Purine ribonucleotides (PNs) are essential for life, powering our metabolism, like ATP, and being monomers of RNA synthesis during expression of genetic material. Interestingly, PNs acts both as an energy supplier molecules and regulators of an ATP biosynthesis efficiency. Mitochondria, the powerhouses of cell, catalyze the fundamental process of oxidative phosphorylation (OXPHOS), coupling the oxygen consumption and ATP production *via* respiratory chain activity. Regulated respiratory chain complexes-mediated recycling of protons through inner mitochondrial membrane is crucial for efficient energy conservation to ATP. However, mitochondrial energy transduction is plastic and incomplete because of proton short-circuit without ATP synthesis. Mitochondrial uncoupling protein (UCP) and adenine nucleotide translocase (ANT) are considered as two main catalysts of futile proton leak in mitochondria. In turn, some mitochondrial kinase, nucleoside-diphosphate kinase (mNDPK), balances PNs pools through transphosphorylation reaction. The common feature of these three proteins, i.e., mNDPK, UCP and ANT, is PN binding with a different purpose/effect. **Therefore, the aim of the project is to establish the role of mNDPK in regulation of mitochondrial proton leak mediated by UCP and ANT**. **The essence of the project is to understand a complicated relationship between mNDPK recruitment, which regulates local concentration of PNs, and the mitochondrial proton leak, which is affected by PNs acting as potential inhibitors of UCP and inhibitors/substrates of ANT.** So far, the crosstalk between mNDPK and the efficiency of protein-mediated proton leak has been completely ignored. The description of physiological substrates, activators and inhibitors of mNDPK, UCP, and ANT is a key point for understanding the contribution of mitochondrial proton leak for the maintenance of cell energy homeostasis. This project, proposing a new function for mNDPK, will clarify the complicated influence of PNs on the energy transduction in the mitochondria. This issue is also significant for future development of drugs (pharmacological targeting of mNDPK, ANT and UCP on clinical scale) normalizing the mitochondrial proton leak in various human and other organisms diseases. According to current knowledge, mNDPK seems to act as a bifunctional nanoswitch, i.e., in bioenergetics and cardiolipin trafficking including pro-apoptotic signaling. In turn, changes of protein-mediated proton leak, like UCP-mediated proton leak, could be implicated in pathogenesis of obesity, type-2 diabetes, cachexia, neurodegenerative diseases (Alzheimer's disease and Parkinson's disease) and tumor.

The research will be carried out on several types of model organisms to draw more general conclusions about the studied problem. The following materials will be used: (i) microorganisms, like amoeba *Acanthamoeba castellanii* and yeast *Saccharomyces cerevisiae* (wild type non-possessing UCP) and (ii) mammalian tissues, i.e., rat tissues (e.g., kidneys and skeletal muscles) and human endothelial cells. As a novelty, measurements of mitochondrial proton leak will be carried out under physiological-like conditions, i.e., those favoring OXPHOS (without OXPHOS inhibitors) and in the presence of high concentration of adenine and guanine nucleotides. The only way to clearly confirm the role of mNDPK in regulation of the UCP- and ANT-mediated proton leaks is to completely preclude (selectively switch off) mNDPK-dependent transphosphorylation in mitochondria without switching off PN import systems into mitochondrial matrix that maintains the possibility of OXPHOS. Therefore, experiments with mNDPK specific inhibitors and mNDPK gene silencing are planned to verify the physiological role of some PNs considered as inhibitors of mitochondrial proton leak. The studies will be focused mainly on GDP, because its inhibitory effect on mitochondrial proton leak is controversial. The project involves the usage of isolated mitochondria with wild bioenergetic phenotype (possessing mNDPK and UCP) as well as mitochondria with changed bioenergetic phenotype (UCP2-knockout rats and mNDPK-silenced human endothelial cells). Therefore, the influence of GDP transphosphorylation process on the UCP-independent mitochondrial proton leak using UCP2-knockout rats will be studied. In turn, gene silencing of mNDPK, using human endothelial cells, will allow us to estimate the GDP effect on mitochondrial proton leak, which is independent of mNDPK-catalyzed transphosphorylation process. The project also involves testing of recognized inhibitors of mNDPK, like cromoglycate (a widely used drug for the treatment of allergic asthma), in wild-type mitochondria (with functioning mNDPK). Measurements of mitochondrial respiration with these inhibitors will validate the findings of mNDPK gene silencing experiments. Furthermore, the project will be focused on the verification of mNDPK substrate affinity under physiological-like conditions and the impact of mitochondrial coenzyme Q redox sate on the mNDPK activity. In turn, functional and molecular characterization of amoeba mNDPK will confirm the existence of mNDPK-dependent transphosphorylation mechanism regulating the mitochondrial proton leak at early evolution stages.