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Virus and host interactions critical for filoviral RNA synthesis as therapeutic targets

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Abstract

Filoviruses, which include Ebola virus and Marburg virus, are negative-sense RNA viruses associated with sporadic outbreaks of severe viral hemorrhagic fever characterized by uncontrolled virus replication. The extreme virulence and emerging nature of these zoonotic pathogens make them a significant threat to human health. Replication of the filovirus genome and production of viral RNAs requires the function of a complex of four viral proteins, the nucleoprotein (NP), viral protein 35 (VP35), viral protein 30 (VP30) and large protein (L). The latter performs the enzymatic activities required for production of viral RNAs and capping of viral mRNAs. Although it has been recognized that interactions between the virus-encoded components of the EBOV RNA polymerase complex are required for viral RNA synthesis reactions, specific molecular details have, until recently, been lacking. New efforts have combined structural biology and molecular virology to reveal in great detail the molecular basis for critical protein-protein interactions (PPIs) necessary for viral RNA synthesis. These efforts include recent studies that have identified a range of host factors and in some instances demonstrated unique mechanisms by which they act. For a select number of these interactions, combined use of mutagenesis, over-expressing of peptides corresponding to PPI interfaces and identification of small molecules that disrupt PPIs have demonstrated the functional significance of virus-virus and virus-host PPIs and suggest several as potential targets for therapeutic intervention.

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1. Significance of filoviruses as human pathogens.

Filoviruses are filamentous, enveloped viruses with non-segmented, negative-sense RNA genomes (Messaoudi et al., 2015). The family *Filoviridae* is comprised of the genus *Ebolavirus* includes *Zaire ebolavirus* (Ebola virus, EBOV) and five other species, the genus *Marburgvirus*, which includes Marburg virus (MARV), and the genus *Cuevavirus* (Afonso et al., 2016; Goldstein et al., 2018). Members of the *Ebolavirus* and *Marburgvirus* genera are zoonotic pathogens that have caused repeated outbreaks with substantial lethality in humans (Rougeron et al., 2015). The largest such outbreak on record was caused by EBOV and occurred in West Africa between 2013-2016. This resulted in upwards of 28,000 infections, more than 11,000 deaths, and the export of infected cases to the United States and Europe (Spengler et al.). In pregnant women, the fatality rate is estimated to be 70% (Hayden et al., 2017). The only treatments available for infected individuals were supportive care and experimental therapies, hampering patient treatment and leaving healthcare workers at risk. Survivors are known to exhibit persistent infections with virus residing in immune privileged sites, including the eye and testes (Jacobs et al., 2016; Uyeki et al., 2016; Yeh et al., 2015; Zeng et al., 2017). The West Africa epidemic reinforced the threat posed by filoviruses and resulted in a major push to provide potential therapeutics and vaccines for infected individuals. The results of a trial with vesicular stomatitis virus (VSV)-based vaccine in which the VSV glycoprotein is replaced by the Ebola virus glycoprotein (GP) were very promising (Henao-Restrepo et al., 2017). However, even if effective vaccines are developed, it is unlikely that universal vaccination will be implemented for EBOV or for other filoviruses. Therefore, there will remain a need for effective complimentary therapies, including small molecule drugs. This reinforces the need for ongoing development of new filovirus therapeutics and continued research into filovirus biology.

2. Overview of filovirus replication.

The filovirus genome is approximately 19 kilobases in length and encodes up to nine translation products from seven separate transcriptional units (Feldmann et al., 2015; Messaoudi et al., 2015) (Fig 1A). These genes encode the viral nucleoprotein (NP), viral protein of 35 kDa (VP35), VP40, a type I transmembrane glycoprotein (GP), VP30, VP24, and the large protein (L), which is the viral polymerase. Members of the *Ebolavirus* genus also produce at least two secreted forms of the GP protein (Messaoudi et al., 2015).

The filovirus replication cycle can be summarized as follows (Fig. 1B). Viral entry is mediated by GP which acts as an attachment factor and mediates fusion of viral and host cell membranes within an endosomal compartment (Davey et al., 2017). A prerequisite for fusion is processing of GP by host cell cathepsin proteases within endosomes, allowing cleaved GP to interact with an intra-endosomal receptor, Niemann-Pick disease, type C1 (NPC-1) (Carette et al., 2011; Chandran et al., 2005; Cote et al., 2011; Miller et al., 2012). The viral genome is released into the cytoplasm as a ribonucleoprotein complex. This complex serves as the template for the RNA synthesis reactions that replicate the viral genomic RNA and transcribe the mRNAs for viral gene expression. Replication of the viral genomic RNA requires NP, which associates with the viral genomic and antigenomic RNAs throughout the course of infection; VP35, a non-enzymatic cofactor for the viral RNA-

dependent RNA polymerase that also serves as a potent suppressor of innate antiviral signaling pathways, and L. L possesses all the enzymatic activities required for viral transcription and genome replication, including RNA-dependent RNA polymerase activity, guanyltransferase, and methyltransferase activities (Muhlberger, 2007). Viral transcription (mRNA synthesis) involves the synthesis of distinct 5'-capped, 3'-polyadenylated mRNAs from each of the viral genes and requires, in addition to NP, VP35 and L, the VP30 protein (Fig. 2) (Muhlberger, 2007). In addition to the required viral proteins, host factors modulate viral RNA synthesis through interaction with viral factors. (Batra et al., 2018; Luthra et al., 2015; Luthra et al., 2013; Smith et al., 2010). However, a complete understanding as to how host factors contribute to viral RNA synthesis remains incomplete.

Other viral functions include filovirus assembly and release (Kolesnikova et al., 2017). The VP40 matrix protein drives the membrane budding events that lead to release of new virus particles. GP is incorporated into the membrane of viral particles and enhances budding. Viral RNPs that contain genomic RNA, NP, VP35, VP30 and VP24 are recruited into the budding particles. In addition to playing roles in replication and assembly, several filovirus proteins counteract host innate antiviral defenses (Olejnik et al., 2017). The filovirus VP35 proteins block interferon (IFN)-α/β production and the VP24 proteins of *Ebolavirus* and *Cuevavirus* genera members and the VP40 proteins of the *Marburgvirus* genus block IFN-induced antiviral signaling (Basler et al., 2000; Cardenas et al., 2006; Leung et al., 2010; Mateo et al., 2010; Reid et al., 2006; Reid et al., 2007; Valmas and Basler, 2011; Valmas et al., 2010; Xu et al., 2014). The MARV VP24 protein also modulates host antioxidant response pathways through interaction with the host protein Kelch-like ECH-associated protein 1 (Keap1) (Edwards et al., 2014; Johnson et al., 2016; Page et al., 2014).

2. Evidence that EBOV RNA synthesis reactions are viable targets for therapeutics

Given their essential role for viral gene expression and viral genome RNA replication, targeting the viral RNA synthesis reactions is a logical strategy for therapeutic development. It is a strategy that also shows substantial promise. As examples, GS-5734 (Remdesivir) and BCX4430, are two nucleoside analogs that target viral RNA synthesis and have proven effective in non-human primate models (Warren et al., 2016; Warren et al., 2014). Other examples of small molecules with anti-EBOV activity include the nucleoside analog favipiravir that is effective in cell culture, can protect mice, reduce viral titers and showed some evidence of efficacy in EBOV- and MARV-infected macaques (Oestereich et al., 2014). Carbocyclic nucleosides, including 3-deazaneplanocin A have broad spectrum activity for non-segmented negative sense RNA viruses including EBOV and MARV (Bray et al., 2000; De Clercq, 1985; Huggins et al., 1999; Ye and Schneller, 2014). This antiviral activity is likely due to inhibition of cellular enzyme S-adenosylhomocysteine (SAH) hydrolase (SAHase), which breaks down the SAH produced from S-adenosylmethionine (SAM), the essential cofactor in macromolecular methylation reactions. Inhibition of SAHase increases intracellular levels of SAH, blocking cellular transmethylation reactions through feedback inhibition, causing diminished methylation of the 5′ cap on viral mRNAs, a reaction carried out by the EBOV L protein, and consequent loss of viral protein synthesis

(De Clercq, 2004). 6-azauridine and azacytidine (5-aza-2′-deoxycytidine) have also been demonstrated to inhibit EBOV replication in cell culture (Edwards et al., 2015; Uebelhoer et al., 2014). In another example, hsp90 inhibitors, which likely act by destabilizing the EBOV L, and are inhibitory towards EBOV in cell culture (Oestereich et al., 2014; Smith et al., 2010; Smither et al., 2014; Warren et al., 2016).

In addition to targeting the enzymatic activities of the L protein or causing L protein degradation, compounds that target VP30 phosphorylation status, which influences viral transcription, or expression can also impair virus replication or RNA synthesis in cell culture. Okadaic acid (OA), an inhibitor of cellular phosphatases, can prevent dephosphorylation of VP30 and inhibit EBOV transcription (Modrof et al., 2002). This observation led to the identification of small molecules that promote VP30 phosphorylation, inhibit EBOV transcription and suppress EBOV replication in cell culture (Ilinykh et al., 2014). In a separate study, small molecule inhibitors of polyamine production were found to inhibit EBOV RNA synthesis measured by minigenome assay. siRNA knockdown of spermidine synthase (SRM) a critical enzyme in polyamine biosynthesis exerted an antiviral effect against an EBOV expressing green fluorescent protein (GFP) (Olsen et al., 2016). Spermidine is required for the hypusination of eukaryotic translation initiation factor 5A (eIF5A), which is modified in this manner at lysine 50. The compounds ciclopirox (CPX) and deferiprone (DEF), which block the enzyme deoxyhypusine hydroxylase (DOHH) required for hypusination, were demonstrated to inhibit minigenome activity and to inhibit both EBOV and MARV growth in cell culture. As eIF5A is a translation factor, the impact of the inhibitors on expression of the EBOV replication complex proteins was assessed in minigenome assays. The results point to inhibition of VP30 expression as an explanation for inhibition of viral replication (Olsen et al., 2016).

Finally, there are examples of host factors identified as modulating viral RNA synthesis that suggest avenues for therapeutic development. For example, genetic and small molecule screens identified inhibition of either host purine or pyrimidine biosynthesis as an effective means of impairing viral RNA synthesis (Edwards et al., 2015; Luthra et al., 2018; Martin et al., 2018). Host RNA binding Staufen was demonstrated to interact with EBOV RNA and with components of the viral replication machinery and to modulate viral RNA synthesis (Fang et al., 2018). DNA topoisomerase 1 was found to interact with and colocalize with EBOV L and its knockdown decrease EBOV replication and viral polymerase function (Takahashi et al., 2013).

3.1 Overview of interactions among EBOV encoded proteins required for viral RNA synthesis

Four viral proteins, NP, VP35, VP30 and L are required to carry out the full complement of EBOV RNA synthesis reactions, with VP30 being required for viral transcription but dispensable for viral genome RNA replication (Muhlberger et al., 1999). Using minigenome assays, which model filoviral RNA synthesis in transfected cells, VP30 was demonstrated to not be required for MARV transcription (Muhlberger et al., 1998). Each of the proteins of the RNA synthesis complex homo-oligomerizes and can interact with RNA. NP consists of an N-terminal domain, a potentially flexible bridge between the N- and C-terminal region

that has low complexity sequences, and a C-terminal domain. NP spontaneously associates with ssRNA and oligomerizes (Kirchdoerfer et al., 2015; Leung et al., 2015; Liu et al., 2017a; Mavrakis et al., 2002; Su et al., 2018; Sugita et al., 2018; Wan et al., 2017; Zhu et al., 2017). VP35s homo-oligomerize through a region in the VP35 N-terminus; EBOV VP35 likely forms various multimers, including tetramers and trimers in solution, whereas the N-terminus of MARV VP35 is reported to form trimers (Figure 3A-B) (Bruhn et al., 2017; Edwards et al., 2016; Luthra et al., 2015; Moller et al., 2005; Reid et al., 2005)(Zinzula et al., 2018). It is not clear at present which forms are critical to mediate each VP35 function. VP35 also possesses a C-terminal domain that binds to dsRNA and is required for inhibition of signaling through retinoic acid-inducible gene I (RIG-I)-like receptor signaling and suppression of IFN and other innate antiviral responses (Bale et al., 2012; Bale et al., 2013; Cardenas et al., 2006; Kimberlin et al., 2010; Leung et al., 2009; Leung et al., 2010; Ramanan et al., 2012). VP30 is a Zn^{2+} binding, phosphoprotein that can also interact with RNA. Zn^{2+} and RNA binding are mediated by an N-terminal domain (John et al., 2007; Modrof et al., 2003). VP30 homo-oligomerization has been demonstrated and VP30-derived peptides that block oligomerization inhibit EBOV growth when introduced into infected cells (Hartlieb et al., 2003). The C-terminal domain of VP30 can dimerize when expressed alone. However, full-length VP30 forms a variety of multimeric complexes, including homohexamers via a domain in the N-terminus (Hartlieb et al., 2007). L polymerase carries out the capping and cap methylation reactions for viral mRNA synthesis as well as RNA-dependent RNA polymerase activities for viral mRNA synthesis and viral genome replication. Studies expressing the N-terminal region of L indicate that L can also homo-oligomerize (Trunschke et al., 2013).

The filovirus RNA synthesis machinery assembles via several protein-protein interactions (PPIs) between different components (Becker et al., 1998). NP associates with and oligomerizes on the viral template RNAs, either negative-sense genomic RNA or positive-sense anti-genomic (complementary) RNA (Kirchdoerfer et al., 2015; Leung et al., 2015; Liu et al., 2017a; Mavrakis et al., 2002; Noda et al., 2010; Su et al., 2018; Sugita et al., 2018; Wan et al., 2017; Watanabe et al., 2006; Zhu et al., 2017). NP also interacts with VP35 and VP30 (Becker et al., 1998; Biedenkopf et al., 2013; Kirchdoerfer et al., 2015; Kirchdoerfer et al., 2016; Kruse et al., 2018; Leung et al., 2015; Liu et al., 2017a; Xu et al., 2017; Zhu et al., 2017). VP35 makes two distinct interactions with NP, one via a sequence in the VP35 N-terminus that interacts with NP near the hinge region between the N- and C-lobes (Kirchdoerfer et al., 2015; Leung et al., 2010; Liu et al., 2017a; Zhu et al., 2017), and one via the C-terminal VP35 IFN inhibitory domain (IID) that interacts with NP, although the exact region is unclear (Prins et al., 2010). VP35 also interacts with L, with an interaction site having been mapped to the first 380 N-terminal residues in L (Trunschke et al., 2013). This presumably brings L to the RNA template via the VP35-NP interaction. The unphosphorylated form of VP30 promotes EBOV transcription and, to a lesser extent MARV transcription (Modrof et al., 2002; Tigabu et al., 2018). VP30 interacts with NP and this occurs via a cleft in the C-terminal domain of VP30 and a proline-rich peptide near residues 610-612 of NP (Hartlieb et al., 2007; Kirchdoerfer et al., 2016; Xu et al., 2017). The interaction with NP, as detected by co-immunoprecipitation assay, is stronger when phosphomimetic mutants of VP30 are used (Biedenkopf et al., 2013). The mechanism by

which VP30 phosphorylation impacts NP interaction remains to be clarified, however. VP30 has also been reported to interact with VP35 and L (Biedenkopf et al., 2013; Tigabu et al., 2018). The former interaction is influenced by the phosphorylation status of VP30 and is suggested to require the presence of RNA but the regions of VP30 and VP35 have not been defined. The VP30-L interaction, as well as evidence of an NP-VP30-L bridge have been demonstrated by co-localization assessed by indirect immunofluorescence studies performed on cells transfected with expression plasmids for individual or combinations of RESTV proteins (Groseth et al., 2009). The domains involved in the VP30-L interaction and the functional significance of the VP30-VP35 and VP30-L interaction remain to be defined.

Filovirus RNA synthesis takes place in cytoplasmic inclusions (Dolnik et al., 2015; Hoenen et al., 2012). These inclusions are also sites of the PPIs described above. NP expressed alone forms inclusion bodies and when co-expressed with NP, VP35, VP30 and L also co-localize in these inclusions (Becker et al., 1998; Nanbo et al., 2013). Further, the ratio of NP to VP35 can alter propensity of NP to form inclusions (Noda et al., 2011). Inclusions are associated with cellular membranes as well as with a variety of cellular factors including endosomal complex required for transport (ESCRT) associated proteins, autophagy-associated proteins such as microtubule associated protein 1 light remain to be defined. chain 3 alpha (LC3)and stress granule proteins (Dolnik et al., 2010; Dolnik et al., 2015; Nelson et al., 2016). Whether these proteins influence filoviral RNA synthesis and by which mechanisms remains to be determined.

4. Recent insights into the protein-protein interactions that modulate Ebola virus RNA synthesis.

Although it has long been known that interactions between NP, VP35, VP30 and L are required for filovirus RNA synthesis, detailed understanding of these interactions has been lacking. Further, several host-viral RDRP complex interactions have been defined. Recently, the combination of structural, biophysical and molecular biology approaches has revealed in substantial detail the molecular basis for protein-protein interactions (PPIs) and defined how specific interactions contribute to the function of the viral RDRP complex (Figure 3). In several cases, disruption of an interaction has been demonstrated to inhibit viral RNA synthesis and EBOV replication.

4.1 Structures of EBOV NP reveal structure and dynamics of NP-RNA and NP-protein interactions critical for viral RNA synthesis and genome RNA encapsidation

Three recent studies reported electron cryo-microscopy and -tomography derived structures of EBOV NP complexes. These data revealed, in great detail, interactions necessary for NP assembly and dynamics that allow NP to participate in EBOV RNA synthesis and nucleocapsid assembly (Su et al., 2018; Sugita et al., 2018; Wan et al., 2017). In one study, a structure of NP residues 1-450, a region sufficient to form α-helical structures and encapsidate RNA was solved, as were structures of an NP-VP35-VP24-VP40 nucleocapsid and nucleocapsids in EBOV and MARV particles. In a second study, NP nucleocapsids formed by the N-terminal domain of NP were examined by a combination of methods that included hydrogen-deuterium exchange mass spectrometry (HDX-MS) and functional

assays coupled with cryoEM. In a third study, cryo-EM and single-particle analysis enabled determination and an atomic model of the NP 1-450 structure. While the studies and conclusion differ in some details, the overall picture that emerges is one where NP is a highly dynamic molecule and that these dynamics play critical roles in EBOV RNA synthesis and assembly. These studies yielded electronic density into which previously-determined EBOV NP X-ray crystal structures could be fitted. These studies therefore clarified how individual NP monomers assemble into helical structures in which rings of NP stack upon one another. Among the findings were the identification of α-helices within NP monomers that mediate NP-NP interactions and NP-RNA interactions. Of particular note is the binding pocket on NP where the VP35 NP-binding peptide (NPBP) binds. In the assembled NP structures, a-helices from nearby NP molecules occupies this groove.

Wan et al. also determined the nucleocapsid structures present in virus-like particles formed by co-expression of full-length NP–VP35, VP24 and VP40 as well as nucleocapsids present in EBOV and MARV particles. Comparative analysis of these structures suggested that NP oligomerization causes structural changes allowing an α-helix in NP to acts as a clamp that facilitates RNA binding in the NP RNA binding cleft. The RNA binding promotes stabilization of the NP assembly (Figure 3D). For the NP N-terminus alone, this is seen to result in a condensed structure. With full-length NP, condensation is incomplete until the nucleocapsid also associates with VP35 and VP24. In this model, NP oligomerization, RNA association and recruitment of other viral proteins to the nucleocapsid are interconnected processes. However, the hierarchy of these processes remains largely unexplored.

Su et al. began their analysis from the perspective of their previously described X-ray crystal structure of the NP N-terminus, where two α-helices at the C-terminus of the NP construct, α21 and α22, made NP-NP contacts. Mutagenesis and HDX-MS studies indicated that NP conformational changes occur due to NP-NP interactions and that these affect accessibility of the VP35-NPBP binding site. RNA binding was also demonstrated to be regulated by the first 24 amino acid residues of NP and the cooperation of this region with the α21 and α22 helices was described as a context-dependent regulatory module (CDRM). The CDRM appears to be a critical regulator of NP intermolecular interactions that couples NP oligomerization, RNA binding and access the viral RNA template for viral RNA synthesis reactions (Figure 3E).

Functional assays that utilized minigenome assays and either wild-type of mutated NPs indicated that viral RNA synthesis is dependent on NP oligomerization. Modeling NP dynamics through the use of Markov state modeling (MSM), which maps the conformational space adopted by dominant structural states, suggested that monomeric NP can sample conformations similar to those seen with the NP oligomer and that oligomerization stabilizes the structure. From these studies, the authors propose a similar model to that proposed by Wan et al. whereby NP assembles into a helical nucleocapsid structure through structural rearrangements in both the N- and the C-termini of the NP N-terminal domain. Interactions within the CDRM, including those involving the N-terminal 24 amino acids and more C-terminal sequences regulate NP oligomerization and through this, NP-ssRNA interaction. These dynamic interactions are likely to play important roles in the regulation of filoviral RNA synthesis. Based on their high resolution structure, Sugita et

al. propose that peptidomimetics could be used to competitively inhibit or cross-link interactions within the NP oligomer to achieve antiviral activity (Sugita et al., 2018).

4.2 Interactions between NP and VP35 critical for viral RNA synthesis

Based on existing data and by analogy to other NNSVs, VP35 and NP interaction has been thought to serve at least 2 functions in the context of viral RNA synthesis. Interaction of VP35 with NP serves a chaperoning function, preventing NP from non-specifically associating with RNA while maintaining NP as a monomer. VP35-NP interaction is also expected to bring the L protein to the NP-encapsidated template viral genomic or antigenomic RNAs. Several studies that included structural, biophysical and functional analyses have now defined in detail how the chaperoning function is mediated by either EBOV VP35 or MARV VP35 (Kirchdoerfer et al., 2015; Leung et al., 2015; Liu et al., 2017a; Zhu et al., 2017).

Leung et al. and Kirchdoerfer et al., in separate studies, employed multi-disciplinary approaches to address the EBOV VP35 chaperoning function for EBOV NP. In each study, core domains of the NP N-terminus and truncations of VP35 were used to define short peptides capable of high affinity binding to the NP N-terminus (Kirchdoerfer et al., 2015; Leung et al., 2015). In the case of Leung et al., a VP35 peptide corresponding to residues 20-48 was sufficient to bind and was named NPBP. In solution, NPBP was found to be intrinsically disordered, but it exhibited a propensity to form helical structures in the presence of trifluoroethanol, suggesting that interaction with NP might occur in the form of an α helix. To further explain the functional consequences of the interaction, the X-ray crystal structure of VP35 NPBP in complex with the NP N-terminal domain (ΔNP_{NTD}) was solved to 3.7 Å resolution (Figure 3F). The NPBP/ΔNP_{NTD} complex formed double stacked rings with eight ΔNP_{NTD} molecules per rung. ΔNP_{NTD} was found to have two lobes, a head lobe and a foot lobe. The two C-terminal α-helices in the foot lobe (α21 and α 22) make contacts with adjacent ΔNP_{NTD} molecules in a ring. In the structure, NPBP interacts with the foot lobe of ΔNP_{NTD} as two orthogonal helices. The NPBP binding site was identified as critical for NP RNA binding and NPBP was able to compete with ssRNA for binding to ΔNP_{NTD} providing evidence that this VP35-derived peptide is a key regulator of NP RNA binding. Further, when NPBP was added to ring structures formed by NP 1-457, the ring structures were disrupted. Size exclusion chromatography confirmed that NPBP dissociated the oligomeric forms of NP. Although approaches differed, Kirchdoerfer et al. similarly identified a peptide derived from the VP35 N-terminus capable of binding to the NP N-terminus. The NP N-terminal construct used was sufficient to bind RNA and to oligomerize. In the presence of the VP35 peptide, however, NP oligomerization was prevented. While oligomerized NP 1-450 binds RNA, the presence of VP35 peptide blocks RNA binding. Their approach to crystallization of the complex was to produce a protein containing VP35 residues 15–60 fused to NP residues 34–367. This also identified a bilobed structure for the NP N-terminus, demonstrated binding of VP35 peptide near the RNA binding site and identified mutations that disrupt VP35 peptide-NP interaction and impair viral RNA synthesis. Subsequent studies on MARV VP35-NP N-terminal domain interactions reached similar conclusions (Liu et al., 2017a; Zhu et al., 2017). Remaining to be clarified are the precise mechanisms by which the VP35-chaperoned NP, upon delivery to assembling viral

nucleocapsid structures, is freed from VP35 allowing NP oligomerization and NP-RNA association. Also, the details by which the viral RNA template, which is tightly associated with oligomerized NP in the nucleocapsid, becomes accessible to the viral replication complex that contains L and VP35 proteins remain to be explained. It is tempting to speculate that NPBP can interact with NP to temporarily disengage the RNA from NP to allow it to be transcribed and replicated.

Data from minigenome assays using mutants that disrupt the NPBP-NP interaction suggest the NPBP-NP interface as an antiviral target. In support of this, Leung et al demonstrated that expression of the NPBP is sufficient to inhibit EBOV RNA synthesis, as assessed by minigenome assays (Leung et al., 2015). In order to take advantage of the mechanistic insights and to aid the development of potential therapies that target the NPBP binding site on EBOV NP, Liu et al. developed a sensitive fluorescence polarization assay (FPA) for high-throughput screening (HTS) of the EBOV NP_{NTD}-NPBP interaction (Liu et al., 2017b). A pilot screen was then performed using a library of 640 FDA approved drugs. This identified Tolcapone, an FDA-approved drug that inhibits catechol-O-methyltransferase (COMT) and is used in the treatment of Parkinson's Disease (Politi et al., 2018), as an inhibitor or NP:NPBP with an IC50 of 2 μM in the FPA. Tolcapone was further demonstrated to inhibit growth or EBOV in cell culture and, consistent with the role of NP:NPBP interaction for EBOV RNA synthesis, to inhibit the EBOV minigenome assay. Because the NPBP sequence is relatively well conserved across filoviruses, the binding of NPBP from SUDV, RESTV and MARV to EBOV NP was assessed and found to yield similar affinities. When Tolcapone was tested against the MARV NPBP interaction, inhibition was demonstrated (Liu et al., 2017b). These findings suggest that the NP:NPBP interaction has potential as a pan-filovirus target.

As noted above, VP35 not only chaperones NP but may mediate delivery of the viral polymerase to the NP-encapsidated template RNA for viral RNA synthesis. It is not clear that the NPBP-NP interaction fully explains this function. A second interaction between VP35 and NP that may serve this role has also been described (Kirchdoerfer et al., 2015; Prins et al., 2010). Present in the C-terminal IID of EBOV VP35 are two basic patches, the central basic patch, which mediates interaction with dsRNA, and the first basic patch which consists of VP35 residues K222, R225, K248 and K251 (Leung et al., 2010; Prins et al., 2010). Mutation of first basic patch residues to alanine disrupted viral RNA synthesis in minigenome assays (Prins et al., 2010). These same mutants exhibited impaired interaction with NP in co-immunoprecipitation experiments and when maltose binding protein-VP35 IID fusions were used to pull-down VP35 from transfected cell lysates (Prins et al., 2010). These data indicate that the IID can mediate an interaction between VP35 and NP. However, pulldowns performed in a separate study suggest that sequences outside of the IID can also contribute to this NP interaction (Kirchdoerfer et al., 2015).

As with the NPBP-NP interactions, mutations that disrupt the IID-NP interaction impair viral RNA synthesis, suggesting this interaction could be a viable target for therapeutics. In further support of this, two strategies that targeted the IID domain of VP35 were able to inhibit EBOV RNA synthesis. In one approach, the X-ray crystal structure of the EBOV VP35 IID was used as a target for in silico-based screening (Brown et al., 2014).

Compounds were sought that could target the IID. Taking hits from the in silico screen and combining NMR, X-ray crystallography and medicinal chemistry, small molecules were identified that target a binding pocket adjacent to the IID first basic patch. NMR mapping experiments and high-resolution x-ray crystal structures showed that select small molecules bind to a region of VP35 IID that is important for replication complex formation through interactions with the viral nucleoprotein (NP). Select compounds from this series could impair VP35 IID-NP interaction in vitro, inhibit EBOV minigenome assay activity and replication of EBOV in tissue culture. These results confirm the ability of compounds identified in this study to inhibit VP35-NP interactions in vitro and to impair EBOV replication in cell culture (Brown et al., 2014). In another study, RNA aptamers that target the EBOV VP35 IID were selected for. These could compete with dsRNA for binding to eVP35 and disrupt VP25-NP interaction and select aptamers could also inhibit EBOV RNA synthesis (Binning et al., 2013).

4.3. VP35-L interactions.

It is generally accepted that VP35 interacts with L and via its interaction with NP, VP35 brings L to the NP-encapsidated template RNAs but the underlying molecular mechanisms remain to be characterized at high resolution. VP35 has been demonstrated to interact with the N-terminal region of L (Shabman et al., 2013; Trunschke et al., 2013). Studies found that co-expression of VP35 can substantially enhance expression of L residues 1-505 ($L_{1\text{-}505}$), compared with when $L_{1\text{-}505}$ is expressed alone, suggesting a possible role of VP35 as a chaperone for L, although further studies using full-length L are required to address this question (Shabman et al., 2013). Further analysis, defined L residues 280-370 as sufficient to mediate a weak VP35-L interaction and L residues 1-370 as sufficient for stronger interaction (Trunschke et al., 2013). Co-immunoprecipitation studies pointed to an L-L interaction domain within the N-terminal 450 amino acid residues, but this interaction was not altered by co-expression of VP35. Supporting a role for these interactions in EBOV RNA synthesis, expression of fragments of L sufficient to interact with VP35 inhibited minigenome assays (Trunschke et al., 2013).

4.4 VP35 interactions with host proteins that impact viral RNA synthesis

In addition to its interactions with viral factors, VP35 interactions with host factors can also modulate viral RNA synthesis. VP35 interaction with host protein 8 kilodalton dynein light chain (DLC8 or LC8) was initially identified based on a yeast two-hybrid screen for VP35 interactors. The interaction mapped to the amino-terminal half of VP35 and requires the motif SQTQT, which matches the LC8 consensus binding motif K/SxTQT (Kubota et al., 2009; Lo et al., 2001). In a separate study, this interaction was demonstrated to be direct and of high affinity with an equilibrium dissociation constant [K_D] = 82.4 ± 18 nM. LC8 forms dimers and VP35 forms tetramers; in complex, two LC8 dimers interact with the VP35 tetramer. Functionally, the interaction was demonstrated to enhance VP35 stability. Mutations to the consensus binding site substantially decrease binding. Further, these mutations negatively impact viral RNA synthesis, as measured by minigenome activity, whereas LC8 over-expression enhances activity, with the main impact being on viral transcription (Luthra et al., 2015). These data suggest that blocking LC8-VP35 interaction would negatively impact EBOV RNA synthesis during infection.

VP35 was demonstrated to interact with the host protein known as protein activator of interferon induced protein kinase eIF2AK2 (PACT) (Luthra et al., 2013). PACT can stimulate innate antiviral defenses through activation of the IFN-induced, dsRNA activated protein kinase (PKR) kinase activity, which phosphorylates translation initiation factor eukaryotic translation initiation factor 2 alpha (eIF-2α) to suppress translation, and by facilitating activation of RIG-I, which leads to induction of type I IFN (IFN) responses (Kok et al., 2011; Patel and Sen, 1998). The interaction with PACT occurs through the C-terminal VP35 IID which is critical for potent suppression of IFN responses (Luthra et al., 2013). It was further demonstrated that interaction with VP35 binding prevent PACT binding to RIG-I to suppress innate immune responses. Because VP35 is also critical for EBOV RNA synthesis, the impact of PACT interaction on this function was also assessed. PACT was demonstrated to inhibit EBOV RNA synthesis in the minigenome assay. The inhibition was relieved when VP35 IID point mutants that lose interaction with PACT were used in place of wild-type VP35 in the same assay. Co-immunoprecipitation assays demonstrated that PACT can interfere with VP35 interaction with the N-terminus of L and that this effect was also lost with the VP35 IID mutant. Consistent with these findings, in EBOV infected cells over-expression of PACT increased IFN responses and decreased viral RNA production; whereas PACT knockdown decreased IFN responses and increased viral RNA synthesis (Luthra et al., 2013). These data provided evidence that host factors can negatively modulate EBOV replication by targeting components of the RNA synthesis machinery. These findings also suggest as therapeutic strategies the identification of molecules that target the VP35-L interaction in a manner similar to PACT or that increase the availability of PACT to target VP35.

Another host interaction with VP35 involves tripartite motif-containing protein 6 (TRIM6), a host cell E3-ubiquitin ligase demonstrated to produce free poly-ubiquitin chains that promote type I IFN responses (Bharaj et al., 2017). VP35 and TRIM6 were found to co-immunoprecipitate, and VP35 was demonstrated to be ubiquitinated in the IID on K309, which was enhanced by over-expression of TRIM6. VP35 was demonstrated to interact with polyubiquitin and to inhibit TRIM-6 induced IFN responses. Significantly, expression of a wild-type but not a catalytically-inactive TRIM6 enhanced EBOV RNA synthesis, as measured by minigenome assay, and TRIM6 knockout cells exhibited reduced EBOV replication. Therefore, while the mechanisms by which TRIM6 modulates EBOV RNA synthesis and propagation require further clarification, strategies that either reduce TRIM6 E3-ubiquitin ligase activity or disrupt VP35-TRIM6 interaction might effectively suppress EBOV growth.

Finally, double-stranded RNA-binding protein, 76 KD (DRBP76;also referred to as NFIL3, NF90 and other names) has been demonstrated to interact with the VP35 IID (Shabman et al., 2011). VP35-IID-DRBP76 interaction was detectable in the presence of exogenously added dsRNA, suggesting the possibility of RNA bridging the protein-protein interaction. However, full-length VP35 bound to DRBP76 when exogenous dsRNA was absent. Regardless of the basis for interaction, when DRBP76 was over-expressed it impaired EBOV minigenome activity, demonstrating a capacity to modulate the function of the EBOV RNA synthesis machinery (Shabman et al., 2011).

4.5 Interactions between VP30 and NP modulate viral RNA synthesis

The C-terminal domain of VP30 interacts with NP and this interaction is important for viral RNA synthesis (Hartlieb et al., 2007). Two studies that combined structural, biophysical and molecular approaches defined the basis and demonstrated the functional significance of VP30-NP interactions for EBOV (Kirchdoerfer et al., 2016; Xu et al., 2017). Xu et al. demonstrated that the C-terminal region of NP (residues 600-739) can bind to the C-terminal domain of VP30 (residue 110-272) and were able to generate co-crystals that revealed NP residues 600-612 in complex with a cleft on the surface of VP30 (110-272) (Figure 3C) (Xu et al., 2017). The interacting NP peptide contains a motif, PPxPxY, conserved across filoviral NPs. A combination of in vitro FPAs and cell-based co-immunoprecipitation assays identified interface loss of binding mutants. Because VP30 and NP each participate in EBOV RNA synthesis, the mutants were assessed in a minigenome assay. Several of the mutants exhibited loss of activity, demonstrating a role for the interaction in viral RNA synthesis. The loss of function of these mutants is related to the role of VP30 as a transcription initiation factor. This was demonstrated by introducing into the minigenome template RNAs that disrupt the stem-loop at the NP gene transcription start site. These render the minigenome assay VP30- dependent and restored function in the presence of the loss of function mutants (Xu et al., 2017).

Kirchdoerfer et al. similarly demonstrated binding of the C-terminus of NP to the C-terminal domain of VP30 and mapped an NP peptide, residues 600-617, sufficient for binding (Kirchdoerfer et al., 2016). For crystallization, to overcome relatively low NP peptide-VP30 affinity, the NP peptide was fused to the N-terminus of the VP30 C-terminal domain, with a short spacer sequence between. The conclusions based on the structure parallel those described above, and mutations designed to alter NP-VP30 affinities also yielded variable results but demonstrated an important role for the interaction for viral RNA synthesis. By comparing VP30-NP binding affinities of various mutants with activity in the minigenome assay, the authors proposed that there is an optimal binding affinity such that too high or too low binding affinities negatively impacts filoviral RNA synthesis (Kirchdoerfer et al., 2016). As both studies implicated the VP30-NP interaction in viral RNA synthesis, disrupting the interaction would be a potential therapeutic target. Consistent with this, Xu et al. demonstrated that expression of the NP peptide as a fusion with GFP impaired RNA synthesis measured by the minigenome assay (Xu et al., 2017).

4.6. An interaction between NP and host protein phosphatase regulatory subunit B56 mediates VP30 dephosphorylation.

VP30 function is modulated by phosphorylation, with the dephosphorylated state promoting viral mRNA synthesis. Protein phosphatases PP1, PP2A and PP2C have been implicated as enzymes that mediate VP30 dephosphorylation (Modrof et al., 2002). However, details as to how the VP30 phosphorylation state is regulated have been unclear. Significant light was shed on this when it was recognized the EBOV NP possesses an LxxIxE motif, a sequence that can serve as a binding site for the cellular PP2A-B56 protein phosphatase (Hertz et al., 2016). PP2A possesses three distinct subunits, one catalytic (PP2A-C), one which serves a scaffolding function (PP2A-A) and any of several B subunits which regulate activity (Shi, 2009). One B subunit family, B56, interacts with the LxxIxE motifs that were noted in

filovirus NPs (Hertz et al., 2016). The NP LxxIxE motifs lie 18-28 amino acids, depending on which filovirus is examined, from the PPxPxY motif that mediates VP30-NP interaction (Kruse et al., 2018). It was demonstrated that NP recruits PP2A-B56 to the LxxIxE motif and that this interaction is needed for VP30 dephosphorylation and, hence, for viral transcription. Further, LxxIxE-containing peptides were developed and were demonstrated to inhibit EBOV RNA synthesis in a minigenome assay and to inhibit EBOV replication (Kruse et al., 2018). These data therefore define a critical NP-host interaction that modulates the balance of VP30 phosphorylation/dephosphorylation and demonstrates that disruption of NP-B56 interaction could serve as a therapeutic approach. A caveat to the approach tested is that the peptides would presumably target other B56 PPIs as well.

4.7 Comprehensive profiling of EBOV-host PPIs identifies novel modulators of EBOV RNA synthesis.

The increasing evidence that host factors engage and modulate filoviral RNA synthesis reactions, suggested that more systematic and comprehensive approaches to identify virus-host protein-protein interactions PPIs, was warranted. A four lab, four institution effort sought to systematically characterize Ebola virus-human PPIs using an affinity purification mass spectrometry (AP-MS) approach (Batra et al., 2018) (Shah et al., 2015). In order to build this interaction network, individual Ebola virus open reading frames for NP, VP35, VP40, GP, VP30, and VP24 were cloned into a mammalian expression vector fusing each viral protein to a 2×Strep affinity tag (Jager et al., 2011b). Tagged plasmids were transfected into HEK293T and Huh7 cells and EBOV-human protein complexes were purified by StrepTactin affinity purification and subjected to quantitative mass spectrometry analysis. Each purification was repeated multiple times in both cell lines and rigorous tests of statistical significance were applied (Verschueren et al., 2015), yielding, a network of 194 virus-host PPIs (Batra et al., 2018). This approach has proven successful in defining a number of virus-host networks, including networks for human immunodeficiency virus 1 (HIV-1), hepatitis C virus (HCV), Kaposi's sarcoma-associated herpesvirus (KSHV), dengue virus (DENV), human papillomavirus (HPV) and now EBOV (Batra et al., 2018; Davis et al., 2015; De Maio et al., 2016; Eckhardt et al., 2018; Jager et al., 2011a; Ramage et al., 2015)(Shah et al., Cell in press). The EBOV network recapitulates known interactions, such as between EBOV VP24 and human karyopherin alpha (KPNA; also knows as importin alpha), which mediates suppression of antiviral signaling by blocking nuclear translocation of tyrosine phosphorylated signal transducer and activator of transcription 1 (STAT1) (Mateo et al., 2010; Reid et al., 2006; Reid et al., 2007; Xu et al., 2014), and between VP35 and LC8 (Kubota et al., 2009; Luthra et al., 2015). The L protein was not included in the analysis because low L expression reduced confidence in hits obtained (data not shown). Therefore, strategies that effectively increase L expression are needed to address host interactions with this critical viral enzyme. Nonetheless, two components of the RNA replication complex, VP35 and VP30, each had very robust interactomes (Figure 4), suggesting the likelihood that host factors capable of influencing viral RNA synthesis reactions had been identified.

For example, we found that VP30 was physically associated with components of the spliceosome and the polyadenylation machinery, two complexes which are functionally linked (Kornblihtt et al., 2004). These connections suggest that VP30 may be impinging on

host transcriptional events, similar to what we have previously observed for DENV and host mRNA splicing (De Maio et al., 2016). Furthermore, further manipulation of the host transcriptional apparatus via VP35 may be occurring, as we identified physical associations with components of both TFIIIC, a transcription factor for RNA polymerase III (Pascali and Teichmann, 2013), as well as the RNA exosome (Morton et al., 2018), a complex involved in a myriad of processes, including RNA degradation during transcription. Interestingly, we recently found that RNA exosome physically associates with the influenza A virus (IAV), a connection required for efficient IAV RNA synthesis (Rialdi et al., 2017). Finally, we also uncovered a connection between VP30 and VPS35 with the mitochondrial and canonical ribosome, respectively, implying a direct connection between EBOV infection to host translational as well (Figure 4). Further work will be required to tease out the functional relevance of these and other physical interactions uncovered by our unbiased AP-MS analysis of the EBOV proteome.

4.8. Host protein RBBP6 interacts with VP30 to modulate EBOV RNA synthesis.

The first EBOV-host PPI derived from the network that was investigated in mechanistic detail was between VP30 and retinoblastoma binding protein 6 (RBBP6), an interaction that was among the highest confidence scores in the entire PPI network, both in HEK293T and Huh7 cells. The interaction could be confirmed in an immunoprecipitation (IP)-Western blot experiment, and IP-Western blotting of other filoviral VP30 proteins demonstrated that the interaction is conserved across all filoviruses tested (EBOV, Reston virus (RESTV), MARV, and Lloviu virus (LLOV)). Truncation mutants of EBOV VP30 and RBBP6 were used to identify specific domains required for the interaction in both cell-based co-immunoprecipitation and in vitro binding assays, the latter using purified VP30 and RBBP6 domains. These efforts mapped the interaction to the C-terminal domain of VP30 and to a proline rich motif within RBBP6, data that enabled the co-crystallization of the VP30 C-terminus with a RBBP6 peptide spanning its interaction domain and the structure of the complex was solved to 1.5 A resolution. Strikingly, the RBBP6 peptide was found to interact with the same cleft on VP30 that interacts with NP (Kirchdoerfer et al., 2016; Kruse et al., 2018; Xu et al., 2017). Quantitative binding measurements demonstrated that the RBBP6 peptide binds the eVP30 C-terminus with ~5 fold higher affinity than the NP-derived peptide. Examination of the NP and RBBP6 peptides that bind eVP30 identified the motif PPxPxY as being present in both. Notably, PPxPxY is conserved among filovirus NPs (Kirchdoerfer et al., 2015) and mutations within this motif can disrupt RBBP6 interaction with eVP30 (Batra et al., 2018).

Because the interaction with NP at this cleft on VP30 is important for EBOV RNA synthesis, experiments were performed to determine whether RBBP6 modulates viral RNA synthesis (Batra et al., 2018). Knockdown of RBBP6 with siRNA stimulated viral RNA synthesis, as measured by minigenome assay, while overexpression of RBBP6 or its VP30-interacting peptide, which was fused to green fluorescence protein (GFP), was strongly inhibitory. That these inhibitory activities are mediated through direct VP30-RBBP6 interaction was supported by the loss of inhibitory activity when RBBP6 or VP30 mutants that were unable to bind were tested. Mechanistically, the inhibition mediated by RBBP6 appears to occur by at least 2 mechanisms. RBBP6 was demonstrated to effectively compete

with NP for binding to VP30 and, at least when over-expressed at high levels, to also promote VP30 degradation. Based on the studies examining VP30-NP interaction, loss of VP30-NP interaction should lead to inhibition of viral transcription because this would be expected to inhibit the eVP30 dephosphorylation which occurs on NP. Further study will be required to determine if this is the case.

4.9. The VP30 cleft that binds NP and RBBP6 as a site for therapeutic targeting.

To determine the impact of RBBP6 on actual EBOV replication a combination of knockdown and over-expression studies were performed (Batra et al., 2018). Knockdown of endogenous RBBP6 expression substantially increased infectivity as measured by GFP expression from an EBOV that encodes GFP (EBOV-GFP). Further, expression of RBBP6 fused to GFP significantly decreased EBOV infectivity compared to cells expressing GFP. These data demonstrated the relevance of the interaction for EBOV replication and suggest that methods, perhaps peptidomimetic or other small molecules, that target the VP30 cleft that binds either NP or RBBP6 could serve as antivirals. Given the conservation of the interaction across multiple filoviruses, such an approach could have pan-filovirus potential.

It is striking that human cells possess a protein that negatively impacts EBOV gene expression. It is possible that the VP30-RBBP6 interaction serves a beneficial, perhaps regulatory role for the virus. Further study will be required to develop insight into this question, to further define the mechanisms by which RBBP6 impacts the viral RNA synthesis machinery and to explore the VP30 cleft as a therapeutic target. Intriguingly, the host-EBOV PPI network contains 3 other VP30 interactors, hnRNP L, hnRNP UL1 and PEG10, possessing PPxPxY motifs (Batra et al., 2018). This raises the intriguing possibility that multiple host factors, potentially with different abundance and affinities for eVP30 might together regulate EBOV replication.

5 Conclusions and Future Directions

The efforts described above have provided substantial insights into the assembly and function of the filovirus RNA synthesis machinery and suggest strategies to interfere with critical viral functions. Continued study should further define in high resolution the basis for critical interactions between viral proteins and host proteins and identify the most promising therapeutic targets. At the same time, it is important to recognize that viral transcription and replication are dynamic processes, with specific interactions playing distinct roles on different aspects of these reactions. For example, the interaction between NP and the NPBP from VP35 are thought to chaperone free NP, preventing it from inappropriately binding to cellular RNAs and facilitating its appropriate oligomerization on viral genomic and antigenomic RNAs (Leung et al., 2015). As these dynamics are revealed in higher resolution, additional opportunities will arise to intervene in critical intra- and intermolecular movements in order to inhibit viral growth. The same should also hold true for virus-host PPIs, and it is likely that the field has only scratched the surface of those that modulate viral RNA synthesis. Finally, it should be recognized that, with the exception of VP30 phosphorylation, the impact of post-translational modifications on relevant PPIs and on viral RNA synthesis reactions has largely gone unaddressed. A full accounting of the host

factors that interact with and impact filoviral RNA synthesis will require that post-translational modifications also be comprehensively defined.

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Highlights

1. Filoviruses remain important human pathogens for which therapeutics are needed.
2. The viral RNA synthesis machinery is appealing as a therapeutic target.
3. Multiple protein-protein interactions (PPIs) are required for viral RNA synthesis.
4. Targeting these PPIs impairs viral RNA synthesis and inhibits virus replication.

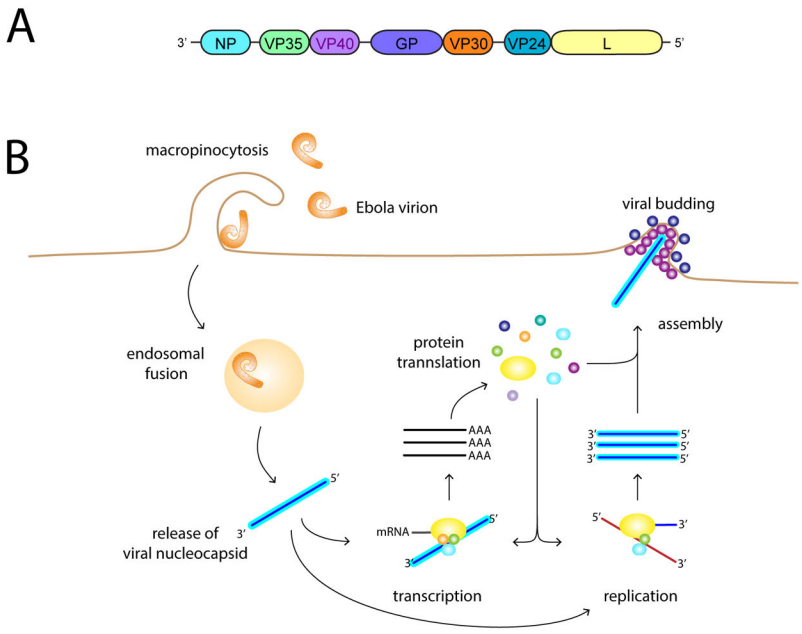

Figure 1. The filovirus genome and replication cycle.

A. A schematic diagram of the EBOV genome showing individual genes, named after the proteins they encode: NP, nucleoprotein; VP35, viral protein 35; VP40, viral protein 40; GP, glycoprotein/soluble glycoprotein; VP30, viral protein 30; VP24, viral protein 24; L, Large protein. The GP gene of members of the Ebolavirus and Cuevavirus genera also encode a soluble glycoprotein (sGP). B. Overview of the filovirus replication cycle. Proteins are indicated by circles and ovals with colors corresponding to those in panel A. Depicted are viral entry via micropinocytosis, release of the genomic RNA into the cytoplasm where the depicted viral mRNA and genome replication take place. Assembly and budding occur at the plasma membrane.

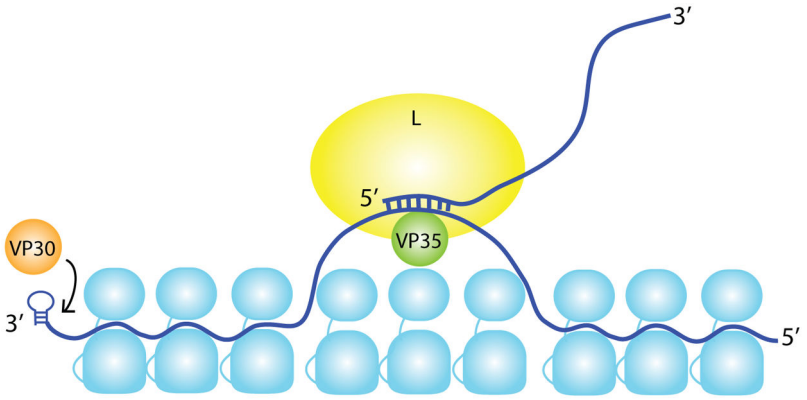

Figure 2. Schematic diagram of the Ebola virus RNA synthesis complex.

The template viral genome RNA is depicted as a blue line. The RNA is encapsidated by the bilobed NP. Viral transcription requires the presence of VP30 and the requirement for VP30 is due to the presence of a stem loop near the transcription start site in the NP gene. VP35 interacts with NP and L and brings L, which possesses the necessary enzymatic activities required for viral transcription and replication, to the template. The interaction of VP35-L with NP presumably exposes the template RNA to allow access to L.

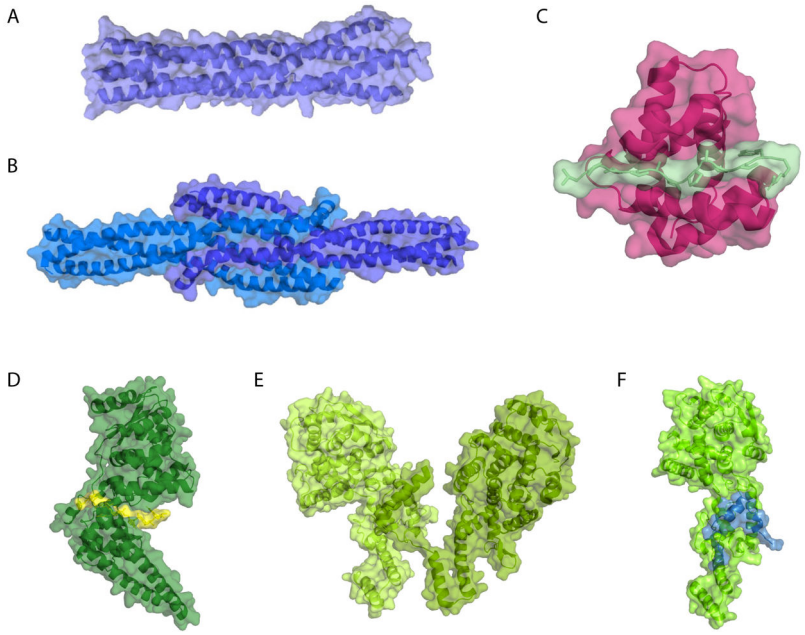

Figure 3. Structural characterization of specific interactions that are important for modulating filoviral replication.

A. Coiled-coil motif of Reston VP35 N-terminal oligomerization domain arranged as a parallel tetramer (slate; PDB 6GBR). B. Coiled-coil motif of Ebola VP35 N-terminal oligomerization domain arranged as an antiparallel dimer of parallel trimers (molecule A, marine; molecule B, tv blue; PDB 6GBO). C. Ebola VP30 C-terminal domain (warm pink) bound to an Ebola NP peptide (pale green) (PDB 5VAP). D. CDRMs modulating Ebola NP-NP interactions (molecule A, limon; molecule B, split pea; PDB 6C54). E. RNA (yellow) bound Ebola NP (forest) (PDB 5Z9W). F. Ebola NP (chartreuse) bound to an Ebola VP35 peptide NPBP (sky blue) (PDB 4YPI). Structures are colored according to filoviral proteins. Different shades of a color are used to indicate different conformational arrangements. Simple named colors used are defined by PyMOL.

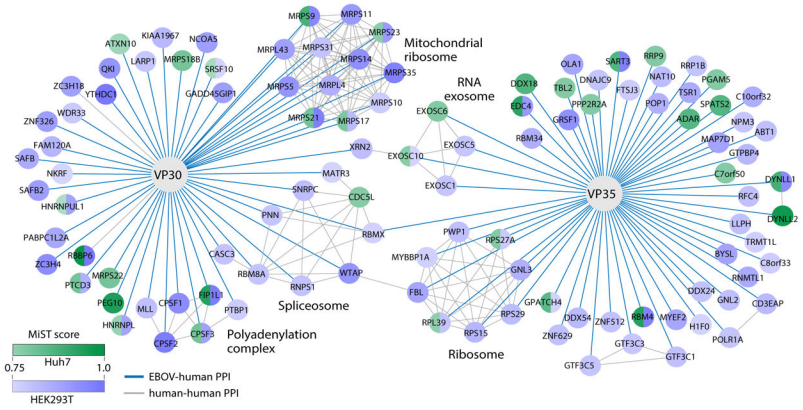

Figure 4. EBOV-human protein-protein interactions with VP30 and VP35.

EBOV-human protein-protein interactions are represented (blue lines) between VP30 and VP35 proteins and human proteins. Each human prey node is colored according to the cell line in which it was identified: blue indicates HEK293T cells, green indicates Huh7 cells, and blue-green bifurcation indicates the protein was identified as an interactor in both cell types. The shade of the prey node correlates with the MiST score of the interaction (scale at lower left). Grey lines correspond to human-human protein-protein interactions curated in the publicly available CORUM database and several human protein complexes are labeled.