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Breach of the nuclear lamina during assembly of herpes simplex viruses

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Abbreviations: INM, inner nuclear membrane; ONM, outer nuclear membrane; HSV, herpes simplex virus; PK, protein kinase; VZV, varicella-zoster virus; PRV, pseudorabies virus; HCMV, human cytomegalovirus; EBV, Epstein-Barr virus; KSHV, Kaposi's sarcoma herpesvirus

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Beneath the inner nuclear membrane lies the dense meshwork of the nuclear lamina, which provides structural support for the nuclear envelope and serves as an important organizing center for a number of nuclear and cytoplasmic constituents and processes. Herpesviruses have a significant and wide-ranging impact on human health, and their capacity to replicate and cause disease includes events that occur in the host cell nucleus. Herpes viruses begin assembly of progeny virus in the nuclei of infected cells and their capsids must escape the confines of the nucleus by traversing the inner nuclear membrane (INM) to proceed with later stages of virion assembly and egress. Access of viral capsids to the INM thus necessitates disruption of the dense nuclear lamina. We review herpesvirus effects on the nuclear lamina and in particular the roles of the herpes simplex virus-encoded nuclear egress complex and viral kinases on phosphorylation and dissociation of lamina components, and nucleocapsid envelopment at the INM.

Viruses are obligate intracellular parasites that must modify the cellular environment and cellular processes to aid their replication. The nuclear lamina poses a major obstacle to survival of viruses that replicate in the nucleus of the cell. Herpesvirus family members, and herpes simplex virus 1 (HSV-1) and HSV-2 in particular, have evolved some similar and unique means to achieve the same fundamental goals of nuclear lamina disruption and nucleocapsid egress essential for virus replication and spread, and ultimately their capacity to cause disease.

Structure and Regulation of the Nuclear Lamina

The double membrane of the mammalian nuclear envelope defines the nuclear rim and separates the nuclear contents from the cytoplasm. The outer nuclear membrane (ONM) is contiguous with the ER membrane and its associated ribosomes. The inner nuclear membrane (INM) surrounds the nucleoplasm and contains integral membrane proteins that secure the nuclear lamina to the nucleoplasmic face of the INM.[1] This lamina comprises a dense meshwork that maintains the architecture of the nuclear envelope, acting as a scaffold for structural support.[2,3] Lamins are type V intermediate filaments that comprise the majority of the nuclear lamina proteins. They consist of several types: Lamins A and C are alternative splice products of the *lmnA* gene.[4,5] B-type lamins, subdivided into lamins B1 and B2, derive from the *lmnB1* and *lmnB2* genes, respectively.[6] As with other intermediate filaments, the structure of nuclear lamins consists of a central alpha-helical rod domain flanked by globular domains at the amino-and carboxy-termini.[5,7] Lamin rod domains intertwine to form dimers that interact with other lamin dimers to form longer filaments.[3] Lamin B filaments assemble in a regular lattice pattern having an average crossover spacing of 15 nm, with lamin A forming irregular bundles beneath.[8] Lamin B associates permanently with the INM and provides essential support for the nucleus.[8] Interactions between lamin B and integral membrane proteins such as the lamin B receptor and emerin tether the meshwork to the INM.[9] Lamins A/C provide additional stiffness to the nuclear envelope and contribute to other

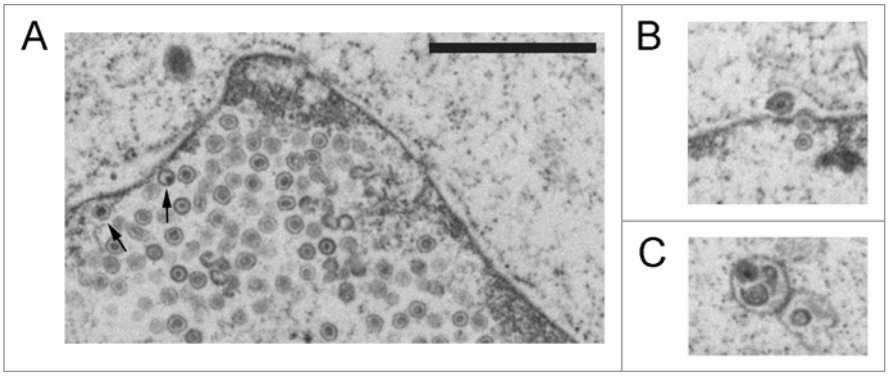

Figure 1. Electron micrographs of steps in herpesvirus egress. (A) Capsids accumulate at the nucleoplasmic face of the INM; arrows indicate DNA-containing C-type capsids. (B) C-type capsid buds through the INM into the perinuclear space, and (C) Trans-Golgi-derived vacuole containing mature enveloped virions moves through the cytoplasm. Black bar represents 1 μm.

specialized functions such as organization of the cytoskeleton and peripheral chromatin, positioning of the nuclear pore complexes, and interaction with certain transcription factors.[3,10] This multitude of interactions implicates the nuclear lamina as a nexus of functional activities within and beyond the nuclear membrane.

To achieve cell division the nuclear lamina must undergo dissolution and reorganization. The lamina disassembles between prophase and metaphase of the cell cycle, and is reconstructed during interphase.[11] Reversible site-specific phosphorylation within the head and tail domains of lamins A/C and B dynamically regulate lamina integrity.[3,11] Protein kinase A (PKA), PKC, MAP kinases and Cdc2 phosphorylate lamins,[12-14] and phosphorylation by Cdc2 of conserved serine residues in the head and tail domains results in lamin depolymerization during mitosis.[12,15] These phosphorylation events interfere with head-to-tail polymerization of lamin dimers, and thus play a critical role in dismantling the otherwise dense lamina.

Herpesvirus Replication and the Problem Posed by the Nuclear Lamina

Herpesviruses are large viruses composed of a capsid surrounding a double-stranded DNA genome, a host cell-derived lipid envelope, and tegument proteins packed between that link the capsid to viral glycoproteins embedded in the envelope.[16] Herpesviruses replicate their genomes and assemble their capsids in the nuclei of infected cells. The large diameter of herpesvirus nucleocapsids (~125 nm) prohibits their exit through standard nuclear pore complexes, which have a typical diameter of ~38 nm.[17-19] Although one group has observed that nuclear pores may dramatically increase in size to 300 nm or more in herpesvirus-infected cells,[20] this does not appear to be an avenue of virus escape.[16,21-23] Instead, a wealth of evidence indicates nucleocapsids undergo an envelopment-deenvelopment-reenvelopment process.[16,18,24] Primary envelopment occurs when capsids bud into and become swathed in the INM.[16,18,19] Among capsids accumulating at the nucleoplasmic face of the INM, those containing viral DNA (C-type capsids) preferentially undergo envelopment (**Fig. 1A**), transit across the perinuclear space (**Fig. 1B**), and lose their envelope upon fusion with the ONM. Capsids acquire some tegument proteins in the nucleus and certain glycoproteins (such as gB and gH) during primary envelopment. Numerous other tegument proteins are added to capsids in the cytoplasm.[18] Reenvelopment is thought to occur as cytoplasmic capsids and associated tegument proteins bud into membranes of the transGolgi containing mature viral glycoproteins (**Fig. 1C**). Fusion of exocytic vesicles with the plasma membrane results in release of mature virions. For more extensive reviews of herpesvirus assembly and egress, see references 16, 18 and 19. To escape from the nucleus through the nuclear envelope, herpesvirus nucleocapsids must gain access to the INM; however, because of the tight spacing in the lamina matrix the nuclear lamina represents a formidable obstacle to nuclear egress of herpesvirus capsids and ultimately maturation and egress of virions from the infected cell.

Nuclear Egress of Herpes Simplex Virus Capsids

Herpesviruses are a family of viruses related more closely by structure and replication strategy than pathogenesis, although all herpesviruses share the capacity to establish latency in the nuclei of host cells.[25] Viruses from this family cause a wide variety of human and animal diseases, from skin rashes to severe neurological infections and certain cancers. Alphaherpesvirus subfamily members include herpes simplex virus (HSV) 1 and 2, as well as varicella-zoster virus (VZV) and the porcine pseudorabies virus (PRV). Human cytomegalovirus (HCMV), and Epstein Barr virus (EBV) and Kaposi's sarcoma herpesvirus (KSHV) are the archetypal members of the beta- and gammaherpesvirus subfamilies, respectively. Alphaherpesviruses target neurons as their site of latency, during which the viral genome exists as an episome in the nucleus of sensory neurons. Reactivation to active infection is particularly interesting because it requires transit of viral capsids and other virion components over the vast distance between the neuronal nucleus and the terminal axon membrane. Maintenance of the nuclear envelope may be especially important for egress from neurons because travel from the cell body to the axon terminus requires both extraordinary time and functional cytoskeletal machinery linked to the nuclear envelope. The remainder of this review will focus on the human alphaherpesviruses, primarily HSV-1 and HSV-2.

Complete dismantling of the nuclear matrix does not occur during alphaherpesvirus infection; however, infection appears to induce partial lamin disassembly or reorganization at the nuclear periphery.[24,26-31] HSV infections do not result in gross loss of lamins A and C. Rather, local disruptions of the lamina occur, accompanied by distortion of the nuclear membrane.[27,32-35] HSV-1 and HSV-2 infections

Table 1. Properties of the human alphaherpesvirus kinases related to nuclear egress

Virus	Kinase	Effect of mutation on virus replication	Potential substrates[a]	Direct substrates[b]	Effect of mutation on nuclear egress
HSV-1	pUS3	Replication reduced ~10-fold in Vero cells[63,65]	emerin,[60] matrin 3[75]	pUL31,[52,65] pUL34,[52,76] gB,[67] lamin A/C[32]	Alters localization of pUL31 and pUL34,[42,63,65,66] lamins,[32,34] emerin[36] and matrin 3.[75] Defect in virion maturation and egress;[38,42,63,64] nucleocapsids accumulate in the perinuclear space[38,63] implying a role in fusion of primary enveloped virions with the ONM.[65]
HSV-2	pUS3	No effect on virus yield in Vero cells[69,77]	emerin,[61]		No effect on localization of pUL31 and pUL34, or efficient nuclear egress of nucleocapsids[69]
VZV	ORF66	No effect in MeWo cells; growth in human T cells reduced[78]	matrin 3[75]		unknown
HSV-1	pUL13	~5-fold reduction in virus yield in Vero cells[72-74]		pUS3[66]	Alters localization of pUL31 and pUL34[66]
HSV-2	pUL13	Severely attenuated in Vero cells(unpublished observation)		lamins A/C and B1[33]	Impairs budding through the INM (unpublished observation)
VZV	ORF47	No effect in MeWo cells;[78] growth restricted in human immature DCs,[79] T cells and skin[78,80]			Appears to impair proper envelopment during nuclear egress[80]

[a]Phosphorylation pattern altered in cells infected with kinase-deficient virus. [b]Direct substrates can be directly phosphorylated by the indicated kinase.

appear to induce conformational changes within the head and tail domains of lamins A and C, leading to masking of the epitopes recognized by selected lamin A/C antibodies.[27,33] In HSV-1-infected cells lamin B is disrupted by phosphorylation[36] and mobility of the lamin B receptor increases as it abandons its role in anchoring the nuclear lamina to the INM and translocates to the ER.[26] This partial disassembly of the lamin meshwork preserves the integrity of the nuclear envelope while also allowing nucleocapsids to collect at the INM, interacting with proteins that will ferry them through the nuclear bilayer.[18]

Nuclear egress of HSV capsids requires the participation of two types of viral proteins, a conserved pair associated with the INM which controls primary envelopment, and kinases that mediate phosphorylation and dispersion of nuclear matrix components (**Table 1**). HSV-1 pUL31 and pUL34 localize to the nuclear rim.[37,38] pUL31 is a nuclear phosphoprotein associated with the nuclear matrix,[39] whereas pUL34 is an integral membrane protein that localizes to the INM.[40] In co-dependent fashion, pUL31 and pUL34 form a complex at the nuclear rim variously termed the nuclear egress complex or nuclear envelopment complex (NEC),[16,18,19] that is required for budding of HSV-1 nucleocapsids through the INM,[37,38,40] possibly by bridging the capsid surface and the INM.[41] The NEC interacts with lamin A and is required to induce the discontinuities in the nuclear lamina observed in HSV-1-infected cells.[27,42] However, HSV-1 pUL34 expressed by itself severely disrupts lamins A/C and B; phosphorylation of pUL34 by the viral kinase pUS3 is required to temper this activity.[34] pUL31 and pUL34 of HSV-2,[43,44] and orthologs in other herpesviruses[19,28,45-48] show similar localization and functions in nucleocapsid egress. The HSV-1 NEC is necessary but not sufficient to cause the alterations to lamins A and C that occur during HSV-1 infection, indicating a role for additional viral protein(s) in this process.[27]

HSV-1 and HSV-2 encode two serine/threonine (Ser/Thr) protein kinases, pUS3 and pUL13, known to play roles in nuclear egress. Beta and gammaherpesviruses encode only orthologs of pUL13.[49,50] pUS3 localizes to the nucleus as well as the cytoplasm,[51] and is packaged into the virion.[38] pUS3 of HSV-1 has broad substrate specificity, phosphorylating numerous proteins containing the core kinase recognition motif RRXS/TR[52-54] as well as additional non-consensus sites in lamin A.[32] HSV-1 pUL13 and its orthologs are also a component of the viral tegument,[55] and are postulated to share a Cdc2-like kinase activity,[56,57] suggesting they could supplant the role of cellular Cdc2 in phosphorylating and dispersing nuclear lamina components.[29,31] HSV-1 and HSV-2 pUL13 homologues share 86% amino acid sequence identity.[58] However, HSV-2 pUL13 is unique in that its kinase activity does not mimic Cdc2, at least on peptide substrates.[59]

Roles for Kinase Activity in Nuclear Lamina Dissolution and Nucleocapsid Egress

Whether partial dissociation of the nuclear matrix is a necessary prerequisite for proper localization of the NEC to the INM in HSV-infected cells is not known. Nonetheless, direct evidence exists for effects of HSV-1 and HSV-2 pUS3 on the nuclear lamina and localization of proteins required for nuclear egress. Hyperphosphorylation and dispersion of emerin occurs in HSV-1- and HSV-2-infected cells.[60,61] Emerin hyperphosphorylation is altered in cells infected with HSV-1 or HSV-2 pUS3 mutants,[60,61] and pUS3 of HSV-1 has been implicated in regulating disruption of emerin.[36] HSV-1 pUS3 also can phosphorylate lamins A and C in vitro at multiple sites and alter localization of lamins in HSV-1-infected cells.[32,34] However, phosphorylation by pUS3 does not account for all of the phosphospecies of emerin or lamins A and C seen in HSV-1 infected cells.[32,61] Cellular

kinases recruited by HSV also facilitate dissolution of the lamin matrix. Recruitment of PKCα and PKCδ to the nuclear rim during HSV-1 infection requires pUL31 and pUL34, and one or both of these cellular kinases mediates phosphorylation of lamin B during infection, which becomes relocated to the cytoplasm.[62] However, lamin B phosphorylation is only partially reduced by PKC inhibitors.[36,62] These observations indicate a role for additional kinase(s) in phosphorylation of lamins during HSV infection.

HSV-1 pUS3 disruption of the nuclear lamina correlates with its effects on virion egress. Cells infected with HSV-1 lacking pUS3 or its kinase activity show multiple defects in virion maturation and egress.[38,42,63,64] Phosphorylation of pUL31 by pUS3 controls capsid egress from the nucleus,[65] and capsids of virus lacking pUS3 kinase activity accumulate in the perinuclear space,[38,63] implying a crucial role for HSV-1 pUS3 in fusion with the ONM. pUS3 kinase activity is required for consistent localization of the NEC at the nuclear rim during HSV-1 egress,[42,63,65,66] which in turn is required for localization of cellular PKC.[62] Inhibition of the PKC family also inhibits nuclear egress and reduces progeny virus production.[36] These results link HSV-1 pUS3 kinase activity to capsid budding through the INM at least indirectly via effects on pUL31 and PKC. HSV-1 pUS3 may play an additional role in nuclear egress by phosphorylation of the cytoplasmic tail of glycoprotein B,[67] because primary enveloped virions of an HSV-1 gH-deficient mutant that also expresses gB which lacks the pUS3 phorphorylation site do not fuse with the ONM.[68] HSV-1 pUL13 can phosphorylate pUS3 in vitro, but this phosphorylation event does not significantly alter the capacity of pUS3 to phosphorylate pUL31 during infection,[66] implying a major role for HSV-1 pUS3 rather than pUL13 in dissolution of the lamin network and egress of nucleocapsids in HSV-1-infected cells.

Interestingly, HSV-2 pUS3 does not control nuclear egress or localization of the NEC to the nuclear rim.[69] Instead, HSV-2 pUL13 appears to play a more prominent role in altering the nuclear lamina than does HSV-1 pUL13. We previously noted changes in nuclear morphology in cells transfected with a plasmid encoding HSV-2 pUL13, but not an enzymatically-inactive pUL13 (pUL13-K176A).[70] In cells transfected with plasmids encoding HSV-2 pUL13 or pUL13-K176A, we found that HSV-2 pUL13 alters the conformation and distribution of nuclear lamins.[33] HSV-2 pUL13 but not the enzymatically-inactive pUL13-K176A mutant induces changes in lamins A and C that result in loss of detection by certain antibodies, presumably due to a conformational change.[33] pUL13 but not pUL13-K176A also causes redistribution of lamin B1 to the nuclear interior.[33] HSV-2 pUL13 directly phosphorylates lamins A, C and B1 in vitro, and the lamin A1 tail domain, suggesting that lamin alterations induced by HSV-2 pUL13 in cells could result from direct phosphorylation by pUL13.[33] Thus, HSV-2 pUL13 kinase activity alters the integrity of the nuclear lamina by targeting the nuclear lamins. In HSV-2-infected cells, some of the lamin alterations observed are consistent with the effects of pUL13 alone, and other alterations are also seen, indicating that additional viral protein(s) play a direct or indirect role in altering lamin distribution during HSV-2 infection.

The Cdc2-kinase phosphorylates lamin A at sequences with the motif S/TPxS/R that are functionally important in lamin disassembly.[71] Although the kinase activity of HSV-2 pUL13 does not mimic Cdc2 on peptide substrates, the amino acid pair SP can be a minimal recognition sequence for HSV-2 pUL13 phosphorylation.[59] Lamins A and C contain three SP sequences in the mature tail domain. Because HSV-2 pUL13 can phosphorylate the tail domain of lamin A in vitro, it is possible that the decreased reactivity seen with the monoclonal antibody recognizing epitopes in the tail domain of lamins A and C reflects a conformational change in this region induced by HSV-2 pUL13 phosphorylation, thus altering the nuclear lamina.

HSV-1 pUL13 mutants replicate to titers only 4 to 5-fold lower than wild-type HSV-1.[72-74] In contrast, HSV-2 pUL13 mutant viruses replicate very poorly. Loss of kinase activity alone severely compromises virus replication and spread, and preliminary results suggest pUL13-deficient HSV-2 is not released into the supernatant (DeLassus GS and Morrison LA, unpublished observations). Capsids accumulate near the INM but are not observed in the cytoplasm and antibody to lamin A reveals an intact nuclear rim in infected cells (DeLassus GS and Morrison LA, unpublished observations), consistent with an inability of HSV-2 pUL13 mutants to directly or indirectly disrupt the nuclear lamina. Thus, unlike HSV-1, HSV-2 pUL13 appears to play a highly significant role in some aspects of nuclear lamina modification and virus replication and egress. To what extent these properties are connected remains an area of intense investigation.

Nuclear egress of HSV is clearly directional. An unresolved question is how capsids that have budded through the INM proceed to fuse with the ONM and enter the cytoplasm, rather than returning from the cytoplasm to fuse with the ONM and INM. Despite their high degree of homology, HSV-1 and HSV-2 apparently rely on different viral and possibly cellular kinases to effect nuclear egress. The full array of proteins phosphorylated by viral and cellular kinases within the nuclei of cells infected with HSV-1 versus HSV-2, and the ramifications of these modifications for directional budding, remains to be established. Finally, herpesviruses replicate in polarized cells, and alphaherpesvirus infection of neurons poses particularly interesting and unresolved questions about directional transport and the mechanisms by which these viruses maintain or manipulate connections between proteins of the nuclear matrix and the cytoskeleton to achieve their ultimate goal of egress from the cell.

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