JCB Journal of Cell Biology

SPOTLIGHT

Painting lysosomes to study organelle heterogeneity

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Like other organelles, the heterogeneity of lysosomes within a single cell has been challenging to capture and study in detail. In this issue, Chen and Gutierrez discuss new work that tackles this question using DNA-PAINT imaging, from Lakadamyali and colleagues (https://doi.org/10.1083/jcb.202403116)

The late endocytic compartment (aka lysosomes) is key for cell function and is a master regulator of cell homeostasis that goes beyond its degradative capacity. Depending on the cell type, lysosomes regulate metabolism, cellular signaling, and innate immunity (1). Due to their endocytic origin, lysosomes exhibit considerable heterogeneity between and within different cell types (2, 3). This heterogeneity is further influenced by their positioning within the cell (4).

In the last decade, significant and numerous advances in the field of lysosomes allowed the identification and characterization of the molecular machinery associated with their function. However, it remains unclear whether lysosomal populations are molecularly uniform or exhibit heterogeneity. To fully understand lysosomal function, it is essential to consider individual lysosomes as distinct functional entities (5). An important challenge lies in correlating and integrating the sources that contribute to lysosomal heterogeneity within a single cell with specific functions at the individual lysosome level.

In this new work (6), Bond et al. used DNA-based Point Accumulation for Imaging in Nanoscale Topography (DNA-PAINT) to study lysosomal heterogeneity. This method exploits the stochastic and transient binding of fluorescently labeled DNA probes, enabling multiplexed labeling of intracellular proteins with a nanoscale spatial resolution (7). By detecting multiple lysosomal proteins with DNA-barcoded antibodies and imaging them sequentially, Bond et al. quantitatively mapped the distribution of proteins on individual lysosomes with exceptional precision. Their outcomes not only identified the protein composition of lysosomes but also demonstrated the spatial relationships between these lysosomal subpopulations and other organelles, such as mitochondria, suggesting the importance of lysosomal heterogeneity in cellular function.

The authors first generated a pipeline to capture the lysosomal features with high spatial resolution by using DNA-PAINT to characterize the distribution of seven key lysosomal proteins: LAMP1, LAMP2, CD63, Cathepsin D, TMEM192, NPC1, and LAMTOR4. These lysosomal proteins play various roles in maintaining lysosome function, including structural stability, cholesterol transport, and enzymatic activity. As well-established lysosomal components, they have been widely utilized as lysosome markers in diverse studies. Strikingly, by using DNA-PAINT, the authors found that not all the lysosomes shared the same protein makeup. Although proteins like LAMP1, LAMP2, and Cathepsin D were abundant across most lysosomes, other lysosomal proteins exhibited more varied distribution patterns. This study in fact identified eight distinct lysosome subpopulations based on different protein compositions (Fig. 1).

Importantly, this study revealed that overexpression of common lysosomal markers skews some of these subpopulations. The authors found that TMEM192 and NPC1 are present in only a subset of lysosomes, while overexpressed TMEM192 associates with all the LAMP1-positive lysosomes. The overexpression of LAMP1—a common approach used in cell biology studies—caused the enlargement of lysosomes and reduced the lysosomal surface density of LAMP2 and NPC1. These findings underscore the need for caution in interpreting data from overexpression experiments since such approaches can significantly alter lysosome composition and possibly function.

Another intriguing finding of this work is that the alteration of intraluminal pH leads to differences in morphology and lysosome protein content. Specifically, blocking V-ATPase activity with Bafilomycin A1 decreases the percentage of LAMTOR4-positive lysosomes and reduces LAMTOR4 protein density on lysosomes. In contrast, enhancing V-ATPase activity with EN6 increases the subpopulation of both NPC1 and LAMTOR4-positive lysosomes as well as the membrane density of these proteins. Although underlying mechanisms and the biological implications of these phenotypes remain unclear, these results highlight the functional regulation of lysosome heterogeneity in response to physiological changes.

The authors then went further and developed a quantitative approach to analyze the relationship between lysosome protein composition and relative position in the context of other organelles. They found spatial proximity of NPC1-positive lysosomes with mitochondria in Hela cells but not in ARPE-19 cells. As NPC1 is a key protein involved in cholesterol transport, this close association with mitochondria suggests a potential role in lipid exchange or metabolic coordination between these two

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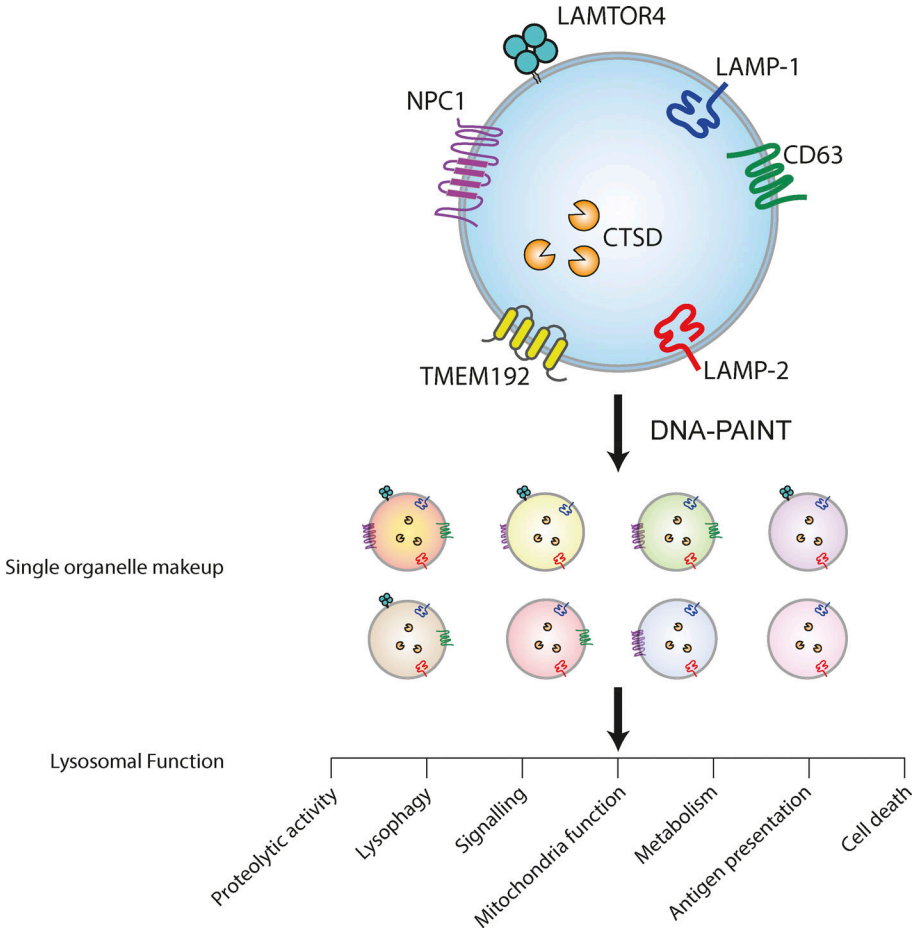

Figure 1. **Lysosome heterogeneity and function.** Schematic shows the distribution of seven lysosomal proteins analyzed by Bond et al. to define lysosomal heterogeneity, highlighting the possibility of this heterogeneity linked to diverse functions of individual lysosomes.

organelles. This finding implicates the involvement of specific lysosomal subpopulations in organelle interactions and highlights the power of this quantitative pipeline in revealing the interrelationships among distinct organelle subpopulations.

Overall, the authors present a novel and promising approach to study organelle heterogeneity. Lysosomes have varying intracellular localization, morphology, and biochemical properties that define their functions. This variability suggests that lysosomal dynamics, quality control, and function should be studied at the single lysosome level. This study introduced a new tool to dissect important questions in the lysosome field—what are the functions of individual lysosomes and how these functions are regulated? Applying this approach to lysosome research, particularly in the context of membrane repair and lysophagy, will shed light on the functional regulation of membrane repair and the fate of damaged lysosomes. While distinct mechanisms and machinery, such as the ESCRT-III complex, stress granules, and autophagy, have been implicated in membrane repair and organelle recycling after damage, the coordination between these repair mechanisms and the transition between repair and recycling remains largely unknown. By leveraging multiplexed labeling of repair and recycling machinery alongside diverse organelle markers, this approach enables the identification of distinct lysosomal subpopulations and their interactions with other organelles, paving the way to distinguish lysosomes that undergo repair from those destined for recycling after damage.

A limitation of this approach is the inability to capture lysosome dynamics. Lysosomes are highly dynamic organelles, constantly undergoing movement, fusion, and fission, not to mention the dynamic interactions with other organelles (1). As this approach relies on fixed-cell imaging, it provides only a snapshot of lysosomal features. The correlative nature of the method, while powerful for linking molecular profiles to spatial positioning, does not directly reveal functional outcomes. Extending this approach to live cell imaging can be challenging due to technical limitations, such as the speed of DNA hybridization, phototoxicity, and photobleaching. The analysis was performed in 2D rather than 3D, which can lead to misclassification of lysosomes or over/underestimation of probe intensity due to overlapping signals. Recent developments in 3D super-resolution imaging and quantitative techniques for interorganelle contact analysis offer promising ways to extend this approach, potentially enhancing its precision (8). Another limitation is the reliance on antibody-based labeling, which can introduce variability in data quality. Antibody specificity and efficiency, particularly across different cell types, can vary significantly. The authors address this issue by employing

nanobodies, which facilitate high-resolution multiplex imaging, but these reagents may not be available for all target proteins.

Considering the concept of distinct "lysosomal states" for characterizing individual lysosomes will be important to study the role of lysosomes in health and disease. With the development of methods described by Bond et al. that enable single-organelle resolution, there will be a substantial increase in our understanding of the lysosomal functions and complexity these organelles present (9). This approach not only provides insights into lysosomal heterogeneity but also establishes a starting point for exploring the function of lysosome heterogeneity in cellular physiology and pathology.

Acknowledgments

Funding in the lab is supported by the Francis Crick Institute (to M.G. Gutierrez), which receives its core funding from Cancer Research UK (CC2081), the UK Medical Research Council (CC2081), and the Wellcome Trust (CC2081). D. Chen is supported by a European Molecular Biology Organization long-term fellowship (ALTF 202-2023).

Author contributions: D. Chen: Writing - original draft, Writing - review & editing, M.G. Gutierrez: Conceptualization, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

Disclosures: The authors declare no competing interests exist.

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