Mitogen-activated protein kinase cascades have emerged as the most common signal transduction mechanism involved in biotic and abiotic stress. Although the MAPKKKs are the largest group of MAP kinases, up to date, only a few of such proteins have been associated with specific biological processes or signaling cascades, even in the model plant Arabidopsis thaliana. The plant hormone ABA functions as a key regulator in many including adaptation to various biotic and abiotic stress conditions. Key elements of the ABA signaling pathway are protein kinases and protein phosphatases. It is known that protein phosphatases type 2C (PP2C) from group A, in particular ABI1 PP2C (ABA-Insensitive 1), are key effectors of ABA signaling. Despite intensive studies little is known about the role of MAP kinases, especially MAPKKK in ABA signaling pathway. Molecular and genetic evidence indicate that MAPKKK18 is an ABA-activated kinase. Importantly, our previous studies showed that MAPKKK18 expression was significantly affected in the ABI1 knockout. Significantly, we confirmed this interaction between MAPKKK18 and ABI1 using yeast-two hybrid, pull-down assay and BIFC. Significantly, recent data have linked ABI1 protein phosphatase to protein degradation by the ubiquitin-proteasome pathway (UPS). It suggested a new meaning on the role of ABI1 in negative regulation of ABA signaling. The ABI1 protein phosphatase might be involved in resetting various signaling pathways to pre-stimulatory status by directing proteins for degradation via the UPS . Very recently we documented that ABI1 PP2C is involved in the regulation the stability of ACC synthase 6 (Acs6). We have documented, that ABI1 interacts with ACS6 and promotes its degradation by dephosphorylation at C- terminally located MPK6 target site . In addition, our unpublished results shows that ABI1 is involved in degradation of ACC synthase 7 (Acs7). Based on this results, we might hypothesize, that ABI1 targets to degradation also other regulators of ABA signaling pathway. Based on these assumption we hypothesize that ABI1 might also control turnover of MAPKKK18. To test this hypothesis we examined the turnover of MAPKKK18 using cell-free degradation assay and results this experiment strongly suggested that ABI1 PP2C promotes degradation of MAPKKK18. However, the exact mechanism of this degradation remains to be determined. Therefore the aim of this project is to resolve the proteasomal degradation mechanism of MAPKKK18. These include 1) identification of MAPKKK18 ubiquitination sites and 2) identification of the ubiquitin ligase E3 involved in MAPKKK18 this process.

Protein degradation by the 26S proteasome pathway is an integral part of ABA signaling, therefore these investigations concern important and universal biological phenomena: UPS-mediated protein degradation of ABA signaling regulators. The results from this study will provide novel findings which will be beneficial for understanding the mechanisms of removing certain ABA effector from the cell. UPS regulate several ABA-responsive transcription factors, which are the positive or negative regulators of ABA signaling. ABA-regulated protein kinases were also identified to be degraded by proteasome 26S pathway. Because understanding of the degradation mechanism of ABA regulators is crucial for resetting ABA signaling, this proposal rise several fundamental questions regarding molecular aspects of the MAPKKK18 degradation. One of them is what is the mechanism of MAPKKK18 turnover? Which specific ligase E3 is involved in this process and finally which lysine residues serves as a signal for MAPKKK18 polyubiquitination? Another expected results of these studies will include a better understanding of protein complexes, molecular basis of protein interactions and the protein interaction networks. It is known, that the key elements of biological systems are represented by the complex, specific or non-specific interactions between molecules, therefore information obtained under this project, will give a better knowledge about the functioning of plant organisms.