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THESIS TITLE:

" IMPACT OF CHANGES IN MITOCHONDRIAL TRANSLATION ON GLOBAL TRANSCRIPTOME, WITH PARTICULAR EMPHASIS ON CHANGES IN THE CHLOROPLAST GENOME EXPRESSION AND COMMUNICATION BETWEEN CHLOROPLAST AND NUCLEUS"

ABSTRACT

Interorganellar communication between nucleus, chloroplast and mitochondria has crucial importance for plant cells. Studies performed in this dissertation concern understanding of communication between organelles in response to changes in the mitochondrial translation.

Arabidopsis thaliana mutants with silenced expression of *RPS10* gene encoding S10 protein, which is a component of the small subunit of mitochondrial ribosome, were biological material used in these studies. Characteristic of *rps10* mutants is presence of heterogeneous population of mitoribosomes. This disturbance in mitoribosomal biogenesis result in changes in mitochondrial translation, leading to increased protein synthesis of mitoribosomes and reduced protein translation of oxidative phosphorylation complexes (OXPHOS).

The microarray data analysis (ATH1, Affymetrix campany) of *rps10* mutants showed, that silencing of *RPS10* caused global changes in nuclear gene expression. Among 2314 and 4955 differentially expressed genes, respectively in P2 and in P3 phenotype of *rps10* mutants, compared to wild type plants, up to 1488 genes were common regulated in both phenotypes. Analysis of the common group of genes in *rps10* mutants using MapMan software with Benjamin-Hochberg correction revealed significant changes in 7 functional groups: "*mitochondrial electron transport/ATP synthesis*", "*cell wall*", "*lipid metabolism*", "*biotic stress", "biotic stress/PR proteins*", "*RNA regulation of transcription - basic Helix-Loop-Helix family*", "*signalling - receptor kinases*".

Detailed analysis of functions of gene products involved in biotic stress response, which were activated in *rps10* mutants, suggested that induction of a biotic stress is associated with the accumulation of salicylic acid (SA). This suggestion was confirmed experimentally. In *rps10* mutants elevated level of SA was observed by liquid chromatography coupled with mass spectrometry analysis. Elevated level of SA probably also plays a role in observed higher resistance of *rps10* plants to drought and increased sensitivity to continuous darkness stress, which induces aging. It was suggested that increased level of SA in *rps10* mutants was associated with

abnormalities in the stomata biogenesis, which probably led to their closing. This in turn had significance not only for the defense mechanism against entring of pathogens, but also contributed to a reduction in transpiration, generating water supply in the cell, which in turn maintained longer the plant alive. It is postulated that perturbations in mitochondria caused by silencing the *RPS10* gene lead to an increase of SA content and consequently activate the expression of biotic-related genes. Most likely reactive oxygen species were involved in this signal transduction.

Comparative transcriptome analysis of *rps10* mutants with transciptomic data of 26 different mitochondrial mutants showed the greatest similarity with *aox1a:rpoTmp* mutant, with impairment of both cytochrome and alternative mitochondrial respiration pathway. It was showed that among 709 commonly regulated genes between *rps10* and *aox1a:rpoTmp* up to 97% showed the same expression pattern. Further studies have shown that in *rps10* mutants similarly to the *aox1a:rpoTmp*, both cytochrome and the alternative respiration pathways in mitochondria, were reduced. Decreased activity of the cytochrome pathway in *rps10* mutants was associated with a decreased amount of OXPHOS complexes as a results of changes in mitochondrial translation. In turn, the presented studies suggest that the decline in activity of alternative respiration pathway in *rps10* mutants is not a consequence of changes in expression of AOX. Performed analysis showed increase in the level of transcript and protein of alternative oxidase, probably as a result of compensation mechanism activated in response to stress occuring in *rps10* cells. Additionally it was documented that reduced activity of an alternative oxidase in *rps10* mutants is not a result from oxidation form of AOX in this plants. Results presented in this thesis also showed, that increase in an active form of AOX observed in *rps10* mutants was not correlated with the amount of their activator in mitochondria - pyruvate. Based on the conducted studies, it was suggested, that one of the probable reasons for the AOX activity reduction in *rps10* mutants was disturbances in the import of pyruvate into mitochondria. These results indicate translational mechanism of regulation of cytochrome pathway activity and the posttranslational mechanism of regulation of alternative respiration pathway activity in *rps10* mutants.

Transcriptomic data analysis showed that beside mitochondria (**3.7%**), the most common affected genes in *rps10* and *aox1a:rpoTmp* mutants encode proteins targeted to the chloroplast (**3.48%**). Among them the largest functional group represents genes encoding proteins, which are involved in regulation of transcription. Studies performed at the transcript level showed changes in chloroplast transcription apparatus (NEP and PEP polymerase, pTAC proteins and sigma factors). Taking into account these results, it was hypothesized that "*the decrease in activity of both cytochrome and alternative respiration pathways leads to a reduction in chloroplast transcription activity*". This hypothesis was confirmed by "*in vitro*" assay, where it was shown that simultaneous inhibition of both complex IV of mitochondrial respiratory chain and alternative oxidase activity in

wild-type plants led to reduced level of chloroplast-encoded transcripts, like in *rps10* and *aox1a:rpoTmp* mutants. Therefore, it was postulated that decrease in the activity of complex IV and AOX is a signal to the reduction of chloroplast transcription efficiency in *Arabidopsis thaliana*. It was also shown, that changes in the transcriptional activity influenced on the level of chloroplast mRNA genes, whose transcription depends on both nuclear (NEP) and chloroplast (PEP) encoded polymerases. It was revealed that decrease in the level of chloroplast mRNAs was not a consequence of changes in number of copies of chloroplast genes. In turn, reduction of the chloroplast mRNA genes was reflected in the decreasing of protein synthesis and steady-state chloroplast protein level. It was also showed that changes in chloroplast transcription initiate anterograde signal from the chloroplast to the nucleus resulting in changes in the level of nuclear-encoded photosynthetic gene transcripts.