

ELECTRON TOMOGRAPHY RECONSTRUCTION OF VACCINIA VIRUS. José L. Carrascosa(1), Cristina Risco(1), Jose J. Fernández(2), Mariano Estéban(1), Marek Cyrklaff(3), and Wolfgang Baumeister(3). (1) Centro Nacional de Biotecnología, CSIC. Campus de la UAM, Cantoblanco. 298049 Madrid, Spain. (2) Departamento de Arquitectura de Ordenadores, Universidad de Almería. 04120 Almería, Spain. (3) Max Planck Institute of Biochemistry. D-82152 Martinsried, Germany. Email: jlcarras@cnb.uam.es.

Vaccinia virus is the best characterized member of the *Poxviridae* family. The viral particle is very complex, including more than 100 different proteins, and it is assembled through a complicated morphogenetic pathway. Viral factories are built by recruitment of both viral and cellular components that lead to the viral crescents. These evolve into the formation of spherical immature virus (IV) that later mature to the brick-shaped intracellular mature virus (IMV). Some IMVs acquire additional membranes to become intracellular enveloped virus (IEV). IEVs are released from the cell by fusion with the plasma membrane, leading to the extracellular enveloped virus (EEV) (1).

For many years, different microscopic techniques have been applied to solve the structure of the different particles related to the morphogenesis of Vaccinia. The accumulation of structural data using different technologies has led to conflicting interpretations and to the proposal of alternative models for the viral particle (2,3). The task to get three-dimensional structural data from vaccinia virus particles at sufficient resolution so as to establish unambiguous structural features has been tackled by Electron Tomography (4).

Purified Intracellular Mature Vaccinia Virus (IMV) were rapidly frozen and observed by cryo-electron microscopy. Tilt series were obtained using energy filtering to remove the inelastically scattered electrons. Direct visualization of the sections from the three-dimensional reconstructed tomograms revealed a consistent morphology based on several layers enclosing an internal core with a lower density area in the inner region. Surface rendering representation revealed viral particles with a homogeneous morphology, with an overall shape of a barrel slightly compressed along the longitudinal axes, and dimensions around 350-370 x 250 x 270 nm. The outer surface of the viral particles presented a moderated corrugation, with features consistent with protrusions of irregular shape extending around 3-5 nm from the viral surface. No special ordering of these corrugations was found in any of the reconstructions.

The tomograms of such large, multicomponent assemblies as Vaccinia particles are difficult to interpret due to their inherent structural complexity that is further obscured by noise derived from the data acquisition procedures. Therefore, denoising is required to increase the signal to noise ratio up to a point that segmentation of consistent structural features can be obtained. We have applied a combination of Gaussian filtering and anisotropic non-linear diffusion (AND) to take advantage of their best properties (5). AND allowed to define a mask preserving those areas containing consistent structural features, while the background noise and discontinuous features were discarded. The mask was then applied to a Gaussian filtered tomogram, thus retrieving all important features at sufficient resolution and without significant modification of the signal.

Tomograms filtered following the above described scheme were more easily interpretable due to the removal of the noise. Features barely visible in raw tomograms were now evident, revealing a consistent structure of the IMVs: An outer layer consisting with a membrane with protrusions, an area below the membrane that contains aggregates of heterogeneous material (previously described as "lateral bodies"), and a complex core containing a double external layer, a dense area filled with fibrous material and an inner region with lower density.

The outer layer of the tomograms was fully consistent with a lipidic membrane (5 – 6 nm thick). A fairly conspicuous feature found in almost all of the reconstructed particles was the presence of one or, most frequently, two lateral bodies build up by a heterogeneous material without apparent ordering or repetitive features. These masses, although located below the membrane were not connected to it.

The internal core revealed a complex architecture. Two layers surrounded it with an overall thickness around 18-19 nm: The inner one was continuous and it was consistent with a lipidic membrane both in thickness (5-6 nm) and in its general continuous aspect. The outer layer was discontinuous, and presented periodicities that fit to the existence of a periodic palisade that connected the inner with the outer layer. The more likely interpretation of this assembly is that the outer, discontinuous core layer is build by the side interaction of T shaped protein spikes that are anchored in the lower membrane. The systematic analysis of tomogram sections revealed an unexpected feature in the presumptive lipidic membrane of the core: there were consistent discontinuities with a constant morphology. Side sections of the membrane showed places where a channel communicated the inner side of the core with the region outside the layer build by the T-shaped spike palisade. The overall morphology of this pore-like structure consisted of a channel (diameter around 7 nm) surrounded by a cylindrical structure with an outer diameter around 20nm. It is tempting to speculate that these pores may represent channels to release mRNA during early transcription (and, eventually, could be also used for exporting genomic DNA).

The core membrane encloses a region that showed two areas with different density. Just below the membrane there was a densely packaged area with aggregated material. This material eventually showed a fiber-like morphology, without apparent periodicities. These fibers probably represent the presumptive nucleoprotein complexes of the virion. In fact, wounded, interlaced fibers were evident suggesting the ordered compaction of this material. Although the aggregates found in this region were closely apposed to the core membrane, there were no consistent connections between the fibers and either the membrane or the pores. The inner region of the core showed a much lower material density in the different tomograms. As the viral particles were rapidly frozen, this area is not likely to be derived from artifactual precipitation and exclusion of core material, but rather represents a distinct region of the core.

References

- (1) B. Moss (2001). *Fields Virology* (Lippincott, Williams & Wilkins, Phi.).
- (2) J. Krinjse-Locker et al. (1996). *J. Biol. Che.* 271, 14950-14958.
- (3) M. Hollinshead et al. (1999). *J. Virol.* 73, 1503-1517.
- (4) M. Cyrklaff et al. (2005). *Proc. Nat. Acad. Sci. USA* 102, 2772-2777.
- (5) J.J. Fernandez et al. (2003). *J. Struct. Biol.* 144, 152-161.