**[O3]**

**Vaccinia Virus Extracellular Virions Exploit Macropinocytosis for Host Cell Entry**

F. I. Schmidt*, J. P. Mercer, A. Helenius

*Institute of Biochemistry, ETH Zurich, Switzerland*

Vaccinia virus (VACV), the model poxviruses, produce two types of infectious particles with distinct roles in virus spread: mature virions (MVs) and extracellular virions (EVs). MVs are surrounded by one membrane; EV particles consist of MVs surrounded by an additional lipid bilayer and therefore require an unusual penetration process. EV infection presumably involves the non-fusogenic disruption of the outermost membrane followed by fusion of the underlying MV with cellular membranes.

Using recombinant strains of Vaccinia virus encoding EGFP or EGFP fusion proteins, we characterized the entry of EV particles into human epithelial cells. By a combination of fluorescence microscopy and flow cytometry, we demonstrate that EV particles are endocytosed and that infection requires actin dynamics, protein kinase C (PKC), P21-activated kinase 1 (PAK1), and $Na^+/H^+$ exchanger activities. This suggests a macropinocytic uptake mechanism. Additionally, EV infection is dependent on the acidification of endosomal vesicles. We find that low pH treatment disrupts the outer EV membrane *in vitro* and can partly rescue infection in the presence of inhibitors of endosomal acidification. This indicates that the disruption of the outer EV membrane is a pH-dependent step.

In conclusion, our data suggest that EV particles are internalized by macropinocytosis. Acidification of macropinosomes is required to disrupt the outermost membrane and expose the viral fusion machinery on the underlying MV. Subsequent fusion of the MV with limiting membranes results in the release of the viral core into the host cell cytoplasm allowing for productive infection.

Keywords: Vaccinia virus, poxvirus, endocytosis, macropinocytosis