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Nuclear envelope breakdown induced by herpes simplex virus type 1 involves the activity of viral fusion proteins

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ABSTRACT

Herpesvirus infection reorganizes components of the nuclear lamina usually without loss of integrity of the nuclear membranes. We report that wild-type HSV infection can cause dissolution of the nuclear envelope in transformed mouse embryonic fibroblasts that do not express torsinA. Nuclear envelope breakdown is accompanied by an eight-fold inhibition of virus replication. Breakdown of the membrane is much more limited during infection with viruses that lack the gB and gH genes, suggesting that breakdown involves factors that promote fusion at the nuclear membrane. Nuclear envelope breakdown is also inhibited during infection with virus that does not express UL34, but is enhanced when the US3 gene is deleted, suggesting that envelope breakdown may be enhanced by nuclear lamina disruption. Nuclear envelope breakdown cannot compensate for deletion of the UL34 gene suggesting that mixing of nuclear and cytoplasmic contents is insufficient to bypass loss of the normal nuclear egress pathway.

Introduction

Herpesviruses morphogenesis starts in the nucleus by formation of DNA-filled capsids and is finalized in the cytoplasm where capsids acquire the tegument proteins and the final envelope. The primary mechanism for nuclear egress is an envelopment/de-envelopment process. Capsids leave the nucleus by budding into the INM (primary envelopment), which results in formation of enveloped virions inside the perinuclear space. These so-called primary virions then quickly resolve by fusing with the outer nuclear membrane (ONM) (de-envelopment) (Johnson and Baines, 2011; Roller, 2008). Several viral proteins are involved in envelopment/de-development at the NE. HSV-1 pUL34 and pUL31, form a complex at the INM (Reynolds et al., 2001, 2002). The alphaherpesviral pUL34/pUL31 complex has a multifunctional role during nuclear egress including lamina disruption (Bjerke and Roller, 2006; Leach et al., 2007; Mou et al., 2007; Park and Baines, 2006; Simpson-Holley et al., 2005) and wrapping the INM around the capsid (Klupp et al., 2007; Roller et al., 2010, 2011). De-envelopment of primary virions is facilitated by the pUS3 serine/threonine kinase and this function appears to be conserved within the alphaherpesviruses (Klupp et al., 2001; Mou et al., 2009; Reynolds et al., 2002; Ryckman and Roller, 2004; Schumacher et al., 2005). In HSV-1, the two glycoproteins gB and gH also participate in de-envelopment. Double deletion of gB and gH results in accumulation of primary virions inside a dilated perinuclear space (Farnsworth et al., 2007; Wisner et al., 2009; Wright et al., 2009). The fusogenic activity of gB is required for its de-envelopment function since mutations that interfere with gB fusion activity also interfere with de-envelopment when gH is also absent (Wright et al., 2009). pUS3 presumably has a regulatory role in de-envelopment that, in HSV-1, includes regulating the fusogenic activity of gB (Wisner et al., 2009). Interestingly, de-envelopment fusion in infection with the related alphaherpesvirus PrV does not require gB and/or gH, since single and double deletions egress from the nucleus as efficiently as wild-type virus, and accumulation of perinuclear virions is not observed (Klupp et al., 2008).

Alphaherpesvirus deletion recombinants that fail to express either of pUL31 or pUL34 still exhibit low levels of replication, suggesting that they can egress from the nucleus by a mechanism independent of pUL34 and pUL31 (Chang et al., 1997; Fuchs et al., 2002; Klupp et al., 2000; Roller et al., 2000). Furthermore, the requirement in HSV-1 for UL31 expression in nuclear egress is apparently cell-specific, indicating that that there is a nuclear

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egress mechanism that does not rely on the pUL31/pUL34 complex (Liang et al., 2004). Extensive passage of UL31 or UL34-null PrV recombinants resulted in variants (called ΔUL31Pass and ΔUL34-pass) that produced virus at titers similar to the wild type PrV (Grimm et al., 2012; Klupp et al., 2011). Both viruses caused nuclear envelope breakdown (NEBD) in infected cells. The observation was quite striking since normally during herpesvirus infection the NE appears intact despite drastic changes in nuclear architecture that include chromatin marginalization, phosphorylation of the lamina components and expansion of the nucleus (Bjerke and Roller, 2006; Camozzi et al., 2008; Hamirally et al., 2009; Leach et al., 2007; Leach and Roller, 2010; Milbradt et al., 2007; Morris et al., 2007; Mou et al., 2007; Muranyi et al., 2002; Park and Baines, 2006; Reynolds et al., 2004; Scott and O'Hare, 2001; Simpson-Holley et al., 2004, 2005). Although the mechanisms by which ΔUL31pass or ΔUL34Pass induced NE breakdown are not known, these studies imply that breakdown might provide an alternative pathway to envelopment/de-envelopment for capsids to exit the nucleus.

The TOR1A gene encodes TorsinA, a member of the AAA+ ATPase superfamily of proteins that perform diverse cellular activities (Ozelius et al., 1997; White and Lauring, 2007). TorsinA is a widely expressed, peripheral membrane protein (i.e., having no transmembrane domain) localized in the lumen of the ER and in the space between the inner and outer nuclear membranes (Callan et al., 2007; Jungwirth et al., 2010; Kustedjo et al., 2000; Vander Heyden et al., 2011). The TorsinA mRNA encodes a polypeptide of 332 amino acids with a cleaved signal sequence that is thought to form hexamers in the ER lumen. Mutation in the TOR1A gene that leads to a loss of a single glutamic acid residue, Glu^{302} or Glu^{303} near the C-terminus of TorsinA (called the ΔE mutation) is associated with dominantly-inherited early-onset torsion dystonia (Ozelius et al., 1997) and reviewed in (Granata et al., 2009; Granata and Warner, 2010). Several functions, including a role as a molecular chaperone and a homeostatic regulator of an induced ER stress response have been suggested for TorsinA, and chaperone activity has been demonstrated in vitro (Burdette et al., 2010; Caldwell et al., 2003; Chen et al., 2010; Hewett et al., 2003; Hewett et al., 2007; McLean et al., 2002). Although TorsinA is ubiquitously expressed, pathology is limited to neuronal cells and in transgenic mice with defects in TorsinA expression or function that pathology is accompanied by changes in the architecture of the nuclear envelope including loss of proper spacing between the INM and the ONM and formation of intranuclear membrane inclusions (Gonzalez-Alegre and Paulson, 2004; Goodchild et al., 2005; Jungwirth et al., 2010; Kustedjo et al., 2000). TorsinA has been found to interact with other host cell proteins that may participate in its function in maintenance of NE architecture, including LAP1 and nesprin 3A (Goodchild and Dauer, 2005; Naismith et al., 2009; Nery et al., 2008).

Overexpression of wild-type torsinA (TA), but not a dominant negative mutant form, impaired herpes simplex virus type 1 (HSV-1) replication and caused a defect in capsid nuclear egress (Maric et al., 2011). To continue our studies on importance of TorsinA for HSV-1 we used transformed wild-type and $Tor1a^{-/-}$ mouse embryonic fibroblasts (MEFs). We show here that wild type HSV-1 can induce NEBD in $Tor1a^{-/-}$ mouse embryonic fibroblast cell line that is morphologically highly similar to that seen in infection with mutant PrV. This demonstrates that, in some host cell environments, extensive NEBD is one possible consequence of a wild-type virus infection. We have used recombinant mutant viruses to explore the mechanism of NEBD and find that it is strongly enhanced by UL34 expression and by expression of the HSV glycoproteins gH and gB that have been implicated in de-envelopment fusion during nuclear egress.

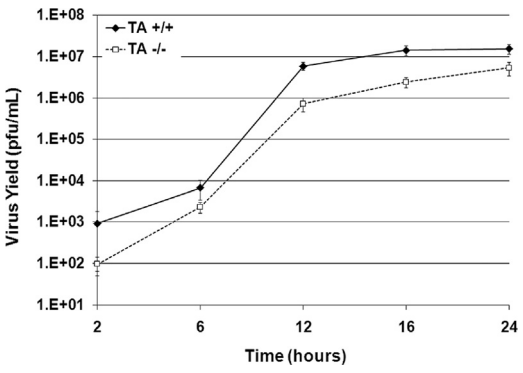

Fig. 1. HSV-1 replication is inhibited in transformed $Tor1a^{-/-}$ MEFs. Single-step growth kinetics of HSV-1 were measured on TA MEFs. $Tor1a^{+/+}$ and $Tor1a^{-/-}$ MEFs were infected with HSV-1(F) at an MOI of 5 and at indicated points virus infectivity of whole cell cultures was determined by plaque assay on Vero cells. Points plotted are the mean of three independent experiments; error bars represent the standard deviation. The difference in infectivity at 2 h.p.i. reflects differences in the amount of residual infectivity left from the inoculum after washes in citrate buffer.

Results

HSV-1 replication is impaired in transformed $Tor1a^{-/-}$ MEFs

We previously showed that overexpression of TA inhibits HSV-1 production in two different cell lines (Maric et al., 2011). As part of an effort to determine whether TA expression was necessary for efficient HSV-1 replication, we tested viral single-step growth on transformed MEFs from $Tor1a^{-/-}$ and $Tor1a^{+/+}$ mouse embryos (Fig. 1). Viral yields in $Tor1a^{-/-}$ MEFs were significantly lower at 12–24 h.p.i. (12 ($p < 0.003$), 16 ($p < 0.008$) and 24 ($p < 0.018$) h.p.i.) in comparison to $Tor1a^{+/+}$ MEFs. At 12 h.p.i. virus production was more than 8-fold less efficient in $Tor1a^{-/-}$ than in $Tor1a^{+/+}$ MEFs. At later times during infection the difference in HSV-1 replication decreased to about than 3-fold.

HSV-1 infection induces NE breakdown in transformed $Tor1a^{-/-}$ MEFs

Since TorsinA is a NE and ER-resident protein, we asked if its loss interferes with HSV-1 morphogenesis. To ensure that events prior to capsid assembly are unaffected by TorsinA loss, $Tor1a^{+/+}$ and $Tor1a^{-/-}$ MEFs were infected with HSV-1(F) at an MOI of 5 and at 12 h.p.i. accumulation of late (VP5 and pUL34), true late gene products (pUS11, gC) and processing of viral glycoproteins (gC, gD, gB) were analyzed by immunoblotting (Fig. 2A). Both cells lines supported accumulation and processing of viral proteins to similar extent, indicating that absence of TorsinA does not interfere with early events in virus life cycle (entry, gene expression, and replication). The impairment in HSV-1 replication is thus likely associated with capsid assembly and/or subsequent events.

To examine the defects in TorsinA-null MEFs, mock- or HSV-1(F)-infected $Tor1a^{+/+}$ and $Tor1a^{-/-}$ MEFs were examined by TEM (Fig. 2B). All stages of virus assembly were observed in both MEF cell lines and the quantification of virus particles in 10 randomly selected cell sections is provided in Table 1. About 3 fold fewer virus particles were counted in $Tor1a^{-/-}$ in comparison to $Tor1a^{+/+}$ MEFs and this reduction was correlated with fewer capsids found in the nucleus and the cytoplasm of $Tor1a^{-/-}$ MEFs. While quantitative measurement of capsid production is uncertain in analysis of EM, due to the low number of cells examined, these results suggest the possibility of a defect in assembly and/or stability of capsids in $Tor1a^{-/-}$ MEFs, which might explain the lower virus titer in these cells. Ultrastructural analysis also revealed breakdown of the NE in