The Ebola Virus Matrix Protein VP40 Interacts With Several Host Protein Networks to Facilitate Viral Replication

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Abstract The Ebola virus (EBOV) is a lipid-enveloped virus that is filamentous in shape and belongs to the *Filoviridae* family. EBOV can cause viral hemorrhagic fever in humans and non-human primates with high rates of fatality. Using a negative sense RNA genome that encodes just eight proteins, the lipid-enveloped EBOV can enter mammalian cells and undergo efficient replication to produce more virions to sustain and spread the infection. Throughout the replication cycle, EBOV inhibits or hijacks several host protein networks to suppress the immune response and to enhance the efficiency of assembly and budding from the host cell plasma membrane. Assembly and budding of EBOV is regulated by its matrix protein VP40, which can form different structures with distinct functions in the viral life cycle. VP40 has been shown to interact with several important host proteins, and although the conformational regulation of these interactions is mostly unknown, the sites of interaction have been somewhat resolved and will serve as the basis of this review article.

Keywords Ebola virus · Filovirus · Protein-protein interaction · VP40 · Virus budding · Virus-host interactions

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Introduction

The Ebola virus (EBOV) causes severe hemorrhagic fever in humans and poses a growing threat to public health [1]. The genome of EBOV consists of a single-stranded, negative sense genome that encodes only eight proteins [2]. The genome is encapsulated by a lipid bilayer membrane that is acquired as the virus replicates and buds away from the plasma membrane of the host cell it has infected. EBOV proteins include the transmembrane glycoprotein (GP), a secreted GP (sGP) product, the matrix protein VP40, minor matrix protein VP24, and four proteins that make up the nucleocapsid: VP30, VP35, nucleoprotein, and the polymerase L. Investigations over the years are beginning to reveal how the EBOV utilizes its limited genome to achieve viral replication with such horrifying efficiency. VP40, which is the most abundant protein in the virions, can single-handedly regulate the assembly and budding step during the late stages of the viral life cycle. This ability showcases EBOV self-sufficiency as well as the sole exit mechanism for the virus, revealing potential targets for antiviral interventions [3–6]. When expressed alone, VP40 is able to assemble into virus-like particles (VLPs) on the plasma membrane, morphologically indistinguishable from authentic virions [7–9], and achieve scission from the host cell. VP40 also plays an equally vital but less understood role in the regulation of viral transcription [10•, 11]. Understanding the mechanisms by which VP40 achieves its role in the cell is of utmost importance.

Recent studies have begun to reveal that the impressive abilities of VP40 lie in its conformational plasticity [10••] as well as its strategic exploitation of host cell machinery [12••, 13, 14]. Most notably, a pivotal study by Bornholdt et al. [10••] has demonstrated that VP40 is a morphecin [15] with a native homodimeric structure as well as higher-order structures it adopts during virion assembly. VP40 harbors an N-

terminal domain that mediates dimerization and a C-terminal domain that forms an interface for extensive oligomerization (see Fig. 1). This breakthrough study revealed an elegant mechanism by which the VP40 homodimer can oligomerize into filaments that drive viral budding as well as form an alternative octameric ring structure not involved in assembly but instead in regulation of viral transcription [10••]. In concert with this structural information, studies are parsing out the mechanisms by which VP40 interacts with the plasma membrane, the critical step in EBOV evacuation of the cell, revealing an emerging model for phosphatidylserine-mediated [16•] and other plasma membrane lipid-mediated assembly and egress [6, 17]. Despite these elegant structural studies, how VP40 interacts with host cell proteins and lipids at the molecular level is mostly unknown.

VP40 Interacts With Several Important Host Protein Components

Although VP40 possesses remarkable self-sufficiency, VP40 requires the host cell machinery in order to achieve egress from the cell (Table 1). In the early stages of viral replication, VP40 interacts with Sec24C of the COPII ER-to-Golgi transport system likely via amino acid residues 303-LTMVI-307 in the VP40 C-terminal domain [14]. This transport mechanism is thought to be necessary for VP40 to localize to the plasma membrane and bud from the cell. It is still unclear how VP40 is later delivered to the plasma membrane after transport to the Golgi by the COPII vesicle system, but it is hypothesized that VP40 may co-opt the COPII system to traffic all the way to the plasma membrane as Sec24C shows co-localization with VP40 at the plasma membrane as does the COPII protein Sec23a [14]. Additionally, dominant negative studies of the COPII transport pathway inhibited VP40 accumulation at the plasma membrane.

Once at the plasma membrane, several important host protein interactions have been discovered for VP40. VP40 harbors three late domains (L-domains) at its N-terminus. This includes a P(T/S)AP motif (7-PTAP-10), a PPxY motif (10-PPEY-13), and a YPx_(n)L/I motif (18-YPx_(n)I-26). VP40 has been shown to physically interact with actin remodeling scaffold protein, IQGAP1, via its late-domain PPxY motif (10-PPEY-13) [18•]. This study revealed that VP40 may exploit the actin cytoskeleton to facilitate viral assembly and egress as knockdown of endogenous IQGAP-precluded VLP production [18•]. Indeed, there is evidence that mCherry-actin coordinates the movement and assembly of partially assembled VLPs as determined by live-cell single-particle tracking experiments [19]. During the late stages of viral replication, VP40 has been shown to interact with the endosomal sorting complex required for transport (ESCRT) machinery via its two overlapping L-domain motifs, PTAP and PPxY [3, 13, 20, 21], as well as through a recently discovered third L-domain motif, YPxL/I [12••], to mediate scission from the cell. There is also evidence that VP40 requires post-translational modifications by the host cell as tyrosine-13 phosphorylation via c-Abl1 is necessary for efficient release of VLPs and productive replication of the EBOV [22]. In addition to all these interactions, there may be other undefined host factors involved in VP40 trafficking and egress as regions critical for VP40 function have been identified [23–25] but the mechanisms remain unknown.

How Does Virus Particle Scission From the Plasma Membrane Occur?

Among the many tasks VP40 needs to accomplish, it is of interest and controversial how VP40 manages to execute the critical scission step from the host cell membrane. VP40 possesses an overlapping L-domain at its

Fig. 1 Different structural conformations of the EBOV matrix protein VP40 in the virus life cycle. VP40 is a dimer (PDB ID: 4LDB) [10••], which is mediated by an N-terminal domain hydrophobic interface. The dimer is necessary for localization and/or transport to the plasma membrane [10••]. Interactions between VP40 C-terminal domain cationic residues and the anionic plasma membrane interface are

purported to induce structural rearrangement into VP40 filaments [6, 10••]. VP40 can also form an octameric ring upon interactions with RNA, and this structure is mediated with a different N-terminal domain interface [10••]. The octameric ring can regulate the rate of viral transcription [10••, 11]

Table 1 Several regions of VP40 have been implicated in selective interactions with host proteins

VP40 amino acids	Interaction partner	Mutational effects	Reference(s)
7-PTAP-10	Tsg101	Lowers budding efficiency	[13, 26]
10-PPEY-13	Nedd4, IQGAP1	Lowers budding efficiency	[18•, 26, 27, 41••]
18-YPx _(n) I-26	ALIX	Greatly reduces budding	[12••]
Tyr ¹³ phosphorylation	c-Abl1 is the enzyme that phosphorylates Tyr. Consequences of Tyr phosphorylation on protein-protein or lipid-protein interactions are unknown	Greatly reduces budding	[22]
303-LTMVI-307	Sec24c	Reduces plasma membrane localization of VP40	[14]

N-terminus (amino acids 7-PTAPPEY-13) containing a PTAP motif and a PPxY motif that have been shown to recruit Tsg101 [26] of the ESCRT I machinery and the Nedd4-1 E3 ubiquitin ligase [27], respectively, both of which are important for VLP production in EBOV VP40 as well as other viral matrix proteins. It has been hypothesized and demonstrated that these L-domains mediate the recruitment of the ESCRT machinery to facilitate scission from the host cell, much as ESCRT facilitates scission during multi-vesicular body formation and cell division [28]. Until recently, it was assumed that these were the only L-domains of VP40 that enabled EBOV scission. However, an experiment using reverse-engineered EBOV with mutations in either the PTAP or PPxY domain of VP40 or both challenged this idea as mutating these L-domains only attenuates EBOV replication in cell culture by 1 log unit compared to WT [29]. This suggested that perhaps an L-domain in one of the other viral proteins may be contributing or possibly VP40 may achieve scission without the aid of the host cell. To the credit of the latter hypothesis, VP40 alone has been shown to mediate budding and scission into synthetic vesicles recapitulating plasma membrane lipid composition [30, 31]. However, it was revealed recently that VP40 possesses a third L-domain, a YPxL/I motif (18-YPVRSNSTI-26), which has been shown to mediate interactions with the early ESCRT protein Alix [12••]. Overexpression of Alix was able to rescue VLP

production of PTAP and PPxY-deficient VP40, providing an explanation as to how L-domain-deficient VP40 in reverse-engineered EBOV could achieve efficient replication. Nevertheless, it remains unclear if these L-domains serve redundant, cooperative, or independent roles in the scission event or if VP40 possesses L-domain-independent scission activity as well.

Timeframe of VP40-Mediated Assembly and Budding

As the assembly of virus particles occurs over a short time-scale and assembly events are asynchronous, which is problematic for conventional biochemical and fixed-cell microscopy techniques, there remain significant gaps in our understanding of the kinetic and chronological mechanisms of these events. However, advances in live-cell fluorescence microscopy are overcoming these obstacles and have already been utilized to study the assembly kinetics of human immunodeficiency virus (HIV) [16•, 32–39] and other retroviruses [39]. These investigators have established an experimental framework with which to study the assembly events of viruses as well as host cell interactions. In order to maximize the signal-to-noise ratio at the plasma membrane, these experiments primarily utilize total internal reflection fluorescence (TIRF) microscopy, which selectively excites fluorophores only at or near the plasma membrane. This has been shown to provide robust spatiotemporal analysis of HIV virion assembly, allowing the identification of markers for different stages of the assembly process and the characterization of host cell participation using dual-fluorescence experimental systems. For instance, it has been revealed in equine infectious anemia virus (EIAV) that ESCRT components are recruited differentially [39]: Alix is gradually incorporated into the EIAV Gag VLP throughout assembly, while ESCRT III components as well as the ATPase VPS4A are recruited transiently in a pulse-like manner at the end of assembly; after which, scission occurs and ESCRT III components dissociate from the assembly site. Additionally, this allows for the system to be perturbed at various levels of the assembly process to assess the effects in a spatiotemporal manner. For instance, expression of dominant-negative VPS4A stalled ESCRT III recycling and prevented HIV Gag VLP scission, suggesting that VPS4A plays a direct role in viral membrane scission [39]. These technologies and experimental methodologies show promise for elucidating the spatiotemporal timeline of EBOV VP40 assembly and egress. Studies to this effect will help fill in the gaps of our understanding of how VP40 structure, lipid binding, and host cell interaction interplay to achieve its task.

Conclusions

Future drug and vaccine development may be tied to understanding how the matrix protein VP40 orchestrates both budding and egress of the virus as well as regulation of viral transcription. Inhibition of either structure would be detrimental to viral replication so understanding the molecular basis of VP40-host interactions may be very important for the development of new therapeutics. Moreover, different types of filoviruses may utilize the same host components for budding or transcriptional control, suggesting that newly developed antivirals may be useful in more than one type of filovirus infection. This approach has been effective for arenaviruses where an inhibitor of Tsg101 interactions with the Junin virus protein's late domain inhibited budding of Junin virus VLPs [40]. Moreover, this inhibitor was effective at blocking EBOV VLPs [40], suggesting that targeting common host cell interactions among viruses could have an impact as a broad-spectrum antiviral. Indeed, small molecules generated to block PPxY-Nedd4 interactions were effective at inhibiting budding of several different viruses [41•].

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Compliance with Ethics Guidelines

Conflict of Interest EBOV research in the Stahelin lab has been supported by the NIH (AI081077).

Human and Animal Rights and Informed Consent This article contains no studies with human or animal subjects performed by the author.

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