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Regulation of Mitochondrial Gene Expression in Metazoa

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ABSTRACT

Mitochondria are essential organelles of eukaryotic cells and their main function is to provide the cell with the ubiquitously used energy currency ATP. Impaired energy conversion caused by mitochondrial dysfunction is a direct cause of several human diseases. Mitochondria evolved from eubacteria-like endosymbionts and they contain their own genome as well as their own machinery for gene expression. Nevertheless, most mitochondrial proteins are nuclear encoded and mitochondrial energy conversion depends on the coordinated expression of the nuclear and the mitochondrial genome. Mitochondrial gene expression is regulated at different levels, including transcription, RNA maturation, RNA stability and translation. However, it is still largely unknown how these processes are coordinated and the involved molecular mechanisms that regulate mitochondrial gene expression are incompletely understood. In this thesis, the role of two important factors, the leucine-rich-pentatricopeptide repeat containing protein (LRPPRC) and the mitochondrial transcription termination factor 3 (MTERF3), in regulation of mitochondrial gene expression were investigated in the fruit fly *Drosophila melanogaster* and in the mouse. RNAi-mediated knockdown was used to study the *in vivo* function of the *Drosophila* LRPPRC homologue called bicoid stability factor (BSF). The results demonstrated that BSF is an essential mitochondrial protein involved in the control of mRNA stability and polyadenylation. In addition, BSF also functions as a coordinator of mitochondrial translation because the lack of BSF resulted in misregulation of mitochondrial translation. While *de novo* translation of some mitochondrial proteins was not changed, the synthesis of others was increased and a specific subset of mitochondrial polypeptides showed increased degradation in BSF mutant flies. To test whether BSF function is specific to flies or conserved among metazoans, we analyzed LRPPRC function in mammals using whole-body and tissue-specific *Lrpprc* knockout mice. Whole body knockout of *Lrpprc* resulted in embryonic lethality, demonstrating that LRPPRC is essential for mouse development. Heart-specific ablation of *Lrpprc* caused severe respiratory chain dysfunction, cardiomyopathy and premature death at 16 weeks of age. On the molecular level, we found that LRPPRC is involved in mitochondrial mRNA maturation and stability. Furthermore, we showed that LRPPRC together with the RNA mitochondrial stem-loop-interacting protein (SLIRP) forms an RNA-dependent complex that stabilizes an extra-ribosomal pool of non-translated mRNAs. The LRPPRC-SLIRP complex is important for coordinating mRNA binding to the active ribosome. Transcription termination is the second molecular checkpoint in the regulation of mitochondrial gene expression. MTERF3 has been suggested to act as a repressor of mitochondrial transcription in mammals. To determine the underlying mechanisms by which MTERF3 represses mitochondrial transcription and to investigate whether its function is evolutionary conserved between flies and mammals, we generated MTERF3 knockout flies and performed MTERF3 RNAi knockdown studies. Lack of MTERF3 in *Drosophila* caused severe respiratory chain dysfunction and lethality at the pupal stage. At the molecular level, MTERF3 deficiency caused increased initiation of mitochondrial transcription, suggesting that the role of MTERF3 as a repressor of mitochondrial transcription is evolutionarily conserved between flies and mice. By analyzing mitochondrial translation during different developmental stages, we identified a novel function for MTERF3 in ribosomal biogenesis in *Drosophila*. Lack of MTERF3 caused aberrant ribosomal biogenesis, due to an impaired assembly of the large ribosomal subunit. Interestingly, this function of MTERF3 in the control of ribosomal biogenesis is evolutionarily conserved in mice, validating the fly as a useful model organism to study mitochondrial gene expression.

Keywords: *Drosophila melanogaster*, *Mus musculus*, mitochondrial gene expression, mitochondria, transcription, RNA maturation, translation, human diseases

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