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Viral entry mechanisms: human papillomavirus and a long journey from extracellular matrix to the nucleus

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Abstract

Papillomaviruses are epitheliotropic non-enveloped double stranded DNA viruses, whose replication is strictly dependent on the terminally differentiating tissue of the epidermis. They induce self-limiting benign tumors of skin and mucosa, which may progress to malignancy like cervical carcinoma. Prior to entry into basal cells, virions attach to heparan sulfate moieties of the basement membrane. This triggers conformational changes, which affect both capsid proteins, L1 and L2, and which are a prerequisite for interaction with the elusive uptake receptor. These processes are very slow resulting in an uptake halftime of up to 14 h. This review summarizes recent advances in our understanding of cell surface events, internalization and subsequent intracellular trafficking of Papillomaviruses.

Introductory remarks

Papillomaviruses (PV) are epitheliotropic nonenveloped small DNA viruses with icosahedral symmetry. Their strict dependence on terminally differentiating keratinocytes for completion of the replication cycle initially made the study of entry processes difficult with regard to two aspects. First, it was impossible to produce virions until the development of organotypic raft cultures based on keratinocytes harboring human papillomavirus (HPV) genomes [1]. Since these culture systems produced only very limited amounts of virions they provided only a partial relief. The limitation was partially overcome by the use of DNA-free virus-like particles and later by pseudovirions harboring marker plasmids, which were generated using heterologous expression systems [2–4]. The observation that codon optimization of capsid genes yielded high level expression of capsid proteins [5,6] and the development of packaging cell lines harboring high copy numbers of packaging plasmids finally allowed large scale productions of pseudovirions [7] as well as quasivirions [8]. This advance further facilitated the investigation of early events of PV infection. Second, until very recently [9], it was not possible to infect either organotypic raft cultures or primary keratinocytes *in vitro* unless pseudovirions had been activated (see below) [10]. The reason for this deficiency is unknown but suggests that taking primary keratinocytes into culture induces enough changes to make them refractory to HPV infection. Therefore, researchers have had to rely on established cell lines, the most commonly used of which is the HaCaT cell line, to study PV binding and uptake. However, the recent development of an *in vivo* mouse model by the Schiller group will allow for the testing of observations made *in vitro*. In this review we will focus on the entry of HPV type 16 (HPV16) and closely related viruses, which are the main cause of various cancers including cervical carcinoma. *In vitro* data backed by recent *in vivo* studies suggest an elaborate sequence of cell surface events

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that may explain the extremely slow uptake of viral particles with reported half times of up to 14 h.

Capsid structure

To fully appreciate viral entry strategies we have to discuss their surface structure. The outer shell of PV is composed of 360 molecules of the major capsid protein, L1 [11]. They are organized into 72 capsomeres, each comprised of a pentameric L1 assembly forming a T=7 icosahedral lattice (Fig. 1A). Twelve and sixty capsomeres are pentavalent and hexavalent, respectively, i.e. they have five and six nearest neighbors. Initial structural information for HPV16 was derived from T=1 capsids composed of only 12 pentamers [12], which was later modified using cryoelectron microscopy and image reconstruction [13]. The core of the capsomeres is mainly composed of an antiparallel β -sandwich to which eight β strands labeled B through I contribute. The outwards facing BC, DE, FG, and HI loops, which connect the β -strands, contain the major neutralizing epitopes [14–18] (Fig. 1B). These loops show the highest sequence variations among different HPV types, which translate into characteristic structural differences and are probably responsible for the type-specificity of neutralizing antibodies [19]. The five L1 molecules within a capsomere are intimately associated, even displaying an interlock of their secondary structures (Fig. 1C). The initial structural information suggested that the C-terminal arm folds back into the core structure from which it emanates. However, cryoelectron microscopy-based image reconstruction [13] points rather to an invading C-terminal arms model similar to Polyomaviruses, which form the principal interpentamer contacts (Fig. 1D). This model implies that a flexible hinge (aa 403–413) bridges the gap between capsomeres forming the base of the protein shell in the intercapsomeric region. The α -helix h4 (aa 419–429) reaches halfway up the wall of the invaded capsomere and brings Cys-428 in close contact with Cys-175 thus allowing disulfide bond formation [13,20,21], which is not essential for virion formation but strongly stabilizes virions [22,23]. Finally, the C-terminus extends further around the circumference of the targeted capsomere (aa 430–446) and inserts between two L1 molecules of the invaded pentamer to firmly link capsomeres (aa 447–474). This model suggests that the majority of the C-terminal arm is surface-exposed, although located within the intercapsomeric cleft. Therefore, it may provide surfaces for receptor binding and for the induction of neutralizing antibodies. Indeed, binding sites of some neutralizing antibodies have been mapped to the C-terminal arm [14].

Under forced expression, up to 72 molecules of the minor capsid protein, L2, are incorporated into a virion suggesting it requires the pentameric L1 structure for interaction [24]. The observation that L2 can occupy binding sites in adjacent capsomeres raises the possibility of homotypic L2 interactions. L2 is mainly hidden inside the capsid and only portions of the N-terminus including residues 60 to 120 are accessible on the capsid surface [25,26]. Additional evidence suggests that the extreme N-terminus folds back into the capsid thus rendering it inaccessible to antibody binding and proteolytic cleavage [27,28]. As discussed later, these regions undergo conformational changes following cell attachment. The N-terminus also contains two highly conserved cysteine residues, which in HPV16 form an intramolecular disulfide bond [29]. L2 density was located at the central internal cavity of each capsomere by cryoelectron microscopy but the majority of the L2 chain was not discernable [24]. L2 residues 396 to 439 (HPV11) probably mediate this likely hydrophobic interaction [30]. However, other regions of L2 also contribute to interaction with L1 as shown for bovine PV type 1 (BPV1) and HPV33 [31,32]. The central cavity of capsomeres is not large enough to allow passage of polypeptide chains. Thus, it is likely that the L2 N-terminus extends to the capsid surface between neighboring capsomeres. This notion is supported by observations that L2 protein stabilizes capsomere interactions under reducing conditions [32].

Receptors

The majority of PV types that have been examined to date use heparan sulfate proteoglycans (HSPGs) as the primary attachment receptors [33,34] (Fig. 2). HSPGs contain unbranched oligosaccharides composed of alternating disaccharide units of uronic acid and glucosamine, which are sulfated and acetylated to various degrees. *O*-sulfation occurs at the 2-*O*, 3-*O*, and 6-*O* position of the uronic acid and at the 3-*O* and 6-*O* position of the amino sugar. The amino group of the glucosamine may be either acetylated or sulfated. The two major families of cell surface HSPGs are the syndecans and glypicans (reviewed in [35,36]). In addition, secreted perlecanins are abundant in the extracellular matrix (ECM). *In vitro* studies have shown that infectious entry of HPV33 requires *N*- as well as *O*-sulfation. However, *O*-sulfation is sufficient for binding suggesting that distinct interactions with HSPGs may occur subsequent to primary cell interaction [37]. This finding was recently confirmed by the use of heparan sulfate (HS) neutralizing drugs applied post attachment. These drugs efficiently blocked infection of prebound virions without inducing their release from the cell surface [38]. HPV16 VLP binding and HPV11 infection does not appear to require a specific HSPG protein core for infection *in vitro* [39]. Since Syndecan-1 is the predominant HSPG in epithelial tissue it was suggested to serve as the primary attachment receptor *in vivo*. This is further supported by its high level of expression in the appropriate target cell and up-regulation during wound healing [35,40,41]. However, the *in vivo* model suggests primary attachment to the basement membrane rather than cell surface indicating that a secreted HSPG must be involved [42]. HPV31 was reported to not require HSPG interaction for infection of keratinocytes *in vitro*, but did interact with COS-7 in an HS-dependent manner [43]. The *in vivo* murine cervicovaginal challenge model yielded results contradicting these observations where HPV31 infection was blocked by heparin and heparinase III treatment similar to HPV16 [44]. Neither heparin nor carrageenan, another sulfated polysaccharide, was found to inhibit HPV5 infection *in vitro* despite having detectable interaction [45]. In contrast to this finding, the *in vivo* model again suggested a role for HSPG in HPV5 attachment and infection, albeit with seemingly different requirements regarding sulfation, as N-desulfated and N-acetylated variants of heparin rather than the highly sulfated form preferentially inhibited infection [44].

In vitro studies have shown that PV can also bind to components of the ECM secreted by keratinocytes and can be transferred from ECM to cells in an infectious manner. One ECM component, Laminin 5 (LN5), has high affinity to HPV11 virions and, in addition to HS, may mediate binding to ECM [38,46,47]. However, HPV16 and HPV18 preferentially utilize HS moieties for binding to ECM and subsequent infectious transfer to cells [38]. Studies using the murine cervicovaginal challenge model suggested that virions bind initially to the basement membrane prior to transfer to the basal keratinocyte cell surface [42]. Thus, the ECM might function as the *in vitro* equivalent of the epithelial basement membrane.

The minimal length requirement for HS binding to HPV16 VLPs is 8 monosaccharide units [48]. For HPV16, positionally conserved lysine residues K278, K356, and K361 located at the rim of capsomeres are involved in primary attachment. Residues from two or more L1 monomers within a capsomere may form a single receptor binding site, five of which are present per capsomere [48]. Lysine residue 443 located at the vertex of capsomeres does not seem to be involved in primary cell attachment. Nevertheless, its exchange for alanine severely impaired infection suggesting that secondary binding events may involve residues found in the cleft between capsomeres. Another study found that the neutralizing monoclonal antibody H16.U4, prevented cell surface but not ECM association of HPV16 and consequently reduced infection [49]. This antibody is specific to a conformational epitope in the intercapsomeric cleft to which the invading C-terminal arm contributes [14],

suggesting that elements located within the cleft contribute to cell binding. Hopefully, determining the structure of HPV particles in complex with its attachment receptor HS combined with a mutational approach will allow solving these apparent discrepancies.

It has become clear in recent years that a secondary non-HSPG receptor is involved in infectious internalization of PV particles [27,38]. The report of HSPG-independent infection of HPV16 pseudovirions pre-cleaved with furin, which processes L2 protein within capsids, has especially provided evidence for this notion [10]. Obviously, the treatment of immature virions with furin induces a conformational change sufficient to bypass the HS-dependent steps. This indirectly suggests that the engagement of HS is primarily required to induce structural changes (see below). The identity of the second non-HSPG binding moiety is still unknown but the availability of activated virions with reduced affinity to HS will hopefully soon allow its identification. Initial cell surface interactions are predominantly L1-dependent. However, the L2 protein may contribute to secondary interactions. Two regions of L2 that have been described to mediate this engagement encompass residues 13 to 31 and 108 to 120 of HPV16 L2, respectively [28,50].

Attachment-induced conformational changes

It is well established that engagement with cellular receptors, likely HSPG, induces conformational changes which affect both capsid proteins. The changes in L1 are not well documented yet but seem to affect the BC loop. Improved recognition of a neutralizing L1 epitope in this loop has been observed after virion attachment to the cell surface [17,37]. Our own unpublished evidence suggests that at least some structural shifts in L1 precede those in L2 (M. B.-H., H. D. Patel, K. F. Richards, and M. S.). Based on the relocation of viral capsids from cells to ECM under conditions that block transfer to the secondary receptor, it was suggested that L1 conformational changes result in reduced affinity of the capsid with HS thus aiding the handover to the secondary receptor [27]. This was suggested to occur following L2 conformational changes [27]. However, no direct evidence for this notion has been provided yet.

Capsid interaction with HSPG also induces a conformational change that results in the exposure of the L2 amino terminus [27]. Consistent with this idea, the N-terminal portion of L2 can induce cross-type neutralizing antibodies as a free protein immunogen, but not when it is assembled into a mature PV capsid [51]. Exposure of the L2 N-terminus allows access to a highly conserved consensus furin convertase recognition site and subsequent cleavage by furin on the cell surface, rendering the cross-neutralizing epitopes accessible to antibody binding [27,52]. Therefore, L2-dependent neutralization must occur following these events and not in solution. Proteolytic cleavage is essential for successful infection. Incorporation of an N-terminally truncated form of L2 into virions cannot bypass the furin dependence. This suggests that the N-terminus is essential for the L2 protein to adopt a correct conformation within the assembled capsid. Correct folding may also require the formation of a disulfide bond between HPV16 L2 residues Cys-22 and Cys-28, which was recently identified. Mutation of the contributing cysteine residues rendered mutant virions non-infectious [29]. However, it is unclear at the moment whether this is due to defects in assembly, which only indirectly affect infection processes similar to the N-terminally truncated forms of L2, or whether it has a direct effect on cell surface and/or subsequent events.

The cellular peptidyl-prolyl cis/trans isomerase Cyclophilin B (CyPB) facilitates the exposure of the HPV16 L2 N-terminus [53]. CyPB has been found on the cell surface in association with HSPG [54]. Inhibitors of CyPB and its siRNA-mediated down-regulation prevented the exposure of the L2 N-terminus. These treatments induced noninfectious virus

internalization with characteristics similar to post-attachment treatment with HS-blocking drugs. Therefore, it was suggested that CyPB acts prior to or mediates the capsid protein rearrangements, which are required for transfer to the non-HSPG receptor [53]. A sequence with homology to a known CyP binding site is present at surface-exposed L2 residues 90-110 in many but not all HPV types. Exchanging the central Gly-99 and Pro-100 of this motif for alanine made exposure of the HPV16 L2 N-terminus CyPB-independent. This indicated that the mutations increase flexibility in this loop. The data also suggested that the L2 protein is the substrate for CyPB. Exposure of L2 was not achieved in solution or attached to ECM after addition of bacterially expressed CyPB, however, indicating that the L2 conformational change requires engagement with the cell surface receptor and possibly L1 conformational change(s).

Taken together, these recent advances suggest a dynamic model of virion-cell surface interactions in which subsequent engagement with cell surface receptors induce conformational changes in capsid proteins. It is tempting to speculate that this complex process evolved to ensure the inaccessibility of critical regions to prevent a host antibody response to conserved virion epitopes that are essential for infection. The remarkable conservation of the requirement for L2 furin cleavage is suggestive that this elaborate process evolved early in the speciation of Papillomaviruses.

Endocytosis

Internalization of HPV16 is highly asynchronous with an unusually protracted residence on the cell surface. Similar observations have been made with other PV types [33,55–57]. In addition to the aforementioned conformational changes, the reported transport along filopodia towards the cell body prior to internalization may contribute to the delayed kinetics [58]. Filopodia assisted transport was demonstrated by live cell imaging using HeLa cells. It was suggested that internalization can only occur at the cell body. Open questions regarding this transport include which receptor is linking viral particles to F-actin for retrograde transport and if these interactions are sufficient to induce the observed structural rearrangements. Consistent with the important role of actin-rich protrusions in HPV16 infection, it was recently demonstrated that transport along filopodia also facilitated HPV31 infection. This report also suggested that particle binding induced the formation of filopodia [59]. Given preferential binding of HPV to the basement membrane this mechanism might have evolved to allow for efficient transfer of virions from ECM to the cell body.

A recent report suggested clathrin- and caveolae-independent internalization of HPV16 pseudovirions in HeLa and HEK 293TT cells [60]. Entry and infection was resistant to combined siRNA-mediated down regulation of caveolin-1 and clathrin heavy chain and to over-expression of dominant-negative mutants of dynamin-2, caveolin-1, and eps-15 (EGF receptor pathway substrate clone No. 15, which plays a role in clathrin coated vesicle formation) [60] (Fig. 3). These findings have now been extended to HaCaT cells (C. Lambert, L. Florin, personal communication). Similar observations were recently presented at the 25th International Papillomavirus Workshop by the Helenius group, who used a large library of siRNA and inhibitors to interfere with known factors of endocytosis. Also, they found that uptake of HPV16 does not occur via micropinocytosis (M. Schelhaas, personal communication). As of yet, this entry pathway has not been characterized any further but may utilize tetraspanin-enriched microdomains as entry platforms [60]. Earlier reports using biochemical inhibitors like chlorpromazine had suggested an internalization via clathrin-mediated endocytosis [55,61], however, these findings were mainly based on the use of small drug inhibitors, which might have unwanted side effects on cell function. In addition, a recent report also suggested partial sensitivity of HPV16 pseudovirus infection of 293TT to dynasore, an inhibitor of dynamin GTPase activity, which is required for clathrin-

mediated endocytosis [62]. BPV1 was reported to utilize a clathrin-dependent endocytic pathway for infectious uptake based on a combination of microscopic analyses and biochemical inhibition of known pathways [61]. This was recently confirmed using pseudovirions by showing sensitivity to chlorpromazine and initial colocalization of virions with the early endosomal antigen (EEA-1) [63] as well as partial sensitivity to dynasore [62]. For HPV33 internalization was suggested to be dependent on the actin cytoskeleton [64]. However, none of these studies could demonstrate an effect of caveolae disruption, via nystatin, methyl- β -cyclodextrin, or filipin treatment, on HPV16, HPV33, or BPV1 infection. In contrast, HPV31 was reported to depend on intact caveolae for internalization [55,65]. However, one study found that treatment with chlorpromazine but not with inhibitors of caveolar uptake prevented HPV31 pseudovirus infection [66]. As previously mentioned HPV31 appears to interact with HSPG similarly to HPV16 during *in vivo* infection. Possibly HPV31 interacts differently with or has a unique co-receptor that shunts it into a different internalization pathway.

Vesicular trafficking

A comprehensive study of intracellular trafficking of different PV types in normal keratinocytes using siRNA-mediated gene knockdown and dominant-negative constructs targeting multiple endocytic mediators is still lacking. Given the divergent reports regarding the endocytic mechanisms, it is not surprising that the subject of intracellular trafficking of PV-containing vesicles and the cellular compartments involved is also highly controversial (Fig. 3). The studies are complicated by the fact that different laboratories are utilizing different virus sources and cell lines. However, there is near consensus that successful infection requires acidification of endocytic vesicles suggesting that PV particles must pass through the endosomal compartment [61,64,67,68]. Colocalization with early endosome marker EEA-1 has not been observed for HPV16 [60] suggesting they traffic to acidified compartments via a different route. HPV31 was found to traffic via caveosomes to early endosomes in a Rab5 GTPase-dependent manner [67]. Since the infection did not require functional Rab7, it was suggested that infectious genomes exit the endocytic pathway prior to transit into late endosomes. However, successful infection required acidification of endosomes. In contrast, it was reported that BPV1 entry via a clathrin-dependent pathway, which led to colocalization with EEA-1, was followed by transport to the caveosome and subsequent entry into the ER in 293TT cells [63,69,70]. Over-expression of dominant-negative caveolin-1 and shRNA-mediated knock down of caveolin-1 significantly inhibited infection without affecting the initial internalization [63]. In addition, over-expression of a dominant-negative caveolin-1 mutant, which is defective for translocation to the plasma membrane, did not block BPV1 infection thus also pointing to a role for caveolin-1 subsequent to internalization. However, other work has shown that the BPV1 genome accumulates in late endosomes or lysosomes if egress from the endocytic compartment to the cytosol is blocked [71] and that it requires acidification of endosomes [61]. Vesicular transport of PV particles may also be influenced by capsid protein interactions with vesicle-resident receptors. It is intriguing that a binding site for syntaxin-18 was mapped to a peptide immediately downstream of the Furin cleavage site. Syntaxin-18 is an ER-resident protein and was found to bind to L2 residues 40 to 44 of BPV1. In addition, over-expression of a dominant negative form of syntaxin-18 impaired BPV1 infection [69,70]. However, it is unclear whether syntaxin-18 is present in endocytic vesicles and the mechanism or consequence of the interaction with L2 has not yet been fully elucidated. Furthermore, up to now no convincing data showing ER localization of PV during infectious entry has been published.

Viral uncoating and egress from endosomes

Following internalization of HPV16 most conformational L1 epitopes are lost or are no longer accessible to antibody binding [38]. L1-specific antibodies to measure uncoating are rare. One such antibody, 33L1-7, which has been used for detection of internalized particles [60], recognizes an epitope that is neither accessible in capsids nor in capsomeres [72]. It is unclear at the moment if this antibody recognizes a specific step in uncoating or reacts with protein in the lysosomal compartment in the process of being completely degraded. Detection of hidden L2 epitopes and encapsidated DNA for examination of the uncoating of papillomaviral pseudoviruses has proved to be more successful. An HA tag at the L2 C-terminus and bromodeoxyuridine (BrdU)-labeled viral pseudogenome were used for this study, respectively [73]. Examination of when these determinants became accessible to antibody staining suggested that uncoating occurs in endocytic vesicles prior to transfer to the cytosol. L1 protein seems to be shed from the viral genome during these events. It could not be detected in the nucleus of infected cells even when fluorescently labeled particles were used. In line with this, linear L1 epitopes are continuously detected in Lamp-3 positive compartments late in infection [60]. Previous reports showing that intact HPV capsids exceed the size capacity for transit across the central nuclear pore complex channel had already suggested that disassembly of the viral particle must occur before nuclear import [74,75]. L2 protein is not essential for viral uncoating as measured by detection of BrdU-labeled genome following infection with L1-only particles [71]. However, L2 protein mediates the escape of viral DNA from endosomes. An L2 C-terminal peptide harboring a stretch of hydrophobic residues adjacent to positively charged amino acids has been demonstrated to contain membrane disrupting activity and to mediate tight association with membranes in the absence of cellular chaperones. Deletion and point mutations within this region yielded non-infectious pseudovirus despite unaffected DNA encapsidation and cell surface interactions. A similar deletion in BPV-1 L2 rendered mutant virus particles non-infectious. Mutant L2 proteins were retained together with the viral genome within the endosomal compartment at late times post-infection [71]. Furin cleavage of L2 is also essential for endosomal escape despite occurring on the cell surface [27,52]. However, it is unclear at the moment how the proteolytic processing contributes to egress from endosomes. One possibility is that furin cleavage enables the release of the L2-genome complex from L1. Alternatively, L2 may promote binding to a specific receptor that directs virions to vesicles that facilitate uncoating and endosomal membrane passage.

Transport to the nucleus

The issue of how the papillomaviral genome transits from the endosome to the nucleus has not been systematically addressed. It is well established that vesicle trafficking occurs along microtubules. Indeed, the microtubule disrupting drug nocodazole inhibits PV infection at a late step [61,64]. However, microtubule-dependent transport may also be required for the post-endosomal step of delivering the viral genome into the nucleus. Cytoplasmic transport along microtubules is mediated by motor protein complexes that use cellular energy to move cargo. The L2 protein of HPV16 and HPV33 was found to interact with the microtubule network via the motor protein dynein during infectious entry [76]. The C terminal 40 amino acids of L2 were found to be essential for interaction with the dynein complex. Other data support co-delivery of L2 and genome to the nucleus for HPV16 and BPV1 L2, perhaps in conjunction with a cell-encoded chaperone [76]. The mechanism by which the viral genome enters the nucleus is not well understood yet. L2 protein harbors two terminal peptides that function as nuclear localization signals when fused with GFP [77–80] raising the possibility that L2 protein provides the nuclear import signals. However, these signals overlap with the furin consensus site and the membrane-destabilizing peptide, making it difficult to study their role in nuclear entry during infection. Recently, a report suggested that nuclear

envelope breakdown is required for establishment of HPV16 infection indicating that active nuclear import via nuclear pore complexes may not be required [81]. It is undisputed that L2 protein accompanies the viral genome to the nucleus. L2 and the viral genome colocalize in the nucleus at ND10 domains (PML nuclear bodies) following infection [73] suggesting that they are translocated to the nucleus as complex. The localization of the genome and L2 at ND10 is critical for the establishment of infection. Efficient early PV transcription as well as transcription of the pseudoviral genome under the control of the CMV immediate early promoter required either intact ND10 or expression of the PML protein [73]. However, at the moment mechanistic explanations for these observations have not been provided.

In summary, our knowledge with regard to PV entry has increased considerably in recent years. This is especially due to the development of systems allowing large scale production of viral particles by bypassing the need for stratified epithelia. However, many controversies remain especially regarding the mode of endocytosis, intracellular trafficking and the vesicular compartments involved in uncoating. The discrepancies may be partially due to the fact that PV types evolved different entry strategies. It is to be hoped that future studies will compare several PV types in order to minimize the effect of different experimental systems on findings. In addition, the recent development of an *in vivo* model will hopefully allow testing the significance of *in vitro* findings.

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Figure 1. Structure of the HPV16 L1 protein

(A) Structure of a T=7 HPV16 capsid according to [12,13]. (B) L1 monomer; α -helices are highlighted in pink; all five surface loops are marked in addition to the internal C-D loop. (C) top view of a L1 pentamer (spacefill); individual L1 molecules are displayed in different colors to highlight the intertwining of the molecules. (D) L1 invading C-terminal arm model as proposed by [13]. Side view of a pentamer in addition to a single L1 molecule from the neighboring capsomere is shown in spacefill. The arrow points to the intercapsomeric disulfide bond. Images were downloaded from the RCSB Protein Data Bank (www.rcsb.org) and modified using RasMol (A, C) and Jmol (B, D) software.

Figure 2. Model of the ECM and the cell surface events of HPV infection

(1) Most virions bind to primary attachment receptors, HSPG1, present in ECM (basement membrane *in vivo*) or on the cell surface. HPV11 capsids have also been shown to bind to ECM-resident laminin 5 (LN5). Viral particles are transported towards the cell body along actin-rich protrusions. (2) Capsids engage with secondary HSPG binding sites present on the cell surface (HSPG2). Whether transfer from primary ECM binding sites to primary cell surface binding sites occur has not been directly investigated. Interaction with the HSPG2 cell surface receptor induces conformational changes in L1 and L2 resulting in the exposure of the L2 amino terminus and subsequent furin cleavage at a conserved cleavage site. Host cell CyPB facilitates the L2 conformation changes. (3) These events may induce an additional conformational change that either reduces the affinity of capsids to HSPG or result in exposure of sites required for handover to a putative non-HSPG uptake receptor, which then triggers endocytosis.

Figure 3. Proposed endocytosis pathways

Schematic diagrams of the entry pathways proposed for various PV types. HPV16 is endocytosed via a clathrin- and caveolin-independent pathway, whereas BPV1 and HPV31 were shown to enter via clathrin-coated pits and caveolae, respectively. For more details see main text.