Annexins are a family of calcium and membrane binding proteins. There were discovered in all known eukaryotic organisms and presently more and more prototypic proteins from this family is found in different bacteria species. The genome of most species usually contain several to tenth genes encoding annexins, and expressing of at least sub-group of them occurs in all cells and tissues at a high level. They were discovered over 30 years ago; so far we can already say something about the biological functions of these proteins in animal cells and organisms but still very little is known about their plant homologues. It has been demonstrated partially thanks to our research that certain annexins are involved in the signal transduction and, in yet unknown way, improves the plant tolerance and its adaptation to sub-optimal external conditions

In our recent work, we showed that increased level of endogenous annexin ANXD36 in the potato has a significant impact on plant physiology and biochemistry. Hormonal homeostasis was changed as well as the relative levels of certain lipids in photosynthetic membranes; photosynthesis during drought was more efficient. We also noticed that during high light stress the level of accumulation of oxygen free radicals was reduced. ROS-mediated signaling is currently one of the most extensively studied processes. The basic metabolic processes are a significant source of ROS are the and during evolution plant cells acquired the ability to understand these signals and activating the relevant changes to adapt and maintain metabolic balance. Since these processes are often a consequence of exposure to the environment it is currently believed that the ROS metabolism - their formation and scavenging - is a platform for interaction between metabolism and environmental factors. In this project we want to examine in detail the role of annexin 1 in the metabolism of free radicals.

Preliminary data suggest that annexin can adjust the redox poise of the most abundant antioxidant - ascorbate - will explore the potential mechanism of this process. To confirm our hypothesis we will examine if ANXD1 have an impact on the balance between oxidized and reduced ascorbate. Observed effect will be confirmed *in vivo* by studying the double mutants with a reduced level of both ascorbate and annexin. Since reduction in ascorbate content results in development of cell death the ultrastructure of a double mutants will be also analyzed. Finally, the possible interaction between annexin and the membrane electron transport system as a potential mechanism for the regeneration of ascorbate will be analyzed.

In the second part of the project we try to answer a question with which of the ROS-mediated signal transduction pathways annexin may interfere. For this purpose Arabidopsis lines which substantially over-accumulate single ROS without significant contribution of other forms will be used. In addition, this accumulation is inducible and thus can be evoked at any time and it is possible to manipulate with its magnitude. Such plants are widely used to study the biological role of the various ROS. They have been modified by us so the level of ANXD1 is increased. We will analyze their transcriptomic footprint by RNA deep sequencing, in order to determine how genetic manipulation we introduced affects the signaling pathway. This study will be carried out as an external service laboratory with extended practice in this field, which guarantees the chance to correctly perform the experiment. The second, equally difficult part of this experiment i.e. results analysis will be held in collaboration with the laboratory where it is performed routinely.

We will also explore other aspects of the biology of mutant plants, depending on the line, in particular the level of individual ROS will be measure, the content of low molecular weight antioxidant, activity of scavenging enzymes, the accumulation of photosynthetic pigments and photosynthesis efficiency under high light stress. We will also analyze the expression of the marker genes and genes, which expression were most affected in parental lines. Because there are indications that the plant from one of these lines have altered chloroplast ultrastructure we planned to perform experiments to verify this hypothesis.

We hope that the planned study will help to better understand the biology of annexin plant.