In This Issue

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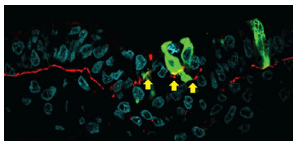
Epiblast cells CLASP onto the basement membrane



The basement membrane (red) is maintained underneath primitive streak epiblasts overexpressing CLASP1α (green).

Nakaya et al. reveal how the microtubule plus-end tracking protein CLASP and the adhesion receptor Dystroglycan combine to regulate epithelial-to-mesenchymal transitions (EMTs) in early embryogenesis.

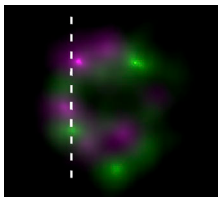
During gastrulation, cells in the primitive streak—part of an epithelial layer called the epiblast—undergo EMT to form the embryonic mesoderm. One of the first steps in this process is the destabilization of basal microtubules that promote epiblast cells' attachments to the underlying basement membrane (BM). As a result, cell adhesion is weakened and the BM disassembles. Nakaya et al. wondered whether microtubule plus end–binding proteins called CLASPs, which anchor microtubules to the cortex of other epithelial cells in culture, might be involved in regulating epiblast adhesion and EMT.

CLASPs were down-regulated in the primitive streak of chick embryos at the onset of gastrulation. Overexpressing CLASP inhibited BM breakdown, whereas depleting the protein prompted BM disassembly in regions outside the primitive streak. Basal microtubules were destabilized in the absence of CLASP and its binding partners LL5α and LL5β.

Nakaya et al. found that CLASP interacted with Dystroglycan and that this transmembrane protein, which binds to BM components such as laminin, was down-regulated in cells lacking CLASP. Overexpressing Dystroglycan prevented BM disassembly, whereas Dystroglycan depletion stimulated BM breakdown. CLASP and Dystroglycan therefore promote epiblast cell–BM attachments by anchoring basal microtubules. Senior author Guojun Sheng now wants to investigate why the BM disassembles when this connection is disrupted at the onset of gastrulation.

Nakaya, Y., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201302075.

Bruchpilot readies synaptic vesicles for release



Super resolution microscopy shows how clusters of two BRP isoforms (green and magenta) alternate within the T bars of fly synapses.

Matkovic et al. describe how a protein matrix may position synaptic vesicles close to the calcium signals that trigger their release.

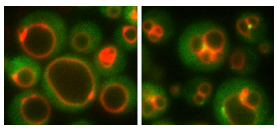
Synaptic vesicles are released at the active zones of presynaptic membranes where voltage-gated calcium channels are clustered together by a dense matrix of cytoplasmic scaffold proteins. In *Drosophila*, these active zone matrices are known as T bars, and their assembly relies on an elongated protein called Bruchpilot (BRP). In the complete absence of BRP and T-bar assembly, calcium channels are dispersed and vesicle release and synaptic transmission are impaired, but BRP's precise function is unclear.

Matkovic et al. found that flies express two isoforms of BRP. Each isoform formed separate clusters that alternated in a circular array to form the T bars of *Drosophila* neuromuscular junctions. In contrast to BRP-null flies, *Drosophila* lacking only one of the isoforms still formed T bars that clustered calcium channels in the presynaptic membrane. These T bars were smaller, however, and synaptic transmission was still impaired because neurons contained a smaller pool of readily releasable vesicles. Accordingly, the researchers saw fewer synaptic vesicles docked near calcium channels at the base of T bars lacking one BRP isoform.

Senior author Stephan Sigrist thinks the alternating arrangement of BRP isoforms helps assemble T bars with a specific number of "release slots" where synaptic vesicles can be coupled to calcium channel activity. Because BRP's distal C terminus can capture synaptic vesicles far from the plasma membrane, Sigrist speculates the protein might transfer these vesicles into their membrane-proximal release slots.

Matkovic, T., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201301072.

Phosphorylation helps Atg18 get the vacuole in shape



Low (left) and high (right) glucose levels alter the morphology of the vacuole (red) by regulating the phosphorylation of Atg18 (green).

Tamura et al. describe how phosphorylation modulates the phospholipid-binding capacity of a protein that regulates autophagy and vacuole morphology.

By binding to the phospholipid PI(3)P, Atg18 promotes assembly of the phagophore, a double-membraned structure that engulfs cytoplasmic contents ahead of their degradation during autophagy. By binding to $PI(3,5)P_2$, on the other hand, Atg18 fragments the vacuole (the yeast equivalent of the lysosome) in response to a variety of environmental stresses. How these two functions are regulated is unclear.

Tamura et al. discovered that the phospholipid-binding domain of Atg18 is partially phosphorylated in the methylotrophic yeast *Pichia pastoris* and that this modification reduced the protein's ability to bind $PI(3,5)P_2$. Atg18 was dephosphorylated in response to conditions—including hyperosmotic stress—that stimulate vacuole fission, prompting the protein to bind the vacuole membrane and promote the organelle's fragmentation. In contrast, conditions that induce vacuole fusion—such as hypo-osmotic stress—stimulated Atg18's phosphorylation and dissociation from vacuole membranes.

Micropexophagy is a specialized form of autophagy that targets peroxisomes for degradation. The membrane that engulfs the peroxisomes is provided by vacuole fission and by the formation of a phagophore-like structure called the MIPA. Tamura et al. found that dephosphorylated Atg18 promoted the vacuole fission required for micropexophagy. However, *Pichia* cells also required a non-vacuole–associated pool of phosphorylated Atg18 to generate the MIPA, suggesting that cells coordinate the two membrane sources by regulating Atg18 phosphorylation levels. Senior author Yasuyoshi Sakai now wants to identify the kinase and phosphatase responsible for Atg18 regulation.

Tamura, N., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201302067.