

## A three-alarm signal for endocytosis?

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A combination of high membrane curvature and two phosphoinositides initiates an actin polymerization pathway that could help cells complete endocytosis when vesicle scission is delayed.

Though actin is essential for clathrin-mediated endocytosis in budding yeast, it is not always required for this process in mammalian cells. One possibility is that actin-dependent forces only become necessary when elevated membrane tension delays or prevents clathrin-coated pits from fully invaginating and separating from the plasma membrane. In this issue, Daste et al. describe an actin polymerization pathway that could be specifically triggered on stalled endocytic intermediates to ensure endocytosis is completed successfully (1).

Actin dynamics help deform membranes during a variety of cellular processes, including cytokinesis and the formation of membrane protrusions. In 2013, Jennifer Gallop and colleagues identified a pathway that prompts the assembly of branched actin filaments on the surface of liposomes placed into frog egg extracts (2). The pathway is triggered by the curvature of the liposome membrane and the presence of the phosphoinositides PI(4,5)P<sub>2</sub> and PI(3)P, and depends on both the GTPase Cdc42 and the adaptor protein SNX9, which, together, stimulate N-WASP-WIP- and Arp2/3-mediated actin polymerization.

All of these factors have been implicated in endocytosis. PI(4,5)P<sub>2</sub>, for example, is enriched at the plasma membrane but is converted, via a series of lipid kinases and phosphatases, to PI(3)P on early endosomes. “We wondered whether this pathway might be specifically activated when you have a stuck, highly-curved endocytic intermediate that is still contiguous with the plasma membrane and contains both of those phospholipids,” explains Gallop, a group leader at the Gurdon Institute, University of Cambridge.

Gallop and colleagues, including Frederic Daste, Astrid Walrant, and Jonathan Gadsby, first confirmed that PI(4,5)P<sub>2</sub>, PI(3)P, Cdc42, N-WASP-WIP, Arp2/3, and SNX9 were both necessary and sufficient to stimulate actin assembly on liposomes in vitro. Whereas Cdc42 was activated by PI(4,5)P<sub>2</sub> alone, SNX9’s activity depended on PI(4,5)P<sub>2</sub>, PI(3)P, and the liposome membrane’s curvature.

SNX9’s adjacent PX and BAR domains specifically bind to a combination of PI(3)P



**Focal Point** A team of researchers, including (left to right) Frederic Daste, Jennifer Gallop, Jonathan Gadsby, and (not pictured) Astrid Walrant and Mikkel Holst, reveal that the simultaneous presence of high membrane curvature and the phospholipids PI(3)P and PI(4,5)P<sub>2</sub> activates a SNX9-dependent actin polymerization pathway that could help cells complete endocytosis when vesicle scission is impaired by membrane tension. In vitro, SNX9 preferentially binds and stimulates the assembly of actin (red) on small (purple) rather than large (green) liposomes containing PI(3)P and PI(4,5)P<sub>2</sub>. The lipid phosphatase INPP4A, which generates PI(3)P, also shows a preference for highly curved membranes. The pathway appears to be hyperactivated in Lowe syndrome, but can be suppressed by inhibiting PI(3)P production. PHOTOS COURTESY OF THE AUTHORS.

and PI(4,5)P<sub>2</sub>, the researchers discovered. “SNX9 does not bind much to PI(3)P alone,” explains Gallop. “The binding is only significant when the BAR domain is also bound to PI(4,5)P<sub>2</sub>.”

SNX9’s PX-BAR domain also preferentially binds to curved membranes, thereby determining the protein’s propensity to stimulate actin assembly on smaller, highly-curved PI(4,5)P<sub>2</sub>/PI(3)P liposomes. Moreover, Daste et al. found that high membrane curvature stimulates the activity of INPP4A, a lipid phosphatase that generates PI(3)P during endocytosis by hydrolyzing PI(3,4)P<sub>2</sub>. Adding INPP4A to the in vitro reaction mixture allowed SNX9 to stimulate actin assembly on small PI(4,5)P<sub>2</sub>/PI(3,4)P<sub>2</sub> liposomes.

To test if this pathway affects endocytosis in cells, Gallop and colleagues collaborated with Mikkel Holst and Richard Lundmark from Umeå University in Sweden. They found that knocking down INPP4A in HeLa cells impaired the recruitment of SNX9 to clathrin-coated pits and reduced the endocytic uptake of transferrin. With the help of Marcel Mettlen from the University of Texas Southwestern Medical Center, the researchers found that INPP4A knockdown inhibited the maturation of clathrin-coated pits, significantly extending the average pit lifetime.

The curvature-sensitive generation of PI(3)P and the subsequent, curvature-sensitive binding of SNX9 might therefore trigger actin assembly at stalled endocytic intermediates, helping these vesicles undergo scission. But the pathway may be hyperactive in Lowe syndrome, a rare genetic disorder caused by mutations in the PI(4,5)P<sub>2</sub> phosphatase OCRL, leading to elevated PI(4,5)P<sub>2</sub>. Patient-derived fibroblasts show defects in endocytosis and clathrin-coated vesicles that are propelled through the cytoplasm at high speed by SNX9-dependent actin comets (3).

Daste et al. found that reducing PI(3)P levels—either by knocking down INPP4A or by inhibiting PI 3-kinases—decreased the number of actin comets in OCRL-deficient cells. Already in development as anti-cancer agents, PI 3-kinase inhibitors could therefore be a new approach for treating Lowe syndrome. Gallop and colleagues now want to further investigate the role of the SNX9 pathway in this disease, as well as test whether its normal function is to complete endocytic events delayed by membrane tension.

1. Daste, F., et al. 2017. *J. Cell Biol.* 10. <https://doi.org/10.1083/jcb.201704061>
2. Gallop, J.L., et al. 2013. *Proc. Natl. Acad. Sci. USA.* 110:7193–7198.
3. Nández, R., et al. 2014. *eLife.* 3:e02975.

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