

Abstract

Mitochondria are semi-autonomous organelles in eukaryotic cells whose main function is to produce energy through cellular respiration. They have their own genome and the molecular machinery that enables replication, transcription and translation. However, mitochondrial DNA encodes only for a few proteins, while the majority are encoded in the nucleus, translated in the cytoplasm and imported into mitochondria. To date, one mitochondrial protein transport pathway has been described, which involves the recognition of mitochondrial localization signal in proteins and directing them to the appropriate mitochondrial compartments by a series of membrane transporters. Recent studies have shown that some proteins undergo translation on the mitochondrial membrane, which facilitates their translocation to the mitochondria. This process most likely occurs via the localization signal, which interacts with the transport machinery. However, many mitochondrial proteins do not have the localization signal and their mode of transport is largely unknown. We have recently shown that in baker's yeast, non-canonical translation produces additional extended protein variants, a significant number of which gain localization signal and are directed to the mitochondria. In addition, our unpublished data strongly suggest that mitochondrial localization of some proteins lacking the localization signal requires a short peptide that is synthesized from the same mRNA upstream of the major protein. These results form the basis of this research project whose main goal is to discover and characterize a new pathway for protein transport into the mitochondria. Our hypothesis is that this pathway uses an additional localization signal that is located either in the extension of the non-canonical protein variant or in the sequence of the short peptide. To achieve this goal, we intend to use a number of modern methods, both bioinformatic and genetic, molecular and biochemical, including high-throughput techniques using next-generation sequencing. First of all, we plan to identify potential substrates of the new transport pathway to estimate its universal character among different organisms. Next, we are going to determine the exact mechanism of this pathway, factors involved in its regulation and circumstances in which it is utilized. We also want to understand the importance of this unconventional transport strategy for mitochondrial functions. These experiments will be carried out not only using yeast as a model system, but also in human cell lines to examine whether the mechanism under investigation is preserved in higher organisms. This comprehensive approach will enable extensive characterization of the alternative targeting mechanism of mitochondrial proteins and will reveal the scope of its activity in eukaryotic cells.