IMAGING CELLULAR DYNAMICS BY CORRELATIVE LIVE CELL – ELECTRON MICROSCOPY (CLEM)

Judith Klumperman
Cell Microscopy Center, Department of Cell Biology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, the Netherlands.
E-mail: j.klumperman@umcutrecht.nl

The visualization of fluorescent proteins in living cells is a powerful approach to study intracellular dynamics. A limitation of fluorescence imaging, however, is that it lacks fine structural information; a fluorescent spot could represent an entire organelle, an organellar subdomain or even aggregates of proteins or membranes. These limitations can be overcome by immuno–electron microscopy (immunoEM), which uniquely combines protein detection with ultrastructural detail. EM, however, requires fixation of the cells, resulting in static images with only limited information on membrane dynamics. To bridge the gap between live-cell imaging and EM, we have developed a novel procedure for correlative light-electron microscopy (CLEM)[1]. Our CLEM method integrates imaging of fluorescent proteins in live cells with the cryo-immunogold technique, which is probably the most optimal method for immunoEM[2].

We apply our CLEM approach to study lysosome biogenesis. Lysosomes are the degradative compartments of the cell, involved in a variety of cellular events that require protein or lipid processing. The biogenesis of lysosomes requires the transport of newly synthesized lysosomal hydrolases and lysosome-associated membrane proteins (LAMPs) from their site of synthesis, the endoplasmic reticulum, to lysosomes. Transport of lysosomal hydrolases from the *trans*-Golgi network (TGN) to the endosomal system is mediated by mannose 6-phosphate receptors (MPRs), through recognition of mannose 6-phosphate (M6P) moieties present on the newly synthesized hydrolases. The MPR-enzyme complexes are packaged in the TGN into clathrin-coated vesicles that transport their cargo to endosomes. However, several lines of evidence indicate that lysosomal hydrolases can also be transported via a MPR-independent pathway. Transport of LAMPs to lysosomes is also independent of MPRs. From the TGN, LAMPs travel to the endosomal system via an indirect (passaging the plasma membrane) or direct (entirely intracellular) pathway.

The objectives of our studies are to characterize the pathways for lysosomal hydrolases and LAMPs that involve direct transport from the TGN to the endosomal system in an MPR-independent manner. The morphological and molecular identity of the membrane carriers and compartments involved in these direct pathways are largely unknown. In order to study these, we combine RNAi, live cell imaging, immunoEM and CLEM. Our present data implicate a novel, direct TGN-to-late endosome pathway for LAMPs.

1. van Rijnsoever C., Oorschot V. and J. Klumperman. A novel correlative light-electron microscopy (CLEM) method integrating live-cell imaging and immunolabeling of ultrathin cryosections. 2008. **Nature Methods**, Nov;5(11):973-80

2. Slot, J.W. & Geuze, H.J. Cryosectioning and immunolabeling. 2007. **Nature Protocols** 2, 2480-2491.