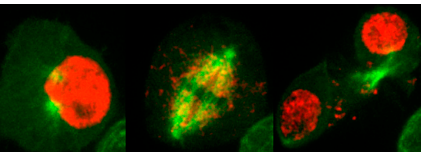
In This Issue



In these time-lapse shots of a mitotic cell, Megator (red) hovers near the spindle.

Spindle's hidden collaborator exposed

The spindle that separates chromosomes during mitosis has a shadowy partner, as Lince-Faria et al. show. The structure serves as a staging area for checkpoint proteins that decide whether the cell can advance through mitosis.

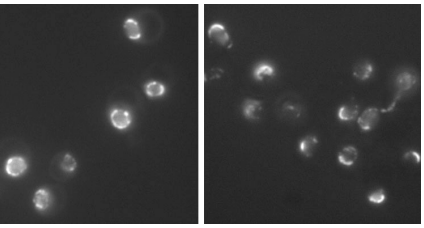
Researchers have long suspected that the microtubular spindle has a companion. This spindle matrix, scientists proposed, houses proteins such as the molecular motors that pull on microtubules and haul the chromosomes apart. However, evidence for such a matrix remains inconclusive.

Lince-Faria et al. tracked a potential spindle matrix protein called Megator. In *Drosophila* cells, they found, the distribution of Megator mirrors the shape of the spindle. Even after a dose of colchicine causes the microtubules to collapse, Megator remains in the spindle's vicinity, suggesting that something holds it in place.

In turn, Megator holds up mitosis while the spindle matures. Without the protein, cells race through mitosis. Megator prompts the delay using the spindle assembly checkpoint, which halts mitosis if spindle fibers aren't correctly fastened to kinetochores on the chromosomes. As the team discovered, Megator links to Mad2, a component of the checkpoint. And when Megator is missing, less Mad2 gloms onto kinetochores that haven't linked to microtubules, which might make it easier for a cell to rapidly enter mitosis even if the spindle isn't correctly connected. This function appears to be conserved in humans.

The researchers conclude that Megator and Mad2 meet the qualifications for spindle matrix proteins. Further studies should probe what draws Megator to the spindle matrix, they say.

Lince-Faria, M., et al. 2009. *J. Cell Biol.* doi:10.1083/jcb.200811012.



Labeled nuclear pores spread around the nuclear membrane in control cells (left), but cluster in cells lacking Rtn1 and Yop1 (right).

Nuclear pore synthesis on a roll

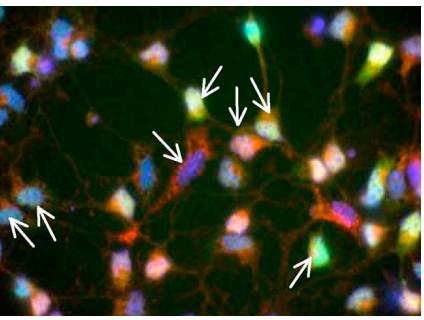
Two proteins that roll the endoplasmic reticulum (ER) into tubes also help make nuclear pores, Dawson et al. reveal.

The pores are passageways into and out of the nucleus. Although researchers have worked out some of the mechanics of nuclear pore construction, they don't understand all of the events. Recent evidence suggested that some proteins that reside in the ER and nuclear envelope are involved. After screening about 70 ER and nuclear envelope proteins, the researchers took a closer look at two, Yop1 and Rtn1, that help curl flat ER membranes into tubes.

Deleting both genes from yeast cells produced obvious flaws in pore position, structure, and function, the researchers found. Normally scattered around the nuclear envelope, the pores clustered in one section of the membrane. And instead of spanning the nuclear envelope, many of the structures were stuck halfway in. These defects disrupted traffic into the nucleus. Using *Xenopus* nuclei, the researchers showed that blocking the vertebrate version of Rtn1 with an antibody also hindered pore formation.

The team concluded that ER-bending proteins are crucial for assembling new nuclear pores. Rolling up ER membranes and building nuclear pores might seem like very different jobs. But in both situations, the proteins have to deal with a curved membrane. At a nuclear pore, the inner and outer nuclear membranes fuse, creating a sharp bend. The researchers hypothesize that Yop1 and Rtn1 help stabilize this curved conformation.

Dawson, T.R., et al. 2009. *J. Cell Biol.* doi:10.1083/jcb.200806174.



Arrows mark fusing mitochondria in Bcl-x_L–lacking cells.

Mitochondrial growth spurt

Mitochondria are restless, continually merging and splitting. But contrary to conventional wisdom, the size of these organelles depends on more than fusion and fission, as Berman et al. show. Mitochondrial growth and degradation are also part of the equation.

Fission is necessary to produce new mitochondria, such as those that power synaptic activity in healthy neurons. Fusion is also important. It goes awry in one form of Charcot-Marie-Tooth disease, in which peripheral nerves deteriorate, and in other neurodegenerative diseases. How cells manage mitochondrial size and number remains unclear.

Berman et al. found a clue when they started refining measurements of mitochondrial dynamics. The team labeled the organelles with a red fluorescent protein and a light-activated green fluorescent protein. By switching on the green marker with a laser and then looking for the mixing of colors, the researchers could distinguish mitochondrial mergers from near misses. To their surprise, they found that in healthy neurons, fission occurs up to six times more often than fusion.

So why aren't the cells cluttered with tiny mitochondria? Because the organelles grow longer, the

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researchers determined. This size increase offsets the higher fission rate. The researchers also surmised that to "balance the books," another process has to be operating—mitochondrial degradation. Together, fusion, fission, growth, and breakdown determine mitochondrial size and shape, Berman et al. propose.

Orchestrating many of these changes is the protein Bcl-x_L. The team found that it spurred mitochondrial elongation and sped up fission and fusion. Without Bcl-x_L, mitochondria became stumpy and seemingly less energy efficient. Bcl-x_L belongs to the Bcl-2 protein family, whose members can protect mitochondria or shatter them to drive apoptosis. Berman et al.'s results suggest that Bcl-x_L manages the number, size, and energy-producing capacity of mitochondria long before the cell is faced with a life-or-death decision. Still a mystery, the scientists say, is how Bcl-x_L sparks mitochondrial growth.

Berman, S.B., et al. 2009. *J. Cell Biol.* doi:10.1083/jcb.200809060.

Lipid rafts get connected

A protein that shackles HIV also helps reorganize the actin cytoskeleton, as Rollason et al. show. The protein, known as tetherin, hitches actin to lipid rafts in the plasma membrane.

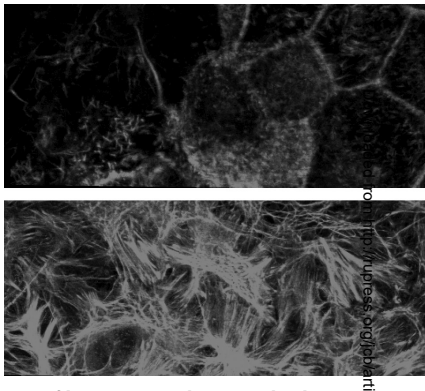
Tetherin made a splash last year when researchers discovered that it snares newly minted HIV particles, preventing them from exiting cells. But tetherin's unusual structure points to other functions. At the cell surface, one end of the protein embeds in a lipid raft. Tetherin then doubles back on itself and protrudes into the cell interior. Researchers have identified several proteins that fasten lipid rafts to the actin cytoskeleton, allowing the rafts to convey signals and alter cell behavior. Rollason et al. tested whether tetherin also serves as one of these links.

Using RNAi, the researchers removed the protein from intestinal epithelial cells. In many ways, the cells seemed normal. They polarized and sported the tight junctions that link them to their neighbors. But epithelial cells normally stand tall and sprout finger-like microvilli from their apical surfaces that are braced by actin filaments. In the absence of tetherin, the cells were squat. No microvilli grew, and the actin filaments bunched up at the base of the cells.

However, the researchers found that tetherin doesn't connect directly to actin. It links through intermediaries called ezrin, EBP50, and RICH2. The involvement of RICH2 might explain how tetherin supports microvilli. By switching off Rac, a GTPase that regulates actin organization, RICH2 probably helps to stabilize the actin filaments that support the microvilli.

The researchers conclude that tetherin does link lipid rafts to the actin cytoskeleton, an interaction that could help control cell shape. Tetherin might exert its impact directly on the cytoskeleton or indirectly through its effects on GTPases like Rac.

Rollason, R., et al. 2009. *J. Cell Biol.* doi:10.1083/jcb.200804154.



Actin fibers in control (top) and tetherin-deficient (bottom) cells.

Turn back, wayward axon

To a growing axon, the protein RGMa is a "Wrong Way" sign, alerting it to head in another direction. As Hata et al. demonstrate, translating that signal into cellular action requires teamwork from two receptors.

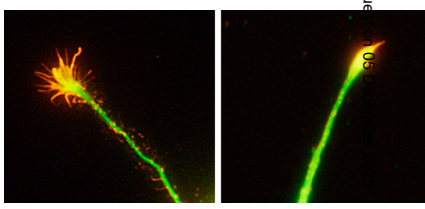
During development, new synapses form when the growing axon of one neuron reaches another neuron. As an axon searches out the path to its destination, it bends toward so-called attractive guidance molecules and veers away from repulsive guidance molecules such as RGMa. For example, if the tip of an axon touches a glial cell instead of a neuron, the extension pulls back. On its membrane the glial cell sports RGMa, which latches onto the receptor neogenin on the axon. Researchers knew that the interaction between RGMa and neogenin halted the axon by activating the GTPase RhoA. However, they didn't know how neogenin switches on RhoA.

Hata et al. discovered that it gets help from another axon membrane receptor called Unc5B. The researchers found that after a dose of RGMa, the tip of a growing axon halted and often retreated. Eliminating Unc5B prevented this collapse.

Neogenin and Unc5B stick together and serve as coreceptors, performing slightly different tasks, Hata et al. conclude. Neogenin's job is to hook up with RGMa. Unc5B, by contrast, never contacts RGMa. Instead, it serves as a docking point for the RhoA activator LARG. Unc5B indirectly switches on RhoA by interacting with LARG.

But that left one further mystery to explain. LARG clings to Unc5B all the time, so why does it fire up RhoA only in response to RGMa? The researchers found that binding of RGMa prodded another protein, the focal adhesion kinase (FAK), to switch on LARG, allowing activation of RhoA. How RGMa binding triggers FAK is the next question the researchers want to answer.

Hata, K., et al. 2009. *J. Cell Biol.* doi:10.1083/jcb.200807029.



An elongating axon tip (left) crumples when it encounters RGMa (right).