Final Report

(PD OTKA 128280)

The main aim of this PD OTKA grant was to unravel the importance of lipids during autophagy, which is a conserved „ self-eating "process of eukaryotic cells that ensures the degradation and recycling of damaged, obsolete materials from cytosol. Accumulation of damaged proteins can lead to a wide range of neurodegenerative diseases, cancer and aging which points to the **importance of autophagy** and its potential relevance for biomedical applications. I am among the coauthors of a review paper summarizing the importance of autophagy in human diseases **(Ref.1)**. Capturing and degradation of the cargo in the lysosomes is governed by membrane surrounded organelles, which are composed mainly from lipids and several proteins. Autophagy research has focused mainly on proteins and very less is known about the lipids composing autophagic membranes and their roles. Therefore the **main targets** of this project were the **lipids** and their interplay with **autophagic proteins**, namely **SNARE Syntaxin17**, which has crucial roles in the fusion step of autophagy, both in animals and humans. The main model organism used in this study was the *Drosophila melanogaster* (an excellent genetic tool to study autophagy in a whole organism), but we applied human cell lines, too. To perform lipid-protein interaction studies first we had to decipher the lipid composition of autophagic membranes from *Drosophila* and human cell line. The obtained results can be summarized in three main points:

I.At the beginning of our project the lipid composition of autophagic membranes was not known in any of the eukaryotic organisms. In the meantime it was discovered the lipids composing autophagic membranes originated from yeast. We successfully established an easy and fast biochemical method for the **isolation of autophagic structures** from *Drosophila melanogaster* and HeLa cells. We used wild type (control) and previously characterized $Atg2^{-}$ mutant flies (accumulating mostly phagophores aka. isolation membranes) to purify early autophagic structures: phagophores and autophagosomes. As we were interested in the main phospholipids composing these autophagic membranes, we optimized the isolation method to downstream **lipidomic investigations**. We paid special attention to the integrity and high purity of the isolated organelles, by applying Western blots of various organelle markers, fluorescence and AFM microscopy. Our lipidomics results show that autophagic membranes are highly unsaturated, contain a high proportion of PE and they likely undergo dynamic lipid changes during their maturation processes. Our mass spectrometry results point to the important *in vivo* function of the Atg2 lipid transport protein in shuttling short fatty acyl chain PE species. We could successfully publish these results in a high repute lipid journal, BBA Molecular and Cell Biology of Lipids **(Ref.2)**. To our knowledge this is the first method for isolation of autophagic structures from *Drosophila*, as well as to report for the first time about the lipid composition of

autophagic membranes originating from a whole multicellular organism. Deciphering the lipid composition of autophagic membranes is crucial to fully understand the mechanism of autophagy.

II. To reveal the role of lipids in the **recruitment of Syntaxin17 proteins** (*Drosophila* and human) to the autophagic membranes we applied a **multidisciplinary approach** by combining several methods: 1) **biochemistry** (recombinant protein expression in *E.coli*, purification of the proteins by affinity chromatography, lipid isolation from various organisms, liposome flotation assay). 2) **biophysics** (liposome generation from isolated and artificial lipids, Dynamic Light Scattering (DLS), Infrared and Electron Paramagnetic Resonance (EPR) spectroscopy, Circular Dichroism). 3) **molecular and cell biology** (cloning, generation of *Drosophila* mutant lines, gene silencing, mosaic clonal system, introducing autophagy markers which enables monitoring the autophagy process by fluorescence microscopy).

Syntaxin17 is an unusual transmembrane protein, as it has been shown to be present in the cytosol and it is recruited later to autophagosomal membranes, promoting the fusion of autophagosomes with lysosomes. According to our knowledge we are the first who could successfully express in *E.coli* and purify from the cytosolic fractions the **full length form of these proteins (*Drosophila* and human)**. By using these recombinant Syntaxin 17 proteins and **liposomes**, mimicking autophagic membranes, we showed that this interesting SNARE protein is able to associate with membranes in the absence of any other co-factors. Among the natural lipids the proteins preferred their own lipids (*Drosophila* and human cell line derived ones). Both proteins exhibited very nice interaction with autophagosome mimicking synthetic lipids what we constructed based on our lipidomic investigations on autophagic membranes. PG, PI, PIPs and cardiolipins are also among favorite lipid environments of these proteins. Results of these investigations are summarized in a manuscript close to submission **(Ref.3).**

III. Based on our ***in vitro*** results we could also show by ***in vivo*** methods the importance of lipids in the recruitment of the Syntaxin17 protein to the autophagosomal membrane. We targeted the *CDS* gene of *Drosophila*, encoding the enzyme necessary for PI, PIPS, PG and cardiolipin synthesis. By gene silencing we could observe a nice phenotype with our mosaic model system. We showed that during disturbed lipid biosynthesis the fusion step of autophagy is defective. This may be due to the insufficient recruitment of Syntaxin17 protein into the changed lipid environment **(Ref.3)**.

Besides the above mentioned published data (3 articles) as well as manuscript in preparation (1) our results were presented at several international and national conferences, advanced courses, symposiums, and scientific events.

Never the less our lipid biochemistry and membrane biophysics knowledge gained during this project was applied in **another international collaborative work**, with a successful outcome of a published article, with a correspondent authorship **(Ref.4)**.

To sum up, we can say that lipids have crucial roles during autophagy, by providing the necessary physico-chemical environment for the recruitment of autophagic proteins into the membranes, which leads to proper autophagic degradation of unnecessary or damaged molecules or organelles, which may prevent the occurrence of several diseases. Therefore we hope that our obtained results may serve in the future as basis for biomedical applications (ex. drug design), too.

Cited references:

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