Unconventional membrane biogenesis of large DNA viruses revealed by electron tomography

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1. INTRODUCTION

Within eukaryotic cells membrane compartments are closed entities and their dynamics underlie regulated fission and fusion reactions. Since enveloped viruses acquire their membrane from the host, virus budding may underlie similar processes. However, some time ago we showed that the poxvirus vaccinia virus (VACV) uses a different, unconventional, pathway of membrane assembly. Using cryo-EM and electron tomography (ET) we concluded that VACV assembles a single, open, membrane, shaped into a sphere by the viral scaffold protein [1]. By combining EM immuno-labeling with ET we could show that the open membrane is derived from small open membranes that accumulate close, and are connected, to the assembling virion (see Figure 1C). Since lipid analysis of the isolated, purified virus suggested that the viral membrane is derived from the ER [2], we postulated that the small open membranes are the result of rupture of ER elements [1].

VACV is a member of the nucleo cytoplasmic large DNA viruses (NCLDVs). We analyzed whether other members of this family also use the unconventional membrane assembly pathway as to search for common denominators. Indeed both Mimivirus [3] and African swine fever virus (ASFV; [4]) use open membrane intermediates to create a single open membrane shaped by their respective scaffold protein. The images below illustrate some of our results.

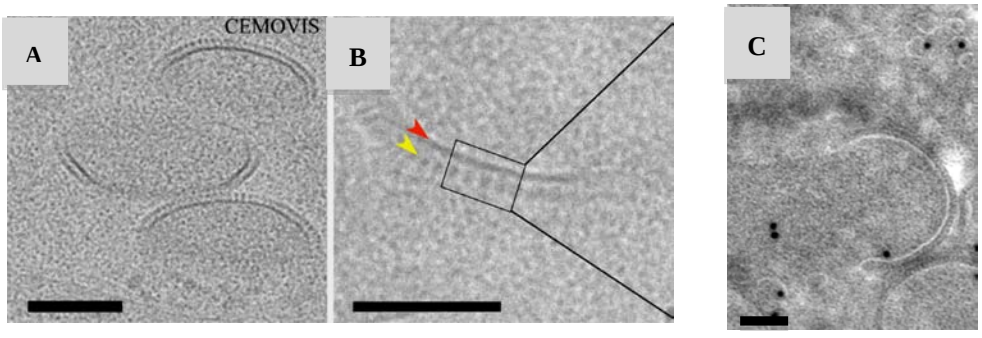
2. RESULTS

2.1 Experimental conditions

To overcome the main limitations in TEM, this is:1. putative embedding artifacts and 2. the loss of one dimension by thin sectioning, we use complementary EM embedding and imaging techniques. Cryo-EM of vitreous sections allows the imaging under native conditions without contrasting and dehydration. Such samples are, however beam-sensitive and the generation of vitreous sections is labor-intensive. To image membranes by electron tomography (ET) we found that Tokuyasu thawed cryo-sections can be a good compromise. Compared to freeze substituted samples membrane continuities are easier to track [4] and the structures of interest can be identified in thick (300nm) sections by EM immuno-labeling. Where the imaging of larger volumes is required we used STEM tomography of 750-1000nm thawed cryo-sections in collaboration with Paul Walther. The images below show some examples.

2.2 Appearance of the viral membrane and scaffold protein of VACV and ASFV

Below are some examples of the appearance of the VACV (A to C) and ASFV (D and E) membrane and scaffold protein with different embedding and imaging methods



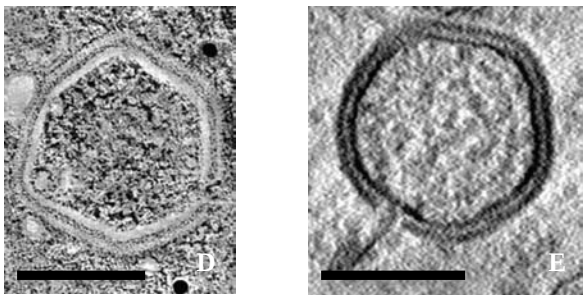


Figure 1. A and B appearance of the VACV precursor membrane by CEMOVIS. The higher magnification in B shows that it is composed of two distinct structures. The structure indicated with a red arrowhead is a membrane, whereas the yellow arrowhead points to the viral scaffold protein shaping the membrane into a half-moon (crescent; see [1]). In C appearance of the crescent in thawed cryo-sections labeled with anti-A14, a major and essential viral membrane protein. The membrane appears white and is coated on its convex side with the spike-like scaffold. The antibody labels the viral membrane as well as small membranes in its vicinity. By ET these membranes accumulate next to viral scaffold assemblies and rendering shows they are open (not shown in this image). Since these membranes are rare and small they are difficult to detect without immuno-labeling. In D and E a slice of a tomogram recorded by TEM (D) or by STEM (E; using a HAADF-STEM filter/ dark field detector) of an immature ASF-virus. Both imaging methods of thawed cryo-sections (300nm and 750nm in thickness in D and E, respectively) display 3 layers; an inner membrane (white by TEM, black by HAADF-STEM) and two layers of the scaffold protein shaping the membrane into an icosahedron. By STEM tomography the surface of the particle displays tiny spikes that are not well resolved by TEM-ET. A and B taken from [1], C taken from [3] and D and E from [4]. Bars-100nm

2.3 VACV, Mimivirus and ASFV assemble from single open membrane precursors as assessed by ET

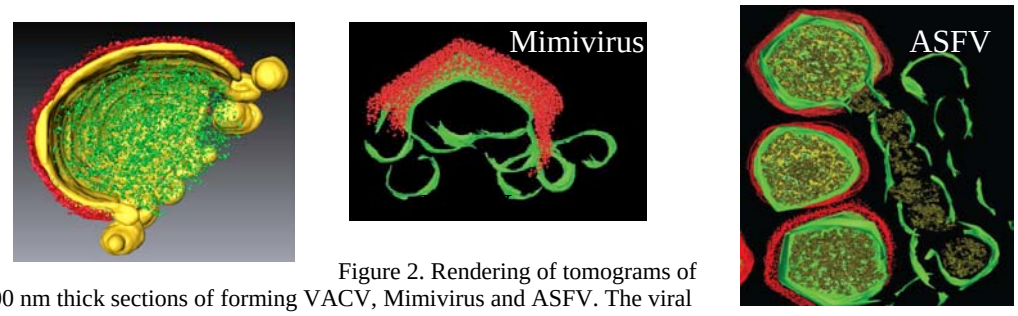


Figure 2. Rendering of tomograms of 300 nm thick sections of forming VACV, Mimivirus and ASFV. The viral membrane (yellow for VACV, green for Mimi and ASFV) is shaped on its convex side with viral scaffold (red) into a sphere for VACV and into icosahedrons for Mimi and ASFV. The membrane is continuous with small membranes from which it is made and that are open.

3. CONCLUSION

Our collective data show that at least three members of the NCLDV-family assemble a single membrane derived from open intermediates. Since it has been postulated that NCLDVs are derived from a common ancestor, we propose that this ancestor acquired this unconventional membrane assembly pathway. We are currently pursuing several interesting candidate molecules that are possibly involved in membrane rupture. Generally, we believe it is important to use complementary EM embedding and imaging techniques, combined with quantification, to study complex questions related to cellular and viral membrane biogenesis.

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