Live cell imaging of membrane recycling using Lattice Light Sheet Microscopy and Multi-Angle (MA) TIRF microscopy

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KEY WORDS: recycling, Light sheet Microscope, Multiangle TIRF, single particle tracking

The study of the whole cell dynamics of exocytic/recycling events has proven difficult until recently because of lack of sensitivity, limited speed, photobleaching and phototoxicity associated with conventional imaging modalities. The Lattice Light Sheet Microscope (LLSM) [1] allows overcoming these difficulties, yet reaching high spatial resolution. This allows 3D images to be captured over long time at a high acquisition frequency, and enables the study of signalling, transport, and stochastic self-assembly in complex environments. In addition, this imaging technique and 3D-tracking allows to look at molecular machinery throughout the full sequence of events that lead to exocytic fusion event, from initial membrane recruitment and budding.

LLSM provides excellent imaging capacities in terms of 3D full cell volume and low photobleaching. Nevertheless, it still shows limitations in terms of z-resolution. Multi-Angle Total Internal Reflection Fluorescence microscopy permits to image events and motion close to the membrane with higher speed and better z-resolution. This technique allows obtaining 30 to 50-nm in axial resolution, up to 500 to 800 nm above the coverslip [2].

Both microscopy techniques give complementary information. LLSM will provide full 3D cell information in a temporal scale of 1-2 seconds per volume with low photobleaching, while the multi-angle TIRF will give a high precision in z-resolution at a temporal resolution of 200 ms per volume of the basal region of the cell (< 500nm).

Our study using 3D MA-TIRF imaging showed that two cargoes that recycle constitutively from endosomes to the PM, the transferrin receptor and the langerin, are recycling in various vesicle populations. These populations exhibit different characteristics in their dynamics of docking/fusion process at the plasma membrane, which depend on Rab11-platforms activities as well as actin dynamics.
Similar experiments performed with LLSM imaging, allow visualization and localization of the same events at the whole cell volume, extend dynamic information using 3D particle tracking and permits the definition of recycling "hot spots" at both the basal and apical part of the PM and their quantification.

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