The Near-to-Native-State Architecture of Measles Virus Assembly Sites and Isolated Measles Virus Particles

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Measles virus (MeV) is a pleomorphic, enveloped, single-stranded RNA virus that belongs to the Paramyxovirus family. Details regarding the coordination and native arrangement of the Measles virus macromolecular complexes at sites of virus assembly and in released virus particles are poorly understood. Three-dimensional structural analyses of Measles viruses by cryo-electron tomography (cryo-ET) have been of purified viruses [1, 2]. To further investigate the structure of assembled, released Measles virus particles and the processes of virus assembly under native-state conditions, we utilized whole cellular cryo-ET.

HeLa and MRC-5 cells were grown directly on gold Quantifoil TEM grids (Quantifoil Micro Tools GmbH, Jena, Germany). Subsequently, cells were infected with MeV strains, including wild type and recombinant strains at a multiplicity of infection of 1, 6 or 10. TEM grids were plunge frozen in liquid ethane using a Cryoplunge 3 system (Gatan, Pleasanton, CA) as previously described [4, 5]. Cryo-grids were imaged with a JEOL JEM-2200FS TEM (JEOL, Ltd., Japan) equipped with an in-column Omega energy filter, operated with a slit with of 20 eV. Image montages were acquired at a magnification of 10,000×, equivalent to a pixel size of 6.14 Å on a DE-20 direct electron detector camera (Direct Electron, LP, San Diego, CA). Bidirectional tilt-series were acquired at 2º increments over an angular range of -62º to +62º using the SerialEM software package [6]. Images were recorded at a magnification of 20,000×, corresponding to a pixel size of 2.94 Å on a DE-20 direct electron detector camera at a rate of 12 frames per second. Data was processed as previously described [3] and tomograms were reconstructed using the IMOD software package [7].

Thin areas along the periphery of Measles virus-infected cells were surveyed to locate sites of virus assembly and to identify released virus particles. Regions of interest were identified and cryo-ET data was acquired. Sites of assembly were identified by the presence of the matrix protein along the plasma membrane (PM). Short and long-range arrays of the matrix protein were present and consisted of subunits in which the spacing was ~8 nm. In regions where the matrix protein was present along the interior of the plasma membrane, well-ordered arrays of the fusion (F) and hemagglutinin (H) glycoproteins were present on the surface. In addition, the ribonucleoprotein complex (RNP) appeared to associate directly with the ordered arrays of the matrix protein, indicating a coordinated recruitment to sites of virus assembly. Direct visualization of MeV-infected cells by cryo-ET has proved valuable for determining the native organization of the M protein, glycoproteins, and ribonucleoprotein (RNP) at both sites of virus assembly and in released virus particles.
References:

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