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In Situ Structural Studies of Paramyxovirus Glycoproteins by Cryo-Electron Tomography

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In an effort to define the viral spatial organization of a paramyxovirus fusion complex, we are investigating the native three-dimensional structure of Measles Virus F and H glycoproteins by cryo-electron tomography. Measles Virus (MeV) is a member of the Paramyxovirus family and mediates its fusion with the host cell using type I viral fusion mechanisms, much like other orthomyxo-, retro- and coronaviruses [1]. The principles that govern the organization of the metastable fusion complexes associated with type I viral fusion and, in the case of MeV, the sophisticated refolding processes of the F(usion) protein are poorly understood. Therefore, we have developed a collaborative strategy to examine the complex structural rearrangements that occur throughout the process of viral fusion.

Our first tomograms of wild type MeV revealed a loosely spooled nucleocapsid within the virus and dense packing of the two glycoproteins on the viral surface (FIG.1.). The extreme concentration of the glycoproteins limits our efforts to resolve the structural arrangement and changes that occur in individual glycoproteins. Therefore, in order to examine isolated glycoproteins individually, an infectious virus with an elongated 'stalk' region of the H glycoprotein was engineered (H118-∇41X) [2]. Tomograms of this mutant virus are consistent with a greater separation between the heads of the two viral membrane proteins. Using this increased staggering effect, our current efforts focus on extracting individual H glycoproteins from the tomograms in order to generate averaged, higher signal-to-noise structures. Once refined, coordinates based on homology models or native crystals of the F and H glycoprotein 'heads' will be docked into the electron densities. Further efforts are underway to computationally map the localization patterns of the glycoproteins on the viral surface.

References
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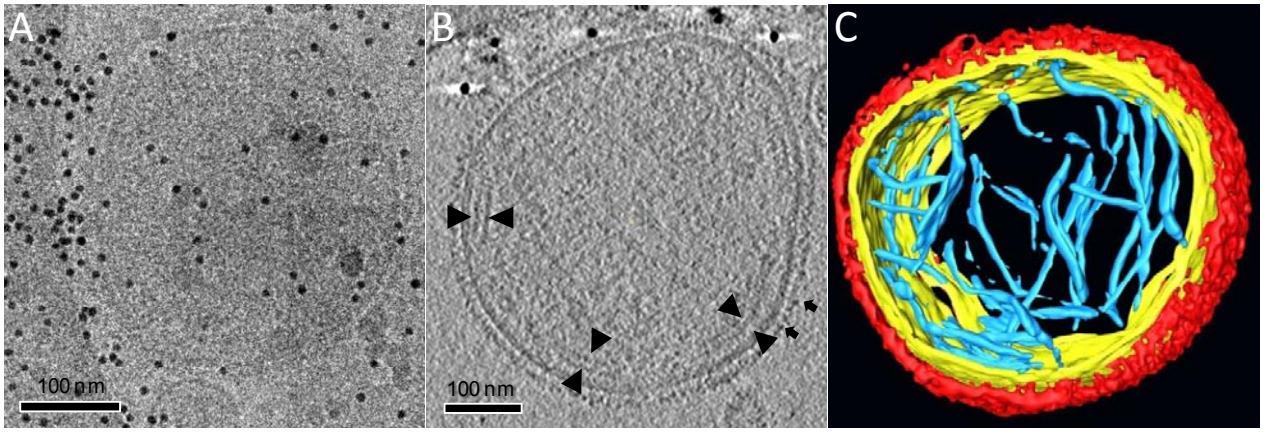

FIG. 1. The figure shows the Cryo-ET analysis of a wild type measles virus (MV) particle. Panel A shows the central image from a tilt series of an isolated MV particle suspended in vitreous ice. In panel B an 11.2 nm averaged slice through a 3D-reconstruction (tomogram, generated with IMOD [3]) of a MV particle is shown. The 300 nm roughly spherical virus contains nucleocapsid (arrowheads) and the envelope glycoproteins are arranged on the surface of the lipid membrane (arrows). Figure C shows a segmentation of the MV particle. The envelope glycoproteins are stained red, the virus membrane yellow. The nucleocapsid (blue) is loosely spooled within the virus.

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