Available online at www.sciencedirect.com
SCIENCE DIRECT
Virology 347 (2006) 261–276
VIROLOGY
www.elsevier.com/locate/yviro

Roles for herpes simplex virus type 1 U_L34 and U_S3 proteins in disrupting the nuclear lamina during herpes simplex virus type 1 egress

Susan L. Bjerke, Richard J. Roller *

Department of Microbiology, The University of Iowa, 3115 Medical Laboratories, Iowa City, IA 52242, USA

Received 11 May 2005; returned to author for revision 17 October 2005; accepted 30 November 2005
Available online 19 January 2006

Abstract

Cells infected with wild type HSV-1 showed significant lamin A/C and lamin B rearrangement, while U_L34-null virus-infected cells exhibited few changes in lamin localization, indicating that U_L34 is necessary for lamin disruption. During HSV infection, U_S3 limited the development of disruptions in the lamina, since cells infected with a U_S3-null virus developed large perforations in the lamin layer. U_S3 regulation of lamin disruption does not correlate with the induction of apoptosis. Expression of either U_L34 or U_S3 proteins alone disrupted lamin A/C and lamin B localization. Expression of U_L34 and U_S3 together had little effect on lamin A/C localization, suggesting a regulatory interaction between the two proteins. The data presented in this paper argue for crucial roles for both U_L34 and U_S3 in regulating the state of the nuclear lamina during viral infection.

Keywords: HSV-1; U_L34; U_S3; Lamin disruption; Egress

Introduction

During primary envelopment herpes simplex virus type 1 (HSV-1) nucleocapsids translocate from the nucleus to the cytoplasm. The capsid must face numerous obstacles before it can reach the inner nuclear membrane (INM). Lining the inside of the INM is the nuclear lamina, which is composed of a meshwork of proteins with spaces too small for the capsid to move through without some disruption of the lamina. The lamina is mainly made up of lamin A/C and lamin B proteins, with smaller amounts of other proteins also present (Gruenbaum et al., 2000). Type A and C lamins are derived from the same gene that is differentially spliced and are expressed primarily in differentiated cells (Goldman et al., 2002). All vertebrate cells express some form of type B lamins (Holaska et al., 2002). The lamins share significant structural similarities to intermediate filaments, and have a characteristic α-helical domain flanked by nonhelical domains, which mediate binding of the lamin proteins to each other to form filaments (McKeon et al., 1986). Lamins associate with themselves, other types of lamins, lamin associated proteins (LAPs) and even chromatin (Gruenbaum et al., 2000; Holaska et al., 2002; Holmer and Worman, 2001). In addition to multiple interactions with cellular proteins, lamins also interact with viral proteins, like the IR6 protein of equine herpesvirus, which is thought to play a role in primary envelopment (Osterrieder et al., 1998). Herpesvirus infections also affect lamin localization and morphology. HSV-1 infection distorts localization of GFP-tagged lamin A, lamin B and lamin B receptor (LBR) constructs, and also disrupts interactions between LBR and the lamina (Scott and O'Hare, 2001). Murine cytomegalovirus M50/p35, the homolog to the U_L34 gene in HSV-1, recruits cellular proteins kinase C to the nuclear membrane and induces lamin phosphorylation (Muranyi et al., 2002). Disruption of the lamina is thought to allow the viral capsid access to the INM, however, there is currently no conclusive experimental evidence to support this hypothesis.

Modifications of the nuclear lamina occur during the normal cell processes of apoptosis and mitosis (Goldman et al., 2002). During apoptosis, cleavage of lamins by caspase 6, as well as cleavage of LAPs by caspase 3, is necessary for progression to cell death (Gruenbaum et al., 2000; Rao et al., 1996). It is

* Corresponding author. Fax: +1 319 335 9006.
E-mail address: richard-roller@uiowa.edu (R.J. Roller).

0042-6822/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2005.11.053

thought that lamin degradation plays a crucial role in shutting down nuclear processes during apoptosis. During mitosis, the lamins are phosphorylated and reversibly depolymerized by various cellular kinases (Gerace and Blobel, 1980; Peter et al., 1990; Thompson et al., 1997). After cell division, phosphatases dephosphorylate the lamins, allowing them to reassemble at the nuclear envelope. It is possible that herpesviruses utilize lamin cleavage, phosphorylation, or both processes to create breaks in the lamina network during viral egress.

Many HSV proteins have been identified as having roles in primary envelopment, including: U_L34, U_L31, U_S3, U_L11, U_L36 and U_L37 (Baines and Roizman, 1992; Brown et al., 1994; Desai, 2000; Desai et al., 2001; Granzow et al., 2000; Hutchinson and Johnson, 1995; Mossman et al., 2000; Reynolds et al., 2001, 2002; Roller et al., 2000). Cellular proteins have not been shown to be required for this process, although it is likely that cellular proteins play a role in targeting viral proteins to specific locations within the cell. The HSV-1 U_L34 gene product is critical for primary envelopment since a recombinant virus that fails to express this protein showed a three- to five-log order of magnitude decrease in replication, and TEM analysis of infected cells showed no evidence of enveloped nucleocapsids (Roller et al., 2000). This critical function is conserved in pseudorabies virus (Klupp and Mettenleiter, 2000) equine herpesvirus type 1 (Neubauer et al., 2002), and Epstein–Barr virus (Farina et al., 2005). The HSV-1 U_L34 gene is a probable type II membrane protein that is phosphorylated by the viral kinase U_S3 (Purves et al., 1991, 1992). In addition to a role in viral egress (Klupp et al., 2001; Reynolds et al., 2002; Wagenaar et al., 1995), the U_S3 gene product protects cells from apoptosis (Asano et al., 1999; Galvan and Roizman, 1998; Leopardi et al., 1997). Recently, U_L34 was shown to also be phosphorylated by an unidentified kinase in specific cell types, although to a lesser extent than phosphorylation by U_S3 (Ryckman and Roller, 2004). The phosphorylation state of U_L34 has different effects on viral growth depending on cell type (Ryckman and Roller, 2004), and it is unknown whether these effects are directly related to problems with primary envelopment. The U_L34 coding sequence is present in β and γ herpesvirus family members and is well conserved among alphaherpesviruses (Davison and Scott, 1986; Dolan et al., 1998; Telford et al., 1992), which may indicate that the role of this protein in primary envelopment is also conserved.

HSV-1 U_L34 forms part of an envelopment complex that also includes the U_L31 and U_S3 proteins. All three proteins colocalize at the nuclear membrane, and each of these is necessary for normal localization of the others in infected cells (Reynolds et al., 2004; Roller et al., 2000). U_L34 also interacts with and forms a complex with the U_L31 protein in vitro (Reynolds et al., 2001).

Alphaherpesvirus U_S3 homologs have been implicated in diverse processes in the infected cell, including inhibition of apoptosis (Leopardi et al., 1997; Asano et al., 1999; Hata et al., 1999; Jerome et al., 1999; Takashima et al., 1999; Asano et al., 2000; Munger and Roizman, 2001; Murata et al., 2002; Benetti et al., 2003; Cartier et al., 2003; Calton et al., 2004; Ogg et al., 2004; Geenen et al., 2005; Matsuzaki et al., 2005; Poon and Roizman, 2005), de-envelopment of capsids at the outer nuclear membrane (Klupp et al., 2001; Reynolds et al., 2002; Granzow et al., 2004; Ryckman and Roller, 2004; Schumacher et al., 2005), and rearrangement of the actin cytoskeleton (Murata et al., 2000; Cartier et al., 2003; Favoreel et al., 2005; Schumacher et al., 2005). Although U_L34 was the first identified substrate of the U_S3 protein kinase activity, it is now clear that there may be many others. It has recently been shown that the viral U_L31, U_S9 and ICP22 proteins can be directly phosphorylated by U_S3 (Kato et al., 2005).

Evidence in the literature supports a role for components of the envelopment complex in disrupting the nuclear lamina. U_L34 and U_L31 proteins have recently been shown to be required for changes in nuclear shape and for changes in the distribution of nuclear lamin A/C protein in infected and transfected cells, and U_L34 has a lamin A/C disrupting activity in transfected cells (Reynolds et al., 2004; Simpson-Holley et al., 2004). U_L34 protein has been shown to interact directly with Lamin A/C in vitro (Reynolds et al., 2004). When U_L34 is overexpressed during transient transfections, the morphology of the nuclear membrane is altered so that the inner and outer membranes are separated (Ye et al., 2000). This distortion of the nuclear membrane resembles the phenotype seen in HSV-1 infected cells. In addition, the location of U_L34 in infected cells at the nuclear membrane places it in an excellent position to interact with and modulate the actions of lamin proteins and LAPs. There is no direct evidence to suggest that U_S3 plays a role is disassembling the nuclear lamina, however, this protein also localizes to the INM during infections and is closely linked to U_L34 localization and function (Leopardi et al., 1997; Reynolds et al., 2002). Here we examine the individual activities and functional relationship of U_L34 and U_S3 in nuclear lamina disruption.

Results

U_L34 and U_S3 proteins are necessary for changes in nuclear contour shape during HSV-1 infection

Previous data have shown that U_L34 and U_L31 are necessary for HSV infection-dependent changes in nuclear shape and lamin A/C distribution (Reynolds et al., 2004; Simpson-Holley et al., 2004). In order to examine the distribution of lamin B, the functional interactions between U_L34 and U_S3 in lamin reorganization, and the role of the U_S3 protein in affecting lamin localization, we determined the localization of nuclear lamins in cells infected with viruses that fail to express either U_L34 or U_S3.

To investigate the roles of U_L34 and U_S3 in disrupting lamins, Vero cells were infected with wild type or mutant viruses at an MOI of 5 for 16 and 24 h, processed for confocal microscopy, and immunostained for lamin A/C or lamin B (Fig. 1). In mock-infected cells, lamin A/C (panels A and O) and lamin B (panels D and R) proteins localized in a tight ring around the nucleus of the cell, showing a smooth, regular distribution of protein on the nuclear envelope. The shape of the