DESCRIPTION FOR THE GENERAL PUBLIC

Autophagy, or self-eating, is a process known from the 60s of the last century. This is a conservative process that occurs in yeast, plants and animals and allows the degradation of the entire organelles, macromolecules and protein complexes. Such objects within the cytoplasm are surrounded by a phospholipid bilayer membrane. Autophagosome is formed, which in the plant is fused with the tonoplast and delivers its contents to the vacuole, to form a autophagic body. Assembly of the autophagosome and its fusion with the tonoplast is controlled by ATG proteins. The autophagic body inside vacuole is rapidly degraded by numerous vacuolar lytic enzymes. Autophagy under normal conditions operates at low intensity, but it is noticeably intensified as a result of various stress conditions (both abiotic and biotic). A model example of stress enhancing autophagy is carbon or nitrogen starvation. In such circumstances, enhanced self-destruction delivers respiratory substrates and enables cell survival. In mammals, autophagy is important for maintaining good health by preventing the development of various diseases (including cancer and neurodegenerative diseases, for example. Huntington's disease). In plants, autophagy participates in re-using of cell components and acts as a quality control mechanism, but it also functions in some developmental processes, such as pollen maturation or aging and cell death (including the PCD). Autophagy was considered for decades as a process in which the cell elements are degraded in a non-selective manner. However, the results of the last 20 years have shown clearly that autophagy is a process during which cell components are degraded in a selective manner, i.e. degraded are only those elements that are damaged or are no longer needed in the cell. An example of selective autophagy is pexophagy, i.e. autophagic, selective degradation of peroxisomes. Nevertheless, the data on selective autophagy in plant cells are not numerous and they are published from a few years.

Our previous research conducted on cultured *in vitro* lupin embryo axes showed that autophagy occurs under sugar starvation conditions. Moreover, in sugar-starved axes the content of storage lipid is clearly higher than in axes fed with sucrose. Such result is completely opposite to numerous literature data. We have also observed that in the sugar-starved axes which were simultaneously fed with asparagine (central amino acid in the metabolism of lupin seed) the degradation of autophagic bodies (the final stage of the course of autophagy) is significantly inhibited. This effect is not described in the literature so far. Thus, the main aim of the project is understanding the mechanism by which asparagine inhibits degradation of autophagic bodies in vacuoles of cells of embryo axes of lupin germinating seeds. The inhibition of degradation of the autophagic bodies by asparagine allows analyze their content under transmission electron microscope. It turns out that inside the preserved autophagic bodies there are organelles that can be recognized as peroxisomes. Therefore, the second important aim of the research is getting unequivocal evidences on pexophagy in embryo cells of plants. At the current stage of research the mechanism of asparagine action is unknown. But the realization of this project and publishing new results will significantly enlarge knowledge in this field. The research will not only explain the mechanism of asparagine action in the regulation of degradation of autophagic bodies in plant cells, but also allows to prove the occurrence of pexophagy in plant embryo cells. Obtaining the evidences on pexophagy and their publication will be a significant success, because there is no data in the literature so far about pexophagy in plant embryo cells.

The research will be conducted on embryo axes which will be isolated from imbibed seeds of white lupin (*Lupinus albus*) and Andean lupin (*Lupinus luteus*) and will be cultured *in vitro* on mineral medium with 60 mM sucrose, without the sugar, and on media enriched with 35 mM asparagine. Lupin species which were selected for the research differ significantly in content of storage lipid in seeds (7-14% lipid in seeds of white lupin and about 20% in Andean lupin seeds). Proteomic analyses are planned in the project. They will be made using 2D electrophoresis and mass spectrometry. Transcriptomic studies will be made using next generation sequencing technique on transcriptomic libraries prepared on mRNA isolated from embryo axes cultured *in vitro*. Numerous experiments with the use of electron and confocal microscopes will be performed. Spectrophotometric and isotopic methods will be used for determination of proteolytic activity. Experiments with antibodies use (Western blot, Immuno-Gold) are also planned. Chromatographic methods will be performed for determination the content of lipid compounds.