

Chapter 2

Forty Years of Ebolavirus Molecular Biology: Understanding a Novel Disease Agent Through the Development and Application of New Technologies

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Abstract

Molecular biology is a broad discipline that seeks to understand biological phenomena at a molecular level, and achieves this through the study of DNA, RNA, proteins, and/or other macromolecules (e.g., those involved in the modification of these substrates). Consequently, it relies on the availability of a wide variety of methods that deal with the collection, preservation, inactivation, separation, manipulation, imaging, and analysis of these molecules. As such the state of the art in the field of ebolavirus molecular biology research (and that of all other viruses) is largely intertwined with, if not driven by, advancements in the technical methodologies available for these kinds of studies. Here we review of the current state of our knowledge regarding ebolavirus biology and emphasize the associated methods that made these discoveries possible.

Key words Ebola virus, Molecular biology, Virus life cycle, Entry, Budding, Replication, Antiviral mechanisms, Host cell interaction, Reverse genetics

1 Introduction

Molecular biology seeks to understand biological phenomena at a molecular level, and achieves this through the study of DNA, RNA, proteins, and/or other macromolecules (e.g., those involved in the modification of these substrates). Indeed, viruses themselves can be viewed as molecular tools, as they themselves represent little more than (relatively) simple assemblages of the above. As such they provide an opportunity to study interactions between these kinds of molecules in a system with a dramatically reduced level of complexity compared to other models. Similarly, as obligate parasites, their intimate interaction with the host cell also provides an opportunity to investigate these host cell processes themselves, in addition to the virus' interactions with them.

With ebolaviruses only having been discovered in 1976, this field has benefitted almost throughout its history from the

availability of molecular methods, and as such can really have been said to have grown up in the era of molecular biology. The result is a continuously shifting focus toward new research areas that is intertwined with, and one could even suggest driven by, advancements in the available technical methodologies.

In this chapter we seek to provide a review of the current state of our knowledge regarding ebolavirus biology, while highlighting some of the hallmark advancements that have been made over the years, and the associated methods that made these discoveries possible, so as to provide a frame of reference within which the individual methods that are presented later in this volume can be more fully appreciated.

2 Phase I: Identifying a New Enemy (1978–1985)

As a novel emerging infectious disease, many of the earliest studies of ebolaviruses unsurprisingly focused on the pathogenic potential of these agents, and on establishing the epidemiological information needed for disease control. These early studies already included the application of the newly developed enzyme-linked immunosorbent assay (ELISA) assay technology to determine antibody prevalence in the affect region prior to the first outbreaks [1], and also outside the outbreak region [2]. However, within a few years research began to focus on the most basic of questions: that of identifying what these viruses were and what they were made of. Fortunately, many of the basic tools for just such investigations had been recently developed. The earliest basic science report on ebolavirus biology was based on transmission electron microscopy imaging, developed already in the 1930s, which allowed the authors to show that ebolaviruses have a physical structure that consists of filamentous particles nearly identical to those of marburgviruses [3] (Fig. 1a). Further, polyacrylamide gel electrophoresis could be used to identify several major virion structural proteins, and also to define the sizes for these proteins based on comparison to Vesicular Stomatitis Virus (VSV) [4]. These first studies also determined that the ebolavirus glycoprotein GP_{1,2} is heavily glycosylated and unusually large [4]. It was not until 5 years later that the same group, again relying on the fundamental method of gel electrophoresis, ultimately succeeded in identifying all seven of the structural proteins known today, namely the RNA-dependent RNA polymerase (L), glycoprotein (GP_{1,2}), nucleoprotein (NP), virion protein (VP) 40, VP35, VP30, and VP24, and assigning them their current naming based on their sizes and/or their presumed functions [5]. Further, this study produced the first estimates of protein abundance in particles, values that remain reasonably consistent (i.e., all estimates within fourfold) with the most recent estimates based on electron

While much of this work now appears rather trivial, in an era reliant on classical biochemical approaches, and before the widespread implementation of (reverse transcription (RT)-) PCR, cloning/expression techniques, and sequencing, these represented major findings. Indeed, these fundamental studies relying on little more than electron microscopy, immunofluorescence analysis, and basic biochemical analysis and gel electrophoresis can be said to have been largely responsible for the classification of ebolaviruses as a sister genus to marburgviruses, which together form the *Filoviridae* family, as well as for our understanding that these unique pathogens are negative-sense single-stranded RNA viruses with unusually long genome lengths of approximately 19 kb that encode seven structural proteins (NP, VP35, VP40, GP, VP30, VP24, and L).

3 Phase II: Focusing on Sequences (1986–1995)

With a workable view of the genetic and protein makeup of the virus particles already in place by the mid/late-80s, the next phase of research into ebolavirus molecular biology shifted its focus to the functions of individual proteins and genetic elements in driving various essential steps of the virus lifecycle. This area remained a subject of intense interest for the next 10–15 years, and saw the development of Sanger sequencing making a huge contribution to developing a more refined view of the virus genome. Similarly, advancements in tools related to antibody detection, expression systems, and reporter systems became available to fuel these kinds of studies.

The first genetic elements identified in the virus were conserved 3' terminal sequences, which based on their conservation between ebolaviruses and marburgviruses and the function of similarly positioned elements in other virus families were proposed to be responsible for polymerase binding and packaging [11]. Indeed, we now know that these elements represent highly conserved terminal hairpin-forming bipartite promoter regions [12].

Subsequent work involving cloning and sequencing of NP mRNAs or terminal fragments of the viral RNA using polyadenylation and RT-PCR-based approaches, similar to those sometimes now used for rapid amplification of cDNA ends (RACE) sequencing of genome ends, then allowed further identification of the transcriptional “start” and “stop” signals responsible for regulating the initiation and termination of transcription [13]. Again, while these sequences have been slightly refined over the years to give a consensus representative of all gene products and ebolavirus species, they have remained essentially unchanged since these early days of filovirus molecular biology research. Further, the authors could determine the sequence and amino acid composition of the

entire NP protein, giving us our first look up close at an ebolavirus protein [13].

Soon to follow was first a partial [14] and then a complete sequence of GP [15]. Here we also saw the first hints of the influence that data repositories such as EMBL, SwissPROT, and GenBank, and the bioinformatics tools that they host, would eventually have in the field, and indeed on molecular biology in general. The availability of these tools allowed the first *in silico* studies of ebolaviruses to be conducted, and lead to the suggestion that the ebolavirus GP_{1,2} possesses a retrovirus-like immunosuppressive motif [14, 16], a finding that when followed up years later yielded evidence that this motif may be functional and influence CD4 and CD8 T cell biology [17]. The availability of these new bioinformatics tools also allowed for the first time genetic and protein level comparisons between ebolaviruses and marburgviruses [18, 19]. The interest in applying bioinformatics also extended to studies of evolutionary rate and pressures, and to assessments looking to understand the origin and evolution of filoviruses [20].

However, in the era of sequencing and genomics there was still room for classical biochemistry-based techniques, including protein sequencing, which was used to establish the protein sequences of NP, VP35, and VP40, and established their order as the first three transcripts encoded by the genome, something that was at that time problematic given that theoretical predictions suggested that VP35 should actually encode the larger of the two proteins, although this is not experimentally observed. Further it was established that VP30 was in fact a distinct protein unique to filoviruses, and not a cleavage product of NP, as had sometimes been suggested [21]. Then, at long last, came the first full-length sequence of an Ebola virus genome, which definitively confirmed the genome organization, although it was already suspected based on an earlier complete sequence for the closely related marburgvirus [22], and established the sequences of the previously missing intergenic regions [23] (Fig. 1b). It also clearly paved the way for the sequencing of complete genomes for other ebolavirus species in the years to follow [24–27], and thus much of our appreciation of the genetic/phylogenetic structure of the family as we know it today—with a clear separation of the family of *Filoviridae* not only into two distinct genera, *Ebolavirus* and *Marburgvirus*, but further subdivision of the ebolaviruses into five species: *Zaire ebolavirus*, *Sudan ebolavirus*, *Reston ebolavirus*, *Tai Forest ebolavirus*, and the recently discovered *Bundibugyo ebolavirus*.

This time span saw also several notable public health events in the timeline of ebolavirus history, including the importation of a new ebolavirus species, *Reston ebolavirus*, into the USA and Italy, as well as the discovery of the another new pathogenic ebolavirus species, *Tai Forest ebolavirus*, in Ivory Coast, and what was until recently the largest and most deadly recorded outbreak of

ebolavirus, in Kikwit in 1995. Thus, while ebolavirus research during this time period had clearly been heavily focused on sequencing, these events encouraged renewed efforts to adapt molecular methods, including those based on electron microscopy and various antibody reactivity-based methods, to develop new and broader specificity diagnostic methods for these viruses (e.g., [28–36]).

4 Phase III: It's All About Function (1995–Present)

The next years were marked by a clear transition away from sequencing and toward the first experiments looking into more functional aspects of ebolavirus biology, and the molecular and cell biological basis for these functions. In retrospect one can see that this transition was clearly triggered by the accumulation of a critical mass of genetic information, and the forthcoming development of a variety of molecular tools/approaches needed to conduct these kinds of experiments. However, it was also clearly fueled by some fascinating discoveries that resulted, at least in part, from examinations of the newly available genetic information itself.

4.1 *The Many Forms of GP*

Possibly one of the most fascinating of such discoveries was also the first, and concerned the ability of the GP gene to undergo transcriptional editing at a stretch of 7 U residues to produce the full length GP_{1,2} mRNA transcript, while in the majority of cases a soluble version of the glycoprotein was produced (sGP) [37, 38] (Fig. 2). Indeed, recently it has been confirmed that other editing products already suggested by this study can in fact also be generated, and lead to the production of an additional small soluble GP (ssGP) [39]. Further studies showed the additional production of a stable C-terminal cleavage product of sGP, known as delta-peptide [40], as well as shed forms of both GP₁ (produced as a result of disulfide bond instability) [41] and the full length GP_{1,2} (produced by tumor necrosis factor α -converting enzyme (TACE) cleavage to yield GP_{1,2ΔTM}) [42]. Interest in the molecular basis for the development of this unusual diversity of GP products, and also the potential relevance of these different forms of GP, helped motivate a transition in ebolavirus research as a whole away from genomics and into studies of protein function in subsequent years. However, even now these questions remain at best partially answered, and thus the biology of these soluble glycoproteins, and their relevance for virus infection, is still being studied today.

The first proposed function of sGP synthesis was simply to serve as a means of regulating GP_{1,2} expression, and thus controlling excessive GP_{1,2}-mediated cytotoxicity [43], as a result of the overlapping open reading frame organization between these two proteins, which strongly favors sGP synthesis. This is supported by some studies that suggest that GP_{1,2}-mediated cytotoxicity is not a

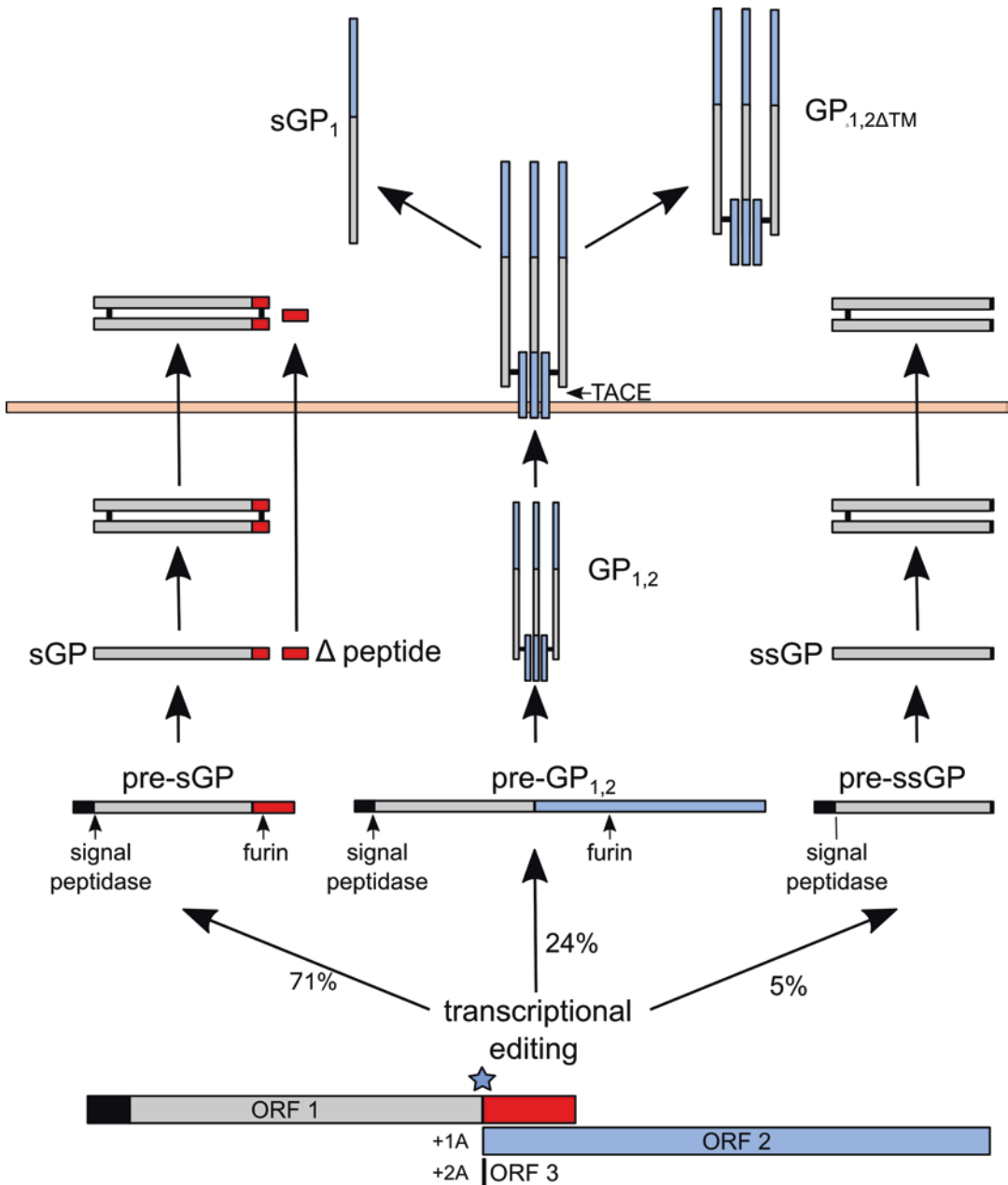


Fig. 2 Glycoprotein processing. The Ebola virus glycoprotein gene contains three open reading frames (ORFs), which can be accessed through transcriptional editing. In cases where no transcriptional editing occurs, only ORF 1 is transcribed, which leads to the expression of pre-sGP, which is processed by signal-peptidase and furin to yield the secreted glycoprotein sGP and Δ peptide. If the second ORF is accessed by insertion of a single nucleotide during transcription of the editing site (highlighted by a *star*), pre-GP_{1,2} is produced, which is processed by the same proteases into the mature GP_{1,2}, the only Ebola virus glycoprotein that is membrane-associated. However, upon cleavage by TACE a secreted version of this protein (GP_{1,2}TM) can also be produced. Further, soluble GP₁ can also be released from GP_{1,2}. Finally, if two nucleotides are inserted in the editing site during transcription, resulting in the third ORF being transcribed after the editing site, pre-ssGP is produced. This protein is processed by signal peptidase only, resulting in the small secreted glycoprotein ssGP

major factor when expression is moderate [44]. More recently, immunological effects such as the inhibition of neutrophil function [45, 46] and antigenic subversion of antibody responses toward nonneutralizing epitopes [47] have also been described. Further, sGP has been suggested to possess an anti-inflammatory function that may help mitigate negative effects of GP_{1,2} on vascular integrity [48]. Similarly, delta-peptide has been proposed to have a modulatory role, in this case by inhibiting the entry of virus into target cells [49]. In the case of GP_{1,2ΔTM}, evidence for a function has only recently been obtained and suggest when properly presented as a trimer, it can bind to and activate uninfected DCs and macrophages [50], and that its shedding directly modulates the levels of EBOV GP_{1,2} expressed at the surface of virus-infected cells, and thus its availability for incorporation into virus particles [51].

There was also tremendous interest starting at this time in the biology of the surface glycoprotein itself. Various aspects including its fusion activity [52], structure [53–55], and processing [56] were studied by a number of different groups, which all contributed to our current view of GP_{1,2} as a heterotrimeric, class I fusion protein which is processed from a single polypeptide by furin cleavage. Intriguingly, already at this time there were the first hints that GP_{1,2} cleavage by furin is not necessary for function and infectivity, in contrast to the situation with many other viruses [57]. However, these findings would not be directly confirmed for many more years until the development of full-length clone systems allowed the production of recombinant viruses with the furin cleavage site knocked out [58, 59]. The role of GP_{1,2} in pathogenesis is more controversial, with early studies indicating that its expression alone causes vascular leakage from vessel explants, and thus that it is the main viral determinant of Ebola pathogenicity [60]. However, more recently studies using recombinant viruses in which the GP_{1,2} from Ebola virus (EBOV, species *Zaire ebolavirus*) and the apathogenic Reston virus (RESTV, species *Reston ebolavirus*) were exchanged have shown that while EBOV GP_{1,2} enhances virulence and contributes to pathological changes, alone it does not confer increased virulence [61]. The role of GP editing in pathogenesis also remains controversial [43, 62].

4.2 Uncovering an Unusual Mechanism of Entry

The mechanism by which Ebola virus enters target cells was for many years a source of tremendous confusion within the field, with numerous binding proteins, including Folate Receptor Alpha [63], a wide variety of C-type lectins (including dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) [64, 65], liver/lymph node-specific ICAM-3 grabbing non-integrin (L-SIGN) [64], other human macrophage calcium-dependent lectins [66]), DC-SIGN receptor [65], and most recently, Tim-1 [67], being identified. However, in many cases binding to these surface receptors, while beneficial, is nonessential

and/or their relevance is restricted to certain cell types. How such a situation is possible may have been clarified by recent evidence identifying Niemann-Pick C1 (NPC-1), which acts at the levels of the endosome, at a stage after both surface attachment and uptake, as an apparently critical receptor for ebolavirus entry [68, 69].

The mechanism of uptake of ebolavirus into host cells also was a controversial topic, with various studies suggesting the involvement of clathrin-mediated endocytosis [70, 71], caveolin-mediated endocytosis [72], or macropinocytosis [73]. However, a major limitation of these studies was that they were performed using virus pseudotypes (VSV, HIV, etc. carrying the ebolavirus GP_{1,2} glycoprotein), which differ significantly in their morphology compared to ebolavirus particles, a factor that could potentially affect uptake by some or all of these pathways. More recently, researchers have begun performing experiments with infectious ebolavirus, or structurally similar ebolavirus-like particles, and these appear to largely demonstrate virus uptake via macropinocytosis [74–76]. However, some studies continue to demonstrate that other pathways could also still be playing a role, and that in fact multiple different mechanisms may actually be used in concert [77–79].

Equally controversial has been the role of protease cleavage of the viral glycoprotein. It appears clear that endosomal cleavage of GP₁ into a low molecular weight form is necessary to obtain a fusion competent conformation. So far, the only proteases identified as participating in this process are the endosomal cysteine proteases Cathepsin L (CatL) and Cathepsin B (CatB) [80, 81]. Interestingly, there appear to be differences in processing in different cell types [82], as well as differences in Cathepsin sensitivity among filovirus species [83–85]. Further, a recent *in vivo* study has also demonstrated that despite the demonstrated importance of Cathepsin cleavage in a number of cell culture systems, during ebolavirus infection in the mouse model neither CatB nor CatL appear to be necessary, and CatB/L double knockout mouse embryonic fibroblast cells remain equally susceptible to virus infection [85], suggesting a role for other proteases under at least some circumstances. Indeed, recent studies with a thermolysin-trimmed GP₁ [86], which structurally resembles that produced by Cathepsin cleavage, appear to support that, at least in principle, a similar effect could be achieved by digestion with other cellular proteases.

Overall, while several issues remain to be fully clarified, what these findings have led to is a current model of virus entry (Fig. 3) in which no specific critical cell surface receptor is required, but rather a number of attachment factors serve to concentrate virus particles at the cell surface to enhance their uptake, which then occurs primarily via macropinocytosis. Following endosome acidification, trimming of GP₁ by Cathepsins, or possibly also other proteases, exposes the receptor binding domain, and following

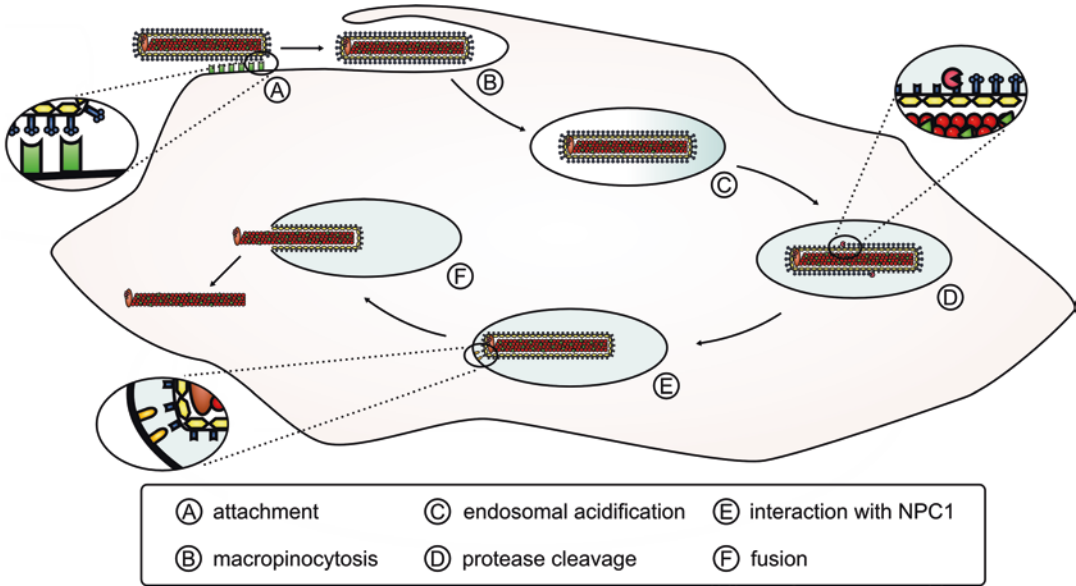


Fig. 3 Model of Ebola virus entry. At the cell surface Ebola viruses attach to C-type lectins through GP_{1,2}, or to phosphatidylserine receptors (e.g., Tim-1) through interactions with the viral envelope (a). Uptake into endosomes occurs through macropinocytosis (b). After endosomal acidification (c) host proteases cleave GP_{1,2} to yield a 19 kDa form (d), which can then interact with the cellular receptor NPC-1 (e), ultimately resulting in membrane fusion (f) and release of nucleocapsids into the cytoplasm

binding to the NPC-1 receptor, fusion occurs to release the viral nucleocapsid into the cytoplasm.

4.3 The Mechanics of Genome Replication and Transcription

The late 90s also saw the first studies of transcription and replication mechanics for filoviruses being conducted (Fig. 4). These studies relied on the development of minigenome systems, which use reporter-expressing viral genome analogues to model viral transcription and replication, and produced findings that define our understanding of the basic viral components necessary for these processes (i.e., NP, VP35, VP30, and L) [87]. While the roles of NP (nucleoprotein), VP35 (polymerase cofactor), and L (polymerase) were easy to rationalize based on better studied analogous virus systems, the need for VP30, which lacks clear homologues in other systems, remained a mystery for several years. Eventually, however, VP30 was shown to serve as a cofactor for EBOV transcription, which is needed to overcome a hairpin structure in the 3' viral noncoding region [88]. The unusual existence of this additional RNP component has led to much interest in the role of VP30 over recent years, with several further studies focusing on the role of its phosphorylation state in the regulation of RNA synthesis. In particular it has been demonstrated that phosphorylation of VP30 regulates its interactions with both VP35 and NP, and must be dynamic in order to allow it to regulate both

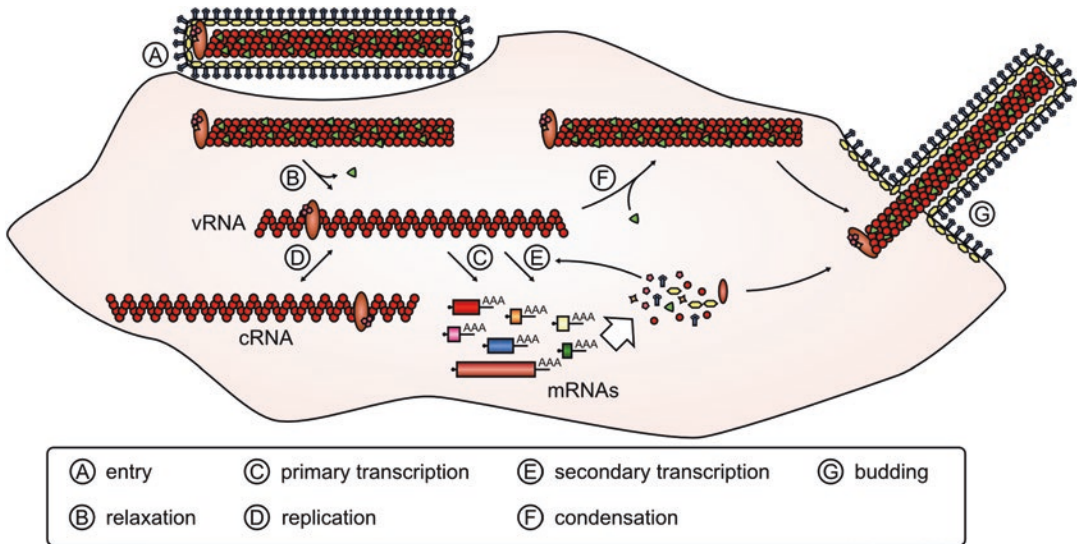


Fig. 4 Model of Ebola virus genome replication and transcription. After entry (a) into the cell, nucleocapsids relax (b) due to dissociation of VP24. This allows the viral polymerase complex that entered the cell within the virus particle to perform primary transcription (c), which produces the viral proteins necessary for genome replication (d) and further, secondary transcription (e) and protein production. Produced VP24 then leads to a condensation of newly formed ribonucleoprotein complexes into packaging-competent nucleocapsids (f), which are transported to the surface, where budding takes place in a process driven by VP40

transcription and replication [89, 90]. Recently, it has also been demonstrated that VP30 is capable of directly binding to the 3'-end of the ebolavirus genome RNA, and that this binding also stabilizes VP35/L RNA binding [90]. As a result, it has been suggested that phosphorylation allows VP30 to modulate the composition of the RNP complex, in order to form either a transcriptase or replicase complex [89], and thereby regulates these two activities of the viral polymerase. Of practical significance, this peculiar dependence of the ebolavirus transcriptional mechanism on VP30 has in recent years also facilitated the development of “biologically contained” Ebola viruses that lack this viral protein in their genome, and whose replication is thus restricted to VP30-expressing cells, allowing them to be studied at lower levels of containment [91]. Most recently, VP30 has also been shown to have a novel function as a trans-acting factor for RNA editing of the GP gene [92]. This process seems to be both sequence and RNA structure dependent and provides some of the first mechanistic information about how this important process in the EBOV lifecycle is being regulated.

Recent discoveries in the field have also clarified the role of the other viral protein unique to filoviruses, VP24, and shown that it too plays an important role in the regulation of viral RNA synthesis. Early biochemical studies suggested that VP24 might function as a minor matrix protein, due to its apparent localization in the

matrix space in salt dissociation experiments [93], as well as a number of properties considered potentially consistent with function as a matrix protein [94]. More recent electron microscopy analyses have, however, demonstrated that VP24 is in fact associated with the outside of the viral RNPs [6], and that its association with the RNP results in rigid RNP forms similar to those found in ebolavirus particles [95]. This observation is further supported by recent work using various minigenome-based tools, and particularly those that have been expanded to include multiple ebolavirus genes, and thus more closely resemble the viral genomes [96]. The findings using these systems suggest that VP24 may serve to condense RNPs from a flexible, accessible form that can undergo transcription/replication to a rigid packaging-competent form, and that this condensation also serves to lock the polymerase at the genome terminus so that it remains competent to perform primary transcription upon virus infection [96]. Thus this also appears to explain previous observations that VP24 serves as an inhibitor of viral RNA synthesis when overexpressed from plasmids [97, 98], and that it is necessary for production of nucleocapsids capable of undergoing primary transcription [99].

Finally, studies have also started to address the question of where exactly in the host cell ebolavirus genome replication and transcription take place. Both IFA-based and reporter virus studies have recently been used to demonstrate inclusion bodies as the sites of virus RNA synthesis, thereby clarifying a long-standing question about whether these structures are biologically relevant, or simply represent functionally dead masses of accumulated protein [100, 101].

4.4 Understanding Particle Morphogenesis and Budding

The biosafety requirements for work on live EBOV have resulted in the development of a large collection of reverse genetics-based tools to study and model aspects of the viral lifecycle (e.g., various monocistronic and multicistronic minigenome and transcription and replication-competent virus-like particle (trVLP) assays) [87, 96, 99, 102, 103]. While these systems were first developed as simple genome analogues to look at viral RNA synthesis, the more complex of these systems are now finding applications for studying other steps in the virus lifecycle, including morphogenesis and budding. Further, as the number of biosafety level 4 facilities worldwide and the availability of high-tech imaging platforms (including live cell imaging) increases, detailed immunofluorescence labeling and reporter viruses studies are now also being used to study the movement of virus proteins and RNAs during the virus lifecycle and are providing important insights. For example, a recent study used VP30-GFP labeled viruses to look at the transport of nucleocapsids within the cytoplasm from sites of replication to the sites of budding at the cell surface and the dependence of this process on the actin cytoskeleton [104]. Interestingly, recent

single-particle tracking studies have shown that actin also directs the movement of the matrix protein (VP40) to the plasma membrane as well [105], where it must eventually meet up with these packaging-ready nucleocapsids. Clearly these kinds of studies demonstrate how powerful these advanced imaging techniques are likely to become in the coming years for looking at these poorly defined interfaces between the different stages in the virus lifecycle, in particular the process of morphogenesis. However, at present our understanding of this process for ebolaviruses still remains limited.

Unlike RNP transport and morphogenesis, the budding process of ebolaviruses has actually been quite intensely studied. The major contributor to budding is the matrix protein VP40, and studies of its function in virus budding began in earnest in 2000. Already these early studies demonstrated that recombinantly expressed VP40 was capable of independently binding to membranes [106], and that it induced the release of lipid enveloped particles [107, 108]. Further, unlike expression of GP_{1,2}, which leads to the production of pleomorphic particles, VP40 expression drives the production of particles having an authentic filamentous structure [109]—all features expected of a bona fide viral matrix protein.

At the same time overlapping proline-rich late domains of both the PT/SAP and PPxY types common to many other viral matrix proteins were identified in VP40, although in ebolaviruses they display an unusual overlapping arrangement (i.e., 7-PTAPPEY-13). As with similar domains in other viruses these were then soon shown to directly support virus budding by mediating interaction with WW domain containing proteins, including the yeast homolog of Nedd4, mammalian Nedd4, Tsg101, and most recently ITCH [110–114], and thereby to facilitate interaction with the Vps4 pathway upon which ebolavirus budding is also dependent [111]. However, while these interactions have been shown to support budding, production of a recombinant ebolaviruses lacking these late-domain motifs has demonstrated that such a virus is still viable, and thus that alternate mechanisms of virus release must also exist [115]. Potentially consistent with this finding, a third YPx_nL/I type late domain (18-YPARSNSTI-26) was recently identified in ebolavirus VP40 and appears to mediate an additional interaction with the ESCRT-III protein Alix [114, 116], which might also contribute to budding, although further studies are still needed to confirm these findings.

Crystal structures were also obtained early during the study of VP40 biology and have revealed a unique duplicated two-domain structure connected by a flexible hinge that controls the transition between monomeric and multimeric states [117, 118]. Others then further demonstrated that hexamerization of VP40 into ring-like structures might be triggered by membrane binding [106,

119], an observation that is supported by biochemical evidence that oligomerization is indeed a prerequisite for the budding activity of VP40 [120].

Another significant area that until recently had remained poorly studied concerns how VP40 is actually able to drive membrane deformation during the budding process. However, a number of recent studies using biochemical and biophysical approaches have demonstrated an intrinsic ability of VP40 to penetrate into lipid bilayers and induce membrane curvature consistent with virus particle formation [105, 121] in a manner that also appears to be dependent on the lipid composition of the membranes themselves [122]. As such these studies provide the first insight into the mechanics of vesiculation as a key step in the budding process.

4.5 Interactions with the Innate Immune Response

In addition to being a time of great interest in virus biology, the late 1990s also saw the first studies begin to investigate the effects of virus infection on the immune system, and specifically interactions with the interferon (IFN) system [123, 124]. It was only a few years later that it was determined that VP35 is responsible for actively inhibiting the IFN production pathway [125], and that this occurs through interaction with IFN regulatory factor 3 (IRF3) [126]. This process was then further shown to be dependent both on trimerization of VP35 through a coil-coiled domain located in the N-terminal portion of the protein [127], and specific basic residues located in the C-terminus of the protein [128], which also harbors a dsRNA-binding domain [129]. Later studies showed that the ability of VP35 to inhibit IFN production is due to direct binding of VP35 to IKK ϵ and TANK-binding kinase 1 (TBK1) in a manner that blocks subsequent IRF3 and IRF7 interaction, and thus their subsequent activating phosphorylation [130]. Recent co-crystal structures of the VP35 RNA-binding domain together with dsRNA have provided interesting insights into the mechanism of dsRNA binding, which for ebolavirus appears to occur efficiently both along the backbone of the dsRNA and at the terminal-free ends, and may block detection of dsRNA templates by RNA helicases, such as retinoic acid inducible gene I (RIG-I) [131].

The relevance of IFN antagonism for virus infection and pathogenesis was quickly recognized, and was highlighted by the finding that unlike immunocompetent mice, IFN receptor α knockout mice are highly susceptible to ebolavirus infection [132]. In addition, the production of recombinant viruses in which the IFN inhibitory activity of VP35 is abolished showed significant attenuation both in vitro [133, 134] and in vivo [135]. Microarray analyses have also suggested that the extent of IFN suppression may correlate with the virulence of different filovirus species [136], a finding that was recently supported by the implementation of highly standardized IFN antagonism assays [137].

More recently, a second point of interference with the IFN system has been described for ebolaviruses, this time with respect to inhibition of IFN signaling and the resulting production of interferon stimulated genes (ISGs) [138]. This work further showed that inhibition was mediated by direct binding of VP24 to karyopherin- α 1, and that this blocks signal transducer and activator of transcription 1 (STAT1) nuclear accumulation, which is essential for subsequent activation of ISG transcription [138]. Further studies eventually showed that VP24 is in fact capable of binding to all members of the nucleoprotein interactor 1 (NPI-1) subfamily (i.e., karyopherins- α 1, - α 5, and - α 6) [139]. Since the VP24-binding site appears to lie within the STAT1-binding site on karyopherin- α , it has been suggested that VP24 may inhibit STAT1 translocation by competing for the same binding site on karyopherin- α [139, 140]. At the same time, biochemical analysis, in conjunction with recently obtained X-ray crystallographic structures and deuterium exchange mass spectrometry analyses, indicate that VP24 can also directly bind to STAT1 itself [141].

Interestingly, an additional point of action for VP24 has also been recently reported, with data suggesting that VP24 can block IFN-stimulated phosphorylation of p38- α in some, but not all, cell lines [142]. While this pathway is known to be involved in the IFN response to other viruses [143, 144], the relevance and further details of this mechanism still need to be established. In any event, this multifaceted approach of targeting both IFN production and signaling is by no means uncommon and again suggests the critical importance of controlling this aspect of the innate immune response for virus survival.

In addition to blocking the production of IFN itself and its subsequent signaling to produce interferon stimulated genes (ISGs) there is some evidence that ebolaviruses are able to specifically counteract the activities of individual ISGs as well. The best studied of these effects is the inhibition of Tetherin (BST-2) by ebolavirus GP_{1,2} [145], which seems to occur via an unusual mechanism that does not involve blocking Tetherin's cell surface expression [146, 147]. Recently this mechanism has been shown to be dependent on the GP_{1,2} transmembrane domain [148], as well as an intact receptor-binding domain and correct N-glycan processing [149], and has been proposed to involve GP_{1,2}s ability to block interaction between VP40 and Tetherin [150].

In addition to interfering with the IFN pathway, ebolavirus infection has been shown to interfere with a variety of other pathways and functions related to innate immune defense. In particular, VP35 has also been described to interfere with and even actively suppress activation of the protein kinase R (PKR) pathway in order to avoid translational shutoff [151, 152]. Further, VP35 has been

shown to act as a suppressor of RNA silencing, a function that is dependent upon its dsRNA-binding activity [153]. Finally, ebolavirus infection has been recently shown to lead to sequestration of stress granule proteins into inclusion bodies, which might help the virus avoid yet another antiviral response, as neither canonical stress granule formation, nor the associated translational arrest, are observed in ebolavirus-infected cells [154]. In addition to all these mechanisms, which inhibit immunity on the cellular level, there are also a number of mechanisms by which ebolaviruses interfere with the immune system on an organismal level. These aspects are discussed in Chapter 3, which addresses the molecular and clinical pathogenesis of Ebola virus.

5 Final Remarks

Taking a step back it is clear that ebolavirus research has seen a number of “trends” over the years with sudden, although sometimes unfortunately short-lived, interest in particular areas. Equally evident is that these shifts in focus (at least as evidenced by publication output) can be driven by specific situations, such as outbreaks, but can also be strongly influenced by the development and accessibility of new technologies. Certainly one of the biggest trends in the last decade or so has been the entry into the field of a wide variety of experts with diverse specialties, something which is in no small part facilitated by advancements in molecular biology approaches that allow studies on biosafety level 4 pathogens like ebolavirus to be conducted outside a high containment environment. As such the number of publications has exploded and the variety of research being conducted has diversified accordingly. It can only be hoped that this trend will continue into the future as new tools and techniques become available to support continued investigations into the many remaining open questions regarding filovirus biology. Finally, it should be noted that it is still the case that the vast majority of ebolavirus studies focus solely on the *Zaire ebolavirus* species, and while it is generally believed that most of the basic biological functions are conserved among all ebolavirus species, this is also clearly not always the case. Thus, there is still much work to do in identifying molecular differences that can help us explain the differences in pathogenicity and geographical distribution observed between different ebolaviruses.

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References

1. Van der Groen G, Pattyn SR (1979) Measurement of antibodies to Ebola virus in human sera from N.W.-Zaire. *Ann Soc Belg Med Trop* 59(1):87–92
2. Saluzzo JF, Gonzalez JP, Herve JP, Georges AJ, Johnson KM (1980) Preliminary note on the presence of antibodies to Ebola virus in the human population in the eastern part of the Central African Republic. *Bull Soc Pathol Exot Filiales* 73(3):238–241
3. Ellis DS, Simpson IH, Francis DP, Knobloch J, Bowen ET, Lolik P, Deng IM (1978) Ultrastructure of Ebola virus particles in human liver. *J Clin Pathol* 31(3):201–208
4. Kiley MP, Regnery RL, Johnson KM (1980) Ebola virus: identification of virion structural proteins. *J Gen Virol* 49(2):333–341
5. Elliott LH, Kiley MP, McCormick JB (1985) Descriptive analysis of Ebola virus proteins. *Virology* 147(1):169–176
6. Beniac DR, Melito PL, Devarennnes SL, Hiebert SL, Rabb MJ, Lamboo LL, Jones SM, Booth TF (2012) The organisation of Ebola virus reveals a capacity for extensive, modular polyploidy. *PLoS One* 7(1):e29608. doi:[10.1371/journal.pone.0029608](https://doi.org/10.1371/journal.pone.0029608)
7. Regnery RL, Johnson KM, Kiley MP (1980) Virion nucleic acid of Ebola virus. *J Virol* 36(2):465–469
8. Van der Groen G, Elliot LH (1982) Lack of cross reactivity of rhabdovirus antibodies with Marburg and Ebola antigens in the indirect immunofluorescent antibody test. *Ann Soc Belg Med Trop* 62(1):67–68
9. Kiley MP, Bowen ET, Eddy GA, Isaacson M, Johnson KM, McCormick JB, Murphy FA, Pattyn SR, Peters D, Prozesky OW, Regnery RL, Simpson DI, Slenczka W, Sureau P, van der Groen G, Webb PA, Wulff H (1982) Filoviridae: a taxonomic home for Marburg and Ebola viruses? *Intervirology* 18(1–2): 24–32
10. Sanchez A, Kiley MP (1987) Identification and analysis of Ebola virus messenger RNA. *Virology* 157(2):414–420
11. Kiley MP, Wilusz J, McCormick JB, Keene JD (1986) Conservation of the 3' terminal nucleotide sequences of Ebola and Marburg virus. *Virology* 149(2):251–254
12. Weik M, Enterlein S, Schlenz K, Muhlberger E (2005) The Ebola virus genomic replication promoter is bipartite and follows the rule of six. *J Virol* 79(16):10660–10671. doi:[10.1128/JVI.79.16.10660-10671.2005](https://doi.org/10.1128/JVI.79.16.10660-10671.2005)
13. Sanchez A, Kiley MP, Holloway BP, McCormick JB, Auperin DD (1989) The nucleoprotein gene of Ebola virus: cloning, sequencing, and in vitro expression. *Virology* 170(1):81–91
14. Volchkov VE, Blinov VM, Netesov SV (1992) The envelope glycoprotein of Ebola virus contains an immunosuppressive-like domain similar to oncogenic retroviruses. *FEBS Lett* 305(3):181–184
15. Will C, Muhlberger E, Linder D, Slenczka W, Klenk HD, Feldmann H (1993) Marburg virus gene 4 encodes the virion membrane protein, a type I transmembrane glycoprotein. *J Virol* 67(3):1203–1210
16. Becker Y (1995) Retrovirus and filovirus “immunosuppressive motif” and the evolution of virus pathogenicity in HIV-1, HIV-2, and Ebola viruses. *Virus Genes* 11(2–3): 191–195
17. Yaddanapudi K, Palacios G, Towner JS, Chen I, Sariol CA, Nichol ST, Lipkin WI (2006) Implication of a retrovirus-like glycoprotein peptide in the immunopathogenesis of Ebola and Marburg viruses. *FASEB J* 20(14):2519–2530. doi:[10.1096/fj.06-6151com](https://doi.org/10.1096/fj.06-6151com)
18. Bukreyev A, Volchkov VE, Blinov VM, Netesov SV (1993) The GP-protein of Marburg virus contains the region similar to the 'immunosuppressive domain' of oncogenic retrovirus P15E proteins. *FEBS Lett* 323(1–2):183–187
19. Bukreyev AA, Volchkov VE, Blinov VM, Netesov SV (1993) The VP35 and VP40 proteins of filoviruses. Homology between Marburg and Ebola viruses. *FEBS Lett* 322(1):41–46
20. Suzuki Y, Gojobori T (1997) The origin and evolution of Ebola and Marburg viruses. *Mol Biol Evol* 14(8):800–806
21. Elliott LH, Sanchez A, Holloway BP, Kiley MP, McCormick JB (1993) Ebola protein analyses for the determination of genetic organization. *Arch Virol* 133(3–4):423–436
22. Feldmann H, Muhlberger E, Randolph A, Will C, Kiley MP, Sanchez A, Klenk HD (1992) Marburg virus, a filovirus: messenger RNAs, gene order, and regulatory elements of the replication cycle. *Virus Res* 24(1):1–19
23. Sanchez A, Kiley MP, Holloway BP, Auperin DD (1993) Sequence analysis of the Ebola virus genome: organization, genetic elements, and comparison with the genome of Marburg virus. *Virus Res* 29(3):215–240
24. Ikegami T, Calaor AB, Miranda ME, Niikura M, Saijo M, Kurane I, Yoshikawa Y, Morikawa S (2001) Genome structure of Ebola virus subtype Reston: differences among Ebola subtypes. Brief report. *Arch Virol* 146(10): 2021–2027

25. Groseth A, Stroher U, Theriault S, Feldmann H (2002) Molecular characterization of an isolate from the 1989/90 epizootic of Ebola virus Reston among macaques imported into the United States. *Virus Res* 87(2):155–163
26. Sanchez A, Rollin PE (2005) Complete genome sequence of an Ebola virus (Sudan species) responsible for a 2000 outbreak of human disease in Uganda. *Virus Res* 113(1):16–25. doi:[10.1016/j.virusres.2005.03.028](https://doi.org/10.1016/j.virusres.2005.03.028)
27. Towner JS, Sealy TK, Khristova ML, Albarino CG, Conlan S, Reeder SA, Quan PL, Lipkin WI, Downing R, Tappero JW, Okware S, Lutwama J, Bakamutumaho B, Kayiwa J, Comer JA, Rollin PE, Ksiazek TG, Nichol ST (2008) Newly discovered ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS Pathog* 4(11):e1000212. doi:[10.1371/journal.ppat.1000212](https://doi.org/10.1371/journal.ppat.1000212)
28. Geisbert TW, Jahrling PB (1995) Differentiation of filoviruses by electron microscopy. *Virus Res* 39(2–3):129–150
29. Becker S, Feldmann H, Will C, Slenczka W (1992) Evidence for occurrence of filovirus antibodies in humans and imported monkeys: do subclinical filovirus infections occur worldwide? *Med Microbiol Immunol (Berl)* 181(1):43–55
30. Geisbert TW, Jahrling PB (1990) Use of immunoelectron microscopy to show Ebola virus during the 1989 United States epizootic. *J Clin Pathol* 43(10):813–816
31. Geisbert TW, Rhoderick JB, Jahrling PB (1991) Rapid identification of Ebola virus and related filoviruses in fluid specimens using indirect immunoelectron microscopy. *J Clin Pathol* 44(6):521–522
32. Rollin PE, Ksiazek TG, Jahrling PB, Haines M, Peters CJ (1990) Detection of Ebola-like viruses by immunofluorescence. *Lancet* 336(8730):1591
33. Kalter SS, Heberling RL, Barry JD, Tian PY (1995) Detection of Ebola-Reston (Filoviridae) virus antibody by dot-immunobinding assay. *Lab Anim Sci* 45(5):523–525
34. Ksiazek TG, Rollin PE, Jahrling PB, Johnson E, Dalgard DW, Peters CJ (1992) Enzyme immunosorbent assay for Ebola virus antigens in tissues of infected primates. *J Clin Microbiol* 30(4):947–950
35. Merzlikin NV, Chepurnov AA, Istomina NN, Ofitserov VI, Vorob'eva MS (1995) Development and application of an immunoenzyme test system for diagnosing Ebola fever. *Vopr Virusol* 40(1):31–35
36. Borisevich IV, Mikhailov VV, Potryvaeva NV, Malinkin Iu N, Kirillov AP, Krasnianskii VP, Markov VI, Makhlaia AA, Lebedinskaia EV (1996) Development of the immunoenzyme test-system for detection of Ebola virus antigen. *Vopr Virusol* 41(5):232–234
37. Volchkov VE, Becker S, Volchkova VA, Ternovoj VA, Kotov AN, Netesov SV, Klenk HD (1995) GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases. *Virology* 214(2):421–430
38. Sanchez A, Trappier SG, Mahy BW, Peters CJ, Nichol ST (1996) The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. *Proc Natl Acad Sci U S A* 93(8):3602–3607
39. Mehedi M, Falzarano D, Seebach J, Hu X, Carpenter MS, Schnittler HJ, Feldmann H (2011) A new Ebola virus nonstructural glycoprotein expressed through RNA editing. *J Virol* 85(11):5406–5414. doi:[10.1128/JVI.02190-10](https://doi.org/10.1128/JVI.02190-10)
40. Volchkova VA, Klenk HD, Volchkov VE (1999) Delta-peptide is the carboxy-terminal cleavage fragment of the nonstructural small glycoprotein sGP of Ebola virus. *Virology* 265(1):164–171
41. Volchkov VE, Volchkova VA, Slenczka W, Klenk HD, Feldmann H (1998) Release of viral glycoproteins during Ebola virus infection. *Virology* 245(1):110–119
42. Dolnik O, Volchkova V, Garten W, Carbonnelle C, Becker S, Kahnt J, Stroher U, Klenk HD, Volchkov V (2004) Ectodomain shedding of the glycoprotein GP of Ebola virus. *EMBO J* 23(10):2175–2184
43. Volchkova VA, Dolnik O, Martinez MJ, Reynard O, Volchkov VE (2015) RNA editing of the GP gene of Ebola virus is an important pathogenicity factor. *J Infect Dis* 212(Suppl 2):S226–S233. doi:[10.1093/infdis/jiv309](https://doi.org/10.1093/infdis/jiv309)
44. Alazard-Dany N, Volchkova V, Reynard O, Carbonnelle C, Dolnik O, Ottmann M, Khromykh A, Volchkov VE (2006) Ebola virus glycoprotein GP is not cytotoxic when expressed constitutively at a moderate level. *J Gen Virol* 87(Pt 5):1247–1257
45. Yang Z, Delgado R, Xu L, Todd RF, Nabel EG, Sanchez A, Nabel GJ (1998) Distinct cellular interactions of secreted and transmembrane Ebola virus glycoproteins. *Science* 279(5353):1034–1037
46. Kindzelskii AL, Yang Z, Nabel GJ, Todd RF 3rd, Petty HR (2000) Ebola virus secretory glycoprotein (sGP) diminishes Fc gamma RIIIB-to-CR3 proximity on neutrophils. *J Immunol* 164(2):953–958
47. Mohan GS, Li W, Ye L, Compans RW, Yang C (2012) Antigenic subversion: a novel mechanism of host immune evasion by Ebola virus.

- PLoS Pathog 8(12):e1003065. doi:[10.1371/journal.ppat.1003065](https://doi.org/10.1371/journal.ppat.1003065)
48. Wahl-Jensen VM, Afanasieva TA, Seebach J, Stroher U, Feldmann H, Schnittler HJ (2005) Effects of Ebola virus glycoproteins on endothelial cell activation and barrier function. *J Virol* 79(16):10442–10450
 49. Radoshitzky SR, Warfield KL, Chi X, Dong L, Kota K, Bradfute SB, Gearhart JD, Retterer C, Kranzusch PJ, Misasi JN, Hogenbirk MA, Wahl-Jensen V, Volchkov VE, Cunningham JM, Jahrling PB, Aman MJ, Bavari S, Farzan M, Kuhn JH (2011) Ebola virus delta-peptide immunoadhesins inhibit marburgvirus and ebolavirus cell entry. *J Virol* 85(17):8502–8513. doi:[10.1128/JVI.02600-10](https://doi.org/10.1128/JVI.02600-10)
 50. Escudero-Perez B, Volchkova VA, Dolnik O, Lawrence P, Volchkov VE (2014) Shed GP of Ebola virus triggers immune activation and increased vascular permeability. *PLoS Pathog* 10(11):e1004509. doi:[10.1371/journal.ppat.1004509](https://doi.org/10.1371/journal.ppat.1004509)
 51. Dolnik O, Volchkova VA, Escudero-Perez B, Lawrence P, Klenk HD, Volchkov VE (2015) Shedding of Ebola virus surface glycoprotein is a mechanism of self-regulation of cellular cytotoxicity and has a direct effect on virus infectivity. *J Infect Dis* 212(Suppl 2):S322–S328. doi:[10.1093/infdis/jiv268](https://doi.org/10.1093/infdis/jiv268)
 52. Ruiz-Arguello MB, Goni FM, Pereira FB, Nieva JL (1998) Phosphatidylinositol-dependent membrane fusion induced by a putative fusogenic sequence of Ebola virus. *J Virol* 72(3):1775–1781
 53. Weissenhorn W, Calder LJ, Wharton SA, Skehel JJ, Wiley DC (1998) The central structural feature of the membrane fusion protein subunit from the Ebola virus glycoprotein is a long triple-stranded coiled coil. *Proc Natl Acad Sci U S A* 95(11):6032–6036
 54. Sanchez A, Yang ZY, Xu L, Nabel GJ, Crews T, Peters CJ (1998) Biochemical analysis of the secreted and virion glycoproteins of Ebola virus. *J Virol* 72(8):6442–6447
 55. Weissenhorn W, Carfi A, Lee KH, Skehel JJ, Wiley DC (1998) Crystal structure of the Ebola virus membrane fusion subunit, GP2, from the envelope glycoprotein ectodomain. *Mol Cell* 2(5):605–616
 56. Volchkov VE, Feldmann H, Volchkova VA, Klenk HD (1998) Processing of the Ebola virus glycoprotein by the proprotein convertase furin. *Proc Natl Acad Sci U S A* 95(10):5762–5767
 57. Wool-Lewis RJ, Bates P (1999) Endoproteolytic processing of the ebola virus envelope glycoprotein: cleavage is not required for function. *J Virol* 73(2):1419–1426
 58. Neumann G, Feldmann H, Watanabe S, Lukashevich I, Kawaoka Y (2002) Reverse genetics demonstrates that proteolytic processing of the Ebola virus glycoprotein is not essential for replication in cell culture. *J Virol* 76(1):406–410
 59. Neumann G, Geisbert TW, Ebihara H, Geisbert JB, Daddario-DiCaprio KM, Feldmann H, Kawaoka Y (2007) Proteolytic processing of the Ebola virus glycoprotein is not critical for Ebola virus replication in non-human primates. *J Virol* 81(6):2995–2998. doi:[10.1128/JVI.02486-06](https://doi.org/10.1128/JVI.02486-06)
 60. Yang ZY, Duckers HJ, Sullivan NJ, Sanchez A, Nabel EG, Nabel GJ (2000) Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. *Nat Med* 6(8):886–889
 61. Groseth A, Marzi A, Hoenen T, Herwig A, Gardner D, Becker S, Ebihara H, Feldmann H (2012) The Ebola virus glycoprotein contributes to but is not sufficient for virulence in vivo. *PLoS Pathog* 8(8):e1002847. doi:[10.1371/journal.ppat.1002847](https://doi.org/10.1371/journal.ppat.1002847)
 62. Hoenen T, Marzi A, Scott DP, Feldmann F, Callison J, Safronetz D, Ebihara H, Feldmann H (2015) Soluble glycoprotein is not required for Ebola virus virulence in guinea pigs. *J Infect Dis* 212(Suppl 2):S242–S246. doi:[10.1093/infdis/jiv111](https://doi.org/10.1093/infdis/jiv111)
 63. Chan SY, Empig CJ, Welte FJ, Speck RF, Schmaljohn A, Kreisberg JF, Goldsmith MA (2001) Folate receptor- α is a cofactor for cellular entry by Marburg and Ebola viruses. *Cell* 106(1):117–126
 64. Alvarez CP, Lasala F, Carrillo J, Muniz O, Corbi AL, Delgado R (2002) C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans. *J Virol* 76(13):6841–6844
 65. Simmons G, Reeves JD, Grogan CC, Vandenberghe LH, Baribaud F, Whitbeck JC, Burke E, Buchmeier MJ, Soilleux EJ, Riley JL, Doms RW, Bates P, Pohlmann S (2003) DC-SIGN and DC-SIGNR bind ebola glycoproteins and enhance infection of macrophages and endothelial cells. *Virology* 305(1):115–123
 66. Takada A, Fujioka K, Tsuiji M, Morikawa A, Higashi N, Ebihara H, Kobasa D, Feldmann H, Irimura T, Kawaoka Y (2004) Human macrophage C-type lectin specific for galactose and N-acetylgalactosamine promotes filovirus entry. *J Virol* 78(6):2943–2947
 67. Kondratowicz AS, Lennemann NJ, Sinn PL, Davey RA, Hunt CL, Moller-Tank S, Meyerholz DK, Rennert P, Mullins RF, Brindley M, Sandersfeld LM, Quinn K,

- Weller M, McCray PB Jr, Chiorini J, Maury W (2011) T-cell immunoglobulin and mucin domain 1 (TIM-1) is a receptor for Zaire Ebola virus and Lake Victoria Marburgvirus. *Proc Natl Acad Sci U S A* 108(20):8426–8431. doi:[10.1073/pnas.1019030108](https://doi.org/10.1073/pnas.1019030108)
68. Carette JE, Raaben M, Wong AC, Herbert AS, Obernosterer G, Mulherkar N, Kuehne AI, Kranzusch PJ, Griffin AM, Ruthel G, Dal Cin P, Dye JM, Whelan SP, Chandran K, Brummelkamp TR (2011) Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. *Nature* 477(7364):340–343. doi:[10.1038/nature10348](https://doi.org/10.1038/nature10348)
 69. Cote M, Misasi J, Ren T, Bruchez A, Lee K, Filone CM, Hensley L, Li Q, Ory D, Chandran K, Cunningham J (2011) Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection. *Nature* 477(7364):344–348. doi:[10.1038/nature10380](https://doi.org/10.1038/nature10380)
 70. Bhattacharyya S, Hope TJ, Young JA (2011) Differential requirements for clathrin endocytic pathway components in cellular entry by Ebola and Marburg glycoprotein pseudovirions. *Virology* 419(1):1–9. doi:[10.1016/j.virol.2011.07.018](https://doi.org/10.1016/j.virol.2011.07.018)
 71. Bhattacharyya S, Warfield KL, Ruthel G, Bavari S, Aman MJ, Hope TJ (2010) Ebola virus uses clathrin-mediated endocytosis as an entry pathway. *Virology* 401(1):18–28. doi:[10.1016/j.virol.2010.02.015](https://doi.org/10.1016/j.virol.2010.02.015)
 72. Empig CJ, Goldsmith MA (2002) Association of the caveola vesicular system with cellular entry by filoviruses. *J Virol* 76(10):5266–5270
 73. Quinn K, Brindley MA, Weller ML, Kaludov N, Kondratowicz A, Hunt CL, Sinn PL, McCray PB Jr, Stein CS, Davidson BL, Flick R, Mandell R, Staplin W, Maury W, Chiorini JA (2009) Rho GTPases modulate entry of Ebola virus and vesicular stomatitis virus pseudotyped vectors. *J Virol* 83(19):10176–10186. doi:[10.1128/JVI.00422-09](https://doi.org/10.1128/JVI.00422-09)
 74. Saeed MF, Kolokoltsov AA, Albrecht T, Davey RA (2010) Cellular entry of ebola virus involves uptake by a macropinocytosis-like mechanism and subsequent trafficking through early and late endosomes. *PLoS Pathog* 6(9):e1001110. doi:[10.1371/journal.ppat.1001110](https://doi.org/10.1371/journal.ppat.1001110)
 75. Nanbo A, Imai M, Watanabe S, Noda T, Takahashi K, Neumann G, Halfmann P, Kawaoka Y (2010) Ebolavirus is internalized into host cells via macropinocytosis in a viral glycoprotein-dependent manner. *PLoS Pathog* 6(9):e1001121. doi:[10.1371/journal.ppat.1001121](https://doi.org/10.1371/journal.ppat.1001121)
 76. Mulherkar N, Raaben M, de la Torre JC, Whelan SP, Chandran K (2011) The Ebola virus glycoprotein mediates entry via a non-classical dynamin-dependent macropinocytic pathway. *Virology* 419(2):72–83. doi:[10.1016/j.virol.2011.08.009](https://doi.org/10.1016/j.virol.2011.08.009)
 77. Aleksandrowicz P, Marzi A, Biedenkopf N, Beimforde N, Becker S, Hoenen T, Feldmann H, Schnittler HJ (2011) Ebola virus enters host cells by macropinocytosis and clathrin-mediated endocytosis. *J Infect Dis* 204(Suppl 3):S957–S967. doi:[10.1093/infdis/jir326](https://doi.org/10.1093/infdis/jir326)
 78. Sanchez A (2007) Analysis of filovirus entry into vero e6 cells, using inhibitors of endocytosis, endosomal acidification, structural integrity, and cathepsin (B and L) activity. *J Infect Dis* 196(Suppl 2):S251–S258. doi:[10.1086/520597](https://doi.org/10.1086/520597)
 79. Hunt CL, Kolokoltsov AA, Davey RA, Maury W (2011) The Tyro3 receptor kinase Axl enhances macropinocytosis of Zaire ebolavirus. *J Virol* 85(1):334–347. doi:[10.1128/JVI.01278-09](https://doi.org/10.1128/JVI.01278-09)
 80. Chandran K, Sullivan NJ, Felbor U, Whelan SP, Cunningham JM (2005) Endosomal proteolysis of the Ebola virus glycoprotein is necessary for infection. *Science* 308(5728):1643–1645
 81. Schornberg K, Matsuyama S, Kabsch K, Delos S, Bouton A, White J (2006) Role of endosomal cathepsins in entry mediated by the Ebola virus glycoprotein. *J Virol* 80(8):4174–4178
 82. Martinez O, Johnson J, Manicassamy B, Rong L, Olinger GG, Hensley LE, Basler CF (2010) Zaire Ebola virus entry into human dendritic cells is insensitive to cathepsin L inhibition. *Cell Microbiol* 12(2):148–157. doi:[10.1111/j.1462-5822.2009.01385.x](https://doi.org/10.1111/j.1462-5822.2009.01385.x)
 83. Misasi J, Chandran K, Yang JY, Considine B, Filone CM, Cote M, Sullivan N, Fabozzi G, Hensley L, Cunningham J (2012) Filoviruses require endosomal cysteine proteases for entry but exhibit distinct protease preferences. *J Virol* 86(6):3284–3292. doi:[10.1128/JVI.06346-11](https://doi.org/10.1128/JVI.06346-11)
 84. Gnirss K, Kuhl A, Karsten C, Glowacka I, Bertram S, Kaup F, Hofmann H, Pohlmann S (2012) Cathepsins B and L activate Ebola but not Marburg virus glycoproteins for efficient entry into cell lines and macrophages independent of TMPRSS2 expression. *Virology* 424(1):3–10. doi:[10.1016/j.virol.2011.11.031](https://doi.org/10.1016/j.virol.2011.11.031)
 85. Marzi A, Reinheckel T, Feldmann H (2012) Cathepsin B & L are not required for ebola virus replication. *PLoS Negl Trop Dis* 6(12):e1923. doi:[10.1371/journal.pntd.0001923](https://doi.org/10.1371/journal.pntd.0001923)
 86. Brecher M, Schornberg KL, Delos SE, Fusco ML, Saphire EO, White JM (2012) Cathepsin cleavage potentiates the Ebola virus glycoprotein to undergo a subsequent fusion-relevant conformational change. *J Virol* 86(1):364–372. doi:[10.1128/JVI.05708-11](https://doi.org/10.1128/JVI.05708-11)

87. Muhlberger E, Weik M, Volchkov VE, Klenk HD, Becker S (1999) Comparison of the transcription and replication strategies of Marburg virus and Ebola virus by using artificial replication systems. *J Virol* 73(3): 2333–2342
88. Weik M, Modrof J, Klenk HD, Becker S, Muhlberger E (2002) Ebola virus VP30-mediated transcription is regulated by RNA secondary structure formation. *J Virol* 76(17):8532–8539
89. Biedenkopf N, Hartlieb B, Hoenen T, Becker S (2013) Phosphorylation of Ebola virus VP30 influences the composition of the viral nucleocapsid complex: impact on viral transcription and replication. *J Biol Chem* 288(16):11165–11174. doi:[10.1074/jbc.M113.461285](https://doi.org/10.1074/jbc.M113.461285)
90. Biedenkopf N, Lier C, Becker S (2016) Dynamic phosphorylation of VP30 is essential for Ebola virus life cycle. *J Virol* 90(10):4914–4925. doi:[10.1128/JVI.03257-15](https://doi.org/10.1128/JVI.03257-15)
91. Halfmann P, Kim JH, Ebihara H, Noda T, Neumann G, Feldmann H, Kawaoka Y (2008) Generation of biologically contained Ebola viruses. *Proc Natl Acad Sci U S A* 105(4):1129–1133. doi:[10.1073/pnas.0708057105](https://doi.org/10.1073/pnas.0708057105)
92. Mehedi M, Hoenen T, Robertson S, Ricklefs S, Dolan MA, Taylor T, Falzarano D, Ebihara H, Porcella SF, Feldmann H (2013) Ebola virus RNA editing depends on the primary editing site sequence and an upstream secondary structure. *PLoS Pathog* 9(10):e1003677. doi:[10.1371/journal.ppat.1003677](https://doi.org/10.1371/journal.ppat.1003677)
93. Becker S, Rinne C, Hofsass U, Klenk HD, Muhlberger E (1998) Interactions of Marburg virus nucleocapsid proteins. *Virology* 249(2):406–417
94. Han Z, Boshra H, Sunyer JO, Zwiers SH, Paragas J, Harty RN (2003) Biochemical and functional characterization of the Ebola virus VP24 protein: implications for a role in virus assembly and budding. *J Virol* 77(3): 1793–1800
95. Bharat TA, Noda T, Riches JD, Kraehling V, Kolesnikova L, Becker S, Kawaoka Y, Briggs JA (2012) Structural dissection of Ebola virus and its assembly determinants using cryo-electron tomography. *Proc Natl Acad Sci U S A* 109(11):4275–4280. doi:[10.1073/pnas.1120453109](https://doi.org/10.1073/pnas.1120453109)
96. Watt A, Moukambi F, Banadyga L, Groseth A, Callison J, Herwig A, Ebihara H, Feldmann H, Hoenen T (2014) A novel life cycle modeling system for Ebola virus shows a genome length-dependent role of VP24 in virus infectivity. *J Virol* 88(18):10511–10524. doi:[10.1128/JVI.01272-14](https://doi.org/10.1128/JVI.01272-14)
97. Hoenen T, Jung S, Herwig A, Groseth A, Becker S (2010) Both matrix proteins of Ebola virus contribute to the regulation of viral genome replication and transcription. *Virology* 403(1):56–66. doi:[10.1016/j.virol.2010.04.002](https://doi.org/10.1016/j.virol.2010.04.002)
98. Watanabe S, Noda T, Halfmann P, Jasenosky L, Kawaoka Y (2007) Ebola virus (EBOV) VP24 inhibits transcription and replication of the EBOV genome. *J Infect Dis* 196(Suppl 2):S284–S290. doi:[10.1086/520582](https://doi.org/10.1086/520582)
99. Hoenen T, Groseth A, Kolesnikova L, Theriault S, Ebihara H, Hartlieb B, Bamberg S, Stroher U, Feldmann H, Becker S (2006) Infection of naive target cells with virus-like particles – implications for the function of Ebola virus VP24. *J Virol* 80(14):7260–7264
100. Hoenen T, Shabman RS, Groseth A, Herwig A, Weber M, Schudt G, Dolnik O, Basler CF, Becker S, Feldmann H (2012) Inclusion bodies are a site of ebolavirus replication. *J Virol* 86(21):11779–11788. doi:[10.1128/JVI.01525-12](https://doi.org/10.1128/JVI.01525-12)
101. Nanbo A, Watanabe S, Halfmann P, Kawaoka Y (2013) The spatio-temporal distribution dynamics of Ebola virus proteins and RNA in infected cells. *Sci Rep* 3:1206. doi:[10.1038/srep01206](https://doi.org/10.1038/srep01206)
102. Brauburger K, Boehmann Y, Tsuda Y, Hoenen T, Olejnik J, Schumann M, Ebihara H, Muhlberger E (2014) Analysis of the highly diverse gene borders in Ebola virus reveals a distinct mechanism of transcriptional regulation. *J Virol* 88(21):12558–12571. doi:[10.1128/JVI.01863-14](https://doi.org/10.1128/JVI.01863-14)
103. Watanabe S, Watanabe T, Noda T, Takada A, Feldmann H, Jasenosky LD, Kawaoka Y (2004) Production of novel ebola virus-like particles from cDNAs: an alternative to ebola virus generation by reverse genetics. *J Virol* 78(2):999–1005
104. Schudt G, Dolnik O, Kolesnikova L, Biedenkopf N, Herwig A, Becker S (2015) Transport of Ebolavirus nucleocapsids is dependent on actin polymerization: live-cell imaging analysis of Ebolavirus-infected cells. *J Infect Dis* 212(Suppl 2):S160–S166. doi:[10.1093/infdis/jiv083](https://doi.org/10.1093/infdis/jiv083)
105. Adu-Gyamfi E, Digman MA, Gratton E, Stahelin RV (2012) Single-particle tracking demonstrates that actin coordinates the movement of the Ebola virus matrix protein. *Biophys J* 103(9):L41–L43. doi:[10.1016/j.bpj.2012.09.026](https://doi.org/10.1016/j.bpj.2012.09.026)
106. Ruigrok RW, Schoehn G, Dessen A, Forest E, Volchkov V, Dolnik O, Klenk HD, Weissenhorn W (2000) Structural characterization and membrane binding properties of the matrix protein VP40 of Ebola virus. *J Mol Biol* 300(1):103–112
107. Timmins J, Scianimanico S, Schoehn G, Weissenhorn W (2001) Vesicular release of

- Ebola virus matrix protein VP40. *Virology* 283(1):1–6
108. Jasenosky LD, Neumann G, Lukashevich I, Kawaoka Y (2001) Ebola virus VP40-induced particle formation and association with the lipid bilayer. *J Virol* 75(11):5205–5214
 109. Noda T, Sagara H, Suzuki E, Takada A, Kida H, Kawaoka Y (2002) Ebola virus VP40 drives the formation of virus-like filamentous particles along with GP. *J Virol* 76(10):4855–4865
 110. Harty RN, Brown ME, Wang G, Huibregtse J, Hayes FP (2000) 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 U S A* 97(25):13871–13876
 111. Licata JM, Simpson-Holley M, Wright NT, Han Z, Paragas J, Harty RN (2003) 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(3):1812–1819
 112. Timmins J, Schoehn G, Ricard-Blum S, Scianimanico S, Vernet T, Ruigrok RW, Weissenhorn W (2003) Ebola virus matrix protein VP40 interaction with human cellular factors Tsg101 and Nedd4. *J Mol Biol* 326(2):493–502
 113. Yasuda J, Nakao M, Kawaoka Y, Shida H (2003) Nedd4 regulates egress of Ebola virus-like particles from host cells. *J Virol* 77(18):9987–9992
 114. Han Z, Sagum CA, Bedford MT, Sidhu SS, Sudol M, Harty RN (2016) ITCH E3 ubiquitin ligase interacts with Ebola virus VP40 to regulate budding. *J Virol* 90(20):9163–9171. doi:[10.1128/JVI.01078-16](https://doi.org/10.1128/JVI.01078-16)
 115. Neumann G, Ebihara H, Takada A, Noda T, Kobasa D, Jasenosky LD, Watanabe S, Kim JH, Feldmann H, Kawaoka Y (2005) Ebola virus VP40 late domains are not essential for viral replication in cell culture. *J Virol* 79(16):10300–10307
 116. Han Z, Madara JJ, Liu W, Ruthel G, Freedman BD, Harty RN (2015) ALIX rescues budding of a double PTAP/PPEY L-domain deletion mutant of Ebola VP40: a role for ALIX in Ebola virus egress. *J Infect Dis* 212(Suppl 2):S138–S145. doi:[10.1093/infdis/jiu838](https://doi.org/10.1093/infdis/jiu838)
 117. Dessen A, Volchkov V, Dolnik O, Klenk HD, Weissenhorn W (2000) Crystal structure of the matrix protein VP40 from Ebola virus. *EMBO J* 19(16):4228–4236
 118. Dessen A, Forest E, Volchkov V, Dolnik O, Klenk HD, Weissenhorn W (2000) Crystallization and preliminary X-ray analysis of the matrix protein from Ebola virus. *Acta Crystallogr D Biol Crystallogr* 56(Pt 6):758–760
 119. Scianimanico S, Schoehn G, Timmins J, Ruigrok RH, Klenk HD, Weissenhorn W (2000) Membrane association induces a conformational change in the Ebola virus matrix protein. *EMBO J* 19(24):6732–6741
 120. Hoenen T, Biedenkopf N, Zielecki F, Jung S, Groseth A, Feldmann H, Becker S (2010) Oligomerization of Ebola virus VP40 is essential for particle morphogenesis and regulation of viral transcription. *J Virol* 84(14):7053–7063. doi:[10.1128/JVI.00737-10](https://doi.org/10.1128/JVI.00737-10)
 121. Soni SP, Adu-Gyamfi E, Yong SS, Jee CS, Stahelin RV (2013) The Ebola virus matrix protein deeply penetrates the plasma membrane: an important step in viral egress. *Biophys J* 104(9):1940–1949. doi:[10.1016/j.bpj.2013.03.021](https://doi.org/10.1016/j.bpj.2013.03.021)
 122. Soni SP, Stahelin RV (2014) The Ebola virus matrix protein VP40 selectively induces vesiculation from phosphatidylserine-enriched membranes. *J Biol Chem* 289(48):33590–33597. doi:[10.1074/jbc.M114.586396](https://doi.org/10.1074/jbc.M114.586396)
 123. Harcourt BH, Sanchez A, Offermann MK (1998) Ebola virus inhibits induction of genes by double-stranded RNA in endothelial cells. *Virology* 252(1):179–188
 124. Harcourt BH, Sanchez A, Offermann MK (1999) Ebola virus selectively inhibits responses to interferons, but not to interleukin-1beta, in endothelial cells. *J Virol* 73(4):3491–3496
 125. Basler CF, Wang X, Muhlberger E, Volchkov V, Paragas J, Klenk HD, Garcia-Sastre A, Palese P (2000) The Ebola virus VP35 protein functions as a type I IFN antagonist. *Proc Natl Acad Sci U S A* 97(22):12289–12294
 126. Basler CF, Mikulasova A, Martinez-Sobrido L, Paragas J, Muhlberger E, Bray M, Klenk HD, Palese P, Garcia-Sastre A (2003) The Ebola virus VP35 protein inhibits activation of interferon regulatory factor 3. *J Virol* 77(14):7945–7956
 127. Reid SP, Cardenas WB, Basler CF (2005) Homo-oligomerization facilitates the interferon-antagonist activity of the ebolavirus VP35 protein. *Virology* 341(2):179–189
 128. Hartman AL, Towner JS, Nichol ST (2004) A C-terminal basic amino acid motif of Zaire ebolavirus VP35 is essential for type I interferon antagonism and displays high identity with the RNA-binding domain of another interferon antagonist, the NS1 protein of influenza A virus. *Virology* 328(2):177–184
 129. Cardenas WB, Loo YM, Gale M Jr, Hartman AL, Kimberlin CR, Martinez-Sobrido L, Saphire EO, Basler CF (2006) Ebola virus VP35 protein binds double-stranded RNA and inhibits alpha/beta interferon

- production induced by RIG-I signaling. *J Virol* 80(11):5168–5178
130. Prins KC, Cardenas WB, Basler CF (2009) Ebola virus protein VP35 impairs the function of interferon regulatory factor-activating kinases IKKepsilon and TBK-1. *J Virol* 83(7):3069–3077. doi:[10.1128/JVI.01875-08](https://doi.org/10.1128/JVI.01875-08)
 131. Bale S, Julien JP, Bornholdt ZA, Krois AS, Wilson IA, Saphire EO (2013) Ebola virus VP35 coats the backbone of double-stranded RNA for interferon antagonism. *J Virol* 87(18):10385–10388. doi:[10.1128/JVI.01452-13](https://doi.org/10.1128/JVI.01452-13)
 132. Bray M (2001) The role of the Type I interferon response in the resistance of mice to filovirus infection. *J Gen Virol* 82(Pt 6):1365–1373
 133. Hartman AL, Dover JE, Towner JS, Nichol ST (2006) Reverse genetic generation of recombinant Zaire Ebola viruses containing disrupted IRF-3 inhibitory domains results in attenuated virus growth in vitro and higher levels of IRF-3 activation without inhibiting viral transcription or replication. *J Virol* 80(13):6430–6440. doi:[10.1128/JVI.00044-06](https://doi.org/10.1128/JVI.00044-06)
 134. Hartman AL, Bird HB, Towner JS, Antoniadou Z, Zaki S, Nichol ST (2008) Inhibition of IRF-3 activation by VP35 is critical for the high level of virulence of Ebola virus. *J Virol* 82(6):2699–2704
 135. Prins KC, Delpout S, Leung DW, Reynard O, Volchkova VA, Reid SP, Ramanan P, Cardenas WB, Amarasinghe GK, Volchkov VE, Basler CF (2010) Mutations abrogating VP35 interaction with double-stranded RNA render Ebola virus avirulent in guinea pigs. *J Virol* 84(6):3004–3015. doi:[10.1128/JVI.02459-09](https://doi.org/10.1128/JVI.02459-09)
 136. Kash JC, Muhlberger E, Carter V, Grosch M, Perwitasari O, Proll SC, Thomas MJ, Weber F, Klenk HD, Katze MG (2006) Global suppression of the host antiviral response by Ebola- and Marburgviruses: increased antagonism of the type I interferon response is associated with enhanced virulence. *J Virol* 80(6):3009–3020
 137. Guito JC, Albarino CG, Chakrabarti AK, Towner JS (2016) Novel activities by ebola-virus and marburgvirus interferon antagonists revealed using a standardized in vitro reporter system. *Virology* 501:147–165. doi:[10.1016/j.virol.2016.11.015](https://doi.org/10.1016/j.virol.2016.11.015)
 138. Reid SP, Leung LW, Hartman AL, Martinez O, Shaw ML, Carbonnelle C, Volchkov VE, Nichol ST, Basler CF (2006) Ebola virus VP24 binds karyopherin alpha 1 and blocks STAT1 nuclear accumulation. *J Virol* 80(11):5156–5167
 139. Reid SP, Valmas C, Martinez O, Sanchez FM, Basler CF (2007) Ebola virus VP24 proteins inhibit the interaction of NPI-1 subfamily karyopherin alpha proteins with activated STAT1. *J Virol* 81(24):13469–13477. doi:[10.1128/JVI.01097-07](https://doi.org/10.1128/JVI.01097-07)
 140. Xu W, Edwards MR, Borek DM, Feagins AR, Mittal A, Alinger JB, Berry KN, Yen B, Hamilton J, Brett TJ, Pappu RV, Leung DW, Basler CF, Amarasinghe GK (2014) Ebola virus VP24 targets a unique NLS binding site on karyopherin alpha 5 to selectively compete with nuclear import of phosphorylated STAT1. *Cell Host Microbe* 16(2):187–200. doi:[10.1016/j.chom.2014.07.008](https://doi.org/10.1016/j.chom.2014.07.008)
 141. Zhang AP, Bornholdt ZA, Liu T, Abelson DM, Lee DE, Li S, Woods VL Jr, Saphire EO (2012) The Ebola virus interferon antagonist VP24 directly binds STAT1 and has a novel, pyramidal fold. *PLoS Pathog* 8(2):e1002550. doi:[10.1371/journal.ppat.1002550](https://doi.org/10.1371/journal.ppat.1002550)
 142. Halfmann P, Neumann G, Kawaoka Y (2011) The Ebolavirus VP24 protein blocks phosphorylation of p38 mitogen-activated protein kinase. *J Infect Dis* 204(Suppl 3):S953–S956. doi:[10.1093/infdis/jir325](https://doi.org/10.1093/infdis/jir325)
 143. Ishida H, Ohkawa K, Hosui A, Hiramatsu N, Kanto T, Ueda K, Takehara T, Hayashi N (2004) Involvement of p38 signaling pathway in interferon-alpha-mediated antiviral activity toward hepatitis C virus. *Biochem Biophys Res Commun* 321(3):722–727. doi:[10.1016/j.bbrc.2004.07.015](https://doi.org/10.1016/j.bbrc.2004.07.015)
 144. Goh KC, Haque SJ, Williams BR (1999) p38 MAP kinase is required for STAT1 serine phosphorylation and transcriptional activation induced by interferons. *EMBO J* 18(20):5601–5608. doi:[10.1093/emboj/18.20.5601](https://doi.org/10.1093/emboj/18.20.5601)
 145. Kaletsky RL, Francica JR, Agrawal-Gamse C, Bates P (2009) Tetherin-mediated restriction of filovirus budding is antagonized by the Ebola glycoprotein. *Proc Natl Acad Sci U S A* 106(8):2886–2891. doi:[10.1073/pnas.0811014106](https://doi.org/10.1073/pnas.0811014106)
 146. Lopez LA, Yang SJ, Hauser H, Exline CM, Haworth KG, Oldenburg J, Cannon PM (2010) Ebola virus glycoprotein counteracts BST-2/Tetherin restriction in a sequence-independent manner that does not require tetherin surface removal. *J Virol* 84(14):7243–7255. doi:[10.1128/JVI.02636-09](https://doi.org/10.1128/JVI.02636-09)
 147. Kuhl A, Banning C, Marzi A, Votteler J, Steffen I, Bertram S, Glowacka I, Konrad A, Sturzl M, Guo JT, Schubert U, Feldmann H, Behrens G, Schindler M, Pohlmann S (2011) The Ebola virus glycoprotein and HIV-1 Vpu employ different strategies to counteract the antiviral factor tetherin. *J Infect Dis* 204(Suppl 3):S850–S860. doi:[10.1093/infdis/jir378](https://doi.org/10.1093/infdis/jir378)
 148. Gnirss K, Fiedler M, Kramer-Kuhl A, Bolduan S, Mittler E, Becker S, Schindler M, Pohlmann

- S (2014) Analysis of determinants in filovirus glycoproteins required for tetherin antagonism. *Virus* 6(4):1654–1671. doi:[10.3390/v6041654](https://doi.org/10.3390/v6041654)
149. Brinkmann C, Nehlmeier I, Walendy-Gnirss K, Nehls J, Gonzalez Hernandez M, Hoffmann M, Qiu X, Takada A, Schindler M, Pohlmann S (2016) Tetherin antagonism by the Ebola virus glycoprotein requires an intact receptor-binding domain and can be blocked by GP1-specific antibodies. *J Virol*. doi:[10.1128/JVI.01563-16](https://doi.org/10.1128/JVI.01563-16)
 150. Gustin JK, Bai Y, Moses AV, Douglas JL (2015) Ebola virus glycoprotein promotes enhanced viral egress by preventing Ebola VP40 from associating with the host restriction factor BST2/tetherin. *J Infect Dis* 212(Suppl 2):S181–S190. doi:[10.1093/infdis/jiv125](https://doi.org/10.1093/infdis/jiv125)
 151. Feng Z, Cerveny M, Yan Z, He B (2007) The VP35 protein of Ebola virus inhibits the antiviral effect mediated by double-stranded RNA-dependent protein kinase PKR. *J Virol* 81(1):182–192
 152. Schumann M, Gantke T, Muhlberger E (2009) Ebola virus VP35 antagonizes PKR activity through its C-terminal interferon inhibitory domain. *J Virol* 83(17):8993–8997. doi:[10.1128/JVI.00523-09](https://doi.org/10.1128/JVI.00523-09)
 153. Haasnoot J, de Vries W, Geutjes EJ, Prins M, de Haan P, Berkhout B (2007) The Ebola virus VP35 protein is a suppressor of RNA silencing. *PLoS Pathog* 3(6):e86. doi:[10.1371/journal.ppat.0030086](https://doi.org/10.1371/journal.ppat.0030086)
 154. Nelson EV, Schmidt KM, DeFlube LR, Doganay S, Banadyga L, Olejnik J, Hume AJ, Ryabchikova E, Ebihara H, Kedersha N, Ha T, Muhlberger E (2016) Ebola virus does not induce stress granule formation during infection and sequesters stress granule proteins within viral inclusions. *J Virol* 90(16):7268–7284. doi:[10.1128/JVI.00459-16](https://doi.org/10.1128/JVI.00459-16)

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