

Coinfection by influenza A virus and respiratory syncytial virus produces hybrid virus particles

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Description of IAV and RSV particles

We identified structures consistent with IAV filaments and pleiomorphic particles that were densely coated with glycoproteins (Fig. 1A). IAV filaments varied in length, with an average diameter of 84.8 (± 6.97) nm. IAV ribonucleoproteins (RNPs) could be observed within pleomorphic virions (Figure 1A). We also identified RSV filaments (Figure 1B) with an average width of 158 (± 14.6) nm, but many irregular shaped virions were also observed (Fig. 2). Differences in glycoprotein distribution as well as RNP structure between RSV and IAV virions were evident. The glycoproteins in RSV virions exhibited sparser distribution along the virion than IAV glycoproteins (Figs. 1C and 1D). RNPs could be clearly seen in many IAV and RSV filaments. While IAV comprised a single ordered set of RNPs in a “7+1” arrangement at the distal end of filaments, multiple RNPs were detected within RSV filaments having herringbone morphology and ring-like structures attributed to be nucleocapsid (N) or N-RNA assemblies (1, 2) (Fig. 1A-D, Fig. 2). Upon closer inspection of glycoproteins on the surface of virions (top and bottom slices in the tomograms), distinct patterns of glycoprotein arrangement were observed between the two viruses. In IAV virions, glycoproteins were densely packed, devoid of any long-range order, with the “triangular-shaped” trimeric head of HA clearly visible (Fig. 1E, 1F, Fig. 3). However, RSV exhibited a more symmetrical helical arrangement of glycoproteins (striped layers of density) with specific clustering in pairs (Fig. 1G, 1H, Fig. 3).

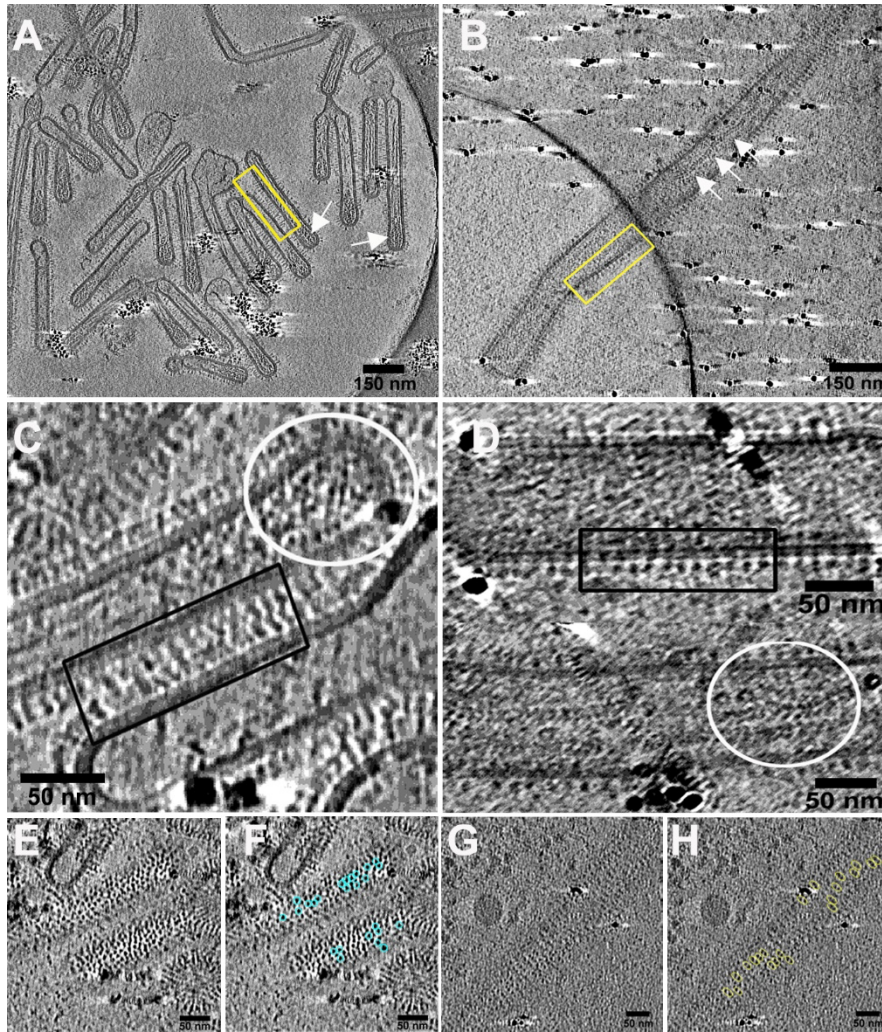


Fig. 1. Cryo-ET shows differences in glycoprotein ordering and RNP organisation between IAV and RSV virions. Cryo-electron tomograms of IAV (**A**) and RSV (**B**) denote pleomorphic morphology alongside differences in glycoprotein and RNP organisation (yellow rectangle and white arrows). IAV virions exhibit a single set of ordered RNPs (**C**, white circle) while multi genomes can be seen in RSV virions (**D**, white circle). Glycoproteins can be seen in a picket fence like distribution along the viral membrane, although more densely packed in IAV than RSV (**C** and **D**, black rectangles). Looking at the glycoproteins from the top and bottom of the virions, significant differences are visible in glycoprotein packing: the triangular hemagglutinin HA spikes on IAV are densely packed with no long-range order (**E** and **F**, cyan circles) while RSV shows clustering of glycoprotein pairs (**G** and **H**, yellow ellipses).

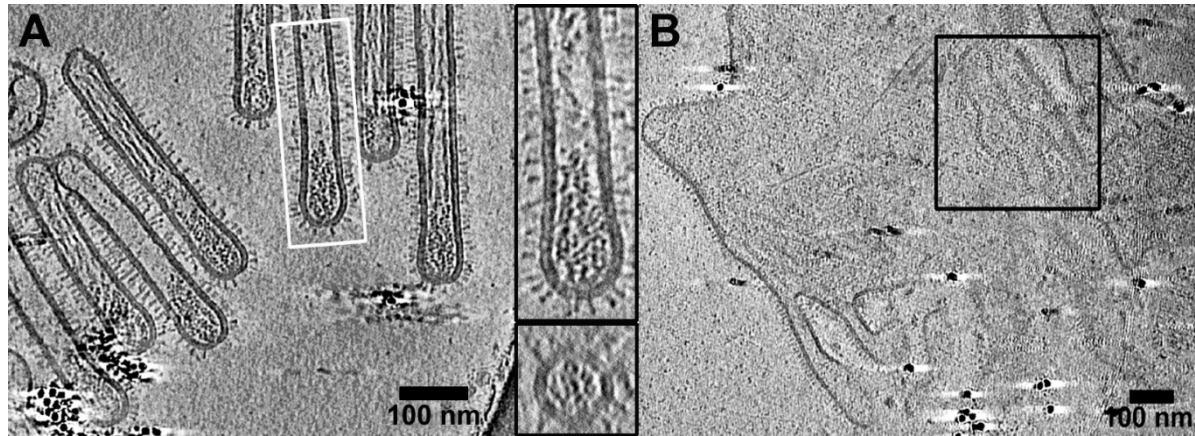


Figure 2. Distinct RNP organization in IAV and RSV virions seen by Cryo-ET. (A) IAV shows a single ordered set of RNP's at the distal end (white rectangle and magnified view of the same) of filaments. Transverse section (bottom image, magnified view of the white rectangle) of the filament denotes the "7+1" arrangement of RNPs in IAV virions. (B) Although RSV can form irregular shaped virions in addition to filaments, they are filled with multiple RNPs inside and decorated with spikes on the outside.

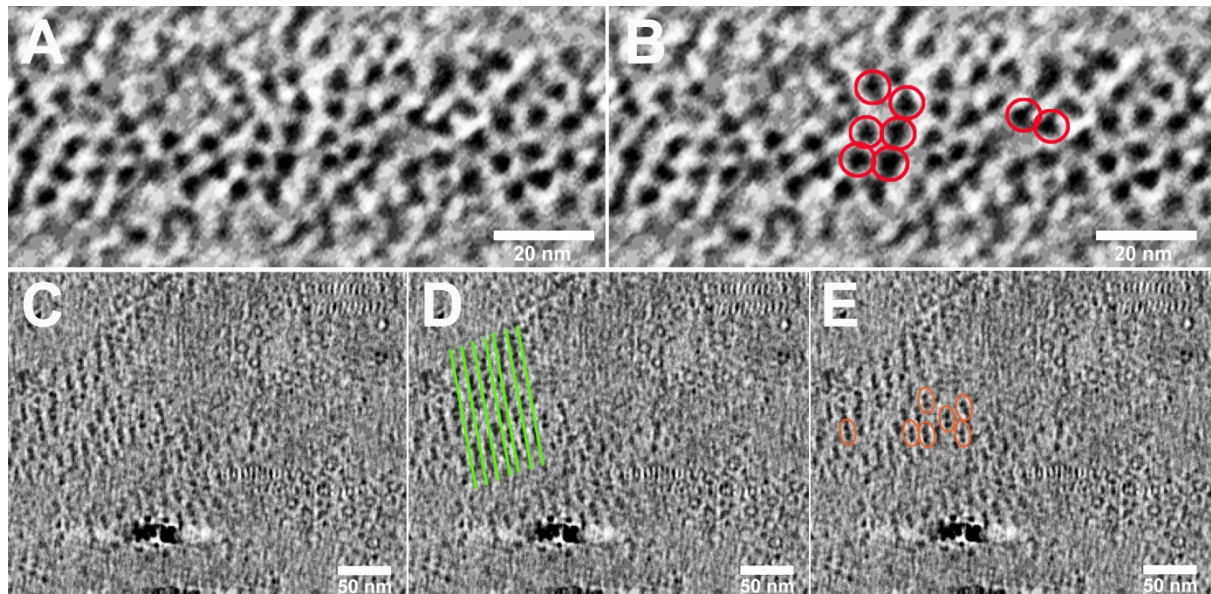


Figure 3. Different glycoprotein ordering between IAV and RSV virions.

Inspection of top and bottom views of glycoprotein spikes (corresponding to top and bottom slices of the tomograms) on IAV and RSV virions indicates a different pattern of organization. (**A** and **B**) While the triangular heads (red circles) of the HA glycoprotein in IAV is clearly visible, the spikes are densely packed and devoid of any long-range order. (**C-E**) On the other hand the spikes on RSV appear to be helically ordered as seen by the striped arrays of density (**D**) and specific (**E**) clustering of glycoproteins in pairs (red ellipses) is clearly evident.

References

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2. D. Bhella, A. Ralph, L. B. Murphy, R. P. Yeo, Significant differences in nucleocapsid morphology within the Paramyxoviridae. *J Gen Virol* **83**, 1831-1839 (2002).