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Significance of host cell kinases in herpes simplex virus type 1 egress and lamin-associated protein disassembly from the nuclear lamina

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ABSTRACT

The nuclear lamina is thought to be a steric barrier to the herpesvirus capsid. Disruption of the lamina accompanied by phosphorylation of lamina proteins is a conserved feature of herpesvirus infection. In HSV-1-infected cells, protein kinase C (PKC) alpha and delta isoforms are recruited to the nuclear membrane and PKC delta has been implicated in phosphorylation of emerin and lamin B. We tested two critical hypotheses about the mechanism and significance of lamina disruption. First, we show that chemical inhibition of all PKC isoforms reduced viral growth five-fold and inhibited capsid egress from the nucleus. However, specific inhibition of either conventional PKCs or PKC delta does not inhibit viral growth. Second, we show hyperphosphorylation of emerin by viral and cellular kinases is required for its disassociation from the lamina. These data support hypothesis that phosphorylation of lamina components mediates lamina disruption during HSV nuclear egress.

Introduction

All herpesviruses assemble newly formed capsids in the nucleus of the host cell. To escape the nucleus, the capsids must traverse the inner and outer nuclear membranes via an envelopment/de-envelopment process at the nuclear envelope (NE) to release un-enveloped capsids into the cytoplasm {Mettenleiter, 2006 #251}.

The inner nuclear membrane (INM) is not, however, freely accessible to large macromolecular complexes like a herpesvirus capsid. The INM is supported by a complex meshwork of proteins called the nuclear lamina (Aebi et al., 1986; Gruenbaum et al., 2005; Worman and Courvalin, 2005). The lamina mesh is primarily composed of intermediate filament-related proteins called lamins of which there are three types: A, B, and C. The lamin A gene is alternatively spliced to generate A and C types while the two B types are encoded by separate genes (Gruenbaum et al., 2003). The lamin meshwork is anchored to the INM via interactions with lamin associated proteins (LAPs) (Worman and Courvalin, 2005). Many LAPs such as emerin, the lamin B receptor (LBR), MAN1, and LAP2β are inner nuclear membrane-bound proteins that bind both lamins and chromatin (Holmer and Worman, 2001). The organization of the nuclear periphery suggests that it may present multiple barriers to herpesvirus envelopment. The presence of chromatin attached to the lamina and the organization of the lamin proteins themselves may each present a steric barrier. The spacing of structural elements of the lamin lattice (about 50 nm) is too small to allow passage of a herpesvirus capsid, and physical measurements suggest that the lamin network is quite stiff and resistant to deformation and therefore unlikely to bend around a capsid during envelopment (Aebi et al., 1986; Panorchan et al., 2004). It is likely that the lamina must be disrupted in order for capsids to gain access to the INM.

Infection with wild-type herpesviruses results in changes in nuclear architecture consistent with disruption of the nuclear lamina, including: (i) enlargement of the nucleus demonstrated for HSV-1 and HCMV (Bjerke and Roller, 2006a; Radsak et al., 1991; Simpson-Holley et al., 2005); (ii) change in the shape of the nucleus from a smooth ovoid to something that more closely resembles a raisin in contour, demonstrated for HSV and HCMV (Bjerke and Roller, 2006a; Hamirally et al., 2009; Radsak et al., 1991; Simpson-Holley et al., 2004, 2005); (iii) changes in the localization of both A and B type lamin proteins from a smooth, even lining of the INM to an uneven distribution showing gross thickening of the lamin layer at some sites and small perforations in the layer at other sites, demonstrated for HSV-1, HSV-2, HCMV, MCMV, and EBV (Bjerke and Roller, 2006a; Camozzi et al., 2008; Cano-Monreal et al., 2009; Hamirally et al., 2009; Lee et al., 2008; Radsak et al., 1991; Reynolds et al., 2004; Simpson-Holley et al., 2004, 2005); (iv) masking and unmasking of monoclonal antibody epitopes on the lamin proteins that indicate a change in the conformation or associations of the lamin proteins, seen with HSV-1 and HSV-2 (Reynolds et al., 2004; Cano-Monreal, 2009); (v) redistribution of LAPs including LBR, LAP2β, and emerin in herpes simplex infections (Bjerke and Roller, 2006b; Leach et al., 2007; Morris et al., 2007; Scott and O'Hare, 2001; Simpson-Holley et al., 2004).

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Mitosis requires disruption of the nuclear lamina (reviewed in (Margalit et al., 2005) which is mediated by phosphorylation of lamins and LAPs by cellular kinases including cyclin dependent kinase 1 (Cdk1), protein kinase C (PKC), mitogen-activated protein kinase (MAPK), protein kinase A (PKA), casein kinase II, and AKT (Gruenbaum et al., 2003, 2005; Margalit et al., 2005). Phosphorylation disrupts the lamina protein–protein and protein–DNA interactions. A growing body of evidence suggests that herpesviruses adapt this mechanism and induce phosphorylation of nuclear lamina components to gain access to the INM. HSV-1, HSV-2 and HCMV infections induce phosphorylation of all three types of lamins (Cano-Monreal et al., 2009; Marschall et al., 2005; Mou et al., 2007, 2008; Park and Baines, 2006). EBV and MCMV infections induce phosphorylation of at least lamin A/C (Lee et al., 2008; Muranyi et al., 2002). LAPs are also phosphorylated during herpesvirus infection. Emerin is hyperphosphorylated and disconnected from the lamina during HSV-1 and -2 infections however the mechanism and significance of this modification remains untested (Leach et al., 2007; Morris et al., 2007). It is unknown which emerin residues are modified during HSV infection and if these modifications are required for its disassociation from the NE.

Phosphorylation of lamina components during infection is mediated by both viral and cellular kinases. Herpesviruses encode a conserved protein kinase called CHPK (Conserved Herpesvirus Protein Kinase), which shows similarities to Cdk1 in function and substrate specificity (Hume et al., 2008; Kawaguchi and Kato, 2003). Cdk1, also known as cdc2, has also been implicated in HSV-2 lamina disruption via its putative role in emerin phosphorylation (Morris et al., 2007). Involvement of the beta- and gamma-herpesvirus CHPKs in nuclear lamina disruption and nuclear egress has been established (Krosky et al., 2003; Lee et al., 2008), and the HCMV CHPK phosphorylates lamin proteins on previously defined CDK-dependent sites (Hamirally et al., 2009). While there is no evidence that HSV-1 CHPK, pUL13, can directly phosphorylate nuclear lamina proteins, the HSV-2 pUL13 can disrupt lamin localization in transiently transfected cells and directly phosphorylate lamins *in vitro* (Cano-Monreal et al., 2009). Alpha-herpesviruses encode a second serine/threonine protein kinase designated pUS3 in HSV. pUS3 mediates phosphorylation of lamina components, including lamin A/C, and emerin, and regulates the degree of lamina disruption (Bjerke and Roller, 2006b; Leach et al., 2007; Mou et al., 2008).

During HSV-1, MCMV, and HCMV infections, PKC isoforms are recruited to the NE by viral proteins that are required for lamina disruption, suggesting that PKC activity may contribute to lamina-disrupting phosphorylation events (Muranyi et al., 2002; Park and Baines, 2006). There are ten PKC isoforms divided into three groups that differ in their activation mechanisms and all isoforms may be involved in herpesvirus-mediated lamina disruption. Conventional PKC isoforms (cPKCs), such as protein kinase c alpha (PKC alpha), require an efflux of calcium and diacylglycerol (DAG) for activation. Novel PKC (nPKC) family members, such as PKC delta (encoded by the PRKCD gene), are activated by DAG in a calcium-independent manner. Atypical PKCs (aPKC) such as PKC zeta do not require either for activation (Reyland, 2009b).

Recruitment of PKCs to the NE appears to be isoform specific. Although not all ten isoforms were tested, both PKC alpha and PKC delta, but not PKC zeta, were recruited to the NE upon HSV-1 infection (Park and Baines, 2006). Treatment of HSV infected cultures with Rottlerin, a widely used putative PKC delta inhibitor, blocked lamin B phosphorylation (Park and Baines, 2006). These data suggested a role for PKC alpha and delta but not zeta in nuclear egress.

Recruitment of PKCs to the NE in herpesvirus infections requires expression of the conserved proteins of the virus nuclear egress complex. In HSV, these proteins are called pUL31 and pUL34, and they form a complex that is required for events in lamina disruption including redistribution of lamin proteins, masking and unmasking of lamin epitopes during infection and full hyperphosphorylation and redistribution of emerin (Leach et al., 2007; Reynolds et al., 2004). In HSV-1 infection, recruitment of both PKC alpha and PKC delta depends on pUL34 expression (Park and Baines, 2006). In MCMV, M50/p38, the pUL34 homolog, is required to recruit cPKCs to the NE (Muranyi et al., 2002). HSV-1 induced emerin phosphorylation is dependent upon both pUS3 kinase activity and pUL34 expression. The pUL34 dependent component of emerin hyperphosphorylation is sensitive to inhibition by Rottlerin suggesting that PKC delta mediates emerin hyperphosphorylation (Leach et al., 2007).

Despite the evidence for herpesvirus-dependent lamina disruption, it should be emphasized that the hypothesis that lamina disruption is necessary for herpesvirus egress has not yet been rigorously tested largely because all of the viral and cellular activities that mediate lamina disruption may also have other functions in infection and in nuclear egress. Experiments that specifically isolate the effects of virus-induced lamina disruption on virus growth have not been performed. Also, no study has yet directly demonstrated that the observed phosphorylation of lamina components causes their disconnection from other components of the lamina. The data presented in this article support both of these hypotheses by showing that (i) PKC family function is required both for efficient replication of HSV-1 and for nuclear egress, and (ii) emerin localization in the infected cell is determined by phosphorylation state. Emerin hyperphosphorylation is not sensitive to pan-PKC or DN-PKC delta inhibition suggesting a role for a non-PKC isoform in emerin phosphorylation. This non-PKC isoform or Rottlerin sensitive kinase (RttSK) is sensitive to Rottlerin but not BIM I treatment.

Results

Inhibition of cellular kinases involved in lamina disruption inhibits viral replication

To test the hypothesis that PKC activity is required for late events of HSV-1 replication, specifically nuclear egress, activity of all PKC isoforms was blocked with the pan-PKC small molecule inhibitor bis-indolylmaleimide I (BIM I) (Toullec et al., 1991). HEp-2 cells were infected with five plaque forming units (PFU)/cell of WT HSV-1(F), treated with 10 μM BIM I or vehicle (DMSO) beginning at 5 h post infection (hpi), and production of infectivity was measured at 24 hpi (Fig. 1). This time of drug addition was chosen to allow at least the entry and IE (immediate early) gene expression phases to occur uninhibited. 10 μM BIM I has been previously shown to inhibit all PKC isoform activity (Toullec et al., 1991). BIM I treatment reduced the infectivity compared to DMSO by nearly five-fold. Similar results were obtained in Vero cells and at 16 hpi (not shown).

Toxicity, determined using the ATPLite system, was not observed at the concentrations used for any of the inhibitors used in this study

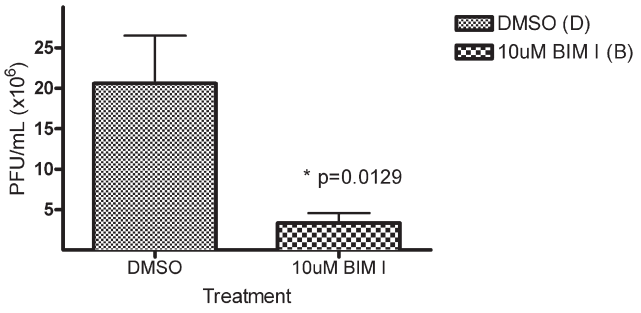

Fig. 1. PKC activity required for HSV-1 replication. Replicate cultures of HEp-2 cells were infected at an MOI of 5 with HSV-1(F) A) Cells were treated with DMSO or 10 μM pan-PKC inhibitor bis-indolylmaleimide (BIM I) beginning at 5 h post infection (hpi). Titer was determined at 24 hpi. Virus yields are expressed as plaque forming units (PFU) per milliliter. Each data point represents the mean of five experiments. Error bars indicate standard deviations. *p*-values were determined via a paired Student *t*-test.