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3D reconstruction of tick-borne encephalitis virus replication complexes

T. Bílý[1,2], M. Vancova[1], D. Ruzek[1,3], M. Palus[1,2], J. Nebesarova[1,4], L. Grubhoffer[1,2]

[1]Biology Centre of ASCR, Institute of Parasitology, Ceske Budejovice, Czech Republic
[2]University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic
[3]Veterinary Research Institute, Department of Virology, Brno, Czech Republic
[4]Charles University, Faculty of Science, Prague, Czech Republic

thomass@paru.cas.cz
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Ixodes ricinus is the main vector of Tick-borne encephalitis virus (TBEV) in Europe. Ticks can be infected during feeding upon infected vertebrates like small rodents [1]. Humans are mostly infected via injected tick saliva. The first phase of the infection has nonspecific influenza-like symptoms. Meningitis, meningoencephalitis or meningoencephalomyelitis occur in the second phase which can be followed by post-encephalitic syndrome.

TBEV is a member of the Flavivirus genus in the family *Flaviviridae*. Flaviviruses are enveloped particles with a 40–60 nm outer diameter and an electron-dense core-nucleocapsid composed of the capsid protein and the positive-sense RNA genome [2]. The RNA genome is translated in a host cell into a polyprotein that is cleaved into structural proteins C (Capsid), M (membrane), E (envelope) and non-structural proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 [3].

This study is focused on 3D reconstructions of structural changes of the rough endoplasmic reticulum (ER) induced by TBEV and the organisation of the replication complexes. We describe the presence of induced vesicles (smooth membrane structures) and convoluted membranes (Figure 1) [4]. This TBEV ER derived induced network is the presumed site of replication [5]. Our results prove the presence of a single pore in the wall of TBEV induced vesicles (Figure 2). Similarly, 3D reconstruction of the replication and assembly sites of another flavivirus, the Dengue virus, has proven presence of one such pore in the virus induced vesicle. The presumed function is in an import of factors required for RNA replication and export of newly synthesized genomes [5].

In TBEV infected human primary astrocytes (prepared by high pressure freezing and freeze substitution method), we observed unique microtubule-like structures within of ER cisterns. The average diameter of structures was 17.9 nm (± 0.15 nm, n = 101). The 3D reconstruction revealed its the detailed organisation. The structures were already described in glutaraldehyde fixed neuroblastoma cells infected with TBEV [4]. Their function is not clear. Tubular elongated structures of a 60-100 nm diameter were observed within the ER of Langat virus (Flavivirus genus) infected mammalian cells [6].

Tilt series electron micrographs were collected from sections thinner than 90 nm which provide clearly distinguishable structures in transmission projection images (without overlapping structures arising from object arrangement in the 3D volume). Sections were examined at 200 kV (JEOL 2100F equipped with high tilt stage and Gatan camera Orius SC 1000) using the Serial EM acquisition software [7]. Tomograms were aligned, reconstructed and 3D models were generated by manually masking the area of interest using the IMOD software package [8].

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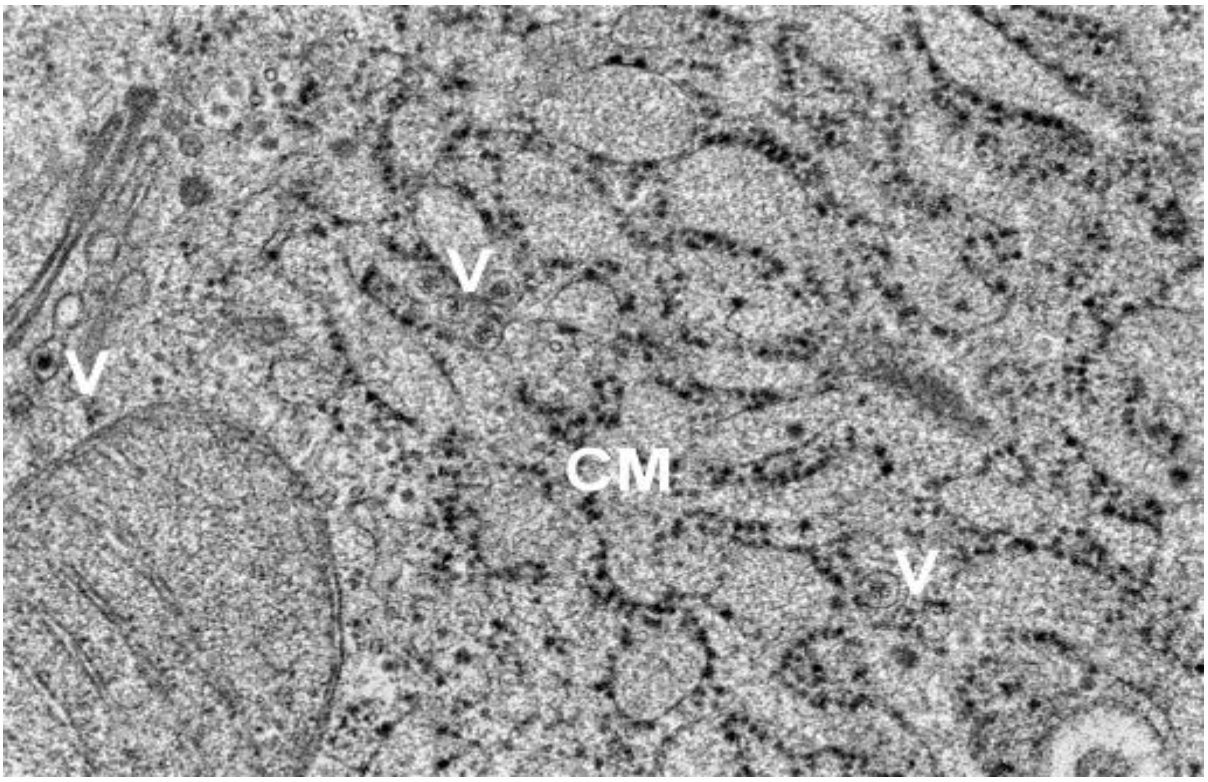

Figure 1: Neuroblast TBEV (V) infected cells, rearranged ER (CM).

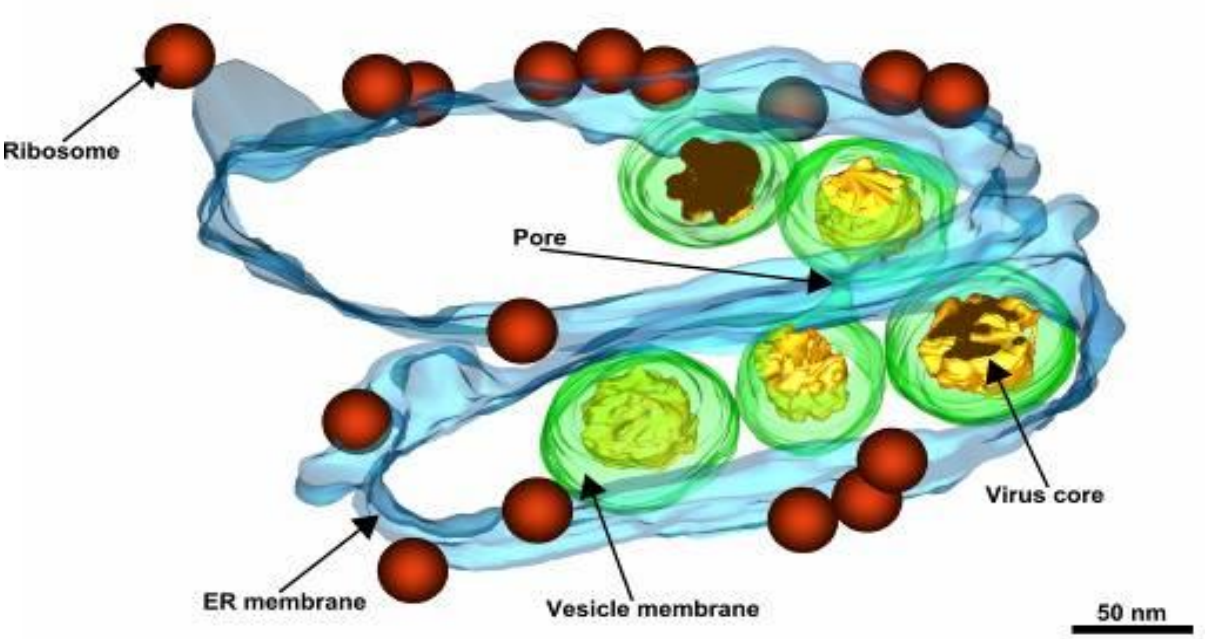

Figure 2: Pore inside induced vesicle.