

Intensive studies on the mechanisms allowing living organisms to minimize the effects of ultraviolet (UV) radiation started in the 1970s, when the depletion of the ozone layer was first observed. This phenomenon, resulting from human activity, leads to an increase in the amount of UVB reaching the surface of the Earth. This represents a threat for both humans, leading to increased risk of skin cancer, and plants, resulting in visibly lower crop yields. Living organisms have evolved two main mechanisms coping with the effects of UVB. The first one is the production of UVB-absorbing compounds which act as natural sunscreens, minimizing the amount of radiation reaching the inside of the cell. The second mechanism allows the fast repair of those lesions which could not be avoided. The negative effects of UVB involve, among others, pyrimidine dimerization resulting from the formation of a stable bond between two adjacent bases in the DNA strand. The presence of such dimers makes the decoding and copying of genetic information impossible. The presence of multiple unrepaired lesions can even lead to cell death. The repair of pyrimidine dimers in human cells is a complex multi-stage process. Many other organisms, including plants, have specialized enzymes, called photolyases. They can use light energy (in the blue and UVA range) to reverse dimer formation in a fast, simple and efficient reaction. DNA repair by photolyases, called photoreactivation, is well understood at the molecular level. However, the data on photolyase functioning in live organisms (*in vivo*) are still scarce. Interestingly, in the course of evolution, photolyases gave rise to cryptochromes, blue light photoreceptors present in animals and plants, involved in circadian rhythm control and magnetoreception in birds. The change of a single amino acid can confer photolyase activity to cryptochromes, which points to a close connection between the photo-perceptive and repair functions in these proteins.

In recent years the simple model of one gene-one protein-one function has become outdated. The newest findings show that there are multiple mechanisms regulating the activity and biological roles of proteins. One of such mechanisms is alternative splicing, leading to the production of proteins differing in structure and function, on the basis of a single gene. Other modifications include phosphorylation (addition of phosphate groups to proteins) or sumoylation (binding of the small SUMO protein). This may lead to changes in protein stability, intracellular localization and interactions. All these modifications are important for DNA repair in animal cells and their perturbation leads to several types of cancer.

The aim of the project is to characterize three genes of the model plant thale cress (*Arabidopsis thaliana*), closely related to many crop species including cabbage, essential for Polish cuisine. These genes encode the known photolyase AtUVR3 and two proteins with putative photolyase activity: AtPHR2 and At4g25290, which have not been described to date. Our research has shown that all three proteins are localized in the chloroplast and AtUVR3 is additionally found in the nucleus and mitochondria. The AtUVR3 photolyase can therefore be responsible for pyrimidine dimer repair in all cellular compartments containing DNA. We also observed a striking phenotype of the *Arabidopsis* mutant lacking AtPHR2. Its seedlings have albino cotyledons and defects in chloroplast ultrastructure. Adult plants are characterized by pale green, strongly serrated leaves, inhibited growth and additional leaf rosettes on the flowering stem. The analysis of the At4g25290 amino acid sequence revealed that apart from a photolyase domain, this protein also has a hydrolase domain, allowing the cleavage of chemical bonds with the participation of water molecules. This hydrolase is homologous to pheophytinase, an enzyme degrading chlorophyll, the green pigment present in chloroplasts. Pheophytinase activity leads to leaf yellowing, in autumn or during plant senescence. A similar effect is elicited by UVB.

Is AtPHR2 a yet unknown protein, controlling chloroplast development, greening of seedlings and plant growth? Blue light influences plant growth and development acting via photoreceptors. Is AtPHR2 an unidentified photoreceptor present in chloroplasts? What kind of reactions can it control? Is At4g25290 involved in both DNA repair and chlorophyll degradation? What do these processes have in common? How is AtUVR3 distribution in different cellular compartments regulated? How do the investigated photolyases affect plant responses to stress conditions such as salinity, drought and high temperature? These are some of the questions which we will answer during this project. We will combine molecular, biochemical and microscopic methods to obtain a comprehensive image of photolyase activity in the plant cell.