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European Research Council
Established by the European Commission

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TITLE Organelle homeostasis: How are membrane fission and fusion machineries coordinated to regulate size and copy number of a lysosomal compartment?
(ERC Advanced Grant FP7)

ACRONYM ORGANELL

DURATION 01.09.2009 – 31.08.2015

BUDGET 2 310 000 €

Yeast vacuoles (lysosomes) serve as an excellent model system: Vacuoles change copy number and size in the cell cycle and upon shifts of media; due to their large diameter (up to 5 ¦m) these changes can be assayed by fluorescence microscopy and are amenable to genetic screening. Moreover, an in vitro system for vacuole fusion exists and we have succeeded in reconstituting also cell-free vacuole fission with purified organelles. We have first built an experimental toolkit for vacuole fission to characterize this reaction in detail. Several approaches have been combined:

(1) Identification of fission proteins by mutant screening, as well as by candidate approaches, and their localization relative to the fission site;
(2) further developing a system reconstituting in vitro fission and efficient methods to quantitate it;
(3) creating organelle chips to synchronously study fission on multiple single vacuoles immobilized in a defined orientation;
(4) time-resolved confocal microscopy of fission proteins in vivo and in vitro;
(5) biochemical characterization of fission protein associations and their changes during fission.

These approaches have identified the vacuolar fission apparatus and helped to elucidate its functioning. In a second step we have explored how the fission apparatus physically and functionally interacts with the already well-defined vacuolar membrane fusion machinery. We have characterized the impact of cell cycle regulators and signaling pathways on these interactions. These studies have been pioneering in that they have led us to a comprehensive description of an organelle fission process and of how membrane fission and fusion components are coordinated to control size and copy number of an organelle.