Regulators of mitochondrial translation in *Saccharomyces cerevisiae*

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Summary

The work presented in this thesis aims to shed more light on the biogenesis of yeast mitochondria. As in all other eukaryotes, yeast mitochondria are important cell organelles. Most of the energy required for all cellular processes is derived from reactions taking place in the mitochondrion and key enzymes for major metabolic routes are localized in this organelle. The inability to assemble a functional mitochondrion underlies a wide spectrum of degenerative diseases in man. So, a better understanding of mitochondrial assembly in yeast might provide us with valuable information about mitochondrial dysfunction in man and other organisms.

For several reasons the yeast *Saccharomyces cerevisiae* is a highly attractive experimental organism to study mitochondrial assembly. Yeast cultures are easily grown and handled under laboratory conditions, and the organism lends itself to classical genetics, transformation with foreign DNA and gene-replacement techniques. Mutations that affect the mitochondrial respiratory function are not lethal, since this organism can still grow on fermentable carbon sources. Therefore, a large number of genes that are involved in the expression of mitochondrial genes could be identified by genetic means. Additionally, the identification of novel mitochondrial proteins and the genome-wide study of changes in gene expression is greatly facilitated by availability of the full genomic sequence data.

The majority of proteins that are needed to assemble functional mitochondria are encoded by nuclear genes and imported into the mitochondria. The mitochondrial genome of *S. cerevisiae* encodes seven protein subunits of the inner membrane, involved in respiration and oxidative phosphorylation. Except Var1p, all mitochondrially encoded proteins are hydrophobic proteins that assemble into the respiratory complexes of the inner membrane. Mitochondrial protein synthesis differs from cytosolic protein synthesis in a number of aspects. Most important characteristic of translation in mitochondria is the large number of factors that seem to be involved in translation initiation of specific mitochondrial mRNAs. For four of the eight major mRNAs one or more nuclear-encoded gene-specific translational activators have been identified. The Introduction of this thesis (Chapter I) describes the specific characteristics of mitochondrial protein synthesis and summarizes the current knowledge on mRNA-specific translational activators in yeast mitochondria. In this chapter a generally accepted model is introduced that places translation of mitochondrial mRNAs at the inner membrane to facilitate co-translational insertion of the newly synthesized proteins.

In this model the mRNA-specific translational activators function as proteins that dock the mRNAs at the inner membrane, however RNA-binding has not yet been demonstrated directly. In contrast, a search for RNA-binding proteins identified NAD\(^+\)-dependent isocitrate dehydrogenase (Idh) as an RNA-binding protein. Disruption of both *IDH* genes led to a strong increase in mitochondrial translation activity, while the newly synthesized products are also more rapidly degraded. From this observation, it was suggested that binding of Idh to mitochondrial mRNAs may suppress inappropriate translation of mitochondrial mRNAs. Chapter II describes further characterization of the RNA-binding activity of Idh by determination of the affinity for RNA and mutagenesis of the RNA-binding site.

In chapter III, we show that the product of the nuclear gene *MSS51* is required to initiate translation of *COX1* mRNA. The Mss51 protein was shown to be firmly associated with the
mitochondrial inner membrane, indicating that translation of COX1 mRNA takes place at the inner membrane analogous to the other mitochondrial mRNAs.

Synthesis of Cox1p is also dependent on Pet309p, the product of the nuclear PET309 gene. Pet309p is an integral inner membrane protein that might interact with the 5' UTR of the COX1 mRNA. In chapter IV, evidence is shown that Mss51p and Pet309p act at distinct levels in the translation of COX1 mRNA. Different genetic and biochemical approaches show that these two translational activators do not physically interact.

Chapter V describes an extensive functional analysis of Mss51p. Mutagenesis of the MSS51 gene (deletion and point mutations) was performed to identify functional domains or amino acids in Mss51p. By isolation of mitoribosomes from a strain expressing HA-tagged Mss51p a possible interaction of Mss51p with the mitoribosome could be shown.

The final chapter of this thesis (chapter VI) summarizes the experimental data from chapters III to V and based on these data two models are presented that speculate on the function of Mss51p. This chapter also discusses the evolutionary conservation of mRNA-specific translational activators.