

Genome encapsidation by orthobunyavirus nucleoproteins

Wenjie Zheng and Yizhi Jane Tao¹

Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77005

All negative-sense, single-stranded RNA (–ssRNA) viruses encode a nucleoprotein (NP), the major function of which is to bind viral RNAs and encapsidate them as ribonucleoprotein complexes (RNPs) (1, 2). Consequently, the genomes of –ssRNA viruses do not exist as naked RNA but rather as protein–RNA complexes with high-order structures. In PNAS, Li et al. (3), Niu et al. (4), and Reguera et al. (5) report the crystal structures of NP–RNA complexes from three orthobunyaviruses, namely the Bunyawera virus (BUNV), Leanyer virus (LEAV), and La Crosse virus (LACV). Meanwhile, the apo-NP structure from another orthobunyavirus, the Schmallenberg virus (SBV), has been reported by Dong et al. (6). The structures of the BUNV NP–RNA complex and SBV apo-NP have also been reported by Ariza et al. (7). Altogether, these findings make a significant impact on our understanding of *Orthobunyavirus* genome encapsidation and condensation.

Bunyaviridae is a large family of viruses encompassing more than 350 species that are organized into five genera, including *Orthobunyavirus*, *Hantavirus*, *Nairovirus*, *Phlebovirus*, and *Tospovirus* (8, 9). *Orthobunyavirus*, which is the focus of study by these five research teams listed above, includes more than 170 species and constitutes the largest genus within the family (9). Except for *Tospovirus*, members from the other four bunyavirus genera infect animals, and many of them are considered as serious human pathogens (e.g., the Crimean-Congo hemorrhagic fever virus, La Crosse virus, Rift valley fever virus, and Sin Nombre virus) (8, 9). For instance, the Yosemite hantavirus outbreak last summer caused ten confirmed infections with three deaths (10).

Bunyaviruses are enveloped viruses that are generally spherical in shape, with a diameter of 80–120 nm (8) (Fig. 1). Their genome consists of three –ssRNA segments that encode four structural proteins (i.e., L,

Gn, Gc, and N) and in some viruses also two nonstructural proteins (i.e., NSm and NSs) (8). Like in other –ssRNA viruses, the genomic RNAs of bunyaviruses are extensively coated by NP and associated with the viral RNA polymerase or L protein to form RNPs, which are responsible for viral RNA synthesis as well as viral genome packaging (1, 8, 9). Electron microscopy (EM) images show that bunyavirus RNPs form closed circular structures that are often loosely coiled without apparent symmetry (3, 4, 8).

These five recent research papers (3–7) reveal that *Orthobunyavirus* NPs adopt a unique structural fold not previously observed in NPs from other –ssRNA viruses or even those from other genera within the same *Bunyaviridae* family (1). Each *Orthobunyavirus* NP folds into a compact structure that can be divided into four parts: the N-arm, N-terminal domain, C-terminal domain, and C-arm. The N-/C-terminal domains, formed by the N-/C-terminal halves of the polypeptide chain, comprise the NP structure core (3–7). The N-/C-arms are short sequences from the N/C termini, and they play important roles in mediating NP–NP interaction. In between the N- and C-terminal domains lies a positively charged RNA-binding groove. Although the core structure of NP is rigid, the N-/C-arms can adopt different conformations due to flexible hinge connections.

The *Orthobunyavirus* NP–RNA complex structures by Li et al. (3), Niu et al. (4), Reguera et al. (5), and Ariza et al. (7) provide valuable insights into the mechanism of NP-mediated viral genome encapsidation. The bound RNAs in these complexes were either derived from the *Escherichia coli* expression host or reconstituted using synthetic oligos. All three *Orthobunyavirus* NP–RNA complexes were crystallized as tetramers, in which each NP interacts with two other NP molecules in a head-to-tail fashion through the N-/C-arms. The positive-charged grooves from neighboring protomers line

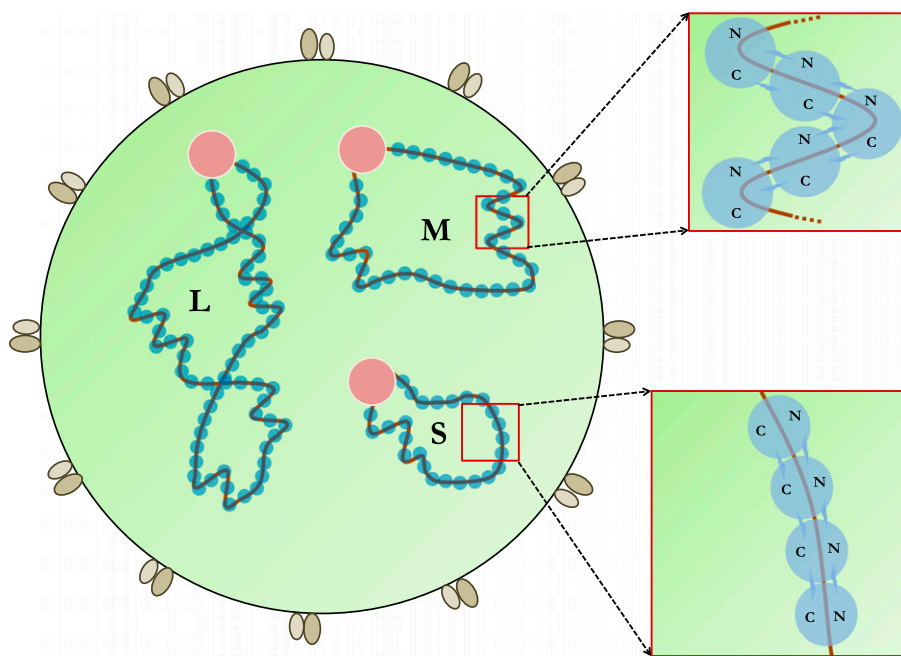


Fig. 1. Schematic drawing of a bunyavirus. The three gene segments are designated as L, M, and S. The two glycoproteins Gn and Gc are associated with the viral envelope. Each RNP is associated with a viral polymerase (pink) and multiple copies of NP (blue). The two enlarged areas highlight a linear and a helical NP–RNA structure, respectively.

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¹To whom correspondence should be addressed. E-mail: ytao@rice.edu.

up sequentially, thereby forming a continuous RNA-binding channel along the inner perimeter of the NP tetrameric ring. Each NP subunit binds 11 nucleotides of RNA according to Niu et al., Reguera et al., and Ariza et al. (4, 5, 7). Bound RNA is largely inaccessible, consistent with the observation that *Orthobunyavirus* NP–RNA complexes are generally resistant to RNase treatment (3–5, 7).

The apo-NP structures by Ariza et al., Dong et al., and Reguera et al. show few structural differences compared with the RNA-bound form in terms of the NP core (5–7). This observation indicates that local structural rearrangement should be sufficient to release the RNA template from RNP during viral RNA synthesis. Furthermore, the N-/C-arms of apo-NP were found to make contacts that are sometimes not the same as in the NP–RNA complexes. NP regions other than the N-/C-arms were also found at the NP–NP interface in an SBV apo-NP structure (6). It is proposed that the different NP–NP contacts observed in apo-NP may reflect structural changes in RNP upon RNA dissociation induced by the viral polymerase (6).

Concrete RNP models have been proposed by Ariza et al., Li et al., Niu et al., and Reguera et al. to explain various RNP structures observed in EM (3–5, 7). In particular, the flexible N-/C-arms of NP should allow *Orthobunyavirus* RNPs to form both linear, 4-nm-wide NP–RNA polymers as well as helical, 10-nm-wide filaments (3–5, 7). Further supercoiling of circularized RNPs would result in highly compact, rod-shaped structures. These three RNP structures condense the associated RNA to different extents, which may be required at different stages of the virus replication cycle. For instance, highly condensed RNPs may facilitate viral RNA packaging into infectious particles whereas extended RNPs may be required for viral RNA synthesis.

Studies by these five research teams (3–7) and others (1) indicate that NP proteins from –ssRNA viruses with segmented genomes exhibit substantial differences in size, sequence, structure, and even function. Considering that NP proteins encoded by bunyaviruses from different genera have non-homologous sequences (8), it is not unexpected that *Orthobunyavirus* NP possesses a

different structural fold than the NPs of Rift Valley fever virus (RVFV, genus *Phlebovirus*) (11–13) and Crimean-Congo hemorrhagic fever virus (CCHFV, genus *Nairovirus*) (14–16). RVFV NP is similar in size to *Orthobunyavirus* NP, but CCHFV NP is much larger with an additional endonuclease activity (14).

NP proteins from –ssRNA viruses with segmented genomes exhibit substantial differences in size, sequence, structure, and even function.

CCHFV NP is structurally related to the NP of Lassa fever virus (LASV), a member of the *Arenaviridae* family with a bisegmented –ssRNA genome (15, 17). LASV NP is associated with multiple activities, including RNA binding, cap snatching, and an exonuclease activity related to immunosuppression (17–19). Lastly, the structure of the influenza virus NP is different from any of

these NPs described above but is conserved within the *Orthomyxoviridae* family (1, 20–22). By comparison, NPs from –ssRNA viruses with nonsegmented genomes are usually related in both structure and function (1).

Now, with the *Orthobunyavirus* RNP structure established, many questions regarding RNP assembly and function start to emerge. For example, how does *Orthobunyavirus* keep its NP in a nonaggregated form that is competent for RNP encapsidation? Does *Orthobunyavirus* selectively package its genomic RNA, and, if so, what mechanism is used to ensure specific packaging of the three RNA segments? Furthermore, how does *Orthobunyavirus* RNP change its structure during RNA synthesis to allow the readout of nucleotide sequences? Such studies are crucial for our understanding of *Orthobunyavirus* biology, and the results will likely lead to better prevention and treatment of *Orthobunyavirus* infection.

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