Lysosomes relax in the cellular suburbs

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Lysosomes support cellular homeostasis by degrading macromolecules and recycling nutrients. In this issue, Johnson et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201507112) reveal a heterogeneity in lysosomal pH and degradative ability that correlates with lysosome subcellular localization, raising questions about the functional implications and mechanisms underlying these observations.

Lysosomes must engage in a dynamic interplay with other organelles of the secretory, endocytic, and autophagy pathways to receive both the hydrolytic enzymes and transmembrane proteins that mediate their catabolic activity as well as to receive cargos destined for degradation. The degradative ability of lysosomes is supported by ∼50 luminal enzymes and cofactors as well as ion channels and transporters that support the efflux of nutrients that are liberated via the lysosomal digestion of diverse macromolecules. This degradative activity of lysosomes is tightly coupled to the acidic pH (4.5–5) within their lumen that is generated by the activity of the vacuolar proton ATPase (V-ATPase; Mindell, 2012). The acidic luminal environment supports the hydrolytic activity of lysosomal enzymes and provides an ion gradient that is harnessed by diverse lysosomal transporters to support secondary active transport of their respective substrates. Lysosomes are thus routinely identified in cell biological studies based on multiple parameters that include acidic luminal pH, ability to concentrate endocytic cargos, and degradative activity toward diverse cargoes, as well as their distinct composition of luminal and integral membrane proteins. Mammalian cells contain hundreds of individual lysosomes that vary considerably in size, shape, and subcellular localization (Fig. 1 A), and it was long assumed that they all shared similar degradative functions. However, in this issue, Johnson et al. reveal a previously unappreciated existence of lysosomal subpopulations in cultured mammalian cells that vary considerably in their subcellular position, intraluminal pH, and degradative activity. These important observations parallel recent findings of neuronal subpopulations of lysosomes defined by varying levels of luminal proteases (Gowrishankar et al., 2015), suggesting that the mechanisms and potential functional implications of localization-dependent lysosome characteristics may be similar and physiologically important in various cell types.

In their new work, Johnson et al. (2016) examined diverse lysosomal markers in cultured HeLa cells combined with quantitative analysis of fluorescently tagged pH-sensitive and -insensitive probes. The experiments revealed that lysosome intraluminal pH varies depending on subcellular localization, such that lysosomes closer to the cell periphery are strikingly less acidic than those in the perinuclear region. Further studies determined that the most peripheral pool of lysosomes comprised ∼20% of the total cellular pool of lysosomes and had a pH near six, as opposed to less than five for lysosomes in the perinuclear region. This finding is important given the central role played by lysosomal pH in supporting the maturation and activity of lysosomal hydrolases and unexpected given that low intraluminal pH (less than five) has long represented a defining feature for lysosomes (Saftig and Klumperman, 2009). C2C12 murine myoblasts and human microvascular endothelial cells displayed a similar heterogeneous distribution of acidic lysosomes as HeLa cells. In contrast, and for reasons that are not yet understood, primary human dendritic cells and Chinese hamster ovary cells exhibited more homogenous lysosomal pH.

The subcellular localization of lysosomes is largely determined by microtubule-based bidirectional transport. This transport is supported by small GTPases that associate with the lysosomal cytoplasmic surface and bind to effectors that interact with kinesin or dynein motors. For example, the Arl8 GTPase binds kinesin 1 through its effector SKIP (SifA and kinesin-interacting protein) and promotes microtubule plus end–directed transport of lysosomes, resulting in their movement toward the cell periphery (Rosa-Ferreira and Munro, 2011). The GTPase Rab7 promotes kinesin-mediated transport of lysosomes toward the cell periphery through interactions with FYCO1 (FYVE and coiled coil domain–containing protein) and dynein-mediated delivery of lysosomes toward the cell interior via interactions with RILP (Rab7-interacting lysosomal protein; Jordens et al., 2001). GTPase effector pairs also represent valuable tools for the experimental manipulation of lysosome positioning. Johnson et al. (2016) tested the link between lysosome position and pH by artificially driving lysosomes to the cell periphery using overexpression of GTPases as well as by inhibiting dynein. Both approaches revealed that the artificial repositioning of lysosomes to the cell periphery resulted in a reduction in their acidification, suggesting that the intracellular position of lysosomes is a major determinant of their pH.

Lysosome pH is regulated by many factors including the density of the V-ATPase proton pump on lysosomes, V-ATPase activity, and other proteins (Mindell, 2012). Consistent with the differences in intraluminal pH, Johnson et al. (2016) observed that the rate of H^+ pumping was decreased in peripheral compared with perinuclear lysosomes. They also found that the rates of proton "leakage," or passive proton permeability, were increased for peripheral lysosomes. These findings raise questions

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The Rockefeller University Press $30.00
J. Cell Biol. Vol. 212 No. 6 617–619
www.jcb.org/cgi/doi/10.1083/jcb.201602082

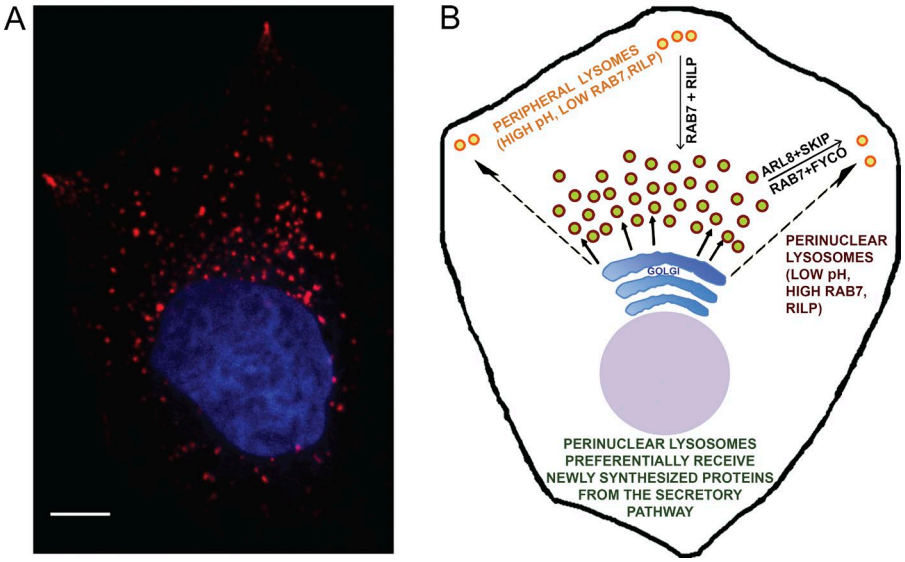

Figure 1. **Overview of the relationship between lysosome subcellular position and function.** (A) Lysosome distribution in a HeLa cell revealed by confocal imaging of the lysosomal marker LAMP1 (red) and nuclear staining with DAPI (blue). This image represents a maximum projection of two confocal sections. Image courtesy of A. Roczniak-Ferguson (Yale University, New Haven, CT). Bar, 10 µm. (B) Schematic diagram of the impact of subcellular localization on the functional properties of lysosomes. The GTPase effector pairs Rab7-RILP, Arl8-SKIP, and Rab7-FYCO control the localization of lysosomes within the cell. Peripheral lysosomes (yellow circles with orange borders) display reduced acidification caused by an increased passive leak of protons and reduced V-ATPase activity. This lysosome population displays reduced Rab7 density, resulting in decreased recruitment of the Rab7 effector RILP, which could both negatively impact the activity of the V-ATPase and limit dynein-mediated transport back toward the cell center. Peripheral lysosomes also exhibit reduced access to material from the secretory pathway. In contrast, perinuclear lysosomes (green circles with red borders) have a more acidic pH and higher Rab7-RILP density. Experimental movement of lysosomes from the perinuclear region to the cell periphery is associated with reduced acidification and impaired proteolytic activity.

about the mechanisms that control the delivery of V-ATPases to lysosomes at the cell periphery and/or their activity at such sites. Multiple subunits of the V-ATPase are assembled early in the secretory pathway before their delivery to lysosomes and disruption of such trafficking is known to result in reduced lysosome acidification (Swetha et al., 2011). Unfortunately, the lack of suitable antibodies precluded the assessment by Johnson et al. (2016) of the relationship between V-ATPase density and lysosome subcellular positioning. Thus, it remains unclear whether subcellular location-dependent lysosomal pH differences reflect reduced density of the V-ATPase or differential V-ATPase activity on more peripheral lysosomes. However, a recent study showed that V-ATPase activity is regulated by the GTP-bound Rab7 effector RILP (De Luca et al., 2014). The researchers found that the levels of both active Rab7 and RILP on peripheral lysosomes were reduced compared with those in the cell center (Fig. 1 B). This observation may explain the reduced acidification of peripheral lysosomes but raises questions about the mechanisms governing the abundance and activation of Rab7 on different lysosomal subpopulations. The increased rate of proton leakage from peripheral lysosomes could also reflect differences in ion channel abundance and/or regulation between peripheral versus centrally located lysosomes.

To investigate the degradative activity of peripheral lysosomes, Johnson et al. (2016) used a fluorescent reporter of cathepsin L protease activity and observed lower levels of cathepsin L activity in peripheral lysosomes. As cathepsin L maturation and activity are optimal at an acidic pH, the effect of lysosome localization on cathepsin L activity could reflect the elevated pH of peripheral lysosomes. Such a change in cathepsin L activity could also reflect a reduction in the delivery of newly synthesized proteases to peripheral lysosomes. Although technical challenges precluded a direct assessment of cathepsin L trafficking, Johnson et al. (2016) examined the lysosomal delivery of other cargos and observed that whereas the delivery of endocytic cargo to peripheral lysosomes was normal, there was reduced delivery of a newly synthesized protein (LIMP-2) to peripheral lysosomes. These findings suggest that the peripheral lysosome population may be generally less accessible to material from the secretory pathway.

Mismatches between the levels of intraluminal hydrolases, endocytic cargo, and integral membrane proteins in peripheral versus central lysosomes (Gowrishankar et al., 2015; Johnson et al., 2016) imply the existence of distinct mechanisms for their respective delivery to lysosomes. Although the best characterized mechanism for the sorting of lysosomal hydrolases from the secretory to the endolysosomal pathway is via interactions with the mannose-6 phosphate receptor (M6PR), some hydrolases are efficiently delivered to lysosomes in the absence of mannose-6 phosphate modification (Braulke and Bonifacino, 2009; Saftig and Klumperman, 2009). Other sorting receptors such as sortilin, lipoprotein receptors, and LIMP2 (also known as SCARB2) have been implicated in M6PR-independent sorting of lysosomal hydrolases (Reczek et al., 2007; Markmann et al., 2015). For example, interactions with the LDL receptor and Lrp1 support the M6PR-independent delivery of cathepsin D to lysosomes (Markmann et al., 2015), whereas glucocerebrosidase, whose loss causes a lysosome storage disorder known as Gaucher's disease, is routed through a LIMP-2–dependent mechanism (Reczek et al., 2007). Additionally, lysosomal integral membrane proteins such as the LAMPs interact with distinct sorting adaptors (Braulke and Bonifacino, 2009) and can traffic directly from the TGN to late endosomes through vesicular intermediates that are distinct from those used by hydrolases (Swetha et al., 2011; Pols et al., 2013). Although it seems plausible that some delivery routes to lysosomes could reach the cell periphery more efficiently than others, questions remain to be answered about the spatial control of the maturation process through which specific lysosomal proteins are transferred within the secretory and endolysosomal pathways.

These new observations of lysosome heterogeneity from mammalian cells in culture parallel recent observations of distinct subpopulations of lysosomes in neurons of the mouse brain that were defined by their varying levels of luminal proteases (Gowrishankar et al., 2015). These distinct populations of neuronal lysosomes also correlated with intracellular location such that the lysosomes within axons (most distant from cell bodies) were strikingly deficient in their luminal protease content compared with those within neuronal cell bodies. Such observations build on a growing understanding that axonal autophagosomes fuse with endosomes before undergoing a coordinated process of retrograde transport and lysosomal maturation (Overly and Hollenbeck, 1996; Maday et al., 2012; Cheng et al., 2015). These studies collectively support a model wherein the maturation of lysosome precursors is coordinated with their transport from distal regions of the axon toward the neuronal cell body. The growing understanding of subcellular location-dependent properties of lysosomes in both neuronal and nonneuronal cells suggests that similar underlying mechanisms may be at play in these different cell types.

Observations of functional differences between lysosomes that depend on their subcellular position raise interesting questions about cellular physiology. For example, a preferential role for peripheral lysosomes in plasma membrane repair, mediated through lysosomal exocytosis (Reddy et al., 2001), would make sense given their relative proximity to the plasma membrane. Alternatively, important signal transduction roles have recently emerged for lysosomes (Ferguson, 2015). For instance, distinct pools of lysosomes could preferentially support specific signaling functions for the integration of nutrient availability with growth factor–derived signals to control cell growth through regulation of mTOR complex 1 (Ferguson, 2015). Indeed, the V-ATPase interacts with components of the mTOR complex 1 signaling pathway and plays a critical role in the ability to sense and respond to changes in amino acid availability (Zoncu et al., 2011). Peripheral pools of lysosomes might also be ideally positioned to most rapidly respond to plasma membrane–derived nutrient and growth factor signals (Korolchuk et al., 2011). Clearly, much remains to be understood about how the subcellular localization and transport of lysosomes affect their various functions. In addition to the large number (>50) of human lysosome storage diseases that are characterized by the lysosomal accumulation of incompletely degraded substrates (Parenti et al., 2015), the identification of pathological conditions, such as Alzheimer's disease (Gowrishankar et al., 2015), which are associated with alterations in the location and proportion of distinct lysosomal pools, provides further motivation for the study of the contribution of peripheral lysosome subpopulations to cell physiology.

Acknowledgments

We are grateful to Agnes Roczniak-Ferguson and Pietro De Camilli for their suggestions on this manuscript.

This work was supported in part by grants from the National Institutes of Health (GM105718 and AG047270).

The authors declare no competing financial interests.

Submitted: 24 February 2016
Accepted: 24 February 2016

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