Purification and structural characterization of EXOG a human mitochondrial inner membrane nuclease

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Mitochondria are the organelles in which the process of oxidative phosphorylation (OXPHOS) takes place. OXPHOS process leads to an increased concentration of reactive oxygen species (ROS) present in these organelles. This phenomenon results in a high incidence of mitochondrial DNA (mtDNA) oxidative damage, which can be about ten times greater than in case of the nuclear DNA. mtDNA is a 16.6 kb circular, double stranded DNA molecule containing genes encoding 13 components of OXPHOS system, 22 tRNAs and 2 rRNAs required for their translation. Mutations in mitochondrial genetic material can affect the replication and the stability of mtDNA and result in vast array of mitochondrial disorders and diseases.

mtDNA repair system is very efficient. It is thought that the main mechanism of mtDNA repair is Base Excision Repair (BER). The existing model for mitochondrial BER is based on the one present in nucleus. One of the proteins involved in this process is EXOG, a nuclease that has both endonuclease and 5'-exonuclease activity and is localized on the inner membrane of mitochondria. Cellular depletion of EXOG results in persistent single-strand breaks in mtDNA, mitochondrial dysfunction and programmed cell death. What is more, EXOG is found in a complex with enzymes involved in mtDNA repair such as: apurinic/apyrimidinic endonuclease 1 (APE1), Polymerase gamma and Ligase III. During the BER process oxidized abasic site (AP) is cleaved by APE1, resulting in a 5'-end nick that is capped by a deoxyribosephosphate (dRP) or its oxidized form 2-deoxyribonolactone (dL) moiety. Both dRP and dL are resistant to the lyase activity of Polymerase gamma, while EXOG has an ability to process and remove dinucleotides from the 5'-end of DNA, including dRP and dL moiety, thus creating a substrate for Polymerase gamma to fill the gap what allows for the next steps of the repair process to occur.

In my project I am focusing on the investigation of the structure and activity of full-length enzyme, which is still unknown. I am investigating different methods of membrane protein purification, extraction and their effect on the protein stability and activity. The long-term goal of the project is to

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understand the structure and function of mitochondrial repairosome, as it is assembled on the mitochondrial inner membrane via EXOG.

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