

# Chapter 8

## Human Papilloma Virus-Infected Cells



Alfredo Cruz-Gregorio and Ana Karina Aranda-Rivera

**Abstract** Human papillomavirus (HPV) is associated with infection of different tissues, such as the cervix, anus, vagina, penis, vulva, oropharynx, throat, tonsils, back of the tongue, skin, the lungs, among other tissues. HPV infection may or may not be associated with the development of cancer, where HPVs not related to cancer are defined as low-risk HPVs and are associated with papillomatosis disease. In contrast, high-risk HPVs (HR-HPVs) are associated with developing cancers in areas that HR-HPV infects, such as the cervix. In general, infection of HPV target cells is regulated by specific molecules and receptors that induce various conformational changes of HPV capsid proteins, allowing activation of HPV endocytosis mechanisms and the arrival of the HPV genome to the human cell nucleus. After the transcription of the HPV genome, the HPV genome duplicates exponentially to lodge in a new HPV capsid, inducing the process of exocytosis of HPV virions and thus releasing a new HPV viral particle with a high potential of infection. This infection process allows the HPV viral life cycle to conclude and enables the growth of HPV virions. Understanding the entire infection process has been a topic that researchers have studied and developed for decades; however, there are many things to still understand about HPV infection. A thorough understanding of these HPV infection processes will allow new potential treatments for HPV-associated cancer and papillomatosis. This chapter focuses on HPV infection, the process that will enable HPV to complete its HPV life cycle, emphasizing the critical role of different molecules in allowing this infection and its completion during the HPV viral life cycle.

**Keywords** HPV infection · L1 protein · L2 protein · Furin · Dynein · Endocytosis

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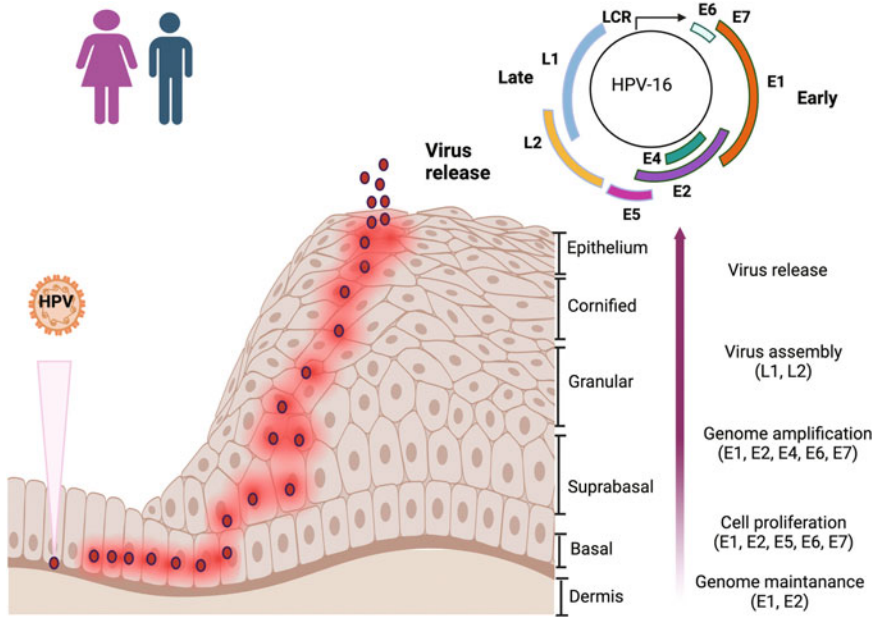
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## Introduction

Human papillomaviruses (HPV) infections can be transmitted by skin-to-skin contact or by sexual intercourse through vaginal, anal, and oral contact. This virus infects different tissues, such as the cervix, anus, vagina, penis, vulva, oropharynx, throat, tonsils, back of the tongue, skin, and lungs, among other tissues (Rubin et al. 2001; Daling et al. 2002; Clark et al. 2004; D'Souza and Dempsey 2011). HPV infection may or may not be associated with the development of cancer. HPVs that are not associated with cancer are defined as low-risk HPVs (LR-HPV) and are associated with papillomatosis disease. In contrast, high-risk HPVs (HR-HPVs) are associated with the development of cancers in areas that HR-HPV infects, such as the cervix. The latter was proved by molecular, clinical, virological, and epidemiological evidence, showing that HPV is the etiological agent that develops cancer (zur Hausen 2002; zur Hausen 2009).

HPV is a nonenveloped virus with a double-stranded DNA genome belonging to the papillomaviridae family. For its study, the HPV genome is divided into three regions: the region that corresponds to the early (E) proteins (E1, E2, E4, E5, E6, and E7), necessary for viral replication, regulation of transcription and immortalization, and cell transformation; the region that encodes the late (L) proteins (L1 and L2) that build the structure of the capsid inducing the release of the virion and the long region (LCR), which contains the origin of replication and the early promoter that allow replication and expression of the viral genome (McMurray et al. 2001). The HPV genome is surrounded by a 55 nm icosahedral capsid composed of two proteins, L1 and L2. The L1 and L2 proteins form 72 capsomeres that build the capsid structure, where L2 is located internally surrounded by the L1 proteins in the capsomeres. L1 is involved in capsid stabilization, endosomal escape of HPV virions, and nuclear transport of the HPV genome (Doorbar et al. 2012). Meanwhile, L2 is associated with the interaction with different substrates to allow HPV to enter the cell and is also necessary for the encapsidation of the viral DNA, in addition to facilitating the endosomal escape of the viral genome after infection, possibly due to the interaction with sorting nexin 17 (Bergant Marušič et al. 2012). The infection of HPV target cells is regulated by L1 and L2 associated with different molecules and receptors that induce conformational changes of the HPV capsid proteins, allowing the activation of HPV endocytosis mechanisms and the arrival of the HPV genome to the cell nucleus. In the nucleus, the replication and transcription of the HPV genome are carried out, increasing its genetic material exponentially. Then the genome is housed in a new HPV capsid, inducing the process of exocytosis of the HPV virions, releasing new HPV viral particles with the potential for a new infection (Doorbar et al. 2012) (Fig. 8.1). Although the HPV infection process has been a topic that researchers have studied and developed, there are many issues to understand about HPV infection. The entire understanding of these HPV infection processes will allow to development of new potential treatments for HPV-associated cancer and papillomatosis. This chapter focuses on HPV infection, the process that allows HPV



**Fig. 8.1** Human Papillomavirus (HPV) life cycle. HPV infects basal cells to induce the transcription of E1 and E2, which trigger the HPV genome replication. Likewise, the E5, E6, and E7 oncoproteins are transcribed, preventing cell death and entry into the cell cycle. In the intermediate layers of the epithelium, the HPV genome is transcribed exponentially, and in the upper layers of the epithelium, E4 is transcribed for keratin breakdown, allowing the virion formed by the encapsidation of the HPV genome by L1 and L2 to exit the cell, and start a new infection

to complete its HPV life cycle. It emphasizes the important role of different molecules in allowing this infection and its completion during the HPV viral life cycle.

### HPV Entry into Its Target Cells

Replication and transcription of HPV require basal epithelial cell differentiation since undifferentiated basal epithelial cells do not express key cellular transcription factors (TFs) like transcription factor IID (TFIID), which induce the expression of the early protein of HPV (Moody 2017; Ribeiro et al. 2018). Thus, the viral transcription of E1, E2, E5, E6, and E7 and genome amplification are maintained at low levels in the basal layer of the squamous epithelium. As the epithelium differentiates, the genes such as E1 and E2 are expressed, inducing amplification of the viral genome and increasing the replication of the HPV genome in high quantities. Because of this dependence, the generation of papillomavirus in vitro has been difficult, so the production of infectious viral particles or virions for its

study *in vitro* was only possible following the development of keratinocyte-based organotypic cultures and mice xerographs (Dollard et al. 1992; Bonnez et al. 1998). These limitations were partially overcome using free virus particles (noninfectious virus-like particles or VLPs) with reporter plasmids (Meyers et al. 1992) for the development of packaging cell lines and codon optimization. Therefore, these technologies permitted the high expression of capsid proteins that occurred in conjunction with the large-scale production of VLPs, leading to a better study of interactions between infected cells and HPV. Since VLPs do not have an HPV genome and are noninfectious, they are used in binding studies. On the other hand, VLPs that harbor reporter plasmids function like HPV viral “genomes” are used to quantify infection levels. Interestingly, VLPs self-assemble into L1-VLPs when L1 is expressed alone or in conjunction with L2, forming the basis of current prophylactic vaccines since VLPs mimic the structure of native HPV virions, and therefore share similar immunological functions to native HPV virions (Wang and Roden 2013). Thus, the development of these HPV virion production systems has helped the understanding of the initial steps of HPV infection and HPV vaccine development.

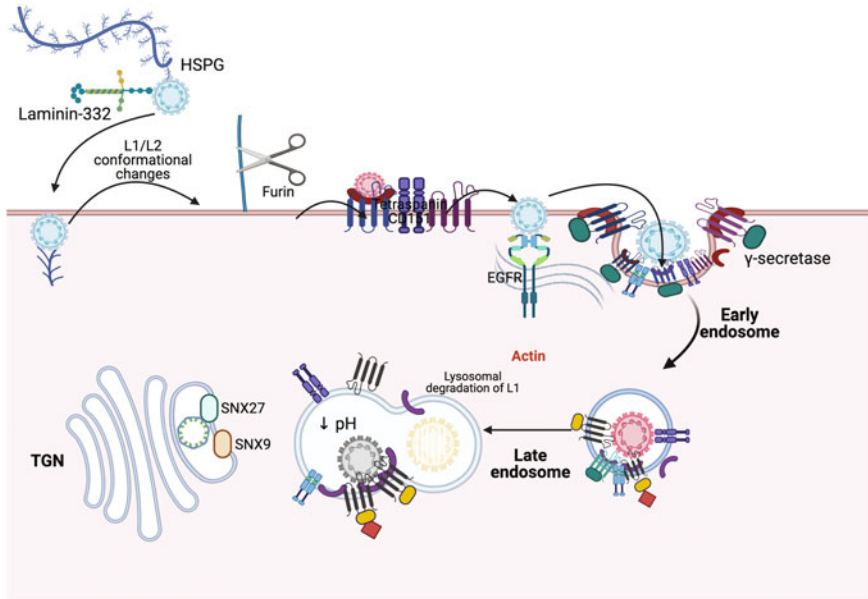
Although most of the knowledge about HPV infection has been discovered from *in vitro* models, the major information can be extrapolated to the *in vivo* infection process. For instance, it has been suggested that before HPV reaches basal epithelial cells, mainly through microlesions, HPV binds to the heparan sulfate (HS) and laminin 332 of the extracellular matrix (ECM). This suggestion was made after researchers reported that *in vitro* essays, VLPs bind to HS and laminin 332 (Shafti-Keramat et al. 2003; Culp et al. 2006a, b). Therefore, it has been suggested that *in vivo*, the virus first uses these molecules to bind to the basement membrane (BM) (which plays the same role as the ECM in *in vitro* models), and then, during wound healing, the HPV is transferred to basal keratinocytes as they migrate to the wounded area (Roberts et al. 2007). Different groups of researchers have discovered that HS modifications, such as O-linked sulfation and N-linked sulfation, induce better binding between viral particles with their target cell and that even the level of HSPG on the cell surface correlates with the number of bound HPVs (Selinka et al. 2003). However, whether these interactions occur *in vivo* remains to be investigated. Knowing if these molecules are key to HPV infection *in vivo* could be key to considering these biomolecules as possible targets to reduce HPV infection. Next, in basal keratinocytes virus binds to the heparan sulfate proteoglycans (HSPG) (Sapp and Day 2009). HSPGs are founded on the membrane surface of the basal cells of the epithelium and in the extracellular matrix. If HSPGs are attached to cell membranes they are called syndecans or if they are attached to the extracellular matrix they are called perlecan. HPGS-syndecans 1 and 4, which are overexpressed during wound healing, serve as the first anchorage receptors on basal cells (Sapp and Day 2009). After that virus is anchored to the cells other bindings are needed, such as  $\alpha\beta4$  integrins plus the tetraspanin CD151 (Abban and Meneses 2010; Scheffer et al. 2014). Moreover, this complex can bind and activate epidermal growth factor receptor (EGFR) signaling, leading to Src kinase phosphorylation of annexin A2, which induces the extracellular translocation of the annexin A2/S100A10

heterotetramer (A2t) (Woodham et al. 2012; Dziduszko and Ozburn 2013; Scheffer et al. 2014). Interestingly, EGFR signaling activation deactivates autophagy through the activation of PI3K signaling. Autophagy is an intrinsic cellular sensor that inhibits HPV infection, permitting an efficient HPV infection process (Surviladze et al. 2013; Griffin et al. 2013). These interactions and activating different molecules allow HPV uptake and successful HPV infection.

The HPV binding with HS also induces a conformational change in the HPV capsid. This conformational change of the capsid weakens the link of the virus with the HPGS, leading to the exposure of the N-terminus of L2. Thus, this conformational change induces L2 to unmask since most of L2 is hidden on the surface of the capsid. When L2 is exposed, it is cleaved by furin (Richards et al. 2006). Cleavage of the N-terminus of L2 by furin exposes the binding site for cell membrane receptors involved in infectious internalization, allowing HPV to enter by endocytosis. Notably, L2 cleavage is essential for the insertion and protrusion of L2 across the vesicular membrane. However, other proteins are needed during L2 insertion; for instance, it is known that the multisubunit intramembrane protease  $\gamma$ -secretase acts as a chaperone that inserts L2 into the cell membrane (Harwood et al. 2020). Only when L2 inserts into the cell membrane is proper downward trafficking of L2/vDNA allowed, as L2 in the cytosol allows for the recruitment of sorting nexins and the retromeric complex. Furthermore, L2 is critical for the retrograde trafficking of L2/vDNA complexes from endosomes to the trans-Golgi network (TGN), which is a required step of the initial infection (Fig. 8.2). Thus, L2 acts as a transmembrane protein that directs HPV DNA trafficking into the TGN and into the cell nucleus for productive infection away from lysosomal compartments that might degrade it.

### ***HPV Endocytosis as a Journey to the Nucleus***

HPV in the cytosol recruits sorting nexin 17 (SNX17), which prevents HPV following a rapid lysosomal sorting and its degradation. This late endosome escape of the viral genome is mediated by L2 (Bergant Marušič et al. 2012). L2 also interacts with the complexes Vps26, Vps29, and Vps35, a trimeric retromer that fulfills endosome tubulation and vesicle formation that induces the retrograde transport of HPV from the endosome to TGN. This showed that different molecules such as Rab-GTPases SNX proteins, TBC1 domain family member 5 (TBC1D5), and the endoplasmic reticulum (ER)-anchored protein vesicle-associated membrane protein (VAMP)-associated protein (VAP) assist this process (Day et al. 2013; Siddiqi et al. 2018; Pim et al. 2021). Where TBC1D5 is recruited to the L2/retromer at the endosomal membrane for stimulating hydrolysis of Rab7-GTP, it induces the disassembly of the retromer from the HPV (Xie et al. 2020). After the retromer is dissociated, vesicles that contain the virus traffic to the TGN, where the cargo is delivered by membrane fusion. This cytoplasmic traffic uses the cellular microtubules as highways mediated by L2 through its binding to the dynein light chain. Dynein is a kinesin and the most crucial motor protein associated with microtubules. Its function is critical in the



**Fig. 8.2** Entry and endocytosis of Human Papilloma Virus. HPV viral particles binds to different receptor and molecules such as heparan sulfate, which induce capsid conformational changes, inducing that furin cleavages L2, this cut is essential for the insertion and protrusion of L2 across the vesicular membrane. However, other proteins are needed during L2 insertion such as  $\gamma$ -secretase, which acts as a chaperone that inserts L2 into the cell membrane. Only when L2 inserts into the cell membrane is proper downward trafficking of L2/vDNA complex from endosomes to the trans-Golgi network (TGN)

retrograde transport of substances within the cell and in inducing chromosome movement during cell mitosis. Thus, this interaction drives the transportation of HPV to the nucleus.

It is important to mention that the study of VLP-associated endocytosis has yielded different endocytosis pathways such as clathrin, caveolin, lipid vesicles, clathrin-independent, and cholesterol-mediated endocytosis mechanisms and, therefore, it is still a matter of scientific debate. This could be because there are different “maturity” states of VLPs. That is when HPV capsids are extracted from replicating cultured cells, capsids can be larger, less regular, and less protease resistant or “immature” in comparison with “mature” capsids; this indicates that immature capsids must suffer a substantial change in conformation during the maturation process (Buck et al. 2005). Thus, this high variability between “immature” and “mature” capsids could direct one process of endocytosis or another. The latter is supported by the fact that HPV that are phylogenetically closely related, such as HPV-16 and -31, follow different endocytosis routes, where HPV-16 activates clathrin-mediated endocytosis while HPV31 activates caveolin-mediated endocytosis (Bousarghin et al. 2003). Furthermore, HPV16 PsV uptake has been reported to

be clathrin-independent, and HPV16 endocytosis occurs via tetraspanin-rich microdomains (Spoden et al. 2008). Thus, it seems that there is not only one endocytosis pathway used by HPV; it will depend both on the microenvironment and the receptors associated with the viral particle. The latter is because the virus enters the cells asynchronously. This means that while some virions enter in minutes, others take hours. It has been proposed that HSPG-binding virions have a slower entry rate than receptor-binding virions, which mediate a high entry rate (Williams and Fuki 1997). We should not forget to mention that these interactions could also be associated with the endocytosis pathway that is activated. Therefore, the entry of the virus will be faster or slower.

## Replication and Transcription of the HPV Genome

The entry of the HPV genome into the cell nucleus is more associated with the nuclear membrane breakdown during mitosis than with the transporters via karyopherin (Pyeon et al. 2009). In this process, L2 remains in a membrane-spanning conformation, interacting with the proteins of the mitotic spindle motor to transport the vesicular HPV DNA genome from the remnants of the TGN to the pericentriolar space in prometaphase, waiting for the chromosomes breakdown in the metaphase (DiGiuseppe et al. 2016). L2 also binds to chromosomes in the delivery of vesicle-bound episomal HPV DNA into the nuclei of daughter cells (Aydin et al. 2017). This vesicle that surrounds L2/vDNA is maintained through mitosis and into the next G1 in the cell cycle (DiGiuseppe et al. 2016). Then, it has been observed that upon G1 re-entry into HPV-infected cells, nuclear entry and nuclear domain 10 (ND10)/promyelocytic leukemia (PML) components bind around L2/vesicular vDNA within nuclei (Guion et al. 2019). Thus, the HPV genome can arrive at the cell nucleus to start replication and transcription of the virus genome. Once the HPV genome is transported to the nucleus, the transcription and replication of the virus begin. The LCR contains several regulatory sites for both viral replication and transcription (Bernard 2013). These cellular processes are induced by the direct interaction between different cellular and viral components with the LCR. For example, the LCR has specific sequences for the association of E1 and E2 that promote virus replication, as well as its transcription. In addition, it contains binding sequences to components of the transcription machinery such as specificity protein 1 (SP1) and TATA-binding protein (TBP), as well as glucocorticoid receptors that induce transcription of the viral genome (Bernard 2013). When these transcription factors associated with HPV genome induce the transcription of its different proteins with the different functions that permit the HPV life cycle to be concluded. For instance, the HPV E1 protein is a helicase that participates in viral DNA replication, it is composed of around 681 amino acids (aa) and is divided into three regions: the carboxyl-terminal region related to helicase activity, the region where the domain is located. Binding to DNA or the DNA Binding Domain (DBD) and an amino-terminal region that is the target of phosphorylation, sumoylation or acetylation



that influence E1 activity and therefore transcription and viral replication. In addition to containing signals that allow the entry and exit from the cell nucleus (Bergvall et al. 2013), E1 associates with the origin of replication on the LCR, inducing the formation of the initiation complex and the unwinding of the DNA to promote replication of the viral genome. It is also known that E1 directly interacts with DNA polymerase, which in turn associates with the replication complex and thus initiates the replication process (Bergvall et al. 2013). Also, E2 is a regulatory protein of viral replication and transcription. This 300–500 amino acid protein consists of two conserved domains: the amino-terminal region where the transactivation domain (TAD) is responsible for transcriptional regulation and viral replication, interacting with different cellular proteins. The other E2 domain is known as DBD. This domain recognizes and binds to specific sequences of ACCGN4CGGT found in the viral genome (Gillitzer et al. 2000). Between the TAD and DBD domains is a hinge region. This region does not participate in the basic functions of virus replication and transcription; however, it provides stability to E2, participates in cytoplasm-nucleus localization, and functions as a spacer between the two domains, avoiding steric hindrance (McBride 2013). When E2 associates with viral DNA, it recruits different cellular proteins involved in viral transcription and replication. E2 participates both in the segregation of the viral genome, anchoring it to the cell chromosomes during cell mitosis, and in the packaging for the formation of virions (McBride 2013). Among other cellular targets of E2 is p53, this interaction induces cell arrest and apoptosis (Desaintes et al. 1999). An important factor in cancer development lies in the antiproliferative role exerted by E2, since it can repress cell growth and induce apoptosis in HPV-positive cells. Partly to the repression of the transcription of E6 and E7, with the consequent increase of p53 and pRb (Demeret et al. 1997). The HPV oncoproteins, E5, E6 and E7, are transcribed to avoid to cell differentiation, cell senescence or cell death, even avoid immune recognition. That is, although E5 is the smallest protein encoded by HPV, with only 83 amino acids and a molecular weight of 9 kDa, it can induce cell proliferation associated with overexposure of the epidermal growth factor receptor (EGFR) receptor (Crusius et al. 1998). It is located mainly in the endoplasmic reticulum (ER) and the Golgi apparatus (GA), inducing stress of these organelles that help viral replication and persistence (Venuti et al. 2011). Likewise, it has been shown that E5 binds to the 16 K subunit of the vacuolar V-ATPase of the endosomes, decreasing its activity and inhibiting its acidification, intervening in vesicular traffic (Di Domenico et al. 2009). Regarding E6 and E7, the most oncoproteins studied so far, they have the ability to induce immortalization, inhibit cell apoptosis, or prevent cell-cell interactions (Mantovani and Banks 2001). For example, it has been found that E6 can associate with human telomerase reverse transcriptase (hTERT), a polymerase that lengthens the telomeric ends of cell chromosomes, inducing their activation and allowing cell immortalization (Oh et al. 2001). Another important characteristic of the E6 proteins of high-risk HPVs is their association with the E6 associated protein (E6AP) ubiquitin ligase, through which it can associate with p53, promoting its degradation via the proteasome and thus blocking cell apoptosis (Thomas et al. 1999). HR-HPV E6 can also bind to proteins with PDZ domains such as human disc



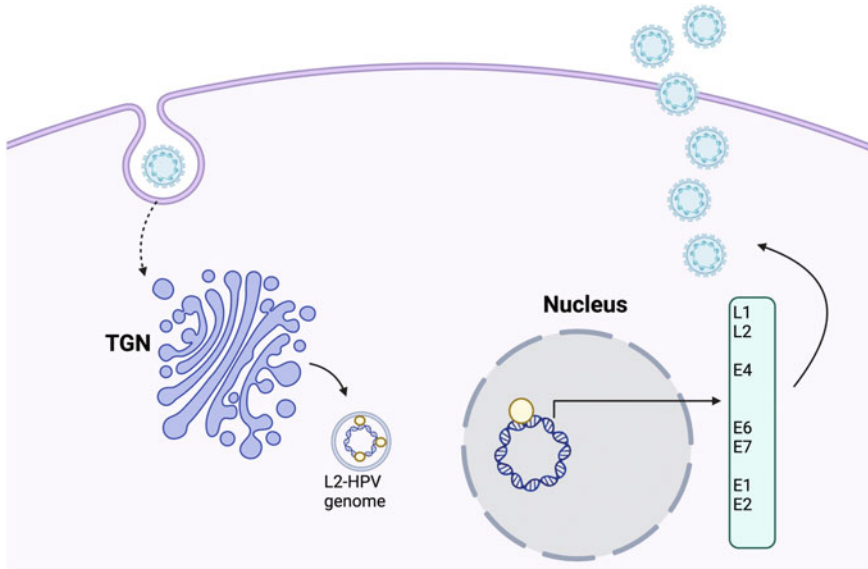
large (hDLG), multi-PDZ domain protein 1 (MUPP) and hSCRIBb for their degradation using the same mechanism as with p53. These proteins are related to cell transformation due to the ability to regulate cell-cell interactions and cell polarity (Mantovani and Banks 2001).

The E7 oncoprotein is a protein with an enormous capacity for transformation due to its ability to induce the degradation of the tumor suppressor protein pRb, via the proteasome (Münger et al. 2001). The degradation of pRb induces the release of E2F, inducing the cells to enter the synthesis phase (S phase) of the cell cycle (Boyer et al. 1996). Thus, these oncoproteins permits that HPV genome is synthesized in huge quantities, thereby avoiding cell death, activation of cell cycle and proliferation.

## Exocytosis and Exit toward a New Infection

After a large amount of the HPV genome has accumulated, it is time to package it; however, HPV virions need make their way back through the intracellular network of cytoskeletal proteins. This process is induced by HPV E4 protein, a protein of approximately 17 kDa. Interestingly, although the E4 open reading frame (ORF) is contained within the E2 ORF, it is expressed late, just before L1 and L2. The late expression of E4 is due to the fact that it is regulated by a specific differentiation promoter (p670 for HR-HPV16), and therefore it only accumulates in differentiated cells of the superficial layers of the epithelium (Doorbar 2013). Thus, the main function of E4 has been associated with the collapse of keratin filaments located in the cell cytoplasm, a collapse necessary for the release of virions. However, E4 has also been associated with the inhibition of Cdk1 activity, inducing cell cycle arrest in the G2 phase (Davy et al. 2002). The L1 and L2 proteins are proteins expressed in the late phase of the viral life cycle. These proteins assemble to build the HPV capsid, a process that requires oxidative stress to produce disulfide bonds between the L1 proteins, permitting the maturation of the capsid (Buck et al. 2005). It is important to mention that HPV proteins differentially regulate the redox state (Cruz-Gregorio et al. 2018a, b), regulating both organelles that produce reactive oxygen species and antioxidants (Cruz-Gregorio et al. 2019, 2020, 2023) of cells and that this provides the appropriate conditions for the virus to infect and conclude its viral life cycle. Thus, five L1 units are associated with each other forming an L1 pentamer. In the gap of each L1 pentamer, an L2 protein associates, this complex is called a capsomer, so the HPV capsid is made up of 72 capsomeres in an icosahedral network. Once the virions are assembled, they are released by shedding, and the viral cycle can be started again through L1, which binds to the heparan sulfate (HS) receptor contained in the extracellular matrix and the HPV target cells (Fig. 8.3).

There remain many questions to be answered to fully resolve HPV infection, and the investigation of the process is likely to provide information to avoid infection that is associated with papillomatosis and cancer development. Moreover, the



**Fig. 8.3** Replication, transcription, and exocytosis of the HPV. As soon as the HPV genome is carried from the TGN to the nucleus, it is transcribed and replicated by the cellular transcription and replication machinery aided by the E1 and E2 proteins. The E5, E6, and E7 proteins are also expressed, which prevent the cells from dying by apoptosis and activate the cell cycle, allowing an exponential increase of the viral genome. With high amounts of the viral genome, it is time to package it in the capsid formed by L1 and L2, which will make their way through the cytoskeleton thanks to the keratin degradation induced by E4 so that the virions can leave the infected cell and start another cycle of infection

majority of the knowledge of papillomavirus infection has developed in HR-HPV, which can be extrapolated to LR-HPV or other papillomaviruses such as canine or feline, as papillomaviruses share characteristics (Cruz-Gregorio et al. 2022); however, it is necessary to study infection in these types of virus and different species.

## Conclusions

Understanding the entire HPV infection process has been a topic that researchers have studied and developed for decades; however, there is still much to understand about papillomavirus infection. A deep understanding of these infection processes by these viruses will allow potential new treatments for papillomavirus-associated cancer and papillomatosis in humans and other species.

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