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Review

Molecular mechanisms of filovirus cellular trafficking

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Abstract

The filoviruses, Ebola and Marburg, are two of the most pathogenic viruses, causing lethal hemorrhagic fever in humans. Recent discoveries suggest that filoviruses, along with other phylogenetically or functionally related viruses, utilize a complex mechanism of replication exploiting multiple cellular components including lipid rafts, endocytic compartments, and vacuolar protein sorting machinery. In this review, we summarize these recent findings and discuss the implications for vaccine and therapeutics development.

Keywords: Filovirus; Assembly; Budding; Viral entry; Rafts; Endocytic pathway

1. Introduction

The filoviruses Ebola (EBOV) and Marburg (MARV) are non-segmented negative-strand RNA viruses that cause severe hemorrhagic fever in humans and non-human primates. High mortality rates, significant transmissibility of disease, and lack of therapeutic and preventive measures make filoviruses a serious potential threat to public health. Natural outbreaks, although still fairly limited, have been on the rise in recent years (www.who.int/disease-outbreak-news/disease_indices/ebol_index.html). Reportedly, the former USSR had developed a weaponized form of MARV, giving rise to the concern that these highly pathogenic viruses can be used as agents of mass casualty or bioterrorism [1].

The 19-kb genome of filoviruses consists of seven genes: nucleoprotein (NP), viral proteins VP35, VP40, glycoprotein (GP), VP30, VP24 and RNA polymerase (L), encoding seven proteins in MARV and eight proteins in EBOV. The additional Ebola protein is a secreted truncated form of the envelope glycoprotein (sGP) generated by reading of unedited mRNA. The ribonucleoprotein complex consists of NP, VP35, VP30, and L [2]. This nucleocapsid is surrounded by an envelope, which originates from cellular membranes. A lattice of the matrix protein VP40 and possibly VP24 lies between the nucleocapsid and this envelope. The only viral protein detected so far on the surfaces of virus and infected cells is GP, which also appears to be the principal (but not the only) target of protective immune responses [2]. One obstacle in the development of preventive and therapeutic strategies against filoviruses is a limited understanding of the function of viral proteins and the molecular details of the interaction of virus and infected host cell. In recent years, more attention has been given to the molecular mechanisms of filovirus pathogenesis. Additionally, many lessons can be inferred from the more intensely studied viruses of the same order, *Mononegavirales*, such as paramyxoviruses and rhabdoviruses. Recent findings on the structure of fusion domains as well as the role of lipid rafts in viral life cycle also suggest interesting similarities between filoviruses and retroviruses. Summarizing the recent progress on filovirus research and related mechanisms in other viruses, in this review, we will focus on the molecular mechanisms of filovirus entry, assembly and budding, as well as modulation of cellular signaling by viral proteins. For the mechanism of RNA replication and transcription, we refer readers to recent publications [3–5].

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14. ABSTRACT

The filoviruses, Ebola and Marburg, are two of the most pathogenic viruses, causing lethal hemorrhagic fever in humans. Recent discoveries suggest that filoviruses, along with the phylogenetically or functionally related viruses, utilize a complex mechanism of replication, exploiting multiple cellular components, including lipid rafts, endocytic compartments, and vacuolar protein-sorting machinery. In this review, we summarize these recent findings and discuss the implications for vaccine and therapeutics development.

15. SUBJECT TERMS

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2. Viral entry

2.1. Viral receptors

Filoviruses appear to enter cells through interaction with specific cell surface receptors [6]. Target cells include myeloid cells such as monocyte/macrophages, dendritic cells (Bosio et al., manuscript submitted), hepatocytes, and endothelial cells [2]. In contrast, lymphocytes are resistant to filovirus infections [2,7]. Although information on the nature of cellular receptors for filoviruses is limited, such receptor(s) appear to be protein(s), most likely glycosylated, with a lineage-restricted expression pattern [8,9]. Due to the highly pathogenic nature of filoviruses and their classification as biosafety level-4 agents, detailed entry studies for these viruses have been only performed by pseudotyping. Using such an approach, folate receptor α (FRα) was recently identified as a cofactor for filovirus entry [7]. FRα renders Jurkat cells permissive to filovirus GP-pseudotyped HIV, although these FRα-expressing cells are poorly infected by live virus [7]. While blocking FRα in this reconstitution system inhibits pseudovirus entry, attempts to block significantly the wild-type filoviral entry of naturally permissive cells were unsuccessful (A. Schmaljohn, unpublished observations). Moreover, some permissive cells such as hepatocytes either do not express FRα or express it at low levels [7]. Therefore, FRα does not seem to be "the" filovirus receptor, but rather a redundant cofactor that might be essential only under certain circumstances. Filoviruses may utilize a combination of receptors and cofactors with a certain level of redundancy, analogously to HIV. It must be noted that data obtained with pseudotypes cannot be entirely extrapolated to the authentic virus. Pseudotypes normally possess a morphology similar to the parental virus rather than the virus from which the envelope protein originates. For example, vesicular stomatitis virus (VSV) pseudotyped with Ebola GP is morphologically identical to VSV [8]. As a result of distinct morphology, the conformation and array of glycoprotein molecules on pseudotyped particles can be quite different from authentic virus, and this may affect their interaction with cellular receptors and the entry process. Therefore, it is important to confirm pseudotype experiments in live virus infections. Taken together, and despite recent progress, the full nature of filovirus receptor(s) remains unresolved. Novel approaches such as proteomics may prove useful in the search for such receptors. The recent finding that filoviruses utilize lipid rafts for entry may restrict this search to a small subset of cellular proteins.

2.2. Mechanism of entry and role of lipid rafts

Most virus families utilize the cellular endocytic machinery for initial entry and trafficking into sites of replication [10]. These mechanisms mainly include clathrin- or caveolae-mediated endocytosis, resulting in the inclusion of the virus in endosomes. These viruses then use penetration or membrane fusion to release their genomes into the cytoplasm. Other enveloped viruses such as HIV fuse directly with the plasma membrane [11]. Two studies performed with pseudotypes suggest that filoviruses use acidified endosomes for viral entry, since neutralization of the endosomal pH significantly reduced infectivity [9,12]. However, direct association of these viruses with endosomes has not been demonstrated. Moreover, these findings have yet to be verified in live virus infections.

Cholesterol-enriched regions in the lipid bilayer have been recently defined that adopt a physical state referred to as liquid-ordered phase (l_o), displaying reduced fluidity and the ability for lateral and rotational mobility [13]. These low-density, detergent-insoluble microdomains, known as lipid rafts, accommodate a selective set of molecules such as gangliosides, glycosphingolipids, glycosylphosphatidylinositol-anchored proteins, and several signaling molecules [13]. By virtue of this specific composition and physical compartmentalization, rafts play a critical role in several cellular functions, including signal transduction [13]. There is accumulating evidence that certain pathogens such as HIV, influenza virus, and SV40 use the ordered environment of cholesterol-enriched lipid rafts on the plasma membrane for cellular entry [14]. A requirement for cholesterol has also been shown for the entry of mycobacterium and Sindbis virus [14].

Our recent studies suggest a critical role for lipid rafts in filovirus entry [15]. Brief treatment of the cells with filipin and nystatin, agents known to disperse cholesterol and disrupt the integrity of lipid rafts, partially inhibited the cellular entry of EBOV and MARV [15]. Our recent unpublished observations also indicate that blocking cholesterol synthesis reduces the infectivity of filoviruses. Using HIV pseudotyped with EBOV and MARV glycoproteins, Empig and Goldsmith [16] also recently reported the association of filovirus entry with caveolae, a specialized form of rafts. In this study, the entry of pseudotypes was inhibited by PMA, an inhibitor of caveolae invagination, as well as raft-disrupting agents [16]. In addition, the kinetics of pseudotype entry was shown to be slower than that of VSV, which enters the cells through clathrin-coated pits. The slow entry rate is a characteristic of caveolae-mediated endocytosis [16]. These authors also demonstrate the colocalization of filovirus pseudotypes with caveolin [16].

The requirement of lipid rafts for viral entry may be a result of localization of receptors and cofactors in these microdomains. The HIV receptor CD4, its coreceptor CXCR4, molecules favoring HIV infection such as glycosphingolipids, and CD44 [14], as well as the SV40 receptor MHC class I [17] reside in lipid rafts. Interestingly, the proposed filovirus cofactor FRα is a raft-associated glycosylphosphatidylinositol-anchored protein [18]. Rafts may therefore promote viral entry by concentrating the viral receptors and facilitating virus binding via efficient ligation of these receptors by multimeric glycoprotein. However, the compartmentalization of viral entry may provide additional advantages for the pathogens. Accumulation of viral recep-

tors in the ordered environment of lipid rafts may promote lateral assemblies at the plasma membrane required for productive infections, concentrate the necessary cytosolic and cytoskeletal components, and enhance the fusion process by providing energetically favorable conditions. Furthermore, lipid rafts may provide a level of regulation for the entry process. It is known that caveolae do not internalize under normal culture conditions [19]. Ligation of caveolae by viral coat proteins can act as a specific trigger for the internalization of these domains as shown for SV40 [20]. It is therefore conceivable that some microorganisms may have evolved to exploit lipid microdomains, thereby acquiring a major survival advantage.

Taken together, the recent data strongly suggest a role for lipid rafts and caveolae in filovirus entry. Importantly, we have demonstrated this requirement for the authentic Marburg and Ebola viruses [15]. However, more detailed studies are needed to fully characterize the underlying mechanisms. The fact that disruption of lipid rafts can interfere with filovirus entry suggests that the integrity of these compartments or their components may be potential therapeutic targets against filovirus infections. Further characterization of the raft composition during host-virus interaction, for instance by proteomic analysis, should aid in identifying such potential targets.

2.3. Structural features of GP and implications for viral fusion

The glycoproteins of EBOV and MARV consist of two components, GP1 and GP2, which are generated by furin-mediated proteolysis and linked by disulfide bonds [21]. GP1 is considered the primary receptor-binding subunit, while GP2 provides the transmembrane anchor as well as the fusion domain [2]. In part, the receptor-binding role of GP1 is inferred from the mapping of most neutralizing antibodies to EBOV and MARV to domains of GP1 [22,23]. The GPs of the four subtypes of EBOV have approximately 60% sequence identity, whereas the identity between the Marburg and Ebola GPs is approximately 30% (Fig. 1). The organization of the two components is similar to that of other glycoproteins involved in membrane fusion such as those of

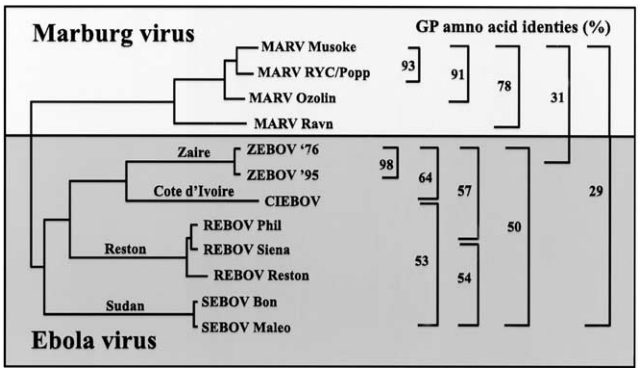

Fig. 1. Overview of sequence identity between glycoproteins of different strains of Marburg and Ebola viruses.

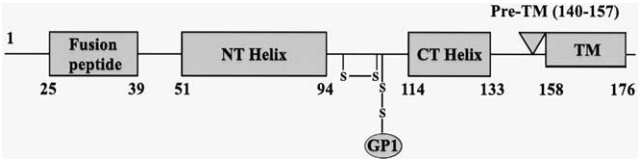

Fig. 2. Organization of structural units of the Ebola GP2 molecule.

paramyxoviruses and retroviruses [21]. According to the current model, the activation energy for membrane fusion is provided by conformational changes in the GP molecule, resulting in the accessibility of the trimeric fusion domain [24]. Consistent with this model, Ebola GP2 has been shown to form a rod-shaped trimer. The structure of the extracellular region of Ebola GP2 has been determined by X-ray crystallography [25]. The ectodomain consists of an N-terminal helix (residues 51–94) and a C-terminal helix (residues 114–133) (Fig. 2). In the trimeric complex, the three N-terminal helices form a coiled coil similar to HIV GP41. The C-terminal helix folds back on this coiled coil by packing along the grooves of the core helices to form a six-helix-bundle hairpin structure [25]. The region connecting the two helices (residues 94–114) contains an eight-residue loop created by a disulfide bridge highly homologous to murine Moloney leukemia virus [25]. This interhelical loop adopts a sophisticated structure, suggesting that its function may go beyond simply linking the two helices. The presence of the putative cysteine residue linking GP1 and GP2 (Cys 108) in this region implies that this structure may relay signals from the receptor-binding subunit to GP2 to induce the necessary conformational changes. Although conformational changes in GP2 have not yet been directly demonstrated, the striking structural homology with retroviral and HA fusion domains strongly suggests that filoviruses may also utilize such a mechanism.

While no direct evidence for membrane fusion between filoviruses and receptive cells has been reported thus far, two alternative models based on homology to HIV and influenza ectodomains were proposed [25]. In the first model, the rod-shaped trimeric conformation observed in the crystal structure represents both the prefusion and postfusion states of the ectodomain. In the prefusion state, the N- and C-termini of the ectodomain are inserted into the cellular and viral membranes, respectively, juxtaposing the two membranes. The membrane fusion then relieves the high level of stress imposed on the structure in this state. A second model, also called the umbrella model, is now commonly accepted as the fusion mechanism for HIV [24]. Evidence for this model stems from the observation that peptides corresponding to the core coiled coil and the C-terminal helix of GP41 are capable of inhibiting viral fusion [24]. According to this model, in the prefusogenic state, the ectodomain is not accessible (Fig. 3). Upon receptor binding, a conformational change is induced, resulting in exposure of the fusion peptide to the cellular membrane and opening of the hairpin structure [24]. In this hypothetical intermediate state, the hydrophobic grooves of the coiled coil as well as the C-terminal helix are

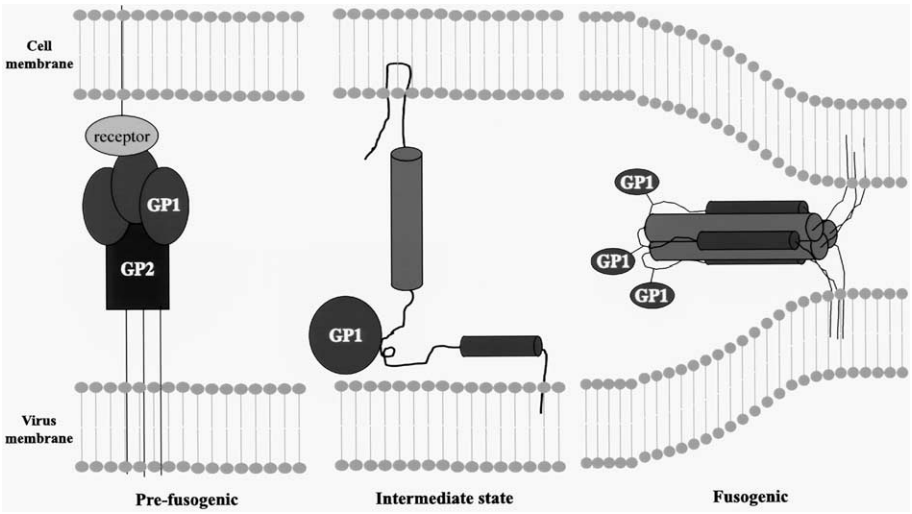

Fig. 3. Hypothetical model for the fusion process of Ebola virus, based on homology with retroviruses.

unoccupied, explaining the ability of the peptide homologs to inhibit fusion. Folding of the C-terminal helix back to the core results in juxtaposition of the viral and cellular membranes, leading to fusion. Based on the high degree of structural conservation between retroviruses and filoviruses in the ectodomain, it is plausible to predict a similar mechanism for filovirus entry. However, more detailed molecular studies are needed to substantiate this model. While the trigger for the conformational switch in HIV (receptor and coreceptor binding) and influenza (low pH) are known, the nature of such a stimulus in filoviruses remains elusive. Identifying the receptor(s) for filoviruses and solving the structure of GP1 will greatly help us understand the mechanism of entry by filoviruses and thus remain a high priority in filovirus research.

Beside the hairpin structure of the ectodomain, recent reports indicate the presence of another structural motif in the ectodomain critical for membrane fusion. A membrane proximal, tryptophan-rich region was identified in gp41 that promotes membrane fusion [26]. Deletion of this region abrogated the viral fusion without affecting the receptor binding [26]. It was recently reported that this region interacts with the raft components cholesterol and sphingomyelin, suggesting a role for this sequence in raft domain targeting of the glycoprotein [27]. Interestingly, a tryptophan-rich sequence also precedes the transmembrane domain of Ebola and Marburg GP2, suggesting another level of functional similarity between filoviruses and retroviruses. Hydrophobic-at-interface moment analysis of the pre-TM region of Ebola GP2 (140–157) suggests that this region may generate one face with strong affinity for membranes, if folded as an α-helix [28]. This study demonstrates that EBOV pre-TM is able to perturb the integrity of sphingomyelin-containing bilayers [28]. This mechanism might be involved in the preparation of viral envelope for fusion with plasma membrane [28]. A previously reported toxic effect of GP expression [29] may also relate to such membrane destabilizing. More direct studies, in particular mutational analyses in the context of virus-like particles (VLPs) or reverse genetics are needed to understand the function of this region in filovirus pathogenesis.

There has been some encouraging progress in the development of peptide-based fusion inhibitors against HIV [24]. Given the structural similarities in the ectodomain of these viruses, the pre-TM and the helical structures of GP2 must be thoroughly evaluated as potential drug targets for therapeutic intervention against filoviruses.

3. Viral assembly and budding

The generation of mature virions requires the assembly of the nucleocapsid (NC), followed by its encapsulation by an envelope derived from cellular membranes that heavily incorporates viral matrix and glycoprotein. In this section, we will first discuss the recent finding on the assembly of the filovirus NC and then the mechanisms involved in packaging and release of the mature virions.

3.1. Assembly of nucleocapsid

Our knowledge of the mechanism of filovirus assembly is very limited, in part due to the restrictions associated with working with these pathogens. The studies published in the past few years suggest that, upon replication in the cytoplasm, the assembled viral particles bud from the plasma membrane [2]. However, the exact site and mechanism of nucleocapsid assembly is poorly understood. The ribonucleoprotein (RNP) complex consists of four proteins: NP, VP30, VP35, and L. NP, VP35 and L are absolutely essential for transcription and genome replication, whereas VP30 seems to play a regulatory function during transcription [3,5]. VP24 has also been suggested to be loosely associated with RNP, since, in contrast to VP40, it was not completely removed from purified RNP under isotonic conditions [30]. The RNP complex can be visualized by electron microscopy [31] and immunofluorescence [32] in infected cells as cytoplasmic

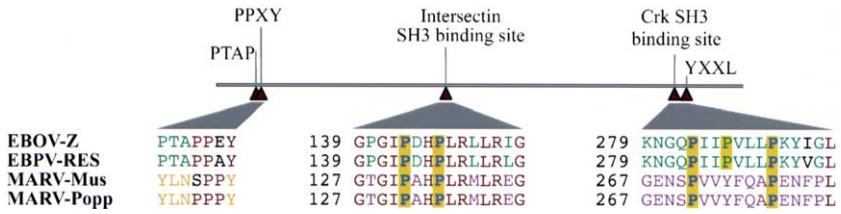

Fig. 4. Organization of functional motifs in EBOV and MARV VP40 proteins.

viral inclusions. However, little is known about the location of these inclusions with respect to subcellular compartments.

A recent study by Huang et al. sheds some new light on the mechanism of NC formation. Using a reconstitution system in 293T cells, these authors demonstrated that the expression of VP35, VP24 and NP is necessary and sufficient for the formation of intracellular Ebola nucleocapsid particles [33]. Expression of these proteins resulted in formation of large numbers of filamentous particles filling most of the cytoplasm that failed to bud from the cells [33]. Fractionation of these particles revealed that NP and VP35 were the main components of the structures [33], suggesting that VP24 may only play a transient role catalyzing the formation of RNP. This is consistent with the reported loose association of VP24 with RNP [30]. Interestingly, the formation of NC particles appeared to require *O*-glycosylation and sialation of NP [33], a posttranslational modification that was not previously recognized. It remains to be seen if the same modifications are critical in the context of live virus infection.

Upon successful nucleocapsid assembly, which is concurrent with the completion of the replication process, it is important to terminate the polymerase activity. It has been shown for respiratory syncytial virus that, late in the infection, the matrix protein (M) associates with the RNP, resulting in termination of polymerase activity. No such studies on filoviruses have been published, in part due to lack of an in vitro enzymatic assay for filovirus RNA polymerase. However, based on the close functional relationship between filo- and paramyxo-viruses, it is conceivable that MARV and EBOV VP40 may also play such a role late in the process of NC formation. Establishing a polymerase assay for filoviruses will greatly enhance our ability to study the detailed mechanisms involved in RNP assembly.

3.2. Assembly and budding of mature virion

Electron microscopy studies strongly suggest that filovirus assembly and budding take place at the plasma membrane [2,31]. Matrix proteins play a critical role in the assembly and budding of many enveloped viruses [34]. A common feature of the negative-strand RNA viruses is that their matrix proteins typically associate both with the membrane and the RNP, providing a link between the assembly of NC and mature virion [34]. Ebola VP40 has been shown to interact with the plasma membrane [35,36], and Marburg VP40 has been detected both at the plasma membrane and endocytic compartments [32]. Presumably, VP40 is associated with cytoplasmic faces of membrane, but a recent report also asserts that two monoclonal antibodies to VP40 are capable of mediating complement-dependent lysis of infected cells [37]. Multiple functional motifs can be recognized in VP40 that point toward a complex set of interactions with cellular machineries. Therefore, understanding the function of VP40 is essential for delineating the mechanism of viral assembly and budding.

3.2.1. Structural features of VP40

Ebola and Marburg VP40 consist of 326 and 303 amino acids, respectively. There is a 29% sequence homology between the two proteins. No significant homology exists to the matrix proteins of other negative-strand RNA viruses. Several motifs of interest can be recognized in VP40. These include the viral late domains PTAP (only in EBOV) and PPXY at the N -terminus, a third putative late-domain motif YXXL in EBOV, and several conserved potential SH3 binding sites (Fig. 4). Several hydrophobic and positively charged residues in the C-terminal half of the protein appear to mediate the membrane association of VP40 [36]. The crystal structure of Ebola VP40 shows that the protein consists of two domains both folding into similar β-sandwich structures [38]. Each domain consists of six antiparallel β-strands arranged in two β-sheets with an α-helix packed laterally against the β-sheets (Fig. 5) [38]. The domains are linked by an untraceable flexible linker (Fig. 5). In addition, two loops linking the β8-α5 and α7-β11 as well as the terminal five amino acids in the C-terminal domain are highly disordered, suggesting high degree of flexibility in these regions [38]. Consistent with biochemical studies [36], the structure of the C-terminal domain exhibits large solvent-exposed hydrophobic residues in areas mostly conserved between EBOV and MARV [38]. However, the N-terminal domain may also contribute to membrane association by providing a positively charged patch (Fig. 5) [38]. More detailed mutational analysis is needed to fully characterize the membrane binding properties of VP40.

The two domains of Ebola VP40 are loosely packed together with only a few side-chain interactions and a hydrophobic zipper [38]. Interestingly, the C-terminus packs onto the interdomain interface (Fig. 5). This loose interface and its peculiar architecture suggest that it may serve an important role in VP40 function. Truncating the C-terminus at position K212 results in spontaneous hexamerization of purified VP40 into ring-like structures and loss of bilayer association [36], suggesting that the C-terminal domain in the resolved monomeric structure may be a hindrance for oligomerization

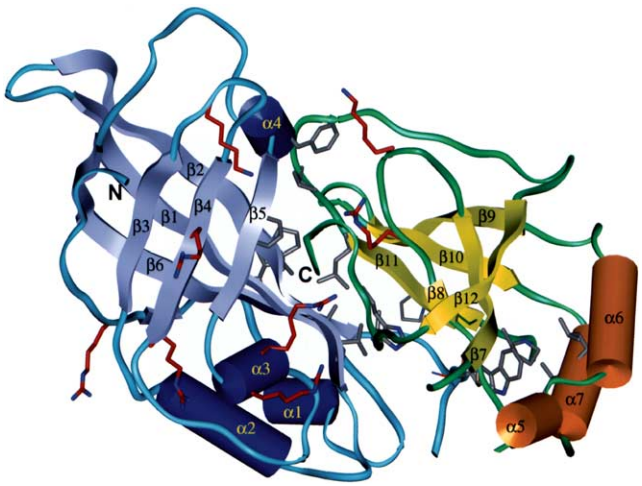

Fig. 5. The crystallographically determined structure of filovirus Ebola VP40 [38]. Structural features of the two domains are shown in different colors. N-terminal domain structural features include helices (dark blue), β sheets (lavender), and random coils/loops (light blue); C-terminal domain structural features include helices (orange), β sheets (yellow), and random coils/loops (green). Carbon atoms of hydrophobic residues lining the interface between the N- and C-terminal domains are colored gray. Carbon atoms of basic residues that may play a role in membrane interactions are colored red. The N- and C- termini are labeled N and C, respectively.

of VP40, believed to be important for viral assembly [38]. There is no evidence for proteolytic cleavage of VP40 during infection; however, conformational changes in vivo may remove this constraint, resulting in VP40 oligomerization during viral assembly. Indeed, Scianimanico et al. [39] recently demonstrated that hexamerization can be induced by destabilization of the interdomain interface by urea treatment or truncation of the C-terminal seven residues that pack onto the interface, or through binding of full-length VP40 to liposomes in vitro. These data suggest that residues 319-326 of VP40 contribute to stability of interdomain interactions, consistent with the structure data, and that a conformational change in vivo may remove this constraint during assembly. Based on these findings, these authors suggest the following three-step model for the involvement of VP40 in EBOV assembly: (i) contact of VP40 with lipid bilayer, (ii) conformational change in VP40 with the movement of the C-terminal domain with respect to the N-terminus, resulting in hexamerization, and (iii) formation of a lattice at the membrane by oligomerized VP40 via contacts between the bridging C-terminal domains and interactions with the cytoplasmic tail of GP. This model has yet to be substantiated by rational mutational analyses, based on the structural data, using VLP formation assays and reverse genetics.

3.2.2. Assembly of GP and VP40 and role of lipid rafts

The process of virus assembly and budding at the cellular membranes requires an accumulation of viral components including nucleocapsid, matrix and envelope glycoprotein in an orchestrated manner, concurrent with structural changes in the plasma membrane [34]. This process is obviously dependent on a precise coordination of the involved components. Recently, several reports indicated the involvement of lipid rafts in the assembly and release of different viruses [14]. Based on a raft-localization signal at the C-terminus of filovirus GPs, we hypothesized a role for lipid rafts in the filovirus life cycle and recently demonstrated that both EBOV and MARV indeed use these microdomains for viral assembly and release [15]. Both after transient expression of filovirus GPs as well as in EBOV- and MARV-infected cells, we observed significant association of viral proteins with lipid rafts [15]. We also demonstrated that the released virions incorporate the raft-associated molecule GM1, but not transferrin receptor, a protein excluded from lipid rafts, suggesting that rafts represent the final gateway for the exit of mature virions [15]. In this regard, the filovirus assembly seems to resemble the assembly of related paramyxoviruses as well as unrelated retroviruses. Rafts have been implicated in the assembly of HIV [14], measles [40], Sendai virus [41], and respiratory syncytial virus [42]. Given the negligible sequence homology in the structural proteins of these viruses, it is conceivable that these viruses may use distinct strategies to take advantage of the specialized environment of lipid rafts.

In retroviruses, the raft localization of the assembly complex is regulated by the association of N-terminally acylated Gag proteins [14]. There is no such acylation site in filovirus VP40. However, filoviral GPs possess dual palmitoylation sites at the end of transmembrane domain (Cys 670 and Cys 672) [43]. Therefore raft localization of VP40 may be dependent on GP or on other structural features of VP40 not previously recognized as raft targeting entities. Consistent with a possible role for GP, we observed a far more efficient release of Ebola VLPs from 293T cells when both GP and VP40 were expressed concurrently, as opposed to expression of VP40 alone [15]. VLPs generated in this manner exhibit a striking similarity to the authentic virus with typical glycoprotein spikes (Fig. 6), suggesting that this process closely resembles the assembly and release of EBOV. It is not known where the assembly of GP and VP40 takes place. Since a fraction of GP resides outside the rafts, probably in a dynamic exchange with the rafts, this pool of GP might be involved in the initial contact with VP40. This interaction and subsequent movement to the rafts may, at the same time, induce a conformational change in VP40, resulting in dissociation of the C-terminal domain from the non-raft membrane and thus removing the constraints on the formation of VP40 hexamers required for viral assembly. In this regard, it is important to know whether the raft-associated VP40 is predominantly hexameric. The unique lipid composition of rafts may facilitate molecular events leading to hexamerization, and hexameric VP40 may expose a surface with increased affinity for raft lipids. More biophysical studies are needed to understand the nature of the interactions between VP40 and raft components.

In several viruses, matrix proteins interact with the cytoplasmic tail of envelope glycoprotein [34]. There are no studies showing a direct interaction between filovirus VP40

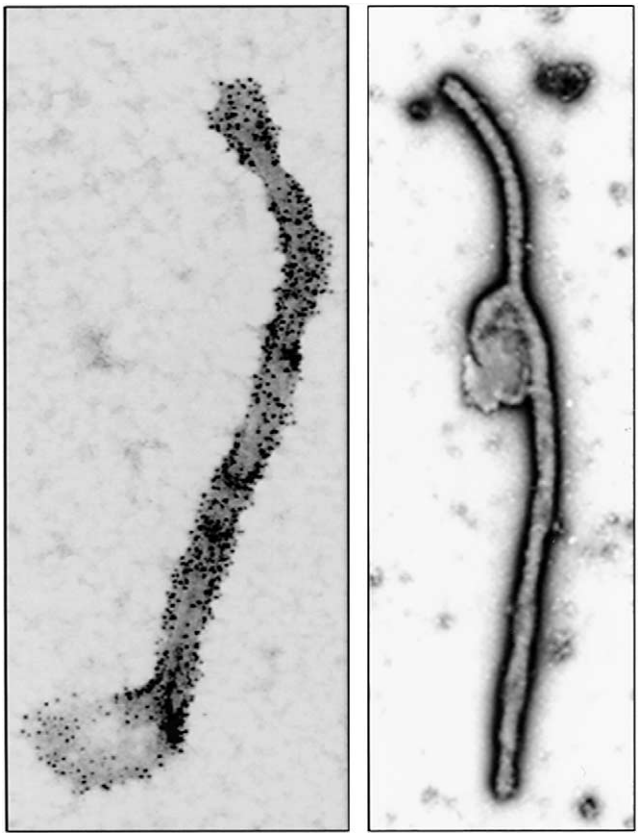

Fig. 6. Electron microscopic picture of VLPs generated by EBOV GP and VP40. In the upper panel, the GPs on the surface of VLPs are visualized by immunogold staining.

and GP. Our recent findings indicate that Ebola GP can form VLPs with Marburg VP40 and vice versa (Swenson et al., manuscript in preparation). Since there is no sequence homology between the short cytoplasmic tails of Ebola and Marburg GPs, these findings make a direct interaction mechanism highly unlikely. However, it is possible that the interaction occurs not with the cytoplasmic tail, but rather with the transmembrane region. This would require an insertion of parts of VP40 into the plasma membrane. Although there is no direct evidence supporting such a mechanism, but given the recent report on the complement-mediated lysis of infected cells with anti-VP40 antibodies [37], this potential mechanism might be worth more in-depth investigation. It is also possible that specific raft-residing cellular proteins are involved in the interaction of VP40 with lipid rafts, resulting in indirect association with GP. VP40 directly interacts with the ubiquitin ligase Nedd4 through its PPXY motif [44]. Interestingly, it has recently been shown that Nedd4 associates with rafts via its lipid-binding C2 domain [45]. Since mutation of PPXY abrogates the ability of VP40 to be released from the cells [46], it is intriguing to speculate that Nedd4 might be involved in raft targeting of VP40. Recently, Licata et al. [47] reported that TSG101 mediates the raft localization of VP40. However, what these authors refer to as rafts is in fact the total detergent-insoluble fraction that contains, besides rafts, the cytoskeletal and some nuclear proteins. Whatever the mechanism of interaction between GP and VP40, it seems that clustering of these two molecules in lipid rafts is an important trigger for the assembly of the virus.

3.2.3. Role of endocytic pathway

Recently, Kolesnikova et al. [32] reported the association of Marburg VP40 with components of the endocytic pathway. In this study, different pools of VP40 were shown to associate with nucleocapsid-containing viral inclusion, with intracellular membranes mainly in the form of multivesicular bodies (MVBs), and the plasma membrane, the latter two pools being free of NP [32]. The amount of VP40 in viral inclusions was seven times less than in mature virions, suggesting that different pools of VP40 contribute to the final particle [32]. It has been shown for VSV that a fraction of the M protein associates with the NC, while another fraction is targeted to the plasma membrane along with the GP [48]. Similarly, influenza M1 protein interacts with both membrane and RNP [49]. In this regard, data published by Kolesnikova et al. may suggest that the different pools of VP40 link the NC formation with the assembly of envelope, exploiting the endocytic machinery in this process. The nature of the interaction between VP40 and NC remains unknown. The association of VP40/NC complex with the VP40 lattice at the membrane might be governed by homotypic interactions between VP40 molecules.

The finding that MARV VP40 is associated with the MVB suggests that filoviruses may use endosomal vesicles as sites of sorting and assembly. However, no association of NC with MVBs was demonstrated [32]. It should be noted that the endosome-associated VP40 may be destined for degradation, although detection of endocytic markers in released virions suggests that this pool may be part of the assembly complex [32]. Although the endocytic pathway is mainly involved in endocytosis and the recycling of receptors, several viral proteins, including CMV proteins [50] and the replicative machinery of alphaviruses, have been shown to associate with endocytic vesicles [51]. The M protein of Sendai virus has also been detected in intracellular membrane fractions [52]. Furthermore, the viral late domain YXXL is known to associate with AP2, a critical component of the endocytic pathway [53]. Another line of evidence for endocytic pathway involvement stems from the observation that ubiquitin ligases interact with viral matrix proteins. MVBs have been proposed to act as intermediates in the receptor recycling pathways [54]. Endosomal recycling and fusion of intracellular vesicles with the plasma membrane can circulate viral assembly complexes between the plasma membrane and the endosomal-lysosomal compartments. Recycling endosomes are also linked to several cellular signaling pathways, such as small GTPases and cytoskeletal proteins [54]. This may enable the VPs to affect the cellular signaling in favor of efficient viral replication. It is intriguing that raft lipids are found in recycling endosomes [55], thus providing a possible link to the raft domains as the site of terminal assembly.

Endocytic compartments are enriched in signaling proteins with multiple protein-protein interaction domains, especially SH3 and PDZ domains. The presence of multiple conserved proline-rich motifs in VP40 suggests that such interactions might be involved in the exploitation of the endocytic pathway by filoviruses. Further evidence for involvement of the endocytic pathway in filovirus assembly stems from the study of viral late domain and is discussed below.

3.2.4. Role of viral late domains and protein sorting machinery

Viral late domains (L) were initially identified in retroviral Gag proteins and later in a number of other viruses, including rhabdo- and filoviruses [56]. Mutation of these domains causes a block late in the assembly process, resulting in inhibition of virus budding. L domains contain characteristic motifs involved in protein-protein interactions [56]. Three motifs have been recognized in L domains: PPXY, PT/SAP, and YXXL [56]. The best-characterized interactions include PTAP/TSG101 [57], PPXY with WW domains [58], and YXXL binding to AP2 [53]. Both Marburg and Ebola VP40 contain PPXY motifs at the N-terminus. Ebola VP40 also has a PTAP motif that overlaps with PPEY at the N-terminus as well as a YXXL motif in the C-terminal domain. A few studies have addressed the role of PPXY and PTAP, but there has been no report on the functional significance of YXXL in Ebola VP40.

The PPEY motif of Ebola VP40 binds to Nedd4 ubiquitin ligase in in vitro Far-Western assays [44], but association in intact cells and in the course of infection has not been demonstrated. Mutation of this motif abrogated both the Nedd4 binding as well as cellular release of VP40, suggesting a role for this interaction in viral budding [44]. This study also shows that VP40 can be ubiquitinated in vitro by Rsp5p, a yeast ortholog of Nedd4 [44]. However, ubiquitination of VP40 has not been demonstrated in vivo. While multiubiquitination, depending on the position of polymeric linkage, can destine proteins either for proteosome-mediated degradation or toward signaling pathways, monoubiquitination is involved in sorting of proteins from endocytic or biosynthetic pathways into budding vesicles such as MVBs and late endosomes [59]. Several viral matrix proteins are evidently ubiquitinated, and depletion of cellular ubiquitin inhibits retrovirus and rhabdovirus budding [56]. More studies are needed to delineate the role of the ubiquitin system in the filovirus replication cycle. However, given the published observations and the similarity between viral budding and intracellular vesicular budding, it seems logical that viruses may exploit this machinery to their advantage.

Recently, TSG101, a ubiquitin enzyme variant (UEV) domain-containing protein, was shown to interact with the PTAP motif of HIV Gag [56]. The N-terminal UEV domain of TSG101 binds PTAP peptide with intermediate affinity (27 μM) [57]. Inhibition of TSG101 expression by RNA interference [57] or overexpression of its UEV domain resulted in significant inhibition of HIV replication [60]. TSG101 is a key component of the vacuolar protein sorting (vps) pathway, in which proteins undergo a process of sorting through endosomal compartments for eventual destination for lysosomal degradation [61]. TSG101 functions as part of a large complex called ESCRT-1 [62]. This complex selects mono- and di-ubiquitinated cargo originating from either the Golgi or the endocytic pathway for incorporation into lumen of MVBs [62]. The ATPase Vps4 provides the energy for disassembly of this complex, allowing multiple rounds of sorting [63]. Interestingly, a dominant-negative Vps4 mutant can inhibit the HIV release from cells [57]. Martin-Serrano et al. [35] recently reported an interaction between Ebola VP40 and TSG101 in yeast two-hybrid assays, and recruitment of TSG101 to plasma membrane in VP40 transfected cells. A recent report shows that both PTAP and PPEY motifs are essential for release of Ebola VP40 VLPs [47]. The authors also assert that the function of VP40 late domain is independent of its position, since it is still functional when moved to the C-terminus [47]. However, these conclusions are complicated by the fact that in the mutant used in this study, the C-terminal 10 amino acids are also deleted, a region known to be important for interdomain interactions of VP40 [39]. Furthermore, the study by Licata et al. shows a role for VPS4 in Ebola release similar to those reported for retroviruses. In contrast to Ebola, Marburg VP40 lacks the PTAP motif. It has been shown that the budding of MLV, which has a PPXY motif but no PTAP, is also sensitive to dominant-negative Vps4 [57]. Therefore, viral matrix proteins and retroviral Gag proteins can enter the *vps* pathway through either late domains. These findings suggest that L domain-containing viruses may take advantage of the cellular protein sorting machinery, ubiquitin system, and the related budding mechanisms associated with MVBs for viral assembly and release. In this regard, the association of Marburg VP40 with MVBs may reflect the usage of this mechanism by filoviruses.

The NMR structure of the TSG101 UEV domain in complex with HIV-derived PTAP peptide was recently resolved [64]. The UEV domain closely resembles ubiquitin-conjugating enzyme with a C→Y substitution in the active site that renders this protein enzymatically inactive. The PTAP peptide binds in a bifurcated groove above this vestigial active site [64]. The binding of Ala and Pro is very similar to the X-Pro (X = any amino acid) binding to the corresponding pockets in WW and SH3 domains, suggesting that this interaction also follows the general rules of the proline-rich recognition domains [64,65]. The recognition site consists in general of an X-proline binding groove and a specificity pocket that bind the upstream or downstream two amino acids. The X-p binding grooves of SH3 and WW domains consist of two aromatic amino acids (Tyr, Trp, or Phe) that embrace the proline [65]. In a similar manner, the last proline of PTAP wedges between Tyr 63 and Tyr 68 of TSG101 [64]. At the same time, the first three residues and several flanking amino acids make important contacts to stabilize the interaction. The striking similarity to the mode of binding of WW and SH3 domains suggests that

PTAP/TSG101 interaction can be targeted in a similar manner. Nguyen et al. [66] discovered that SH3 domains do not exclusively recognize prolines, but would accept a variety of amide N-substituted residues. Based on this fact, a number of peptide-based and peptoid inhibitors were developed, some of which bind SH3 domains with 100-fold increased affinity [66]. It is conceivable that similar strategies can be used to target TSG101/PTAP interaction. The resolution of the NMR structure of the complex also paves the way to rational drug design. Given that abrogation of TSG101 expression dramatically inhibits HIV replication [57], targeting its interaction with PTAP may prove a promising therapeutic approach. The interaction is of moderate affinity (Kd 27 μM), and its disruption by small molecules is entirely realistic. If a critical role for TSG101 in the filovirus life cycle can be established, the disruption of TSG101/VP40 interaction must be seriously pursued as a therapeutic approach.

4. Filoviruses and cellular signaling

Viral proteins are often viewed as serving the sole function of viral replication in the cells. While this is certainly the main concern of the virus, these pathogens often have to modify cellular signaling environment to acquire a growth advantage. Due to the importance of the interferon (IFN) signaling pathway for cellular antiviral defense, we briefly review the recent findings on the inhibition of this pathway by filoviruses.

In many viruses, certain viral proteins subvert the immune system to evade detection and/or destruction by the host. Although there are several branches of the immune response targeted by viruses, one of the best-studied targets is the IFN signaling pathway. Both type I (IFN-α and IFN-β) and type II (IFN-γ) interferons are essential for effective antiviral responses, and their production relies on a complex pathway within the host cell. Different stages of the IFN signaling pathway can be blocked by different viruses—from binding and sequestration of IFN receptor homologs, as seen in vaccinia species, to the formation of specific protein complexes such as interferon-stimulated-gene factor (ISGF) and gamma-activated factor (GAF), as observed in infection of paramyxoviruses and human parainfluenza virus [67].

Recent reports have indicated that EBOV also targets the IFN pathway, inhibiting the formation of both ISGF and GAF, as concluded by reduced binding of IFN-stimulating response elements and gamma-activated sequences and decreased secretion of IFN-α [68]. Further studies have shown that much of this inhibition can be attributed to VP35. Expression of VP35 in cells infected with an influenza deletion mutant lacking the IFN inhibitory protein NS1 restored the inhibition of IFN-α signaling [69].

To date, the specific mechanism by which VP35 disrupts IFN signaling has not been identified; however, based on observations of the mechanisms used by other viral proteins there are several possibilities. For example, the V protein of simian virus 5 targets STAT1 for proteosome-mediated degradation, while CMV reduces the levels of both JAK1 and p48, preventing the formation of ISGF3 and GAF [67]. On the other hand, VP35 may bind to double-stranded RNA, an intermediate species during viral replication that activates the IFN signaling pathway, resulting in its sequestration, similarly to influenza NS1 and the E3L protein of vaccinia virus [67]. In the absence of double-stranded RNA, protein kinase R (PKR) does not phosphorylate its substrates, ultimately resulting in the inability to activate NF-κB. Lack of NF-κB activation may lead to inability of the infected cell to produce other key cytokines crucial for mounting an effective antiviral response.

In addition to VP35, it is possible that other EBOV proteins may interfere with IFN signaling. Although immunomodulatory functions have not been assigned to other EBOV proteins, given the complexity of the IFN pathway, it is possible that EBOV proteins interact and disrupt evolution and maintenance of an antiviral state induced by IFN as well as other cytokines and host immune molecules. Further study of possible interference of filovirus proteins with cellular signaling pathways is therefore of paramount importance for understanding the filoviral life cycle.

5. Concluding remarks

Although the molecular details of the function of filovirus genes remain widely unknown, recent molecular studies summarized in this review unravel the mysteries of these highly pathogenic viruses. In particular, the functional similarities with other members of the order *Mononegavirales* and the distant retroviruses provide a great opportunity to perform more targeted studies on the filovirus life cycle. New findings on the cellular factors involved in viral replication open a new avenue of research both for understanding the replication mechanisms as well as for devising new therapeutic strategies. The parallels across the evolution spectrum from filoviruses to retroviruses, in utilizing such mechanisms, indicate the exciting possibility of developing wide-spectrum antiviral agents targeting the common cellular pathways.

References

[1] K. Alibek, S. Handelman, Biohazard: the chilling true story of the largest covert biological weapons program in the world, told from the inside by the man who ran it, Random House, New York, NY, 1999, p. 319.

[2] H. Feldmann, M.P. Kiley, Classification, structure, and replication of filoviruses, Curr. Top. Microbiol. Immunol. 235 (1999) 1–21.

[3] E. Muhlberger, M. Weik, V.E. Volchkov, H.D. Klenk, S. Becker, Comparison of the transcription and replication strategies of marburg virus and Ebola virus by using artificial replication systems, J. Virol. 73 (1999) 2333–2342.

[4] G. Neumann, M.A. Whitt, Y. Kawaoka, A decade after the generation of a negative-sense RNA virus from cloned cDNA—what have we learned? J. Gen. Virol. 83 (2002) 2635–2662.

[5] M. Weik, J. Modrof, H.D. Klenk, S. Becker, E. Muhlberger, Ebola virus VP30-mediated transcription is regulated by RNA secondary structure formation, J. Virol. 76 (2002) 8532–8539.

[6] H. Feldmann, V.E. Volchkov, V.A. Volchkova, H.D. Klenk, The glycoproteins of Marburg and Ebola virus and their potential roles in pathogenesis, Arch. Virol. 15 (Suppl) (1999) 159–169.

[7] S.Y. Chan, C.J. Empig, F.J. Welte, R.F. Speck, A. Schmaljohn, J.F. Kreisberg, M.A. Goldsmith, Folate receptor-α is a cofactor for cellular entry by Marburg and Ebola viruses, Cell 106 (2001) 117–126.

[8] A. Takada, C. Robison, H. Goto, A. Sanchez, K.G. Murti, M.A. Whitt, Y. Kawaoka, A system for functional analysis of Ebola virus glycoprotein, Proc. Natl. Acad. Sci. USA 94 (1997) 14764–14769.

[9] S.Y. Chan, R.F. Speck, M.C. Ma, M.A. Goldsmith, Distinct mechanisms of entry by envelope glycoproteins of Marburg and Ebola (Zaire) viruses, J. Virol. 74 (2000) 4933–4937.

[10] S.B. Sieczkarski, G.R. Whittaker, Dissecting virus entry via endocytosis, J. Gen. Virol. 83 (2002) 1535–1545.

[11] P.J. Maddon, J.S. McDougal, P.R. Clapham, A.G. Dalgleish, S. Jamal, R.A. Weiss, R. Axel, HIV infection does not require endocytosis of its receptor, CD4, Cell 54 (1988) 865–874.

[12] R.J. Wool-Lewis, P. Bates, Characterization of Ebola virus entry by using pseudotyped viruses: identification of receptor-deficient cell lines, J. Virol. 72 (1998) 3155–3160.

[13] K. Simons, D. Toomre, Lipid rafts and signal transduction, Nat. Rev. 1 (2000) 31–39.

[14] S.M. Campbell, S.M. Crowe, J. Mak, Lipid rafts and HIV-1: from viral entry to assembly of progeny virions, J. Clin. Virol. 22 (2001) 217–227.

[15] S. Bavari, C.M. Bosio, E. Wiegand, G. Ruthel, A.B. Will, T.W. Geisbert, M. Hevey, C. Schmaljohn, A. Schmaljohn, M.J. Aman, Lipid raft microdomains: a gateway for compartmentalized trafficking of Ebola and Marburg viruses, J. Exp. Med. 195 (2002) 593–602.

[16] C.J. Empig, M.A. Goldsmith, Association of the caveola vesicular system with cellular entry by filoviruses, J. Virol. 76 (2002) 5266–5270.

[17] E. Stang, J. Kartenbeck, R.G. Parton, Major histocompatibility complex class I molecules mediate association of SV40 with caveolae, Mol. Biol. Cell 8 (1997) 47–57.

[18] B.J. Nichols, A.K. Kenworthy, R.S. Polishchuk, R. Lodge, T.H. Roberts, K. Hirschberg, R.D. Phair, J. Lippincott-Schwartz, Rapid cycling of lipid raft markers between the cell surface and Golgi complex, J. Cell. Biol. 153 (2001) 529–541.

[19] P. Thomsen, K. Roepstorff, M. Stahlhut, B. van Deurs, Caveolae are highly immobile plasma membrane microdomains, which are not involved in constitutive endocytic trafficking, Mol. Biol. Cell 13 (2002) 238–250.

[20] L. Pelkmans, J. Kartenbeck, A. Helenius, Caveolar endocytosis of simian virus 40 reveals a new two-step vesicular-transport pathway to the ER, Nat. Cell Biol. 3 (2001) 473–483.

[21] V.E. Volchkov, H. Feldmann, V.A. Volchkova, H.D. Klenk, Processing of the Ebola virus glycoprotein by the proprotein convertase furin, Proc. Natl. Acad. Sci. USA 95 (1998) 5762–5767.

[22] J.A. Wilson, M. Hevey, R. Bakken, S. Guest, M. Bray, A.L. Schmaljohn, M.K. Hart, Epitopes involved in antibody-mediated protection from Ebola virus, Science 287 (2000) 1664–1666.

[23] M. Hevey, D. Negley, J. Geisbert, P. Jahrling, A. Schmaljohn, Antigenicity and vaccine potential of Marburg virus glycoprotein expressed by baculovirus recombinants, Virology 239 (1997) 206–216.

[24] D.M. Eckert, P.S. Kim, Mechanisms of viral membrane fusion and its inhibition, Annu. Rev. Biochem. 70 (2001) 777–810.

[25] W. Weissenhorn, A. Carfi, K.H. Lee, J.J. Skehel, D.C. Wiley, Crystal structure of the Ebola virus membrane fusion subunit, GP2, from the envelope glycoprotein ectodomain, Mol. Cell 2 (1998) 605–616.

[26] K. Salzwedel, J.T. West, E. Hunter, A conserved tryptophan-rich motif in the membrane-proximal region of the human immunodeficiency virus type 1 gp41 ectodomain is important for Env-mediated fusion and virus infectivity, J. Virol. 73 (1999) 2469–2480.

[27] A. Saez-Cirion, S. Nir, M. Lorizate, A. Agirre, A. Cruz, J. Perez-Gil, J.L. Nieva, Sphingomyelin and cholesterol promote HIV-1 gp41 pretransmembrane sequence surface aggregation and membrane restructuring, J. Biol. Chem. 277 (2002) 21776–21785.

[28] A. Saez-Cirion, M.J. Gomara, A. Agirre, J.L. Nieva, Pretransmembrane sequence of Ebola glycoprotein interfacial hydrophobicity distribution and interaction with membranes, FEBS Lett. 533 (2002) 47–53.

[29] Z.Y. Yang, H.J. Duckers, N.J. Sullivan, A. Sanchez, E.G. Nabel, G.J. Nabel, Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury, Nat. Med. 6 (2000) 886–889.

[30] M.P. Kiley, N.J. Cox, L.H. Elliott, A. Sanchez, R. DeFries, M.J. Buchmeier, D.D. Richman, J.B. McCormick, Physicochemical properties of Marburg virus: evidence for three distinct virus strains and their relationship to Ebola virus, J. Gen. Virol. 69 (Pt 8) (1988) 1957–1967.

[31] T.W. Geisbert, P.B. Jahrling, Differentiation of filoviruses by electron microscopy, Virus Res. 39 (1995) 129–150.

[32] L. Kolesnikova, H. Bugany, H.D. Klenk, S. Becker, VP40, the matrix protein of Marburg virus, is associated with membranes of the late endosomal compartment, J. Virol. 76 (2002) 1825–1838.

[33] Y. Huang, L. Xu, Y. Sun, G.J. Nabel, The assembly of Ebola virus nucleocapsid requires virion-associated proteins 35 and 24 and posttranslational modification of nucleoprotein, Mol. Cell 10 (2002) 307–316.

[34] H. Garoff, R. Hewson, D.J. Opstelten, Virus maturation by budding, Microbiol. Mol. Biol. Rev. 62 (1998) 1171–1190.

[35] J. Martin-Serrano, T. Zang, P.D. Bieniasz, HIV-1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress, Nat. Med. 7 (2001) 1313–1319.

[36] R.W. Ruigrok, G. Schoehn, A. Dessen, E. Forest, V. Volchkov, O. Dolnik, H.D. Klenk, W. Weissenhorn, Structural characterization and membrane binding properties of the matrix protein VP40 of Ebola virus, J. Mol. Biol. 300 (2000) 103–112.

[37] I.A. Razumov, E.F. Belanov, N.I. Bormotov, E.I. Kazachinskaia, Detection of antiviral activity of monoclonal antibodies, specific to Marburg virus proteins, Vopr. Virusol. 46 (2001) 33–37.

[38] A. Dessen, V. Volchkov, O. Dolnik, H.D. Klenk, W. Weissenhorn, Crystal structure of the matrix protein VP40 from Ebola virus, EMBO J. 19 (2000) 4228–4236.

[39] S. Scianimanico, G. Schoehn, J. Timmins, R.H. Ruigrok, H.D. Klenk, W. Weissenhorn, Membrane association induces a conformational change in the Ebola virus matrix protein, EMBO J. 19 (2000) 6732–6741.

[40] S.N. Manie, S. Debreyne, S. Vincent, D. Gerlier, Measles virus structural components are enriched into lipid raft microdomains: a potential cellular location for virus assembly, J. Virol. 74 (2000) 305–311.

[41] A. Ali, D.P. Nayak, Assembly of Sendai virus: M protein interacts with F and HN proteins and with the cytoplasmic tail and transmembrane domain of F protein, Virology 276 (2000) 289–303.

[42] G. Brown, H.W. Rixon, R.J. Sugrue, Respiratory syncytial virus assembly occurs in GM1-rich regions of the host-cell membrane and alters the cellular distribution of tyrosine phosphorylated caveolin-1, J. Gen. Virol. 83 (2002) 1841–1850.

[43] H. Ito, S. Watanabe, A. Takada, Y. Kawaoka, Ebola virus glycoprotein: proteolytic processing, acylation, cell tropism, and detection of neutralizing antibodies, J. Virol. 75 (2001) 1576–1580.

[44] R.N. Harty, M.E. Brown, G. Wang, J. Huibregtse, F.P. Hayes, A PPxY motif within the VP40 protein of Ebola virus interacts physically and functionally with a ubiquitin ligase: implications for filovirus budding, Proc. Natl. Acad. Sci. USA 97 (2000) 13871–13876.

[45] P.J. Plant, F. Lafont, S. Lecat, P. Verkade, K. Simons, D. Rotin, Apical membrane targeting of Nedd4 is mediated by an association of its C2 domain with annexin XIIIb, J. Cell. Biol. 149 (2000) 1473–1484.

[46] L.D. Jasenosky, G. Neumann, I. Lukashevich, Y. Kawaoka, Ebola virus VP40-induced particle formation and association with the lipid bilayer, J. Virol. 75 (2001) 5205–5214.

[47] J.M. Licata, M. Simpson-Holley, N.T. Wright, Z. Han, J. Paragas, R.N. Harty, Overlapping motifs (PTAP and PPEY) within the Ebola virus VP40 protein function independently as late budding domains: involvement of host proteins TSG101 and VPS-4, J. Virol. 77 (2003) 1812–1819.

[48] L.D. Chong, J.K. Rose, Membrane association of functional vesicular stomatitis virus matrix protein in vivo, J. Virol. 67 (1993) 407–414.

[49] F. Baudin, I. Petit, W. Weissenhorn, R.W. Ruigrok, In vitro dissection of the membrane and RNP binding activities of influenza virus M1 protein, Virology 281 (2001) 102–108.

[50] A. Fraile-Ramos, A. Pelchen-Matthews, T.N. Kledal, H. Browne, T.W. Schwartz, M. Marsh, Localization of HCMV UL33 and US27 in endocytic compartments and viral membranes, Traffic 3 (2002) 218–232.

[51] S. Froshauer, J. Kartenbeck, A. Helenius, Alphavirus RNA replicase is located on the cytoplasmic surface of endosomes and lysosomes, J. Cell. Biol. 107 (1988) 2075–2086.

[52] R.A. Lamb, P.W. Choppin, The synthesis of Sendai virus polypeptides in infected cells. II. Intracellular distribution of polypeptides, Virology 81 (1977) 371–381.

[53] B.A. Puffer, L.J. Parent, J.W. Wills, R.C. Montelaro, Equine infectious anemia virus utilizes a YXXL motif within the late assembly domain of the Gag p9 protein, J. Virol. 71 (1997) 6541–6546.

[54] I. Mellman, Endocytosis and molecular sorting, Annu. Rev. Cell Dev. Biol. 12 (1996) 575–625.

[55] R. Gagescu, N. Demaurex, R.G. Parton, W. Hunziker, L.A. Huber, J. Gruenberg, The recycling endosome of Madin-Darby canine kidney cells is a mildly acidic compartment rich in raft components, Mol. Biol. Cell 11 (2000) 2775–2791.

[56] E.O. Freed, Viral late domains, J. Virol. 76 (2002) 4679–4687.

[57] J.E. Garrus, U.K. von Schwedler, O.W. Pornillos, S.G. Morham, K.H. Zavitz, H.E. Wang, D.A. Wettstein, K.M. Stray, M. Cote, R.L. Rich, D.G. Myszka, W.I. Sundquist, Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding, Cell 107 (2001) 55–65.

[58] V. Kanelis, D. Rotin, J.D. Forman-Kay, Solution structure of a Nedd4 WW domain-ENaC peptide complex, Nat. Struct. Biol. 8 (2001) 407–412.

[59] L. Hicke, A new ticket for entry into budding vesicles-ubiquitin, Cell 106 (2001) 527–530.

[60] D.G. Demirov, A. Ono, J.M. Orenstein, E.O. Freed, Overexpression of the N-terminal domain of TSG101 inhibits HIV-1 budding by blocking late domain function, Proc. Natl. Acad. Sci. USA 99 (2002) 955–960.

[61] S.K. Lemmon, L.M. Traub, Sorting in the endosomal system in yeast and animal cells, Curr. Opin. Cell Biol. 12 (2000) 457–466.

[62] D.J. Katzmann, M. Babst, S.D. Emr, Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I, Cell 106 (2001) 145–155.

[63] N. Bishop, P. Woodman, ATPase-defective mammalian VPS4 localizes to aberrant endosomes and impairs cholesterol trafficking, Mol. Biol. Cell 11 (2000) 227–239.

[64] O. Pornillos, S.L. Alam, R.L. Rich, D.G. Myszka, D.R. Davis, W.I. Sundquist, Structure and functional interactions of the Tsg101 UEV domain, EMBO J. 21 (2002) 2397–2406.

[65] A. Zarrinpar, W.A. Lim, Converging on proline: the mechanism of WW domain peptide recognition, Nat. Struct. Biol. 7 (2000) 611–613.

[66] J.T. Nguyen, C.W. Turck, F.E. Cohen, R.N. Zuckermann, W.A. Lim, Exploiting the basis of proline recognition by SH3 and WW domains: design of N-substituted inhibitors, Science 282 (1998) 2088–2092.

[67] S. Goodbourn, L. Didcock, R.E. Randall, Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures, J. Gen. Virol. 81 (2000) 2341–2364.

[68] B.H. Harcourt, A. Sanchez, M.K. Offermann, Ebola virus selectively inhibits responses to interferons, but not to interleukin-1beta, in endothelial cells, J. Virol. 73 (1999) 3491–3496.

[69] C.F. Basler, X. Wang, E. Muhlberger, V. Volchkov, J. Paragas, H.D. Klenk, A. Garcia-Sastre, P. Palese, The Ebola virus VP35 protein functions as a type I IFN antagonist, Proc. Natl. Acad. Sci. USA 97 (2000) 12289–12294.