Mitochondria are cellular powerhouses and physiological regulators with many features resembling their prokaryotic ancestors. They maintain their own mitochondrial DNA (mtDNA) encoding 13 inner mitochondrial membrane proteins of oxidative phosphorylation machinery, 22 transfer RNAs and two ribosomal RNAs. Healthy mitochondria in normal cells form a dynamic network consisting of highly interconnected tubules. The mitochondrial disorders are very heterogeneous and difficult to diagnose and cure. It is due to combining products of two genomes, the complexity of mitochondrial structure and due to insufficiency of current state of knowledge.

To understand the mitochondrial nucleic acid species distribution, we have developed and established new techniques to visualize mitochondrial network, nucleoids and different RNA species together with qPCR techniques for monitoring the mitochondrial intactness. We determined nucleoid distribution and mtDNA amount following rotenone mediated respiratory inhibition or following degeneration of the electrical component of the protonmotive force by valinomycin treatment. Native mitochondria were mostly tubular with average nucleoid spacing 1.1 +/- 0.2μm which we termed as a nucleoid code. Subsequently induced fission resulted in mitochondrial network fragmentation and each mitochondrial fragment contained several nucleoids. Only rarely the fragments contained one nucleoid and barely were empty. It indicates that mitochondrial fission is nucleoid-centric implicating existence of factor connecting nucleoids with fission machinery. As a model of mitochondrial pathology, we employed a model of type 2 diabetes, the Goto Kakizaki rats, where we measured amount of mtDNA in pancreatic β-cells as well as respiration of pancreatic islets. We observed dramatic decrease in the amount of mtDNA in beta cells of ageing rats and also in livers and heart muscle. Moreover Goto Kakizaki pancreatic islets displayed slightly impaired response to glucose in comparison with Wistar rats. Our findings support the hypothesis, that mitochondria play a key role in the development and progression of type 2 diabetes. Finally, we have developed techniques for monitoring several mitochondrial nucleic acid species for both fluorescence *in situ* or *in vivo* hybridization (FISH or FIVH). Our developed *in situ* and *in vivo* fluorescent hybridization showed mitochondrial mRNAs localization pattern in cancer cells and also pattern of mitochondrial non-coding RNAs. The 5S rRNA enables import of desired small RNA sequence into mitochondria. Both techniques have potential to find an application in diagnostics and in the future can be upgraded for importing larger RNA sequences to compensate point mutations in mtDNA genes or inhibit translation of mutated mRNA.

In conclusion we have established and tested several techniques for monitoring mitochondrial nucleic acid species, which can be potentially used in clinical diagnostics.