## Abstract

## Cross – talk between mitochondrial and nuclear genome in response to perturbation of mitochondrial translation in Arabidopsis

Crosstalk between mitochondrial and nuclear genomes in response to changing developmental and environmental conditions and also in response to stress occurring within the mitochondria is crucial for biogenesis of mitochondrial complexes. In plants, all mitochondrial oxidative phosphorylation (OXPHOS) complexes, except complex II, as well as mitochondrial ribosomes (mitoribosomes) comprise of subunits encoded by both the mitochondrial and the nuclear genomes. So far, studies have failed to exactly define the level of coordination between expression of mitochondrial and nuclear genomes in plants. Some reports indicate that this coordination occurs at the transcriptional level, whereas the global analysis of *Arabidopsis* showed that posttranslational regulation plays a major role in this coordination. The results presented in this work confirm that during biogenesis of OXPHOS and mitoribosomal complexes in plants the coordination of expression between mitochondrial and nuclear genomes occurs at the posttranslational level. Moreover, it suggests that translation of mitochondrial transcripts can be differentially affected by alterations in mitochondrial ribosomes.

In order to determine the level/mechanism coordinating the expression of nuclear and mitochondrial genomes, the *Arabidopsis thaliana* mutants with silenced expression of *RPS10* gene encoding S10 protein, a component of the small subunit of mitoribosomes, were used. Studies showed perturbations in biogenesis of mitoribosomes in *rps10* mutants. Unexpectedly, although *RPS10* transcript abundance was reduced, S10 protein levels were similar in wild type and *rps10* mutants. These results suggest that the decreased *RPS10* transcript abundance induced a feedback mechanism to maintain S10 protein levels and led to increased production of both mitoribosomal subunits. However, the upregulation in biogenesis of large and small subunits of mitoribosomes was imbalanced due to the S10-deficient small subunits being unstable and therefore prone to degradation, resulting in an excess of large subunits.

The compensation of defect in biogenesis of mitoribosomes which led to their intensified biogenesis results in alterations at different level of mitochondrial genome expression. Analysis showed amplification of the whole mitochondrial genome in *rps10* cells, which was then reflected in an elevated level of transcripts of the mitochondrial OXPHOS and ribosomal genes. The level of nuclear-encoded transcripts of mitoribosomes and

OXPHOS complexes seemed to be less affected. Examination of mitochondrial translation activity by analysis of polysomal fractions in the *RPS10*-silenced lines showed that OXPHOS components generally had fewer ribosomes per mitochondrial transcripts as well as a lower proportion of these transcripts bound to ribosomes. By contrast, most mitoribosome components were more translationally active. Nuclear-encoded subunits of both OXPHOS and mitoribosomes were generally unchanged. *In organello* protein synthesis provides independent evidence for aberrant mitochondrial translation which led to overaccumulation of mitoribosomal proteins and reduction of OXPHOS proteins in *rps10*. Moreover, at the steady state protein level the reduction of both mitochondrially- and nuclear-encoded components of OXPHOS complexes I, III and IV was similar. In turn, despite the observed disproportion of mitochondrially- and nuclear-encoded subunits of complex V, the stoichiometry of the complex V subunits was virtually identical in *rps10* mutants to that in wild-type mitochondria. These data suggest that coordination of expression of the nuclear and mitochondrial genomes occurs at the complex assembly level and the excess of non-assembled complex subunits is likely to be degraded by the mitochondrial ATP-dependent proteases, which were upregulated in the *rps10* mutants.

The most innovative finding of these studies is to demonstrate that in consequence of the perturbation in biogenesis the altered mitoribosomes have differential effects on translation of individual mitochondrial proteins. This result suggests that translation in plant mitochondria can be regulated by heterogeneity in mitoribosome population. This is the first documented case showing that plant mitoribosomes may control gene expression by selective translation of a specific group of transcripts. Altogether, this work provides important evidence not only for the predominance of translational regulation in plant mitochondrial gene expression but also confirms that coordination of nuclear and mitochondrial genomes is achieved at the posttranslational level, probably at the level of protein complex assembly.