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How eisosomes help the plasma membrane get organized

Proteins combine into filaments that hug and modify the membrane.

Karotki et al. (1) reveal how two proteins found in structures called eisosomes enable a yeast cell to parcel its plasma membrane into specialized domains. These proteins wind into helices and stick to the membrane, bending a portion of it into shape.

Researchers agree that the proteins and lipids in the plasma membrane can sort into domains that perform particular functions such as signaling. But the question of how these domains arise has spurred plenty of controversy, as researchers tussled over issues such as whether or not lipid rafts exist. In yeast, the plasma membrane consists of at least three compartments: MCC, MCP, and MCT (2, 3). The cellular roles of these domains aren't entirely clear. Besides signaling, their tasks could include regulating protein turnover and even providing spare membrane for cell growth. The MCC domain, which is a furrow in the plasma membrane, is generated by eisosomes, protein complexes that assemble into regular structures beneath the membrane (4). The MCC domain disappears in cells lacking Pil1, one of the main eisosome components. However, researchers haven't determined how eisosomes form, what links them to the cell membrane, and how they create the MCC furrow.

Karotki et al. discovered that, in vitro, Pil1 and Lsp1 proteins assemble into helical filaments. The researchers also saw similar filaments when they isolated eisosomes from yeast cells. Using a technique called freeze-fracture deep-etching EM, Karotki et al. took a close look at the inner surface of the plasma membrane. Helical filaments containing Pil1 and Lsp1 were prevalent on the furrows there as well. "That they form these self-assembling scaffolds is surprising," says senior author Tobias Walther.

Pil1 and Lsp1 contain BAR domains, which are polypeptide regions that can bend the membrane (5). To determine whether the filaments have membrane-remodeling power, the researchers combined the proteins with liposomes in vitro. Lsp1 and Pil1wrapped around the membrane spheres and squeezed them into tubes, suggesting they might similarly reshape the plasma membrane.

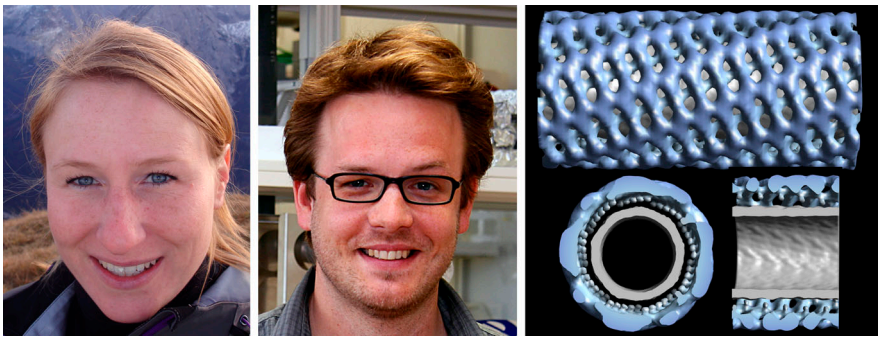
The proteins connect to the membrane by latching onto the phospholipid $PI(4,5)P_2$. The researchers followed eisosomes in yeast that carry a temperature-sensitive version of an enzyme that helps make $PI(4,5)P_2$. When the team raised the temperature to inactivate the enzyme, the cells harbored less $PI(4,5)P_2$ in their plasma membranes—and their eisosomes loosened their membrane attachments. However, the opposite happened in yeast lacking two enzymes that break down $PI(4,5)P_2$. Trapped by an excess of $PI(4,5)P_2$, tangles of Pil1 piled up on the inner side of the plasma membrane and stuck out into the cytoplasm.

> "[This] gives a mechanistic insight into how plasma membrane domain formation occurs."

The researchers then delved further into the structure of the BAR domains, which link Pil1 and Lsp1 to the membrane. By studying truncated proteins and mutant versions that have alterations in the sequence, Karotki et al. narrowed down which parts of the BAR domains were necessary for attachment to the membrane. A solid connection requires a group of positively charged amino acids on each protein's surface that lie within the BAR domain. But it also requires a region in each protein's N terminus that lies outside of the BAR domain.

Walther says that the study "gives a mechanistic insight into how plasma membrane domain formation occurs." The researchers envision that dimers or small strands of Pil1 and Lsp1 gather on the inner surface of the plasma membrane, attaching to an area rich in $PI(4,5)P_2$. The proteins interconnect to weave a lattice that cups and distorts a section of the membrane, causing it to pucker. Although the team studied each protein separately, they suspect that Lsp1 and Pil1 combine to form a mixed lattice. Researchers still need to nail down the details of how the proteins bend the membrane, Walther says. A further mystery, he adds, is what cellular signals spur eisosomes to trigger membrane remodeling. The answer to that question might help researchers figure out the functions of the domains.

Focal Point



PHOTOS COURTESY OF THOMAS HAUSER (KAROTKI) AND EVA-MARIE DIEHL (WALTHER); IMAGE COURTESY OF LENA KAROTKI

To find out how eisosomes mold the plasma membrane, Lena Karotki (left), Tobias Walther (center), and colleagues (not pictured) determined the structures of two eisosome proteins, Pil1 and Lsp1. Both proteins form helical lattices. As this reconstruction shows (right), the lattice of Lsp1 (blue) encircles a liposome (gray) and presses it into a tube, suggesting that the protein might also deform the plasma membrane.

1. Karotki, L., et al. 2011. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201104040.
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