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Review

Papillomavirus assembly: An overview and perspectives

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ABSTRACT

Papillomavirus life cycle is tightly coupled to epithelial cell differentiation, which has hindered the investigation of many aspects of papillomavirus biology, including virion assembly. The development of in vitro production methods of papillomavirus pseudoviruses, and the production of "native" virus in raft cultures have facilitated the study of some aspects of the assembly process. In this paper we review the current knowledge of papillomavirus assembly, directions for future research, and the implications of these studies on new therapeutic interventions.

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1. Introduction

The main evolutionary driving force for a virus is to infect a cell in order to replicate and spread. To accomplish this, viruses need to produce infectious virions, and therefore assembly and genome packaging are often highly regulated processes. The capsid assembly reaction must be orchestrated from a limited number of virion proteins and result in the encapsidation of the viral genome. Viruses with DNA genomes have the specific problem of selectively packaging their genomic DNA in the presence of a vast excess of cellular DNA. Virus assembly is an important subject, not only for the understanding of the basic molecular mechanisms that underlie the process, but also to provide insight into ways that pharmacological inhibitors might interrupt the process, thereby inhibiting viral replication and transmission. The study of virus assembly has also been valuable in the development of gene delivery techniques, since viruses can be used as vectors to selectively package specific nucleic acids and efficiently transfer them into cells.

In vivo, papillomavirus replication and assembly is dependent on an integrated with the terminal differentiation of stratified squamous epithelium, making the study of the process particularly challenging. However, considerable progress had been gained during the past years, primarily by employing simplified in vitro experimental systems. These systems include pseudoviruses, papillomavirus capsids that have encapsidated reporter genes in monolayer culture, and also production of "native" papillomaviruses in organotypic raft cultures. Herein we review the current knowledge on the assembly of papillomaviruses.

2. Papillomavirus structure

Papillomaviruses are small non-enveloped viruses that package an approximately 8 kb circular dsDNA genome. The viral capsid is

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composed by only two proteins, L1, the major capsid protein and L2, the minor capsid protein.

The T = 7 icosahedral viral capsid is primarily comprised of 360 copies of L1 that are arranged in 72 pentamers, of which 12 are pentavalently coordinated and 60 pentamers are hexavalently coordinated (Baker et al., 1991; Klug and Finch, 1965; Trus et al., 1997). Interpentameric disulfide bonds, and also intrapentameric in the case of bovine papillomavirus 1 (BPV1), between L1 molecules stabilize the papillomavirus capsids (Buck et al., 2005a; Cardone et al., 2014; Conway et al., 2011; Ishii et al., 2003; Li et al., 1998; Modis et al., 2002; Sapp et al., 1998; Wolf et al., 2010). The capsid is also stabilized by C-terminal arms that invade the neighboring pentamers and fold back to the original pentamer (Wolf et al., 2010). L1 can self-assemble into virus like particles (VLPs) in the absence of L2 (Kirnbauer et al., 1992).

Atomic level structures of L1/L2 capsids have not been generated. However, cryoelectron microscopy reconstructions and antibody binding studies suggest that each L1 pentamer interacts with a single molecule of L2 (i.e., 72 copies of L2 per virion) (Buck et al., 2008; Cardone et al., 2014). L2 fills the central lumen of the L1 pentamer and a stretch of about 60 N-terminal amino acids may be exposed on the capsid surface (Kawana et al., 2001; Kondo et al., 2007; Liu et al., 1997; Roden et al., 1994). Whether this portion of L2 protrudes from the central lumen of the pentamer or from elsewhere in the capsid shell is unknown. The interactions between the two viral capsid proteins appear to be mostly hydrophobic (Finnen et al., 2003; Okun et al., 2001). L2 plays key roles in the infectious entry process (Aydin et al., 2014; Day et al., 2004; Holmgren et al., 2005; Unckell et al., 1997) and, for some papillomavirus types, for genome packaging (Buck et al., 2004; Holmgren et al., 2005; Zhao et al., 1998; Zhou et al., 1993).

3. Papillomavirus replication

5 Papillomaviruses have a specific tropism for stratified squamous epithelial cells. Viral replication is tightly linked to the differentiation of the epithelium and has traditionally been divided into two phases: an early phase where replication of the viral genome occurs and a late phase where capsid protein production and assembly occurs (Doorbar et al., 2015). To establish a persistent infection, papillomavirus must infect the mitotically active basal cell layer. In these cells, steady state viral genome replication can occur, but vegetative genome replication, characterized by high copy number amplification, only occurs after the daughter of a dividing cell has moved away from the basement membrane into the upper layers of the epithelium and commenced the differentiation process. Capsid assembly occurs in the more differentiated keratinocytes and virus release is thought to be a passive process dependent on nuclear envelope breakdown and desquamation of dying terminally differentiated keratinocytes of the outermost layers of the stratified epithelium. Despite the absolute dependence on epithelial differentiation for virus production in vivo, most functional studies of papillomavirus assembly have been conducted in replicating cultured cells using heterologous vectors to express the capsid proteins and, in many cases, to replicate the target genome for packaging. Aspects of the assembly process that are controlled by the differentiation process cannot be addressed by these systems and may remain to be discovered. Differentiation-dependent mechanisms might be address using organotypic “raft” cultures (Ryndock et al., 2015).

4. Papillomavirus capsid assembly

Both papillomavirus structural proteins, L1 and L2, contain canonical nuclear localization sequences that facilitate translocation of the proteins into the nucleus where assembly occurs (Darshan et al., 2004; Rose et al., 1993; Zhou et al., 1991). For most high-risk human papillomaviruses (HPVs), L2 expression and nuclear localization in the suprabasal cells appears to precede L1 expression (Florin et al., 2002). In contrast, HPV1 L1 seems to be expressed and located in the nucleus throughout the epithelium, while L2 was detected only in the upper epidermal cell layers (Egawa et al., 2000). For *Mus musculus* papillomavirus 1 (MusPV1) L1 is expressed throughout the epithelium in the cytoplasm and only upon L2 expression in the upper layers it is transported to the nucleus (Handisurya et al., 2013). Therefore, in vivo, MusPV1 L2 appears to recruit L1 to the nucleus.

The transport of the L1 proteins of HPV11, 16 and 45 from the cytoplasm into the nucleus was shown to depend on karyopherins (Bird et al., 2008; Darshan et al., 2004; Merle et al., 1999; Nelson et al., 2000, 2002, 2003). L1 is imported into the nucleus as pentamers (Bird et al., 2008; Florin et al., 2002; Merle et al., 1999; Nelson et al., 2002), and assembly into capsids only occurs in the nucleus. Karyopherin binding has been implicated in inhibiting capsid assembly in the cytoplasm (Bird et al., 2008). Hsp70 family members can trigger disassembly of BPV capsids into pentamers (Chromy et al., 2006), which may also help prevent premature assembly in the cytoplasm. L2 nuclear localization is required for its incorporation into capsids (Becker et al., 2004). Karyopherins and Hsc70 have been suggested to be involved in the transport of L2 protein into the nucleus (Bordeaux et al., 2006; Florin et al., 2004). Hsc70 has also been proposed to play a role in L2 incorporation into the capsid or genome packaging (Florin et al., 2004). During assembly, the interaction between L1 and L2 appears to be regulated by sumoylation of L2, such that sumoylation inhibits L1 interactions (Marusic et al., 2010). Under some experimental condition, L2 also promotes L1 capsomer assembly into capsids (Ishii et al., 2005). The cellular protein nucleophosmin, also interacts with L2 protein to promote the correct assembly of infectious capsids, possibly providing a scaffold for the early processes, as it is displaced from L2 by L1 expression, but the precise molecular mechanisms involved are not understood (Day et al., 2015). Studies of raft-derived virions suggest that the L2 of some HPV types, e.g. HPV16 and 45, may be cleaved by furin during virion production in differentiating epithelium, rather than during infection, as has been observed for all types of pseudovirion produced in replicating cells that have been examined (Cruz et al., 2015; Richards et al., 2006)

The assembly kinetics of purified L1 pentamers into VLPs have been investigated in cell-free reactions. Investigations involving multi-angle light scattering concluded that the assembly reaction has a concentration-dependent lag phase with pentamer dimerization being the key nucleating event. This nucleation was followed by rapid sequential addition of single pentamers to form the complete VLP (Casini et al., 2004). It has not been possible to study the kinetics of intracellular assembly.

Taken together, the findings suggest a general mechanism for papillomavirus assembly where (i) papillomavirus genome is replicated to high copy number in the nucleus (ii) L2 and L1 pentamers are recruited to the nucleus and (iii) the capsid assembles once all components are present in the nucleus. The major difference among papillomavirus types appears to be in the relative timing of L1 and L2 expression: for some papillomavirus types L2 seems to precedes L1 and for others L1 precedes L2. It is unclear why these different patterns of L1 and L2 expression have evolved.

After capsid assembly in the nucleus, the redox gradient in the upper layers of the differentiating epithelium in raft cultures allows disulfide bond formation between L1 molecules, leading to capsid maturation (Conway et al., 2009). Based upon studies of monolayer culture cell-derived capsids, this maturation is a slow process that does not seem to be dependent on L2 incorporation into the capsid