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Negative and ambisense RNA virus ribonucleocapsids: more than protective armor

Kimberly R. Sabsay,[1,2] **Aartjan J. W. te Velthuis**[1]

AUTHOR AFFILIATIONS See affiliation list on p. 16.

SUMMARY Negative and ambisense RNA viruses are the causative agents of important human diseases such as influenza, measles, Lassa fever, and Ebola hemorrhagic fever. The viral genome of these RNA viruses consists of one or more single-stranded RNA molecules that are encapsidated by viral nucleocapsid proteins to form a ribonucleoprotein complex (RNP). This RNP acts as protection, as a scaffold for RNA folding, and as the context for viral replication and transcription by a viral RNA polymerase. However, the roles of the viral nucleoproteins extend beyond these functions during the viral infection cycle. Recent advances in structural biology techniques and analysis methods have provided new insights into the formation, function, dynamics, and evolution of negative sense virus nucleocapsid proteins, as well as the role that they play in host innate immune responses against viral infection. In this review, we discuss the various roles of nucleocapsid proteins, both in the context of RNPs and in RNA-free states, as well as the open questions that remain.

KEYWORDS negative sense RNA virus, ambisense virus, influenza A virus, RNA polymerase, t-loop, nucleoprotein, innate immune, RNP, RNA structure, NP, RdRp

INTRODUCTION

Negative RNA viruses (NSV) and ambisense RNA viruses (ASV) cause detrimental human diseases, including Ebola hemorrhagic fever, influenza, Lassa fever, and measles. The genomes of negative sense RNA viruses consist of single-stranded RNA that can be segmented or non-segmented. We will refer to this viral genomic RNA as the viral RNA (vRNA). The vRNA molecules are bound by viral nucleoproteins (abbreviated NP or N, depending on the nomenclature used in each field; we will not abbreviate

Editor Stanley Perlman, University of Iowa, Iowa City, Iowa, USA

Address correspondence to Aartjan J. W. te Velthuis, aj.te.velthuis@princeton.edu.

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nucleoprotein here for clarity) and a viral RNA polymerase, forming viral ribonucleoprotein (vRNP) complexes (1–4). Upon entry into the host cell, the vRNPs must first be transcribed into capped and polyadenylated mRNAs by the RNA polymerase before host cell ribosomes can produce viral proteins. Following viral protein synthesis, the process of viral replication generates new vRNA molecules via a positive sense, complementary RNA (cRNA) intermediate. cRNA molecules are also bound by newly translated nucleoproteins and a copy of the viral RNA polymerase, forming cRNPs.

The RNA polymerase of most NSVs and ASVs is called the large (L) protein. In addition to an RNA-dependent RNA polymerase (RdRp) domain, the L protein can contain combinations of cap-binding and endonuclease, or capping and methyltransferase domains and may bind oligomers of the viral phosphoprotein (P) (1). In orthomyxoviruses, the RdRp domain resides in a heterotrimer of the viral proteins polymerase basic 1 (PB1), polymerase basic 2 (PB2), and polymerase acidic (PA) or polymerase 3 (P3). The PB2 and PA/P3 subunits of this protein complex also contain cap-binding and endonuclease domains (5). In contrast to the host cell's DNA-dependent polymerases, the above RNA polymerases lack proofreading and robust error-repair mechanisms. This results in high mutation rates, which in turn help RNA viruses evade host immune defenses and adapt to host environments more quickly.

The nucleoprotein-encoding gene is present in all NSV and ASV genomes (6, 7). Nucleoprotein gene transcripts are typically the most abundant mRNAs during a negative and ambisense RNA virus infection, making nucleoproteins one of the most abundant viral proteins (8). Upon expression, nucleoproteins can self-oligomerize into symmetric helical and double-helical complexes in the presence or absence of vRNA or cRNA (9–12). These helical nucleoprotein multimers are inherently multifunctional and have evolved to balance a protective role that shields the vRNA of some NSVs, while allowing the vRNA to be accessed and copied by the RNA polymerase (8). Moreover, nucleoproteins minimize RNA secondary structure, especially long-range interactions, within the vRNP to maintain a contiguous organization that supports efficient replication, while at the same time allowing the folding of packaging signals (13, 14). Without this function, a vRNA may form secondary structures, such as template-loops (t-loops), that stall viral RNA synthesis and affect the generation of aberrant RNA molecules that activate the innate immune system (15–19).

Although nucleoproteins vary in length and sequence among different negative and ambisense RNA viruses, the structural principles of nucleoprotein-nucleoprotein interactions, as well as the structural symmetry of helical chain formation are mostly conserved. However, at a biochemical level, different NSVs and ASVs have evolved different solutions to coordinate nucleoprotein multimerization with RNA synthesis, requiring nucleoproteins to interact with P in non-segmented NSV infections, or host cell proteins, such as ANP32A, importins, kinases, and phosphatases in infections with segmented NSVs (20–22). Finally, nucleoproteins can also affect the antiviral immune response by modulating the function of key proteins within the immune signaling pathways, like TBK1 and IRF3, or by triggering host cell apoptosis and autophagy (23–26). In this review, we will discuss the vast number of functions of nucleoproteins from a structural perspective.

NUCLEOPROTEIN ARCHITECTURE

The overall size of nucleoproteins ranges greatly, from 233 amino acids in Bunyamwera virus (BUNV) to 739 amino acids in Zaire ebolavirus (EBOV). However, the core of all nucleoproteins has a high structural homology among NSV families (Fig. 1). Nucleoproteins are composed of two core domains that are connected via a positively charged region that is critical for RNA binding. The N-terminal domain and C-terminal domain of nucleoproteins form a conserved structural pattern, with five alpha-helices in the N domain (5 hours motif) and three alpha-helices in the C domain (3 hours motif) (note that the orientation of the helices varies) (27, 28). Many nucleoproteins also have intrinsically disordered N- or C-terminal regions (or tails) that play a key role in

oligomerization by enabling head-to-tail or side-by-side interactions between nucleoprotein monomers, such as the paramyxovirus nucleoproteins (29–32). Oligomerization between nucleoprotein molecules is further stabilized through other, more dynamic interactions.

NUCLEOPROTEIN OLIGOMERIZATION

Nucleoproteins interact to form large oligomeric structures (Fig. 2) in the presence and absence of RNA. Non-viral RNA can be encapsidated if nucleoprotein oligomerization is not modulated by additional (weak) interactions, such as with P (20, 32, 33). Nucleoprotein oligomerization appears to occur via two mechanisms: head-to-tail interactions and side-by-side interactions, with one of the two mechanisms driving the reaction, depending on the virus. Recent structural data suggest that nucleoproteins molecules in non-segmented NSV RNPs interact via side-by-side oligomerization [EBOV, rabies virus (RABV), vesicular stomatitis virus (VSV), measles virus (MeV), respiratory syncytial virus (RSV), cetacean morbillivirus (CeMV)], whereas segmented NSV RNPs oligomerize via head-to-tail interactions (e.g., IAV and BUNV) (Fig. 3) (34–41). Additional structures of multimeric nucleoproteins, some in complex with RNA, have been solved for several additional segmented NSVs, including BUNV, Rift Valley fever virus (RVFV), Toscana virus (TOSV), Leanyer virus (LEAV), tomato spotted wilt virus (TSWV), and La Crosse virus (LACV). These new structures also point toward a head-to-tail oligomerization pattern in native RNPs (30, 42–47). However, nucleoprotein molecules of Hantaan orthohantavirus (HTNV), a segmented NSV, do not fall within either discrete oligomerization pattern. Instead, HTNV nucleoproteins undergo a unique conformational fold upon oligomerization that results in an interaction between the N-terminal and C-terminal arms, creating a closed crescent-like nucleoprotein monomer with little lateral separation between the "head" and "tail" (Fig. 2) (48).

Electron micrographs reported for NSV RNPs show that NSV RNP structures fall on a flexibility spectrum (Fig. 4); for an overview see Table S1 (31, 41, 49–60). The side-by-side oligomerization pattern of the nucleoprotein molecules in the RNPs of non-segmented NSVs represents the most rigid and tight structure, whereas the head-to-tail oligomerization pattern of the nucleoprotein molecules of segmented NSV RNPs represents a more flexible structure (Fig. 3 and 4). The side-by-side interactions have a larger interaction area, which limits the dynamics of the nucleoprotein chain. Limited nucleoprotein dynamics would likely deform the RNP, such that it would be energetically unfavorable for the RNA polymerase to bind to both ends of the vRNA. The head-to-tail interaction pattern is inherently more flexible, allowing for the formation of loop-like or double helical RNPs, in which both the 5′ and 3′ ends are held close together by the RNA polymerase.

Once nucleoproteins are in a RNP-like oligomeric state, it is likely sterically difficult for these nucleoproteins to subsequently bind vRNA, especially for the NSV nucleoproteins which sequester the vRNA on the interior of the RNP helix. Given the current data, it is likely that the oligomerization of nucleoproteins and the binding of nucleoproteins to vRNA occurs in tandem, rather than sequentially, and that both processes are highly regulated during the viral infection cycle. Indeed, *in vitro* experiments with IAV nucleoprotein have shown that oligomerization states can be controlled by adjusting the salt concentration (10, 61, 62): at 50 mM NaCl, IAV nucleoproteins remain monomeric, whereas at 300 mM NaCl IAV nucleoproteins forms trimers and tetramers. Interestingly, the trimers/tetramers bind to RNA with threefold higher affinity than monomeric nucleoprotein, but nucleoproteins remain in trimer/tetramer form after binding. When multimeric nucleoprotein is added to RNA in 300 mM NaCl and the salt concentration is decreased to 50 mM NaCl, RNP-like structures form spontaneously, comparable to when monomeric nucleoprotein is added to RNA during viral replication (62). These results suggest that IAV nucleoprotein oligomerization is in equilibrium between multimeric and monomeric states and that only monomeric nucleoprotein can form RNP structures.

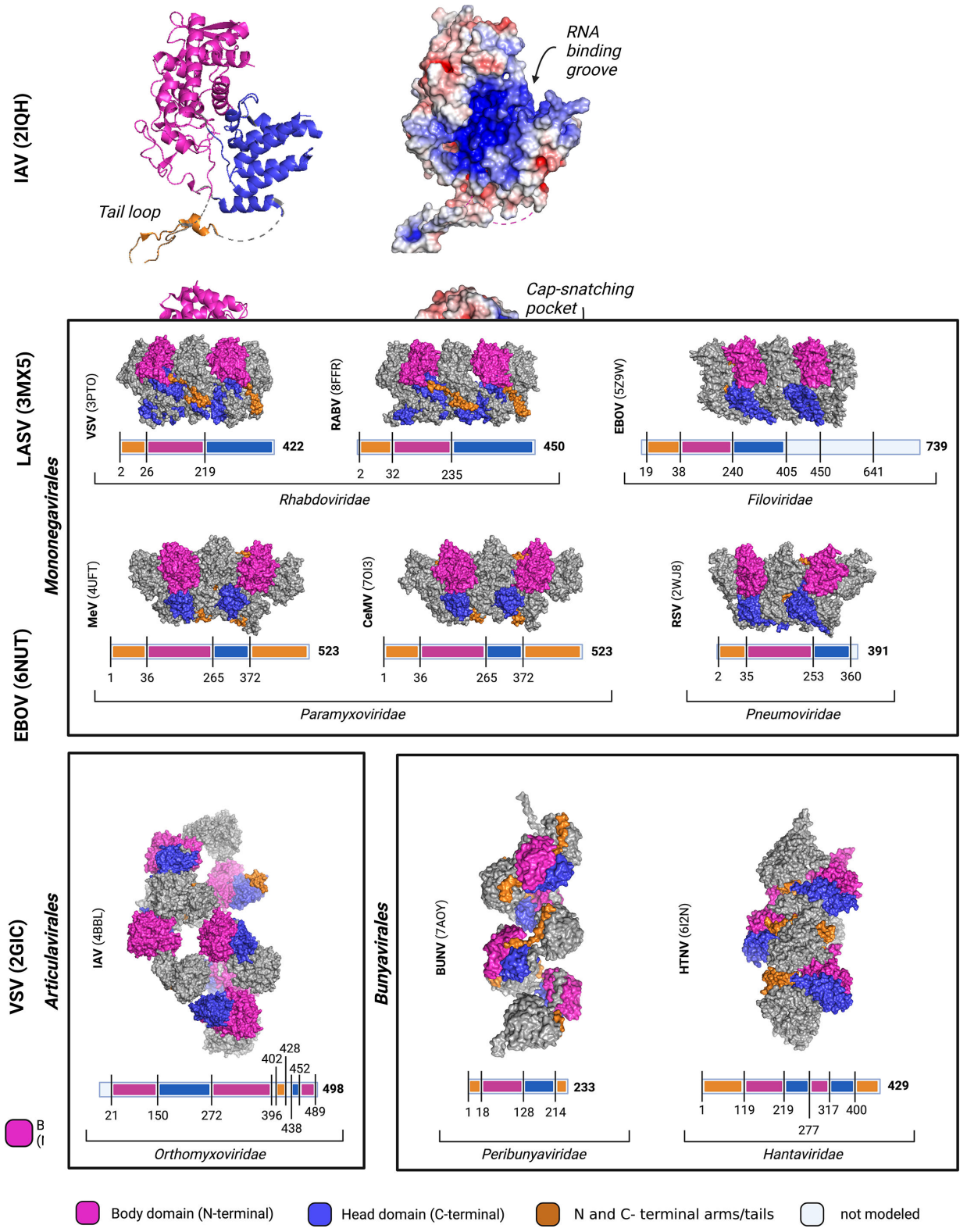

FIG 2 Oligomerization of nucleoproteins into RNPs. Non-segmented (top) and segmented (bottom) NSV RNP structures modeled in surface representation. Alternating nucleoproteins in each structure are colored according to the domains indicated in the corresponding sequence maps below each individual structure. The non-segmented virus RNPs (top) are each shown as one turn of the rod-like helix. From left to right, the non-segmented virus RNPs illustrated are from: vesicular stomatitis virus (VSV), rabies virus (RABV), Zaire ebolavirus (EBOV), measles virus (MeV), cetacean morbillivirus (CeMV), and respiratory syncytial virus (RSV). The segmented virus RNPs (bottom) are shown as portions of the rod-like helices. For the influenza A virus (IAV) RNP, only one twist of the double helix is shown. For both Bunyamwera virus (BUNV) and Hantaan orthohantavirus (HTNV), roughly two twists of the single helices are shown.

FIG 1 (Continued)

FIG 1 Nucleoprotein structures. Examples of nucleoprotein structures showing a conserved bi-lobed core (left row) and positively charged RNA binding regions (right row). The influenza A virus (IAV) NP has non-contiguous head/body domains, whereas other nucleoproteins have terminally contiguous head/body domains, like the Lassa fever virus (LASV), Zaire ebolavirus (EBOV), and vesicular stomatitis virus (VSV) nucleoproteins. The EBOV and VSV nucleoproteins also have extended N-terminal tails. PDB accession numbers used to generate the models are indicated on the left of the panels, and colors are explained in the bottom legend.

Lassa virus (LASV) nucleoproteins form trimers in mammalian cells but these multimers do not bind to RNA (63). It has been hypothesized that the observed trimeric form of LASV nucleoproteins is a regulatory mechanism to keep nucleoprotein RNA-free until it is needed for nascent RNA binding (64). However, by contrast to IAV, regulation is more complex than reducing nucleoprotein to a monomeric state as mutant LASV nucleoprotein that cannot form trimers does not support viral replication and transcription (63). Molecular dynamics studies have shown that upon disruption of the LASV nucleoprotein trimer into nucleoprotein monomers, the individual nucleoprotein subunits undergo conformational changes that are conducive to RNA binding as well as RNP formation (65). Together, these findings suggest that the oligomerization patterns of LASV nucleoprotein have multiple functions, preventing premature RNA binding and regulating the viral infection cycle.

To maintain nucleoproteins in conformations conducive to RNA binding and RNP formation, non-segmented NSVs [RABV, VSV, MeV, EBOV, RSV, and Nipah virus (NiV)] utilize chaperone proteins (P or VP35), which phosphorylate oligomerization grooves

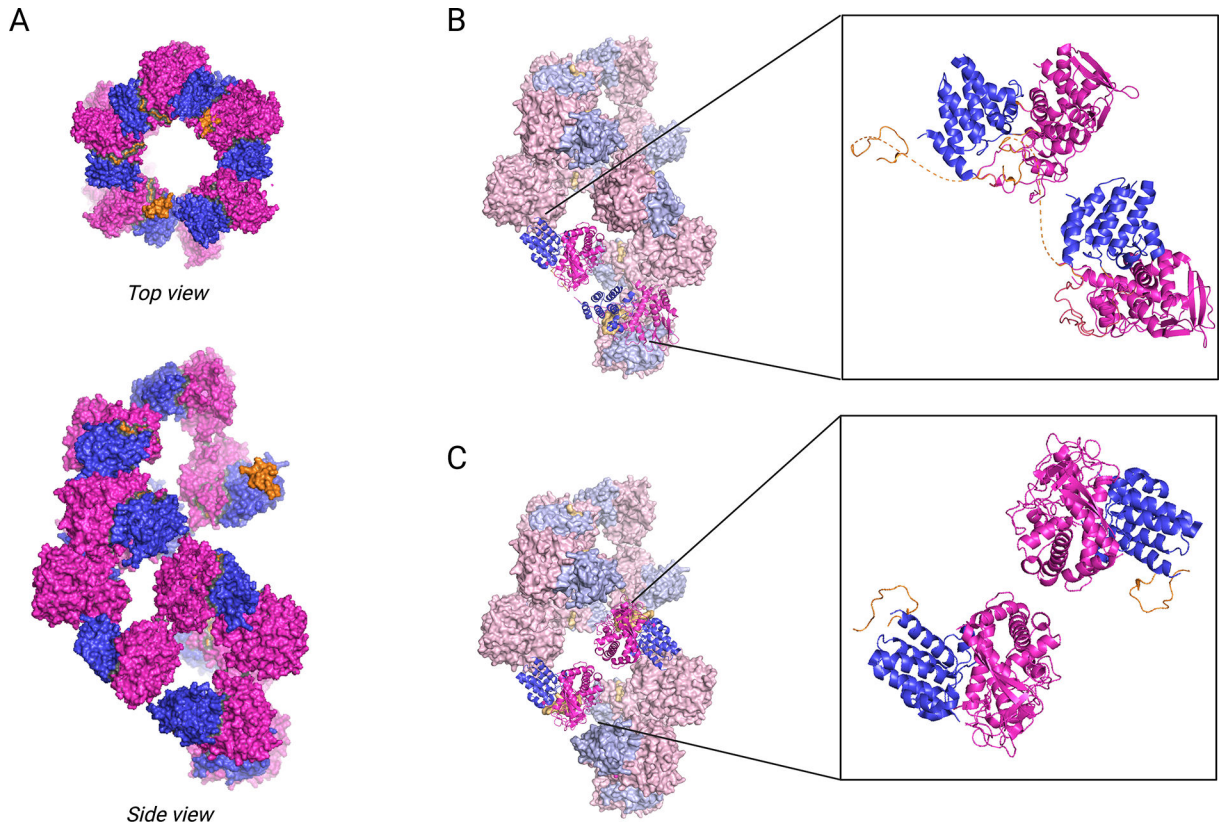

FIG 3 IAV nucleoprotein oligomerization. IAV ribonucleoprotein complex (PDB: 4BBL) with (A) 12 nucleoprotein monomers shown from two opposing strands of the double helix and (B) nucleoprotein head-to-tail oligomerization via the tail loop between adjacent same-strand nucleoprotein subunits and (C) opposing-strand nucleoprotein interaction interface. Domains are colored according to Fig. 2.

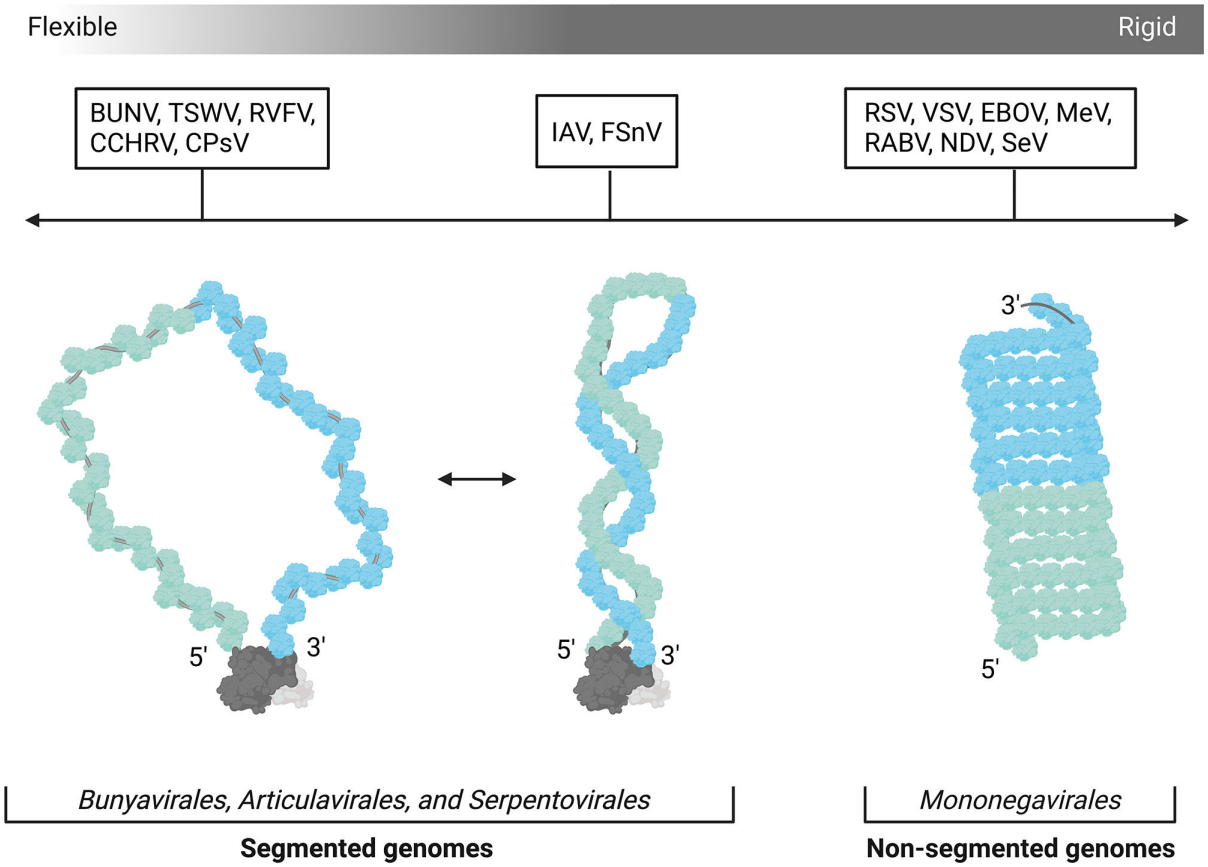

FIG 4 Flexibility of NSV RNPs. Cartoon illustrating RNP structural variability observed by negative stain electron microscopy (Table S1). The flexibility continuum is indicated at the top of the cartoon. The RNA polymerase is depicted in dark gray. The nucleoprotein monomers forming the 5′ consecutive half of the RNP are depicted in light green and the nucleoprotein monomers forming the 3′ consecutive half are depicted in light blue. The leftmost structure represents the typical *bunyavirales* RNP, the middle structure the typical *articulavirales* RNP, and the rightmost structure a typical *mononegavirales* RNP. In the latter structure, no RNA polymerase is depicted. Virus abbreviations are as follows: Bunyamwera virus (BUNV), tomato spotted wilt virus (TSWV), Rift Valley fever virus (RVFV), Crimean-Congo hemorrhagic fever virus (CCHRV), citrus Psorosis virus (CPsV), influenza A virus (IAV), freesia sneak virus (FSnV), human respiratory syncytial virus (RSV), vesicular stomatitis virus (VSV), Ebola virus (EBOV), measles virus (MeV), rabies virus (RABV), Newcastle disease virus (NDV), and Sendai virus (SeV).

and/or competitively block RNA binding or nucleoprotein oligomerization through interactions between adjacent monomers (9, 11, 66, 67). These chaperone proteins are also responsible for regulating the replicase and transcriptase complexes and are strongly associated with full length vRNPs and weakly associated with monomeric nucleoprotein via their disordered arms (1, 20).

EBOV VP35 chaperones nucleoprotein in the RNA-free state. The N-terminal 80 residues of VP35 bind to nucleoprotein and prevent oligomerization as well as premature RNA binding. VP35 competes with the N-terminal arm of a potential neighboring nucleoprotein monomer (9). Since oligomerization induces the necessary conformational change in a nucleoprotein to make it capable of RNA binding, VP35 is thought to modulate RNA binding indirectly by preventing oligomerization. A crystal structure of a nucleoprotein-VP35 fusion protein from which the VP35 disordered regions were removed shows that VP35 straddles an EBOV nucleoprotein-specific beta-hairpin in the C-terminal domain of the nucleoprotein. Removal of the VP35 peptide using TEV cleavage and anion exchange chromatography revealed that monomeric nucleoproteins form oligomers in the absence of VP35. Moreover, addition of VP35 can reverse nucleoprotein oligomerization in the absence of RNA, but not when RNA is present. In other words, once the EBOV RNP is formed, it is stable. These observations are in line with the hypothesis that RNA is not necessary for oligomerization of viral nucleoprotein but

important for stabilizing the vRNP to prevent spontaneous nucleoprotein dissociation. VP35 must dissociate from the monomeric nucleoprotein in order for it to oligomerize and bind vRNA (9). The driving force for this handoff is currently unknown, but it may be nucleoprotein-concentration dependent. The interaction between the EBOV nucleoprotein and VP35 is structurally distinct from distantly related NSVs.

The P protein, analogous to EBOV VP35, is known to act as chaperone for VSV, MeV, and NiV nucleoproteins and to bind to the RNA polymerase as an oligomer. These P proteins are primarily alpha-helical and bind nucleoproteins via disordered arms, whereas the EBOV VP35 is primarily unstructured (9, 11, 68–70). This may be attributed to the unique beta-hairpin structure in EBOV nucleoprotein with which VP35 interacts. Despite the significant structural distinction, the general mechanism of regulation is similar. Solved structures of VSV nucleoprotein-P complexes show that P blocks both RNA-binding and NP oligomerization, whereas in NiV nucleoprotein-P complexes, P blocks nucleoprotein oligomerization (68, 69).

NSVs with segmented genomes do not rely on a chaperone protein for the regulation of nucleoprotein oligomerization. Instead, they use phosphorylation and/or conformational changes to control nucleoprotein oligomerization (3). As previously mentioned, IAV nucleoprotein is present in both monomeric and multimeric states, dependent on the salt concentration and must be monomeric before RNA binding can occur. Mechanistically, the phosphorylation and dephosphorylation of S165 of the IAV nucleoprotein is crucial for the regulation of oligomerization. S165 is located in the tail-loop insertion groove and the phospho-mimic mutant S165E remains mostly monomeric. This suggests that phosphorylation of S165 prevents oligomerization by blocking the oligomerization insertion groove, and that dephosphorylation needs to occur before nucleoprotein molecules can oligomerize (61). Recruitment of nucleoprotein may involve the intrinsically disordered tail of host protein ANP32, analogous to the non-segmented NSV *P* protein (22, 70, 71). ANP32 is bound by a replicase complex consisting of two RNA polymerases, which are held together by ANP32. How a phosphatase is recruited to this complex is presently unknown.

A structural exception to the above descriptions of NSV RNPs is the Newcastle Disease virus (NDV) RNP. This paramyxovirus forms self-capped "double-RNPs" that contain two single-stranded spirals packed with their 5′ ends held in close proximity (31). Minigenome analysis shows that elimination of the nucleoprotein residues that create the 5′ seam between two helical spirals results in non-functional, single-filament RNPs. This self-capped organization means that multiple genome copies can be present in a single RNP and therefore a virion particle with only one RNP can contain multiple genome copies. Paramyxoviruses can contain multiple genome copies within a single virion particle and some segmented viruses, like RVFV, have also been found to contain duplicated genome segments within a single virion particle (72–74). However, NDV is the only known case where the duplicate genome can be encapsidated within the same RNP structure (31). While this is the only known case of self-capping NSV RNPs, this molecular mechanism has been observed in other filamentous systems, such as microtubule assembly (75).

RNA BINDING PROPERTIES OF NUCLEOPROTEINS

The molecular manner by which nucleoproteins interact with vRNA varies greatly. Typically, the nucleoproteins have a positively charged RNA-binding groove and undergo a conformational change that effectively clasps the vRNA after or during binding. The basic structure of an NSV nucleoprotein is made up of a C-terminal domain (CTD) and an N-terminal domain (NTD) (Fig. 2). In VSV and RABV nucleoprotein-RNA ring-like structures, 9 nucleotides (nt) are tightly clasped between the CTD and NTD of individual nucleoprotein subunits (76, 77). The RSV nucleoprotein-RNA ring-like complex showed a similar interaction, with 7 nt of RNA clasped between the CTD and NTD of each individual NP subunit (38). The same mechanism of vRNA binding between the CTD and NTD of individual nucleoprotein subunits was observed in cryo-EM analyses of helical

MeV RNP structures and helical EBOV RNP structures, suggesting that the previously observed nucleoprotein-RNA binding interactions in a ring-like formation are similar to the interactions present in the virion (34, 37).

The structure of IAV nucleoprotein-RNA deviates from the relatively conserved RNA-binding interactions observed in *mononegavirales*. IAV nucleoproteins hold the RNA between the CTD and NTD, but biochemical analysis suggests IAV encapsulates 24 nt per nucleoprotein subunit and that this interaction does not involve the extensive base-stacking observed in RABV and VSV nucleoproteins (78, 79). In the IAV nucleoprotein-RNA complex, the RNA is instead held more flexibly in the basic groove of nucleoprotein subunits through polar interactions and hydrogen bonding and the bases are accessible to nucleases. The flexibility of the RNA winding around each nucleoprotein is hypothesized to be the reason why crystal structures of complete IAV nucleoprotein-RNA interactions have not yet been resolved (80). The only structural evidence currently available for IAV nucleoprotein-RNA comes from a crystal structure of a monomeric H5N1 IAV nucleoprotein in complex with a 9-nt long synthetic RNA molecule, of which only 3-nt could be modeled in the observed density (79).

The only other segmented NSV for which the interactions between nucleoprotein and RNA have been revealed at a structural level is BUNV (47). A crystal structure for a tetrameric ring-like BUNV nucleoprotein-RNA complex shows that the bound RNA is buried deep in a narrow cleft on the internal surface of the ring. The number of bases that is covered by a single nucleoprotein is 11 nt. Unlike IAV nucleoprotein-RNA interactions, the RNA bases are completely isolated from solvent exposure and held in place more rigidly. While this may not necessarily illustrate native NP-RNA interactions within a BUNV RNP, electron micrographs of BUNV RNPs show that the width of the native RNP is comparable to that of the tetrameric BUNV nucleoprotein-RNA ring structure. This is noteworthy because the width of nucleoprotein tetramers of the closely related RVFV do not match the width of RVFV RNPs seen in electron micrographs, suggesting that NP multimers can adopt several different conformations distinct from RNA-bound NP multimers (42, 44).

The shortest estimated RNA-binding footprint is 6 nt for the nucleoproteins in both MeV and EBOV RNPs (34, 37). This is followed by a 7 nt RNA-binding footprint in RSV nucleoprotein, 9 nt in VSV and RABV nucleoproteins, and 11 nt in BUNV, LACV, and LEAV nucleoproteins (30, 38, 45, 47, 76). The longest RNA-binding footprint observed in NSV nucleoproteins is 24 nt observed for IAV (78, 79). Although the number of known nucleoprotein footprints is small, the current data indicate that the RNA-footprint is not correlated with nucleoprotein size or the number of amino acids that make up a nucleoprotein molecule.

The location of the vRNA in the RNP also varies among NSVs. In both RABV and VSV RNP structures, the vRNA is encapsidated in the center of a left-handed conical helix (81). Paramyxovirus RNPs also adopt left-handed helices, but in contrast to the *Rhabdoviridae* (RABV and VSV) RNP structures, the vRNA is encapsidated on the outside of the helix, as seen in RSV (27). The nucleoprotein-RNA binding mechanism influences the amount of protection that the RNP offers. Most paramyxoviruses form RNPs that can protect RNA from RNase digestion despite the vRNA being more exposed. However, EBOV RNA-nucleoprotein complexes are sensitive to RNase digestion (82). IAV RNPs have fully exposed RNA bases which are also susceptible to RNase cleavage (83). The location of vRNA is important because vRNA that resides on the outside of the RNP can form RNA secondary structures. Recent SHAPE-MAP experiments have shown that IAV vRNA has less RNA secondary structure in a virus particle (in complex with nucleoproteins) than it does as naked vRNA (13, 14). The remaining RNA secondary structures seen in virions involve short-range interactions (spanning less than 100 nt) and segment-segment interactions. These observations support the hypothesis that nucleoproteins can minimize RNA secondary structure and facilitate the selective formation of those structures that are critical, like in the packaging of segmented NSVs.

Several experimental approaches have been used to determine the strength of the nucleoprotein-RNA interactions in influenza viruses, including filter binding assays, surface plasmon resonance, and polarization of fluorescence. These analyses show that the RNA binding affinity of nucleoproteins at room temperature ranges from 4 nM to 70 nM, depending on the salt concentration and type/length of RNA used (84). Divergent affinity measurements were observed at colder temperatures (380 nM at 4°C) and with monomeric mutants (1–10 uM). In comparison to other known RNA binding proteins (RBPs), which have Kd values ranging from <10 nM to uM, viral nucleoprotein bind RNA with moderate strength (85). The RNA binding affinity of other NSV NPs has not been determined.

DYNAMICS OF RIBONUCLEOPROTEIN COMPLEXES

As depicted in Fig. 4, NSV RNPs fall on a flexibility spectrum, with nonsegmented NSVs adopting the most rigid RNP structures and bunyaviruses the most flexible RNP structures. However, during infection, RNPs likely adopt additional conformations, each conducive to a given stage of NSV replication or transcription. During NSV replication, these RNP structures must allow the RNA polymerase to access the vRNA. A still unanswered question is how the RNA polymerase gains access to the vRNA template that is otherwise protected by oligomerized viral NPs. NPs likely need to dissociate from the RNA to allow the RNA polymerase to copy the vRNA, and then rebind the vRNA as the RNA polymerase translocates to avoid exposure of naked vRNA to nucleases or other host proteins. Presently, the exact mechanisms of nucleoprotein dissociation and reassociation during replication and transcription remain unknown for all NSVs.

In paramyxoviruses, it has been shown that transcription is efficient only when the vRNA genome consists of a multiple of 6 nucleotides (i.e., 6 n nucleotides and known as the "rule of six") (86). This correlates with the number of nucleotides that each nucleoprotein subunit binds to. More specifically, it has been shown that in the Sendai virus (SeV) nucleoprotein the exact position of each nucleotide is critical for recognition by the RNA polymerase (87). Essentially, the precise way that the nucleoprotein holds each nucleotide in the RNA binding groove is distinct and important for transcription. Furthermore, many paramyxoviruses are known to have bipartite promoters, which are non-contiguous regions in the vRNA that can be recognized by the RNA polymerase only when they are in complex with nucleoprotein and in close physical proximity (88). This highlights the importance of the nucleoprotein structure, the helical arrangement of the RNP, and the resulting interactions of individual nucleotides of the vRNA with the RNP (89).

Structural analysis of IAV RNPs suggests that the RNP retains a double helical RNP conformation throughout transcription, and that the RNA polymerase remains bound to both the 3′ and 5′ ends of the vRNA (90). Using nucleozin, a known influenza virus inhibitor that causes nucleoprotein aggregation that can stall IAV transcription, further insight into RNP dynamics was obtained (90). Cryo-EM analysis of the nucleozin-stalled RNPs showed that nucleozin binds to two adjacent nucleoproteins from opposite strands within the RNP, acting like a staple (90). This inhibition mechanism is cooperative: once one nucleozin molecule binds, it brings the adjacent strand nucleoproteins closer together, allowing for the crosslinking to spread throughout the entire helix and resulting in a more rigid RNP structure. Importantly, the stapled nucleoprotein strands impair viral RNA synthesis, which implies that the opposing nucleoprotein strands need to "slide" past each other to allow the RNA polymerase to copy the template (Fig. 5A) (90). In this 'processive helical track' model, IAV transcription occurs when the RNA polymerase "pulls" the vRNA template locally from the nucleoprotein chain, detaching the RNA from the nucleoprotein upstream of the RNA polymerase entry channel, but likely leaving the nucleoprotein attached to rest of the nucleoprotein chain. The RNA polymerase subsequently pulls the RNA into the active site of the RNA polymerase for template-dependent RNA synthesis. The template vRNA next reassociates with the

RNA-free nucleoprotein in the nucleoprotein chain upon exit from the RNA polymerase active site (91, 92).

Similar to articulaviruses, bunyavirus RNA polymerases hold onto the 5′ and 3′ ends of the vRNA while transcribing and replicating the vRNA segments (93). However, unlike articulaviruses, the RNPs of bunyaviruses form a more flexible, spiraling loop, instead of a double helix (41). It is likely that the bunyavirus vRNA also dissociates from the nucleoprotein molecules in the RNP before entering the active site of the RNA polymerase (Fig. 5B). The hypothesized dynamics are therefore less complex than the double helical track, as "sliding" is likely unnecessary due to the open loop structure of the RNP. The illustrated mechanism in Fig. 5B follows the same nucleoprotein handling mechanism as in articulaviruses (Fig. 5A).

Structural data for articulavirus and bunyavirus transcription elongation and termination is limited, and the only data available for IAV transcription, elongation, and termination has been obtained in the absence of nucleoprotein (5, 92–94). Following polyadenylation near the terminal 5′ ends of the vRNA segments, termination likely involves a "reset" of the RNP complex. In the current model, the 5′ end remains bound, while the polyadenylation sequence is released from the active site of the RNA polymerase (93). Nucleoproteins, still in a chain-like oligomerization state, are proposed to reassociate with the released template vRNA upon exit from the RNA polymerase.

A nucleoprotein-vRNA dissociation mechanism similar to the segmented NSVs has been proposed to take place during replication and transcription of the genome of RABV and other members of *mononegavirales*. Oligomerized nucleoprotein molecules are thought to remain connected, like links in a chain, as the RNA polymerase (L protein) copies the vRNA. The template then reattaches to the nucleoprotein near the exit channel of the RNA polymerase. Unlike IAV and other orthomyxoviruses, this process requires the viral P protein. A P-dimer, -trimer, or -tetramer attaches to the L protein, creating an L-P transcriptase complex that can bind to the individual nucleoprotein subunits within the RNP and trigger a conformational change in the nucleoprotein subunits that releases the vRNA (Fig. 5C) (95). It is unknown if the RNA polymerase dissociates from the vRNA upon reaching the end of the transcript or remains attached.

The above models of NSV RNP dynamics during RNA synthesis assume that the vRNA can be released from the nucleoprotein chain and "snap-back" onto the nucleoprotein chain after being copied by the RNA polymerase. In EBOV, monomeric nucleoproteins cannot bind RNA and RNA-binding is dependent on nucleoprotein oligomerization (96). It has been hypothesized that nucleoprotein oligomerization induces conformational changes that facilitate RNA binding. This idea suggests that the "snap-back" model may also be used during EBOV RNA synthesis.

The above model is not fully consistent with experimental results showing that IAV nucleoproteins form RNP-like oligomers without vRNA present and that these IAV oligomers, once formed, cannot turn into functional RNPs by binding vRNA. IAV nucleoprotein trimers/tetramers can readily bind RNA but they cannot form native oligomeric RNP structures, likely due to the closed loop interactions (61). It is possible that a transient conformational change exists within a vRNA-free nucleoprotein in the context of an RNP, while the vRNA is being copied by the RNA polymerase. In HTNV, RNA binding to HTNV nucleoproteins is followed by a conformational change to completely surround the template, creating a protective tunnel (48). Other NSVs simply form a "jaw" between the head and body domains that effectively "clamps" down the RNA post binding (8). The force on the vRNA template, as it is pulled into the RNA polymerase, may be sufficient to "open" these clamp-like structures, but calculations have not been done to support this idea.

ROLE OF NUCLEOPROTEINS IN REGULATION OF TRANSCRIPTION AND REPLICATION

In addition to nucleoprotein's role in vRNA protection and acting as a scaffold, nucleoprotein can function as a mediator of gene regulation in several viruses. A recent study of

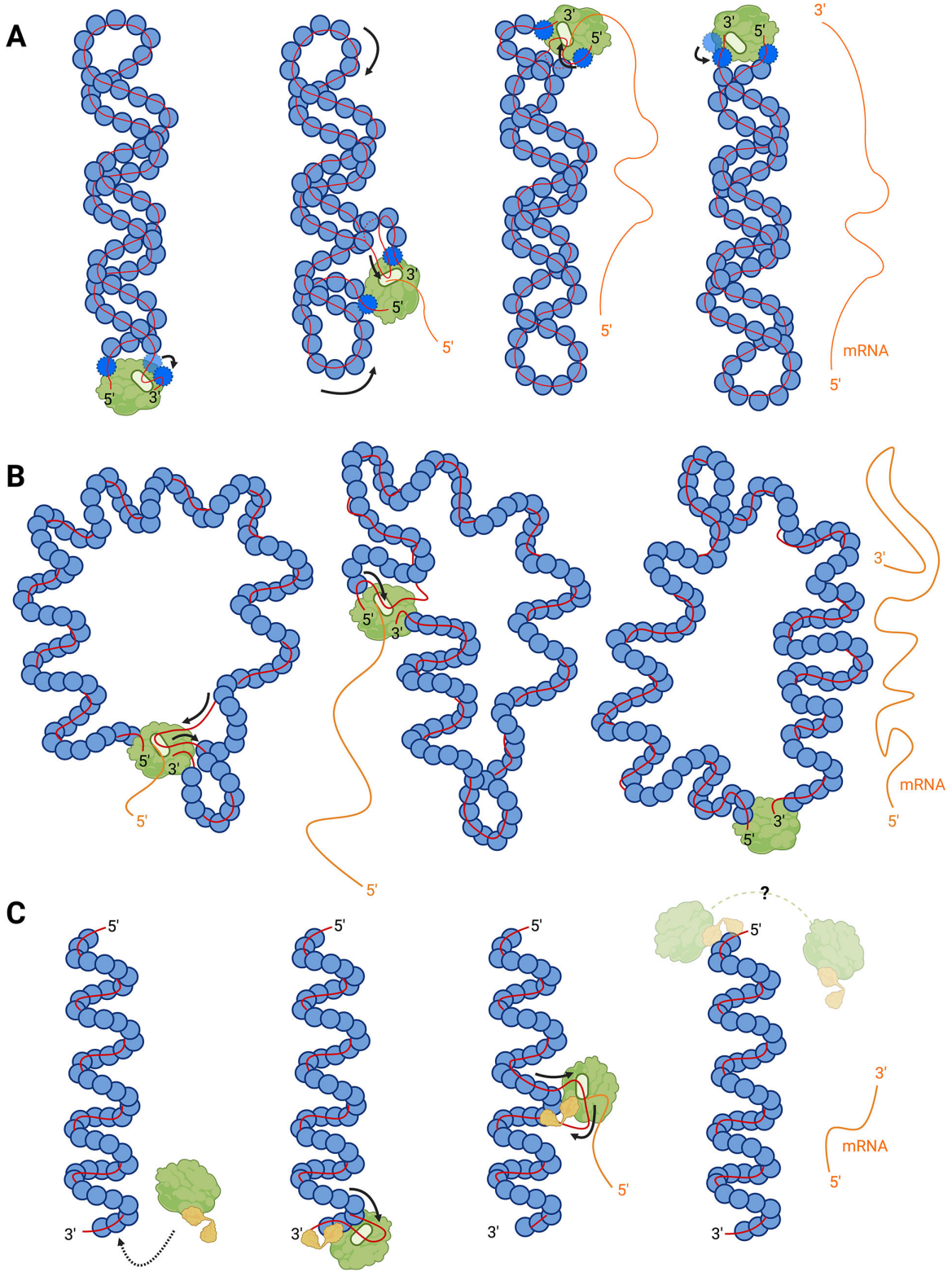

FIG 5 RNP dynamics during NSV transcription. (A) Articulavirus RNPs form a double-helical complex in which both the 3′ and 5′ ends of the vRNA template (red line) are bound to the RNA polymerase (green). In the model shown, IAV transcription (mRNA product shown as orange line) occurs when the RNA polymerase "pulls" the vRNA template locally from the nucleoprotein (blue) chain and into the active site of the RNA polymerase (denoted as a light green oval). The template vRNA next reassociates with the nucleoprotein chain upon exit from the RNA polymerase active site. Black arrows denote the movement of the template vRNA and associated nucleoproteins. The 3′ and 5′ terminal nucleoprotein subunits are denoted in dark blue with dashed edges to illustrate

(Continued on next page)

FIG 5 (Continued)

the proposed handling by the RNA polymerase. (B) The bunyavirus RNP is a spiraling, circular RNP in which the 3′ and 5′ ends of the vRNA are attached to the RNA polymerase (green). Transcription and replication involve local dissociation of the template vRNA (red line) from the nucleoprotein (blue) chain as the vRNA enters the active site of the RNA polymerase (denoted as a light green oval) and reassociation of the vRNA to the nucleoprotein chain upon exit of the RNA polymerase. Black arrows denote the movement of the template vRNA and associated nucleoproteins. (C) The mononegavirus RNP forms a tight helical rod-like structure with no permanent attachment point for the RNA polymerase (green). Phosphoprotein (yellow, shown here as a dimer) binds to the RNA polymerase and forms the transcriptase complex which associates (dashed arrow) with the initiation sites on the template vRNA (red line). Phosphoprotein interacts with the nucleoproteins (blue) eliciting conformational change which induces the release of template vRNA from the nucleoprotein chain as it enters the RNA polymerase active site (denoted as a light green oval). The nucleoprotein re-associates with the template vRNA upon exit of the RNA polymerase. Exact handling of nucleoprotein during the transient RNA-free state is currently unknown, but nucleoprotein is shown here to remain in chain-like form. Black arrows denote the movement of the template vRNA and associated nucleoproteins. It is currently unknown whether the RNA polymerase dissociates from or remains attached to the template vRNA after producing the mRNA (orange line).

IAV replication found that a single mutation in nucleoprotein can selectively modulate neuraminidase (NA) gene segment expression in the A/Puerto Rico/8/1934 (PR8) strain. One amino acid substitution, F346S, decreased the ability of nucleoproteins to promote NA segment vRNA synthesis while not altering the RNA binding or oligomerization function of nucleoprotein (97). The strain-specific effect of F346S was determined to derive from a motif within the UTR of the PR8 NA segment. It was subsequently found that the neighboring aromatic residues in the IAV nucleoprotein, Y385 and F479, also affect NA gene expression. Together, the pi-stacking aromatic residues F346, Y385, and F479 stabilize the nucleoprotein tail-loop. When disrupted through the F346S mutation, the tail-loop may adopt a less stable, alternative conformation that affects how segments are replicated. Presently, the underlying mechanism is not understood.

In the nucleoprotein of human parainfluenza virus 2 (hPIV2), amino acid residue Q202 is 1 of 10 nucleoprotein residues that interact with RNA and the only residue that interacts directly with the base of a nucleotide (98). Experiments have shown that mutation Q202A results in increased levels of RNA polymerase activity without affecting nucleoprotein interactions with phosphoproteins, adjacent nucleoproteins or RNA binding (98). Furthermore, hPIV2 RNA templates with disrupted bipartite promoters were found to be rescued by the Q202A nucleoprotein mutation (98). Together, these fascinating observations suggest that Q202 is necessary for the selective initiation of bipartite promoters and for enforcing the rule of six in the hPIV2 genome and underlining the interplay among RNP structure, nucleoprotein-RNA interactions, and viral RNA synthesis.

Post translational modifications (PTMs) regulate cellular activities as well as NSV replication. Host ubiquitin ligase CNOT4 has been found to ubiquitinate IAV nucleoprotein K184. Ubiquitination of K184 leads to an increase in RNA polymerase activity, whereas lack of or removal of by host deubiquitinase USP11 reduces RNA polymerase efficiency (99). K184 resides in the nucleoprotein RNA-binding groove, suggesting that ubiquitination enhances the RNA binding efficiency of nucleoproteins or the processivity of the viral RNA polymerase. In many other molecular contexts, protein ubiquitination is known to trigger protein degradation, however the amount of nucleoprotein does not decrease upon CNOT4 expression, which suggests that this ubiquitination does not alter the stability of the nucleoprotein (99).

While non-segmented NSVs use the viral P protein to regulate nucleoprotein oligomerization and facilitate the formation of an active replicase complex, segmented NSVs do not encode a protein analogous to P. Influenza viruses rely on host protein acidic nuclear phosphoprotein 32 (ANP32) for some of the roles that P performs in the replicase complex of other NSVs (22). The C-terminal region (LCAR) of both human and chicken ANP32 mediates a direct interaction with the IAV nucleoprotein. It is hypothesized that this flexible tail-like region of ANP32 recruits monomeric nucleoprotein to newly formed RNA, thereby facilitating encapsidation. ANP32-LCAR is acidic and interacts with the basic RNA-binding groove of the IAV nucleoprotein. This prevents any nucleoprotein already bound to RNA from getting recruited to the replicase complex. Furthermore,

mutant ANP32 proteins that did not contain an LCAR domain did not support replication of full-length IAV genome segments, suggesting that LCAR is critical for the replication of IAV.

Lymphocytic choriomeningitis virus has a bi-segmented ambisense genome. Each segment has two genes encoded in opposite orientations and separated by structured intergenic regions (IGRs) (100). Early in infection, the RNA structure of the IGR functions as a terminator for the RNA polymerase, leading to the transcription of only the gene upstream of the IGR in the vRNA. As infection progresses, replication of the vRNA results in an encapsidated cRNA. It has been proposed that increasing levels of intracellular nucleoprotein concentrations may help unfold the secondary structure within the IGRs, allowing the RNA polymerase to replicate past the IGR (100). However, in this model, it is unclear if these nucleoproteins would only help melt the RNA structure or also insert into the existing nucleoprotein chain. Alternatively, in replication mode, triggered by the production of new nucleoprotein and RNA polymerase subunits, the RNA polymerase may be more processive and pass through the structure, creating a cRNP. Indeed, once the cRNP has been formed, transcription of the cRNA template occurs upstream of the IGR but not downstream of the IGR, whereas replication of the cRNP leads to the production of new vRNPs (100). An increase in nascent nucleoprotein levels may thus be a key signal to initiate the switch from transcription to replication. Interestingly, this simple regulatory switch from transcription to replication also occurs in many paramyxoviruses, like VSV and SeV (101). In IAV, the switch is dependent on nascent RNA polymerase levels, RNA polymerase dimer formation, and stabilization of newly formed RNA by nucleoprotein (71, 102, 103).

Recent work has shown that nucleoprotein concentration is vital for viral replication across many NSVs (including IAV, RSV, VSV, EBOV, LASV, and MeV) (17, 18). Limiting the availability of the viral nucleoprotein by gene knock-down induces a strong host immune response despite the reduced amount of vRNA present in infected cells. The lack of nucleoprotein reduces the processivity within IAV replication and as a result more aberrant defective mRNA particles are formed, which act as a trigger for the host interferon (IFN) response. This novel discovery is foundational for new antiviral drug design for potentially all NSVs. By targeting nucleoproteins, not only will viral replication be inhibited but the heightened immune response will prime neighboring uninfected cells, creating an antiviral that could be more effective than most existing therapeutics that target surface glycoproteins and the RNA polymerase.

INTERACTIONS WITH OTHER VIRAL PROTEINS

The viral matrix protein (M) is a crucial protein for enveloped NSVs. The M protein interacts with viral RNPs, envelopes glycoproteins, and facilitates virion assembly during the late stages of infection. In many enveloped NSVs, nucleoproteins directly interact with M to mediate assembly and release of virion particles. In human parainfluenza virus 3 (hPIV3), nucleoproteins interact directly with M. Data suggest that hPIV3 nucleoprotein regulates virion assembly via a single interaction with M protein residue L305 (104). Similarly, in EBOV infection, the matrix protein, VP40, has been found to directly interact with the C-terminal 50 amino acids of the EBOV nucleoprotein (105). In contrast, the RSV M protein does not directly interact with vRNPs, but is still connected by an interaction with the viral protein M2-1 (106).

Apart from involvement in the release of new virion particles, the M protein of Borna disease virus (BoDV) has also been found to colocalize with RNPs. Minireplicon assays have shown that this interaction surprisingly does not inhibit RNA polymerase activity (107). In most other enveloped NSVs, the binding of the RNPs to the M proteins inhibits RNA polymerase activity, likely to ensure that virus particles can efficiently form and egress from the host cell. Given that the interaction between M and RNPs within BoDV does not inhibit RNA polymerase activity, it is likely that the interaction is also important for infection steps prior to virion assembly and egress.

The viral life cycle in IAV is distinct from other NSV life cycles in that replication occurs in the host cell nucleus. This is advantageous because it allows the virus to utilize the nuclear environment as protection and a resource for efficient replication and transcription. On the other hand, the import of vRNPs to the nucleus adds a layer of complexity and the need for additional virus-host interactions (108). The segmented nature of the IAV genome likely facilitates the nuclear import/export processes, as smaller RNPs may transition through the nuclear pore complex more efficiently. Import of vRNPs is initiated by the recognition of the nuclear localization sequences of the IAV nucleoproteins by host protein importin-α (109). Once bound to the vRNP, importin-α is recognized by host protein importin-β, which traffics the vRNP through the nuclear pore complex. This complex process can occur rapidly (within about an hour of infection) suggesting that this process has evolved to be highly efficient and effective (108).

After the IAV genome is delivered to the nucleus and replicated, new vRNPs must exit the nucleus and be transported to the cell membrane for virion budding (110). IAV genome segment eight encodes two non-structural proteins (NS1 and NS2/NEP), which perform a variety of functions during infection. NS1 has been shown to inhibit both interferon production and the nuclear export of host mRNA, likely to ensure that viral mRNA does not have to compete with host mRNA for translation by host ribosomes (111). NS2/NEP is a small 121 amino acid protein that is produced from a spliced genome segment eight mRNA. Although NS2/NEP does not appear to have a significant structural role within the virion, it has been observed to regulate the export of vRNPs from the host nucleus and it is therefore also referred to as IAV nuclear export protein (NEP) (110, 112). Evidence suggests that IAV uses NEP to aid in hijacking the host protein chromosome region maintenance 1 (Crm1) for nuclear export of vRNPs. In this model, NEP acts as a linker between Crm1 and IAV matrix 1 protein (M1)-bound viral RNPs. This complex is then shuttled out of the nucleus via the host nuclear export pathway. Other data show that IAV nucleoprotein can interact directly with Crm1 through a Crm1-specific nuclear export signal, independent of both M1 and NEP (113). Interestingly, M1 also contains a nuclear export signal for Crm1, which suggests that it is possible that M1 plays a regulatory role in nuclear export of vRNPs independent of NEP (114).

The above discussion describes several known interactions between NSV nucleoproteins with other viral proteins of the same species. A more complete list of known interactions can be found in Table S2. The core interactions are visually summarized in Fig. 6 (68, 105–107, 110, 115–124). While the *mononegavirales* encode a viral P protein to chaperone nucleoproteins, it is notable that segmented NSVs (both in *articulavirales* and *bunyavirales*) do not encode such a protein. As previously mentioned, it has been observed that human host protein ANP32 acts as a P protein analog in influenza virus infections. No host analog has been identified for *bunyavirales*, which suggests that the regulation of nucleoprotein multimeric states in bunyaviruses is performed via an alternative mechanism or is not needed at all due to currently unidentified structural differences within the nucleoproteins. Furthermore, there have been no direct interactions observed between mononegavirus nucleoproteins and the associated RNA polymerase (L protein) to date. Interactions are instead coordinated through the encoded *P* protein, maintaining indirect attachment. It is possible that the lack of an encoded P protein in the segmented orders of NSVs (*articulavirales* and *bunyavirales*) is compensated for by the direct interactions of the nucleoproteins with the RNA polymerase.

ROLE OF NUCLEOPROTEINS IN THE DISRUPTION OF HOST CELL IMMUNE PATHWAYS

The host innate immune system uses multiple mechanisms to detect and inhibit a viral infection. One method is the recognition of pathogen-associated molecular patterns (PAMPS) by human host pattern recognition receptors (PRRs). Recognition of such PAMPS activates antiviral signaling cascades, including type I and III IFN expression. Retinoic acid-inducible gene I (RIG-I) is a host pathogen receptor that binds di- or

tri-phosphorylated double-stranded RNA (dsRNA) molecules in the host cell cytoplasm (125). The (partially) complementary 5′ and 3′ terminal ends of segmented NSV vRNAs are substrates for RIG-I and activation of RIG-I induces IFN production which limits the spread of NSV particles (126). NSV nucleoproteins are critical for protecting viral RNA from the detection by RIG-I, and reduced nucleoprotein expression has been shown to increase RIG-I activation (17, 18).

Arenavirus nucleoproteins suppress the IFN response by degrading PAMP RNAs through a C-terminal DEDDh-like exonuclease domain (64, 127). While seemingly counterintuitive, this exonuclease activity is pro-viral and required for the anti-IFN activity observed in arenavirus infections. To prevent virus detection by the host innate immune system, dsRNA formed during a LASV infectious cycle is digested by the LASV nucleoprotein. Only one other subfamily of mammalian RNA viruses is known to encode a protein with exonuclease activity: coronaviruses. For instance, severe acute respiratory syndrome coronavirus nsp14 is also a member of the DEDDh family and targets both ssRNA and dsRNA during proofreading, but nsp14 is not known to be directly involved in IFN suppression (128).

MeV infection is followed by severe immunosuppression, a process that involves many viral proteins. The MeV nucleoprotein plays a critical role in inhibiting antibody production by binding to host B cells, although the structural specifics of this interaction are still unclear (129, 130). Additionally, the MeV nucleoprotein binds to eukaryotic translation initiation factor 3 (eIF3) and inhibits host cell translation of host mRNA by upwards of 90% at 36 hours post infection (131, 132).

In many NSVs, nucleoprotein-P complexes can form cytoplasmic inclusion bodies (IBs), which serve as hubs for viral replication and shield the virus from detection by the host innate immune system. This tactic has been observed in many non-segmented NSVs including RABV, MeV, and EBOV (133–135). NiV is distinct from other non-segmented NSVs in that it forms two types of IBs, neither of which require the viral P protein (136). NiV can induce formation of perinuclear IBs with expression of NP alone as well as plasma membrane IBs when nucleoprotein is in complex with the viral M protein. This is the first observed instance of an NSV forming two IB populations for

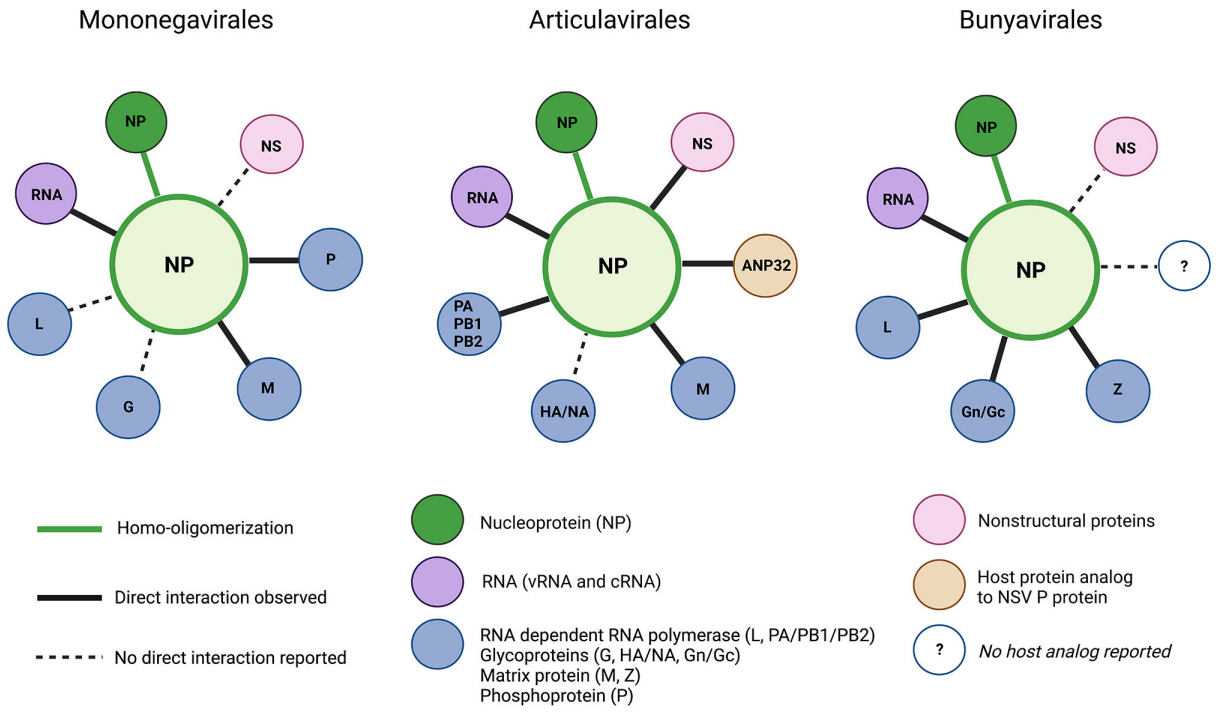

FIG 6 Key nucleoprotein interactions with other viral proteins by phylogenetic order. (Left) Mononegavirales NP interactions, (middle) articulavirales, (right) bunyavirales.

two separate functions. Interestingly, the RSV nucleoprotein can sequester immunostimulatory proteins MDA5 and MAVS into small cytoplasmic IBs (118). Contrary to the IBs discussed above, these are not viral replication hubs, but rather these IBs serve to hold the host immune system hostage while RSV takes over the cell. Alternative roles of NSV nucleoprotein-induced IBs are highlighted in the recent work in NiV and RSV, which suggests that NSV IBs may contribute to the viral life cycle in more ways than originally postulated.

There are likely many more viral-host interactions that are modulated by viral nucleoproteins, and the above discussion only represents the current understanding of the most well studied NSVs. A list of NSV nucleoprotein interactions with host proteins that was assessed for this review can be found in Table S3 (22, 25, 26, 30, 99, 113, 116, 118, 122, 130, 132, 137–143).

DISCUSSION AND OUTLOOK

NSV nucleoproteins are key structural and scaffolding components of NSV RNPs and critical to NSV infection. Recent advances in structural biology techniques and analysis procedures have made it possible to obtain new insight in NSV RNP structures and their dynamics during viral replication. The structural homology of the nucleoprotein core may offer an opportunity to connect the conserved and unique functions of nucleoproteins to the evolution of NSVs. In addition, NSV nucleoproteins are now considered possible antiviral targets and our understanding of how the function of nucleoproteins can be inhibited is better understood.

However, many key questions concerning nucleoprotein function and inhibition remain. In particular, it is unclear why NSVs that employ similar RNA synthesis mechanisms use nucleoproteins of a different size and with different RNA binding properties. It is also unclear why they form different RNP structures with different dynamics. At another level, it remains poorly understood how RNPs are assembled during viral RNA synthesis, whether differences exist between the RNP dynamics during NSV replication or transcription, and whether a dysregulation in RNP dynamics contributes to detection of viral RNA by the host innate immune response. Finally, the role of PTMs on nucleoproteins and RNP dynamics has only just begun to be explored and their exact function in the regulation of viral RNA synthesis, or how they may potentially perturb it, will require further attention in the future.

Overall, the studies discussed in this review highlight our current knowledge of the many mechanisms by which NSV nucleoproteins are active players in the NSV infection cycle. Further investigations into the role of nucleoproteins in lesser studied NSVs will undoubtedly uncover even more fascinating and diverse aspects. Studying the complex functions of viral nucleoproteins offers the benefits of finding novel therapeutic targets while also fortifying our understanding of viral evolution as it pertains to structural conservation.

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AUTHOR AFFILIATIONS

[1]Lewis Thomas Laboratory, Department of Molecular Biology, Princeton University, Princeton, New Jersey, USA

[2]Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey, USA

AUTHOR ORCIDs

Kimberly R. Sabsay http://orcid.org/0000-0003-2204-2693
Aartjan J. W. te Velthuis http://orcid.org/0000-0002-5129-3953

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Kimberly R. Sabsay, Conceptualization, Investigation, Writing – original draft, Writing – review and editing | Aartjan J. W. te Velthuis, Project administration, Resources, Supervision, Writing – review and editing

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Table S1: NSV Taxonomy and structure accession data (MMBR00082-23-s0001.pdf). Supplementary Table 1: NSV Taxonomy and structure accession data.
Table S2: NSV NP interactions with other viral proteins (MMBR00082-23-s0002.pdf). Supplementary Table 2: NSV NP interactions with other viral proteins.
Table S3: NSV NP interactions with host proteins (MMBR00082-23-s0003.pdf). Supplementary Table 3: NSV NP interactions with host proteins.

REFERENCES

1. Te Velthuis AJW, Grimes JM, Fodor E. 2021. Publisher correction: structural insights into RNA polymerases of negative-sense RNA viruses. Nat Rev Microbiol 19:220. https://doi.org/10.1038/s41579-021-00524-9
2. Ouizougun-Oubari M, Fearns R. 2023. Structures and mechanisms of nonsegmented, negative-strand RNA virus polymerases. Annu Rev Virol 10. https://doi.org/10.1146/annurev-virology-111821-102603
3. Ruigrok RWH, Crépin T, Kolakofsky D. 2011. Nucleoproteins and nucleocapsids of negative-strand RNA viruses. Curr Opin Microbiol 14:504–510. https://doi.org/10.1016/j.mib.2011.07.011
4. Zhu Z, Fodor E, Keown JR. 2023. A structural understanding of influenza virus genome replication. Trends Microbiol 31:308–319. https://doi.org/10.1016/j.tim.2022.09.015
5. Fodor E, Te Velthuis AJW. 2020. Structure and function of the influenza virus transcription and replication machinery. Cold Spring Harb Perspect Med 10:a038398. https://doi.org/10.1101/cshperspect.a038398
6. Strauss JH, Strauss EG. 2008. Minus-strand RNA viruses. Viruses Hum Dis:137–191. https://doi.org/10.1016/B978-0-12-373741-0.50007-6
7. Nguyen M, Haenni A-L. 2003. Expression strategies of ambisense viruses. Virus Res 93:141–150. https://doi.org/10.1016/s0168-1702(03)00094-7
8. Šantak M, Matić Z. 2022. The role of nucleoprotein in immunity to human negative-stranded RNA viruses—not just another brick in the viral nucleocapsid. Viruses 14:521. https://doi.org/10.3390/v14030521
9. Kirchdoerfer RN, Abelson DM, Li S, Wood MR, Saphire EO. 2015. Assembly of the ebola virus nucleoprotein from a chaperoned VP35 complex. Cell Rep 12:140–149. https://doi.org/10.1016/j.celrep.2015.06.003
10. Tarus B, Bakowiez O, Chenavas S, Duchemin L, Estrozi LF, Bourdieu C, Lejal N, Bernard J, Moudjou M, Chevalier C, Delmas B, Ruigrok RWH, Di Primo C, Slama-Schwok A. 2012. Oligomerization paths of the nucleoprotein of influenza A virus. Biochimie 94:776–785. https://doi.org/10.1016/j.biochi.2011.11.009
11. Guseva S, Milles S, Blackledge M, Ruigrok RWH. 2019. The nucleoprotein and phosphoprotein of measles virus. Front Microbiol 10:1832. https://doi.org/10.3389/fmicb.2019.01832
12. Gonnin L, Richard C-A, Gutsche I, Chevret D, Troussier J, Vasseur J-J, Debart F, Eléouët J-F, Galloux M. 2022. Importance of RNA length for *in vitro* encapsidation by the nucleoprotein of human respiratory syncytial virus. J Biol Chem 298:102337. https://doi.org/10.1016/j.jbc.2022.102337
13. Dadonaite B, Gilbertson B, Knight ML, Trifkovic S, Rockman S, Laederach A, Brown LE, Fodor E, Bauer DLV. 2019. The structure of the

influenza A virus genome. Nat Microbiol 4:1781–1789. https://doi.org/10.1038/s41564-019-0513-7
14. Mirska B, Woźniak T, Lorent D, Ruszkowska A, Peterson JM, Moss WN, Mathews DH, Kierzek R, Kierzek E. 2023. *In vivo* secondary structural analysis of influenza A virus genomic RNA. Cell Mol Life Sci 80:136. https://doi.org/10.1007/s00018-023-04764-1
15. French H, Pitré E, Oade MS, Elshina E, Bisht K, King A, Bauer DLV, Te Velthuis AJW. 2022. Transient RNA structures cause aberrant influenza virus replication and innate immune activation. Sci Adv 8:eabp8655. https://doi.org/10.1126/sciadv.abp8655
16. Morin B, Liang B, Gardner E, Ross RA, Whelan SPJ. 2017. An *In vitro* RNA synthesis assay for rabies virus defines ribonucleoprotein interactions critical for polymerase activity. J Virol 91:e01508-16. https://doi.org/10.1128/JVI.01508-16
17. Te Velthuis AJW, Long JC, Bauer DLV, Fan RLY, Yen H-L, Sharps J, Siegers JY, Killip MJ, French H, Oliva-Martín MJ, Randall RE, de Wit E, van Riel D, Poon LLM, Fodor E. 2018. Mini viral RNAs act as innate immune agonists during influenza virus infection. Nat Microbiol 3:1234–1242. https://doi.org/10.1038/s41564-018-0240-5
18. Nilsson-Payant BE, Blanco-Melo D, Uhl S, Escudero-Pérez B, Olschewski S, Thibault P, Panis M, Rosenthal M, Muñoz-Fontela C, Lee B, tenOever BR, Schultz-Cherry S. 2021. Reduced nucleoprotein availability impairs negative-sense RNA virus replication and promotes host recognition. J Virol 95:e02274-20. https://doi.org/10.1128/JVI.02274-20
19. Rigby C, Sabsay K, Bisht K, Eggink D, Jalal H, Te Velthuis AJW. 2023. Evolution of transient RNA structure-RNA polymerase interactions in respiratory RNA virus genomes. bioRxiv:2023.05.25.542331. https://doi.org/10.1101/2023.05.25.542331
20. Longhi S, Bloyet L-M, Gianni S, Gerlier D. 2017. How order and disorder within paramyxoviral nucleoproteins and phosphoproteins orchestrate the molecular interplay of transcription and replication. Cell Mol Life Sci 74:3091–3118. https://doi.org/10.1007/s00018-017-2556-3
21. Dolnik O, Gerresheim GK, Biedenkopf N. 2021. New perspectives on the biogenesis of viral inclusion bodies in negative-sense RNA virus infections. Cells 10:1460. https://doi.org/10.3390/cells10061460
22. Wang F, Sheppard CM, Mistry B, Staller E, Barclay WS, Grimes JM, Fodor E, Fan H. 2022. The C-terminal LCAR of host ANP32 proteins interacts with the influenza A virus nucleoprotein to promote the replication of the viral RNA genome. Nucleic Acids Res 50:5713–5725. https://doi.org/10.1093/nar/gkac410
23. Pythoud C, Rodrigo WWSI, Pasqual G, Rothenberger S, Martínez-Sobrido L, de la Torre JC, Kunz S. 2012. Arenavirus nucleoprotein targets interferon regulatory factor-activating kinase IKKε. J Virol 86:7728–7738. https://doi.org/10.1128/JVI.00187-12
24. Zhu L, Jin J, Wang T, Hu Y, Liu H, Gao T, Dong Q, Jin Y, Li P, Liu Z, Liu X, Cao C. 2023. Ebola virus sequesters IRF3 in viral inclusion bodies to evade host antiviral immunity. Microbiology. https://doi.org/10.1101/2023.04.20.537734
25. Lerolle S, Freitas N, Cosset F-L, Legros V. 2021. Host cell restriction factors of bunyaviruses and viral countermeasures. Viruses 13:784. https://doi.org/10.3390/v13050784
26. Zhu X, Guan Z, Fang Y, Zhang Y, Guan Z, Li S, Peng K. 2023. Rift valley fever virus nucleoprotein triggers autophagy to dampen antiviral innate immune responses. J Virol 97:e0181422. https://doi.org/10.1128/jvi.01814-22
27. Green TJ, Cox R, Tsao J, Rowse M, Qiu S, Luo M. 2014. Common mechanism for RNA encapsidation by negative-strand RNA viruses. J Virol 88:3766–3775. https://doi.org/10.1128/JVI.03483-13
28. Luo M, Terrell JR, Mcmanus SA. 2020. Nucleocapsid structure of negative strand RNA virus. Viruses 12:835. https://doi.org/10.3390/v12080835
29. Chan W-H, Ng A-L, Robb NC, Lam M-H, Chan P-S, Au S-N, Wang J-H, Fodor E, Shaw P-C. 2010. Functional analysis of the influenza virus H5N1 nucleoprotein tail loop reveals amino acids that are crucial for oligomerization and ribonucleoprotein activities. J Virol 84:7337–7345. https://doi.org/10.1128/JVI.02474-09
30. Niu F, Shaw N, Wang YE, Jiao L, Ding W, Li X, Zhu P, Upur H, Ouyang S, Cheng G, Liu Z-J. 2013. Structure of the leanyer orthobunyavirus nucleoprotein-RNA complex reveals unique architecture for RNA encapsidation. Proc Natl Acad Sci U S A 110:9054–9059. https://doi.org/10.1073/pnas.1300035110
31. Song X, Shan H, Zhu Y, Hu S, Xue L, Chen Y, Ding W, Niu T, Gu J, Ouyang S, Shen Q-T, Liu Z-J. 2019. Self-capping of nucleoprotein filaments protects the newcastle disease virus genome. Elife 8:e45057. https://doi.org/10.7554/eLife.45057
32. Milles S, Jensen MR, Lazert C, Guseva S, Ivashchenko S, Communie G, Maurin D, Gerlier D, Ruigrok RWH, Blackledge M. 2018. An ultraweak interaction in the intrinsically disordered replication machinery is essential for measles virus function. Sci Adv 4:eaat7778. https://doi.org/10.1126/sciadv.aat7778
33. Warnes A, Fooks AR, Dowsett AB, Wilkinson GW, Stephenson JR. 1995. Expression of the measles virus nucleoprotein gene in *Escherichia coli* and assembly of nucleocapsid-like structures. Gene 160:173–178. https://doi.org/10.1016/0378-1119(95)00227-w
34. Sugita Y, Matsunami H, Kawaoka Y, Noda T, Wolf M. 2018. Cryo-EM structure of the ebola virus nucleoprotein-RNA complex at 3.6 Å resolution. Nature 563:137–140. https://doi.org/10.1038/s41586-018-0630-0
35. Gérard FCA, Bourhis J-M, Mas C, Branchard A, Vu DD, Varhoshkova S, Leyrat C, Jamin M. 2022. Structure and dynamics of the unassembled nucleoprotein of rabies virus in complex with its phosphoprotein chaperone module. Viruses 14:2813. https://doi.org/10.3390/v14122813
36. Green TJ, Rowse M, Tsao J, Kang J, Ge P, Zhou ZH, Luo M. 2011. Access to RNA encapsidated in the nucleocapsid of vesicular stomatitis virus. J Virol 85:2714–2722. https://doi.org/10.1128/JVI.01927-10
37. Gutsche I, Desfosses A, Effantin G, Ling WL, Haupt M, Ruigrok RWH, Sachse C, Schoehn G. 2015. Near-atomic cryo-EM structure of the helical measles virus nucleocapsid. Science 348:704–707. https://doi.org/10.1126/science.aaa5137
38. Tawar RG, Duquerroy S, Vonrhein C, Varela PF, Damier-Piolle L, Castagné N, MacLellan K, Bedouelle H, Bricogne G, Bhella D, Eléouët J-F, Rey FA. 2009. Crystal structure of a nucleocapsid-like nucleoprotein-RNA complex of respiratory syncytial virus. Science 326:1279–1283. https://doi.org/10.1126/science.1177634
39. Zinzula L, Beck F, Klumpe S, Bohn S, Pfeifer G, Bollschweiler D, Nagy I, Plitzko JM, Baumeister W. 2021. Cryo-EM structure of the cetacean morbillivirus nucleoprotein-RNA complex. J Struct Biol 213:107750. https://doi.org/10.1016/j.jsb.2021.107750
40. Arranz R, Coloma R, Chichón FJ, Conesa JJ, Carrascosa JL, Valpuesta JM, Ortín J, Martín-Benito J. 2012. The structure of native influenza virion ribonucleoproteins. Science 338:1634–1637. https://doi.org/10.1126/science.1228172
41. Hopkins FR, Álvarez-Rodríguez B, Heath GR, Panayi K, Hover S, Edwards TA, Barr JN, Fontana J. 2022. The native orthobunyavirus ribonucleoprotein possesses a helical architecture. mBio 13:e0140522. https://doi.org/10.1128/mbio.01405-22
42. Ferron F, Li Z, Danek EI, Luo D, Wong Y, Coutard B, Lantez V, Charrel R, Canard B, Walz T, Lescar J, Rey FA. 2011. The hexamer structure of the rift valley fever virus nucleoprotein suggests a mechanism for its assembly into ribonucleoprotein complexes. PLoS Pathog 7:e1002030. https://doi.org/10.1371/journal.ppat.1002030
43. Baklouti A, Goulet A, Lichière J, Canard B, Charrel RN, Ferron F, Coutard B, Papageorgiou N. 2017. *Toscana virus* nucleoprotein oligomer organization observed in solution. Acta Crystallogr D Struct Biol 73:650–659. https://doi.org/10.1107/S2059798317008774
44. Raymond DD, Piper ME, Gerrard SR, Skiniotis G, Smith JL. 2012. Phleboviruses encapsidate their genomes by sequestering RNA bases. Proc Natl Acad Sci U S A 109:19208–19213. https://doi.org/10.1073/pnas.1213553109
45. Reguera J, Malet H, Weber F, Cusack S. 2013. Structural basis for encapsidation of genomic RNA by la crosse orthobunyavirus nucleoprotein. Proc Natl Acad Sci U S A 110:7246–7251. https://doi.org/10.1073/pnas.1302298110
46. Komoda K, Narita M, Yamashita K, Tanaka I, Yao M. 2017. Asymmetric trimeric ring structure of the nucleocapsid protein of *Tospovirus*. J Virol 91:e01002-17. https://doi.org/10.1128/JVI.01002-17
47. Ariza A, Tanner SJ, Walter CT, Dent KC, Shepherd DA, Wu W, Matthews SV, Hiscox JA, Green TJ, Luo M, Elliott RM, Fooks AR, Ashcroft AE, Stonehouse NJ, Ranson NA, Barr JN, Edwards TA. 2013. Nucleocapsid protein structures from orthobunyaviruses reveal insight into

ribonucleoprotein architecture and RNA polymerization. Nucleic Acids Res 41:5912–5926. https://doi.org/10.1093/nar/gkt268
48. Arragain B, Reguera J, Desfosses A, Gutsche I, Schoehn G, Malet H. 2019. High resolution cryo-EM structure of the helical RNA-bound hantaan virus nucleocapsid reveals its assembly mechanisms. Elife 8:e43075. https://doi.org/10.7554/eLife.43075
49. Modrego A, Carlero D, Arranz R, Martín-Benito J. 2023. CryoEM of viral ribonucleoproteins and nucleocapsids of single-stranded RNA viruses. Viruses 15:653. https://doi.org/10.3390/v15030653
50. Kormelink R, Garcia ML, Goodin M, Sasaya T, Haenni A-L. 2011. Negative-strand RNA viruses: the plant-infecting counterparts. Virus Res 162:184–202. https://doi.org/10.1016/j.virusres.2011.09.028
51. Raymond DD, Piper ME, Gerrard SR, Smith JL. 2010. Structure of the rift valley fever virus nucleocapsid protein reveals another architecture for RNA encapsidation. Proc Natl Acad Sci U S A 107:11769–11774. https://doi.org/10.1073/pnas.1001760107
52. Wang X, Li B, Guo Y, Shen S, Zhao L, Zhang P, Sun Y, Sui S-F, Deng F, Lou Z. 2016. Molecular basis for the formation of ribonucleoprotein complex of crimean-congo hemorrhagic fever virus. Journal of Structural Biology 196:455–465. https://doi.org/10.1016/j.jsb.2016.09.013
53. García ML, Bó ED, da Graça JV, Gago-Zachert S, Hammond J, Moreno P, Natsuaki T, Pallás V, Navarro JA, Reyes CA, Luna GR, Sasaya T, Tzanetakis IE, Vaira AM, Verbeek M. 2017. ICTV virus Taxonomy profile: *Ophioviridae*. J Gen Virol 98:1161–1162. https://doi.org/10.1099/jgv.0.000836
54. Jennings PA, Finch JT, Winter G, Robertson JS. 1983. Does the higher order structure of the influenza virus ribonucleoprotein guide sequence rearrangements in influenza viral RNA? Cell 34:619–627. https://doi.org/10.1016/0092-8674(83)90394-x
55. MacLellan K, Loney C, Yeo RP, Bhella D. 2007. The 24-angstrom structure of respiratory syncytial virus nucleocapsid protein-RNA decameric rings. J Virol 81:9519–9524. https://doi.org/10.1128/JVI.00526-07
56. Barge A, Gaudin Y, Coulon P, Ruigrok RWH. 1993. Vesicular stomatitis virus M protein may be inside the ribonucleocapsid coil. J Virol 67:7246–7253. https://doi.org/10.1128/jvi.67.12.7246-7253.1993
57. Peng R, Zhu T, Oladejo BO, Musyoki AM, Cui Y, Shi Y, Wang P, Gao GF. 2016. *In vitro* assembly of ebola virus nucleocapsid-like complex expressed in *E. coli*. Protein Cell 7:888–898. https://doi.org/10.1007/s13238-016-0314-1
58. Liljeroos L, Huiskonen JT, Ora A, Susi P, Butcher SJ. 2011. Electron cryotomography of measles virus reveals how matrix protein coats the ribonucleocapsid within intact virions. Proc Natl Acad Sci U S A 108:18085–18090. https://doi.org/10.1073/pnas.1105770108
59. Riedel C, Vasishtan D, Pražák V, Ghanem A, Conzelmann K-K, Rümenapf T. 2019. Cryo EM structure of the rabies virus ribonucleoprotein complex. Sci Rep 9:9639. https://doi.org/10.1038/s41598-019-46126-7
60. Egelman EH, Wu SS, Amrein M, Portner A, Murti G. 1989. The sendai virus nucleocapsid exists in at least four different helical states. J Virol 63:2233–2243. https://doi.org/10.1128/JVI.63.5.2233-2243.1989
61. Turrell L, Hutchinson EC, Vreede FT, Fodor E. 2015. Regulation of influenza A virus nucleoprotein oligomerization by phosphorylation. J Virol 89:1452–1455. https://doi.org/10.1128/JVI.02332-14
62. Chenavas S, Estrozi LF, Slama-Schwok A, Delmas B, Di Primo C, Baudin F, Li X, Crépin T, Ruigrok RWH. 2013. Monomeric nucleoprotein of influenza A virus. PLoS Pathog 9:e1003275. https://doi.org/10.1371/journal.ppat.1003275
63. Lennartz F, Hoenen T, Lehmann M, Groseth A, Garten W. 2013. The role of oligomerization for the biological functions of the arenavirus nucleoprotein. Arch Virol 158:1895–1905. https://doi.org/10.1007/s00705-013-1684-9
64. Hastie KM, Kimberlin CR, Zandonatti MA, MacRae IJ, Saphire EO. 2011. Structure of the lassa virus nucleoprotein reveals a dsRNA-specific 3' to 5' exonuclease activity essential for immune suppression. Proc Natl Acad Sci U S A 108:2396–2401. https://doi.org/10.1073/pnas.1016404108
65. Pattis JG, May ER. 2020. Markov state model of lassa virus nucleoprotein reveals large structural changes during the trimer to monomer transition. Structure 28:548–554. https://doi.org/10.1016/j.str.2020.03.002
66. Yang J, Koprowski H, Dietzschold B, Fu ZF. 1999. Phosphorylation of rabies virus nucleoprotein regulates viral RNA transcription and replication by modulating leader RNA encapsidation. J Virol 73:1661–1664. https://doi.org/10.1128/JVI.73.2.1661-1664.1999
67. Decool H, Gonnin L, Gutsche I, Sizun C, Eléouët J-F, Galloux M. 2021. Interactions between the nucleoprotein and the phosphoprotein of pneumoviruses: structural insight for rational design of antivirals. Viruses 13:2449. https://doi.org/10.3390/v13122449
68. Leyrat C, Yabukarski F, Tarbouriech N, Ribeiro EA, Jensen MR, Blackledge M, Ruigrok RWH, Jamin M, Rey FA. 2011. Structure of the vesicular stomatitis virus N^0-P complex. PLoS Pathog 7:e1002248. https://doi.org/10.1371/journal.ppat.1002248
69. Yabukarski F, Lawrence P, Tarbouriech N, Bourhis J-M, Delaforge E, Jensen MR, Ruigrok RWH, Blackledge M, Volchkov V, Jamin M. 2014. Structure of nipah virus unassembled nucleoprotein in complex with its viral chaperone. Nat Struct Mol Biol 21:754–759. https://doi.org/10.1038/nsmb.2868
70. Staller E, Sheppard CM, Neasham PJ, Mistry B, Peacock TP, Goldhill DH, Long JS, Barclay WS. 2019. ANP32 proteins are essential for influenza virus replication in human cells. J Virol 93:e00217-19. https://doi.org/10.1128/JVI.00217-19
71. Carrique L, Fan H, Walker AP, Keown JR, Sharps J, Staller E, Barclay WS, Fodor E, Grimes JM. 2020. Host ANP32A mediates the assembly of the influenza virus replicase. 7835. Nature 587:638–643. https://doi.org/10.1038/s41586-020-2927-z
72. Rager M, Vongpunsawad S, Duprex WP, Cattaneo R. 2002. Polyploid measles virus with hexameric genome length. EMBO J 21:2364–2372. https://doi.org/10.1093/emboj/21.10.2364
73. Beniac DR, Melito PL, Devarennes 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:e29608. https://doi.org/10.1371/journal.pone.0029608
74. Wichgers Schreur PJ, Kortekaas J, Palese P. 2016. Single-molecule FISH reveals non-selective packaging of rift valley fever virus genome segments. PLoS Pathog 12:e1005800. https://doi.org/10.1371/journal.ppat.1005800
75. Zehr EA, Kraemer JA, Erb ML, Coker JKC, Montabana EA, Pogliano J, Agard DA. 2014. The structure and assembly mechanism of a novel three-stranded tubulin filament that centers phage DNA. Structure 22:539–548. https://doi.org/10.1016/j.str.2014.02.006
76. Albertini AAV, Wernimont AK, Muziol T, Ravelli RBG, Clapier CR, Schoehn G, Weissenhorn W, Ruigrok RWH. 2006. Crystal structure of the rabies virus nucleoprotein-RNA complex. Science 313:360–363. https://doi.org/10.1126/science.1125280
77. Green TJ, Zhang X, Wertz GW, Luo M. 2006. Structure of the vesicular stomatitis virus nucleoprotein-RNA complex. Science 313:357–360. https://doi.org/10.1126/science.1126953
78. Ortega J, Martín-Benito J, Zürcher T, Valpuesta JM, Carrascosa JL, Ortín J. 2000. Ultrastructural and functional analyses of recombinant influenza virus ribonucleoproteins suggest dimerization of nucleoprotein during virus amplification. J Virol 74:156–163. https://doi.org/10.1128/JVI.74.1.156-163.2000
79. Tang Y-S, Xu S, Chen Y-W, Wang J-H, Shaw P-C. 2021. Crystal structures of influenza nucleoprotein complexed with nucleic acid provide insights into the mechanism of RNA interaction. Nucleic Acids Res 49:4144–4154. https://doi.org/10.1093/nar/gkab203
80. Tarus B, Chevalier C, Richard C-A, Delmas B, Di Primo C, Slama-Schwok A, Digard P. 2012. Molecular dynamics studies of the nucleoprotein of influenza A virus: role of the protein flexibility in RNA binding. PLoS ONE 7:e30038. https://doi.org/10.1371/journal.pone.0030038
81. Jenni S, Horwitz JA, Bloyet L-M, Whelan SPJ, Harrison SC. 2022. Visualizing molecular interactions that determine assembly of a bullet-shaped vesicular stomatitis virus particle. Nat Commun 13:4802. https://doi.org/10.1038/s41467-022-32223-1
82. Noda T, Hagiwara K, Sagara H, Kawaoka Y. 2010. Characterization of the Ebola virus nucleoprotein-RNA complex. J Gen Virol 91:1478–1483. https://doi.org/10.1099/vir.0.019794-0
83. Klumpp K, Ruigrok RWH, Baudin F. 1997. Roles of the influenza virus polymerase and nucleoprotein in forming a functional RNP structure. EMBO J 16:1248–1257. https://doi.org/10.1093/emboj/16.6.1248

84. Labaronne A, Swale C, Monod A, Schoehn G, Crépin T, Ruigrok RWH. 2016. Binding of RNA by the nucleoproteins of influenza viruses A and B. Viruses 8:247. https://doi.org/10.3390/v8090247
85. Helder S, Blythe AJ, Bond CS, Mackay JP. 2016. Determinants of affinity and specificity in RNA-binding proteins. Curr Opin Struct Biol 38:83–91. https://doi.org/10.1016/j.sbi.2016.05.005
86. Kolakofsky D, Pelet T, Garcin D, Hausmann S, Curran J, Roux L. 1998. Paramyxovirus RNA synthesis and the requirement for hexamer genome length: the rule of six revisited. J Virol 72:891–899. https://doi.org/10.1128/JVI.72.2.891-899.1998
87. Vulliémoz D, Roux L. 2001. Rule of six": how does the sendai virus RNA polymerase keep count J Virol 75:4506–4518. https://doi.org/10.1128/JVI.75.10.4506-4518.2001
88. Bhella D, Ralph A, Murphy LB, Yeo RP. 2002. Significant differences in nucleocapsid morphology within the *Paramyxoviridae*. J Gen Virol 83:1831–1839. https://doi.org/10.1099/0022-1317-83-8-1831
89. le Mercier P, Kolakofsky D. 2019. Bipartite promoters and RNA editing of paramyxoviruses and filoviruses. RNA 25:279–285. https://doi.org/10.1261/rna.068825.118
90. Coloma R, Arranz R, de la Rosa-Trevín JM, Sorzano COS, Munier S, Carlero D, Naffakh N, Ortín J, Martín-Benito J. 2020. Structural insights into influenza A virus ribonucleoproteins reveal a processive helical track as transcription mechanism. Nat Microbiol 5:727–734. https://doi.org/10.1038/s41564-020-0675-3
91. Wandzik JM, Kouba T, Karuppasamy M, Pflug A, Drncova P, Provaznik J, Azevedo N, Cusack S. 2020. A structure-based model for the complete transcription cycle of influenza polymerase. Cell 181:877–893. https://doi.org/10.1016/j.cell.2020.03.061
92. Te Velthuis AJW, Fodor E. 2016. Influenza virus RNA polymerase: insights into the mechanisms of viral RNA synthesis. Nat Rev Microbiol 14:479–493. https://doi.org/10.1038/nrmicro.2016.87
93. Malet H, Williams HM, Cusack S, Rosenthal M. 2023. The mechanism of genome replication and transcription in bunyaviruses. PLoS Pathog 19:e1011060. https://doi.org/10.1371/journal.ppat.1011060
94. Te Velthuis AJW, Oymans J. 2018. Initiation, elongation, and realignment during influenza virus mRNA synthesis. J Virol 92:e01775-17. https://doi.org/10.1128/JVI.01775-17
95. Albertini AAV, Ruigrok RWH, Blondel D. 2011. Rabies virus transcription and replication. Adv Virus Res 79:1–22. https://doi.org/10.1016/B978-0-12-387040-7.00001-9
96. Lin AE, Diehl WE, Cai Y, Finch CL, Akusobi C, Kirchdoerfer RN, Bollinger L, Schaffner SF, Brown EA, Saphire EO, Andersen KG, Kuhn JH, Luban J, Sabeti PC. 2020. Reporter assays for ebola virus nucleoprotein oligomerization, virion-like particle budding and minigenome activity reveal the importance of nucleoprotein amino acid position. Viruses 12:105. https://doi.org/10.3390/v12010105
97. Diefenbacher M, Tan TJC, Bauer DLV, Stadtmueller BM, Wu NC, Brooke CB, Lowen AC. 2022. Interactions between influenza A virus nucleoprotein and gene segment untranslated regions facilitate selective modulation of viral gene expression. J Virol 96:e0020522. https://doi.org/10.1128/jvi.00205-22
98. Matsumoto Y, Ohta K, Kolakofsky D, Nishio M. 2017. A point mutation in the RNA-binding domain of human parainfluenza virus type 2 nucleoprotein elicits abnormally enhanced polymerase activity. J Virol 91:e02203-16. https://doi.org/10.1128/JVI.02203-16
99. Lin Y-C, Jeng K-S, Lai MMC, Goff SP, Jung J, Kawaoka Y. 2017. CNOT4-mediated ubiquitination of influenza A virus nucleoprotein promotes viral RNA replication. mBio 8:e00597-17. https://doi.org/10.1128/mBio.00597-17
100. Pinschewer DD, Perez M, de la Torre JC. 2003. Role of the virus nucleoprotein in the regulation of lymphocytic choriomeningitis virus transcription and RNA replication. J Virol 77:3882–3887. https://doi.org/10.1128/jvi.77.6.3882-3887.2003
101. Vidal S, Kolakofsky D. 1989. Modified model for the switch from sendai virus transcription to replication. J Virol 63:1951–1958. https://doi.org/10.1128/JVI.63.5.1951-1958.1989
102. Vreede FT, Ng A-L, Shaw P-C, Fodor E. 2011. Stabilization of influenza virus replication intermediates is dependent on the RNA-binding but not the homo-oligomerization activity of the viral nucleoprotein. J Virol 85:12073–12078. https://doi.org/10.1128/JVI.00695-11
103. Vreede FT, Jung TE, Brownlee GG. 2004. Model suggesting that replication of influenza virus is regulated by stabilization of replicative intermediates. J Virol 78:9568–9572. https://doi.org/10.1128/JVI.78.17.9568-9572.2004
104. Zhang G, Zhong Y, Qin Y, Chen M, Schultz-Cherry S. 2015. Interaction of human parainfluenza virus type 3 nucleoprotein with matrix protein mediates internal viral protein assembly. J Virol 90:2306–2315. https://doi.org/10.1128/JVI.02324-15
105. Licata JM, Johnson RF, Han Z, Harty RN. 2004. Contribution of ebola virus glycoprotein, nucleoprotein, and VP24 to budding of VP40 virus-like particles. J Virol 78:7344–7351. https://doi.org/10.1128/JVI.78.14.7344-7351.2004
106. Kiss G, Holl JM, Williams GM, Alonas E, Vanover D, Lifland AW, Gudheti M, Guerrero-Ferreira RC, Nair V, Yi H, Graham BS, Santangelo PJ, Wright ER. 2014. Structural analysis of respiratory syncytial virus reveals the position of M2-1 between the matrix protein and the ribonucleoprotein complex. J Virol 88:7602–7617. https://doi.org/10.1128/JVI.00256-14
107. Chase G, Mayer D, Hildebrand A, Frank R, Hayashi Y, Tomonaga K, Schwemmle M. 2007. Borna disease virus matrix protein is an integral component of the viral ribonucleoprotein complex that does not interfere with polymerase activity. J Virol 81:743–749. https://doi.org/10.1128/JVI.01351-06
108. Dou D, Revol R, Östbye H, Wang H, Daniels R. 2018. Influenza A virus cell entry, replication virion assembly and movement. Front Immunol 9:1581. https://doi.org/10.3389/fimmu.2018.01581
109. Wu WWH, Sun Y-H, Panté N. 2007. Nuclear import of influenza A viral ribonucleoprotein complexes is mediated by two nuclear localization sequences on viral nucleoprotein. Virol J 4:49. https://doi.org/10.1186/1743-422X-4-49
110. Paterson D, Fodor E. 2012. Emerging roles for the influenza A virus nuclear export protein (NEP). PLoS Pathog 8:e1003019. https://doi.org/10.1371/journal.ppat.1003019
111. Kochs G, García-Sastre A, Martínez-Sobrido L. 2007. Multiple anti-interferon actions of the influenza A virus NS1 protein. J Virol 81:7011–7021. https://doi.org/10.1128/JVI.02581-06
112. Esparza M, Bhat P, Fontoura BM. 2022. Viral–host interactions during splicing and nuclear export of influenza virus mRNAs. Curr Opin Virol 55:101254. https://doi.org/10.1016/j.coviro.2022.101254
113. Elton D, Simpson-Holley M, Archer K, Medcalf L, Hallam R, McCauley J, Digard P. 2001. Interaction of the influenza virus nucleoprotein with the cellular CRM1-mediated nuclear export pathway. J Virol 75:408–419. https://doi.org/10.1128/JVI.75.1.408-419.2001
114. Cao S, Liu X, Yu M, Li J, Jia X, Bi Y, Sun L, Gao GF, Liu W. 2012. A nuclear export signal in the matrix protein of influenza A virus is required for efficient virus replication. J Virol 86:4883–4891. https://doi.org/10.1128/JVI.06586-11
115. Jiang Y, Qin Y, Chen M. 2016. Host–pathogen interactions in measles virus replication and anti-viral immunity. Viruses 8:308. https://doi.org/10.3390/v8110308
116. Jain S, Martynova E, Rizvanov A, Khaiboullina S, Baranwal M. 2021. Structural and functional aspects of ebola virus proteins. Pathogens 10:1330. https://doi.org/10.3390/pathogens10101330
117. Masatani T, Ito N, Shimizu K, Ito Y, Nakagawa K, Sawaki Y, Koyama H, Sugiyama M. 2010. Rabies virus nucleoprotein functions to evade activation of the RIG-I-mediated antiviral response. J Virol 84:4002–4012. https://doi.org/10.1128/JVI.02220-09
118. Van Royen T, Rossey I, Sedeyn K, Schepens B, Saelens X. 2022. How RSV proteins join forces to overcome the host innate immune response. Viruses 14:419. https://doi.org/10.3390/v14020419
119. Biswas SK, Boutz PL, Nayak DP. 1998. Influenza virus nucleoprotein interacts with influenza virus polymerase proteins. J Virol 72:5493–5501. https://doi.org/10.1128/JVI.72.7.5493-5501.1998
120. Hu Y, Sneyd H, Dekant R, Wang J. 2017. Influenza A virus nucleoprotein: a highly conserved multi-functional viral protein as a hot antiviral drug target. Curr Top Med Chem 17:2271–2285. https://doi.org/10.2174/1568026617666170224122508
121. Kaukinen P, Vaheri A, Plyusnin A. 2005. Hantavirus nucleocapsid protein: a multifunctional molecule with both housekeeping and ambassadorial duties. Arch Virol 150:1693–1713. https://doi.org/10.1007/s00705-005-0555-4
122. Liu L, Celma CC, Roy P. 2008. Rift valley fever virus structural proteins: expression, characterization and assembly of recombinant proteins. Virol J 5:82. https://doi.org/10.1186/1743-422X-5-82

123. Snippe M, Willem Borst J, Goldbach R, Kormelink R. 2007. Tomato spotted wilt virus GC and N proteins interact *in vivo*. Virology 357:115–123. https://doi.org/10.1016/j.virol.2006.06.037
124. Neuman BW, Adair BD, Burns JW, Milligan RA, Buchmeier MJ, Yeager M. 2005. Complementarity in the supramolecular design of arenaviruses and retroviruses revealed by electron cryomicroscopy and image analysis. J Virol 79:3822–3830. https://doi.org/10.1128/JVI.79.6.3822-3830.2005
125. Rehwinkel J, Gack MU. 2020. RIG-I-like receptors: their regulation and roles in RNA sensing. Nat Rev Immunol 20:537–551. https://doi.org/10.1038/s41577-020-0288-3
126. Kok K-H, Lui P-Y, Ng M-H, Siu K-L, Au SWN, Jin D-Y. 2011. The double-stranded RNA-binding protein PACT functions as a cellular activator of RIG-I to facilitate innate antiviral response. Cell Host Microbe 9:299–309. https://doi.org/10.1016/j.chom.2011.03.007
127. Shao J, Huang Q, Liu X, Di D, Liang Y, Ly H, Dermody TS. 2018. Arenaviral nucleoproteins suppress PACT-induced augmentation of RIG-I function to inhibit type I interferon production. J Virol 92:e00482-18. https://doi.org/10.1128/JVI.00482-18
128. Tahir M. 2021. Coronavirus genomic Nsp14-ExoN, structure, role, mechanism, and potential application as a drug target. J Med Virol 93:4258–4264. https://doi.org/10.1002/jmv.27009
129. Ravanel K, Castelle C, Defrance T, Wild TF, Charron D, Lotteau V, Rabourdin-Combe C. 1997. Measles virus nucleocapsid protein binds to FcγRII and inhibits human B cell antibody production. J Exp Med 186:269–278. https://doi.org/10.1084/jem.186.2.269
130. Watanabe A, Yoneda M, Ikeda F, Terao-Muto Y, Sato H, Kai C. 2010. CD147/EMMPRIN acts as a functional entry receptor for measles virus on epithelial cells. J Virol 84:4183–4193. https://doi.org/10.1128/JVI.02168-09
131. tenOever BR, Servant MJ, Grandvaux N, Lin R, Hiscott J. 2002. Recognition of the measles virus nucleocapsid as a mechanism of IRF-3 activation. J Virol 76:3659–3669. https://doi.org/10.1128/JVI.76.8.3659-3669.2002
132. Sato H, Masuda M, Kanai M, Tsukiyama-Kohara K, Yoneda M, Kai C. 2007. Measles virus N protein inhibits host translation by binding to eIF3-p40. J Virol 81:11569–11576. https://doi.org/10.1128/JVI.00570-07
133. Lahaye X, Vidy A, Pomier C, Obiang L, Harper F, Gaudin Y, Blondel D. 2009. Functional characterization of negri bodies (NBS) in rabies virus-infected cells: evidence that NBS are sites of viral transcription and replication. J Virol 83:7948–7958. https://doi.org/10.1128/JVI.00554-09
134. Zhou Y, Su JM, Samuel CE, Ma D, Dutch RE. 2019. Measles virus forms inclusion bodies with properties of liquid organelles. J Virol 93:e00948-19. https://doi.org/10.1128/JVI.00948-19
135. Miyake T, Farley CM, Neubauer BE, Beddow TP, Hoenen T, Engel DA, Heise MT. 2020. Ebola virus inclusion body formation and RNA synthesis are controlled by a novel domain of nucleoprotein interacting with VP35. J Virol 94:e02100-19. https://doi.org/10.1128/JVI.02100-19
136. Ringel M, Heiner A, Behner L, Halwe S, Sauerhering L, Becker N, Dietzel E, Sawatsky B, Kolesnikova L, Maisner A. 2019. Nipah virus induces two inclusion body populations: identification of novel inclusions at the plasma membrane. PLoS Pathog 15:e1007733. https://doi.org/10.1371/journal.ppat.1007733
137. Dou D, Hernández-Neuta I, Wang H, Östbye H, Qian X, Thiele S, Resa-Infante P, Kouassi NM, Sender V, Hentrich K, Mellroth P, Henriques-Normark B, Gabriel G, Nilsson M, Daniels R. 2017. Analysis of IAV replication and co-infection dynamics by a versatile RNA viral genome labeling method. Cell Rep 20:251–263. https://doi.org/10.1016/j.celrep.2017.06.021
138. Digard P, Elton D, Bishop K, Medcalf E, Weeds A, Pope B. 1999. Modulation of nuclear localization of the influenza virus nucleoprotein through interaction with actin filaments. J Virol 73:2222–2231. https://doi.org/10.1128/JVI.73.3.2222-2231.1999
139. Eisfeld AJ, Neumann G, Kawaoka Y. 2015. At the centre: influenza A virus ribonucleoproteins. Nat Rev Microbiol 13:28–41. https://doi.org/10.1038/nrmicro3367
140. Zhang X, Glendening C, Linke H, Parks CL, Brooks C, Udem SA, Oglesbee M. 2002. Identification and characterization of a regulatory domain on the carboxyl terminus of the measles virus nucleocapsid protein. J Virol 76:8737–8746. https://doi.org/10.1128/JVI.76.17.8737-8746.2002
141. Brasier AR, Spratt H, Wu Z, Boldogh I, Zhang Y, Garofalo RP, Casola A, Pashmi J, Haag A, Luxon B, Kurosky A. 2004. Nuclear heat shock response and novel nuclear domain 10 reorganization in respiratory syncytial virus-infected A549 cells identified by high-resolution two-dimensional GEL electrophoresis. J Virol 78:11461–11476. https://doi.org/10.1128/JVI.78.21.11461-11476.2004
142. Oliveira AP, Simabuco FM, Tamura RE, Guerrero MC, Ribeiro PGG, Libermann TA, Zerbini LF, Ventura AM. 2013. Human respiratory syncytial virus N, P and M protein interactions in HEK-293T cells. Virus Res 177:108–112. https://doi.org/10.1016/j.virusres.2013.07.010
143. Patil G, Xu L, Wu Y, Song K, Hao W, Hua F, Wang L, Li S. 2020. TRIM41-mediated ubiquitination of nucleoprotein limits vesicular stomatitis virus infection. Viruses 12:131. https://doi.org/10.3390/v12020131