JCB Journal of Cell Biology

SPOTLIGHT

Neuronal endosomes to lysosomes: A journey to the soma

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How are lysosomal degradation pathways spatially organized in the complex landscape of a neuron? Cheng et al. (2018. *J Cell Biol.* https://doi.org/10.1083/jcb.201711083) and Yap et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201711039) characterize the distribution and function of endolysosomal organelles in neurons, providing insights into compartment-specific mechanisms regulating the neuronal proteome.

A fundamental question in the field of neuronal cell biology is: how are intracellular trafficking pathways orchestrated within the extreme morphology of the neuron to facilitate neuronal development, homeostasis, and functionality? Axons can extend up to 1 m in length, and dendrites develop elaborate and complex branching patterns. This architecture poses a spatial challenge to the directed traffic of cargo throughout the neuron such as the shuttling of cellular material to lysosomes for degradation and recycling. As endosomes and autophagosomes mature into degradative lysosomes, a continuum of intermediates is generated through the exchange of cargoes and membranes as well as insertion of proteolytic enzymes. However, the identity and distribution of this spectrum of endolysosomal organelles throughout the neuron has remained incompletely understood. Dysregulation of lysosomal degradation pathways and protein/organelle homeostasis is linked to various neurodegenerative disorders (Wang et al., 2018), and a comprehensive understanding of these pathways and how they impact neuronal viability and functionality is essential to understanding disease pathogenesis. Cheng et al. and Yap et al. help build a detailed roadmap of how lysosomal degradation is coordinated in the neuron to regulate the proteome.

Cheng et al. (2018) performed a quantitative analysis of the compartment-specific distribution (axon versus dendrites versus soma) of lysosomal-associated membrane protein 1 (LAMP1) in various types of neurons using fluorescence and electron microscopy in vitro and in vivo. Correspondingly, Yap et al. (2018) used fluorescence microscopy to characterize the distribution of endolysosomal organelles along the dendrites of primary hippocampal neurons from proximal to distal regions. LAMP1 is often used as a lysosomal marker; however, work from both teams quantitatively shows that LAMP1 is not restricted to lysosomes alone. Rather, LAMP1 localizes to a heterogeneous population of endolysosomal organelles including predegradative species. The two groups show that while nearly all cathepsin B/D–positive compartments colocalize with LAMP1, less than half of LAMP1-positive organelles colocalize with cathepsins (Cheng et al., 2018; Yap et al., 2018). The remaining LAMP1-positive organelles represent intermediates of the endocytic and autophagy pathways (Cheng et al., 2018; Yap et al., 2018). Thus, the two groups define lysosomes as LAMP1-positive, cathepsin B/D–positive, proteolytically active compartments. Cheng et al. (2018) caution interpreting LAMP1 analysis as a sole readout for lysosome functionality; indeed, deficits in lysosome function in primary motor neurons isolated from a familial amyotrophic lateral sclerosis mouse model were detected only with decreased cathepsin D signal relative to LAMP1 and not with LAMP1 alone (Cheng et al., 2018).

Both teams find that lysosomes are differentially distributed within the neuron, creating a gradient of degradative activity (Fig. 1 A; Cheng et al., 2018; Yap et al., 2018). Yap et al. (2018) demonstrate that EEA1-positive early endosomes and Rab7-positive late endosomes are localized throughout the dendrite (Fig. 1 B; Yap et al., 2018). By contrast, more mature compartments within the endolysosomal spectrum are enriched in proximal regions of the major dendrites and the soma. These organelles include mature versions of late endosomes, termed "late" late endosomes, that are distinguished from "early" late endosomes by the presence of LAMP1 on Rab7-positive vesicles but are not yet degradative and lack proteolytic enzymes (Yap et al., 2018). Degradative lysosomes exhibit a steep spatial gradient and decline sharply in abundance past the proximal dendrites of both young (7–10 d in vitro) and mature neurons (aged hippocampal cultures and cortical neurons from P28 mouse brain sections), consistent with degradative activity concentrated in the cell body and proximal dendrite (Yap et al., 2018). This gradient is

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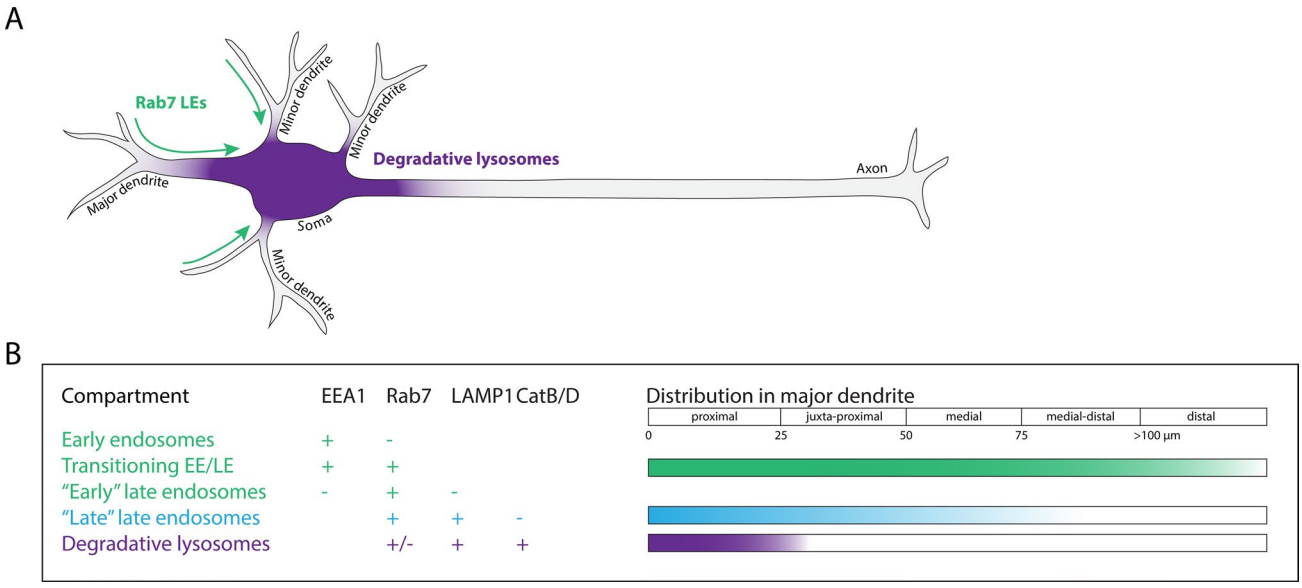

Figure 1. **Spatial gradient of endolysosomal organelles in neurons. (A)** Distribution of degradative lysosomes (defined as LAMP1-positive compartments containing active cathepsins) in the neuron. Arrows denote directionality of transport of Rab7-positive late endosomes to the soma for degradation. **(B)** Distribution of endolysosomal subpopulations in major dendrites. EE, early endosome; LE, late endosome.

steeper in thinner (or minor) dendrites, where degradative lysosomes are rarely present even in proximal regions (Fig. 1 A). Yap et al. (2018) confirmed the degradative nature of specific compartments by analyzing the distribution of endocytosed cargoes: Alexa Fluor 647 BSA was used to label all endocytic compartments versus DQ-BSA, which fluoresces only upon proteolytic cleavage. While Alexa Fluor 647 BSA was detected throughout dendrites, signal generated from proteolytic processing of the DQ-BSA was enriched in the soma and proximal regions of the major dendrites (Yap et al., 2018). They also used pharmacological inhibition of degradative activity with the protease inhibitor leupeptin to show the preferential accumulation of endocytic cargo only in the soma and not in dendrites (Yap et al., 2018). Data from Cheng et al. (2018) and Yap et al. (2018) establish a spatial gradient of organelle maturation in the endolysosomal pathway in neurons. LAMP1 labels a heterogeneous population of organelles ranging from predegradative endosomal species to degradative lysosomes, with mature compartments enriched in the soma relative to dendrites and the axon.

Yap et al. (2018) additionally demonstrate that Rab7 is required for the transport of distal dendritic cargoes to the proximal dendrite and soma for degradation (Fig. 1 A). Interference with Rab7 activity decreases the motility of dendritic cargoes, resulting in their accumulation in predegradative endosomes along the dendrite (Yap et al., 2018). This study, combined with previous research from their group, generates a model for endosome maturation along dendrites whereby early endosomes mature locally to Rab7-positive late endosomes (Yap et al., 2017, 2018). Rab7 is then required for the transport of medial and distal dendritic cargos to proteolytically active lysosomes in the soma and proximal dendrite for degradation (Yap et al., 2018).

The studies by Cheng et al. (2018) and Yap et al. (2018) integrate well into recent work also indicating a gradient of degradative activity within the neuron. Gowrishankar et al. (2015) showed in the mouse cerebral cortex and primary cortical neurons that cathepsin B/L–positive, LAMP1-positive compartments are enriched in the neuronal cell bodies, whereas peripheral regions of neurites are enriched for LAMP1-positive compartments that lack cathepsins (Gowrishankar et al., 2015). Lee et al. (2011) showed a gradient of cathepsin B activity in the axon that is concentrated in the soma and proximal axon and that progressively declines toward the distal axon (Lee et al., 2011). Some groups, however, observed a population of proteolytically active lysosomes in peripheral regions of dendrites (Goo et al., 2017; Padamsey et al., 2017). Nonetheless, the dramatically higher concentration of degradative lysosomes in the soma relative to dendrites has consistently been observed across systems (Goo et al., 2017; Padamsey et al., 2017; Cheng et al., 2018; Yap et al., 2018). In further support of this gradient of degradative activity, our work, along with others, showed that autophagosomes mature into degradative autolysosomes as they travel from distal regions of the axon toward the soma (Lee et al., 2011; Maday et al., 2012). Following autophagosome formation in the distal axon, autophagosomes initially fuse with LAMP1-positive late endosomes and undergo retrograde transport to the soma (Maday et al., 2012). Complete acidification and maturation into degradative autolysosomes occurs predominantly in the proximal axon and cell body (Lee et al., 2011; Maday et al., 2012). Blocking lysosome function with bafilomycin A1 results in the accumulation of autophagosomes specifically within the soma and not in the axon or dendrites (Maday and Holzbaur, 2016). Thus, the soma is likely the primary site of autophagosome deposition and cargo degradation, indicating a gradient of organelle maturation within the autophagy pathway in primary neurons.

In summary, Cheng et al. (2018) and Yap et al. (2018) provide comprehensive analyses of the identity, distribution, and function of endolysosomal organelles in the neuron to establish compartment-specific capacities for degradation, which are en-

riched in the soma. Trafficking to lysosomes in the soma likely represents the primary route for bulk degradation in neurons, providing an efficient mechanism to recycle biosynthetic building blocks to primary sites of protein synthesis. Evidence also supports degradative activity in the periphery that may serve localized and specialized functions, particularly at synaptic connections (Goo et al., 2017; Padamsey et al., 2017). The high frequencies with which neurons fire action potentials renders synaptic proteins and organelles vulnerable to damage. Furthermore, local regulation of the synaptic proteome is needed for its remodeling in response to activity. In fact, lysosomes are recruited to postsynaptic sites in an activity-dependent manner and play an important role in plasticity of the synapse (Goo et al., 2017; Padamsey et al., 2017). By contrast, presynaptic activity appears to stimulate the axonal flux of cargo to somal lysosomes (Wang et al., 2015).

The advances made by Cheng et al. (2018) and Yap et al. (2018) highlight key questions that lie ahead. How are these polarized distributions established during development, and how do alterations in endolysosomal trafficking lead to neurodevelopmental and neurodegenerative disorders? How do interactions between neurons and neighboring cells impact degradative processes? In models of Alzheimer's disease, protease-deficient LAMP1-positive organelles accumulate in axonal distensions near extracellular Aβ plaques, linking defects in lysosome maturation with disease pathogenesis (Gowrishankar et al., 2015). Davis et al. (2014) provide evidence for the intercellular transfer of organelles from neurons to neighboring glia for degradation by glial lysosomes (Davis et al., 2014). Future work is needed to examine how these degradative pathways are coordinated in the context of the brain with surrounding glia to maintain neuronal health and functionality.

Acknowledgments

We thank Aditi Kulkarni and Swathi Ayloo for helpful comments on the manuscript.

Research in the Maday laboratory is supported by grants from the National Institutes of Health (R00NS082619), the University of Pennsylvania Alzheimer's Disease Core Center, and the Intellectual and Developmental Disabilities Research Center at the Children's Hospital of Philadelphia and the University of Pennsylvania.

The authors declare no competing financial interests.

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