The GST-HPV16 L2-(1–128)-GFP (but not GST-GFP) fusion protein also bound to the 43-kDa cellular protein (Fig. 3D), showing that this interaction is conserved in two evolutionarily distant papillomavirus types, BPV1 and HPV16. When testing smaller N-terminal subfragments of HPV16 L2, only residues 25–45 co-immunoprecipitated with the 43-kDa cellular protein from detergent lysates of radiolabeled SiHa cells (Fig. 3C), suggesting that this motif is sufficient for interaction.

To determine the distribution of the cellular protein that interacts with the HPV16 L2-GFP fusion protein, we examined, by confocal fluorescence microscopy (Fig. 4, A1.1–A1.4) and flow cytometry (A2.1–A2.4), the binding to detergent-permeabilized human cervical carcinoma-derived cell lines of GST-GFP chimeric proteins either with or without HPV16 L2 residues 1–128. Whereas the GST-GFP fusion protein failed to bind to SiHa cells, GFP fusion proteins containing either residues 1–128 or 25–45 of HPV16 L2 bound to a similar extent within the cytoplasm of the detergent-permeabilized SiHa cells (Fig. 4). To eliminate the possible effects of GST in the binding of the fusion protein to cells, the GST-HPV16 L2-GFP fusion proteins were digested with PreScission protease (Fig. 4B1, PreS.P) to release the GST tag (37). Thus, the binding to SiHa cells of PreScission protease-digested (Fig. 4B3.2) and undigested (Fig. 4B3.4) GST-HPV16 L2-(25–45)-GFP fusion protein was compared. L2-GFP (but not GFP alone) bound to SiHa cells to a similar extent either with or without GST, indicating that neither GST nor GFP mediates binding. Flow cytometric analysis showed that HPV16 L2 residues 1–128 bound to both HPV-positive cervical carcinoma-derived cell lines HeLa (data not shown) and SiHa (Fig. 4A2.2) to a similar extent as the HPV-negative human cervical carcinoma-derived cell line C33A (Fig. 4A2.4), indicating that HPV16 L2 binds to a cytoplasmic component that is not derived from papillomavirus.

To identify the 43-kDa cellular protein, a detergent lysate of SiHa cells was passed over a GST-HPV16 L2-(1–128)-GFP-coated column. After extensive washing, the proteins bound were eluted and visualized by SDS-PAGE and silver staining (data not shown). The 43-kDa protein band recovered was excised and subjected to in-gel trypsin digestion and MALDI-TOF analysis. This analysis resulted in the identification of 13 peptides whose protein sequences were all consistent with β-actin (Fig. 5, A and B).

Because peptides of L2 were used for the actin binding experiments, it is possible that the truncations resulted in exposure to a nonphysiologic cryptic epitope. To demonstrate interaction between full-length L2 and actin, a detergent lysate of HeLa cells was passed over glutathione-Sepharose beads pre-coated with GST fused to full-length HPV16 L2. The bound proteins were separated by electrophoresis and subjected to Western blot analysis using a mouse monoclonal antibody to β-actin (Fig. 3D). Full-length HPV16 L2 and its fragments 1–128 and 25–45 bound to actin, whereas fragment 299–333 and the GFP control did not (Fig. 3D). Thus, binding to actin is a property of full-length L2.

It is unclear whether L2 binds directly to actin. Therefore, to address this question, purified GST-GFP fusion proteins containing HPV16 L2 residues 25–45 or, as a negative control, residues 299–333 were incubated for 1 h at ambient temperature with actin purified from rabbit muscle (A-2522, Sigma).
Upon pull-down with glutathione-Sepharose, actin copurified with the GST-GFP fusion protein containing residues 25–45 (but not residues 299–333) of HPV16 L2 (Fig. 3E). This observation strongly supports the existence of a direct interaction between HPV16 L2 residues 25–45 and actin.

L2 Residues 25–45 Are Necessary for Efficient Transport to the Perinuclear Region and HPV16 Infection—Studies in other viral systems suggest that interaction with actin can facilitate the intracellular transport of viral particles and infection. Therefore, to address the biologic significance of the putative actin-binding domain in HPV16 L2, we compared the uptake of HPV16 VLPs comprising wild-type L1 alone, L1 and L2, and L1 and L2 with residues 25–45 deleted (L1/L2Δ25–45). Whereas wild-type HPV16 L1/L2 VLPs were rapidly transported to the perinuclear region along radial tracts (Fig. 2A1), L1/L2 VLPs lacking residues 25–45 failed to align along radial tracts in the cytoplasm and did not reach the perinuclear region during a 6-h time course (Fig. 2, C1–C3). Rather, L1/L2Δ25–45 VLPs remained widely distributed throughout the cell as described for L1 VLPs (Fig. 2, B1–B3).

Because papillomavirus exhibits a high particle to infectivity ratio in vitro (22), it is possible that the uptake of HPV16 VLPs shown in Fig. 1 does not represent the true infectious pathway. Therefore, to examine the significance of this interaction between L2 and β-actin to the infectious process, we generated HPV16 pseudovirions lacking the conserved β-actin-binding domain, viz. residues 25–45 (Fig. 6A), and tested their infectivity using a previously described system. Briefly, the hamster fibroblast cell line BPHE-1 harbors 50–200 episomal copies of the bovine papillomavirus genome/cell (29), but it produces no virus because the L1 and L2 genes are not expressed. However, ectopic expression of HPV16 L1 and L2 in BPHE-1 cells via infection with recombinant defective Semliki Forest viruses results in the generation of infectious HPV16 pseudovirions containing the BPV genome within capsids formed of HPV16 L1 and L2 (22). Like native BPV1 virions, the infectivity of HPV16 (BPV1) pseudovirions can readily be quantified using the in vitro focal transformation of mouse C127 cells (36). HPV16 pseudovirions lacking residues 25–45 of L2 showed dramatically reduced infectivity (Fig. 6), suggesting that interaction between L2 and β-actin indeed plays a critical role in the infectious process of papillomavirus.

Cytoplasmic Overexpression of HPV16 L2 Residues 25–45 Disrupts the Actin Architecture—Actin is one of most abundant proteins in eukaryotic cells, and its role as a primary determinant of cell shape, cytoplasmic structure, and locomotion is highly regulated by a plethora of binding proteins. Given the ability of L2 to bind to actin and the karyophilic nature of full-length L2, we hypothesized that an overabundance of L2 within the cytoplasm might induce changes in the cytoskeleton and cell morphology. To test this hypothesis, the fragment of HPV16 L2 encoding residues 25–45 was inserted 3' of the GFP gene in the mammalian expression vector pEGFP-C2 to form pEGFP-L2-(25–45). COS-7 cells were transfected with either pEGFP-C2 or pEGFP-L2-(25–45). Three days after transfection, equivalent expression of GFP alone and fused to HPV16 L2 residues 25–45 was confirmed by Western blot analysis with a monoclonal antibody to GFP (data not shown). The subcellular localization of actin (upon staining with rhodamine-phalloidin) and either GFP alone or fused to HPV16 L2
residues 25–45 was examined by confocal fluorescence microscopy. Transient expression of GFP fused to HPV16 L2 residues 25–45 within the cytoplasm of COS-7 cells induced a dramatic retraction of transfected cells from the culture surface (Fig. 7B1). In contrast, expression of GFP alone in the transfected cells did not noticeably influence cell morphology (Fig. 7A1) compared with the parental untransfected cells (data not shown). Interestingly, staining revealed apparent reorganization of filamentous actin into cytoplasmic bundles, which colocalized with the GFP protein band that bound to residues 1–128 of HPV16 L2 was excised and digested with TPCK-treated sequencing grade trypsin as previously described (28). Masses of the resulting peptides were measured by MALDI-TOF analysis on a Voyager DE STR apparatus. Positive ion mass spectra were analyzed using Data Explorer (Version 3.5). Mass accuracy was better than 100 ppm. B, the predicted and measured peptide masses are listed. β-Actin was identified by using the acquired monoisotopic masses to search the NCBI Non-redundant Database using the MS-Fit search engine on the Protein Prospector Web site (see Footnote 2).

![Fig. 5. Tryptic mass fingerprint identifies the 43-kDa band as β-actin. A, the 43-kDa protein band that bound to residues 1–128 of HPV16 L2 was excised and digested with TPCK-treated sequencing grade trypsin as previously described (28). Masses of the resulting peptides were measured by MALDI-TOF analysis on a Voyager DE STR apparatus. Positive ion mass spectra were analyzed using Data Explorer (Version 3.5). Mass accuracy was better than 100 ppm. B, the predicted and measured peptide masses are listed. β-Actin was identified by using the acquired monoisotopic masses to search the NCBI Non-redundant Database using the MS-Fit search engine on the Protein Prospector Web site (see Footnote 2).](image)

During the infectious process, an intracellular pathogen hijacks the normal cellular function of its receptor molecules for transportation to its site of replication. Thus, study of such infectious pathways can both enhance our understanding of normal cellular transport as well as identify targets for intervention. Although intracellular pathogens use diverse primary and secondary receptors to gain entry to the cell, the mechanisms employed for intracellular transport are more restricted. For example, microorganisms as diverse as Listeria, Shigella, Richettsia, vaccinia, and SV40 employ different receptors to gain entry to a cell, but a similar mode of intracellular transport (6, 34). However, despite their structural similarity to SV40 (18), the papillomaviruses use a distinct cell-surface receptor and entry pathway, about which little is known. Indeed, three different surface molecules (12–14) have been proposed to bind to the major capsid protein L1 and to function as the primary receptor for papillomavirus. Papillomavirus L1 pseudovirions prepared in vitro are infectious, although significantly less so than those containing L2 (38–40). We have provided genetic evidence that L2 also plays a critical role during papillomavirus infection, but after the initial binding of
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The high degree of sequence conservation of the L2 motif (Fig. 6A) and the ability of both BPV1 and HPV16 L2 to bind actin suggest that this interaction is common to other papillomavirus genotypes and therefore may represent a useful target for the development of pan-papillomavirus-preventative treatments. Indeed, antibody to L2 (but not L1) neutralizes diverse papillomavirus genotypes, suggesting that a conserved functional domain of L2 is displayed on the capsid surface (25, 43). Furthermore, L2-specific neutralizing antibodies predominantly recognize its N terminus and do not prevent virions from binding to the cell surface (26), suggesting that neutralization may occur by a blockade of virion uptake or intracellular transport.

Several pathogens have independently evolved mechanisms to harness the power of actin polymerization to get into and out of cells (34). Indeed, the entry of a wide variety of viruses, including papillomavirus, human immunodeficiency virus, vaccinia, Autographa californica M nucleopolyhedrovirus, adenovirus type 2, and echoviruses, is dependent upon actin polymerization as demonstrated by blockade with the inhibitor cytochalasin D (44). However, this does not necessarily reflect a direct interaction between the virus and actin because actin function is necessary for receptor-mediated endocytosis and many other cellular processes.

A. californica M nucleopolyhedrovirus induces the formation of thick actin cables that frequently project toward the nucleus. These actin cables are transiently formed in association with the viral nucleocapsids prior to viral gene expression and concomitantly with nucleocapsid transport to the nucleus (45). Two virus-encoded capsid proteins, p39 and p78/83, in A. californica M nucleopolyhedrovirus were found to bind to actin directly and therefore could be involved in the observed acceleration of actin polymerization by viral actin-binding proteins (46). It is unclear how this effect relates to the changes in actin structure produced by overexpression of L2 residues 25–45 within the cytoplasm because neither papillomavirus L1/L2 VLPs nor L2 induces such actin cables.

Both endosomes and diverse pathogens are able to recruit host cytoskeletal factors to induce the polymerization of actin filaments from their surface into a structure known as a comet tail for intracellular propulsion (34). However, the presence of L2 or L1/L2 VLPs during infection did not promote the formation of such actin comet tails, suggesting that interaction of L2 with β-actin facilitates particle transport by another mechanism.

Actin is an ATPase, and ATP hydrolysis affects the kinetics of polymerization (47). In vivo, actin polymerization is a highly regulated process controlled both by ATP binding and hydrolysis and by the action of a number of actin-binding proteins that initiate, cleave, cross-link, stabilize, or destabilize the filaments (48). Actin comet tails result from a depolymerization of filamentous actin and re-polymerization behind the particle (34). Interestingly, overexpression of the β-actin-binding motif comprising residues 25–45 of HPV16 L2 in the cytoplasm was associated with the redistribution of actin in COS-7 cells and altered cell morphology. This is consistent with a functional interaction between L2 and actin in vivo that orchestrates the intracellular motility of papillomavirus during infection.

In addition to promoting uptake, the actin cytoskeleton facilitates egress of vaccinia from infected cells (8, 34). The spread of vaccinia is enhanced by actin tail formation that is triggered via tyrosine phosphorylation of A36R. Herpesvirus type 1 VP22 exploits microfilaments to promote intercellular spreading (49). Furthermore, interaction of the Black Creek Canal virus N protein with actin microfilaments is required for virion morphogenesis and release, but not infection (50). However, papillomavirus accumulates in microcrystalline arrays within the nucleus of productively infected cells, and there is currently no evidence for such an egress pathway mediated by L2-actin interaction.

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References

1. Lub-view, K. (2000) Int. Rev. Cytol. 192, 189–221
2. Sodeik, B. (2000) Trends Microbiol. 8, 465–472
3. Tarasuk, J., Rowing, B. A., Coughlin, M. L., Wu, M., Moon, R. T., Mitchison, T. J., and Larabell, C. A. (2000) J. Cell Biol. 148, 519–530
4. Dramsi, S., and Cossart, P. (1998) Annu. Rev. Cell Dev. Biol. 14, 137–166
5. Cudmore, S., Reckmann, I., and Way, M. (1997) Trends Microbiol. 5, 142–148
6. Polkman, L., Puntener, D., and Helenius, A. (2002) Science 296, 535–539
7. Rietdor, J., Ploubidiou, A., Reckmann, I., Holmstrom, A., Frischknecht, F., Zettl, M., Zimmermann, T., and Way, M. (2001) Nat. Cell Biol. 3, 992–1000
8. Moss, B., and Ward, B. M. (2001) Nat. Cell Biol. 3, E245–E246
9. Walmsley, J. M., Jacobs, M. Y., Manos, M. M., Bosch, F. X., Kummer, J. A., Shah, K. V., Snijders, P. J., Peto, J., Meijer, C. J., and Munoz, N. (1999) J. Pathol. 182, 10–17
10. Pisani, P., Parkin, D. M., Bray, F., and Ferlay, J. (1999) Int. J. Cancer 3, 18–29
11. zur Hausen, H. (2002) Nat. Rev. Cancer 2, 342–350
12. Joyce, J. G., Tung, J. S., Przysecki, C. T., Cook, J. C., Lehman, E. D., Sands, J. A., Jansen, K. U., and Keller, P. M. (1999) J. Biol. Chem. 274, 5810–5822
13. Evander, M., Faer, J. H., Payne, E., Qi, Y. M., Hengst, K., and McMillan, N. A. (1997) J. Virol. 71, 2449–2456
14. Da Silva, D. M., Velders, M. P., Nieland, J. D., Schiller, J. T., Nickoff, J. B., and Kast, R. M. (2001) Int. Immunol. 13, 623–641
15. Baker, T. S., Newcomb, W. W., Olson, N. H., Cowsert, L. M., Olson, C., and Brown, J. C. (1991) Biophys. J. 60, 1445–1456
16. Hagenesse, M. E., Olson, N. H., Baker, T. S., and Galloway, D. A. (1994) J. Virol. 68, 4503–4505
17. Doorbar, J., and Gallimore, P. H. (1987) J. Virol. 61, 2793–2799
18. Trus, B. L., Roden, R. B., Greenstone, H. L., Vrabl, M., Schiller, J. T., and Bovy, F. P. (1997) Nat. Struct. Biol. 4, 413–420
19. Kirnhauser, R., Bovy, F., Cheng, N., Lowy, D. R., and Schiller, J. T. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 12180–12184
20. Roden, R. B., Kirnhauser, R., Jensen, A. B., Lowy, D. R., and Schiller, J. T. (1994) J. Virol. 68, 7260–7266
21. Zhou, J., Stenzel, D. J., Sun, X. Y., and Faer, I. H. (1993) J. Gen. Virol. 74, 573–586
22. Roden, R. B., Greenstone, H. L., Kirnhauser, R., Bovy, F. P., Jessie, J., Lowy, D. R., and Schiller, J. T. (1996) J. Virol. 70, 5875–5883
23. Roden, R. B., Day, P. M., Bronzo, B. K., Yu, W. Y., IV, Yang, Y., Lowy, D. R., and Schiller, J. T. (2001) J. Virol. 75, 10493–10497W
24. Kawana, Y., Kawana, K., Yoshikawa, H., Taketani, Y., Yoshikawa, K., and Kanda, T. (2001) J. Virol. 75, 2331–2336
25. Kawana, K., Yoshikawa, H., Taketani, Y., Yoshikawa, K., and Kanda, T. (1999) J. Virol. 73, 6188–6199
26. Roden, R. B., Weisenger, E. M., Henderson, D. W., Bovy, F., Kirnhauser, R., Mushinski, J. F., Lowy, D. R., and Schiller, J. T. (1994) J. Virol. 68, 7570–7574
27. Kirnhauser, R., Taub, J., Greenstone, H., Roden, R., Durst, M., Gissmann, L., Lowy, D. R., and Schiller, J. T. (1993) J. Virol. 67, 6929–6936
28. Chevenchenko, A., Wilm, M., Vorm, O., and Mann, M. (1996) Anal. Chem. 68, 850–858
29. Zhang, Y. L., Lewis, A., Jr., Wade-Glass, M., and Schlegel, R. (1987) J. Virol. 61, 2924–2929
30. Christensen, N. D., Dillner, J., Eklund, C., Carter, J. J., Wipf, G. C., Reed, C. A., Cledal, N. M., and Galloway, D. A. (1996) Virology 223, 174–184
31. Roden, R. B., Armstrong, A., Haderer, P., Christensen, N. D., Hubbert, N. L., Lowy, D. R., Schiller, J. T., and Kirnhauser, R. (1997) J. Virol. 71, 6247–6252
32. Liu, W. J., Qi, Y. M., Zhao, K. N., Liu, Y. H., Liu, X. S., and Fraer, I. H. (2001) Virology 282, 237–244
33. Zhou, J., Gissmann, L., Zentgraf, H., Muller, H., Picken, M., and Muller, M. (1995) Virology 214, 167–176
34. Frischknecht, F., and Way, M. (2001) Trends Cell Biol. 11, 30–38
35. Meyers, C., Prattini, M. G., Hudson, J. B., and Lainains, L. A. (1992) Science 257, 971–973
36. Dvoresky, I., Shober, R., Chattpahiy, S. K., and Lowy, D. R. (1980) Virology 103, 369–375
37. Cordingley, M. G., Callahan, P. L., Sardana, V. V., Garsky, V. M., and Colonna, R. J. (1990) J. Biol. Chem. 265, 9062–9065
38. Tov, L., and Courasge, P. (1998) Nucleic Acids Res. 26, 1317–1323
39. Kawana, K., Yoshikawa, H., Taketani, Y., Yoshikawa, K., and Kanda, T. (1998) J. Virol. 72, 10298–10300
40. Ungcel, F., Streeck, R. E., and Sapp, M. (1997) J. Virol. 71, 2934–2939
41. Merle, E., Rose, R. C., Lefoux, L., and Moransio, J. (1997) J. Cell. Biochem. 74, 628–637
42. Zhou, J., Sun, X. Y., Louis, K., and Fraer, I. H. (1994) J. Virol. 68, 619–625
43. Roden, R. B., Yu, W. Y., IV, Fallon, R., Inglis, S., Lowy, D. R., and Schiller, J. T. (2000) Virology 270, 254–257W
44. Ploubidhos, A., and Way, M. (2001) Curr. Opin. Cell Biol. 13, 97–105
45. Charlton, C. A., and Volkman, L. E. (1993) Virology 197, 245–254
46. Lanier, L. M., and Volkman, L. E. (1998) Virology 243, 167–177
47. Korn, E. D., Carlier, M. F., and Pancelloni, D. (1987) Science 238, 638–644
48. Schmidt, A., and Hall, M. N. (1998) Annu. Rev. Cell Dev. Biol. 14, 305–338
49. Elliott, G., and O’Hare, P. (1997) Cell 88, 223–223
50. Barkov, E. V., Nichol, S. T., Peters, C. J., and Compana, R. W. (1998) J. Virol. 72, 2865–2870