Application of FIB-SEM tomography, serial sectioning TEM and STEM tomography gives insight into herpesvirus egress dynamics and the process of secondary envelopment

Villinger, C.[1,2], Neusser, G.[3], Schauflinger, M.[4], Mertens, T.[2], von Einem, J.[2] and Walther, P.[1]

[1] Central Facility for Electron Microscopy, Ulm University, Germany, [2] Institute of Virology, University Medical Center Ulm, Germany, [3] Institute of Analytical and Bioanalytical Chemistry, Ulm University, Germany, [4] Electron Microscopy Core Facility, University of Missouri, Columbia, United States

Electron microscopy (EM) of high-pressure frozen and freeze-substituted samples allows unambiguous identification and detailed analysis of the various stages during human cytomegalovirus (HCMV) maturation. However, EM *per se* cannot provide dynamic information. Nevertheless, quantitative structural imaging provides some insight into HCMV egress dynamics since EM captures slow and/or frequent events with a higher chance than quick and/or rare events. Additionally, application of 3D EM techniques enables quantification with high accuracy.

By application of FIB-SEM tomography with TEM-like resolution[1,2] and serial sectioning transmission electron microscopy (TEM)[3] we evaluated the number of HCMV capsids present at different stages of egress: (I) within the nucleoplasm, (II) budding (primary envelopment) into the perinuclear space (PNS), (III) within the PNS, (IV) leaving the PNS and (V) at the viral assembly complex (vAC), which is the site of secondary envelopment and therefore final virus maturation. The capsids at the vAC were also quantified regarding their stage of secondary envelopment (*free*, *budding* and *enveloped*). The numbers were then summarized in a snapshot model including dynamic data from life cell imaging studies[4,5]. First, analysis of the snapshot model suggested nuclear egress and release of virions at the plasma membrane as bottlenecks for the production and release of infectious virions. This knowledge could be useful for development of new antiviral drugs. Second, the high number of capids in the process of secondary envelopment or enveloped at the vAC demonstrates the importance of the vAC for secondary envelopment at host cell membranes by concentrating these membrane systems at a distinct cytoplasmic site. Last, we applied scanning TEM (STEM) tomography to gain a more detailed insight into the process of secondary envelopment at the vAC.

1. Villinger et al., 2012. Histochem Cell Biol 10.1007/s00418-012-1020-6.

2. Villinger et al. 2015. Viruses 7:5686 - 5704.

3. Schauflinger et al. 2013. Cell Microbiol 15:305 - 314.

4. Sampaio et al. 2005. J Virol 79:2754 - 2767.

5. Bosse et al. 2014. mBio 5:e01909-14.