Regulation of cell death and OXPHOS activity by Fmp40 AMPylase in yeast *S. cerevisiae*

Mitochondria, besides the key role in generation of energy by oxidative phosphorylation system OXPHOS, carry out a lot of functions essential for cell physiology and viability, such as biosynthesis of Fe-S clusters and heme, metabolism of amino acids, nucleotides, lipids or carbohydrates and induction of the programmed cell death – apoptosis in mammalian cells. Impairment of OXPHOS functioning results in deficit of metabolites and increase of the reactive oxygen species production. This leads to a wide spectrum of severe abnormalities, which in humans are known as mitochondrial diseases, and are associated with cancer development. Apoptosis, a process defective in cancers, is preceded by the increase of reactive oxygen species and increased permeability of the mitochondrial inner membrane, caused by the formation of so cold Permeability Transition Pore (PTP). The composition of this pore is a subject of debate, however it seems now certain that ATP synthase (last enzyme of OXPHOS) dimers form a core of this pore.

The activity of proteins and their localization is tightly regulated in the cell on different levels. One of regulation is achieved by the post-translational modifications of proteins leading to diversification of their functions and activity. The most prominent modification is phosphorylation, when the γ phosphate (third phosphate group) of adenosine triphosphate - ATP is attached to the protein. This modification is carried by the protein kinases. Many proteins classified to the kinase family have no kinase activity and are named a “pseudokinases”. One of such pseudokinase is human selenoprotein O (SelO), characterized by the presence of an atypical amino acid residue, selenocysteine at the C-terminus. SelO is one of the most highly conserved members of either the human protein kinase families or the various selenoprotein families. Most recently we have discovered that human SelO, and its bacterial and yeast homologs (YdiU and Fmp40 proteins, respectively) have activity of AMPylase. During AMPylation the adenosine monophosphate - AMP is attached through its α-phosphate (first phosphate in the ATP) to proteins. SelO AMPylates proteins involved in the detoxification of reactive oxygen species which are toxic for the cell, among substrates in *E. coli* cells we have found the glutaredoxin protein, a small thioredoxin-like protein involved in reducing cellular disulfides using the reducing power of glutathione. One of the modification of cysteine residues in proteins is attachment of a molecule of glutathione (S-glutathionylation). This is a ubiquitous mechanism for protecting proteins exposed to oxidative conditions. The SelO deficient mutants showed a modest decrease in global S-glutathionylation. We proposed the biological role of SelO which is regulation of protein S-glutathionylation levels by AMPylation of the glutaredoxin family and other, yet unknown proteins during oxidative stress.

Interestingly in the screen of substrates of yeast SelO we have found the subunits of ATP synthase and another respiratory complexes, regulators of the permeability transition Por1 and Om45 and a small protein Prx1 – a member of the thioredoxin family. Moreover we have found that yeast cells deficient in SelO die much faster than the control wild type cells after inducing the oxidative stress by hydrogen peroxide treatment and this death is carried on the programmed cell death pathway (PCD). Moreover yeast Prx1 protein was shown also to be involved in this death pathway.

To find out the role of AMPylation for mitochondrial functions we plan to study in yeast mutant missing SelO following mitochondrial functions: oxygen consumption, ATP synthesis and hydrolysis, assembly and stability of ATP synthase and complex IV. We will undertake the systematic investigation of AMPylation of subunits of ATP synthase, respiratory complex IV, Prx1, Por1 and Om45 proteins. Moreover we will study the programmed cell death in these cells.

We are using a set of biochemical methods for measuring the mitochondrial activities, AMPylation and the permeability transition. The genetic and cell biology methods will be used to understand the yeast SelO role in cell death.