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ABSTRACT

Functional analysis of mitochondrial AtFtsH4 protease with emphasis on the proteomic studies.

It has already been 125 years ago since the Swiss anatomist and physiologist Rudolph Albert von Kölliker discovered mitochondria in the muscles of insect wings. Following this discovery many researchers have been working on understanding the structure and specific functions of these organelles of eukaryotic cells. Mitochondrial homeostasis requires constant regulation of the mitochondrial proteome in response to changing environmental and developmental conditions. This regulatory work is fulfilled by the mitochondrial protein quality control (mtQC) system which is composed of membrane-bound and soluble AAA proteases (***A****TPases* ***a****ssociated with various cellular* ***a****ctivities*) named FtsH. These enzymes are ATP-dependent proteases consist of two domains: an AAA domain with chaperone activity and a proteolytic domain responsible for protein degradation.

In this doctoral dissertation a broad functional analysis of *Arabidopsis thaliana* AtFtsH4 protease was conducted. AtFtsH4 is an inner membrane-embedded metalloprotease, which belongs to *i*-AAA family with catalytic domains faced the intermembrane space. We showed that the loss of AtFtsH4 alters plant growth and development in short day (SD), 22°C as well as in LD, 30°C conditions. Similar morphological and developmental abnormalities, such as an asymmetric shape and an irregular serration of expanding leaf blades and the inflorescence development were found for *ftsh4* plants in both conditions, named conditions promoting phenotype. The dynamics of spatial and temporal morphological abnormalities resembled AtFtsH4 expression profile observed on transcript and protein levels in wild type (WT) plants. This finding was also confirmed by monitoring the *AtFtsH4* promoter activity. The full phenotype complementation of *ftsh4*-Ftsh4 revertant line was observed. It should be mentioned that AtFtsH4 does not respond like a typical heat shock protein under short heat stress.

We have previously shown that accumulations of ROS and carbonylated proteins in *ftsh4* mutants suggest strong oxidative stress. Using common known oxidative stress markers, like

transcript levels of *UPOX*, *AOX1a* and high abundance of low molecular weight antioxidants, we proved that this stress was accumulative and was the strongest in the generative phase of growth.

To understand better the connection between the lack of AtFtsH4 and oxidative stress we performed “omnics” like quantitative comparative analyses of mitochondrial total proteomes and oxyproteomes of two *ftsh4* mutants and wild-type plants under LD, 30°C and SD, 22°C conditions. These proteomic analyses revealed disturbances in numerous metabolic pathways in *ftsh4* mitochondria. The loss of AtFtsH4 is associated with lower abundance and activity of complexes I and V at both studied conditions, except complex II, where subunit of succinate dehydrogenase was found in a higher abundance. Moreover, subunits of oxidative phosphorylation (OXPHOS) complexes I, II and V were found to be oxidatively damaged. Most of the enzymes of the TCA cycle and photorespiration were changed according to their abundance and most of them were more excessively oxidized in mutants than in wild type mitochondria. Thus, alternative electron transport pathways as well as extensive protein degradation feeding electrons to the mitochondrial respiratory chain were induced in *ftsh4* mutants.

Disturbed mitochondrial homeostasis in the *ftsh4* mutant was also observed together with accumulation of chaperones and stress related proteins. Manganese superoxide dismutase (MSD1), which is a key enzyme involved in the removal of ROS was also intensively carbonylated. Therefore we suggest that this enzyme was inactive in *ftsh4* mutants, because of the lack of AtFtsH4 which may also have an impact on the ROS-scavenging systems. We assume that mitochondrial membrane permeability for ROS is greater in *ftsh4* mutant comparing to WT.

Combining proteomic and transcriptomic data we selected several proteins, among them PHB and AtFtsH10, which seemed to be the most likely substrates for AtFtsH4. Both proteins were identified by co-immunoprecipitation like physiological partners of AtFtsH4, but the increased *in vitro* stability was observed exclusively for AtFtsH10 in *ftsh4* mutant comparing to WT. In transgenic lines with chaperone domain (*ftsh4*-FtsH4^{CH}) much higher abundance of PHB in comparison to the *ftsh4* mutant was observed. However, in *ftsh4*-FtsH4^{P}, the line with active proteolytic domain lower abundance of AtFtsH10 was detected. Thus AtFsH4 may have a proteolytic activity towards AtFtsH10.

In conclusion, we proved using wide genetic and proteomic techniques that the absence of AtFtsH4 protease causes broad oxidative stress, which results in impairment of most of the

metabolic pathways in *Arabidopsis* mitochondria. The increased oxidative stress is caused by the defects of ROS production or/and scavenging pathways. Moreover, all approaches described in this thesis indicate that AtFsH10 is a natural proteolytic substrate for AtFtsH4 protease. This finding is particularly interesting in the context of interactions between mitochondrial ATP-dependent proteases and due to the fact that this can be the first identified substrate of a plant ATP-dependent protease.