Text by Mitch Leslie
mitchleslie@comcast.net

Making sure late endosomes are on time

Researchers discover two proteins that help maturing endosomes shed phosphatidylinositide.

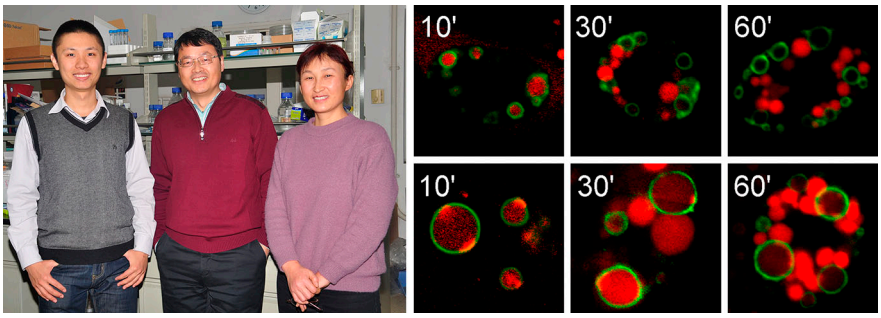
Early endosomes collect and sort cargoes before maturing into late endosomes that ferry those cargoes to the lysosome. Liu et al. identify two proteins that spur early endosomes to transform and thus help ensure that cellular deliveries occur on schedule (1).

As an early endosome morphs into a late endosome, it revamps its molecular composition. Early endosomes carry the GTPase Rab5, which attracts components, such as Vps34 and the CORVET/HOPS tethering complex, that enable them to fuse with other early endosomes (2). Early endosomes also carry phosphatidylinositol 3-phosphate (PtdIns3P), a phospholipid that promotes fusion with other early endosomes and helps sort cargoes (3). When early endosomes begin to convert into late endosomes, they swap Rab5 for the GTPase Rab7. And instead of PtdIns3P, late endosomes sport the phosphatidylinositide PtdIns(3,5)P_2 (4). Replacement of PtdIns3P is a key step, but researchers are still trying to work out which proteins control its levels on maturing endosomes.

Liu et al. followed endosome maturation in *C. elegans* coelomocytes, the worm's version of macrophages. The researchers had previously discovered that knocking out VPS-18, a component of the CORVET/HOPS complex, impairs fusion between endosomes and lysosomes. When they screened worms for mutations that could partially overcome this defect, the researchers identified two genes they named *sorf-1* and *sorf-2*.

Loss of either gene resulted in oversized early endosomes carrying extra amounts of PtdIns3P. To determine the genes' roles in endosome dynamics, the researchers injected a red dye into worms. In normal nematodes, coelomocytes absorb the dye, which then briefly appears in early endosomes before moving on to late endosomes. In worms lacking either *sorf-1* or *sorf-2*, however, the dye remained longer in early endosomes.

FOCAL POINT



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(Left to right) Kai Liu, Chonglin Yang, Youli Jian, and colleagues investigated how maturing early endosomes control levels of PtdIns3P. They determined that *sorf-1* and *sorf-2* spur early endosomes to replace PtdIns3P. If endosomes don't swap out PtdIns3P, intracellular trafficking is disrupted. Time-lapse imaging shows that normal endosomes (green) rapidly pass on the red dye TR-BSA (top row). But enlarged endosomes lacking *sorf-1* and *sorf-2* (bottom row) retain the compound.

Next, the researchers tracked the levels of Rab7 and PtdIns3P on maturing endosomes. In cells from normal worms, the amount of PtdIns3P on endosomes rose and stabilized. The level then plunged at about the same time as the level of Rab7 peaked. But in cells from worms missing *sorf-1*, *sorf-2*, or both genes, PtdIns3P remained on endosomes for about twice as long.

PtdIns3P didn't leave the endosomes at all if Liu et al. also deleted one of the genes involved in replacing Rab5 with Rab7. So the researchers asked what happens to early endosomes if PtdIns3P doesn't depart. Although early endosomes often merge with each other, endosomes that retained PtdIns3P fused even more often, producing giant organelles. These results indicate that SORF-1 and SORF-2 team up with the Rab-switching proteins to reduce PtdIns3P levels on maturing endosomes, thereby preventing them from growing too large.

"We've discovered a mechanism for PtdIns3P regulation on endosomes."

To determine which proteins help SORF-1 and SORF-2 perform this job, the researchers deleted genes that alter PtdIns3P abundance. They discovered that Beclin1, which is part of the PI3K complex that synthesizes PtdIns3P on early endosomes, interacts with SORF-1 and SORF-2. Their experiments suggest that *sorf-1* and *sorf-2* act through Beclin1 to reduce PtdIns3P synthesis on early endosomes.

Humans carry their own versions of SORF-1 and SORF-2, WDR91 and WDR81. Liu et al. determined that these proteins gather on early endosomes in human cells and interact with Beclin1. By inhibiting the PI3K complex, WDR91 and WDR81 diminish PtdIns3P levels and promote intracellular trafficking.

Prompt endosome transport is important for many cellular functions, including signaling, and the study suggests that *sorf-1* and *sorf*-2 speed the process along by promoting endosome maturation. "We've discovered a mechanism for PtdIns3P regulation on endosomes," says senior author Chonglin Yang. The findings might also provide insight into the mechanism behind a rare disease. Mutations in WDR81 trigger CAMRQ2 syndrome. The symptoms of the illness, which include mental retardation and the inability to walk, could stem from faulty endosome transport. The results suggest that researchers should check whether mutations in WDR91 also cause disease, Yang says.

1. Liu, K., et al. 2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201506081
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3. Di Paolo, G., and P. De Camilli. 2006. *Nature.* 443:651–657.
4. Huotari, J., and A. Helenius. 2011. *EMBO J.* 30:3481–3500.