

Publication

A novel live cell imaging assay reveals regulation of endosome maturation

Discussion paper / Internet publication

ID 4621781

Digital Object Identifier DOI 10.1101/2021.06.28.450147

Author(s) Podinovskaia, Maria; Prescianotto-Baschong, Cristina; Buser, Dominik P.; Spang, Anne

Author(s) at UniBasel [Spang, Anne](#) ;

Year 2021

Month and day 06-21

Year: comment 2021

Title A novel live cell imaging assay reveals regulation of endosome maturation

Pages 53

Publisher / Institution Cold Spring Harbor Laboratory

Keywords endocytosis, small GTPases, Rab proteins, mammalian cells, CRISPR, Rab conversion, endosome maturation, endosomes, acidification, V-ATPase

Mesh terms Endosomes, metabolism; HeLa Cells; Humans; Lysosomes, metabolism; Microscopy, Fluorescence, methods

Cell-cell communication is an essential process in life, with endosomes acting as key organelles for regulating uptake and secretion of signaling molecules. Endocytosed material is accepted by the sorting endosome where it either is sorted for recycling or remains in the endosome as it matures to be degraded in the lysosome. Investigation of the endosome maturation process has been hampered by the small size and rapid movement of endosomes in most cellular systems. Here, we report an easy versatile live-cell imaging assay to monitor endosome maturation kinetics, which can be applied to a variety of mammalian cell types. Acute ionophore treatment led to enlarged early endosomal compartments that matured into late endosomes and fused with lysosomes to form endolysosomes. Rab5-to-Rab7 conversion and PI(3)P formation and turn over were recapitulated with this assay and could be observed with a standard widefield microscope. We used this approach to show that Snx1- and Rab11-dependent endosomal recycling occurred throughout endosome maturation and was uncoupled from Rab conversion. In contrast, efficient endosomal acidification was dependent on Rab conversion. The assay provides a powerful tool to further unravel various aspects of endosome maturation.

edoc-URL <https://edoc.unibas.ch/83803/>

Full Text on edoc Available;

ISI-Number WOS:000730815400001