CELL BIOLOGY

Lipids under scrutiny

Researchers have developed a system to rapidly deplete or increase specific phosphatidylinositide species at the plasma membrane.

If a marketing firm were to take on the promotion of phosphatidylinositides (PIPs) an appropriate slogan might be: "You name it, they do it." PIPs are involved in a plethora of biological processes such as intracellular signaling, vesicle trafficking and cell migration; they function either by targeting essential proteins to membranes or by providing a source for soluble second messenger molecules. Unfortunately, the importance of PIPs is directly proportional to the challenges their study presents.

PIPs are made up of a fatty acid, which anchors them to the plasma or intracellular membrane, and an inositol ring, which can be modified by lipid kinases or phosphatases. Overexpressed lipid-modifying enzymes are one tool to investigate the role of specific PIPs, but this instrument is blunt and left scientists like Tobias Meyer at Stanford University dissatisfied. He says, "What we want to look at is a really quick signaling process, but by overexpressing the enzyme we manipulate PIP levels over many hours. So we were never really sure if we interrupted a lot of downstream housekeeping general functions in the cell." His goal was to develop a tool for the rapid and selective modification of one PIP species at a time.

Meyer's team modified a method Takanari Inoue, a postdoctoral fellow in the laboratory, had previously used to target proteins to the plasma membrane. It required three components: the chemical rapamycin and the two proteins FRB and FKBP12, which heterodimerize upon rapamycin addition. The researchers constitutively targeted FRB to the plasma membrane and fused FKBP12 to a lipid kinase or phosphatase. Adding rapamycin to cells overexpressing the two proteins brings the enzyme to the plasma membrane, where it modifies its target PIP (**Fig. 1**).

With Meyer and his group thus armed with a way to rapidly deplete a specific PIP, the door to asking questions about some of its roles was opened. The team first used the technique to investigate how GTPases with polybasic amino acid stretches are anchored to the plasma membrane. They selectively depleted both $PI(4,5)P_2$ and $PI(3,4,5)P_3$—the former using their new lipid-phosphatase targeting method, the latter by inhibiting the kinase that creates $PI(3,4,5)P_3$—and showed that these two lipids are sufficient to anchor GTPases at the plasma membrane (Heo *et al.*, 2006).

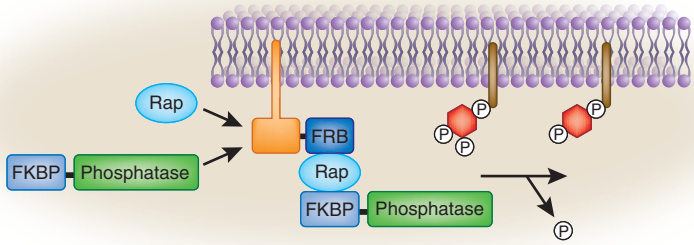

Figure 1 | Rapamycin (Rap) induces the heterodimerization of two rapamycin-binding peptides—FRB anchored to the plasma membrane via a membrane protein (orange), FKBP fused to a lipid phosphatase—and brings the enzyme to the plasma membrane, where it modifies the lipids.

In collaboration with Bertil Hille from the University of Washington, Meyer used the technique to investigate the role of PIPs in the activation of a K^+ channel (Suh *et al.*, 2006). They used a $PI(4,5)P_2$ phosphatase to deplete $PI(4,5)P_2$ and a lipid kinase to increase $PI(3,4,5)P_3$ levels and showed that $PI(4,5)P_2$ depletion is sufficient to inhibit channel activity.

In parallel to the Meyer and Hille work, another group at the US National Institutes of Health led by Tomas Balla developed a very similar strategy to show the importance of $PI(4,5)P_2$ for Ca^{2+} signaling and endocytosis (Varnai *et al.*, 2006).

Although this technique has many applications, Meyer also stresses that it is not yet an off-the-shelf method but requires careful optimizing for each new experimental setup. Furthermore, he emphasizes that an appropriate system to monitor the PIP that is being created or depleted is important. Meyer cautions that without such reporters the efficacy of the method will be harder to establish.

An effective control is a reciprocal approach. Meyer says, "It would be good to have two enzymes, one that makes a species, the other that degrades it. That gives you more confidence that you really see the right effect."

With these controls in place, Meyer sees two developments for the near future: "One is to make more specific enzymes for [plasma membrane] signaling processes and the other to create targeted species for particular membrane compartments inside the cell, like endosomes, Golgi or ER, where signaling and regulatory processes are also known to occur."

Although this technique is not yet high throughput and requires some diligent optimization, it promises to shine light on the role of PIPs in important cellular processes in every membrane inside the cell.

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RESEARCH PAPERS

Heo, W.D. *et al.* $PI(3,4,5)P_3$ and $PI(4,5)P_2$ lipids target proteins with polybasic clusters to the plasma membrane. *Science* **314**, 1458–1461 (2006).

Suh, B.C. *et al.* Rapid chemically induced changes of $PI(4,5)P_2$ gate KCNQ ion channels. *Science* **314**, 1454–1457 (2006).

Varnai, P. *et al.* Rapidly inducible changes in $PI(4,5)P_2$ levels influence multiple regulatory functions of the lipid in intact living cells. *J. Cell. Biol.* **175**, 377–382 (2006).