1306
doi:10.1017/S1431927622005360
Microsc. Microanal. 28 (Suppl 1), 2022

Resolving Subcellular Organelle Interactions with Correlative Live-Cell – Volume Electron Microscopy Analysis

Sergey Loginov[1], Job Fermie[1,2], Cecilia de Heus[2], Hans C. Gerritsen[1], Judith Klumperman[2], Nalan Liv[2*]

[1]Molecular Biophysics, Debye Institute for Nanomaterials Science, Utrecht University, Utrecht, the Netherlands
[2]Center for Molecular Medicine, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

[1.] Department of Cell Biology, University Medical Centre Groningen- Groningen, Netherlands.
[2.] Delmic B.V. - Delft, Netherlands.
* Corresponding author: N.Liv@umcutrecht.nl

Eukaryotic cells are compartmentalized into a complex array of membrane-bound organelles, each with a specific function. The maturation and exchange of contents between organelles are tightly regulated, and relies on vesicle transport and membrane contacts between organelles [1]. Misregulation of organelle biogenesis, function and interactions have been associated with numerous neurological disorders, metabolic disorders including diabetes, and cancer.

Visualizing cellular organelles and their interactions with high spatio-temporal resolution is crucial for understanding their function and regulation. Fluorescence microscopy (FM) provides an extensive and sensitive toolbox for molecular localization and functional imaging. Electron microscopy (EM) on the other hand delivers high-resolution structural information. However, none of these state-of-the-art microscopy methods can bridge molecular and functional information to ultrastructural information with molecular (sub-10nm) resolution, resulting in a gap between the structure, functional and dynamic characteristics of subcellular organelles [2].

Recent advances in correlative light and electron microscopy (CLEM), in particular the novel live-cell single organelle methods [3], [4], provide elegant means to directly link molecular composition and dynamic behavior of organelles to their morphology at nanometer resolution. Mastering the challenge to retrace single, especially small, organelles in 3 dimensions (3D), we have recently presented an approach where we have used live-cell volumeCLEM to link the dynamics and ultrastructural context of individual, highly dynamic organelles. We presented that live-cell imaged lysosomes can be reliably retraced in 3D EM data, and fusion, fission and trafficking dynamics of single organelles can be linked to their ultrastructure.

Here, we present the Correlative Organelle Microscopy workflow, for targeted EM imaging of live-cell imaged organelles for analysis of their motile characteristics within the ultrastructural context. We have visualised functional characteristics and interactions of cellular organelles with live-cell imaging, and analyzed their dynamic behaviors. After collection of the live-cell data, the cells are prepared for electron microscopy; the exact organelles are retraced, and imaged in volumeEM. Using this live-cell volumeCLEM approach, we link rare (e.g. membrane contact sites) and transient (e.g. organelle interactions) cellular events to the underlying 3D ultrastructure. We relate motility of single lysosomes to their morphology, and the contact sites they have with endoplasmic reticulum (ER). We show that extensive ER-lysosome interactions restrict lysosome motility, highlighting the unique capabilities of the

correlative organelle microscopy pipeline to link dynamic data to high-resolution ultrastructural detail in 3D. Live-cell CLEM provides a unique prospect for organelle biology research to study the spatial and temporal regulation of inter-organelle processes with respect to their 3D ultrastructure.

References

[1] S Cohen, AM Valm, and J Lippincott-Schwartz, Current Opinion in Cell Biology **53** (2018), p. 84. doi: 10.1016/j.ceb.2018.06.003.
[2] X Huang, C Jiang, L Yu, and A Yang, Front. Cell Dev. Biol. **8** (2020). doi: 10.3389/fcell.2020.00195.
[3] J Fermie et al., Traffic (2018). doi: 10.1111/tra.12557.
[4] S Loginov et al., bioRxiv (2021), p. 2021.03.29.437317, doi: 10.1101/2021.03.29.437317.

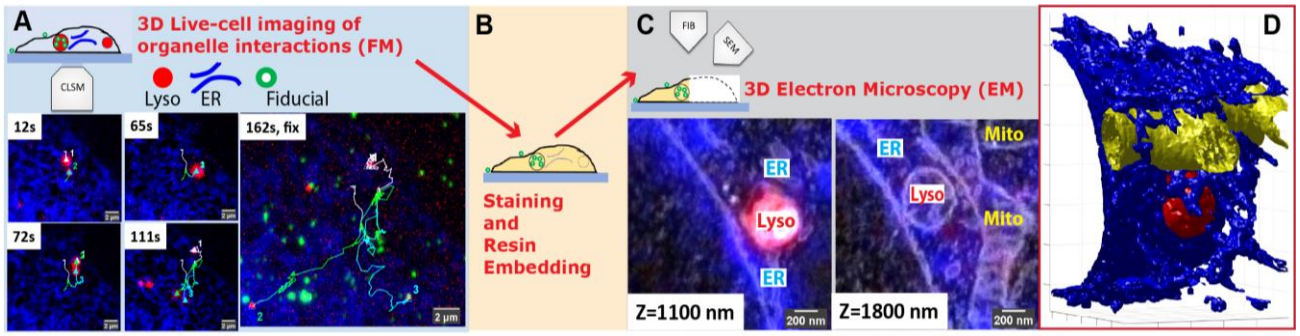

Figure 1. Correlative Organelle Microscopy workflow provides a direct link between organelle interaction dynamics and 3D ultrastructure. (A) The cell, labelled for lysosomes, endoplasmic reticulum and endocytic fiducial markers, is imaged live in the FM, tracking time-resolved organelle interactions, followed by in-situ fixation. (B) The same cell is prepared for EM. (C) Organelles are retraced, and imaged in volumeEM (FIB-SEM) visualizing the ultrastructure of organelle interactions with 5nm isotropic resolution. The panel shows two EM planes overlayed with the FM data. (D) 3D segmentation of the region showing Lyso-ER and Lyso-Mito interactions.

https://doi.org/10.1017/S1431927622005360 Published online by Cambridge University Press