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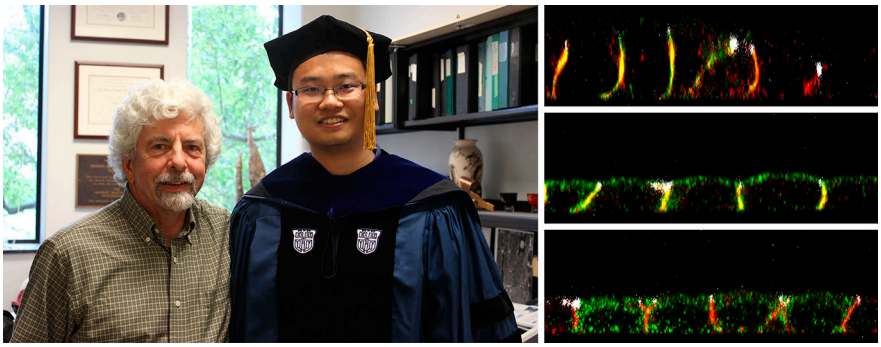
Lipids help epithelia stand tall

Palmitoylation and phosphoinositides target ankyrin-G/βII-spectrin network to lateral membranes.

Focal Point



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Vann Bennett (left), Meng He (right), and Khadar Abdi (not pictured) describe how the scaffold proteins ankyrin-G and βII-spectrin are targeted to the lateral membrane of columnar epithelial cells in order to promote lateral membrane assembly. The palmitoyltransferases DHHC5 and DHHC8 target ankyrin-G by palmitoylating the protein's N-terminal region. βII-spectrin, in turn, associates with ankyrin-G but must also bind to phosphoinositides in order to specifically localize to the lateral membrane. Wild-type βII-spectrin (green, top right) colocalizes with ankyrin-G (red), allowing MDCK cells to obtain their normal height. Replacing βII-spectrin with mutant versions unable to bind ankyrin-G (middle right) or phosphoinositides (bottom right) results in the protein's mislocalization and a dramatic shortening of the epithelial cells.

Ankyrin proteins organize the plasma membrane into specialized domains by binding to transmembrane proteins and anchoring them to a submembranous network of spectrin and actin (1). Ankyrin-G, for example, helps build the axon initial segment of neurons and also promotes assembly of the lateral membrane of columnar epithelial cells (2). He et al. reveal that lipids play an essential role in targeting ankyrin-G and its binding partner, βII-spectrin, to the right location (3).

"Ten years ago, we found that if you eliminate ankyrin-G from columnar epithelial cells you lose the entire lateral membrane," says Vann Bennett from Duke University School of Medicine in Durham, NC. "The cells go from being 10 microns in height to being less than a micron." βII-spectrin also controls epithelial cell height (4), but how the two proteins promote lateral membrane assembly remains unclear. "We have to understand how they target to the lateral membrane," says Bennett. "How does ankyrin-G know where to go?"

Bennett and colleagues, led by grad student Meng He, recently discovered that ankyrin-G is palmitoylated and that blocking this lipid modification by mutating a specific cysteine residue near the protein's N terminus prevented ankyrin-G from promoting lateral membrane assembly (5). He et al. now checked all 23 members of the DHHC family of palmitoyltransferases and found that two of them—DHHC5 and DHHC8—localized to the lateral membrane of MDCK epithelial cells and palmitoylated ankyrin-G (3). "Knocking down both of these enzymes eliminated ankyrin-G palmitoylation and targeting to the lateral membrane," says Bennett. As a result, the cells became shorter, just as they do in the complete absence of ankyrin-G.

He et al. then turned their attention to βII-spectrin, which, in addition to binding ankyrin-G, can associate with membrane phospholipids through a phosphoinositide-binding pleckstrin homology domain. A βII-spectrin mutant unable to bind ankyrin-G lost its polarity, localizing to both the apical and lateral cell membranes. Surprisingly, however, a βII-spectrin mutant unable to bind phosphoinositides localized intracellularly, despite its continued ability to bind with high affinity to ankyrin-G. Both mutants failed to promote lateral membrane assembly.

"So it looks like this system requires palmitoylation of ankyrin-G, which, in turn, imposes polarity on βII-spectrin. But βII-spectrin requires both ankyrin and phosphoinositide binding [to localize specifically to the lateral membrane]." βII-spectrin is thus a "coincidence detector" that is targeted to the correct membrane by simultaneously recognizing both a protein and a phospholipid (probably PI(3,4)P2 or PI(3,4,5)P3). "Lipids have a major role in determining where these polarized, membrane-associated proteins localize," Bennett says. "Protein–protein interactions by themselves aren't sufficient to explain how protein complexes assemble in cellular environments."

> **"Lipids have a major role in determining where these... proteins localize."**

A final surprise came when the researchers examined the protein's localization in closer detail using 3D deconvolution microscopy. Instead of localizing all over the membrane as their homologues do in red blood cells, ankyrin-G and βII-spectrin concentrated in small microdomains scattered around the lateral membranes of MDCK cells. The palmitoyltransferases DHHC5 and DHHC8 localized to these membrane subdomains as well. "We're very excited by this," says Bennett. "These structures are in the range of 0.5 to 2 microns, much larger than lipid rafts, and we think that they require both protein and lipid interactions. Ankyrin-G has to bind to βII-spectrin to colocalize in these domains."

The researchers now want to investigate how these ankyrin–spectrin microdomains promote lateral membrane assembly. One possibility is that they enhance intercellular contacts by inhibiting the endocytosis of E-cadherin, a cell-adhesion molecule that binds to ankyrin-G.

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