In This Issue


Cytochrome b5 (green) normally localizes in the ER, but without its targeting sequence is redirected to the outer segment (right).

No direction? Join the crowd

Sometimes you can get to your destination just by following the crowd. Baker et al. report that photoreceptor proteins lacking targeting sequences end up in the right place thanks to their more abundant, and more goal-oriented, fellow travelers.

Rod and cone cells are divided into four compartments: a synaptic terminal, a nuclear region, an inner segment for protein and lipid synthesis, and an outer segment that contains a highly folded membrane packed with proteins such as light-sensitive rhodopsin. Rhodopsin and some other outer-segment proteins contain targeting signals directing them there, but most outer-segment proteins do not.

To understand how these signal-lacking proteins reach their destination, the authors examined the trafficking of two proteins: R9AP, which is almost exclusively found in the outer segment, and the structurally similar syntaxin 3, localized to the inner segment. Switching the transmembrane domains of the two had no effect on their final destinations, and neither did whittling away at R9AP's cytoplasmic domain. Removing the cytoplasmic domain of syntaxin-3, on the other hand, led the protein to accumulate in the outer segment, whereas adding this domain to R9AP led the protein to accumulate in the inner segment. Syntaxin's cytoplasmic domain thus specifically targets proteins to the inner segment, and without it proteins head to the outer segment by default. The case for the outer segment as the default destination was clinched by removing signal sequences from multiple proteins, all of which then ended up in the outer segment.

R9AP and other signal-lacking proteins might be randomly packaged into vesicles bound for all destinations, but since rhodopsin-bearing vesicles headed for the outer segment are so abundant, "directionless" proteins will wind up where they belong just by going with the crowd.

Baker, S.A., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200806009.
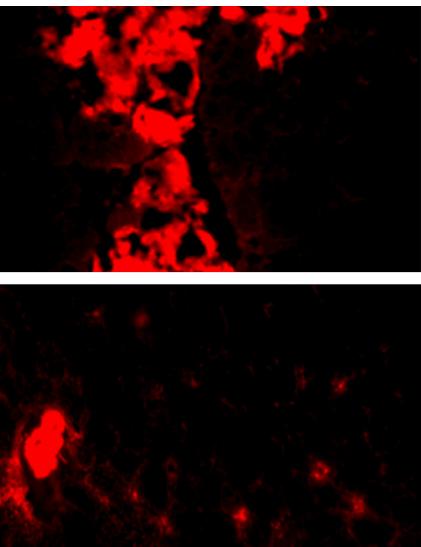
Unstable muscle membranes in the *mdx* mouse (top), indicated by infiltration of a dye, are stabilized by sarcospan (bottom).

Sarcospan, a little protein for a big problem

The overlooked and undervalued protein, sarcospan, just got its moment in the spotlight. Peter et al. now show that adding it to muscle cells might ameliorate the most severe form of muscular dystrophy.

In Duchenne muscular dystrophy (DMD), the mutated dystrophin protein fails to anchor correctly to its membrane glycoprotein complex. Without this anchoring, muscle cells experience severe contraction-induced damage. Sarcospan is part of the anchoring complex, but because mice without sarcospan don't seem any worse for its absence, it hasn't received much attention. Sarcospan's structure, however, suggests it might help stabilize the membrane complex, so the authors decided to test the effects of increasing sarcospan expression in a DMD mouse model.

The increase did not improve the dystrophin–glycoprotein interaction, but instead, the team was surprised to find sarcospan coaxed a dystrophin relative called utrophin to spread out on the muscle membrane. Utrophin is normally restricted to the neuromuscular junction, where it serves a function similar to that of dystrophin.

The extra sarcospan prompted higher levels of utrophin in the cell, but not by increasing its expression. Sarcospan instead stabilized extrajunctional utrophin complexes, which normally form early in development and then disappear after the first few weeks of life.

Mouse muscle cells were protected by sarcospan, but the true importance of this discovery will lie in its potential for human therapeutics, specifically gene therapy. In that regard, sarcospan's small gene size is significant—at 600 bp, it is easily packaged into the safest viral vectors, unlike either dystrophin or utrophin, which are about 700 times larger and require more immunogenic vectors.

Peter, A.K., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200808027.

BBB building

Construction of the brain's border fence is supervised by Wnt/β-catenin signaling, report Liebner et al.

Like many a nation, the brain requires tight border security to maintain levels of nutrients and keep out toxic substances. The blood–brain barrier (BBB) is a virtually impermeable network of

tight junctions between endothelial cells that prevents paracellular flow of materials. Because Wnt/β-catenin signaling is a major pathway regulating other aspects of brain development, the authors examined its potential involvement in constructing the BBB.

In brain endothelial cells, Wnt signaling was active during the time of maximum vascular development, but not after the BBB matured. Activation of the Wnt signaling pathway in vivo and in vitro promoted BBB development, and inactivation prevented it. In vitro, increasing Wnt signaling also strengthened junctions between non-brain endothelial cells.

This suggests that Wnt signaling might be tweaked to mend the BBBs in patients where it has failed—such as in stroke—or to temporarily open the BBB to deliver drugs that would normally be shut out.

Liebner, S., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200806024.
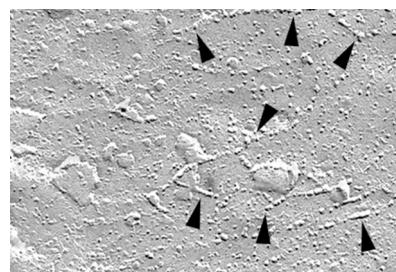
Wnt signaling promotes tight junction formation (arrowheads).

If you build it, leukocytes will come (in)

As leukocytes rush by in the blood, the cells of the vessel wall are ready with a convenient handhold in the form of tetraspanin-enriched membrane microdomains, say Barreiro et al.

To fight infection, circulating leukocytes must exit the bloodstream, a process known to involve tetraspanins, membrane proteins that bring together other membrane proteins such as adhesion receptors.

To discover how tetraspanins assist leukocytes out of the blood, the team visualized them in live vascular endothelial cells by FRET-FLIM. Even in the absence of leukocytes, tetraspanins interacted with each other and with adhesion receptors such as VCAM-1 and ICAM-1 to form sticky nanoplatforms. The nanoplatforms then coalesced into larger structures, which presented the leukocytes with a plethora of adhesion receptors to grab onto. The platforms likely increase efficiency of leukocyte adhesion and bloodstream exit in the face of the high shear stress within the bloodstream, says author Francisco Sánchez-Madrid.

Barreiro, O., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200805076.
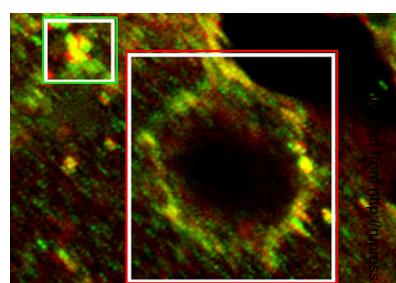
Adhesion receptors cluster into adhesive platforms (small box) on the surface of endothelial cells before engaging leukocytes at the docking structure (large box).

Microtubule failure assists cleavage success

When it comes to positioning the cleavage furrow for cytokinesis, microtubules that fall apart are as important as stable ones, according to two papers by Foe and von Dassow and Odell and Foe.

The cleavage furrow forms at the equatorial region of the cell, when the underlying cortex transiently accumulates active myosin II that pulls on cortical actin filaments, constricting the membrane. It's clear that positioning the furrow is one job of the mitotic apparatus, and probably its microtubules, but exactly how has been a mystery. To investigate this, von Dassow and Foe looked at microtubule dynamics and active myosin II distribution at various points during cytokinesis. They found that just prior to cleavage, the entire cortex was transiently depleted of activated myosin, setting the stage for its focused repositioning later on. Then, during anaphase, a set of stable microtubules formed, stretching from the centrosome to the equator, while short-lived dynamic microtubules spread to contact other regions of the cortex. Active myosin accumulated only where the stable microtubules contacted the cortex. The factors responsible for stability of the equatorial microtubules are not yet known.

These results were accounted for in a computer model by Odell and Foe when both types of microtubules bound centralspindlin, a complex of kinesin motor and Rho modulators that assembles on microtubules and motors toward the cortex. The stable microtubules acted as long-lasting rails, allowing time for centralspindlin to reach the cortex and activate myosin II. Meanwhile, excess centralspindlin remained sequestered on dynamic microtubules, but couldn't reach the cortex before those microtubules depolymerized, thus reducing contractility everywhere else.

Foe, V.E., and G. von Dassow. 2008. *J. Cell Biol.* doi:10.1083/jcb.200807128.
Odell, G.M., and V.E. Foe. 2008. *J. Cell Biol.* doi:10.1083/jcb.200807129.
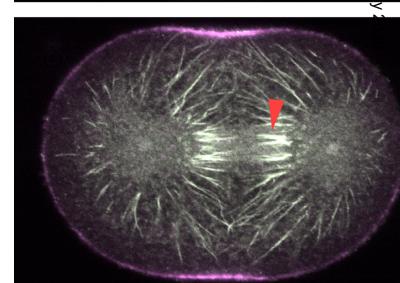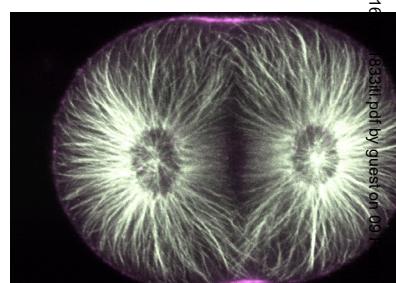
Nocodazole treatment to remove dynamic microtubules (below) reveals stable microtubules (arrowhead) that deliver centralspindlin to the equatorial cortex, activating myosin II (lavender).