Application of the electron tomography to the study of membrane biogenesis of Mimivirus

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Acanthamoeba polyphaga Mimivirus, a virus infecting the common amoeba *A. polyphaga* was discovered in 2009. The subsequent analysis of its size, gene content and phylogeny of this virus challenged many accepted ideas about what a virus should look like as well as about their origin [4, 9].With an icosahedral capsid of approximately 500 nm Mimivirus is the largest virus known. The capsid is covered with a 140 nm-thick layer of closely packed fibers forming a 750 nm spherical entity. Its genome is a 1.2 million base pairs linear, double-stranded DNA coding for 911 genes exceeding by far the number of genes encoded by other large DNA viruses. Among these genes many are not expressed by any other virus, but only by living cells. These include, for example, tRNA synthetases, enzymes for amino acid, carbohydrate, and lipid metabolism [7,6].

Comparative genome analyses suggest that Mimivirus shares a common origin with the members of NCLDVs [11]. NCLDV are double-stranded DNA viruses with genomes sizes in the range from 150 kb to 1.2 Mb. They form a super-family of viruses infecting various eukaryotic host and include the *Poxviridae*, *Iridoviridae*, *Phycodanviridae* and *Asfarviridae* [8]. Thus, similar to *Pox*- and *Asfarviridae*, Mimivirus may deliver its DNA to the host cytoplasm where it is replicated and packaged inside an icosahedral capsid [7]. For VACV and ASFV assembly starts with a precursor membrane, which is crescent-shaped for VACV and icosahedral for ASFV, and the origin and biogenesis of this membrane is subject to debate since a long time. For both viruses one model postulates that the precursor membrane is a collapsed double membrane derived from the endoplasmic reticulum (ER) [1,10, 3]. Another postulates that the precursor is a single membrane of unknown origin [5, 2]. Recently Chandla and col., has proposed an unconventional, not observed before, way of membrane acquisition. The data showed how pleiomorphic ER-derived membranes rupture to create open membrane sheets that then contribute to the formation of the VACV membrane. This membrane is then shaped into a crescent by a viral scaffold protein and remains open until the viral DNA has been inserted [3].

In contrast Mimivirus assembly and structure is poorly understood. We have recently established HPF/FS protocols for Amoeba infected with Mimivirus that enable us to preserve and image viral membranes in a way not seen before. We detected large virosomes surrounded by mature virions. At the immediate periphery of the virosome we detect icosahedral-shaped membranes, coated on their convex side with scaffold protein, connected to a membrane without coat (Figure 1). Additional uncoated membrane structures collect close to the growing icosahedral membrane strongly suggestion that they contribute to its formation. Thus, by thin section TEM Mimivirus membrane assembly strikingly resembles VACV.

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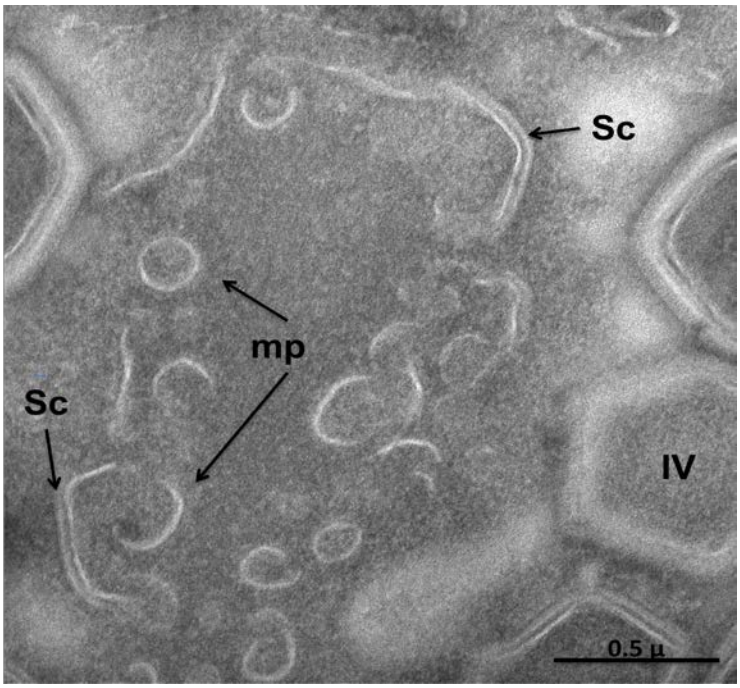

Figure 1. *Partly formed icosahedral-shaped membranes coated on the outside with scaffold protein are connected to a membrane without scaffold. In their vicinity small membranes assemble, someof which are seemingly open in 2D sections and which are likely connected to the growing icosahedral precursor membrane.*