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SEARCHING FOR PROTEASES SIGNIFICANT FOR FUNCTIONALITY AND MORPHOLOGY OF PLANTS MITOCHONDRIA

Abstract

The functioning of mitochondria depends on composition and quality of mitochondrial proteome, which is maintained by the inner proteolytic system. It consist of ATP-dependent and ATP-independent proteases. Currently, our knowledge about ATP-indpendent proteases is rather scarce.

In the presented project the role of ATP-indepedent proteases in mitochondrial metabolism and their impact on plant development and morphology were defined. There were identified and characterized 6 unknown untill now ATP-indpendent proteases in plant mitochondria, based on known yeast homologs: Oct1 (AtOCT1), Oma1 (AtOMA1), Icp55 (AtICP55), Atp23 (AtATP23), Imp2 (AtIMP2) and Imp1 (AtIMP1a). Moreover, all of studied proteases were localized in Arabidopsis mitochondria. Further, similarly to their yeast counterparts AtATP23 was localized in soluble fraction, while AtOMA1 and AtIMP1a were localized in membrane fractions. Whereas AtOCT1, contrary to its yeast homolog, was localized in a membrane mitochondrial fraction. Among these proteases only AtICP55 and AtOMA1 complemented respiratory function of yeast counterparts. With the exception of AtOMA1, the lack of the other analyzed proteases neither caused any changes in plant development and morphology, nor under molecular level in ATP synthase functionality under optimal growth conditions. Furthermore, the lack of AtOMA1 protease caused notable reduction of complex V activity under optimal growth conditions. Increase in the temperature for plants growth casued further decrease in the activity of complex V together with a slight reduction of its amount in *oma1-1* mutants. Moreover, these stress conditions induced abberation in functionality in other OXPHOS complexes in plants deprived of AtOMA1 protease. The above results suggest that AtOMA1 protease regulates primarily the activity of complex V. The putative mechanism of this regulation can presumably take place under

proteolytic activity of AtOMA1, which influence the plant specific factor engaged in the functionality of compex V.

The second aim of this project was to determine if two proteases ATP-independent (AtATP23) and ATP-dependent (FTSH4) have an influence on the lipid metabolism and mitochondrial morphology. Both proteases were chosen for studies according to the literature, where yeast and mammalian homologs were shown to be involved in lipid metabolism. However, results from this project revealed, that only FTSH4 protease influences phosholipid content in Arabidopsis mitochondria. With the use of novel technonology Shotgun lipidomics, detailed lipid profile of plant mitochondria deprived of FTSH4 protease was conducted. One of the most important result was the reduction of cardiolipin (CL) together with the increase of phosphatidylglycerol (PG) and diacylglycerol (DAG) in mitochondria of mutant plants. Simultaneuosly, with the use of confocal microscopy, it was shown that the lack of FTSH4 protease caused appearance of heterogeneous mitochondrial population which consisted of abnormal, giant mitochondria, among normal ones. The correlation of reduced CL amount with the giant mitochondria occurance could suggest that FTSH4 protease contributes to the mitochondrial phospholipid metabolism and thus influences mitochondrial morphology and dynamics.