Global warming is gaining significance as a threat to viability of plants, while our knowledge on plant response to heat stress has, so far, mostly focused on the effects of short acute heat stress. It is therefore of utter importance to increase our understanding of the molecular basis of plant response to chronic moderately elevated temperature stress. Based on our previous study we know that a lack of either mitochondrial protease, AtFTSH4 or AtOMA1, has no noticeable effect on the morphology of mutant plants at optimal growth temperature of 22°C in the model plant Arabidopsis. However, a prolonged exposure to an elevated temperature of 30°C affects mutant plants, as they are noticeably shorter. Such behavior was not observed regarding the other seven tested mitochondrial proteases, suggesting that AtFTSH4 and AtOMA1 are involved in the same process of a distinct, yet rudimentarily known type of thermotolerance to chronic moderately high temperature. To circumvent the expected functional redundancy between AtFTSH4 and AtOMA1 we are going to use in our study not only single loss-of function mutants (*ftsh4*, *oma1*), but also a double mutant lacking both proteases (*ftsh4oma1*) in comparison to wild-type.

A major challenge of this project will be to elucidate the role and interplay of AtFTSH4 and AtOMA1 under chronic moderately elevated temperature conditions. Two hypotheses, not mutually exclusive, will be tested. The first hypothesis assumes that the examined proteases control the abundance/processing of specific substrates which act as heat protectors under stress conditions, while, according to the second hypothesis, both proteases are involved in the removal of toxic, unfolded proteins generated during long-term stress of moderately elevated temperature. Looking for the answer to the question about the biological function of the examined proteases we decided to perform an extensive search for their substrates under optimal as well as moderately high temperature. Knowledge of the substrates is the key to understanding the role of a protease *in vivo*. Three advanced, mass spectrometry-based, methodological approaches (iTRAQ, COFRADIC, TRAP) are planned to be used to identify substrates degraded or processed by AtFTSH4 and AtOMA1.We also expect to identify interactors of the examined proteases, particularly by utilizing the TRAP approach as well as BN-PAGE native electrophoresis combined with mass spectrometry. Validation of candidate substrates will be performed by a variety of research methods *in silico*, *in vivo* and *in vitro*. Particular attention will be devoted to substrates that could be involved in Arabidopsis response to heat stress. This involvement will be tested by thermotolerance phenotyping of mutants lacking the putative protease substrate. In addition, several approaches will be conducted to analyze the predicted accumulation of misfolded proteins in plants lacking AtFTSH4 and/or AtOMA1, such as: misfolded protein aggregation assay in protoplasts, *in vivo* monitoring of protein unfolding or *in vitro* degradation assay of unfolded model substrate. To prove that AtFTSH4 and OMA1 mutually complement each other's function, at least under long-term moderately elevated temperature, we are going to introduce AtFTSH4cDNA in *oma1* plants and *vice versa* AtOMA1cDNA in *ftsh4* plants. We believe that the multi-directional approach proposed in this project is a chance to reveal mechanisms affected by loss of the investigated proteases, and to what extend these mechanisms are independent of those already known.