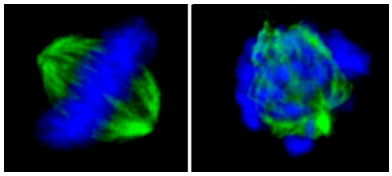
In This Issue

Text by Mitch Leslie
mitchleslie@comcast.net

Clathrin teams up to strengthen the spindle



The mitotic spindle (green) is orderly in a normal cell (left) and chaotic in a cell missing clathrin heavy chain (right).

When it's not busy making endocytic vesicles, the clathrin heavy chain stabilizes the mitotic spindle. Lin et al. reveal that the heavy chain performs this job by recruiting another key spindle-supporting protein.

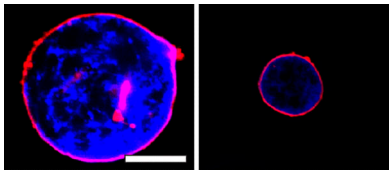
The spindle has important work to do during mitosis, helping to arrange and separate the chromosomes, and cells can't afford a premature microtubule collapse. Loss of the clathrin heavy chain causes kinetochore fibers to break down, prevents the chromosomes from lining up properly, and slows mitosis. But how the protein reinforces the spindle has eluded researchers.

Lin et al. discovered that the heavy chain interacts with the spindle-stabilizing protein TACC3 after the latter is phosphorylated by the aurora A kinase. TACC3 attracts the protein ch-TOG, which spurs microtubules to assemble. Without the clathrin heavy chain, little TACC3 reached the spindle, which became jumbled.

The next step, the researchers say, is determining how the clathrin heavy chain maneuvers TACC3 into position. Another question is whether the heavy chain's partner in endocytosis, the clathrin light chain, also affects spindle dynamics.

Lin, C.-H., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200911120.

Secret passage to the inner nuclear membrane



A swollen nucleus lacking Nup188 and Nup93 (left) dwarfs a normal nucleus (right).

Proteins enter the inner nuclear membrane through a side door in the nuclear pore complex, Theerthagiri et al. report.

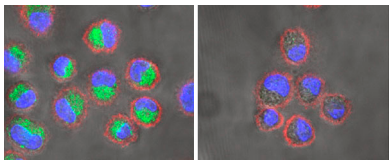
The double membrane that envelops the nucleus causes delivery problems for the cell. Newly made membrane-embedded proteins can travel directly to the outer nuclear membrane (ONM) because it connects to the endoplasmic reticulum. But how do such proteins reach the inner nuclear membrane (INM)? Researchers have suggested that the ONM and INM temporarily fuse to allow protein transfer or that vesicles ferry the proteins across the inter-membrane gap. Another possibility is that the proteins slip across via the nuclear pore complexes that span the two membranes.

Theerthagiri et al. found the latter case to be true using nuclei from *Xenopus* egg extracts lacking certain nucleoporins, or Nups. Nup93 and Nup188 belong to a complex that anchors the nuclear pore to both membranes, and the team showed that nuclei missing these pore components balloon to 4 or 5 times their normal volume. The nuclei enlarge because their INM accumulates more protein than normal.

Loss of the two Nups didn't affect transport through the main nuclear pore channel, but it sped up entry of the INM proteins, indicating that they use an alternative portal. The researchers think that INM proteins pass through accessory channels in the nuclear pore complex that flank the main entranceway. Nup93 and Nup188 could serve as gatekeepers for these side channels, opening them to allow certain molecules through. How the Nups determine which molecules to admit is still unclear.

Theerthagiri, G., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200912045.

Lysosomes don't accentuate the negative



Lysosomes can maintain their acidity in macrophages that carry the chloride transporter ClC-7 (green, left image) and in macrophages lacking the transporter (right).

Like the stomach, lysosomes soak their contents in an acid bath. Using two new techniques, Steinberg et al. show that lysosomes can hike their acidity because they shed positive ions.

To keep its internal pH between 4 and 5, a lysosome actively pumps in protons. But as more positively charged hydrogen ions enter, continued importation becomes harder. The organelle needs to balance the electrical charge across its membrane. Researchers assumed that lysosomes counteract the increase in positive charge by allowing in negatively charged chloride ions through channels such as the cystic fibrosis transmembrane conductance regulator (CFTR) channel, the protein that's defective in cystic fibrosis.

But Steinberg et al. found that lysosomes could still acidify in mice lacking CFTR or another key chloride transporter, ClC-7. By temporarily punching holes in the cell membrane, the researchers replaced chloride ions in the cytosol with bulky, negatively charged molecules that can't diffuse into the lysosome. Even after this substitution, lysosomes still reduced their pH, indicating that a chloride influx isn't necessary.

Instead, the lysosomes offset the entering protons by losing positively charged sodium and potassium ions. To explore this effect, the researchers temporarily permeabilized the lysosome membrane, allowing them to adjust the organelle's contents. Steinberg et al. replaced the internal potassium with a positively charged molecule that can't pass through the lysosome membrane after it reseals. Organelles altered in this way couldn't increase their acidity.

The results don't mean that chloride has no role, the researchers say. They suggest that lysosomes probably lose some positive ions and gain some chloride, thus avoiding any osmotic swelling or shrinking.

Steinberg, B.E., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200911083.