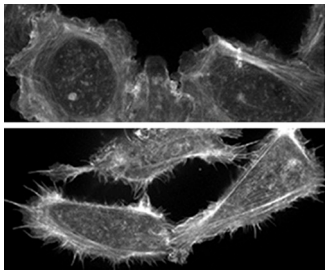
In This Issue

Text by Mitch Leslie
mitchleslie@comcast.net

NIK flips the switch on Arp2/3



EGF spurs control cells (top) to extend protrusions but not cells that carry an unphosphorylatable form of Arp2/3 (bottom).

LeClaire et al. identify a kinase that helps activate the actin-nucleating Arp2/3 complex.

The Arp2/3 complex spurs membrane protrusion and cell movement by triggering formation of branched actin filaments. WAVE and WASP proteins switch on the Arp2/3 complex, but the complex's activation also requires phosphorylation of Arp2. However, the kinase responsible for this phosphorylation was unknown.

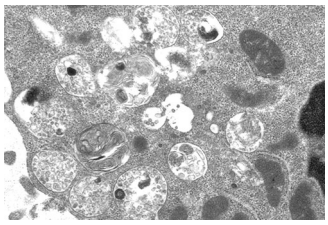
LeClaire et al. tested several candidates and found that Nck-interacting kinase (NIK, also known as MAP4K4) phosphorylated Arp2 and other subunits of the complex in vitro. It wasn't the only one, however. Src also phosphorylated the Arp2 and Arp3 subunits. The researchers therefore put the two kinases through a further test by stripping away all phosphate groups from the complex. NIK restored the Arp2/3 complex's ability to polymerize actin in vitro but Src did not, suggesting that phosphorylation by NIK helps to activate the complex.

The team also gauged NIK's effects in cells. Epidermal growth factor (EGF) spurs actin assembly in cancer cells. LeClaire et al. discovered that a dominant-negative version of Arp2/3 that can't be phosphorylated prevented EGF-induced actin polymerization in these cells. Knocking down NIK with shRNA also curbed polymerization and inhibited membrane protrusion.

The researchers suggest that Arp2/3's two on-switches—WASP/WAVE and phosphorylation of Arp2—serve as a coincidence detector. The Arp2/3 complex remains inactive unless two stimuli arrive within a short time of each other.

LeClaire, L.L., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201404095.

Mannose 6-phosphate steers B cells on the right course



In a B cell from a mouse that mimics symptoms of mucolipidosis II patients, lysosomes are packed with undigested material.

B cells need mannose 6-phosphate to proliferate, differentiate, and present antigens, Otomo et al. show.

Immune cells slice up antigens in lysosomes and attach the fragments to MHC II complexes, which then display them on the cell surface. To ensure that they can cut up the antigens, cells steer newly made proteases to the organelles by tagging them with mannose 6-phosphate molecules. In the rare disease mucolipidosis II, patients can't synthesize mannose 6-phosphate, and their lysosomes fill up with undigested molecular junk known as storage material.

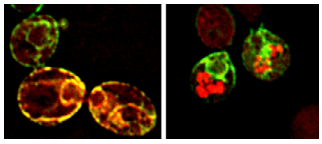
To find out how loss of mannose 6-phosphate affects different kinds of immune cells, Otomo et al. used knock-in mice that mimic symptoms of mucolipidosis II patients. The animals' B cells were abnormal. Their lysosomes bulged with storage material, and they were poor at dismembering antigens and breaking down CD74, a protein that prevents the MHC II complex from binding antigen fragments.

B cells show off antigenic peptides to $CD4^+$ T cells, and the interaction spurs B cells to mature, proliferate, and differentiate into antibody-making plasma cells. All three processes were impaired in B cells from the mutant mice. The researchers found that B cells from mucolipidosis II patients were also defective and produced fewer IgG, IgM, and IgA antibodies than normal.

However, other types of immune cells in the mice weren't as severely affected. For example, dendritic cells broke down antigens normally and didn't accumulate storage material, although their ability to present antigens to T cells was below par. Thus, B cells are more dependent on mannose 6-phosphate than are other immune cells. The enzymes in these other cells presumably reach the lysosome through mannose 6-phosphate–independent pathways.

Otomo, T., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201407077.

Erv41–Erv46 complex recaptures wayward ER proteins



In control cells (left), Gls1 (green) resides in the ER (red), but not in cells lacking Erv41 (right).

Like cows wandering away from the herd, ER proteins sometimes stray. Shibuya et al. identify a protein complex that helps return them to the ER.

ER resident proteins occasionally break free and end up in the Golgi apparatus, where they may be shunted into the secretory pathway. Researchers already knew about one mechanism for retrieving these escapees. Proteins carrying the KDEL motif ride back to the ER in COPI-coated vesicles, which sport a KDEL receptor. How cells recapture ER proteins that lack the KDEL motif was unclear.

Shibuya et al. found that a complex of two Golgi proteins, Erv41 and Erv46, performs this function. Deleting Erv41 from yeast cells caused a 60% decrease in the levels of one ER protein, Gls1. In these cells, the escaped Gls1 was eventually secreted or directed to the vacuole for destruction.

The early portions of the Golgi apparatus are slightly acidic. By altering pH levels in vitro, Shibuya et al. determined that the Erv41–Erv46 complex releases Gls1 if the pH rises much above 6. The team confirmed the pH effect by dosing yeast cells with bafilomycin A1, which makes the Golgi less acidic. The cells began to secrete Gls1, indicating the protein wasn't being recovered.

Erv46 carries a sequence that binds to the COPI coat, and the complex returns Gls1 to the ER by corralling it into COPI vesicles. The researchers found that the complex also retrieves several other ER proteins, including Fpr2 and Vps62.

Shibuya, A., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201408024.