

In This Issue

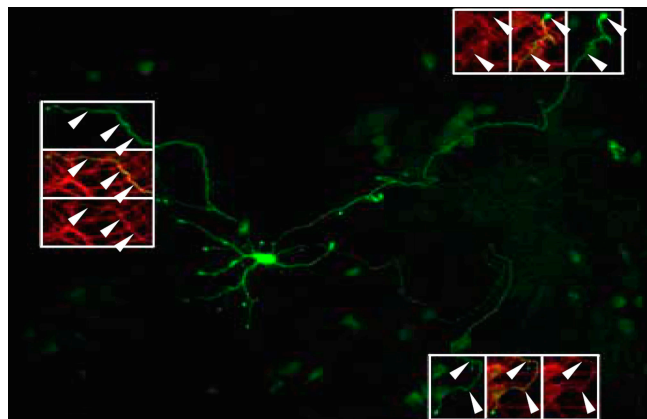
A protein long shot

In one race at least, having a chemical advantage over the competition is okay. This race determines which branch from a developing neuron becomes an axon. As Toriyama et al. report on page 147, the winning extension gets a boost from a previously undescribed protein that spurs axon growth.

Although it sports an axon at one end and a fringe of dendrites at the other, a neuron starts out symmetrical. The imbalance develops because the branches, or neurites, that sprout from a youthful cell compete with each other. The fastest-growing extension typically morphs into the axon, and the stragglers become dendrites. Researchers have identified some of the molecular events that dictate which neurite transforms into an axon—the enzyme PI 3-kinase accrues in the winning branch, for example. But they do not understand what sets up the asymmetry.

Toriyama et al. identified one candidate, a new protein they dubbed shootin1, whose levels soar in axons and during cell polarization. Early in a neuron's development, shootin1 quantities fluctuate in neurites. But they shoot up and remain high in the neurite that will become the axon, while plummeting in the losers. When the team genetically modified cells to over-express shootin1, multiple axons sprouted; slashing shootin1 production blocked cell polarization.

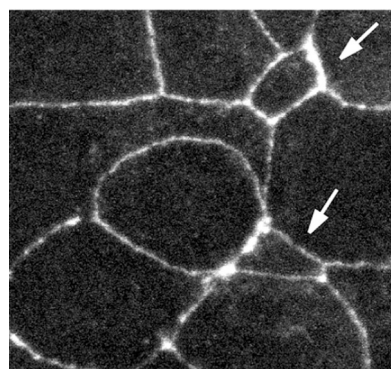
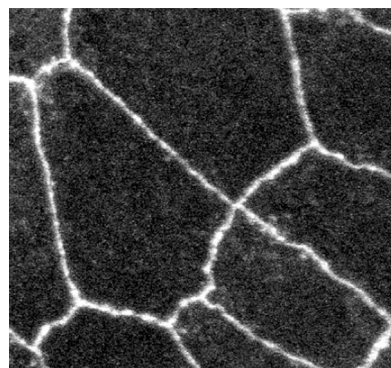
To determine how the protein interacts with PI 3-kinase, the team suppressed shootin1 levels. The kinase no longer accumulated



Too much shootin1 (green) results in a neuron with a surplus of axons (red; arrowheads).

in growing axon tips. The findings suggest that shootin1 spurs neurons to polarize by controlling the location of PI 3-kinase.

The researchers hypothesize that random fluctuations in shootin1 levels unleash a positive feedback loop that promotes polarization. A neuron actively transports shootin1 into the neurites, and the protein diffuses back to the cell body. If by chance one neurite gets a little more shootin1 than the other branches, it will outgrow them. As the lucky neurite extends, shootin1's diffusion time stretches. The protein would thus remain in the neurite longer, propelling even more growth. **JCB**



When Tuba goes silent (bottom), the plasma membrane droops (arrows).

Not so close

Like passengers in a crowded subway car, cells try to limit contact with their neighbors. Otani et al. on page 135 identify a protein that helps cells minimize their closeness by keeping their membranes taut.

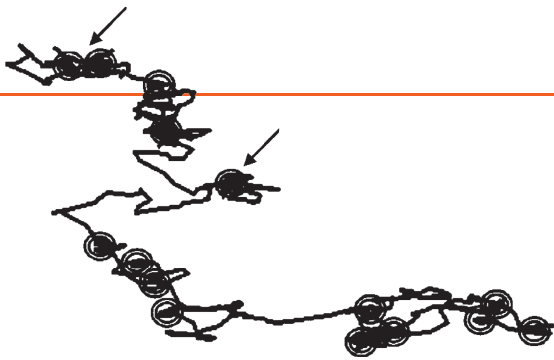
Subway passengers can pull in their elbows, but cells in an epithelial layer cannot avoid touching other cells. Instead, they tend to assume a hexagonal shape in which the membrane is stretched tight. Previous studies suggest that the proteins cadherin and actin help structure the adherens junctions that form at the borders where cells meet. The mystery is, what controls the membrane's tension between these intersections?

To find out, Otani et al. investigated the little-studied protein Tuba, which belongs to a group of factors that indirectly regulate actin. The researchers found that Tuba built up at cell edges. Cutting its levels with RNAi caused the cell membrane to sag, indicating that the molecule

helps tighten the structure. In normal cells, a mesh of cadherin and actin runs along the inside of the membrane and connects to the adherens junction. But in the Tuba-depleted cells, the cadherin network fractured, and fewer strong, thick actin fibers were present.

Tuba switches on Cdc42, one of the RhoGTPases that regulates actin. Like Tuba, Cdc42 accumulated at cell-cell junctions, the researchers discovered. Reducing Cdc42 activity in cells produced the same cadherin and actin defects as RNAi. But if the Tuba knock-down cells made a hyperactive version of Cdc42, their membranes were taut.

The results suggest that Tuba controls the tension of the cell membrane by adjusting actin polymerization through Cdc42. The finding makes sense, the researchers note, because actin helps regulate the elasticity of the cell surface. The actin network might keep cells in shape by bracing the membrane and helping the boundaries remain straight. **JCB**



The journey of a cluster of GPI-anchored proteins around the cell membrane includes occasional stops (arrows).

membrane. Scientists suspect that these structures permit communication, but they do not understand the mechanism.

By treating fibroblasts with gold particles coated in antibodies, Chen et al. forced two kinds of GPIAPs to clump. These clusters halted for up to 10 seconds, a behavior dependent on cholesterol and Src kinases.

The authors hypothesize that, during the stops, proteins in the patches connect to the underlying cytoskeleton. Supporting that conclusion, the cystic fibrosis transmembrane conductance regulator, which does span the membrane, also shows the intermittent stops. But a version that cannot bind to the cytoskeleton just keeps drifting. The researchers propose that clusters unite GPIAPs with a membrane-bridging molecule that links to the cytoskeleton. The identity of this connector remains elusive, however. **JCB**

Division of labor

More than 100 proteins collaborate to build the kinetochore. Using RNAi, Liu et al. (page 41) sort out the responsibilities of key proteins in this process.

The kinetochore is a three-layered disc that sits on either side of the centromere. It links to spindle fibers and helps align and separate mitotic chromosomes. Although researchers have teased out the roles of some kinetochore proteins, they lacked a comprehensive picture of how these molecules interact to assemble the structure.

Liu et al. picked 20 putative kinetochore big shots and knocked them down, one at a time, using RNAi. The team then merged its results with past findings to sketch a map of the connections. Sitting atop the protein hierarchy is CENP-A, which permanently resides on centromeres. At the next level, three branches split off: two are headed by other centromere fixtures, CENP-I and CENP-C, and the third under the direction of the Aurora B kinase, a passenger protein crucial for chromosome separation.

Each branch takes on a different task. CENP-I establishes the three-layered organization, for example. Multiple cross-links tie the branches together, however, so there is no linear chain of command. The researchers are now investigating whether interacting proteins make direct contact or whether other molecules serve as intermediaries. The interaction map might be useful to pharmaceutical researchers developing anticancer drugs that disrupt kinetochore proteins. The map may point the way to biomarkers for monitoring the drugs' effects. **JCB**

Brief encounter

At first glance, some of the cell's communication proteins seem ill-suited for the job. They are embedded in the outer layer of the plasma membrane and have no direct connection to the cell interior. Yet they manage to relay signals across the membrane. The proteins tend to congregate, and on page 169, Chen et al. show that this gregariousness might enable them to link to the cytoskeleton, a step necessary to pass on messages.

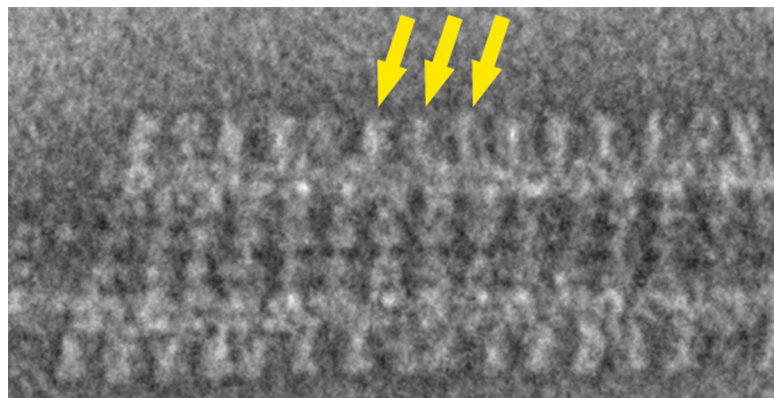
The study focused on GPI-anchored proteins (GPIAPs), some of which interconnect and spur cell division. Cross-linked GPIAPs band together with lipids and other molecules to form clusters that travel together around the

Ring around the tubule

The kinesin protein family is in the trucking business, hauling vesicles and other cellular cargo along the microtubules. The family's black sheep is kinesin-13, which disassembles the filaments while riding on them. Tan et al. report on page 25 that kinesin-13 is unusual in another way. The protein forms rings and spirals around microtubules. The structures might help it maintain its position as a tubule shrinks.

By shortening microtubules, kinesin-13 helps chromosomes to go their separate ways during mitosis. But how kinesin-13 performs the job is mysterious. The researchers spotted the rings and spirals when they combined microtubules with the motor domain of kinesin-13, the protein segment that latches onto the filament. Other types of kinesin did not coil up, the scientists showed. Molecular reconstructions suggest that each ring consists of several kinesin-13 molecules encircled by a strand of free tubulin, the building block of microtubules.

Why kinesin-13 gets into a twist is not certain, but Tan et al. speculate that the formation slides along the microtubule like a sleeve, keeping kinesin-13 at the end of the shortening tubule. To test that possibility, the researchers tagged kinesin-13 with green fluorescent protein. As the microtubule depolymerized, its tip glowed brighter, indicating that the rings were bunching up at the end. **JCB**



Coils of kinesin-13 (arrows) surround a microtubule.