Localization of Vaccinia virus proteins by Tokuyasu technique

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KEY WORDS : Electron microscopy, Immuno-labelling, Vaccinia virus, Membrane assembly.

The *Poxviridae* constitutes a family of viruses that includes small pox and several zoonotic viruses, such as monkeypox or cowpox. Members of this family are DNA viruses that replicate their double-stranded DNA in the cytoplasm of the host cells. Vaccinia Virus (VACV) is the best characterized poxvirus and is used in most studies as a model-poxvirus infection.
We have shown some time ago that VACV acquires its membrane in an unconventional way from open membrane intermediates derived from the ER of the host cell. These build an open membrane sphere in the cytoplasm and upon DNA uptake they form the fully infectious mature virion (MV). [1]
Our research focuses on the molecular details of this unconventional membrane biogenesis mechanism.
Two classes of proteins have been identified as essential for VACV membrane assembly: the structural proteins A14, A13 and A17, and the conserved viral membrane associated proteins (VMAPs) A11, A6, H7, L2, A30.5.

The aim of our work is to decipher the function and localization of these viral proteins by using a combination of different approaches ranging from biochemical techniques to fluorescence and electron microscopy. In order to precisely and quantitatively localize these viral proteins, we use EM immuno-labelling of thawed cryo-sections (so called Tokuyasu technique). Since anti-A14 antibody has been studied in detail, we are using A14 immuno-labeling to find the optimal conditions for investigations on VMAPs localization and quantification. [2]

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