Antiviral Research 81 (2009) 189–197

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Review

No exit: Targeting the budding process to inhibit filovirus replication

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ARTICLE INFO

Article history:
Received 30 October 2008
Received in revised form
25 November 2008
Accepted 1 December 2008

Keywords:
Filovirus
Ebola virus
Marburg virus
Viral budding
VP40 matrix protein
Virus-like particles
Antiviral therapy

ABSTRACT

The filoviruses, Ebola and Marburg, cause severe hemorrhagic fever in humans and nonhuman primates, with high mortality rates. Although the filovirus replication pathway is now understood in considerable detail, no antiviral drugs have yet been developed that directly inhibit steps in the replication cycle. One potential target is the filovirus VP40 matrix protein, the key viral protein that drives the budding process, in part by mediating specific virus–host interactions to facilitate the efficient release of virions from the infected cell. This review will summarize current knowledge of key structural and functional domains of VP40 believed to be necessary for efficient budding of virions and virus-like particles. A better understanding of the structure and function of these key regions of VP40 will be crucial, as they may represent novel and rational targets for inhibitors of filovirus egress.

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1. Introduction

Ebola (EBOV) and Marburg (MARV) viruses are the sole members of the *Filoviridae* family and are important pathogens of humans and nonhuman primates (Ascenzi et al., 2008; Bray and Murphy, 2007; Casillas et al., 2003; Feldmann et al., 1993; Peters and Khan, 1999). EBOV and MARV have been the cause of sporadic and deadly outbreaks of hemorrhagic fever in many countries since their initial outbreaks in 1976 and 1967, respectively (Ascenzi et al., 2008; Bray and Murphy, 2007; Feldmann et al., 1993; Peters and Khan, 1999). Depending on the virus strain initiating the outbreak, the mortality rate is variable and can be as high as 90%. The filoviruses have been classified by the CDC as Category A bioterrorism agent, and a Category A NIAID priority pathogen (Bray, 2003). Currently, there are no approved vaccines, nor antiviral drugs available to prevent or treat filovirus infections (Bausch et al., 2008; Bray and Paragas, 2002).

One of the major obstacles toward development of filovirus vaccines and therapeutics is that live virus experiments can be conducted only under Biosafety Level-4 (BSL-4) conditions.

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doi:10.1016/j.antiviral.2008.12.003

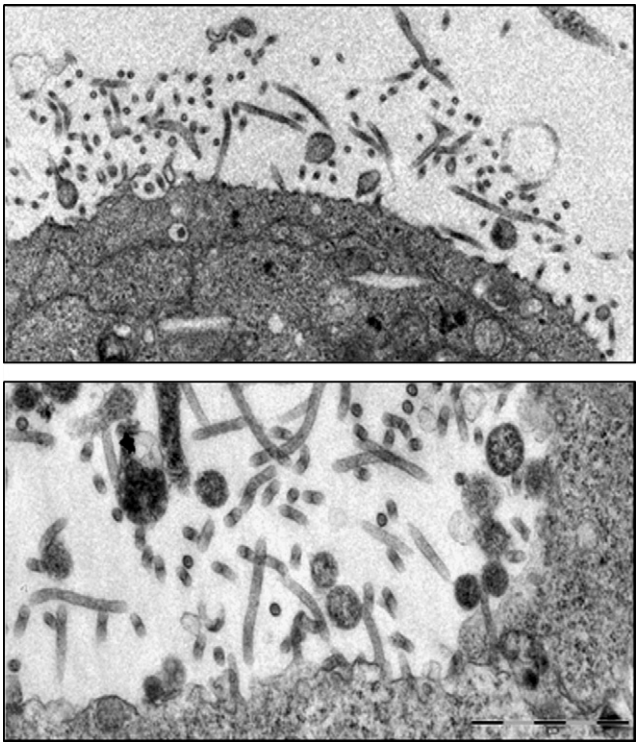

Fig. 1. Electron micrographs of EBOV VP40 VLPs budding from the surface of human 293T cells. Ultrathin sections were examined with a Philips CM-100 transmission electron microscope equipped with a KeenView digital camera system.

Nevertheless, much progress has been made toward our understanding of the molecular aspects of filovirus replication by investigating the structure and function of the viral proteins independently under less stringent conditions. A better understanding of the molecular events that govern filovirus replication will be essential for future development of vaccines and/or therapeutics. For example, our understanding of the budding process and identification of important virus–host interactions that contribute to efficient virus egress has progressed rapidly over the last decade (Chen and Lamb, 2008; Hartlieb and Weissenhorn, 2006; Jasenosky and Kawaoka, 2004; Schmitt and Lamb, 2004). One of the key approaches that has helped provide us with an abundance of valuable insight into filovirus budding has been the use of virus-like particle (VLP) budding assays, which are relatively straightforward to perform under BSL-2 conditions and accurately mimic the budding process of authentic, infectious virus. For example, human 293T cells are transfected with a plasmid encoding the filovirus VP40 matrix protein, and both cell lysates and cell culture media are harvested 24–48 h post-transfection. The media sample is then layered onto a 20% sucrose cushion, and the VLPs are pelleted through the cushion by high-speed centrifugation. VLPs can be purified further by floatation gradient centrifugation. The amount of VP40 present in the VLPs can be quantitated by immunoprecipitation and SDS-PAGE analyses, and the budding VLPs can also be visualized by electron microscopy (Fig. 1) (Johnson et al., 2006; Noda et al., 2002). Co-expression of additional filovirus proteins (*e.g.* GP and NP) along with VP40 results in their incorporation into budding VLPs and enhances the release of VLPs over that observed by expressing VP40 alone (Licata et al., 2004). Thus, this late stage of filovirus replication represents a viable and promising target for development of novel antivirals as our fundamental understanding of the budding process grows.

There are precedents for targeting late stages of virus assembly, maturation, and release with antiviral drugs. One example is Bevirimat, a novel anti-HIV-1 drug currently in clinical trials and designed to inhibit virion maturation (Salzwedel et al., 2007). A second example includes the family of neuraminidase inhibitors of influenza viruses (Tambyah, 2008). These drugs were designed to block neuraminidase activity, which is required for efficient release and spread of influenza viruses. Antivirals targeting filovirus budding would be predicted to dampen or slow down virus budding and spread in an infected host, thus allowing more time for the individual's immune system to respond and control the infection. The filovirus VP40 late (L)-domain/host interaction represents a particularly attractive target since many additional human pathogens (*e.g.* HIV-1, Lassa fever virus, and Nipah/Hendra viruses) utilize L-domains for efficient budding, and thus inhibitors of this process could potentially have broad-spectrum activity and application.

2. Functional domains of viral matrix proteins

Early studies on retroviral Gag proteins paved the way for identification of functional protein domains required for virus budding. Pioneering work from Wills and Craven as well as others helped to identify three modular domains within the Gag proteins of Rous sarcoma virus and HIV-1 that were crucial for the budding process (Accola et al., 2000; Craven and Parent, 1996; Gottlinger et al., 1991; Patnaik and Wills, 2002). The M (membrane-binding), I (interaction), and L domains were determined to be the minimal essential components of Gag required for budding (Patnaik and Wills, 2002). The M-domains of RSV and HIV-1 Gag mapped to their respective N-termini, the I-domains mapped to the region of the Gag polyprotein of RSV and HIV-1 that is involved in nucleocapsid (NC) formation, and the L-domains mapped to the N-terminal p2b region of RSV Gag and the C-terminal P6 region of HIV-1 Gag (Patnaik and Wills, 2002). The working model was that Gag localized and bound to the plasma membrane (M-domain), began to self-interact or oligomerize (I-domain), and then budded or "pinched off" (L-domain) from the cell surface (Patnaik and Wills, 2002). Results from subsequent studies supported this model of budding, not only for retroviruses, but also for other RNA viruses (Craven et al., 1999; Harty et al., 1999; Licata et al., 2003; Martin-Serrano et al., 2001; Noda et al., 2002; Schmitt and Lamb, 2004; Timmins et al., 2001). Thus, it is fairly well accepted that RNA viral matrix proteins (*e.g.* filovirus VP40) that are functional homologues of Gag and that can bud independently as VLPs must possess domains equivalent to M, I, and L to promote efficient budding of VLPs and mature virions.

3. VP40 matrix protein

VP40 is the most abundant protein in mature filovirus virions and is the key building block for virion maturation and subsequent egress (Feldmann et al., 1993). Functional homologs of filovirus VP40 include the Gag polyprotein of retroviruses, and the M proteins of rhabdoviruses and paramyxoviruses. Like the Gag proteins of Rous sarcoma virus and HIV-1 and the M protein of vesicular stomatitis virus (VSV), Ebola VP40 was found to bud from mammalian cells in the absence of other viral proteins (Harty et al., 2000). VP40 is believed to possess at least three domains essential for efficient budding: the M, I, and L-domains (Fig. 2). While the L-domain region of Ebola VP40 has been characterized extensively, precise identification of the more complex M and I domains of VP40 remains to be determined. However, recent studies have provided new insights into these structural and functional regions of filovirus VP40.