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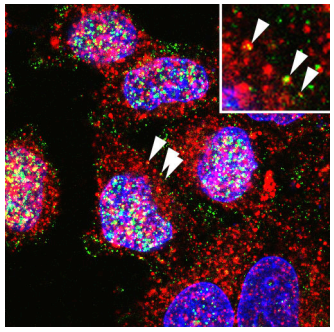
Signaling specifically from the endosome

For Akt signaling, location matters, according to Annette Schenck, Marino Zerial (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany), and colleagues.

Akt (protein kinase B) influences a wide variety of pathways in the cell, but how it divides its activity between them was unclear. Akt is known to signal from the plasma membrane, but an upstream regulator of Akt called APPL1, which is known to interact directly with Akt, has been reported to reside on endosomes.



ZERIAL/ELSEVIER

Akt (green) and APPL1 (red) colocalize (arrows) on endosomes, where APPL1 triggers Akt signaling.

Here, the authors showed that loss of APPL1 affected the activity of some downstream targets of Akt, but not others. For example, the activity of Gsk-3β, an Akt target involved in cell survival, was diminished by APPL1 loss, while the activity of Tsc2, an Akt target that promotes growth, was unaffected.

These differential effects were tied to differences in location: Gsk-3β, but not Tsc2, linked up with Akt and APPL1 on the endosome. And the endosomal location was clearly important, since neither nuclear-targeted nor cytosolic APPL1 could rescue cells with depleted endosomal APPL1.

"This is the first time Akt signaling has been shown from the endosome," Zerial says. "We think these results should make the signaling community take seriously the contribution of endocytic trafficking to the quality and quantity of signaling." JCB

Schenck, A., et al. 2008. *Cell.* 133:486–497.

Quick flip = one-way proton trip

One amino acid's quick flip maintains a one-way flow of protons across the mitochondrial membrane, according to Ville Kaila, Marten Wikström (University of Helsinki, Finland), and colleagues.

ATP production in mitochondria depends on maintaining a proton gradient across the inner membrane. To establish this gradient, protons are drawn up from the mitochondrial matrix through the interior of cytochrome c oxidase, to a glutamic acid residue in the core of the protein (position 242). This glutamic acid was known to act as a switch—in its down position it accepts protons, while in its up position it sends most protons up the concentration gradient to accumulate in the intermembrane space (it also diverts some protons to the enzyme's heme group, where they react with oxygen, making water and powering the whole process). But a key question has been how the enzyme keeps protons from flowing backward, down their concentration gradient.

Using molecular dynamics simulations, the team now shows that the preferred orientation of the glutamic acid switch depends heavily on its protonation state. With a proton attached, it was equally stable pointing either up or down. But once the proton detached, the side chain flipped back down in a picosecond. "The unprotonated glutamic acid prefers the down state by a factor of at least ten thousand," Wikström says. Its rapid movement takes it quickly out of reach of the proton it has just dropped off, and thus it serves as a one-way valve for proton transfer. JCB

Kaila, V.R.I., et al. 2008. *Proc. Natl. Acad. Sci. USA.* doi:10.1073/pnas.0800770105.

Take a big gulp of pox

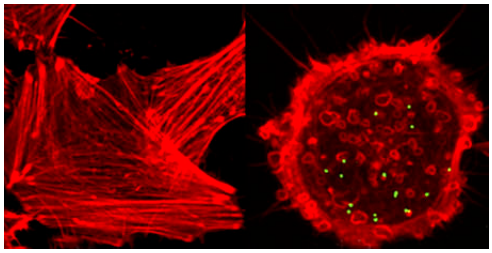
A poxvirus tricks cells into drinking it up, say Jason Mercer and Ari Helenius (Institute of Biochemistry, Zurich). Vaccinia, studied here, is a prototypical poxvirus, whose members also include the human smallpox virus.

In studying how vaccinia enters cells, the authors observed that mature virus particles bound to filopodia and surfed toward the cell. After arriving at the cell body, the virus particles induced the entire cell surface to erupt into blebs, which, when retracting, engulfed the virus. To the authors' amazement, a single virus particle was sufficient to induce this dramatic behavior.

Entry could be inhibited by the myosin II inhibitor, blebbistatin, as well as by inhibitors of actin dynamics and endosomal fusion. These and other clues, such as the requirement of p21-activated kinase and Na^+/H^+ exchangers, pointed to a central role for macropinocytosis in viral entry. As confirmation, the authors showed that fluid-phase, but not clathrin-mediated, markers were internalized along with the virus. "This is the first connection of blebbing and macropinocytosis in eukaryotes," says Mercer.

One function of macropinocytosis is engulfment of apoptotic debris, which is triggered by contact between the engulfing cell and membranes with exposed phosphatidylserines (PS), which are normally hidden on the inner membrane surface of cells. The vaccinia membrane is known to be rich in PS, and when the authors blocked PS, infectivity dropped; the virus still bound to cells, but no blebbing or entry occurred. "It's a beautiful way to invade a lot of different cell types," Mercer says, "because uptake of phosphatidylserine is such a general mechanism. The virus is taking advantage of a system the cell can't get rid of." JCB

Mercer, J., and A. Helenius. 2008. *Science.* 320:531–535.



MERCER/AAAS

A cell exposed to vaccinia (green dots) forms widespread blebs on its surface.