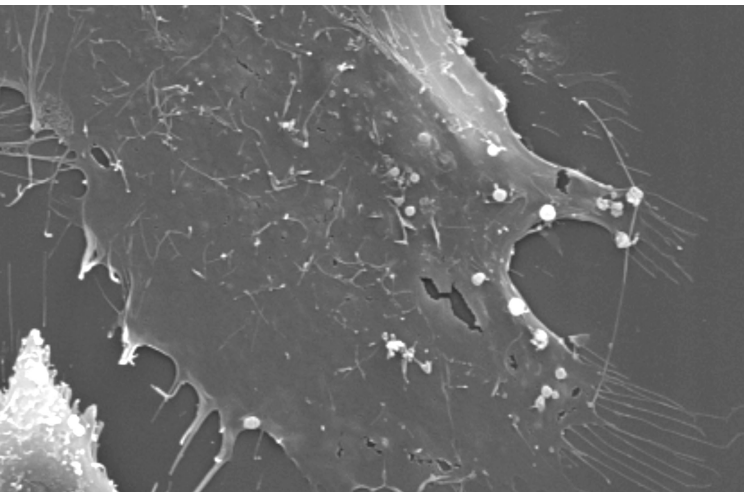
Research Roundup

Tumor cells share oncogenic receptors

Mutant receptors made in one tumor cell can be passed to tumor cells lacking them, say Khalid Al-Nedawi, Janusz Rak (McGill University, Montreal), and colleagues, increasing oncogenicity of the entire tumor.

In a certain type of human brain cancer called glioma, the gene encoding epidermal growth factor receptor is often mutated, creating a version of the receptor that is truncated and overactive. The presence of this mutant version, called EGFRvIII, can signify a more aggressive disease state, even if many cells in the tumor don't express the receptor gene. Expressing and nonexpressing cells both display the mutant protein, however, and both contribute to malignancy. "It was hard to understand how receptor expression in one small set of cells could upgrade the entire tumor" to the more aggressive form, Rak says.



RAK/MACMILLAN

Microvesicles (bright dots) containing mutant receptor are shed by glioma cells.

The authors found that glioma cells expressing EGFRvIII transferred this errant receptor to nonexpressing cells via microvesicles—small plasma membrane buds. The microvesicles were produced in abundance by the mutant expressing cells and were widely taken up by receptor-negative cells. Within 24 hours, these recipient cells had increased receptor-triggered downstream signaling and, compared with cells without receptors, could form twice as many colonies in agar—a standard sign of increased malignancy.

"We propose there is a much greater level of communication between cancer cells than is usually appreciated," Rak says. Microvesicles aren't just shared among cells; previous work has shown that they're also shed into the bloodstream. The finding that glioma cells are sending out microvesicles could therefore potentially lead to a less invasive means of brain tumor characterization. JCB

Al-Nedawi, K., et al. 2008. *Nat. Cell Biol.* doi:10.1038/ncb1725.

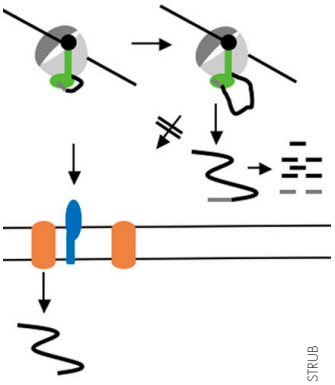
Too long for translocation?

Peptides that are made too quickly can't get into the ER, say Asvin Lakkaraju, Katharina Strub (University of Geneva, Switzerland), and colleagues. It seems that the peptides become too long for the translocation machinery to deal with.

The signal sequence at the amino terminal of a growing peptide chain is required for translocating the peptide into the ER. The signal sequence binds a signal recognition particle (SRP), which in turn binds to an SRP receptor on the endoplasmic reticulum and guides the nascent chain to a translocon—a channel that feeds the new peptides into the ER. Mammalian SRP has been known to delay elongation in vitro, but the significance of the delay, and its occurrence in vivo, was unknown.

To explore SRP's role in vivo, the authors prepared a mutated version of human SRP14 that specifically lacked the delaying function. SRP14's ability to bind to nascent peptides and to the SRP receptor remained intact. In cells that carried the mutant SRP, elongation sped up, but the final concentration of secreted protein dropped and growth suffered. Closer inspection revealed that translocation in these cells had slowed down and nascent peptide chains were being degraded.

The effects of mutant SRP could be mitigated with antibiotics that slowed elongation. They could also be mitigated by increasing the number of SRP receptor molecules. Together, the data suggest that overly long peptides, produced as a result of faster elongation, can still bind to the SRP receptor but do so unproductively—perhaps by inhibiting the receptor's ability to engage with the translocon.



STRUB

SRP (light green) targets nascent chains via the SRP receptor (blue) to the translocon (orange) for translocation into the ER. Chains that become too long before translocation are degraded.

Translation elongation, it appears, has the ability to go at a much faster pace than the normal cellular translocation machinery can cope with, and thus requires SRP to put the brakes on. Why elongation has evolved to be so super speedy, however, is not yet clear. JCB

Lakkaraju, A.K.K., et al. 2008. *Cell.* 133:440–451.

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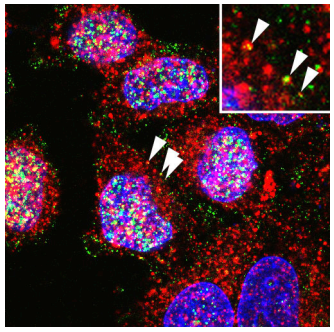
Signaling specifically from the endosome



ZERIAL/ELSEVIER

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

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.

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

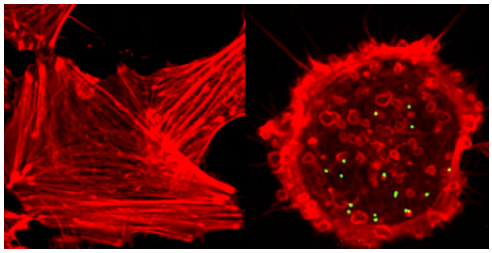
A poxvirus tricks cells into drinking it up, say Jason Mercer and Ari Helenius (Institute of Biochemistry, Zurich). Vaccinia, studied here, is a prototypical poxvirus, whose members also include the human smallpox virus.

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.