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On The Structure of Coronaviruses: Cryo-electron Tomography of Mouse Hepatitis Virus

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Coronaviruses (CoV) are large enveloped plus-strand RNA viruses that principally cause respiratory and enteric diseases in mammals and avians [1-2]. The traditional perception of CoVs as of mainly veterinary importance changed drastically after the outbreak of SARS (Severe Acute Respiratory Syndrome) in 2002-2003, when the causative agent was found to be a member of this group.

In spite of the renewed interest in CoVs, many fundamental aspects, which might be essential for the development of new antiviral strategies, remain to be elucidated. For example, a direct ultrastructural characterization of CoVs is still missing. The viral organization inside the envelope has been particularly elusive. The information available comes from studies in which the viral envelope was disrupted and the released content examined. However, two types of structures have been observed in this way and consequently lead to two different structural models: (a) a helical capsid that would be formed by the ribonucleoprotein complex (RNP) and (b) a shelled core, possibly even icosahedral, that would enclose the RNP [3].

To directly investigate this and other structural viral elements and analyze their global 3D structural organization in intact viruses, we have applied cryo-electron tomography on purified samples of murine hepatitis virus (MHV), the prototype for CoVs. Seven tilt series were collected in a 300 kV FEI Polara microscope equipped with a post-column energy filter. Each tilt series covered an angular range of ~130° in 2° increments. The nominal underfocus value used was 4 μm, which ensured no contrast inversions due to the contrast transfer function up to about 3 nm. The pixel size was 0.58 nm at the specimen level. In the tomograms, a total of about 140 viruses were reconstructed and independently examined in 3D.

The reconstructed MHVs show a distinct spherical shape and a relatively homogenous size (85 nm envelope diameter, SD ± 6 nm), although viruses from 65% to 200% the volume of the average MHV were measured. Club-shape spikes of ~20 nm length stem from the envelope, but the spike coverage showed to be quite limited in a variety of preparation conditions. Since the infectivity of the virions was confirmed by plaque assays, this observation suggests that few spikes on the virus could be sufficient to trigger infection. As for the interior of the virus, no evidence of shelled core structures could be found in the reconstructions. Instead, loosely arranged coiled structures and tubular fragments were observed inside the envelope, in agreement with a helical RNP nucleocapsid

model. The helical RNP seems to be extensively folded upon itself in a hank-like compact structure that closely follows the membrane and occasionally contacts the viral envelope. Most of the viral MHV envelope showed to be exceptionally thick for a lipid bilayer (~8 nm). We interpret this extra thickness as the result of an additional layer formed by the C-terminal domain of the most abundant membrane protein, M. Local striation patterns are observed in regions of the envelope, suggesting the presence of local M lattices. Our results suggest a model for the structure of coronaviruses based on local structural motifs that might serve as the basic framework for the global architecture of the virion.

References

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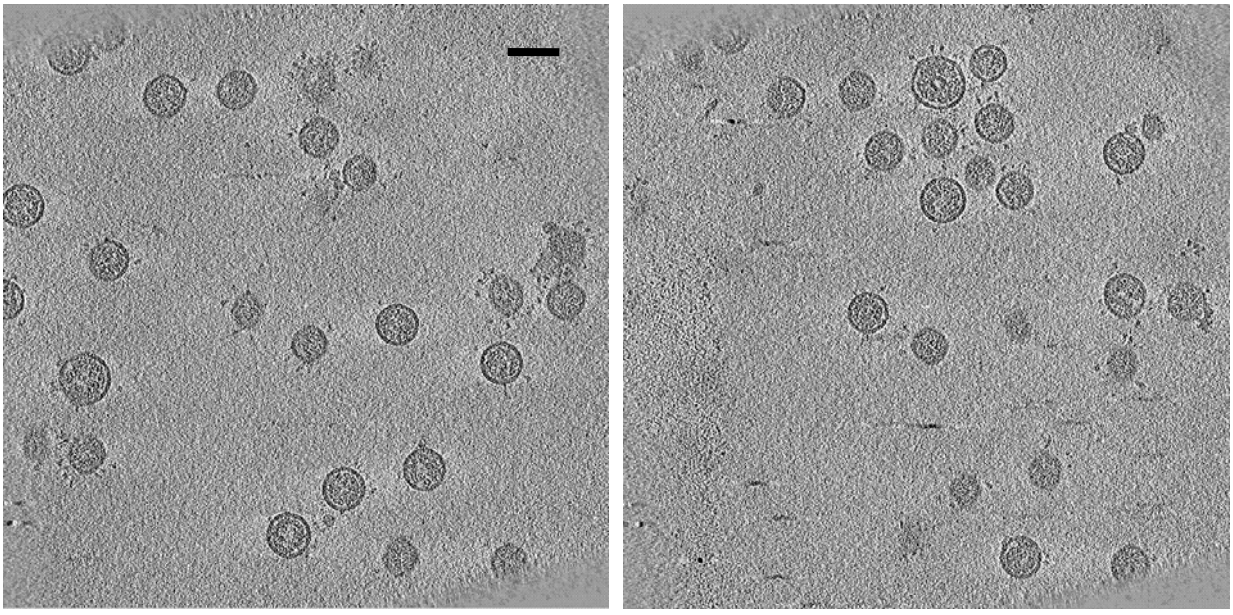

Figure 1. Two 4.6 nm thick virtual slices at different heights (20 nm apart) of one of the tomograms from vitrified samples of isolated MHV. Scale bar, 100 nm.