1708
doi:10.1017/S1431927621006243
Microsc. Microanal. 27 (Suppl 1), 2021

Visualization of intracellular Ebola virus nucleocapsid assembly by cryo-electron tomography

Reika Watanabe[1], Guillaume Castillon[2], Ruben Diaz Avalos[1], Mark Ellisman[3] and Erica Saphire[4]

[1]La Jolla Institute for Immunology, La Jolla, California, United States, [2]UCSD, United States, [3]UCSD, La Jolla, California, United States, [4]La Jolla Institute for Immunology, United States

Ebola virus (EBOV) and related filoviruses cause severe hemorrhagic fever with high mortality 1. Up to now, there are no universal treatments against filovirus infection. Elucidating the conserved mechanism of viral replication mechanism is vital to the development of antiviral drugs. The long, flexible, and filamentous EBOV virion contains a central coiled nucleocapsid (NC) that comprises nucleoprotein NP and two cofactors, Virion protein (VP) 24 and VP35. During NC assembly inside cells, the NP first polymerizes into a loose coil that condenses upon binding to other molecules (presumably VP24 and VP35) in the intracellular viral replication factory. The NCs then gather into bundles that travel to the plasma membrane, where they separate to allow incorporation into budding virions. TEM techniques used to view these processes in previous studies involved multiple treatments (e.g., fixation, heavy metals) that can compromise resolution and structural integrity 2–5. Thus, many of the structural aspects of the nucleocapsid assembly processes remain unclear.

Cryo-ET combined with cryo-focused ion beam (FIB) milling (cryo-FIB-ET) is now an essential technique for structure determination of macromolecules in their native cellular environment and requires no chemical treatment. EBOV requires containment at biosafety level-4, where FIB-milling instrumentation is not currently available. To overcome this limitation, we confirmed that HEK 293T cells transfected with EBOV NP, VP24, and VP35 exhibit NC-like bundles that are indistinguishable from those seen in EBOV-infected cells (Fig. 1)4,6. To perform in situ cryo-ET analysis, transfected cells were deposited and plunge-frozen on TEM grids (Fig.2 A). We performed focused-ion beam (FIB) milling in SEM/FIB dual-beam microscopy to prepare thin slices of cells (Fig.2 B, C) and tomography using a cryo-transmission electron microscope equipped with an energy filter and direct electron detector camera. In the tomograms, we observe condensed EBOV NC bundles (Fig.2D purple arrowhead) as well as the helical pattern of individual NCs, confirming that we can obtain high-quality, physiologically relevant data using this workflow. NC cross-sections have a ~50 nm diameter (Fig. 2E), consistent with NC found in EBOV virions7. We also observe loosely coiled NP (cyan arrowhead) resembling purified, full-length

NP expressed and isolated from mammalian cells, and the form that is the precursor to the fully assembled NC7.

We are collecting more data to perform subtomogram averaging to generate an in situ structure of the Ebola nucleocapsid assembly process, and I will present the outcomes and challenges of these studies.

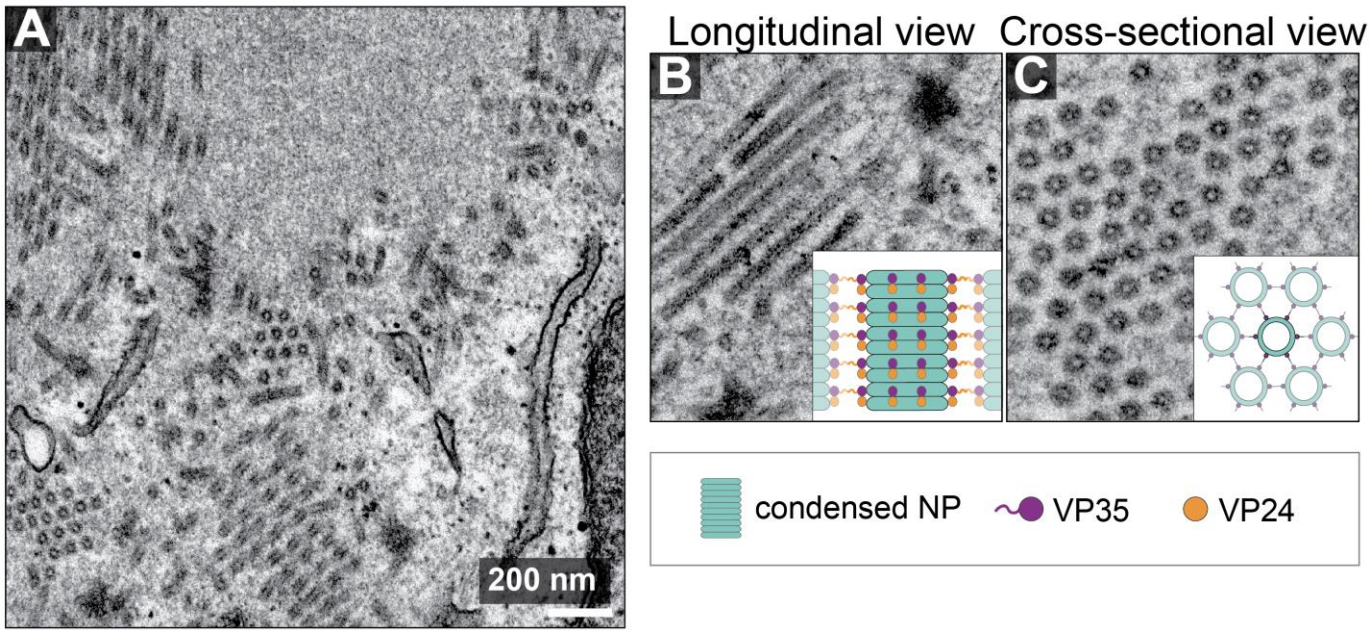

Figure 1. EBOV NC-like assembly observed in cells transfected with EBOV NP, VP24, and VP35. (A) Ultrathin section image of a HEK293T cell transfected with EBOV NP, VP24, and VP35 shows the assembly of nucleocapsids, indistinguishable from those in EBOV-infected cells4,5. Longitudinal (B) and cross-sectional views (C) of the nucleocapsid bundles are shown.

https://doi.org/10.1017/S1431927621006243 Published online by Cambridge University Press

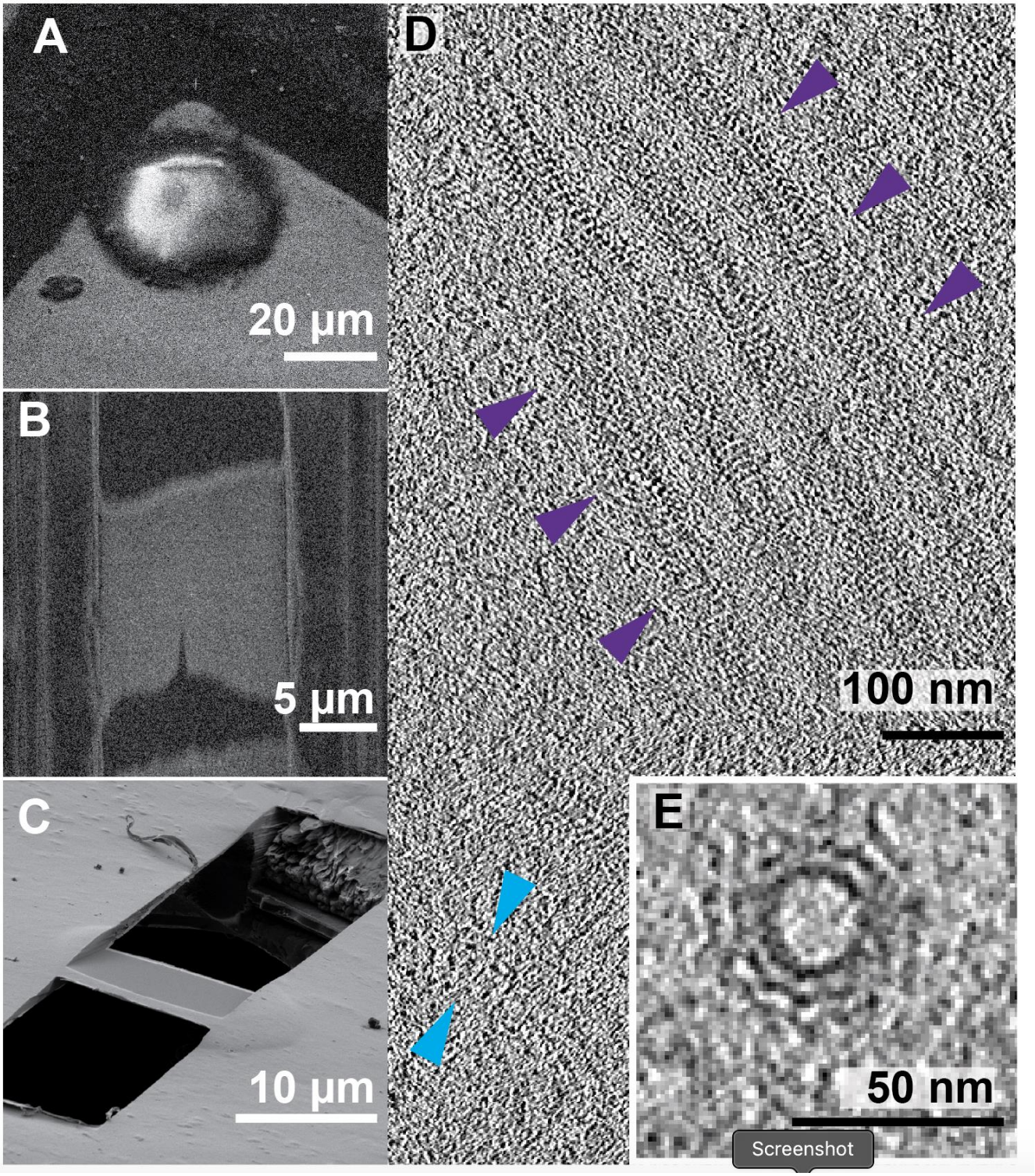

Figure 2. In situ cryo-FIB-ET workflow to visualize EBOV NC-like assembly in cells.SEM images of cells before (A) and after FIB milling acquired by dual-beam cryo-FIB/SEM (B). (C) Cells containing lamella as visualized by FIB. (D) Representative tomogram showing preassembled NC bundles (purple arrowhead) and loosely-coiled NP (cyan arrowhead). (E) Cross-section view of preassembled NC.

https://doi.org/10.1017/S1431927621006243 Published online by Cambridge University Press

References

1. Baseler, L., Chertow, D. S., Johnson, K. M., Feldmann, H. & Morens, D. M. The Pathogenesis of Ebola Virus Disease. Annu. Rev. Pathol. **12**, 387–418 (2017).

2. Ellis, D. S. et al. Ebola and Marburg viruses: II. Thier development within Vero cells and the extra-cellular formation of branched and torus forms. J. Med. Virol. **4**, 213–225 (1979).

3. Geisbert, T. W. & Jahrling, P. B. Differentiation of filoviruses by electron microscopy. Virus Res. **39**, 129–150 (1995).

4. Noda, T. et al. Assembly and budding of Ebolavirus. PLoS Pathog. **2**, e99 (2006).

5. Olejnik, J., Ryabchikova, E., Corley, R. B. & Mühlberger, E. Intracellular events and cell fate in filovirus infection. Viruses **3**, 1501–1531 (2011).

6. Huang, Y., Xu, L., Sun, Y. & Nabel, G. J. The assembly of Ebola virus nucleocapsid requires virion-associated proteins 35 and 24 and posttranslational modification of nucleoprotein. Mol. Cell **10**, 307–316 (2002).

7. Bharat, T. A. M. et al. Structural dissection of Ebola virus and its assembly determinants using cryo-electron tomography. Proc. Natl. Acad. Sci. U. S. A. **109**, 4275–4280 (2012).

https://doi.org/10.1017/S1431927621006243 Published online by Cambridge University Press