In This Issue

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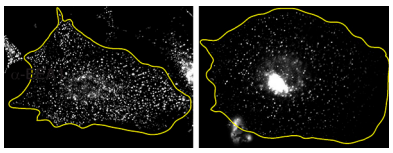
Misdirection leads the way to vesicle transport proteins



Compared with a control cell (left), AP21967 causes early endosomes to cluster in the center of a cell expressing FRB-KIF13A and an FKBP-tagged dynein adaptor (right).

Bentley et al. use a novel assay to identify the kinesin motor proteins that bind to different types of endosomes.

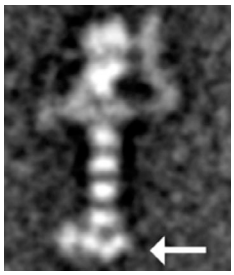
Membrane trafficking is controlled by a large number of proteins that bind to specific vesicles and organelles. But defining which proteins bind to which membranes within a crowded cell—by fluorescence colocalization, for example—is often difficult. Bentley et al. therefore developed an assay that can directly test whether a protein binds to a specific vesicle population in vivo.

The researchers fused candidate proteins to an FRB tag that, in the presence of a rapamycin-like drug called AP21967, binds to an FKBP-tagged version of the dynein motor adaptor molecule Bicaudal D2. If a candidate protein binds to a specific vesicle population, dynein will be recruited to these organelles in the presence of AP21967 and subsequently transport them to the minus ends of microtubules, which, in most cell types, are clustered around a microtubule-organizing center (MTOC) in the middle of the cell.

Bentley et al. verified their assay by showing that an FRB-tagged version of the small GTPase Rab5 could misdirect early endosomes to microtubule minus ends, whereas FRB-Rab7 caused late endosomes to cluster around the MTOC. The researchers then systematically analyzed the endosome-binding properties of Kinesin-3 family members and found that KIF13A and KIF13B bound to early endosomes, whereas KIF1A and KIF1Bβ associated with late endosomes and lysosomes. Bentley et al. think that the approach should be applicable to many other families of membrane trafficking proteins as well.

Bentley, M., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201408056.

Nup82 complex puts the P in NPC



An electron micrograph shows the P-shaped structure of a modified Nup82 complex. Arrow indicates the position of Nup159's β-propeller domain.

Gaik et al. describe the P-shaped structure of a key protein complex at the cytoplasmic face of nuclear pores.

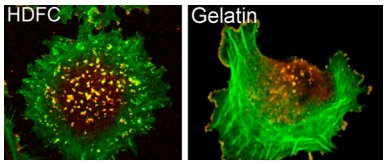
The nuclear pore complex (NPC) consists of ∼30 different nucleoporins, most of which are organized into stable subcomplexes. The Nup82 subcomplex, comprising the nucleoporins Nup82, Nup159, Nsp1, and the dynein light chain Dyn2, localizes to the cytoplasmic face of NPCs where it promotes mRNA export. How the subcomplex assembles and fits in to the rest of the NPC is unclear, however.

Gaik et al. purified the Nup82 complex from yeast and analyzed its structure using several different techniques. Most Nup82 complexes contained 2 copies of each nucleoporin and 10 Dyn2 molecules. Although Nup82, Nup159, and Nsp1 all contain α-helical domains predicted to form coiled-coil dimers, electron microscopy showed that the complex didn't adopt an extended, rod-like conformation. Instead, the complex was shaped like the letter P, whose globular head domain was formed by the C-terminal coils of Nup159 and Nsp1 packed together with the entirety of Nup82. The structure's tail consisted of Nup159's N-terminal domains bound to Dyn2.

Fitting the Nup82 complex's structure into a map of the entire NPC suggested that the FG repeats of Nup159 and Nsp1 could be oriented toward the pore's central channel, where they would interact with transport factors moving through the pore. At the same time, Nup159's N-terminal β-propeller domain would be exposed to the cytoplasm, allowing it to recruit the RNA helicase Dbp5 to disassemble messenger RNPs once they arrive in the cytoplasm.

Gaik, M., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201411003.

Dense collagen kindles invadopodia formation



A cancer cell invading HDFC (left) forms many more invadopodia (yellow dots) than a cell invading gelatin (right). The cells are labeled for actin (green) and cortactin (red).

Artym et al. reveal that a type of ECM commonly found in advanced metastatic tumors is a potent inducer of invasive cell protrusions.

Cells remodel and invade the ECM by forming invadopodia, actin-rich protrusions containing matrix metalloproteinases such as MT1-MMP. Studies using artificial gelatin-based matrices have shown that increasing ECM stiffness enhances invadopodia formation, but how more physiologically relevant matrices influence the process is unclear. Advanced-stage tumors often deposit large amounts of fibrillar collagen into their stroma, so Artym et al. tested the ability of high-density fibrillar collagen (HDFC) matrices to induce invadopodia.

HDFC matrices induced numerous cancer cell lines, and even normal fibroblasts, to form many more invadopodia than they did on stiffer gelatin-based matrices. Unlike the protrusions formed on gelatin, HDFC-induced invadopodia didn't require the activity of any growth factors for their formation. Nor did their induction involve any changes in gene or protein expression. Instead, the researchers found, HDFC stimulated invadopodia formation by activating the collagen-binding integrin α2β1 and a downstream network of signaling proteins that regulate cell adhesion and the actin cytoskeleton. For example, HDFC stimulated phosphorylation of the integrin-activating protein kindlin2. Knocking down kindlin2, or expressing a nonphosphorylatable version, inhibited invadopodia induction, whereas phosphomimetic kindlin2 mutants stimulated invadopodia formation.

The dense collagen fibers found in malignant tumors may therefore stimulate ECM remodeling and cell invasion. Lead author Vira Artym now wants to identify ways of blocking invadopodia formation in tumor cells without affecting the function of healthy cells.

Artym, V.V., et al. 2015. J. *Cell Biol.* http://dx.doi.org/10.1083/jcb.201405099.