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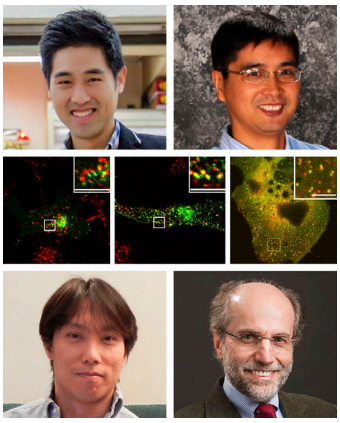
PI(4)P gets Sac-rificed in the name of endocytic recycling

Two studies reveal that Sac2 acts as a phosphoinositide 4-phosphatase on early endosomes.

Focal Point

Two groups find that the enzyme Sac2 is a PI 4-phosphatase that localizes to early endosomes and hydrolyzes PI(4)P. (Top row) FoSheng Hsu (left), Yuxin Mao (right), and Fenghua Hu (not pictured) reveal that, compared with control cells (middle row, left), PI(4)P (green) accumulates on endosomes (red) in the absence of Sac2 (center), which inhibits endocytic recycling and slows cell migration. (Bottom row) Fubito Nakatsu (left), Pietro De Camilli (right), and colleagues (not pictured) demonstrate that the small GTPase Rab5 promotes the formation of a complex between (middle row, right) Sac2 (green) and the PI 5-phosphatase OCRL (red) that might serve to sequentially dephosphorylate endosomal PI(4,5)P_2 in a similar manner to the synaptojanin family of dual PI phosphatases.



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Phosphoinositide (PI) lipids regulate a wide variety of cellular processes, from cell signaling to cytoskeletal dynamics, by controlling the identity and properties of cellular membranes. A large number of PI kinases and phosphatases restrict the distribution of PI species and give each cellular compartment its own, distinct PI signature. When vesicles are transported from one compartment to another, therefore, their PI composition must be modified accordingly. Two papers by Hsu et al. and Nakatsu et al. reveal that the phosphatase Sac2 promotes endocytic trafficking by dephosphorylating PI(4)P (1, 2).

Sac2 is one of five vertebrate proteins that contain a Sac1 phosphatase domain. Other members of the family, including Sac1 itself, have been shown to dephosphorylate PI(4)P, but Sac2 was initially reported to remove the 5′ phosphate group from both PI(4,5)P_2 and PI(3,4,5)P_3 (3). "That puzzled us," says Yuxin Mao from Cornell University in Ithaca, New York, "because the residues forming the phosphatase catalytic site in both Sac1 and Sac2 are almost identical."

Mao and colleagues, led by graduate student FoSheng Hsu, purified human and mouse Sac2 and retested its ability to dephosphorylate different PIs in vitro (1). "It only hydrolyzed PI(4)P, and not the lipids it had previously been reported to target," Mao says. That made sense from a biochemical point of view, but it raised the question of what Sac2 does in cells. Mice lacking Sac2 develop cardiac hypertrophy, a phenotype attributed to the phosphatase's ability to dephosphorylate PI(3,4,5)P_3 and thereby attenuate PI3 kinase signaling (4). Hsu et al.'s findings suggested that Sac2 might have another function, so the researchers examined the phosphatase's intracellular distribution and found that it localized to early endosomes containing endocytic cargoes such as the transferrin receptor. Moreover, PI(4)P accumulated on these organelles in the absence of Sac2. "So we thought Sac2 might function in endocytic recycling," Mao explains.

> "Nature has split these properties into two different proteins."

Sure enough, endocytic trafficking was inhibited in cells lacking Sac2, delaying the recycling of both the transferrin receptor and the adhesion molecule β1 integrin to the cell surface. As a result, *Sac2*-deficient neuroblastoma cells migrated more slowly than wild-type cells.

Meanwhile, at Yale University in New Haven, Connecticut, Pietro De Camilli and colleagues had also discovered that Sac2 was a PI 4-phosphatase that dephosphorylated PI(4)P and localized to early endosomes (2). During endocytosis, PI(4,5)P_2, which is enriched at the plasma membrane, must be dephosphorylated and converted to PI(3)P, the predominant PI on early endosomes. Synaptojanins, which are well conserved from yeast to mammals, play a major role in this process, as they contain a PI 5-phosphatase domain as well as a Sac1 domain that removes the 4′ phosphate group. Unlike yeast, however, mammals also express PI 5-phosphatases, such as OCRL and INPP5B, that localize to endosomes but lack Sac1 domains. De Camilli and colleagues, led by postdoc Fubito Nakatsu, therefore wondered whether Sac2 might work in conjunction with these 5-phosphatases to mimic the activity of synaptojanins and dephosphorylate endosomal PI(4,5)P_2.

Nakatsu et al. found that Sac2 colocalized with OCRL and INPP5B on endosomal membranes. The small GTPase Rab5, which recruits OCRL to early endosomes, also recruited Sac2 and promoted the formation of a complex containing the two phosphatases, suggesting that, like the two phosphatase modules of synaptojanins, Sac2 and OCRL cooperate to sequentially dephosphorylate PI(4,5)P_2. "Nature has split these properties into two different proteins," De Camilli says, explaining that this might help fine tune endocytic recycling in some cell types, whereas synaptojanin 1 is optimally adapted for synaptic vesicle recycling in neurons. Partial redundancy between the phosphatases might explain the relatively mild phenotype of *Sac2* knockout mice, however, and mutations in both the Sac2 and synaptojanin 1 genes have been linked to Parkinson's disease. De Camilli and Mao want to investigate these mice in more detail and to learn more about the role of PI(4)P in endocytic trafficking and how Sac2 is targeted to early endosomes in order to remove it.

1. Hsu, F., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201408027.
2. Nakatsu, F., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201409064.
3. Minagawa, T., et al. 2001. *J. Biol. Chem.* 276:22011–22015.
4. Zhu, W., et al. 2009. *Circ. Res.* 105:1240–1247.