Phosphatidylserine (PtdSer) and phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) have been implicated in the maintenance of caveolae, but direct evidence that these lipids are required for normal caveolar structure and dynamics in living cells has been lacking. A new study by Fairn and colleagues uses sophisticated tools to perturb specific lipids in living cells to assess the consequences for caveolae. This study demonstrates disparate roles for these lipids in the stability and mobility of caveolae and points the way for future work to understand how these lipids contribute to the biology of caveolae.

Caveolae, literally “little caves,” have been known since the 1950s as specialized invaginations of the plasma membrane (1, 2). They have since been proposed to participate in a wide variety of cellular processes, including lipid metabolism, cell signaling, trafficking, and migration. The range of biology involving caveolae is reflected in the fact that mutations in caveolar structural proteins result in human diseases involving multiple organ systems.

Despite their discovery almost 70 years ago, the molecular details of the composition and assembly of these structures have only recently begun to emerge (1, 2). Caveolin proteins Cav1, Cav2, and Cav3 are the core components of caveolae. The caveolins self-assemble into disk-shaped oligomers composed of ~7–14 monomers. Caveolae also contain the cavin proteins, cav1, cavin2, cavin3, and cavin4. cavin1 self-assembles into trimers. The minimal structural unit of caveolae is thought to contain ~144 caveolin monomers associated with ~20 cavin trimers. Cryoelectron tomography indicates that these proteins assemble on the membrane to produce a dodecahedral structure. The flat faces of this structure are proposed to be occupied by disks of caveolin, whereas the vertices are proposed to be composed of trimers of cavins.

Given their placement at the membrane, it is perhaps no surprise that caveolar components interact with lipids. The core protein Cav1 has been observed to bind to phosphatidylserine (PtdSer) in vitro and also interacts with other anionic lipids such as phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) (3, 4). Likewise, cav1 can bind PtdSer in vitro with high affinity and specificity (5). Furthermore, interrogation of membrane lipid composition suggests that there is enrichment of PtdSer and PtdIns(4,5)P₂ at caveolae (6, 7). Although in vitro experiments predict a role for PtdSer and PtdIns(4,5)P₂ in caveolae, testing this prediction in live cells is not straightforward and thus has remained an important gap in our knowledge. The current study by Fairn and colleagues (8) bridges that gap, using sophisticated tools to allow perturbation of specific lipids, together with quantitative imaging methods to assess the effects on caveolae.

To characterize caveolae, the authors produced cell lines that stably express low levels of Cav1-GFP or cav1-GFP. Control experiments indicate that these GFP-tagged proteins behave similarly to endogenous, untagged proteins. As expected for caveolar proteins, both proteins are seen to localize to puncta. Using automated image analysis, the authors assess not only the number of puncta per cell but also the integrated intensity of each puncta, providing an indication of the number of GFP molecules incorporated into each puncta, and thus the relative molecular size of each puncta. In addition, time-lapse imaging allows for particle tracking to determine the motion of each puncta.

The authors then use a few tricks to interfere with specific lipids. To mask PtdSer present in the cell, they make use of a dimer of the C2 domain from the protein lactadherin, which binds with high affinity and specificity to PtdSer and competes for interaction of this lipid with other proteins (9). Expression of high levels of this dimer results in a decrease in the number of caveolae (assessed by electron microscopy), a decrease in the number and intensity of puncta (assessed by imaging of either Cav1-GFP or cav1-GFP), and increased mobility of the puncta (Table 1). Similar results are observed in a CHO cell line deficient in PtdSer due to mutations in PtdSer biosynthetic enzymes. A limitation of these experiments is that they do not allow for acute depletion of PtdSer, and it remains possible that the effects observed are indirect. Nevertheless, taken in the context of the prior in vitro studies, these experiments provide a valuable demonstration of a requirement for PtdSer to maintain normal caveolae in living cells.

The authors also investigate the role of plasma membrane phosphoinositides in maintaining caveolae in cells. Here, they make use of a series of rapamycin-regulatable phosphoinositide phosphatases (10). These phosphatases can be inducibly recruited to the plasma membrane in response to rapamycin, resulting in rapid depletion of PtdIns(4,5)P₃, PtdIns(4)P, or both, from the plasma membrane. The authors observe that depleting these phosphoinositides from the plasma membrane has no significant effect on the number of caveolae or...
their fluorescence intensity (Table 1). However, depletion of PtdIns(4)P, PtdIns(4,5)P2, or both, causes a dramatic increase in the mobility of the caveolae.

The results from Fairn and colleagues (8) provide the first direct evidence that perturbation of specific lipids has consequences for caveolae, yet many questions remain to be answered. Perhaps, most importantly, a solid mechanistic understanding of the system is not yet forthcoming. The role of phosphoinositides in limiting the mobility of caveolae could be mediated by a direct interaction with caveolar proteins or could be more indirect, for example via effects on cortical actin, which is known to be regulated by phosphoinositides. Likewise, the role of PtdSer in caveolae could involve direct binding to caveolins and/or cavins, as observed in vitro, or could be mediated by a more indirect mechanism. The methods reported here, combined with the use of mutational analysis, open new experimental avenues to shed light on the subject. The development of constructs that allow for rapidly inducible masking or depletion of PtdSer may also be instructive. Fairn and colleagues have assembled the rope, headlamps, and compass for descent into the caveolar “little caves,” enabling exploration of the roles of lipids in their assembly, maintenance, and anchoring.

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Table 1
Summary of a few of the key findings of Hirama et al. (8). These results are described in more detail in the main text

| Perturbation          | Caveolae Density | Caveolae Intensity | Caveolae Mobility |
|-----------------------|------------------|--------------------|-------------------|
| Interfere with PtdSer | ↓                | ↓                  | ↑                 |
| Deplete PtdIns(4)P    | –                | –                  | ↑                 |
|                       | PtdIns(4,5)P2    |                    |                   |

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