

Dissertation topic: „Characteristics of mitochondrial changes arising in response to disturbed biogenesis of mitoribosomes”.

ABSTRACT

Research indicates that ribosomes are not simple nonselective translation machines, but can also act as regulatory elements in protein synthesis. This view is supported by our previous data showing that silencing of the gene encoding the mitoribosomal S10 protein in *Arabidopsis* produces a unique population of mitoribosomes with different effects on mitochondrial transcript translation compared to the wild type.

Ribosome profiling, an innovative method that allows monitoring of translation „in vivo” by sequencing ribosome-protected mRNA fragments (so-called „ribosome footprints”), was performed to understand the molecular bases of the altered translation observed in the mutant with decreased abundance of the mitoribosomal protein S10 (*rps10*). This research showed that mainly transcripts encoding oxidative phosphorylation system (OXPHOS) proteins were translated in wild-type mitochondria. However, this feature is lost in the mutant. The *rps10* mitoribosomes show a slightly reduced translational efficiency of most OXPHOS proteins, but the synthesis of other mitochondrial proteins, including the ribosomal proteins, MatR and TatC is performed with significantly increased efficiency. The obtained data indicate that mitoribosomes without the S10 protein protect shorter fragments of the transcript and exhibit a lower 3-nt periodicity characteristic for translating ribosomes compared to the wild type. Interestingly, the decrease in triplet periodicity is especially drastic for genes that contain introns. In addition, splicing has been shown to be significantly less effective in the mutant, indicating an unexpected relationship between S10 deficiency and mitochondrial transcript assembly.

Analysis of the reads matched outside the coding sequences revealed the presence of large amounts of small RNAs, especially in the *rps10* mutant, likely derived from the protection of RNA against nuclease degradation by RNA binding proteins (RBPs). Some of them (mostly from the 3' untranslated region) resemble clustered organellar sRNAs (cosRNAs).

In the course of research it was also shown that in the *rps10* mutant, the maturation of 18S-5S rRNA precursor is impaired. Apart from the mature forms of 18S and 5S rRNA in the mutant, the presence of polyadenylated precursors containing 18S rRNA, ITS (intergenic sequence) and 5S rRNA, as well as 18S rRNA with ITS of various lengths were identified.

The latter forms were identified as polyadenylated, but forms without a polyA tail have been found, as well. Although, it is unclear, what role the S10 protein plays in rRNA maturation two options are considered that are not mutually exclusive. S10 has extra-ribosomal functions and/or its presence as a constituent of mitoribosomes is necessary for the proper, hierarchical maturation of rRNA. It should be emphasized that the deficiency of each mitoribosomal protein does not lead to significant disturbances in the biogenesis of the mitoribosomes and changes in mitochondrial translation. This conclusion is confirmed by my results on the *Arabidopsis mrpl11* mutant with the decreased level of transcript coding the L11 protein from large subunit of mitoribosome.

In addition, during the implementation of my doctoral dissertation, I uncovered that the proper functioning of the alternative and cytochrome mitochondrial respiration pathways is disturbed in the *rps10* mutant.