In This Issue

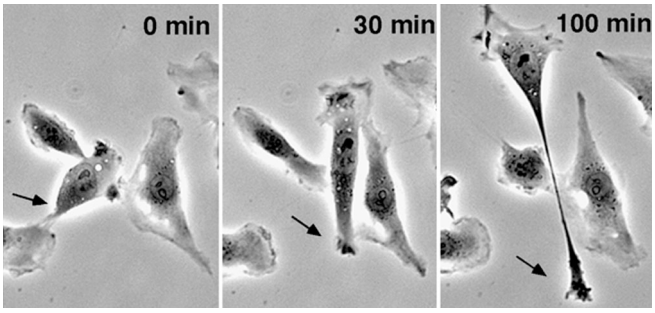
Backtracking on FAK

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.

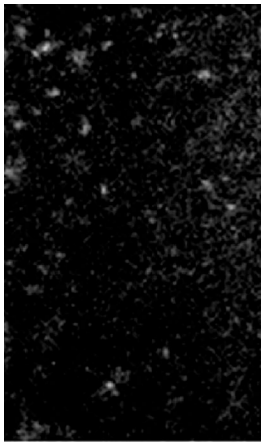
Exactly why Pyk2 gets up-regulated in FAK-deficient cells, and how overexpression of PyK2 causes constitutive RhoA expression, is unclear. In any case, the work suggests that researchers should take a second look at conclusions based on FAK-deficient cells. JCB

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.