

## A lifeline for lipid rafts?

Study finds raft-like domains in yeast vacuole membrane.

So far, definitive proof of the existence of lipid rafts *in vivo* has slipped through researchers' fingers. Although their results probably won't settle the debate over whether such structures exist, Toulmay and Prinz reveal that yeast produce raft-like domains in a surprising place (1).

The original raft hypothesis held that the plasma membrane harbors orderly, long-lasting lipid subdomains (2). Rich in sterols and sphingolipids, these subdomains purportedly cluster proteins and promote activities such as trafficking and signaling. Studies have detected what appear to be rafts in giant unilamellar vesicles, lab-made lipid spheres that are so large researchers can scrutinize them with fluorescence microscopy (3), but it has been tricky to pinpoint rafts in cells without resorting to techniques, such as detergent extraction, that meddle with the membrane. Super-resolution microscopy suggests that, instead of coalescing into large, durable structures, lipids convene to form small, ephemeral clusters (4). Thus, whether rafts are real remains a matter of debate.

To hunt for raft-like structures *in vivo*, Toulmay and Prinz took advantage of a specific cellular membrane: the one that swaddles the yeast vacuole. The organelle is similar to the lysosome and helps the cell manage pH and osmolarity. The researchers started by tracking the protein Vph1, part of a pump that acidifies the vacuole interior. In well-fed cells, Vph1 was dispersed throughout the vacuole membrane. But when cells were under stress, the protein converged into a few clumps. The team next tracked the distributions of a further fourteen vacuole membrane proteins. Twelve of the proteins copied Vph1, whereas two proteins formed a reciprocal pattern, avoiding Vph1 patches. This difference suggested that two distinct

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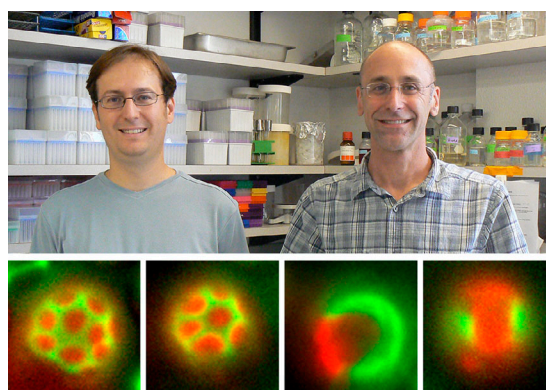


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### FOCAL POINT

Lipid rafts have proven elusive *in vivo*, motivating Alexandre Toulmay (left) and Will Prinz (right) to look for these structures in the membrane of the yeast vacuole. The organelle balloons when a cell is hungry or stressed, and, as the researchers found, its membrane segregates into domains. (Bottom row) In these images of the vacuole membrane, the proteins Ivy1 (red) and Vph1 (green) mark these domains.

domains assemble in the vacuole membrane during stress.

The Vph1-lacking domains share several characteristics with rafts. For example, they weren't just the result of brief molecular encounters. Toulmay and Prinz found that they could endure for more than three hours and were rich in sterols, as envisaged by the original raft model. The primary sterol in these domains was ergosterol, the yeast substitute for cholesterol. To gauge the importance of ergosterol for the clumps, the scientists added fenpropimorph, which blocks ergosterol synthesis. The number of domains dwindled in cells that received the treatment.

The researchers found other structural similarities to previously described rafts. The Vph1-lacking domains were more orderly than the surrounding lipids. In giant unilamellar vesicles, researchers can observe seams at the junctions between domains, where the membrane bends slightly because one lipid domain is slightly taller than its neighbor. Toulman and Prinz also saw these seams in the yeast vacuole membrane.

To determine what spurs the clusters to form, the researchers subjected yeast cells to an assortment of stresses. Glucose scarcity, disruption of protein synthesis, and low pH prompted the vacuole domains to

assemble. To investigate the influence of pH further, the researchers observed yeast cells in a neutral medium and noted that the domains failed to form. If cells already sported domains, raising the medium's pH caused them to dissipate. By testing various mutants, the researchers determined that genes involved in several processes, including phospholipid homeostasis and vesicle delivery to the vacuoles, were essential for domain formation.

The work shows that "membrane domains can form in live, unperturbed cells," says senior author Will Prinz. He adds that "raft" is a loaded term that he hesitates to apply to the domains but says, "I do think they are very similar to the classical idea of rafts." A key question now, Prinz says, is how changes in the vacuole membrane's lipid and protein content are responsible for the waxing and waning of the clusters. Researchers also need to nail down what benefits the domains provide to cells.

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