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Insights into the structure of RNPs from segmented negative-sense RNA viruses

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Summary

The genome of segmented negative-sense single-stranded RNA viruses, like influenza virus and bunyaviruses, is coated by viral nucleoproteins (NP), forming a ribonucleoprotein (RNP). In this issue of Structure, Dick et al.[1] expand our knowledge on the RNPs of these viruses by solving the structures of Thogoto virus NP and RNP.

The group of negative-sense segmented RNA viruses includes several members that cause significant disease in plants, humans and animals, with perhaps the most prominent groups being the influenza viruses and the bunyaviruses. A common feature of these viruses is that their segmented genomes are never naked, likely an adaptation to prevent activation of the host immune response. Instead, their RNA segments are always coated by the viral nucleoprotein (NP), forming a ribonucleoprotein (RNP), which is also associated with the viral RNA dependant RNA polymerase (RdRp). RNP functionality is critical to many fundamental viral processes including transcription, replication and assembly (reviewed in[2,3]) and thus RNPs represent appealing targets for antivirals. As a consequence, a significant effort is focused on the characterisation of their structure/function relationship.

Structural information of negative-sense segmented RNPs is limited due to the difficulties of recombinantly generating functional RNPs from the two protein constituents, namely NP and RdRp. Most previous work has focussed on understanding the structure/function relationship of these two RNP components in isolation, with crystal structures of recombinantly-expressed NP in both monomeric or multimeric forms, with and without bound RNA, being solved for many species within both influenza A virus (IAV) and bunyavirus groups (e.g.[4]). More recently, X-ray crystallography and cryo-electron microscopy (cryo-EM) derived structures of the corresponding but considerably more complex RdRps have been solved (reviewed in[5,6]), revealing the polymerase to be a dynamic molecular machine for which transitions between several conformations are required for its multiple activities.

To characterise the structure/function of assembled RNPs, the majority of information has been gained using native RNPs purified from infectious virions and characterising their structure by cryo-EM. However, the heterogeneity and pleiomorphicity of the RNPs represents a challenge when aiming for high-resolution structures. So far this issue has been dealt with computationally, by discarding a large proportion of the available RNP particles to focus on those most straight and homogeneous. This has resulted in medium-resolution averages of RNPs from IAV (e.g.[7,8]) and of the prototypical Bunyamwera bunyavirus, for

which the RNPs are considerably more flexible[9]. This has demonstrated the striking difference between RNPs of these two virus groups, with influenza viruses exhibiting a rod-like double-helical arrangement and bunyavirus RNPs a more flexible loop-like structure.

In this issue of Structure, Dick *et al.*[1] expand our understanding of segmented negative-sense RNA virus RNPs by characterising Thogoto virus (THOV) NP and RNPs. After recombinantly expressing THOV NP and solving its structure by X-ray crystallography, they found that THOV NP is remarkably similar to IAV NP, with both NPs containing globular 'head' and 'body' domains. Dick *et al.*, further identify the RNA binding cleft within THOV NP by mutating key residues involved in NP-RNA interactions and assessing NP-RNA binding by isothermal titration calorimetry; the region they identify is again similar to the RNA binding cleft in IAV NP. They also identify an extended 'tail loop' and confirm its essential role in mediating NP oligomerization, and hence RNP formation, by interacting with adjacent NP monomers; a feature shared with other known structures of negative-sense RNA virus NPs (namely IAV, and the bunyaviruses Toscana virus and Hantaan virus). Finally, they solve the structure of THOV RNPs purified from infectious virions by subtomogram averaging, resulting in a model similar to one of the models previously proposed for IAV[8].

Overall, while the recent progress in cryo-EM have significantly advanced the field of RNPs of segmented negative-sense RNA viruses, and we now have information for many viruses, we still lack a model in which the high-resolution structure of NP can be confidently fitted into the RNP average. Potentially this will be solved in the near future either by improving the computational approaches that handle sample heterogeneity and/or by developing a system that allows to recombinantly express functional RNPs that are more homogeneous than RNPs purified from virions. The latter approach has been applied recently for IAV, enabling higher resolution of RNP-like structures to be obtained, albeit with a different overall architecture to IAV derived RNPs[10]. These structures could then aid to tackle the burden caused by these viruses.

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Declaration of Interests
The authors declare no competing interests.

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