Available online at www.sciencedirect.com
ScienceDirect
Virology 369 (2007) 263–280
VIROLOGY
www.elsevier.com/locate/yviro

Incorporation of the herpes simplex virus type 1 tegument protein VP22 into the virus particle is independent of interaction with VP16

Kevin J. O'Regan, Michael A. Murphy, Michelle A. Bucks, John W. Wills, Richard J. Courtney *

Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, 500 University Drive, Hershey, PA 17033, USA

Received 12 February 2007; returned to author for revision 12 March 2007; accepted 16 July 2007
Available online 20 September 2007

Abstract

Herpes simplex virus type 1 (HSV-1) virions contain a proteinaceous layer termed the tegument that lies between the nucleocapsid and viral envelope. The mechanisms underlying tegumentation remain largely undefined for all herpesviruses. Using glutathione *S*-transferase (GST) pulldowns and coimmunoprecipitation studies, we have identified a domain of the tegument protein VP22 that facilitates interaction with VP16. This region of VP22 (residues 165–225) overlaps the glycoprotein E (gE) binding domain of VP22 (residues 165–270), which is sufficient to mediate VP22 packaging into assembling virus particles. To ascertain the contribution of the VP16 and gE binding activities of VP22 to its virion incorporation, a transfection/infection based virion incorporation assay, using point mutants that discern between the two binding activities, was utilized. Our results suggest that interaction with VP16 is not required for incorporation of VP22 into virus particles and that binding to the cytoplasmic tail of gE is sufficient to facilitate packaging.

Keywords: HSV-1; VP22; U_L49; VP16; U_L48; Virion incorporation; Dileucine motif; Tegument; Tegumentation; Membrane association

Introduction

Herpesviruses share common virion morphology; icosahedral capsids containing the viral genome are surrounded by an amorphous layer of at least 20 proteins known as the tegument, which is in turn enclosed by a lipid bilayer composed of 11 or more virally encoded glycoproteins (Roizman and Sears, 2001). While it is well established that capsid assembly and packaging of the viral genome occur in the nucleus, the compartment(s) in which the tegument and envelope are acquired is less well-defined (Enquist et al., 1998; Mettenleiter, 2000, 2002). As with other herpesviruses, the current model for herpes simplex virus type 1 (HSV-1) assembly and egress suggests that nucleocapsids are shuttled to the cytoplasm via a budding/fusion event that occurs across the inner and outer membranes of the nucleus, respectively. The unenveloped nucleocapsids then travel through the cytoplasm until they reach a *trans*-Golgi network (TGN)-derived vesicle. While at this site, nucleocapsids are thought to acquire their final lipid bilayer during a budding event that also results in the acquisition of tegument and viral glycoproteins (Gershon et al., 1994; Granzow et al., 1997, 2001; Harley et al., 2001; Mettenleiter, 2002, 2004; Sanchez et al., 2000; Skepper et al., 2001; Van Genderen et al., 1994; Whealy et al., 1991; Whiteley et al., 1999; Zhu et al., 1995). The mature virions subsequently follow the secretory pathway to the cell surface, where they are released into the extracellular milieu (Mettenleiter, 2002).

In contrast to nucleocapsid assembly, the molecular mechanisms of tegumentation and the process of final envelopment are poorly understood (Mettenleiter, 2002, 2004; Roizman and Sears, 2001). Tegumentation of nucleocapsids can theoretically occur at various stages in the egress pathway: in the nucleus, at the nuclear membrane, in the cytoplasm or during budding at the TGN. Recent studies have demonstrated that a subset of the tegument proteins are added to the capsid prior to nuclear egress; however, the mechanism behind the addition of the majority of the tegument components remains elusive (Bucks et al., 2007; Naldinho-Souto et al., 2006). Understanding how the process of tegumentation occurs is important as evidence indicates that tegument proteins, possibly in concert with certain viral

* Corresponding author. Fax: +1 717 531 6522.
E-mail address: rcourtney@psu.edu (R.J. Courtney).

0042-6822/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2007.07.020

glycoproteins, contain all of the functions required for budding at the TGN (McLauchlan and Rixon, 1992; Rixon et al., 1992; Szilagyi and Cunningham, 1991). Our studies have focused on defining the protein–protein interactions of one specific HSV-1 tegument protein, VP22, and the role these interactions play in facilitating the incorporation of VP22 into assembling virions.

Encoded by the U_L49 gene of HSV-1, VP22 is a highly phosphorylated, 301-amino-acid protein which is one of the most abundant tegument components, with an estimated 2000 copies of the protein packaged per virion (Elliott and Meredith, 1992; Heine et al., 1974; Leslie et al., 1996). Despite its abundance, the role of VP22 during HSV-1 assembly and the mechanism of its incorporation, remain undefined.

VP22 interacts with VP16, another abundant tegument protein, which is essential for secondary envelopment and egress (Elliott et al., 1995, 2005; Mossman et al., 2000; Weinheimer et al., 1992). Transmission immunoelectron microscopy (TIEM) studies suggest that during viral assembly, detectable amounts of VP16 are added to the capsid in the nucleus, with additional VP16 added as the nucleocapsid moves through the cytoplasm, prior to final envelopment (Miranda-Saksena et al., 2002; Naldinho-Souto et al., 2006). In contrast, VP22 is packaged into virions during final envelopment as nucleocapsids bud into TGN-derived vesicles (Miranda-Saksena et al., 2002). Consistent with this observation, previous studies have shown that VP22 associates with membranes and localizes to acidic compartments of the cell including the TGN (Brignati et al., 2003).

Although little is known about the molecular details of final tegumentation and envelopment, it is likely that protein–protein interactions between tegument proteins or between tegument proteins and the cytoplasmic tails of virally encoded glycoproteins, facilitate the process and may result in the incorporation of viral proteins into the assembling particle. Two VP22 null viruses have recently been described and demonstrate a variety of cell-specific replication defects with altered virion composition including decreased packaging of both glycoprotein D (gD) and glycoprotein E (gE) (Duffy et al., 2006; Elliott et al., 2005; Pomeranz and Blaho, 2000). VP22 binds to the cytoplasmic tails of both gD and gE, and may facilitate the interaction of viral nucleocapsids with glycoproteins lining up on the membranes of TGN-derived vesicles, perhaps through its interaction with VP16 (Chi et al., 2005; O'Regan et al., 2006). In HSV-1, simultaneous deletion of gD and gE results in accumulation of unenveloped capsids in the cytoplasm that are embedded in tegument-like material, a similar phenotype to that seen with a VP16 null virus (Farnsworth et al., 2003; Mossman et al., 2000; Weinheimer et al., 1992). In pseudorabies virus (PrV) the cytoplasmic tails of the envelope glycoproteins gE and gM bind to VP22 in a yeast two-hybrid study (Fuchs et al., 2002). Interestingly, simultaneous deletion of gM and the gE/gI heterodimer results in reduced amounts of VP22 in the mature PrV particle, and in the formation of capsid-bound tegument aggregates in the cytoplasm (Brack et al., 1999, 2000; Fuchs et al., 2002). Furthermore, the Bartha stain of PrV, which lacks the glycoproteins gI and gE, fails to package VP22 (Lyman et al., 2003).

Previous studies from our laboratory demonstrated that the gE binding domain of VP22 (residues 165–270) competes efficiently with wild-type VP22 for packaging into assembling virus particles (O'Regan et al., 2006). Interestingly, a recent study suggested that a similar domain of VP22 may facilitate binding to VP16, and reported a correlation between the ability of VP22 to bind VP16 and its incorporation into virus particles (Hafezi et al., 2005). The focus of the current study was to elucidate the role these protein–protein interactions play in the virion packaging of VP22.

Deletion mutagenesis was used to identify the minimal domain of VP22 that is required for interaction with VP16. The experiments presented in this report extend the findings of Hafezi et al. (2005) and reveal a central region that is both necessary and sufficient to facilitate interaction with VP16. Membrane flotation experiments suggest that this region of VP22 tagged with the green fluorescent protein (GFP), has the ability to associate with cellular membranes; however, this activity is not sufficient to facilitate virion incorporation of VP22 and additional protein–protein interactions appear to be required. Using site-directed point mutagenesis to discern between the VP16 and gE binding activities of VP22, virion incorporation studies suggest that VP16 binding is not a requirement for incorporation of VP22 into assembling virus particles. This report extends our knowledge of the network of protein–protein interactions that facilitates the process of final tegumentation and envelopment and further defines the mechanism by which VP22 is incorporated into virus particles.

Results

Mapping the domain of VP22 that facilitates interaction with VP16

To ascertain the domain of VP22 that facilitates interaction with VP16, a series of N-terminal and C-terminal truncation mutants was made in the context of a GST-VP22 fusion protein (Fig. 1A). All deletions were designed to avoid major disruptions in protein folding that could result from truncating the protein in the middle of a hydrophobic region. The truncation mutants were evaluated in a glutathione *S*-transferase (GST) pulldown assay for their ability to bind to VP16. HSV-1-infected cell lysates were incubated with equivalent amounts of purified GST fusion proteins bound to glutathione-Sepharose beads. After a 3-h incubation, beads were washed extensively with lysis buffer, and bound material was separated by sodium dodecyl sulfate (SDS)–10% polyacrylamide gel electrophoresis (PAGE) and then analyzed on Western blots. Unlike GST alone, the GST-VP22 fusion protein reacted with a 65 kDa protein that was recognized by the anti-VP16 antiserum (Fig. 1B). Upon deletion of the N-terminal 43, 86, 120 or even 164 amino acids of VP22, VP16 binding was still detectable; however VP22.87-301 bound to VP16 weakly, a reproducible observation that may suggest problems in protein folding with this truncation mutant. However, when the N-terminal 225 or 270 residues of VP22 were deleted, binding was abolished. These results indicate that the region of VP22 that facilitates interaction with VP16 lies within the C-terminal 137 amino acids.