In This Issue

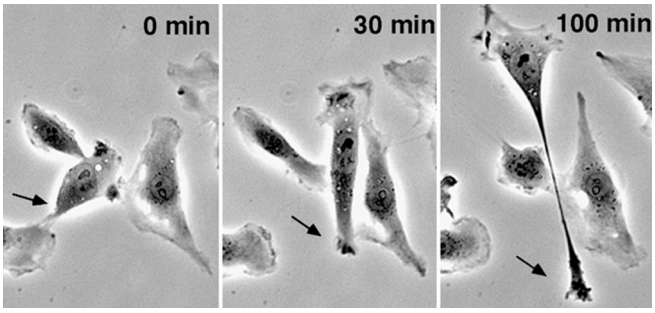
Backtracking on FAK

To decipher how cells crawl, researchers have knocked out a key signaling protein known as focal adhesion kinase (FAK), which relays messages from the cell surface. But the results of these knockout studies have been confused by the fact that a FAK paralogue gets up-regulated in these cells. Lim et al. now clear up the picture.

As a cell crawls along a surface, it temporarily attaches at sites called focal adhesions. FAK concentrates at focal adhesions and passes on signals from integrins that have latched onto molecules in the extracellular matrix. Fibroblasts missing FAK remain rounded up instead of flattening out on a surface and crawl sluggishly. Both of which might be expected if focal adhesions are impaired. However, FAK-lacking cells actually have an inordinate number of focal adhesions and have increased RhoA activity—a factor that spurs focal adhesion and stress fiber formation, thus promoting the rounded shape by increasing the internal contractile force.

Lim et al. now show that some of these observations can be explained by the fact that when FAK is lacking, fibroblasts manufacture more of FAK's paralogue, proline-rich kinase (Pyk2).

To sort out Pyk2's contributions, Lim et al. used RNAi to knock down the protein in fibroblasts that also lacked FAK. This reduction restored normal focal adhesions and RhoA activity pattern and reverted cells to a flattened shape.



Because their back ends get stuck, cells missing FAK and Pyk2 stretch until they break.

The FAK- and Pyk2-lacking cells continued to have a motility problem, however. The cells couldn't detach their rear ends from the surface, the team found. The cells stretched and stretched until their anchored back ends broke off and the rest of their cell bodies snapped forward like rubber bands. That result indicates that the immobility of FAK-lacking cells isn't due to extra Pyk2.

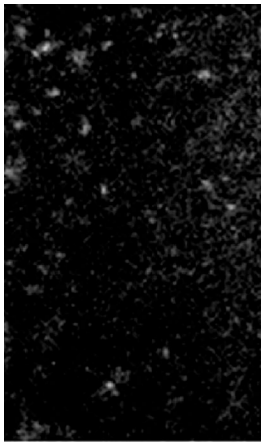
Exactly why Pyk2 gets up-regulated in FAK-deficient cells, and how overexpression of PyK2 causes constitutive RhoA expression, is unclear. In any case, the work suggests that researchers should take a second look at conclusions based on FAK-deficient cells. JCB

Reference: Lim, Y., et al. 2008. *J. Cell Biol.* 180:187–203.

Traffic control in the ER

The endoplasmic reticulum (ER) contains the equivalent of speed bumps to prevent newly synthesized proteins from traveling too fast and crashing into one another, Nagaya et al. report. The researchers were the first to observe individual proteins moving in the ER.

Freshly made proteins fold into shape as they travel through the ER. But if proteins run into one another before they finish the job, they can end up misshapen or stick together to form potentially toxic aggregations. Chaperones inside the ER, such as the lectin calnexin, help some proteins avoid this fate. But Nagaya et al. wanted to test whether the ER also has a mechanism to put the brakes on speeding proteins.



Imaging single proteins (white) traveling through the ER reveals traffic control.

The researchers found that they could stall movement of some proteins in the ER by exposing cells to a hyperosmotic solution. Molecules that got stuck usually sported oligosaccharides called N-glycans, suggesting that these attachments help slow the proteins down. To get a closer look at events inside the ER, the team turned to total internal reflection fluorescence microscopy. Researchers had previously applied this technique to observe molecules on the cell surface, but Nagaya et al. were able to use it to track proteins moving through the ER just beneath the cell membrane. Instead of progressing smoothly through the tubules, the proteins appeared to be catching. However, they didn't seem to be getting stuck in the ER exit sites.

The researchers also discovered that this slowing required actin but not microtubules. That finding suggests that the actin cytoskeleton exerts control over movements of proteins in the ER. Nagaya et al. hypothesize that lectins cluster on the inner wall of the ER and then grab a passing protein's N-glycans, temporarily detaining it. Actin might help the lectins congregate by corralling them or by serving as a platform where they can gather. JCB

Reference: Nagaya, H., et al. 2008. *J. Cell Biol.* 180:129–143.

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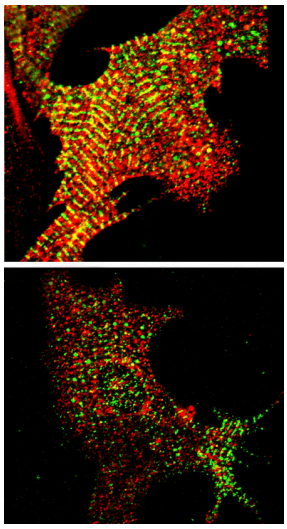


Normal levels of a sodium channel (red, top) are reduced in cardiac cells with too little ankyrin-G (green, bottom).

Putting channels in their place

Without voltage-gated sodium channels, heart muscle cells can't keep a beat. Lowe et al. show how these cells, known as cardiomyocytes, direct channels to the right domain of their plasma membrane.

Opening $Na_v1.5$ sodium channels allows an inrush of sodium ions that depolarizes a cardiomyocyte. Patients who carry a faulty version of the channel are at risk for potentially lethal heart arrhythmias. Rather than scattering around the membrane, the channels home in on the junctions between cardiomyocytes. In neurons, the membrane skeletal protein ankyrin-G helps direct sodium channels to specific membrane domains, and Lowe et al. tested whether this ankyrin-based pathway performed the same function in heart cells.

When the researchers knocked down ankyrin-G using RNAi, they found that $Na_v1.5$ resided around the nucleus instead of in the membrane. The amount of $Na_v1.5$ in the cell also fell, possibly because wayward channels get degraded. After stimulation, cardiac muscle cells lacking ankyrin-G produced a smaller-than-normal current.

The knockdown of ankyrin-G had no effect on the location or activity of the cells' primary voltage-gated calcium channel, suggesting that the targeting pathway in heart cells is specific for $Na_v1.5$. By testing mutant forms of ankyrin-G that can't bind to the channel, the team showed that interactions between the two proteins are necessary to get $Na_v1.5$ into position.

The big question now is how ankyrin-G does the job. The protein might haul $Na_v1.5$ to the membrane or stabilize channels that have already made it there. JCB

Reference: Lowe, J.S., et al. 2008. *J. Cell Biol.* 180:173–186.

Genetic feng shui for cancer cells?

One of the first things a cancer cell does is move around some of its genes, Meaburn and Misteli show.

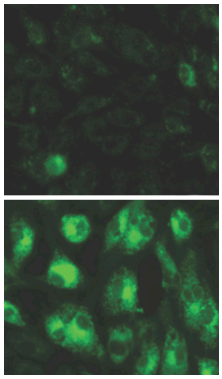
Cells rearrange their DNA during certain diseases. In neurons from people with epilepsy, for instance, the X chromosome tends to be closer to the middle of the nucleus than in neurons from nonepileptics. Researchers have also observed chromosomal relocation in the few cancers they have examined. However, these observations involved late-stage disease, and no one knew what happened early in tumor development.

Meaburn and Misteli scrutinized mammary cells that they had coaxed to grow abnormally. When the researchers pinpointed 11 genes, they found that four—including the antiapoptotic gene BCL2—were in different positions in the cancer cells than in normal cells. Three of the genes were closer to the edge of the nucleus in the tumor cells, and one was closer to the center.

Some studies suggest that active genes tend to shift into the interior of the nucleus, whereas inactive genes are marginalized. But the team determined that the location of a particular gene in the nucleus didn't depend on its expression level. For example, activity of the gene for the extracellular matrix protein MMP1 shot up in the cancerous cells, but the gene remained in place. The researchers conclude that early in tumor formation, cancer cells reposition certain genes. Why the cells go to the trouble is a mystery, but the discovery might lead to new diagnostic tests. JCB

Reference: Meaburn, K.J., and T. Misteli. 2008. *J. Cell Biol.* 180:39–50.

Tuning up mitochondria for angiogenesis



Fewer mitochondria depolarize (green) in control cells (top) than in cells lacking prohibitin-1 (bottom).

During angiogenesis, the protein prohibitin-1 doesn't live up to its name. Instead of stopping some cellular process, it encourages new blood vessel growth by ensuring that mitochondria run efficiently, as Schleicher et al. reveal.

For lower organisms, prohibitin-1 is an asset. It keeps mitochondria functioning smoothly, and its loss pushes cells into the low-activity, slow-dividing state called senescence. The researchers suspected that prohibitin-1 would do a similar job in mammals and might be particularly important for vascular homeostasis, since mitochondrial maintenance is known to be important for vascular health—reactive oxygen species (ROS) that spill out from malfunctioning mitochondria can damage endothelial cells and prompt atherosclerosis.

The team knocked down the protein in mammalian endothelial cells and found that the cells' ROS levels shot up and several signs of senescence became apparent. Without the protein, cells also moved less and wouldn't roll up into tubes, suggesting that angiogenesis as well as homeostasis was affected.

To gauge prohibitin-1's effects on angiogenesis in vivo, the team injected mice with small amounts of a gel that contains the blood vessel growth promoter VEGF. Endothelial cells swarmed into the gel—unless it also contained RNAi that blocks prohibitin-1. The rapid increase in ROS following prohibitin-1 loss was caused by depolarization of the mitochondria membranes, suggesting that prohibitin-1 somehow maintains membrane integrity. JCB

Reference: Schleicher, M., et al. 2008. *J. Cell Biol.* 180:101–112.