In This Issue

ESCRT rings around the membrane

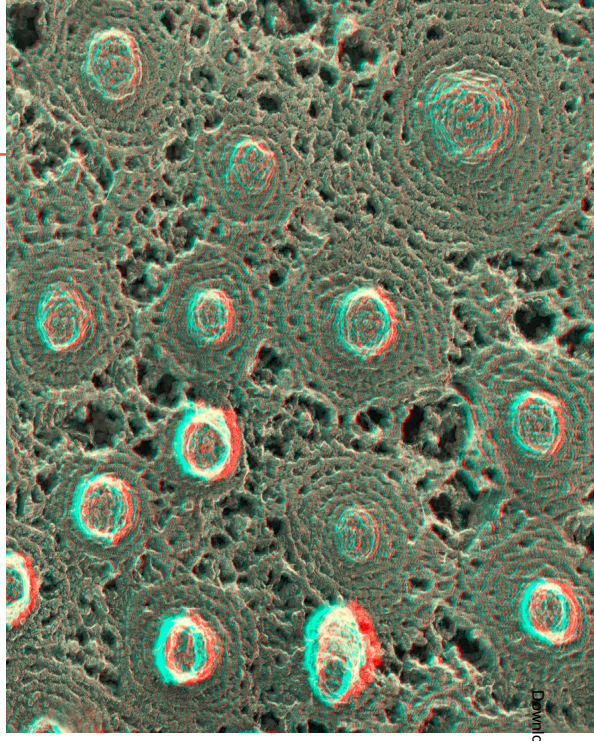
Vesicles within vesicles might be made with the help of rings of membrane-bending polymers that are revealed in images from Hanson et al.

The budding of small vesicles into larger endosomes—known as multivesicular bodies (MVBs)—is directed by the sequential action of the ESCRT I, II, and III complexes. ESCRTs I and II bind to ubiquitinated cargo and to endosome-defining phospholipids and probably concentrate the soon-to-be budded cargo on the membrane of the endosome.

The function of ESCRT III has been more elusive, owing to its tendency to form large and insoluble complexes, but mutant phenotypes suggest it acts after the other ESCRTs. As a late player, the authors imagined, ESCRT III might deform the outer membrane to create inward-budding vesicles. Their new striking images support this idea.

Using deep-etch EM, Hanson and colleagues captured images of large ESCRT III polymers. The authors expressed various ESCRT III proteins at high levels, causing them to accumulate on both endosomes and the plasma membrane, where their structures could be more easily viewed.

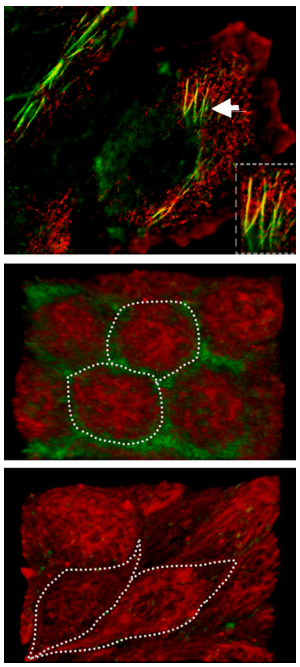
The images revealed curved 5-nm filaments of ESCRT III polymers tightly associated with the membrane. Upon deletion of the C-terminal half of the protein, rings of the filaments induced curvature in the surrounded membrane, which pushed bud- and tubule-like structures outwards. On endosomes, such budding would result in the creation of internal vesicles. The deletion mutant probably lacks a self-inhibitory domain that normally limits its oligomerization and thus its ability to bend the membrane. The authors suggest that in wild-type cells, accessory proteins probably relieve this inhibition.



Rings of ESCRT III push out buds of membrane (shown in 3D).

Artificially high levels of the ESCRT III proteins were used to obtain the images, but the authors argue that the images represent an exaggerated version of the normal process. The group has yet to figure out, however, how the ESCRT III rings produce buds without themselves getting trapped inside the resultant vesicles. Perhaps either the rings are initially very large and then tighten as the vesicles form (akin to a purse string) or sequential rings form as buds grow, with internal rings disassembling as new outer ones form. JCB

Reference: Hanson, P.I., et al. 2008. *J. Cell Biol.* 180:389–402.



SEPT2 (green) turns polyglutamated microtubules (red, top) into high speed tracks. Epithelial cells (middle) no longer polarize when SEPT2 is missing (bottom).

Septins clear high speed tracks

The road to polarity is paved with septins, say Spiliotis et al. Septin-decorated microtubule tracks, the group finds, provide polarity proteins with a high-speed path to the plasma membrane.

Septins were first linked to polarity in budding yeast, where they form diffusion barriers and scaffolds for congregating polarity proteins at the yeast cortex. But, based on the new findings, mammalian septins may work differently by altering microtubule properties.

In epithelial cells, septins bound along microtubules near the trans-Golgi network, at exit sites for vesicles carrying polarity proteins to the apical or basolateral plasma membrane. Disruption of one septin, SEPT2, caused vesicles to accumulate in the cytoplasm and thereby prevented cell polarization.

SEPT2 seemed to help polarity proteins get to the surface by removing impediments on the microtubule tracks. These cytoskeletal speed bumps are created by microtubule-associated proteins such as MAP4. But MAP4 was less likely to hop onto SEPT2-bound tubulin, the group found. The two proteins probably compete for similar binding sites on microtubules.

Without MAP4, the SEPT2-decorated tracks are presumably freed for high speed transport. Such speed is probably needed for the intense, dynamic membrane expansion that drives polarization.

Only some microtubule tracks harbored SEPT2, which preferred filaments containing polyglutamated tubulin. In turn, SEPT2 was needed to maintain polyglutamation. Increases in this post-translational modification might boost trafficking in developing epithelia and in other cell types that perform heavy amounts of regulated vesicle secretion, such as neurons and immune cells.

In addition to the microtubule pool, some SEPT2 was also seen on vesicles. As septins form oligomers, the two pools might velcro together vesicles and microtubules. The authors are also considering the possibility that different mammalian septins, like the various Rab GTPases, may define distinct post-Golgi trafficking pathways—to the apical versus basolateral membranes, for example. JCB

Reference: Spiliotis, E.T., et al. 2008. *J. Cell Biol.* 180:295–303.

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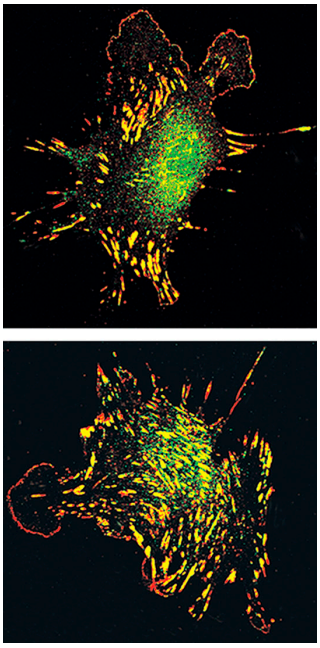
ICAP for sensing matrix density

Competition for the cytoplasmic tail of an integrin allows cells to read different matrix densities, say Millon-Frémillon et al.

The tail region of β1 integrin is tiny—only 47 amino acids long. That doesn't leave much room for the binding of cytoplasmic accessory proteins that help control the integrin's activation state. When talin holds this position, it flips open integrin as a first step toward full activation. Active integrins then cluster into patches called focal adhesions, which stick a cell to its surface. Signals from the adhesions then relay matrix information to the rest of the cell.

In the new paper, the authors show that talin's competitor for integrin binding—a protein called ICAP-1—ensures that integrin activation does not occur prematurely. They found that fibroblasts and osteoblasts that lack ICAP-1 had overzealous focal adhesions: the adhesions were larger, more widespread, and formed more rapidly than those of normal cells grown on the β1 integrin substrate fibronectin.

In the mutant cells, talin was recruited to integrins prematurely. Their integrins were thus more adhesive, fooling cells into sensing a denser matrix than was actually present. The ICAP-1 mutant cells spread and migrated on lower densities of fibronectin and collagen (another β1 integrin substrate). The authors reproduced these effects by artificially activating β1 integrins in wild-type cells.

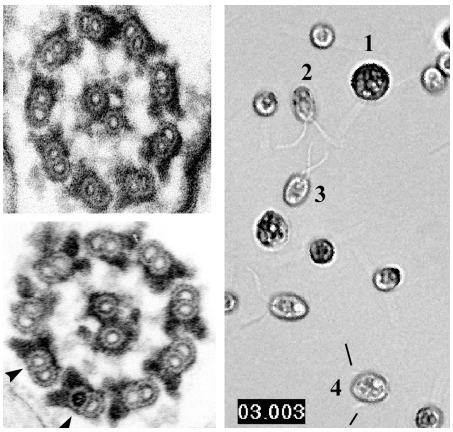


Clusters of integrin (green) grow and adhesions (yellow) are redistributed in fibroblasts lacking ICAP-1 (bottom).

Matrix sensing is important for determining migration speed, cell spreading, and lineage specification in progenitor cell lines. The authors previously showed that mice lacking ICAP-1 have structural bone defects but are viable. Evidently, some cell types—such as bone forming osteoblasts, which start out on a smooth, flexible substrate that later hardens—are more sensitive to their environment than others.

Although delayed by ICAP-1, talin must eventually make its way onto the integrin tail for adhesions to form. The authors are now searching for signals that trigger the dismissal of ICAP-1, allowing talin to take its place. JCB

Reference: Millon-Frémillon, A., et al. 2008. *J. Cell Biol.* 180:427–441.



Normal flagellar structure (left, top) is only slightly perturbed by the lack of HSP40 (left, bottom), but the mutants (right) beat their flagella erratically.

Spokes coordinate flagella

Like a good relationship, communication is key for properly beating flagella. According to results from Yang et al., coordinated movements require the radial spoke, which keeps the center of the flagellum in touch with its outer parts.

Most flagella have a pair of central microtubules surrounded by nine outer microtubule doublets. The central pair is transiently connected to each outer pair by a protein complex called the radial spoke. But not all flagella have spokes and a central pair, leaving scientists to wonder just what these structures do.

The creation of mutant flagella to address this question has been less than successful. Most of the flagellum assembles in units, so loss of one protein demolishes a chunk of the structure and leaves it paralyzed. Yang et al. averted this problem by getting rid of a protein that is added on its own, late in spoke assembly. The latecomer, a chaperone assistant called HSP40, hooks onto the spoke. Using RNAi, the group fully depleted HSP40 from *Chlamydomonas* and created mutants with only subtle structural defects in the spoke, near the central pair.

The loss of HSP40 resulted in herky-jerky flagella that paused sporadically midstroke and occasionally switched directions prematurely. The authors conclude that the spoke helps time the beating movements.

For *Chlamydomonas*, this timing coordination probably entails the sequential activation—from flagellum base to tip—of dynein motors along outer doublets. Dyneins slide one doublet past another to drive bending. The team imagines that mechanical or molecular signals travel along the spoke from the outer doublet to the central pair, then back out to the outer pair, and so forth. Near the head of the spoke, HSP40 is well-positioned to stabilize other spoke proteins into a rigid structure that might transduce these signals.

Flagella that naturally lack the central pair have a different beating pattern and evidently use an alternative coordination system. The nodal flagella that establish left–right asymmetry, for example, move in a swirling pattern rather than the more powerful breaststroke-like movements of *Chlamydomonas* flagella. Spoke-driven coordination might thus be the basis for this extra power. JCB

Reference: Yang, C., et al. 2008. *J. Cell Biol.* 180:403–415.