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## Understanding mitochondrial biogenesis through gene relocation

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### Abstract

The yeast mitochondrial genome encodes seven hydrophobic subunits of oxidative phosphorylation enzymes and Var1p, an essential protein in the small ribosomal subunit. Expression of the membrane proteins is dependent on nuclear, mRNA-specific regulatory genes, several of which specify translational activators that recognize sites within the 5' untranslated leaders (UTLs) of their target mRNAs. At the onset of this work, it was not known if the expression of the Var1p also requires mRNA-specific regulatory genes. To investigate this and other aspects of Var1p synthesis and function, I developed a novel system in which Var1p is supplied from a recoded gene in the nucleus (VAR1<sub>u</sub>) and the VAR1 coding sequence in mtDNA is replaced by a recoded nuclear gene for an arginine biosynthetic enzyme (Arg8p), thus creating a reporter gene designated  $\text{ARG}_{\text{m}}^{\text{1V}}$ . This system has been used to address the following objectives: (1) Genetic screens were conducted to identify nuclear mutants defective in  $\text{ARG}_{\text{m}}^{\text{1V}}$  expression. One such gene, SOV1, was identified and cloned. SOV1 is specifically required for the stable accumulation of VAR1 mRNA. (2) To determine whether the targeting information for mitochondrial membrane proteins is contained in UTLs of their mRNAs, I examined the ability of chimeric mRNAs containing the VAR1 UTL to direct expression of COX2 and COX3. Although cells expressing these chimeric mRNAs synthesized both proteins, they were deficient in the accumulation of Cox2p and Cox3p. These data suggest that translation of Var1p is different from that of the membrane proteins, and support the physiological importance of interactions between the translational activators and the 5' UTLs of the COX2 and COX3 mRNAs for localizing synthesis of hydrophobic proteins to the inner membrane. (3) Heretofore, it has not been possible to produce respiratory

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competent  $\rho$  haploids by mating two  $\rho$  haploids. An explanation for this lack of functional complementation is that  $\rho$  cells are devoid of small ribosomal subunits and translation cannot be restored without a source of Var1p. I have shown that respiratory competent diploids can be obtained in crosses between two complementing  $\rho$  strains, but only when Var1p is supplied from  $\rho$ .

## Subject Area

Biology, Molecular|Biology, Genetics

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