

## **Abstract for the general public**

Every living creature is a highly organized system that carries out a series of physical and chemical processes requiring a constant energy supply. People are not unique in this respect, and, as is well known, we need food and oxygen to live. These two components provide our body the energy necessary for its functioning, thanks to the process of cellular respiration. At the level of a single cell, specialized organelles - the mitochondria - are responsible for cellular respiration. They are present in every cell (except red blood cells) of the human body.

Human mitochondria are fascinating, among other things, because they have their own genome in the form of a circular DNA molecule. Mitochondrial DNA is small and encodes only a dozen proteins, while other about 1,500 mitochondrial proteins are encoded by genes stored in the cell nucleus. Thus, mitochondria result from the cooperation of two spatially separated genomes - the nuclear and the mitochondrial. Proper expression of mitochondrial DNA is essential for the life of the cell and organism. Mutations in mitochondrial DNA or abnormal expression of information encoded therein are the cause of a wide variety of diseases, such as mitochondrial diseases, neurodegenerative diseases, and diseases related to the immune system. Despite many years of research on the functioning of the human mitochondrial genome, many aspects related to its expression (i.e., transferring information encoded in DNA into mRNA and further translating it into a protein sequence) remain unknown.

The aim of the proposed project is to identify factors responsible for the regulation of the level of individual mitochondrial mRNAs (mt-mRNAs) in human cells; in particular, we aim to identify proteins involved in the degradation of these RNA species. We will examine the role of mitochondrial mRNA maturation and translation in the regulation of their steady-state levels, and we will check whether the uridylation of mitochondrial transcripts is linked with their quality control.

We intend to achieve the above goals by carrying out high-throughput screening of the human genome using a siRNA library that enables the silencing of the expression of 6,395 genes. Small interfering RNAs (siRNA) will be transfected into human cells in 384-well plate format, and then, using fluorescence microscopy, we will check how the lack of individual proteins affects the level of mitochondrial mRNA and its degradation. The function of several identified proteins will be carefully examined. Simultaneously, we will carry out studies involving the profiling of mitochondrial ribosomes, which will allow to examine to what extent mitochondrial RNA maturation (excision of mRNA from primary transcripts, 3' end processing) and its translation contribute to the regulation of the level of mature mitochondrial mRNAs.

The implementation of the proposed project will provide a lot of valuable scientific information. Identification of the protein factors involved in the regulation of mt-mRNA levels and degradation will fill the gap in our knowledge about the regulation of the expression of genetic information present in the mitochondrial DNA. In addition, the proposed research will allow to determine the role of mitochondrial RNA translation and processing in the control of mt-mRNA levels. We will also find out whether uridylation of mtRNA is involved in its quality control. As the proper functioning of the mitochondrial genome is crucial to human health, our studies may reveal genes with potential diagnostic significance.