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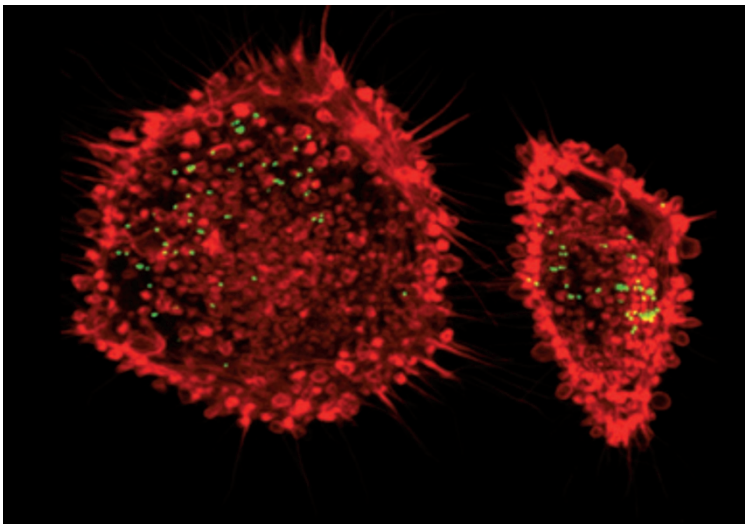
CELLULAR MICROBIOLOGY

Virus plays dead

Vaccinia virus is a large, complex, enveloped DNA virus that belongs to the Poxviridae family of viruses, which includes variola, the causative agent of smallpox. The infectious mature virus (MV) form of vaccinia has been shown to bind to actin-containing finger-like protrusions (filopodia) of the host cell and enter the cell in a pH-dependent manner. In a new study published in *Science*, Jason Mercer and Ari Helenius report that the MV form of vaccinia virus enters host cells using macropinocytosis and apoptotic mimicry.

The authors prepared fluorescent MV particles and used live imaging to follow the entry of individual particles into host cells that expressed fluorescent actin. Virus particles that bound to filopodia moved towards the cell body in an actin-dependent manner. Once they reached the cell body, membrane blebs formed at the site of contact with virus, followed by the formation of further blebs along the entire cell body. The blebs eventually retracted, which coincided with virus entry. Inhibiting membrane blebbing caused a large reduction in infection, which suggests that blebbing is required for infection.

The Ser/Thr kinase p21-activated kinase-1 (PAK1) is essential for MV infection; indeed, knockdown of PAK1 reduced infection significantly. MV infection is accompanied by phosphorylation of residue Thr423 of PAK1, which is known to be essential for macropinocytosis. PAK1 knockdown studies also showed that PAK1 is required for events that occur prior to viral fusion, including blebbing. Perturbance of several other factors, including the PAK1 activator GTPase Rac1 and Na^+/H^+ exchangers, inhibited both blebbing and infection.



Two infected cells that are blebbing. Actin is shown in red and virus particles are shown in green. The blebs appear in cross-section as circles. Image courtesy of Jason Mercer, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland.

This further confirmed that these processes are linked. Sensitivity to Na^+/H^+ exchangers is also characteristic of macropinocytosis. Indeed, MVs co-internalized with fluid-phase cargo, but not clathrin-dependent cargo, in a macropinocytosis-type endocytic process.

Apoptotic bodies are macropinocytosed by phagocytes and other cell types, and uptake of apoptotic debris is triggered by the presence of exposed phosphatidylserine (PS) on the plasma membrane. The MV membrane is known to be enriched in PS, which is required for infection. The authors showed that PS is exposed on the MV surface and that virus particles extracted with a detergent that removes all lipids fail to induce blebbing and infection. Reconstitution of the extracted virus particles with PS restored infection. Because MVs are comparable in size to apoptotic bodies and the uptake mechanisms are the same, Mercer and Helenius concluded that viral PS might be analogous to cellular PS and triggers the uptake of virus particles by mimicking apoptotic bodies. Consistent with this, late-stage vaccinia-infected cells were shown to undergo apoptosis.

The clever use of apoptotic mimicry and subsequent entry by macropinocytosis allows large particles such as vaccinia virus to infect many different potential host cells. And by posing as apoptotic bodies, MVs may also avoid immune detection.

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ORIGINAL RESEARCH PAPER Mercer, J. & Helenius, A. Vaccinia virus uses macropinocytosis and apoptotic mimicry to enter host cells. *Science* **320**, 531–535 (2008)

FURTHER READING Gruenberg, J. & van der Goot, G. Mechanisms of pathogen entry through the endosomal compartments. *Nature Rev. Mol. Cell Biol.* **7**, 495–504 (2006)