Detection of cytoplasmic proteins translated by mitochondrial ribosomes

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Abstract

Background: The mitochondria has its own genome that is translated by its own unique translation machinery. This translation machinery is highly specialized to translate the mitochondrial genome, and has its own intriguing properties, especially, its ability to translate polycistronic mRNAs. This research is an attempt to find if the mitochondrial translation machinery is responsible for the translation of other poly-peptides that are encoded outside the mitochondrial genome.

Methods: The design of this research was dependent on the selective inhibition of the cytoplasmic ribosomes activity in primary hepatocytes, by using Cycloheximide, followed by labeling of mitochondrially synthesized proteins, using Puromycin. These labeled proteins are to be detected by the use of immunoblotting using specific antibodies for Puromycin.

Results: The resulted blots had a lot of noise, and the uptake of puromycin by the cells seems to be minimum, rendering the use of the designed method not to be optimum, especially when considering an unaccounted for interaction between cycloheximide and puromycin.

Conclusion: The used method is not suitable for such investigation, and the use of Mass spectrometric analysis, accompanied with the use of a label other than puromycin is required for an optimum detection of the proteins of interest.