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FIB-SEM 3D CLEM of Cultured Cells

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In the biological sciences, images from light microscopy (LM) can be correlated with those from electron microscopy (EM) to specifically localize fluorescence biomolecules within the cellular ultrastructure and/or analyze the underlying nature of the fluorescence signal. Several "volume" EM imaging techniques are available for 3D-reconstruction of biological specimens. Among these, focused ion beam scanning electron microscopy (FIB-SEM) tomography is the only tool that can image large volume with high isotropic resolution in all three dimensions (x, y, z) [1] [2]. Here we combine FIB-SEM with correlative fluorescence microscopy (CLEM) [3] to examine the ultrastructure of fluorescence signals throughout the entire 3D volume of mammalian cells.

More specifically, we used FIB-SEM 3D CLEM to explore the complex interplay of different organellar membranes during cellular autophagy. Autophagy is an intracellular stress response that hinges on the rapid de novo formation of a new organelle called the autophagosome. Neither the precise membrane source nor the mechanisms of membrane expansion used to grow the autophagosome are yet understood, with many cellular organelles implicated in this process. A key protein in autophagy, often used as a marker of autophagic intermediates is LC3 [4]. Here we used whole cell FIB-SEM 3D CLEM to examine accumulation of GFP-LC3 positive autophagic intermediates in gene-edited cell lacking an important autophagic protein. The fluorescent GFP-LC3 puncta were shown to represent areas of high vesicle density, identifying such vesicles as intermediates in the process of autophagosome formation (Fig 1B).

Gene-edited HEK293 cells expressing a GFP-LC3 were cultured in gridded glass-bottom dishes, fixed with 4% PFA, and the 3D fluorescence signal of GFP-LC3 was analyzed by confocal microscopy to identify the positive cells before further FIB-SEM imaging, we use coordinate maps of the target cell to navigate between two imaging methods. Basically, cells were fixed with 2.5% glutaraldehyde in 0.1 M Na Cacodylate buffer, post-fixed with 2% OsO_4 + 1% $K_4Fe(CN)_6$ in 0.1 M Na Cacodylate buffer, *en block* stained in 2% aqueous UA, dehydrated with graded EtoH, Epon infiltrated, Epon flat embedded, and cured at 60 degrees for 48 hr. The flat embedded monolayer cell block was glued onto aluminum studs with Epo-TEK H20S (EMS), the space between the edge of the block and the stud was sealed with silver paint (EMS) and to prevent charging effect, samples were coated with a 10 nm-thick platinum layer. The selected cell(s) was located with a secondary electron detector (SE2) in Zeiss Gemini 2 SEM according to the coordinate maps and cell morphology. Whole cell FIB-SEM image acquisition was done with an ESB detector in a Zeiss Crossbeam 550 system and imaging software of SmartSEM and Atlas 5. The FIB-SEM dataset was aligned using Atlas 5 automatically or manually and further processed with IMOD. Fluorescence LM dataset and FIB-SEM EM dataset were processed with Icy eC-CLEM or Bigwarp (Fiji plugin).

References:

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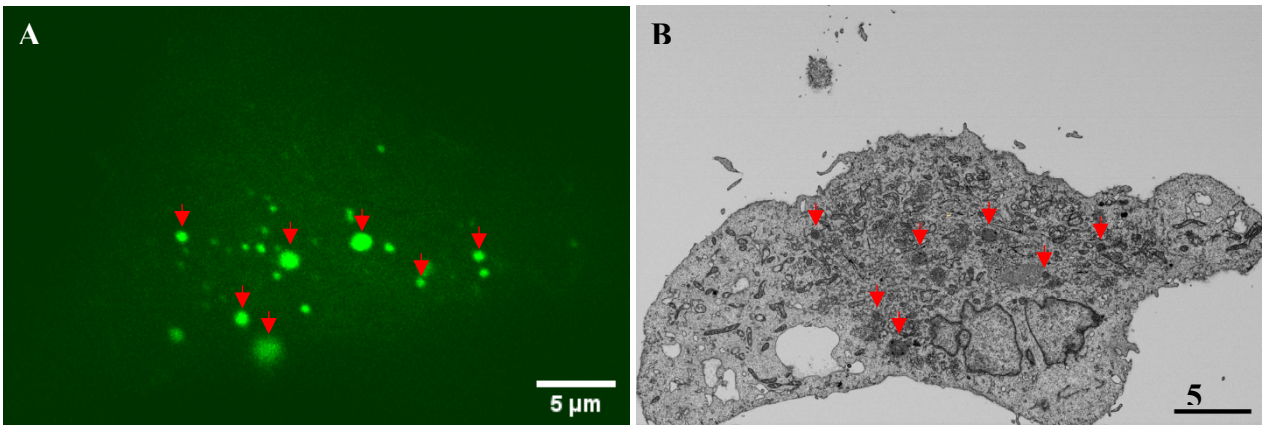

Figure 1. Correlative whole cell imaging of LM (A) and FIBSEM (B). **A**, GFP-LC3 puncta (arrows). **B**. a snap-view from a reconstructed whole-cell volume (same cell). Red arrows indicate regions that correspond to fluorescence signals.