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Molecular mechanism of autophagy in neurodegenerative diseases and cancer

Autophagy is a major recycling pathway that operates in all eukaryotic cells to maintain cellular homeostasis. It degrades damaged or superfluous cytoplasmic compartments by a de novo formed membrane, termed phagophore. Starvation and cytosolic stresses induce non-selective autophagy that recycles bulk cytoplasm instead of defined cargo to free resources. The molecular switch from selective to nonselective autophagy is not well understood but triggering one or the other pathway is of major interest to develop targeted therapies to treat cancer and neurodegenerative diseases. We are reconstituting autophagy in the test tube using purified components and model membranes to investigate molecular functions of key autophagy factors. We combine this with biochemical, cell biological and structural approaches in vivo using cell culture, primary cells and stem cells to reveal insights into the regulation of autophagy in cells.
The major goal of the project is to investigate how specificity in autophagy is controlled and how cells switch from selective to non-selective autophagy. The project involves reconstitutions of a key step in autophagy from purified and fluorescent labelled proteins on supported lipid bilayers and on liposomes of various sizes. One example of a reconstitution reaction is shown in the figure below, in which we rebuilt phagophores using purified proteins from the autophagy pathway and model membranes. We could demonstrate that only a small subset of more than 40 proteins that are functioning in autophagy are sufficient to generate membrane cups in vitro that resemble phagophores. In cells, such membrane cups collect and enclose non-functional or unwanted cytoplasmic material to deliver it to lysosomes for degradation.

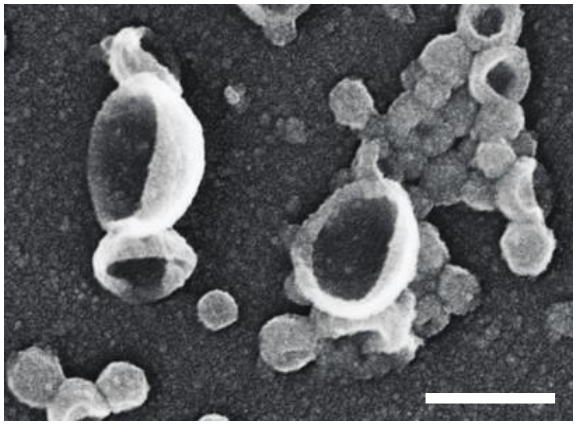

Fig.: Reconstitution of phagophores using purified proteins in vitro. Electron micrograph of a supported lipid bilayer on which proteins from the autophagy pathway were reconstituted. The proteins remodelled the flat membrane into cup shaped structures which are identical to phagophores that were observed in cells during autophagy. The major function of these phagophores is to collect non-functional cytoplasmic material.

Apart from electron microscopy, we are using fluorescence microscopy and fluorescent lifetime imaging (FLIM) to investigate protein-protein interactions on model membranes. Observed interactions will be confirmed by co-immunoprecipitation experiments and by FLIM experiments in cells.

We provide a stimulating working atmosphere and a broad training in various in vitro and in vivo techniques. Our work at the intersection of physics and biology through combination of biophysical and cell biology techniques allow us to reveal principle functions of components of the autophagy pathways and to define interactions that are involved. Based on this knowledge, we will develop novel activators and inhibitors that stimulate or inhibit autophagy to treat neurodegeneration and cancer.

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