Abstract

Virions disassemble during viral entry, they must expertly navigate and manage the complex and unwelcoming environments they encounter in order to successfully infect host cells. Herpesviruses incorporate proteins into their virions in a layer between the capsid and envelope termed the tegument to assist in this hostile takeover. When delivered to infected cells subsequent to membrane fusion, tegument proteins begin to facilitate viral infection after entry but before the immediate-early (IE) gene expression (referred to as the pre-IE stage of infection). Tegument-delivered proteins mediate capsid migration through the cytoplasm to nuclear pore complexes and the transmission of the genome into the nucleus. Furthermore, tegument proteins modulate viral transcription, and help infected cells avoid all three classes of immune function (intrinsic, innate and adaptive). While they are most often studied during lytic infections, a new appreciation for the role that the proper regulation of tegument-delivered protein function may play during viral latency is emerging. Here the pre-IE functions of tegument proteins during both lytic and latent infections are reviewed and analysed.

Introduction

Tegument proteins are those incorporated into herpesvirus virions between the genome-containing capsid and the glycoprotein-containing envelope. They are deposited into the cytoplasm of the infected cell upon fusion of the viral and cellular membranes, and as such are the very first viral proteins that act intracellularly during viral infections. As discussed below, they have important activities in priming cells for viral gene expression during a productive, lytic infection (a function that must be suppressed to allow for the establishment of latency) and in helping the infected cell avoid immune surveillance.

Herpesviruses are prominent human pathogens, with human cytomegalovirus (HCMV) likely the most ubiquitous. Primary HCMV infection of the unborn causes birth defects, and reactivations of latent HCMV causes severe disease in immunocompromised or immunosuppressed populations, such as AIDS, cancer, and transplant patients (Mocarski et al., 2007). HCMV infection is also associated with chronic conditions such as immunosenescence (Pawelec and Derhovanessian, 2011), atherosclerosis, and restenosis (Caposio et al., 2011). The virus is also present in glioblastoma multiforme tumours (Ranganathan et al., 2012; Sorceanu and Cobbs, 2011), though the consequences of these infections for tumour biology remain unclear. In addition to the productive, lytic infection in which high levels of the majority of HCMV proteins are synthesized and infectious progeny virions are assembled and released, HCMV also establishes persistent and latent infections. Persistent infections are productive, however lower levels of proteins and infectious particles are produced, and the infected cell survives significantly longer than a lytically infected cell (Britt, 2008). During latency viral lytic gene expression, specifically the immediate-early (IE) genes, must be silenced, and the genome is maintained over time without the production of infectious virions (Sinclair, 2010). In response to certain stimuli, latent virus can reactivate and complete a productive, lytic infection.

This chapter briefly covers the genesis and organization of the tegument, but focuses on the biological effects of tegument proteins prior to the onset of viral gene expression. As the first viral genes to be expressed are the IE genes, the time period before their synthesis is called the pre-immediate-early (pre-IE) stage of infection. Please note that in addition to the pre-IE functions of the tegument-delivered proteins discussed below, de novo expressed tegument proteins also play substantial roles throughout all phases of a productive HCMV infection. For those seeking further information, readers are directed to a previous review of this topic (Kalejta, 2008a) as well as to a more thorough review of tegument protein
function throughout the course of viral infection (Kalejta, 2008b).

**Tegument composition**

Approximately 35 viral proteins are consistently found in HCMV teguments (Kalejta, 2008b). Many are phosphorylated (and named for their apparent molecular mass with a ‘pp’ prefix that stands for phosphoprotein) although for most, any significance of their phosphorylation status to their function is unknown. Tegument proteins act throughout the infectious cycle, including during entry, gene expression, immune evasion, assembly, and egress. Along with viral proteins, cellular proteins and viral and cellular RNAs are also packaged within virions.

**Tegument assembly**

Mechanisms through which tegument proteins are packaged into virions are poorly understood. Bioinformatic and experimental searches for short, linear amino acid motifs that could mediate tegument localization, akin to a nuclear localization signal, have failed to reveal a universal packaging sequence. Sporadic examples of specific tegument proteins being underrepresented in the virions of recombinant viruses lacking a different tegument protein have been reported, but they fail to illuminate any conserved mechanism of tegument protein incorporation, calling into question whether or not such a mechanism exists. Interestingly, recent evidence appears to indicate that independent derivatives of the same viral strain (i.e., different isolates of AD169) can have substantially different tegument protein compositions (Reyda et al., 2011) further confounding issues dealing with tegument assembly.

In the absence of mechanistic data addressing tegument assembly, a stepwise protein–protein interaction cascade is the current working model for tegumentation. This model is based on the observations that some tegument proteins are nuclear while others are cytoplasmic, and that capsids form in the nucleus but are enveloped with their final membrane in the cytoplasm. In attempts to formulate a more defined model, high-throughput tegument protein interaction yeast two-hybrid screens have been employed to identify those tegument proteins that interact with each other, and those that interact with capsids or envelope glycoproteins (Phillips and Bresnahan, 2011; To et al., 2011). The two screens detected a core set of overlapping interactions, as well as unique binding pairs found in one screen but not the other. Both screens confirmed binary interactions between pp71, pp150, UL35 and UL94. It will be interesting to see if these proteins form a higher order complex that might be essential for tegument assembly or for proper tegument protein function. One screen (To et al., 2011) detected a substantial number of interactions between UL24, UL25, UL89 and other virion proteins, suggesting that these three proteins may serve as organizing centres during tegument assembly. However, both UL24 and UL25 are non-essential for viral replication in vitro (Dupuy et al., 2003; Yu et al., 2003) making them unlikely to be significant tegument organizers. Likewise, the UL89 protein that is part of the terminase complex required for genome cleavage and packaging is present in vanishingly small quantities within the tegument (Varnum et al., 2004) and thus unlikely to play a significant role in tegument organization. In summary these interaction screens have provided a wealth of data to fuel and inspire future research efforts, but by themselves do not provide any specific insight into the process of tegumentation.

**Tegument structure**

Visualization by cryo-electron tomography (cryoET) and biochemical fractionation has differentiated two general classes of tegument proteins, those that are densely packed and tightly associated with the capsid, and those that are loosely packed and not detectably associated with capsids (Yu et al., 2011). The tegument proteins found strongly associated with capsids were pp150 (UL32) and UL48, with UL47 also substantially capsid-associated, but apparently not as tightly bound as pp150 or UL48. CryoET experiments combined with pp150 antibody labelling detected at least some structurally ordered pp150 molecules directly adjacent to the capsid. Thus it appears that tegument proteins involved in capsid stability and movement (see below) are strongly capsid associated, whereas those that have other cytoplasmic and nuclear functions (see below) are weakly or not at all associated with capsids.

**Tegument disassembly**

Just as the tegument must assemble upon virion egress, it must also disassemble upon viral entry. How the tegument disassembles after entry is unknown, as is whether or not this process is similar in instances where HCMV enters by membrane fusion or by endocytosis (Ryckman et al., 2006). Specific proteins such as pp150, UL96 (Tandon and Mocarski, 2011) and UL26 (Munger et al., 2006) that appear to regulate virion stability may, in part, function through effects on the tegument, and thus may impact tegument disassembly. One clear observation is that upon infection of fibroblasts, some tegument proteins such as pp150 remain capsid associated and pp65 and pp72 tegument-delivered upon infection and Kalejta, Z.E. Albright (2007) infected cells where infections, re localization of defect in traffic components o appearance remain apparent.
associated and cytoplasmic, whereas others, such as pp65 and pp71 rapidly enter the nucleus. Interestingly, tegument-delivered pp65 and pp71 fail to enter nuclei upon infection of uninfected NT2 cells (Penkert and Kaleda, 2010) and CD34+ cells (Saffert et al., 2010; E.R. Albright and R.E. Kaleda, unpublished observations) where HCMV establishes quiescent or latent infections, respectively. Whether this cytoplasmic localization of normally nuclear proteins represents a defect in trafficking of these two individual tegument components or a more global defect in tegument disassembly remains to be determined.

Capsid delivery to nuclear pores
Membrane fusion deposits the genome-containing capsid into the cytoplasm, where it along with closely associated tegument proteins, makes its way along cellular microtubules to nuclear pore complexes (Ogawa-Goto et al., 2003; Kaleda, 2008b), allowing for entry of the viral genome into the nucleus during the initiation of a lytic infection (Fig. 1.9.1). If microtubules promote genome delivery to the nucleus during the establishment of latency, or if tegument proteins promote HCMV capsid movement during the entry process in any cell type has not been directly studied. However, during herpes simplex virus type 1 (HSV-1) lytic infections, the VP1/2 (UL36) tegument protein is responsible for trafficking capsids along microtubules and mediating the release of genomes into the nucleus (Roberts et al., 2009; Abaitua et al., 2011).

The HCMV orthologue of VP1/2 is the UL48 protein. UL48 is a deubiquitinating protease (Wang et al., 2006) with both ubiquitin-specific carboxy-terminal hydrolase and isopeptidase activity (Kim et al., 2009). Catalytic site mutants show only mild growth phenotypes at low multiplicities of infection (Wang et al., 2006; Kim et al., 2009), so the significance of the deubiquitinating activity of this protein for viral replication is not currently appreciated. The protein itself however is critical for viral infection, as shown by independent mutational analysis (Dunn et al., 2003; Yu et al., 2003).

UL48 interacts with another tegument protein, UL47, as well as with the major capsid protein (Bechtel and Shenk, 2002). UL47-null mutant viruses replicate 100-fold less than wild type viruses and show delays in viral IE gene expression, implying a role for this protein during the pre-IE stage of virus infection. However, UL47-null viruses contain less UL48 within their teguments (Bechtel and Shenk, 2002), so it is premature to assign a pre-IE function to UL47 based on these experiments. Interestingly, recent studies with HSV-1 argue that only the UL48 orthologue (HSV-1 VP1/2, UL36), and not the UL47 orthologue (HSV-1 UL37) is required for capsid trafficking to nuclei during the entry process (Roberts et al., 2009). Further experimentation is required to determine if the same holds true for HCMV, and if UL47 and/or UL48 play roles during the assembly and egress of infectious HCMV virions.

Recently, the UL77 protein has been implicated in pre-IE events. UL77 is orthologous to the HSV-1 UL25 protein, overexpression of which impairs HSV-1 IE gene expression without disrupting the targeting of incoming capsids to the nuclear pores (Rode et al., 2011). Fewer HSV-1 genomes were found in the nuclei of cells overexpressing UL25 as compared to control cells, leading to the speculation that UL25 plays some role in the efficient delivery of viral genomes to the nucleus. Quantitative immunofluorescence studies concluded that overexpression of UL77 in HSV-1 infected cells reduced ICP8 expression, although the magnitude of inhibition appeared to be minor. In this experiment, effects on nuclear pore docking or genome delivery to the nucleus were not analysed (Rode et al., 2011).
Nevertheless, it is likely that HCMV UL77 participates in the efficient nuclear delivery of infecting viral genomes. Little is more known about UL77 (Kalejta, 2008b) except that it is an essential gene.

**Activation of IE gene expression**

Upon entry into the nucleus, the HCMV genome is immediately silenced by a cellular intrinsic immune defence (Fig. 1.9.2A) mediated in large part by proteins that localize to PML nuclear bodies (Kalejta, 2008a; Tavalai and Stamminger, 2011). Intrinsic immune defences (Bieniasz, 2004; Neil and Bieniasz, 2009) are mediated by constitutively expressed proteins, and were originally discovered as retroviral restriction factors. HCMV was the first DNA virus found to be subject to intrinsic immunity (Saffert and Kalejta, 2006), but now it is appreciated that other DNA viruses are also controlled by such defences (Tavalai and Stamminger, 2008).

HCMV DNA is bereft of histones in virions, but becomes rapidly chromatinized upon entry into the nucleus (Neveils et al., 2011). Initially, the histones associated with incoming viral genomes bear post-translational modifications consistent with transcriptionally inactive heterochromatin (Sinclair, 2010; Reeves, 2011). The cellular intrinsic defence against HCMV plays a role in the formation of this repressive chromatin structure (Woodhall et al., 2006). However, an open chromatin structure indicative of active transcription soon replaces the initial repressive histone markings, and the tegument protein pp71 is responsible for initiating the transactivation of pp71-deficient virus), the histones associated with viral genomes retain het al., 2006), (Centrella et al., 2006). By c intrinsic IE gene ex important IE gene ex: immune (Zydek et al., 2011).

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retain heterochromatic markings (Woodhall et al., 2006), and viral IE gene expression is silenced (Cantrell and Bresnahan, 2006; Saffert and Kalejta, 2009). By degrading Daxx, pp71 inactivates a cellular intrinsic defence that would otherwise prevent viral IE gene expression and inhibit viral replication. It is important to note that, in addition to Daxx, HCMV IE gene expression is also inhibited by other intrinsic immune (Adler et al., 2011) and cell cycle-related (Zydek et al., 2011) mechanisms that do not appear to be targets of tegument-delivered proteins.

The mechanism through which pp71 induces protein degradation is unknown, but has been characterized as proteasome-dependent yet ubiquitin-independent (Kalejta and Shenk, 2003; Hwang and Kalejta, 2007). While pp71 induces the covalent addition of the small ubiquitin-like modifier (SUMO) protein to Daxx, this does not appear to be required for pp71-mediated Daxx degradation (Hwang and Kalejta, 2009). Recently, the IRS regulatory particle, a prominent proteasome activator, has been shown to be required for pp71-mediated protein degradation (Winkler and Kalejta, 2011), a unique finding among the characterized examples of virus protein-mediated proteasome-dependent, ubiquitin-independent protein degradation (Hwang et al., 2011a).

In addition to Daxx, pp71 also induces the degradation of all three members of the retinoblastoma (Rb) family of tumour suppressors (Kalejta et al., 2003; Hume et al., 2008), as well as the BclAF1 protein (Lee et al., 2012). Furthermore, pp71 disrupts the association of ATRX with Daxx (Lukashchuk et al., 2008). While Rb family member degradation by HCMV does not appear to modulate IE gene expression nor be required for efficient HCMV replication in fibroblasts in vitro (Cantrell and Bresnahan, 2005), BclAF1 degradation and ATRX dispersal by pp71 do stimulate IE gene expression (Fig. 1.9.B).

Both pp71 function and IE gene expression appear to be modulated by the viral UL35 proteins (Kalejta, 2008b). The UL35 gene is expressed as two isoforms, UL35 and UL35a (Liu and Biegalke, 2002), both of which interact with pp71 (Schierling et al., 2004), but likely modulate its activity in different ways. The full-length protein (UL35) forms dot-like structures within nuclei of transfected cells that recruit co-transfected pp71 and the cellular PML, Sp100 and Daxx proteins (Salsman et al., 2011). In contrast, transfected UL35a (which consists of the C-terminal 193 amino acids of UL35) redirects co-transfected pp71 from the nucleus to the cytoplasm, which may be important during virion egress for the proper incorporation of pp71 into the tegument (Schierling et al., 2005). UL35a is not a tegument protein (Liu and Biegalke, 2002), and UL35 is only found in the tegument in very small quantities (Varanum et al., 2004). Thus, how UL35 might affect the pre-IE stage of HCMV infection is currently unclear. However, recent experiments indicate that UL35 is required for the ability of pp71 to efficiently degrade the cellular BclAF1 protein (Fig. 1.9.B) that acts as a restriction factor to inhibit IE gene expression (Lee et al., 2012). The mechanisms of pp71 and UL35 cooperation and the BclAF1-mediated repression of IE gene expression are unknown. Interestingly, pp71-mediated degradation of Daxx does not require UL35, indicating that pp71 has both UL35-dependent and -independent functions. Likewise, it is possible that UL35 has pp71-independent functions in addition to its role in the pp71-dependent degradation of BclAF1.

While pp71-dependent processes clearly de-repress the IE genes, other tegument proteins also appear to activate them (Fig. 1.9.C). The pp65 protein was recently shown to interact with the cellular IFI16 protein and recruit it to the major immediate-early locus where these proteins stimulated IE gene expression through an unknown mechanism (Cristea et al., 2010). Knockdown of IFI16 inhibited IE gene expression and productive viral replication. Interestingly, IFI16 was also recently identified as an innate DNA sensor that induces the production of interferon-β, an antiviral cytokine (Unterholzer et al., 2010). As IFI16 is required for efficient IE gene expression and replication of not only HCMV (Cristea et al., 2010) but also murine CMV (Hertel et al., 1999; Rolle et al., 2001), it appears that the positive effects of this protein may be more important for cytomegaloviruses than its presumptive negative effects as a DNA sensor and interferon inducer.

Another tegument protein, UL29/28, also activates immediate-early gene expression (Fig. 1.9.C). Exons produced from the UL29 and UL28 genes are spliced together in a transcript that is translated to generate a protein termed UL29/28 (Mitchell et al., 2009). This protein interacts with the nucleosome remodelling and deacetylase (NuRD) complex (Terhune et al., 2010). UL29/28 and NuRD complex components are found at the MIEP, and the loss of either UL29/28 or a functional NuRD complex inhibits IE gene expression. As the HDAC inhibitor trichostatin A (TSA) fails to rescue the growth defect of a UL29/28 null virus, it appears that this viral protein activates IE gene expression through an HDAC-independent mechanism (Terhune et al., 2010).

Finally, while much of the regulation of IE protein production occurs at the level of transcriptional activation, later steps can also be modulated. For example, it is likely that enhancement of transcriptional
elongation, probably through histone monoubiquitination by an Elongin B-containing ubiquitin ligase complex, also plays a role in IE gene expression during HCMV infection (Hwang et al., 2011b). Furthermore, although most of the viral IE transcripts are spliced, the U63 mRNA is not (Mocarski et al., 2007), indicating perhaps that alterations to nuclear mRNA export might also facilitate the expression of at least this IE gene. To date, there have been no reports of translation control of IE gene expression. Whether or not tegument-delivered proteins regulate IE mRNA elongation and export during viral infection remains to be determined. However, pp71 has been shown to interact with the Elongin B protein (R.F. Kalejta, unpublished observations) that appears to promote transcriptional elongation of at least one HCMV late gene (Hwang et al., 2011b). Furthermore, the UL69 tegument protein that binds RNA and shuttles between the nucleus and the cytoplasm may (Zielke et al., 2011) or may not (Kronemann et al., 2010) play a critical role during HCMV infection by facilitating the export of unspliced HCMV messenger RNAs.

Immune evasion
Avoiding detection by host immune defences is another likely activity of tegument proteins, with several having demonstrated immune-evasive activities. However, very little evidence exists to indicate whether or not virion-delivered (as opposed to de novo expressed) tegument proteins actually perform these functions at pre-IE times. Regardless, the known roles that tegument proteins play in preventing the infected cell from being detected and killed by host immune functions are listed below.

Host immunity is divided into intrinsic, innate, and adaptive/acquired branches. Intrinsic immunity is mediated by constitutively expressed proteins. As discussed above, it is clear that during lytic infection, tegument-delivered pp71 (and likely tegument-delivered UL35) inactivates intrinsic defences that would otherwise silence viral IE gene expression (Figs. 1.9.2B and 1.9.3A). Innate immunity is a rapid and varied response to viral infection. Multiple tegument protein domains can thwart individual innate immune functions during lytic infection (Fig. 1.9.3A). The viral proteins UL35 (McCormick et al., 2010) and UL38 (Xuan et al., 2009) inhibit apoptosis, which can be considered an arm of the innate immune system. The pp65 protein blocks natural killer cell-mediated cytotoxicity by interacting with the NKP30 activating receptor (Arnon et al., 2005), and has been reported to diminish the interferon response to HCMV infection (Brown and Shenk, 2003; Abate et al., 2004), although that result has been challenged (Taylor and Bresnahan, 2006). Furthermore, two similar proteins, IRS1 and TRS1, bind double stranded RNA and inhibit the ability of PKR to shut down translation in HCMV infected cells (Marshall et al., 2009). Finally, pp65 may modulate adaptive immune recognition of lytically infected cells (Fig. 1.9.3A) by causing the degradation of the HLA-DR alpha chain by mediating the accumulation of HLA class II molecules in the lysosome (Odeberg et al., 2003). The biological significance of the reported ability of pp65 to block the presentation of IE peptides through MHC class I molecules (Gilbert et al., 1996), debatable due to the strong IE-protein specific immune response mounted by infected individuals.

Tegument proteins and latency
Tegument proteins are also delivered to cells that will establish a latent infection (Penkert and Kalejta, 2011). Such cells are less differentiated (often referred to as 'undifferentiated') than the fibroblasts where lytic infection is most often studied. Several undifferentiated cell types are used to study quiescent (e.g. NT2 and THP-1 cells) and latent (e.g. CD34+ cells) infections. Quiescent and latent infections are similar in all aspects examined to date except for one important difference, namely that quiescent infections have as yet been shown to amenable (i.e. to initiate IE gene expression) or reactivate (i.e. to produce infectious progeny virions) efficiently in response to the proper stimulus. This separates them from true latent infections, for which animation and reactivation have been unequivocally demonstrated. The pre-IE stages of lytic and quiescent/latent infections have conserverved similarities and important differences. While the process of viral entry and the delivery of viral genomes to the nucleus has not been studied during latency, it is expected that these events occur similarly as they do during lytic infection. For example, the efficiency of viral genome delivery to the nucleus of THP-1 cells where a quiescent infection is established was found to be similar to that in THP-1 derived macrophage where a lytic infection is initiated (Saffert and Kalejta, 2007). However, the next steps, de-repression and activation (Fig. 1.9.2B,C) of IE gene expression do not take place in latently infected cells due to alternative subcellular localization of key tegument-delivered proteins.

Upon entry into NT2, THP-1, or CD34+ cells, tegument-delivered pp71 and pp65 remain in the cytoplasm and do not enter the nucleus (Saffert and Kalejta, 2007; Saffert et al., 2010; E.R. Albright and R.F. Kalejta, unpublished observations). In these cells, Daxx is not degraded and thus the intrinsic defence is not inactivated but allowed to silence viral IE gene expression (Fig. 1.9.3A). RNA interference a small molecule, (Saffert and Kalejta, 2007) has been examined as a possible effect of AT. Although it has been shown to play different roles fibroblasts. The proteins in the list are established in the Daxx-mediated prevention of the activity while it is likely that the proteins (e.g. UL123) upon cytoplasm has not yet been
expression (Fig. 1.9.4). If DaXX levels are depleted with RNA interference, or if HDAC activity is inhibited with a small molecule, viral IE gene expression is initiated (Saffert and Kalejta 2007; Saffert et al., 2010). Repressive effects of ATRX, BclAF1, PML or Sp100 have not been examined during quiescent/latent infections (Fig. 1.9.4), although there is no reason to suspect that they play different roles in these cell types than they do in fibroblasts. The sequestering of tegument-delivered proteins in the cytoplasm of cells in which latency will be established not only inhibits the deactivation of the DaXX-mediated intrinsic defence, but should also prevent the activation of IE gene expression by pp65. While it is likely that other key tegument-delivered proteins (e.g. UL35 and UL29/28) also localize to the cytoplasm upon infection of undifferentiated cells, this has not yet been examined.

Mechanisms for the differentiation-dependent subcellular localization of tegument-delivered proteins such as pp71 and pp65 remain unexplained. Interestingly, it appears the cytoplasm is the default localization for tegument-delivered proteins. Heterogeneous (NT2/fibroblast) cell fusion experiments indicate that tegument-delivered proteins found in the cytoplasm of undifferentiated cells can be driven into the nucleus by one or more factors expressed in differentiated cells (Penkert and Kalejta, 2010). Whether this unidentified factor or factors work by modulating tegument protein nuclear trafficking or simply facilitate tegument disassembly remains to be determined. The observation (Saffert and Kalejta, 2007; Saffert et al., 2010) that de novo expressed pp71 and pp65 localize to the nucleus in the same undifferentiated cells where the tegument-delivered proteins remain cytoplasmic is provocative, but does not provide mechanistic details.

Figure I.9.3 Functions of tegument-delivered proteins during the pre-IE stage of lytic infection. A. Flowchart depicting the viral and cellular proteins that execute immune evasion and gene expression functions during the pre-IE stage of a lytic infection. See text and Fig. 1.9.2 for details. B. Flowchart depicting the viral and cellular proteins that execute immune evasion and gene expression functions during the establishment of latency. Grey text indicates expected but unconfirmed protein functional roles. Question marks indicate processes that have yet to be examined. See text and Fig. 1.9.4 for details.
Figure I.9.4 IE gene de-repressing and activating tegument proteins are inactive during the establishment of latency due to their cytoplasmic localization. During the establishment of latency, tegument proteins that de-repress (pp71) or activate (pp65) IE gene expression are found in the cytoplasm and are thus unable to perform their normal activities. Other tegument proteins with similar functions may also be cytoplasmic, but their localization in latently infected cells has yet to be examined (depicted as blurred grey symbols to indicate expected but untested location). This allows the Daax mediated intrinsic defence to silence IE gene expression. Other intrinsic defence proteins may also participate in this silencing, although their activities during latency have not been tested (depicted as blurred grey symbols to indicate expected but untested activity). Furthermore, a clinical strain-specific, HDAC-independent trans-dominant inhibition of IE gene expression (X) is also observed during the establishment of latency.

activities are muted during the establishment of latency by their cytoplasmic localization, an HDAC-independent, trans-dominant inhibition of IE gene expression (Fig. I.9.4) is present in cells infected with clinical viral strains, but not with the laboratory-adapted AD169 strain (Saffert et al., 2010). The identity and mechanism of this clinical strain-specific restriction to IE gene expression during latency (that exists in addition to the host-mediated intrinsic defence that also silences IE gene expression) is not known, but could conceivably be mediated by a tegument-delivered protein. It is also unclear whether or not tegument proteins participate in the animation and/or reactivation of latent HCMV genomes. Depending on how long the genome remains latent, it might seem unlikely that tegument proteins delivered during viral entry would remain functional to allow their participation in these later processes. However, recent evidence indicates that proteins commonly incorporated into virion teguments could be produced at some from latent genomes, and as such might initiate latent genome animation as the critical first step leading to productive reactivations (Penkert and Kalejsa, 2011).

Finally, the potential immune evasion roles of tegument-delivered proteins during latency have generally not been examined (Fig. I.9.3B). One exception, of course, is the intrinsic defence that is not inactivated during latency because tegument-delivered pp71 remains cytoplasmic. Conceivably the ability of UL36 and UL38 to block apoptosis, IRIS1 and TRS1 to promote translation, and pp65 to interact with the NKP30 activating receptor would remain in undifferentiated cells. Thus, tegument-delivered proteins could in fact inhibit some innate immune functions during latency. Whether or not pp65 could dampen the interferon response is questionable, but it might possibly modulate antigen presentation from the cytoplasm.

Conclusion
Activities and roles of individual tegument proteins at pre-IE times are coming into focus (Fig. I.9.3). They play roles in delivery of the capsid to the nuclear pores and genomes into the nucleus (Fig. I.9.1), de-repression and activation (Fig. I.9.2) of IE gene expression, and likely immune evasion as well. While information about binary tegument protein interactions has increased, mechanistic information about tegument assembly or disassembly is lacking. Finally, the previously unexplored roles of tegument proteins during the early event highlighted (Fig. I.9.2) were not considered in this chapter. More mechanistic studies are required.

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References
Abaitu, F., Dalio, O' Hare, P., (2011) temperature-sens v the VP-1-2 prote 2024-2036.
Abati, D.A., Watanabe human cytomeg (ppUL83) proves in the interferon.
during the early events of latency are beginning to be highlighted (Fig. 1.9.4). Interventions that could modulate tegument protein function at the beginning of lytic replication, latency, or animation/reactivation could prevent replication cycles at very early (pre-IE) time points, and thus might prove to be effective antivirals. More mechanistic knowledge about how tegument proteins work after entry but prior to viral gene expression will be required to design such treatments.

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References


