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PIPing on lysosome tubes

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The role of lysosomes in important cellular responses, including phagocytosis, cell surface repair, and autophagy underlies a number of human diseases. Furthermore, the role of the lysosomal surface in TORC1 signalling has revealed unexpected properties of these organelles. In this issue, Sridhar *et al* (2013) uncover an important role for PI(4)P for lysosome function under normal nutrient conditions and after prolonged nutrient deprivation. Ana Maria Cuervo, the late Dennis Shields, and colleagues (Sridhar *et al*, 2013) conclude that PI4 kinase IIIβ on the surface of the lysosome controls the fidelity of sorting from the lysosome, and is required for autophagic lysosome reformation (ALR). These novel findings provide important insights into the complexities of the lipid composition of the lysosome, and how these lipids may control lysosome function.

Lysosomes, traditionally thought of as inert, terminal endocytic compartments, are in fact dynamic organelles. In addition to their function in degradation of cargo ranging from small vesicles to whole phagosomes and autophago-

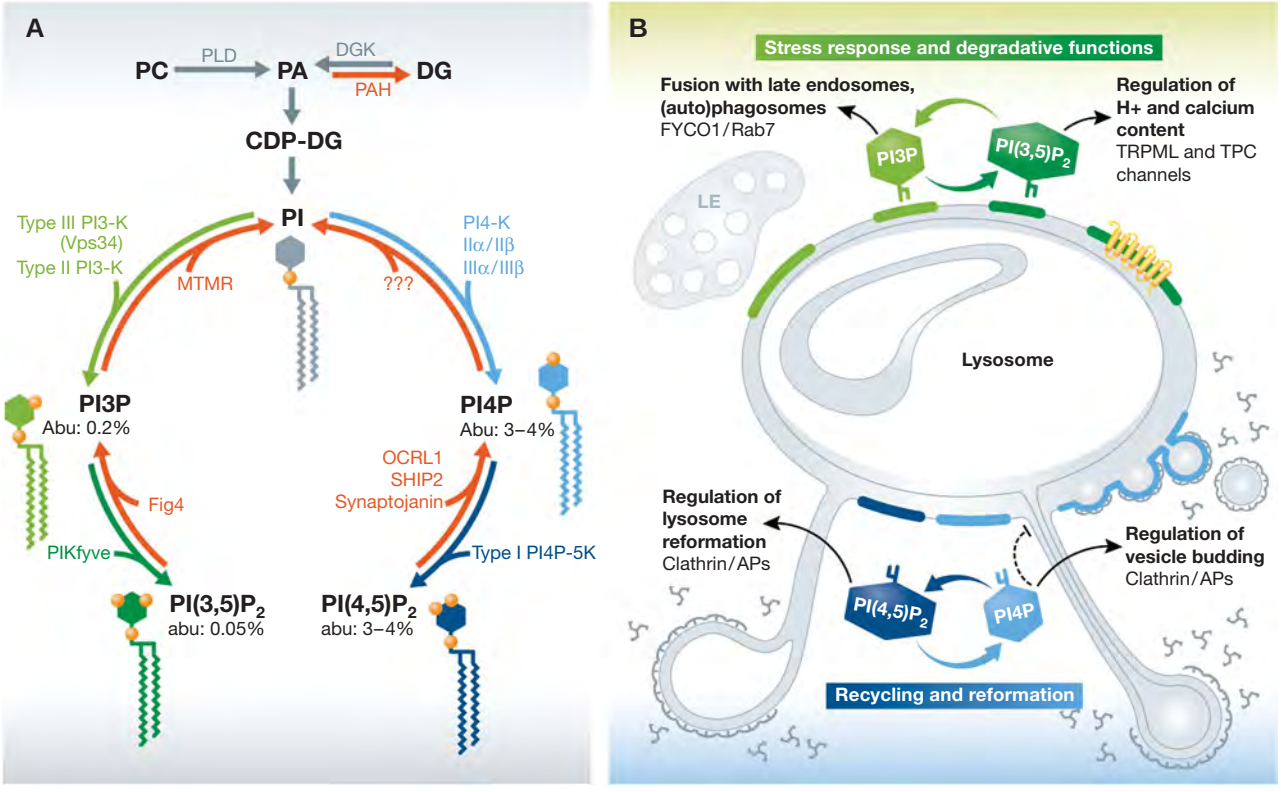

Figure 1 Two phosphoinositide cycles regulate aspects of lysosome function. (**A**) Pathway of synthesis and consumption of PI3P, PI(3,5)P_2, PI(4)P and PI(4,5)P_2 from PA via PI. The relative abundance of the various lipid species is shown, alongside the main enzymes responsible for their synthesis and consumption. (**B**) Stress responses of lysosomes following extracellular or intracellular challenge are regulated in part by PI3P and PI(3,5)P_2, with the former involved in fusion with other organelles and the latter in regulation of ion channels (shown in green). A second phosphoinositide cycle dependent on PI(4)P and PI(4,5)P_2 was uncovered in the paper by Sridhar *et al* (2013), which regulates traffic from the lysosomes either in the form of vesicles or tubules (shown in blue, PI(4)P favouring vesicular intermediates). Recent work by Yu and colleagues has also suggested that these lipids regulate lysosome reformation during enhanced flux, also thought to proceed via a tubular intermediate favouring PI(4,5)P_2 (shown in blue).

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somes, they respond dynamically to extracellular ion fluxes, and they can generate potent intracellular calcium (and other ionic) signals (Saftig and Klumperman, 2009; Morgan *et al*, 2011; Shen *et al*, 2011). The cytoplasmic surface of the lysosome mediates the delivery of cargo-containing vesicles and organelles via SNAREs and tethers, but is also a platform for TORC1 signalling and transcriptional regulation by TFEB (Settembre *et al*, 2011; Efeyan *et al*, 2012). The membrane of the lysosome, protected from the degradative enzymes contained in the lumen by highly glycosylated lysosomal membrane proteins (LAMPs, LIMPs, etc.), contains the machinery for its acidification (V-ATPase), as well as a variety of transporters and channels for ions and amino acids. LAMP2A also serves as the receptor for chaperone-mediated autophagy (CMA) that together with the chaperone protein Hsc70 delivers individual proteins into the lysosome (Orenstein and Cuervo, 2010). A key feature of lysosomes is their ability to reform (Luzio *et al*, 2007; Yu *et al*, 2010) and noteworthy here is the enhancement of reformation that occurs during prolonged nutrient deprivation described as ALR (Yu *et al*, 2010).

Many of these functions are thought to be dependent on PI(3,5)P_2 (phosphatidylinositol (PI)-3,5-bis phosphate), and the cycle of PI-3 phosphate (PI3P) to PI(3,5)P_2 (Figure 1A; Michell *et al*, 2006; Ho *et al*, 2012). In broad terms, PI3P is required during the fusion of vesicles/(auto)phagosomes with lysosomes, whereas PI(3,5)P_2 appears to regulate ion channels that maintain acidification and calcium content optimal for digestive functions (Figure 1B). Indeed, deficiency of PI(3,5)P2 caused by loss of lipid-modifying enzymes (both kinases and phosphatases) is the cause of several human neuropathologies (Ho *et al*, 2012), and at the cellular level, deficiency in the levels of either PI(3)P or PI(3,5)P2 leads to swelling and loss of lysosomal function (Michell *et al*, 2006; Ho *et al*, 2012). This phenotype underscores the fact that defects in lipid-modifying enzymes do not always produce clearly distinct effects because, in addition to lipid interconversion (see Figure 1A), deficiencies of one lipid species may feedback to other enzymes, leading to accumulation of unexpected lipids.

While PI(3,5)P_2 is present at very low levels (Figure 1A) it has a well-documented role in lysosome function (Figure 1B). Thus, identification of a role for a PI-4 kinase (PI4KIIIβ), and the more abundant lipids PI(4)P and PI(4,5)P_2, in the regulation of lysosome function, in particular vesicle-mediated exit from the lysosome, is surprising and unexpected (Sridhar *et al* (2013), this issue; Rong *et al* (2012)). PI(4)P, which is produced in cells by four different enzymes (Figure 1A), plays an important role at the plasma membrane and the trans-Golgi network (TGN), and out of these four enzymes PI4KIIIβ is most important for trafficking out of the TGN (Santiago-Tirado and Bretscher, 2011). Cuervo and colleagues (Sridhar *et al*, 2013) now show that loss of PI4KIIIβ causes constitutive formation of tubules from lysosomes and loss of retention of lysosomal cargo (Figure 1B). Dynamic LAMP1-positive tubules emanate from lysosomes in the absence of PI4KIIIβ which contain lysosomal content such as cathepsin D, a number of clathrin-coat components, including adaptor protein (AP)-2, kinesin motor Kif13b, and Rab9 (a small GTPase implicated in traffic to late endosomes and lysosome). Sridhar *et al* (2013) demonstrate the presence of these molecules, some of which are the core machinery for vesicle formation, using elegant fractionation approaches combined with cryoimmunogold labelling and live-cell imaging. Furthermore, preliminary data from two-dimensional electrophoresis suggest that a subpopulation of PI4KIIIβ may undergo a post-translational modification, and it is this subpopulation which is recruited to the lysosome.

Importantly, this work relates to a recent paper from Rong *et al* (2012), which demonstrates a role for PI(4,5)P_2 in ALR, supporting the notion that PI4P-PI(4,5)P_2 cycling regulates traffic out of lysosomes and reformation during enhanced flux. Rong *et al* (2012) used similar approaches including isolation and proteomic analysis of the tubules found during ALR to identify the components required for ALR, which includes clathrin and AP-2, AP-4 and PI(4)P5K1B and 1A. Rong *et al* (2012) suggested that PI(4)P5K1B was required during ALR for tubule formation, while PI(4)K1A was required for tubule extension and/or fission.

Sridhar *et al* (2013) recapitulate these findings and show, using LAMP1-RFP and fluorescent pepstatin A (which labels cathespin D) labelling, that loss of PI4KIIIβ results in inefficient sorting in particular into the tubules produced by loss of PI(4)P5K1A. It is proposed that PI(4)P levels control ALR; loss of PI4P causes constitutive formation of tubules and loss of retention of lysosomal cargo, and is required for ALR (Figure 1B). Cuervo and colleagues hypothesize that PI4P is not just a substrate for PI(4)P5Ks (the scenario proposed by Rong *et al* (2012)) but that it is in fact a critical molecule for determining the balance between tubulation and vesicular transport out of the lysosome during ALR and in normal conditions.

The work of Cuervo and her colleagues following on from Rong *et al* (2012) shows a masterful control of techniques needed to identify the machinery needed for ALR and efflux from the lysosome. Importantly, both papers looked at the lipid species present using lipid probes, and showed that kinase dead mutants of the kinases do not rescue. However, neither paper actually measured changes in lipid levels as a result of their manipulations. As pointed out above, this may change the interpretation. Some lipid effectors were identified, but no lipid binding mutants of effectors were employed, and no lipid phosphatases were used or identified. These unexplored issues leave open the possibility that further complex regulation is yet to be discovered which may involve effector proteins, and proteins that activate or inhibit the kinases. In summary, Cuervo and her team have provided a clue to how ALR may be mediated by PI(4)P, which combined with data provided by Rong *et al* (2012), clearly implicates a cycle of PI4P and PI4,5P_2 synthesis and hydrolysis in the trafficking out of lysosomes and ALR. Further exciting developments in understanding the dynamic nature of the lysosome have the potential to truly advance our knowledge of cellular homeostasis in both health and disease.

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Conflict of interest

The authors declare that they have no conflict of interest.

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