Abstract of PhD thesis entitled: “Phosphodiesterases in higher plants - a missing link in cyclic nucleotide signal transduction”

Signal transduction is a process in which chemical or physical stimuli reaching the cell are registered by specialized sensors and transferred through the cell membrane to the cytoplasmic elements of the effector system. Binding of an extracellular ligand to a receptor triggers a cascade of events of a biochemical and genetic nature leading to a specific physiological response. The process of transmitting information contained in a stimulus activates a series of signal particles. Cyclic nucleotides (cNMP), classified as secondary messengers, are one of such particles. Appearing in the response to receptor activation, they play key roles in many physiological and developmental processes in both prokaryotes and eukaryotes. For many years, signal transduction with the participation of cNMP in plants was controversial, mainly due to the technical impossibility of their determination in plant cells. With the discovery of techniques such as LC-MS/MS, allowing the determination of concentrations of substances at the trace amounts, the position of cNMP as a secondary messenger in plants was stabilized, and the enzymes responsible for their synthesis began to be discovered. However, the enzymes responsible for the degradation of cNMP, phosphodiesterases (PDEs), remained elusive in higher plants. Taking into account the fact that the termination of cNMP signaling is possible only by the action of PDE, I have hypothesized that PDEs are present in plants, and the difficulty in their discovery is related to a structure different from that known for animal PDEs.

Based on the analysis of the sequence and structural properties of the catalytic sites of phosphodiesterases from various organisms, I have developed a unique, universal amino acid sequence that allows the identification of plant PDEs, based on the key amino acids involved in ligand binding and hydrolysis. This led to the discovery of over 30 PDEs in the proteome of the model plant *Arabidopsis thaliana*. The implementation of protein structure visualization and ligand docking simulation techniques allowed to state that plant PDEs are not stand-alone enzymes, but their domains are embedded in larger, multifunctional proteins. By analyzing the structure of PDE orthologs of various plant species in which the original PDE motif was absent and by using a mutagenesis tool, I modified the motif by adding new, unique amino acids involved in PDE catalytic activity. The resulting tool significantly increased the amount of PDEs found in higher plants. The *in vitro* biochemical studies carried out with the use of the LC-MS/MS detection system confirmed the correctness of the PDE motifs, because both

proteins that were tested showed hydrolytic activity against the cyclic nucleotides cAMP and cGMP.

In summary, implementation of the tandem motif based - molecular docking approach, led to a significant expansion of our knowledge about plant PDEs. The analysis of individual candidates shows that plant PDEs, unlike their animal orthologs, are not individual proteins, but small domains embedded in the structure of larger proteins. This distribution of enzymes responsible for cNMP metabolism sheds new light on the different evolution of cNMP metabolism in plants, which in the future will directly contribute to a better understanding of the processes in which these secondary messengers are involved.