In This Issue

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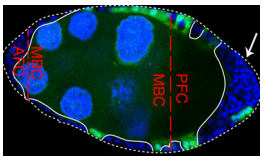
Mitochondrial morphology shapes differentiation



Follicle cells at the posterior of egg chambers (arrow) hyperproliferate in the absence of DRP1.

Mitochondrial fission regulates the development of *Drosophila* egg chambers, Mitra et al. reveal.

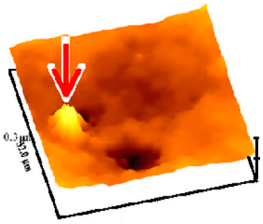
Fusion and fission proteins shape mitochondria into either long, interconnected tubules or shorter fragments dispersed throughout the cytoplasm. Mitra et al. previously found that tissue culture cells must fuse their mitochondria into a large network in order to enter S phase and progress through the cell cycle. To determine whether mitochondrial morphology has a similar function in vivo, the researchers investigated how follicle cells—the outer layer of *Drosophila* egg chambers—develop in the absence of the mitochondrial fission protein DRP1.

Most follicle cells proliferate until the Notch signaling pathway induces cell cycle exit and differentiation into a polarized epithelium. In the absence of DRP1, however, the follicle cells nearest to the oocyte maintained a highly fused mitochondrial network and continued to proliferate instead of differentiating. The Notch receptor wasn't activated in DRP1-null cells, but Notch signaling and differentiation were restored if the mitochondrial network was dispersed by simultaneously depleting the mitochondrial fusion protein Marf-1.

When Marf-1 alone was depleted—leaving the fission activity of DRP1 unopposed—follicle cells differentiated prematurely, at a stage when they should have continued to proliferate. This suggests that DRP1-dependent mitochondrial fission initiates follicle cell differentiation, perhaps because cells' energetic requirements change when they stop proliferating, necessitating a change in mitochondrial morphology. The authors, from Jennifer Lippincott-Schwartz's lab, now want to investigate whether mitochondrial organization also regulates the differentiation of pluripotent stem cells and to determine whether cancer cells are affected by changes in the organelle's morphology.

Mitra, K., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb. 201110058.

Putting a "cap" on clathrin-mediated endocytosis



ICM generates a topographical map of the cell surface, revealing a membrane protrusion (arrow) as it caps a clathrin-coated pit.

Shevchuk et al. use ion conductance microscopy (ICM) to examine how clathrin-coated pits (CCPs) close as they internalize from the cell surface.

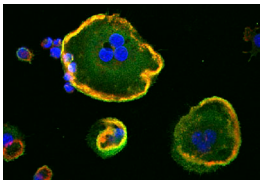
Electron microscopy can provide high-resolution snapshots of CCPs budding from the plasma membrane, but observing these dynamic structures in living cells has proved to be much more challenging. Shevchuk et al. followed the topologies of CCPs in live cells by combining fluorescence imaging with ICM, a technique in which the cell is rapidly scanned by a probe that detects nanoscale bumps and dips in the plasma membrane.

CCPs are generally thought to seal off from a flat region of the plasma membrane, but Shevchuk et al. found that 70% of CCPs were capped by a membrane protrusion that grew from one side of the pit. These protrusions contained the actin-binding protein Abp1, and their growth was blocked by the actin polymerization inhibitor latrunculin B. Abp1 wasn't associated with the 30% of pits that closed via the canonical mechanism, however, perhaps explaining why other researchers have reported conflicting findings on the involvement of actin in clathrin-mediated endocytosis.

Both types of CCP required the GTPase dynamin-2 to separate from the plasma membrane. Vesicles formed by the capping method moved more quickly into the cell interior, however, perhaps due to their greater association with the actin cytoskeleton. Author Andrew Shevchuk now wants to use ICM to investigate other forms of endocytosis and—if the temporal resolution of the technique can be improved even further—to study exocytic events as well.

Shevchuk, A.I., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201109130.

Protrusion drives osteoclast fusion



Tks5 (green) colocalizes with actin (red) in the podosome belt of a multinucleate osteoclast.

Oikawa et al. describe how the adaptor protein Tks5 promotes the formation of invasive podosomes in order to induce osteoclast cell fusion.

As precursor cells differentiate into bone-resorbing osteoclasts, they assemble rings of actin-rich membrane protrusions called podosomes. The precursors then fuse with each other to form giant, multinucleate osteoclasts that seal to the surface of bones through a stable podosome belt. How osteoclast precursors fuse is unknown, but Oikawa et al. saw that fusion occurred at sites where protrusions from one cell's podosome ring extended into a neighboring cell.

These podosome-associated protrusions contained the phospholipids PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, as well as the phospholipid-binding adaptor protein Tks5, which promotes the formation of invasive protrusions called invadopodia in cancer cells. Osteoclast precursors lacking Tks5 formed fewer podosomes and failed to fuse with each other. Podosome assembly and cell fusion was rescued by wild-type Tks5 but not by a mutant lacking the protein's phospholipid-binding domain. In addition, Tks5 was phosphorylated by the tyrosine kinase Src, and a phosphomimetic mutant of Tks5 partially rescued the podosome organization and fusion defects seen in Src-null osteclasts.

Src and phospholipid binding may therefore activate Tks5 to stimulate podosome-based protrusions that destabilize the plasma membrane of neighboring cells to induce osteoclast fusion. Tks5 also promoted the fusion of osteoclasts with melanoma cells to produce a type of hybrid cell found in cancer patients. Author Tsukasa Oikawa now wants to investigate how such hybrid cells contribute to bone metastasis.

Oikawa, T., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201111116.