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Herpesvirus nuclear egress: Pseudorabies Virus can simultaneously induce nuclear envelope breakdown and exit the nucleus via the envelopment–deenvelopment-pathway

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ABSTRACT

Herpesvirus replication takes place in the nucleus and in the cytosol. After entering the cell, nucleocapsids are transported to nuclear pores where viral DNA is released into the nucleus. After gene expression and DNA replication new nucleocapsids are assembled which have to exit the nucleus for virion formation in the cytosol. Since nuclear pores are not wide enough to allow passage of the nucleocapsid, nuclear egress occurs by vesicle-mediated transport through the nuclear envelope. To this end, nucleocapsids bud at the inner nuclear membrane (INM) recruiting a primary envelope which then fuses with the outer nuclear membrane (ONM). In the absence of this regulated nuclear egress, mutants of the alphaherpesvirus pseudorabies virus have been described that escape from the nucleus after virus-induced nuclear envelope breakdown. Here we review these exit pathways and demonstrate that both can occur simultaneously under appropriate conditions.

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1. Introduction

Herpesviruses are an extensive family of large DNA-viruses. Within the order *Herpesvirales*, the family *Herpesviridae* can be further divided into the three subfamilies *Alpha-*, *Beta-* and *Gammaherpesvirinae* (Davison, 2010; Davison et al., 2009), which differ in their host specificity, replication efficiency and target cells for establishment of latency (Pellett and Roizman, 2013; Pellett and Roizman, 2007). However, all herpes virions have a similar structure. The double-stranded DNA-genome, which is enclosed in an icosahedral capsid, is surrounded by proteinaceous tegument, and an envelope, in which viral glycoproteins are embedded (McGeoch et al., 2006).

Herpesviruses encode far more genes than many other viruses. Approximately 40 of them are conserved within the *Herpesviridae* and are designated as “core” genes. These genes encode proteins which are important for general aspects of lytic replication, like DNA-replication, -processing and -encapsidation, capsid assembly and nuclear egress, or for structural proteins of capsid, tegument or envelope (McGeoch et al., 2006).

The complex structure of Herpesviruses is also reflected by their replication cycle. The nucleocapsid enters the host cell after fusion of the virion envelope with the plasma membrane, or with the endosomal membrane if endocytosis occurs. For this process the conserved core fusion machinery is essential. It consists of glycoprotein (g)B and the heterodimeric gH/gL complex. Other glycoproteins, which mediate attachment of the virion to cellular surface receptor proteins, enhance the fusion process (Eisenberg et al., 2012). Subsequently the virion is transported along microtubules to the nuclear pore (Sodeik et al., 1997; Zaichick et al., 2013), where the viral genome is released and enters the nucleus through the nuclear pore. Following circularization of linear viral DNA (Strang and Stow, 2005), the viral genome is transcribed and replicated. Protein translation occurs in the cytoplasm and, after transport of capsid proteins into the nucleus, the capsid is assembled around a protein scaffold which is autoproteolytically cleaved and extruded when DNA is packaged to form nucleocapsids (Homa and Brown, 1997).

These nucleocapsids exit the nucleus through the nuclear membranes. Final maturation of the virion then takes place in the cytosol. Acquisition of proteins of the inner tegument during or immediately following nuclear egress is followed by addition of an outer

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tegument layer in the trans-Golgi region and final envelopment by budding into trans-Golgi vesicles. Interactions between tegument and capsid proteins, among tegument proteins, as well as between tegument proteins and cytoplasmic domains of glycoproteins drive virion formation. Subsequently, the enveloped intravesicular virions are transported to the plasma membrane and released into the extracellular space (Johnson and Baines, 2011; Mettenleiter, 2004, 2006; Mettenleiter et al., 2009).

1.1. Nuclear exit

Exit from the nucleus is an essential and highly regulated step in herpesviral replication. In contrast to the plasma membrane, the nuclear envelope is a formidable barrier consisting of two membranes with high complexity. The two membrane layers surround the periplasmic space and are linked at nuclear pores, which allow transport between nucleus and cytoplasm (Beck et al., 2004, 2007). Although nuclear pores have an outer diameter of ~125 nm, the open transport canal only averages to a diameter of ~50 nm (Frenkiel-Krispin et al., 2010; Pante and Kann, 2002). Interestingly, although both the inner (INM) and outer nuclear membrane (ONM) are contiguous, they contain different sets of proteins (Hetzer, 2010). Below the INM lies the nuclear lamina, a meshwork of intermediate filaments composed of type A and B lamins. The lamina has been shown to be important for nuclear stability, genome organization, transcription, DNA repair and signal transduction (Dittmer and Misteli, 2011).

It has long been disputed how herpesvirus nucleocapsids leave the nucleus, considering their diameter of 125 nm. Initially three different mechanisms were proposed, the single-envelopment-pathway, egress through dilated pores and the envelopment–deenvelopment-pathway (recently reviewed in Mettenleiter et al. (2013)).

Q6 For the single-envelopment-pathway, it was assumed that the capsid buds into the INM obtaining a lipid envelope from the INM which are maintained during transport of the enveloped virion through the endoplasmic reticulum and the secretory pathway. However, even though some experimental data was interpreted in favor of this pathway (Johnson and Spear, 1982; Torrisi et al., 1992), it was shown that the composition (Klupp et al., 2000; Kopp et al., 2002; Reynolds et al., 2002; Skepper et al., 2001; van Genderen et al., 1994) and the appearance (Granzow et al., 2001; Mettenleiter et al., 2009) of primary and mature virions differs considerably. Moreover, fusion events between primary enveloped virions and the ONM, as well as naked cytoplasmic capsids, were observed for different herpesviruses, contradicting the single-envelopment-pathway (Granzow et al., 1997, 2001; Stackpole and Mizell, 1968). Therefore, it is highly unlikely that herpesviruses use the single-envelopment-pathway to exit the nucleus. Alternatively it was proposed that herpesviruses dilate nuclear pores to exit the nucleus (Wild et al., 2005, 2009). However, no solid evidence for herpesvirus transfer through nuclear pores has been provided. In contrast, it has been shown that reorganization of the nuclear pore network is not required for herpesviral nuclear egress (Nagel et al., 2008). The same holds true for the proposed combination of both postulated pathways (Leuzinger et al., 2005).

Meanwhile, it is accepted that nuclear egress of herpesviruses proceeds via a two-step mechanism designated as "envelopment–deenvelopment-pathway" (Fig. 1A; Skepper et al., 2001). Here, the nucleocapsid buds at the INM into the perinuclear space followed by fission of the INM-derived vesicle to form a primary, transient envelope. This primary envelope then fuses with the ONM, thereby releasing the nucleocapsid into the cytosol (Johnson and Baines, 2011; Mettenleiter et al., 2009, 2013). Mechanistically, this process equals a vesicular (=primary envelope mediated) transport of cargo (=the nucleocapsid) through the nuclear envelope, a process so far unknown in cell biology.

1.2. The nuclear egress complex

The conserved heterodimeric nuclear egress complex (NEC) is an essential component of this pathway (Mettenleiter, 2004). It is anchored in the nuclear envelope by its transmembrane protein component which has been designated as pUL34 in herpes simplex virus 1 (HSV-1) (Reynolds et al., 2001; Roller et al., 2000) and pseudorabies virus (PrV) (Klupp et al., 2000), pUL50 in human (HCMV) (Milbradt et al., 2007) and M50 in murine cytomegalovirus (MCMV) (Muranyi et al., 2002) and BFRF1 in Epstein–Barr Virus (Farina et al., 2005; Lake and Hutt-Fletcher, 2004). The transmembrane protein interacts with a second protein designated as pUL31 in HSV-1 and PrV (Fuchs et al., 2002; Reynolds et al., 2001), pUL53 or M53 in HCMV and MCMV (Dal Monte et al., 2002; Muranyi et al., 2002) and BFLF2 in EBV (Gonnella et al., 2005; Lake and Hutt-Fletcher, 2004). When expressed separately, the type II tail-anchored pUL34 localizes intrinsically to the nuclear membrane, whereas pUL31 is distributed diffusely throughout the nucleus and is recruited to the nuclear membrane only by interaction with pUL34 (Mettenleiter, 2004). Until now little is known about the structure of the NEC, since no crystal structure is available. However, HSV-1, PrV, MCMV and HCMV pUL34 homologs consist of a non-conserved carboxy-terminal part and a highly conserved amino-terminal part, which can be subdivided in two (Milbradt et al., 2012) or three (Haugo et al., 2011) conserved regions (CR), depending on the selection of sequences used for the comparison and the alignment algorithm.

The pUL31 interaction domains have been located within the conserved parts of the pUL34 homologs, although the exact location may differ considerably between the different homologs (Bubeck et al., 2004; Fuchs et al., 2002; Liang and Baines, 2005; Milbradt et al., 2012; Passvogel et al., 2013). For HSV-1 pUL34 the pUL31 interaction domain was localized to amino acids 137 to 181 (Liang and Baines, 2005), whereas in PrV pUL34 it is located between aa 5 and 161 (Passvogel et al., 2013) and the pUL53 interaction domain of HCMV pUL50 was mapped between aa 10 to 169 (Milbradt et al., 2012). However, also residues that are not part of the identified interaction domains may influence NEC formation (Roller et al., 2010).

To further examine the pUL31–pUL34 interaction several mutational studies were performed to identify amino acids important for interaction with pUL31 homologs and NEC function. For the MCMV M50 amino acids Glu56 and Tyr57, which are strictly conserved among herpesviruses (EY motif), have been shown by random-transposon mutagenesis to be important for complex formation (Bubeck et al., 2004). Using site-directed mutagenesis, the same two amino acids were also shown to be required for interaction of HCMV pUL50 with pUL53 (Milbradt et al., 2012) and PrV pUL34 with pUL31 (Passvogel et al., 2013). Remarkably, a charge cluster mutation at that position in HSV-1 pUL34 resulted in a mutant protein which did not localize correctly to the nuclear membrane and was unable to complement replication of a UL34-null virus, despite continuing interaction with pUL31 (Bjerke et al., 2003).

Recently, site directed-mutagenesis of PrV pUL34 has been used to identify other amino acids important for interaction with pUL31. In transfection experiments NEC complex formation was impaired after mutation of Asn75 and Gly77. However during infection, pUL34 carrying a mutation in Gly77 was still able to form a complex with pUL31 and largely complemented replication of a UL34 deficient virus mutant, indicating that other viral components might be involved in NEC formation. Both, Asn75 and Gly77 are part of the conserved NTG motif, which, together with the conserved EY motif, may form a structure necessary for pUL31 binding (Passvogel et al., 2014). Interestingly, mutation of Asn103 or a dileucine motif

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