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REVIEW

Role of tegument proteins in herpesvirus assembly and egress

Haitao Guo[1,2], Sheng Shen[1,2], Lili Wang[1,2], Hongyu Deng[1] ✉

[1] CAS Key Laboratory of Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China
[2] Graduate School of the Chinese Academy of Sciences, Beijing 100080, China
✉ Correspondence: rrain6@yahoo.com
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ABSTRACT

Morphogenesis and maturation of viral particles is an essential step of viral replication. An infectious herpesviral particle has a multilayered architecture, and contains a large DNA genome, a capsid shell, a tegument and an envelope spiked with glycoproteins. Unique to herpesviruses, tegument is a structure that occupies the space between the nucleocapsid and the envelope and contains many virus encoded proteins called tegument proteins. Historically the tegument has been described as an amorphous structure, but increasing evidence supports the notion that there is an ordered addition of tegument during virion assembly, which is consistent with the important roles of tegument proteins in the assembly and egress of herpesviral particles. In this review we first give an overview of the herpesvirus assembly and egress process. We then discuss the roles of selected tegument proteins in each step of the process, i.e., primary envelopment, deenvelopment, secondary envelopment and transport of viral particles. We also suggest key issues that should be addressed in the near future.

KEYWORDS herpesvirus, tegument, assembly, egress, transport

INTRODUCTION

The family *Herpesviridae* consists of three subfamilies including *Alphaherpesvirinae, Betaherpesvirinae* and *Gammaherpesvirinae* whose members infect a broad range of animal species including mammals, birds and reptiles (Davison et al., 2009). Until now, more than 130 herpesviruses have been identified, among which there are eight human pathogens, including the *Alphaherpesvirinae* members herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2) and varicella zoster virus (VZV), the *Betaherpesvirinae* members human cytomegalovirus (HCMV), human herpesvirus type 6 (HHV-6) and human herpesvirus type 7 (HHV-7), and the *Gammaherpesvirinae* members Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV). HSV most commonly cause mucocutaneous infections, resulting in recurrent orolabial or genital lesions (Roizman et al., 2007). HCMV infection is responsible for approximately 8% of infectious mononucleosis cases and is also associated with inflammatory and proliferative diseases (Söderberg-Nauclér, 2006; Steininger, 2007). EBV and KSHV are associated with several malignancies, such as Burkitt's lymphoma and Kaposi's sarcoma (Chang et al., 1994; Kutok and Wang, 2006).

All members of the *Herpesviridae* family comprise two distinct stages in their life cycle: lytic replication and latency (Sunil-Chandra et al., 1992; Decker et al., 1996a, b; Dupin et al., 1999; Flaño et al., 2000). Members of the *Alphaherpesvirinae* subfamily establish latency in neurons, members of the *Betaherpesvirinae* subfamily establish latency in a range of nonneuronal cells, whereas members of *Gammaherpesvirinae* subfamily members establish latency mainly in B and T lymphocytes. Latency provides the viruses with advantages to escape host immune surveillance so as to establish lifelong persistent infection and thus contributes to transformation and development of malignancies. However, it is through lytic replication that viruses propagate and transmit among hosts to maintain viral reservoirs. Both viral latency and lytic replication play important roles in herpesvirus pathogenesis.

All herpesvirions have a characteristic multilayered architecture (Fig. 1). An infectious virion contains a double-stranded DNA genome, an icosahedral capsid shell, a thick, proteinaceous tegument compartment, and a lipid bilayer

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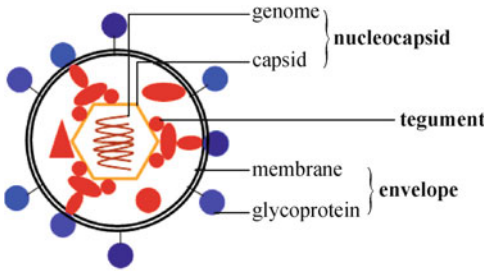

Figure 1. Diagram of herpesvirion structure. The virion particle is approximately 200 nm in diameter. A linear double-stranded DNA genome is packaged within an icosahedral capsid to form the nucleocapsid. The capsid is embedded in a matrix known as the tegument layer, which contains many virus coded proteins. The tegument is itself surrounded by the envelope, a lipid membrane containing several viral glycoproteins.

envelope spiked with glycoproteins (Rixon, 1993; Roizman and Pellett, 2001; Liu and Zhou, 2006; Dai et al., 2008). As a unique structure of herpesviruses, the tegument plays important roles in multiple aspects of the viral life cycle, including translocation of nucleocapsids into the nucleus as well as virion assembly and egress (Subak-Sharpe and Dargan, 1998; Fuchs et al., 2002a). As components that are carried into a host cell during viral infection, tegument proteins can also transactivate the expression of viral immediate-early genes and cellular genes as well as modulate, host signal transduction and innate immunity (Bresnahan and Shenk, 2000; Castillo and Kowalik, 2002; Ishov et al., 2002; Zhu et al., 2002).

Virion assembly and egress is an essential stage of herpesviral lytic replication, hence contributes indirectly to herpesvirus pathogenesis. In this review we summarize the growing range of functions attributed to tegument proteins during the course of herpesvirus assembly and egress. We mainly focus on tegument proteins from the *Alphaherpesvirinae* subfamily, in particular HSV-1 and the related porcine pathogen pseudorabies virus (PrV), since they are the most extensively studied. Meanwhile, some well studied tegument proteins from the *Betaherpesvirinae* and *Gammaherpesvirinae* subfamilies are also discussed.

OVERVIEW OF HERPESVIRUS ASSEMBLY AND EGRESS

During herpesvirus lytic infection, after attachment and penetration, capsids are transported to the nucleus via interaction with microtubules, docking at the nuclear pore where the viral genome is released into the nucleus (Döhner et al., 2002; Wolfstein et al., 2006), where transcription of viral immediate-early, early and late genes and genome replication take place. Then virion assembly and egress proceed from the nucleus to the cytoplasm. Several models have been proposed to explain this process, among which the most widely accepted is the envelopment-deenvelopment-reenvelopment model: capsid formation and viral DNA packaging in the nucleus, primary envelopment at the inner nuclear membrane and deenvelopment at the outer nuclear membrane, association with tegument proteins and secondary envelopment (i.e., reenvelopment) in the cytoplasm, and budding of complete virions into Golgi body-derived compartments for egress via the cellular secretory pathway (Mettenleiter, 2002, 2004). In each stage of virion assembly and egress, tegument proteins are found to play important roles. There are also lines of evidence suggesting direct exit from the nucleus of unenveloped nucleocapsids via enlarged nuclear pores or transport of perinuclear enveloped nucleocapsids through a continuum of outer nuclear, rough ER and Golgi membranes (Wild et al., 2002; Leuzinger et al., 2005; Wild et al., 2005, 2009). In this review, the functional roles of tegument proteins will be discussed in the context of the envelopment-deenvelopment-reenvelopment model of virion assembly.

TEGUMENT PROTEINS

Composition of tegument in all three *Herpesviridae* subfamilies has been studied. At least 26, 38 or 17 virus-encoded tegument components are recruited into an HSV-1, HCMV or EBV virion, respectively (Johannsen et al., 2004; Varnum et al., 2004; Zhu et al., 2005; Loret et al., 2008). Some of these proteins, such as pUL36, pUL47, pUL48 and pUL49, are classified as major, structurally significant components (Heine et al., 1974), whereas some, such as vhs and the protein kinase pUL13, are minor but nonetheless important components (Schek and Bachenheimer, 1985; Overton et al., 1992). Some tegument proteins are conserved in all herpesvirus members (Table 1), and their functions are progressively unrevealed.

As an indispensable part of mature virion, tegument plays important roles in herpesviral particle production. Much effort has been placed on elucidating the molecular interactions between various tegument proteins with the hope of understanding the mechanisms by which this complex virus is assembled. Tegument addition is thought to originate in the nucleus, then more tegument proteins are added to capsids in the cytoplasm following nuclear egress and finally at trans-Golgi network (TGN)-derived membranes during maturation budding (Mettenleiter, 2006). The complexity of virion assembly route clearly raises the issue that different tegument proteins may be incorporated into herpesviral particles at different stages of egress and that the composition of virions in the perinuclear space may not be the same as that of the mature extracellular viruses. For example, the UL31 and UL34 gene products of PrV are present in viral particles in the perinuclear space but are absent from virions at later stages of assembly and mature virions (Fuchs et al., 2002c). In contrast, the pUL36, pUL37, pUL46, pUL47, pUL48 and

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Table 1 Conservation of herpesvirus tegument proteins

HSV-1 tegument proteins	nomenclature of HSV-1 tegument homologs		
	HCMV	EBV	KSHV
UL7	UL103	BBRF2	ORF42
UL11	UL99	BBLF1	ORF38
UL13	UL97	BGLF4	ORF36
UL14	UL95	BGLF3	ORF34
UL16	UL94	BGLF2	ORF33
UL21	UL87	BcRF1	ORF24
UL23	NA	BXLF2	ORF21
UL36	UL48	BPLF1	ORF64
UL37	UL47	BOLF1	ORF63
UL51	UL71	BSRF1	ORF55
UL41	NA	NA	NA
UL46	NA	NA	NA
UL47	NA	NA	NA
UL48	NA	NA	NA
UL49	NA	NA	NA
UL50	NA	NA	NA
UL55	NA	NA	NA
US2	NA	NA	NA
US3	NA	NA	NA
US10	NA	NA	NA
US11	NA	NA	NA
RL1 (ICP34.5)	NA	NA	NA
RL2 (ICP0)	NA	NA	NA
RS1 (ICP4)	NA	NA	NA

NA: not applicable; HSV: herpes simplex virus.

pUL49 tegument proteins of PrV are found in only cytoplasmic enveloped virions and mature PrV particles and are therefore presumably not recruited into the virion during primary envelopment (Fuchs et al., 2002a; Klupp et al., 2002; Kopp et al., 2002). The PrV pUS3 protein kinase, on the other hand, is a component of both primary and mature enveloped virions (Granzow et al., 2004). However, some researchers showed conflicting data on the related HSV-1 homologs, in which HSV tegument proteins pUL36, pUL37, pUL48 and pUL49 have been found associated with intranuclear capsids or existing in primary enveloped virions (Mossman et al., 2000; Naldinho-Souto et al., 2006; Bucks et al., 2007; Padula et al., 2009). These results indicate that the sequence of tegument protein addition and the regions in which capsid acquires the tegument are very complicated. The inner tegument proteins may interact with the capsid while the outer tegument proteins may be recruited by interacting with the cytoplasmic tails of viral glycoproteins. Several molecular interactions have been implicated in linking the viral capsid, tegument and membrane during the envelopment process. Examples include pUL25 (capsid)-pUL36 (tegument), pUL49 (tegument)-gD (membrane), pUL48 (tegument)-gH (membrane), and pUL11 (membrane)-pUL16 (tegument/capsid) interactions (McNab et al., 1998; Mettenleiter, 2002, 2004; Coller et al., 2007). These interactions will be elaborated in the following sections.

PRIMARY ENVELOPMENT AND DEENVELOPMENT

After capsid assembly, the nucleocapsids will bud at the inner nuclear membrane (NM). There, the viruses encounter the nuclear lamina, a rigid network of lamin proteins lining the inner NM, which is a major obstacle for budding of the nucleocapsids. Herpesviruses disrupt the nuclear lamina in order to bud into the perinuclear space and acquire the inner NM as the envelope, the so called primary envelopment (Scott and O'Hare, 2001; Muranyi et al., 2002; Leach et al., 2007; Mou et al., 2007; Mou et al., 2008). HSV-1 and PrV disrupt the lamina through two viral proteins, pUL31 and pUL34, which form a complex colocalized to the inner NM (Reynolds et al., 2002; Reynolds et al., 2004; Simpson-Holley et al., 2004; Simpson-Holley et al., 2005; Bjerke and Roller, 2006). Research suggested that pUL31 and pUL34 promote viral nuclear egress by several mechanisms, including (1) affecting maturation of viral replication intermediates so that capsids assemble adjacent to the nuclear envelope, making it convenient for primary egress (Simpson-Holley et al., 2004, 2005), (2) causing displacement of and conformational changes in lamins A/C and B (Reynolds et al., 2004; Simpson-Holley et al., 2004; Bjerke and Roller, 2006), and (3) mislocalizing the membrane lamin receptors, such as the lamin B receptor and emerin, which tether lamins to the inner NM (Scott and O'Hare, 2001; Leach et al., 2007; Morris et al., 2007; Mou et al., 2008). Amazingly, expression of PrV pUL31 and pUL34 in RK-13 cells resulted in formation of vesicles in the perinuclear space, indicating that they may compose the essential budding machinery (Klupp et al., 2007).

pUL31 and pUL34 are conserved in all three *Herpesviridae* subfamilies. In EBV, the pUL34 positional homolog BFRF1 and the pUL31 positional homolog BFLF2 also interact with each other. HSV-1 UL34 or EBV BFRF1 mutant viruses showed a phenotype of nuclear retention (Lake and Hutt-Fletcher, 2004; Farina et al., 2005; Gonnella et al., 2005; Calderwood et al., 2007). EBV BFLF2 functions in both viral DNA packaging and primary envelopment, as deletion of BFLF2 led to sequestration of nucleocapsids in the nucleus and production of more empty capsids without viral genomes (Granato et al., 2008).

Alphaherpesviruses also express a viral serine/threonine protein kinase, pUS3, that phosphorylates lamins and lamin receptor emerin, which are thought to be involved in maintaining nuclear integrity (Leach et al., 2007; Morris et al., 2007; Mou et al., 2008). In addition, both HSV-1 and PrV pUL31 and pUL34 have been shown to be phosphorylated in a pUS3-dependent manner, and all three proteins have been found to be incorporated into primary virions (Purves et al.,

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1991; Granzow et al., 2004; Ryckman and Roller, 2004; Kato et al., 2005; Mou et al., 2007, 2009). pUS3 also regulates the localization of the pUL31/pUL34 complex, as deletion of pUS3 disrupts their localization from a smooth nuclear rim distribution to distinct foci (Wagenaar et al., 1995; Klupp et al., 2001a; Reynolds et al., 2002; Ryckman and Roller, 2004). These observations indicate that pUS3 might function in virion primary envelopment.

The other tegument protein kinase, pUL13, may also play a role in regulating the localization of pUL31 and pUL34 in HSV-1 either directly or indirectly through phosphorylation of pUS3 (Kato et al., 2006, 2008), indicating its participating in primary envelopment. The HCMV homolog of HSV-1 pUL13, pUL97, which is also a kinase, is incorporated into virions (Michel et al., 1996). pUL97 has been reported to play an important role in virion nuclear egress, as a marked decrease in the number of mature capsids was observed in the cytoplasm of cells infected with UL97 null mutant compared to the wild type virus (Wolf et al., 2001; Krosky et al., 2003). Deletion of HCMV UL97 causes an aggregation of tegument and other structural proteins in nuclear inclusion, suggesting that pUL97 kinase is required for the normal function of tegument proteins in the nucleus and may function in initiation of tegumentation or primary envelopment (Prichard et al., 2005). Although it is not clear where pUL97 is added onto the nucleocapsid, interaction between pUL97 and the HCMV major tegument protein pp65 is required for the incorporation of pUL97 into viral particles (Kamil and Coen, 2007; Chevillotte et al., 2009).

HSV-1 tegument proteins pUL36, pUL37, pUL48 and pUL49 have been found to be associated with intranuclear capsids or exist in primary enveloped virions, though their specific functions in nuclear egress are not clear yet (Mossman et al., 2000; Naldinho-Souto et al., 2006; Bucks et al., 2007; Padula et al., 2009). pUL36 contains a capsid binding domain and interacts with a minor capsid protein pUL25 previously found to participate in DNA encapsidation (McNab et al., 1998; Coller et al., 2007). Such an interaction is required for assembly of pUL36 onto capsid, indicating that pUL36 can bind to capsid directly. This result is consistent with those from electron microscope studies, which suggest presence of HSV-1 pUL36 in the inner most layer of the tegument, attached to the vertices of the capsid (McNabb and Courtney, 1992; Zhou et al., 1999). pUL36 is conserved throughout the *Herpesviridae*. It forms a complex with pUL37 as part of the capsid-associated inner tegument. Deletion of either of the genes encoding pUL36 or pUL37 blocks further tegumentation of the capsid in the cytoplasm (Desai, 2000; Desai et al., 2001; Klupp et al., 2001b; Fuchs et al., 2004; Leege et al., 2009; Roberts et al., 2009). By interacting with pUL48, pUL36 is a major factor for recruiting pUL48 onto capsids (Ko et al., 2010). However, in PrV, pUL36 and pUL48 only exist in mature virions, and are undetectable from nuclear or primary enveloped virions, indicating that these two tegument proteins are acquired in the cytoplasm (Naldinho-Souto et al., 2006; Möhl et al., 2009). Deletion of pUL48 does not affect the capsid assembly and nuclear egress of PrV (Fuchs et al., 2002a). These conflicting results suggest that pUL36 and pUL48 of HSV-1 and PrV may function differently.

Deenvelopment is a step of herpesvirus nuclear egress whose details are poorly understood. pUL48 might play a role in this process for HSV-1, as large numbers of enveloped particles accumulate between the inner and outer NM in the absence of pUL48 (Mossman et al., 2000). In addition, HSV-1 gB and gH/gL have redundant or overlapping roles in fusion with the outer NM (Farnsworth et al., 2007b). Consistent with this notion, EBV, KSHV and PrV gB null mutants all exhibit defects in nuclear egress (Peeters et al., 1992; Lee and Longnecker, 1997; Krishnan et al., 2005). In the primary enveloped virions, pUS3 can phosphorylate the gB cytoplasmic domain and this modification is important for gB-mediated fusion of the virion envelope and the outer NM (Wisner et al., 2009). Thus, pUS3 plays important roles in both primary envelopment and deenvelopment.

SECONDARY ENVELOPMENT

Most tegument proteins are recruited onto the nucleocapsid in the cytoplasm. The addition of outer tegument is accompanied with virion secondary envelopment. During this process, several molecular interactions, such as pUL11 (membrane)-pUL16 (tegument), pUL48 (tegument)-gH (membrane) and pUL49 (tegument)-gE (membrane), have been implicated in linking the viral capsid, tegument and membrane (Mettenleiter, 2002, 2004). pUL16 is conserved throughout the *Herpesviridae*, and plays a pivotal role in virion morphogenesis in the cytoplasm. pUL16 binds to the capsid by interacting with pUL21 which is also a tegument protein associated with capsid in both the nucleus and the cytoplasm (de Wind et al., 1992; Takakuwa et al., 2001; Klupp et al., 2005). pUL16 has another binding partner, named pUL11, which is a membrane-bound tegument protein (Loomis et al., 2003). Two distinct binding sites exist in pUL16 for pUL11 and pUL21, as covalent modification of its free cysteines with *N*-ethylmaleimide blocks its binding to pUL11 but not to pUL21 (Yeh et al., 2008; Harper et al., 2010). This observation suggests that pUL16 may interact with pUL21 and pUL11 in sequence and both interactions contribute to efficient incorporation of pUL16 into virions (Meckes et al., 2010). The pUL11 tegument protein is also conserved among all herpesviruses, and each homolog contains amino acid motifs that allow covalent modifications with two fatty acids, myristate and palmitate (MacLean et al., 1989; Loomis et al., 2001). These two modifications allow pUL11 accumulating at TGN-derived vesicles in the absence of other viral proteins (Loomis et al., 2001). In addition, there exist leucine-isoleucine and acidic cluster motifs, responsible for recovering pUL11 from plasma membrane to the internal

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membrane. This might enable the recruitment of other membrane proteins (virus or host encoded) from the plasma membrane for secondary envelopment occurring around the TGN-vesicles (Loomis et al., 2001).

Similar to the conservation of pUL16 and pUL11, their interaction is also conserved among all herpesviruses. In HCMV, the pUL11 homolog pUL99 has been found to interact with the pUL16 homolog pUL94 (Liu et al., 2009). Consistent with the previously described model of capsid transport and budding, herpesviruses lacking pUL16, pUL21 or pUL11 (or their homologs) have defects in virus egress, resulting in decreased amounts of extracellular viruses produced and the accumulation of non-enveloped capsids in the cytoplasm (MacLean et al., 1989, 1992; Baines and Roizman, 1992; Baines et al., 1994; Kopp et al., 2003; Schimmer and Neubauer, 2003; Silva et al., 2003; Britt et al., 2004; Jones and Lee, 2004; Silva et al., 2005; Seo and Britt, 2006; Guo et al., 2009).

In murine gammaherpesvirus-68 (MHV-68), the homolog of pUL16 is ORF33. Our laboratory has confirmed that ORF33 indeed encodes a tegument protein for MHV-68. Through constructing and analyzing an ORF33 null mutant, we demonstrated that ORF33 is not required for viral genome replication or gene expression, but is essential for virion morphogenesis and egress. More interestingly, ORF33 null mutation caused a partial retention of nucleocapsids in the nucleus, and maturation of virions was arrested at a cytoplasmic stage of partially tegumented nucleocapsids, indicating that ORF33 participates in both primary envelopment of nucleocapsids and morphogenesis of virions in the cytoplasm (Guo et al., 2009). We also found that ORF33 localizes in the nucleus during early infection and interacts with another nuclear tegument protein (Guo et al., unpublished data). We hypothesize that such interaction may facilitate the nuclear budding or egress of nucleocapsids, although such an interaction is relatively redundant.

A number of interactions have been identified involving HSV-1 pUL48 and inner tegument proteins, outer tegument proteins or the cytoplasmic tails of glycoproteins, supporting the concept that pUL48 contributes to linking capsid and envelope during virion formation. For instance, pUL48 has been shown to interact with the outer tegument proteins pUL41, pUL46, pUL47 and pUL49, through a combination of yeast two-hybrid, *in vitro* pull-down and co-immunoprecipitation studies (Smibert et al., 1994; Elliott et al., 1995; Vittone et al., 2005). HSV-2 pUL48 has also been shown to copurify and colocalize with pUL46 during the course of infection (Kato et al., 2000). HSV-1 pUL46 is associated with cellular membrane independent of the presence of other viral proteins. Surprisingly, membrane association within the cell does not translate to association of this protein with the viral envelope, as pUL46 exhibits a strong affinity for the capsid of purified viral particles (Murphy et al., 2008). However, deletion of pUL46 in HSV-1 and PrV does not block virion assembly (Zhang et al., 1991; Kopp et al., 2002). Although deletion of pUL47 causes an approximately 10-fold decrease in viral titer, pUL47 is not essential for virion assembly in the cytoplasm (Kopp et al., 2002). HSV-1 pUL49 interacts with pUL48, but this interaction is not required for incorporation of pUL49 into viral particles (O'Regan et al., 2007). Similar to pUL46, deletion of pUL49 in HSV-1 does not have a significant effect on virus assembly (Elliott et al., 2005). In the case of PrV, deletion of any of pUL46, pUL47, pUL48 or pUL49 does not prevent incorporation of the remaining three proteins into the tegument (Fuchs et al., 2002a; Michael et al., 2006). Furthermore, an increased incorporation of cellular actin into virions was observed when pUL46, pUL47 or pUL49 was deleted, suggesting that actin may fill the void left by the absence of these tegument proteins (del Rio et al., 2005; Michael et al., 2006). These observations indicate that redundancy exists in tegument assembly. In addition to tegument proteins mentioned above, HSV-1 pUL48 also interacts with the cytoplasmic tails of HSV-1 glyoprotein gB, gD and gH, as identified by pull-down, co-immunoprecipitation and chemical cross-linking assays (Zhu and Courtney, 1994; Gross et al., 2003; Kamen et al., 2005). However, the role of these interactions in HSV-1 assembly requires further investigation.

pUL49, besides an interaction with pUL48, also interacts with a number of viral envelope proteins. Several studies have shown that pUL49 interacts with the cytoplasmic tails of HSV-1 gE, gD and envelope protein pUS9 (Chi et al., 2005; Farnsworth et al., 2007a; O'Regan et al., 2007). In PrV, interactions of pUL49 with the cytoplasmic tail of both gE and gM were identified (Fuchs et al., 2002b). Furthermore, for both HSV-1 and PrV, gE or gM is sufficient for recruitment of pUL49 into virions through a direct interaction (Fuchs et al., 2002b; Michael et al., 2006; O'Regan et al., 2007; Stylianou et al., 2009).

Other tegument proteins promoting secondary envelopment have also been identified. ORF52 is a tegument protein conserved in gammaherpesviruses. The MHV-68 ORF52 localizes in TGN-derived vesicles in both transfection and viral infection. Furthermore, ORF52 was found to specifically function in virus budding into the vesicles in the cytoplasm (Bortz et al., 2007). The crystal structure of ORF52 has been determined at 2.1 Å resolution, revealing a dimeric association of this protein. The self-association was confirmed by co-immunoprecipitation and fluorescence resonance energy transfer experiments. Functional complementation assay demonstrated that both the N-terminal α-helix and the conserved Arg^{95} residue are critical for ORF52 function (Benach et al., 2007). The detailed mechanism responsible for involvement of ORF52 in secondary envelopment is currently under investigation in our laboratory.

In HCMV, the tegument protein pUL32 has been reported to be important in cytoplasmic maturation of virions, especially in virus egress (Tandon and Mocarski, 2008). pUL32

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interacts with Bicaudal D1 (BicD1), a protein thought to play a role in trafficking within the secretory pathway (Indran et al., 2010). This interaction helps pUL32 to traffic to the virus assembly compartment, suggesting a primary contribution of this protein in the morphogenesis and/or cytoplasmic transport of progeny virion particles to sites of virion secondary envelopment.

TRANSPORT AND RELEASE

How do nucleocapsids or partially tegumented nucleocapsids transport to the site where secondary envelopment takes place? Microtubule has been found to be involved in transporting non-enveloped viral particles. Several tegument proteins have been identified as candidates linking deenveloped nucleocapsids to the anterograde cellular microtubule-dependent molecular motor kinesin. In alphaherpesviruses, pUS11 was found to interact with both kinesin-1 and the kinesin-related protein PAT-1 (Diefenbach et al., 2002; Benboudjema et al., 2003). An association between pUL21 and microtubules was also detected (Takakuwa et al., 2001), raising the possibility that pUL21 could be involved with capsid transport to the TGN-derived vesicles, where pUL16 interacts with pUL11, facilitating the budding process by linking capsids to the membrane. In gammaherpesviruses, an interaction between KSHV tegument protein ORF45 and kinesin-2, determined by yeast two-hybrid and co-immunoprecipitation assays, has been reported (Sathish et al., 2009). Disrupting the interaction between KSHV ORF45 and kinesin-2 by a dominant negative mutant of ORF45 leads to a decreased final production of mature viruses. This result supports the idea that the association between a viral tegument protein and a cellular molecular motor is important for the intracellular movement of deenveloped nucleocapsids. The HSV-1 pUL36 is a multifunctional large tegument protein. As part of the inner tegument, pUL36 is also important for the transport of enveloped virions. A potential mechanism was proposed that, during viral infection, pUL36 resides on the surface of organelles which nucleocapsids bud into, and pUL36 would directly or indirectly recruit kinesin motors to the organelles to enable motility (Shanda and Wilson, 2008).

CONCLUSIONS AND PERSPECTIVES

Through interacting with and manipulating the host microenvironment, tegument proteins are multifunctional during the complete cycle of herpesvirus lytic replication. In particular, they play key structural roles during virion primary envelopment, secondary envelopment and virion trafficking by forming a bridge between capsid or capsid-associated proteins and membrane-associated viral proteins or cellular

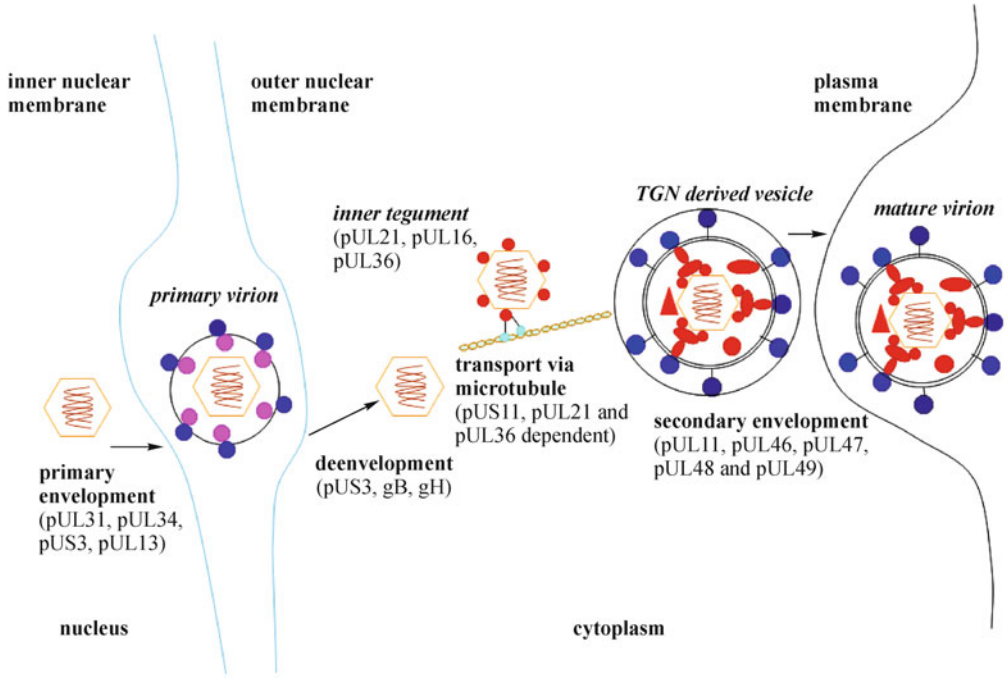

Figure 2. Diagram summarizing the roles of selected tegument proteins in herpesvirus assembly and egress. Viral genomes are packaged into preformed capsids in the nucleus. Through primary envelopment at the inner nuclear membrane and deenvelopment at the outer nuclear membrane, the nucleocapsid is transported into the cytoplasm from the nucleus. Acquisition of tegument proteins onto the nucleocapsid is completed in the cytoplasm. The immature virion is transported via the microtubules into a trans-Golgi derived vesicle containing viral glycoproteins. After transport of the vesicle to the cell surface, the vesicle and plasma membrane fuse, resulting in the egress of a mature, enveloped virion.

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molecular motors (Fig. 2). The herpesvirion assembly and egress process is complex and dynamic, and so are the roles of tegument proteins. As a complex process, herpesvirion assembly and egress involves many protein–protein interactions with marked redundancy. Indeed, interactions between tegument proteins and other viral and cellular proteins are increasingly reported *in vitro* or *in vitro*. However, future research on tegument will require identifying herpesviral protein–protein interaction and herpesviral protein-cellular protein interaction maps during the course of infecting host cells and validating such interactions by viral genetics approach. This has been done to some extent with the *Alphaherpesvirinae* subfamily member VZV and the *Gammaherpesvirinae* subfamily members KSHV and EBV (Uetz et al., 2006; Calderwood et al., 2007; Rozen et al., 2008). Such information will improve our understanding of the biology of herpesvirus and the roles of tegument proteins. More importantly, by examining the importance of each pair of protein–protein interaction, useful therapeutic targets may be identified. The current antiviral strategy for treatment of herpesviruses employs inhibitors of viral DNA replication which have varying efficacies depending on the *Herpesvirinae* subfamily being treated. Therefore, new targets for therapeutic interventions through different mechanisms are useful, especially when combined with the viral DNA replication inhibitors.

The herpesvirion assembly and egress is also a dynamic process, and dissecting the roles of tegument proteins in such a dynamic process calls for integration of different technical approaches. Recently, combinations of molecular tags visible in light and electron microscopes have become particularly advantageous in the analysis of dynamic events at the cellular level (Martin et al., 2005; Gaietta et al., 2006; Lanman et al., 2008). Engineering such tags into herpesvirus tegument proteins as well as capsid/envelope proteins will enable optical live cell imaging and correlated ultrastructural analysis by electron microscopy, and help provide high-resolution information for the dynamic process of herpesvirion assembly and egress.

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