Functions of Human Cytomegalovirus Tegument Proteins Prior to Immediate Early Gene Expression

R. F. Kalejta

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Abstract Proteins within the tegument layer of herpesviruses such as human cytomegalovirus (HCMV) are released into the cell upon entry when the viral envelope fuses with the cell membrane. These proteins are fully formed and active, and they mediate key events at the very start of the lytic infectious cycle, including the delivery of the viral genome to the nucleus and the initiation of viral gene expression. This review examines what is known about tegument protein function prior to the immediate early (IE) phase of the viral lytic replication cycle and identifies key questions that need to be answered to better understand how these proteins promote HCMV infection so that antiviral treatments that target these important viral regulators can be developed.

Introduction

Human cytomegalovirus (HCMV) is a significant human pathogen that infects the majority of the world's population. Viral infection causes birth defects and severe disease in patients with suppressed immune function and is associated with age-related immunosenescence, cancer, and cardiovascular disease (Mocarski

R.F. Kalejta
Institute for Molecular Virology and McArdle Laboratory for Cancer Research, University of Wisconsin-Madison, Madison, WI 53706-1596, USA
rfkalejta@wisc.edu

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et al. 2007). Within mature virions, the HCMV genome is housed in an icosahedral protein capsid that is surrounded by a layer of proteins called the tegument, which in turn is enclosed within a lipid membrane termed the envelope. Virally encoded glycoproteins in the envelope function as mediators of viral entry through a membrane fusion event (see the chapter by M.K. Isaacson et al., this volume) that releases both the DNA-containing capsids and the tegument proteins into the cell (Fig. 1).

As many as 59 viral proteins have been found in the viral tegument, although only about 35 are incorporated at significant levels (Baldick et al. 1996; Varnum et al. 2004). Virions also contain a sampling of cellular proteins (Varnum et al. 2004), as well as viral and cellular RNA molecules (Terhune et al. 2004). Bioinformatic and experimental approaches have failed to detect a tegument localization signal (i.e., a sequence necessary and sufficient to direct macromolecules into the tegument) on either proteins or RNAs. The process of assembling the tegument upon viral egress, as well as the disassembly of the tegument upon viral entry into cells, is poorly understood. Likewise, the structure of the tegument within the virion is not known. Although mostly amorphous, there appears to be some structuring of tegument proteins that are closely associated with the capsid (Chen et al. 1999; Trus et al. 1999). Many tegument proteins are phosphorylated (Irmiere and

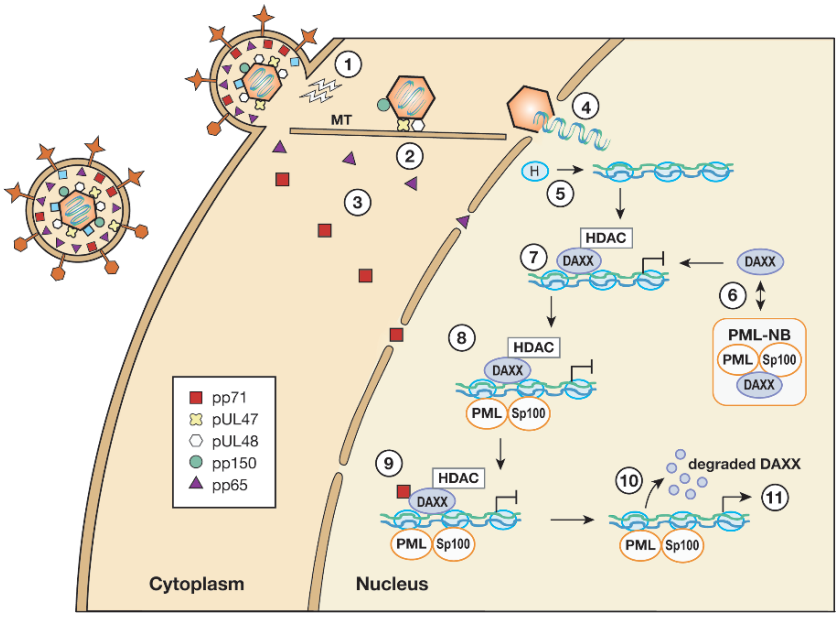

Fig. 1 Postfusion, preimmediate early events during lytic replication of human cytomegalovirus. Schematic representation of delivery of viral genomes and tegument proteins to the nucleus (1.1–1.4), the generation of a silencing complex (PML-NB) on infecting viral genomes (1.5–1.8), and the initial step in the destruction of that complex by tegument-delivered pp71 (1.9–1.11). See the text for further details

Gibson 1983), but the significance of this or other posttranslational modifications to these proteins remains largely unexplored.

Table 1 Human cytomegalovirus tegument proteins with known or predicted functions

Gene, protein	Phenotype	Function(s)	References
UL26	A	Increases stability of virion proteins	Munger et al. 2006 Lorz et al. 2006
UL32 - pp150	E	Directs capsid to site of final envelopment	AuCoin et al. 2006
UL35	A	Activates viral gene expression	Schering et al. 2005
UL36	D	Inhibits apoptosis	Skaletskaya et al. 2001
UL38	A	Inhibits apoptosis	Terhune et al. 2007
UL45	A	Inactive (?) ribonucleotide reductase subunit	Patrone et al. 2003
UL47	A	Release of viral DNA from capsid	Bechtel and Shenk 2002
UL48	E	Deubiquitinating protease	Wang et al. 2006
		Release of viral DNA from capsid	Bechtel and Shenk 2002
UL69	A	Nuclear export of unspliced mRNAs	Lischka et al. 2006
		Arrests cell cycle in G1	Lu and Shenk 1999
UL77	E	Putative pyruvoyl decarboxylase	Yoakum 1993
UL82 - pp71	A	Degrades Daxx, facilitates IE gene expression	Saffert and Kalejta 2006
		Degrades Rb, stimulates cell cycle progression	Kalejta et al. 2003
		Prevents cell surface expression of MHC	Trgovcich et al. 2006
UL83 - pp65	D	Endogenous kinase activity	Yao et al. 2001
		Associated kinase activity	Gallina et al. 1999
		Evasion of adaptive immunity	Gilbert et al. 1996
		Evasion of innate immunity	Arnon et al. 2005
UL94	A/E	Putative DNA-binding protein	Wing et al. 1996
		Similar to autoantigen in systemic sclerosis	Lunardi et al. 2000
UL97	A	Kinase that phosphorylates ganciclovir	Littler et al. 1992 Sullivan et al. 1992
		Stimulates DNA replication, assembly/egress	M. Prichard et al. 1999
UL99 - pp28	E	Directs enclosure of enveloped particles	Silva et al. 2003
IRS1/TRS1	A/E	Inhibits PKR antiviral response	Hakki et al. 2006
		Virion assembly	Adamo et al. 2003
US24	A	Activates viral gene expression	Feng et al. 2006

Genes that encode tegument proteins along with commonly accepted protein names (if applicable) are shown in column 1. Phenotype (column 2), listed as augmenting (A), dispensable (D) or essential (E), refers to the requirement of the gene for lytic replication in human fibroblast cells in vitro as determined in either the provided reference (column 4), the two global mutational analyses of human cytomegalovirus (Dunn et al. 2003; Yu et al. 2003) or as described in a recent review (Mocarski et al. 2007). Column 3 displays either demonstrated or inferred functions for these proteins

Activities for less than half of the tegument proteins have been determined or suggested (Table 1). The phenotypes of recombinant viruses with null mutations in genes encoding tegument proteins demonstrate that some are absolutely essential for viral replication, others are required for efficient replication (often termed augmenting genes), while still others are dispensable for lytic replication in vitro (Mocarski et al. 2007; Dunn et al. 2003; Yu et al. 2003). Many tegument proteins play important roles during the later stages of viral replication, and are required for proper viral assembly and egress (see the chapter by W. Gibson, this volume). This review focuses on the functions of tegument proteins during the initial intracellular events of a lytic infection, after fusion of the viral and cellular envelopes, but prior to the transcription of the first viral genes to be expressed from the infecting viral genome, the immediate early (IE) genes. Thus, what are described here are called postfusion, preimmediate early events.

Tegument Proteins Known to Act at the Very Start of HCMV Infection

The pp65 phospho-protein is the major constituent of HCMV particles (Irmiere and Gibson 1983) and is delivered to the nucleus of permissive cells after fusion of the viral and cellular membranes (Revello et al. 1992). pp65 is either itself a protein kinase (Yao et al. 2001) or associates with a cellular kinase (Gallina et al. 1999) or perhaps both. If pp65 does have intrinsic kinase activity, it is an unusual example of a kinase because it shows poor homology to the catalytic domain sequences of other kinases (Yao et al. 2001). The UL83 gene that encodes pp65 is completely dispensable for replication in cultured fibroblasts (Schmolke et al. 1995), but is likely maintained despite the presence of strong, targeted immune response (Grefte et al. 1992; Wills et al. 1996) because of the ability of pp65 to modulate multiple levels of immune surveillance (Mocarski et al. 2007). Monitoring the delivery of tegument-incorporated pp65 to the nucleus is a common method used to assay for viral entry. Recently, pp150, the second most abundant tegument protein, has also been used to track viral entry (see Sect. 3 below). Encoded by UL32, a gene absolutely essential for lytic replication in vitro (Dunn et al. 2003; Yu et al 2003), pp150 interacts with preformed capsids (Baxter and Gibson 2001), and appears to be required for the incorporation of capsids into forming virions, perhaps because of its ability to stabilize capsids and/or direct their movement within the cytoplasm (Aucoin et al. 2006).

The UL47 and UL48 tegument proteins form a complex with each other and the major capsid protein (Bechtel and Shenk 2002) that appears to play a prominent role during viral entry (see Sect. 3 below). pUL47 has no known enzymatic activity, but pUL48 is a deubiquitinating protease (Wang et al. 2006). While the deubiquitinating activity is not absolutely essential for viral replication, clones with active site mutations in pUL48 show temporal delays in virion release (Wang et al. 2006). Thus, along with their roles in viral entry, pUL47 and pUL48 also likely function during viral maturation and/or egress. UL47 is an augmenting gene, disruption of

which results in a 100-fold reduction in viral titers after infections at either high or low multiplicities (Bechtel and Shenk 2002). The UL48 gene has been classified as either augmenting (Yu et al. 2003) or essential (Dunn et al. 2003).

The UL82 gene encodes the pp71 tegument protein that localizes to the nucleus in both HCMV-infected and UL82-transfected cells (Hensel et al. 1996). Although pp71 is not absolutely essential, it is required for efficient viral replication (Bresnahan and Shenk 2000) because of its ability to facilitate viral IE gene expression (see Sect. 4 below). Other tegument proteins such as pUL26 and pUL35 may assist in the pp71-mediated activation of IE gene expression (see Sect. 4 below). pp71 also targets the hypophosphorylated forms of the Rb family of tumor suppressors for proteasome-dependent, ubiquitin-independent degradation, leading to cell cycle stimulation (Kalejta et al. 2003; Kalejta and Shenk 2003), and decreases the cell surface expression of MHC class I proteins by slowing their intracellular transport (Trgovcich et al. 2006).

Delivery of the Genome to the Nucleus

Once in the cytoplasm, HCMV genome-containing capsids and some tegument proteins must make their way to the nucleus. Although a seemingly simple task, this journey is a difficult one due to the size of the viral particle and the density of the cytoplasm. HCMV overcomes these obstacles using strategies that are also employed by other viruses (Dohner et al. 2005; Greber and Way 2006), namely hijacking the intracellular transport machinery. Cells contain an organized network of microtubules (MTs) that extend from the microtubule-organizing center (MTOC) near the nucleus all the way to the periphery, ending near the cell membrane. This network, along with other mechanisms, allows for the temporal and spatial control of the transport of large cargoes to help establish and maintain cell polarity as well as the uneven distributions of proteins, RNAs, and organelles (Welte 2004). MTs are composed of ordered, head-to-tail associations of tubulin monomers, and thus have a distinct polarity, with their negative ends near the MTOC and their positive ends near the cell surface. Cytoplasmic dynein is a minus-end-directed motor protein, which, along with dynactin, uses power generated from ATP hydrolysis to transport cargo along microtubules toward the MTOC (Malik and Gross 2004).

An intact microtubule network is required for the transport to the nucleus of capsids deposited in cells upon HCMV infection (Ogawa-Goto et al. 2003). Nocodazole, a drug that de-polymerizes microtubules, inhibits IE gene expression, likely by preventing infecting HCMV from depositing its DNA in the nucleus. Entering capsids in transit to the nucleus can be localized by detecting the tightly associated tegument protein pp150 through indirect immunofluorescence (Sinzger et al. 2000). In the absence of nocodazole, incoming pp150 is found associated with MTs and concentrated near the nucleus, but is diffusely distributed in the cytoplasm in the presence of the drug (Ogawa-Goto et al. 2003). Transmission electron microscopy (TEM) also showed entering DNA-containing capsids in the

cytoplasm that co-localized with MTs (Ogawa-Goto et al. 2003). These capsids displayed a dense outer layer that likely represents tightly associated tegument proteins. This compelling study strongly suggests that entering HCMV capsids and tightly associated tegument proteins travel through the cytoplasm on MTs toward the nucleus, and that this process is important for viral gene expression and replication. One of these tightly associated tegument proteins appears to be pp150, and the recent generation of infectious virus containing a pp150-GFP fusion protein (Sampaio et al. 2005) should allow for the visualization and quantitation of capsid transport to the nucleus in live cells.

Additional candidates for HCMV tegument proteins tightly associated with entering capsids are pUL47 and pUL48. Circumstantial evidence suggests that the UL47/UL48 protein complex (Bechtel and Shenk 2002) may play significant roles in the transport of infecting HCMV capsids to the nucleus and/or in the injection of viral DNA into the nucleus through the nuclear pore complex. The pseudorabiesvirus (PRV) orthologs of pUL47 and pUL48 (pUL37 and pUL36, also called VP1/2, respectively) also form a complex (Klupp et al. 2002) and, as visualized in live cells, travel with entering capsids toward the nucleus and accumulate with them at the nuclear rim (Luxton et al. 2005). The herpes simplex virus type 1 (HSV-1) orthologs (pUL37 and pUL36, also called VP1/2, respectively) also interact with each other (Vittone et al. 2005) and may be the tegument proteins required for the in vitro (and presumably in vivo) transport of viral capsids along MTs (Wolfstein et al. 2006). Interestingly, a temperature-sensitive mutant in HSV-1 UL36 docks at the nuclear pore complex during entry but fails to release viral DNA into the nucleus (Batterson et al. 1983).

Direct evidence of a role for pUL47 and pUL48 in the delivery of viral genomes to the nucleus also exists. Experiments with an HCMV UL47-null mutant revealed a decrease in the overall accumulation of the UL48 protein and in its incorporation into virions (Bechtel and Shenk 2002). Therefore, the UL47-null virus is also hypomorphic for pUL48. Upon infection of permissive fibroblasts with a UL47-null virus, viral immediate early gene expression is delayed, but entry, as assayed by the delivery of the tegument proteins pp65 and pp71 to the nucleus, appears to be normal (Bechtel and Shenk 2002). Thus, viruses lacking UL47 have a defect that is postfusion, but prior to immediate early gene expression. A model in which the HCMV pUL47/pUL48 complex binds to viral capsids and perhaps microtubule motors to mediate the delivery of the capsid to the nuclear pore with the subsequent release of the viral DNA into the nucleus appears to be consistent with the current data. The pp150 protein may also participate in this process. A similar scenario likely occurs for PRV and HSV-1.

Many intriguing questions remain about how tegument proteins and DNA-containing capsids are delivered to nuclear pores, and how the viral genome enters the nucleus. For example, how does the tegument disassemble before, during or after entry? There is a clear example of one tegument protein (pp65) that transits into the nucleus without an intact MT network (Ogawa-Goto et al. 2003), and one, pp150, that remains tightly associated with the capsid (Sinzger et al. 2000) and fails to migrate toward the nucleus in the absence of MTs (Ogawa-Goto et al. 2003). The subcellular localization of other tegument proteins including pp71,

pUL47, pUL48 and pUL69 should also be examined during viral entry in both the presence and absence of MT inhibitors to determine which tegument-delivered proteins remain associated with the capsid and thus travel along MTs during viral entry. In addition, a closer examination of the UL26-null virus that appears to have a general defect in tegument formation or stability (Lorz et al. 2006; Munger et al. 2006) could be informative. Is dynein required for the cytoplasmic transport of HCMV capsids toward the nucleus as it is for HSV-1 (Dohner et al. 2002)? If so, to which tegument protein does it bind? Also, how is the viral DNA released from the capsid, through the nuclear pore and into the nucleus? Empty HCMV capsids docked at the nuclear pore complex that presumably had already released their DNA into the nucleus have been observed by TEM (Ogawa-Goto et al. 2003). For HSV-1, the pUL36 protein that is required for DNA release also binds to viral DNA (Chou and Roizman 1989), but it is not clear if this binding plays a role during the release of the genome into the nucleus. Cellular proteins may also play a role in this process, as they do for other viruses (Greber and Fassati 2003). Finally, does one or more of the many cellular signaling pathways induced upon HCMV infection (see the chapter by A. Yurochko, this volume) help during the transport of capsids to the nucleus? Kaposi's sarcoma associated herpesvirus (KSHV) and adenovirus also induce host cell-signaling pathways upon infection, and the activation of these pathways facilitates the MT-directed transport of viral capsids to the nucleus (Naranatt et al. 2005; Suomalainen et al. 2001). Most of the work on the very early stages of HCMV infection has focused on prefusion membrane events or mechanisms of IE gene expression. Although those are defining events in the viral life cycle and certainly merit intense investigation, the time period in between them represents an important, understudied stage of HCMV infection.

Initiating Viral IE Gene Expression

Once viral genomes enter the nucleus, a subset of them associate with subnuclear structures (Ishov et al. 1997) called PML nuclear bodies (PML-NBs), which are sometimes called PODs for PML oncogenic domains or ND10 for nuclear domain 10 (see the chapter by G. Maul, this volume). PML-NBs are visualized as numerous dot-like structures in nuclei, and are built around the PML (promyelocytic leukemia) protein. Other prominent PML-NB proteins include Sp100 and Daxx (Everett and Chelbi-Alix 2007). In the absence of PML, other PML-NB proteins do not co-localize with each other, but are dispersed throughout the nucleus, indicating that the PML protein is required for the integrity of PML-NBs (Ishov et al. 1999). The role of these structures in HCMV-infected cells is beginning to emerge.

Only the HCMV genomes located next to PML-NBs appear to be transcribed, leading to the hypothesis that PML-NBs represent a preferred site for viral gene expression (Ishov et al 1997). However, the proteins that localize to PML-NBs act as transcriptional repressors (Everett and Chelbi-Alix 2007), and many viruses, including HCMV, disrupt PML-NB structures at very early times after infection

(Maul et al. 1993; Korioth et al. 1996). These findings indicate that PML-NBs may actually be detrimental, not helpful, to viral infections. Thus a controversy exists as to whether PML-NBs are pro-virus or anti-virus. Several recent studies from multiple laboratories summarized below paint an incomplete yet quickly resolving portrait of the nuclear events that precede HCMV IE gene expression and argue that PML-NBs are not preferred sites for viral transcription but represent repressive subnuclear domains that are sequentially dismantled during HCMV infection.

Most fluorescent images of PML-NBs give the impression that they are static structures. However, the major constituent proteins of PML-NBs, PML, Sp100 and especially Daxx, actually have a dynamic association with these structures, with high rates of association and disassociation (Wiesmeijer et al. 2002; Everett and Murray 2005). Using synchronized and polarized infections with HSV-1, an elegant series of experiments (Everett and Murray 2005) showed that new PML-NBs are formed de novo around infecting viral genomes (the expected result if PML-NB proteins represent a cellular antiviral defense), and argue against viral genomes migrating through the nucleus to sites of preformed PML-NBs (the expected result if localization to these sites provided an advantage to the virus). The rapidity with which Daxx enters and leaves PML-NBs makes it an obvious candidate for the initial cellular sensor of infecting viral genomes, and this appears to be the case for HCMV. In cells in which the level of PML has been reduced by RNA interference, there are no PML-NBs, and the Daxx and Sp100 proteins are diffusely distributed throughout the nucleus. However, Daxx and Sp100 co-localize to form punctate spots reminiscent of PML-NBs upon HCMV infection (Tavalai et al. 2006). The newly synthesized viral IE2 protein is also found in these aggregates, implying that transcriptionally active viral DNA is located there as well. This significant study showed that in the absence of PML-NBs, Daxx and Sp100 can sense and apparently migrate to infecting HCMV genomes (Tavalai et al. 2006). Any effects of Sp100 on HCMV infection have yet to be established, but it is becoming increasingly clear that Daxx inhibits HCMV infection, and is the very first PML-NB component whose antiviral activities must be neutralized in order for HCMV to express its immediate early genes.

At the low multiplicities presumed to mimic an in vivo infection, the viral pp71 protein, which is delivered from the tegument to the nucleus of infected cells (Hensel et al. 1996), is required for immediate early gene expression and subsequent viral replication (Bresnahan and Shenk 2000). pp71 binds to Daxx through two Daxx-interaction-domains, termed DIDs (Hoffman et al. 2002), and through this interaction partially and transiently localizes to PML-NBs (Hoffman et al. 2002; Marshall et al. 2002; Ishov et al. 2002). Recombinant HCMVs expressing DID-mutant pp71 proteins (and not wild type) have the same phenotype as the pp71-null mutant, indicating that pp71 binding to Daxx is required for efficient viral IE gene expression (Cantrell and Bresnahan 2005). This series of experiments was important because it defined the role of a single function of the multifunctional pp71 protein during viral infection by examining the phenotype of a recombinant virus expressing a mutant pp71 protein, and because it identified Daxx as a critical determinant of HCMV IE gene expression.

The finding that the ability of pp71 to bind Daxx was required for viral IE gene expression at the start of an HCMV infection did not distinguish between two disparate models for pp71 action: the cooperation of pp71 and Daxx to activate IE gene expression (expected if PML-NBs were pro-virus) or the relief of Daxx-mediated repression by pp71 (expected if PML-NBs were anti-virus). The subsequent observation that Daxx levels are dramatically reduced after HCMV infection due to pp71-mediated proteasomal degradation (Saffert and Kalejta 2006) strongly implicated Daxx as a repressor of HCMV IE gene expression and PML-NBs as anti-virus. Multiple approaches by several laboratories have confirmed this. Inhibition of Daxx degradation by a proteasome inhibitor (Saffert and Kalejta 2006) or overexpression of Daxx (Cantrell and Bresnahan 2006; Woodhall et al. 2006) inhibit IE gene expression in HCMV-infected cells, and knockdown of Daxx by RNA interference (Cantrell and Bresnahan 2006; Preston and Nicholl 2006; Saffert and Kalejta 2006; Woodhall et al. 2006) enhances IE gene expression in HCMV-infected cells, especially when pp71 activity is absent or inhibited.

Exactly how Daxx inhibits IE gene expression is still unclear. Daxx is not known to bind directly to DNA, but is recruited to promoters by DNA-binding transcription factors (Salomoni and Khelifi 2006). The cellular factors that mediate the association of Daxx with infecting HCMV genomes and the viral sequences required to recruit Daxx need to be identified. Also, while it is clear that Daxx binds to histone deacetylases (HDACs) and that Daxx-mediated antiviral effects against HCMV requires HDAC activity (Saffert and Kalejta 2006; Woodhall et al. 2006), the specific HDAC or HDACs utilized by Daxx to silence HCMV IE gene expression have not been identified. Also, as chromatin structure plays an important role in HCMV gene expression, the process of chromatin assembly on infecting viral genomes should be explored. In the virion, viral DNA is not associated with histones, but becomes rapidly bound by histones after entering the nucleus (Woodhall et al. 2006). Determining when and how viral genomes acquire a chromatin structure could reveal further insights into the regulation of IE gene expression. For example, chromatin structure may explain why only the viral genomes located at PML-NBs appear to be transcribed (Ishov et al. 1997). Perhaps only a subset of infecting viral genomes becomes properly chromatinized, and only those can be transcribed. By sending PML-NB proteins only to infecting viral genomes competent for transcription, the cell may focus these valuable resources where they are most needed.

Additional tegument proteins may cooperate directly or indirectly with pp71 to stimulate IE gene expression. The pUL35 protein interacts with pp71 (Schierling et al. 2004), and UL35-null viruses have delayed IE gene expression and dramatically reduced production of the early UL44 protein (Schierling et al. 2005), perhaps indicating that while pUL35 has a modest effect on IE gene expression, it may have a much more significant effect on early gene expression. Interestingly, an interaction between pUL35 and pp71 may play a prominent role during viral egress. In cells infected with the UL35-null virus, pp71 (and pp65) remain in the nucleus at late times during infection and do not enter the cytoplasm with egressing capsids

(Schierling et al. 2005). Other assembly/egress defects were also noted. Thus, pUL35 may control the incorporation of other viral proteins (such as pp71) into the tegument. Lower levels of pp71 in UL35-null virions could explain the delay in IE gene expression observed after infection with the mutant virus. A quantitative comparison of tegument proteins incorporated into wild type and UL35-null virions is thus an essential experiment. As pUL35 is only a minor component of the tegument (Varnum et al. 2004), it may have a catalytic (as opposed to stoichiometric) role in tegument assembly, perhaps by influencing nucleocytoplasmic transport pathways, as has been hypothesized (Schierling et al. 2005).

pUL26 is also a minor component of virions (Varnum et al. 2004) that is required for efficient viral replication (Dunn et al. 2003; Yu et al. 2003; Lorz et al. 2006; Munger et al. 2006) and may have an indirect role in the activation of IE gene expression by pp71. In the absence of pUL26, the phosphorylation and stability of at least one tegument protein (Munger et al. 2006) and the stability of virions themselves (Lorz et al. 2006) is reduced. Thus pUL26 appears to play a role in tegument assembly/disassembly, and/or tegument protein/virion stability. How pUL26 may modulate the functions of tegument proteins that act at postfusion, preimmediate early times such as pUL47, pUL48, and pp71 remains to be determined.

In addition to Daxx, the PML protein itself also inhibits HCMV IE gene expression (Tavalai et al. 2006). However, the newly synthesized IE1 protein disrupts PML-NBs (Korioth et al. 1996) and neutralizes the repressive effects of PML and perhaps other PML-NB proteins (Tavalai et al. 2006). Interesting points for further study include determining if PML can be recruited to infecting HCMV genomes in the absence of Daxx, and if Sp100 or any other PML-NB protein also represses HCMV gene expression. We know that at least two PML-NB proteins (Daxx and PML) can repress HCMV IE gene expression, that these structures are sequentially dismantled during HCMV infection, and that HCMV replicates to higher titers in the absence of at least two PML-NB proteins (Daxx and PML), which strongly argues that PML-NBs are not preferred sites of viral transcription and replication, but that the proteins that localize to these structures have antiviral functions. Because these proteins are constitutively expressed, they have been characterized as mediators of intrinsic immunity against HCMV (Saffert and Kalejta 2006; Tavalai et al. 2006), analogous in their effects to retroviral restriction factors (Bieniasz 2004).

Model for Postfusion, Preimmediate Early Events

This section describes an overly simplified model for postfusion, preimmediate early events during HCMV lytic infection of fully permissive fibroblasts (Fig. 1). The model is based on experiments with HCMV, data from other herpesviruses, and a certain amount of speculation on the part of the author. It is not meant as a comprehensive, definitive picture, but as a working model that needs to be refined and built upon.

Figure 1 illustrates that signals initiated on receptor binding may prime the cell for subsequent events during viral entry (1). Mediated by tightly associated tegument proteins such as pUL47, pUL48 and perhaps pp150, capsids are transported along microtubules (MTs) toward the nucleus (2). Cellular motor proteins such as dynein likely assist this transport. Other tegument proteins not directly associated with capsids (such as pp65 and pp71) are transported independently to the nucleus (3). Through unknown mechanisms, capsids dissociate from MTs, dock at nuclear pores, and release their DNA into the nucleus (4). Viral genomes associate with cellular histones (H; 5) and are packaged into chromatin. The Daxx protein, which rapidly dissociates from and reassociates with PML-NBs (6) interacts, in an uncharacterized way, with the viral genome (7), presumably at the major immediate early promoter (MIEP) and perhaps other IE promoters as well. Daxx recruits an HDAC and silences viral gene expression (8) by establishing a repressive chromatin structure. Other PML-NB components are also recruited and participate in the silencing of viral gene expression (8). pp71 binds to Daxx in the newly formed PML-NBs that are silencing infecting viral genomes (9) and induces Daxx degradation (10), thus de-repressing viral IE gene expression (11). The viral IE1 gene product subsequently dismantles PML-NBs and neutralizes the repressive effects of one or more of the proteins that localize to these structures (not shown).

Perspectives

Future work should focus on identifying how, where and when during entry the tegument disassembles, the process of histone association with viral genomes, how Daxx and other PML-NB proteins are recruited to viral genomes, how other tegument proteins cooperate with pp71 (and then IE1) to inactivate the cellular defenses mediated by the PML-NB proteins and how signal transduction cascades induced upon viral entry impact on each of these processes.

Additionally, the question as to whether PML-NBs are either pro-virus or anti-virus needs to be answered. Interestingly, recent evidence suggests that the real answer may be that PML-NBs have both negative and positive effects on the HCMV life cycle. While these proteins clearly inhibit lytic replication (and thus are anti-virus), recent evidence suggests that Daxx may be absolutely necessary to silence expression from the viral genome when latency is established and thus avoid an abortive infection in undifferentiated cells where productive lytic replication cannot be completed (Saffert and Kalejta 2007). Thus, for latent HCMV infections, Daxx could be considered to be pro-virus as well. More work is needed to explore the possibility that HCMV uses the same cellular defense to establish latency that it easily and systematically inactivates at the start of lytic infections.

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