Synthesis of Cox1 in yeast mitochondria is highly regulated. Pet309 and Mss51 are translational activators of the mitochondrial COX1 mRNA. Newly made Cox1 interacts with Mss51 to form high-molecular weight complexes (COA complexes) with proteins like Cox14 and Coa3. If assembly of the cytochrome c oxidase is blocked by mutations on structural subunits or assembly factors, then synthesis of Cox1 is dramatically reduced. In this context, the C-terminal end of Cox1, Cox14 and Coa3 are involved in stabilizing the interaction of Mss51 with the COA complexes, making it unavailable for more rounds of translational activation of the COX1 mRNA [1, 2]. Pet54 was first described as translational activator of the mitochondrial COX3 mRNA [3], and later was found to be necessary to splice an intron on the COX1 transcript [4]. Our group found that Pet54 has an additional role on Cox1 synthesis that is independent of the two previously described functions. In the absence of Pet54, synthesis of Cox1 decreased, however it did not recover when the Cox1 C-terminal end, Cox14 or Coa3 was deleted. This contrasts with the phenotype observed for the majority of assembly mutants. Mss51 has at least two states: one involved in the assembly of Cox1 and one involved in translational activation of the COX1 mRNA (reviewed in [2]). In contrast to what is usually observed for mutants that block assembly, by blue native gel electrophoresis we observed that in pet54Δ mutants, Mss51 is enriched as the translational activator form. However, our results suggested that Mss51 might not be competent for translation of the COX1 mRNA. In addition we observed that the reduced amount of Cox1 that is present on pet54Δ mutants is highly unstable. We conclude that Pet54 has a positive role on Cox1 synthesis, probably by converting Mss51 to a competent form to activate translation of the COX1 mRNA. In addition, the lack of Pet54 renders Cox1 protein very unstable. Together these results indicate that Pet54 is a multifunctional protein with an additional role on Cox1 biogenesis.

References

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S5.P4

Pet54 is a positive regulator of Cox1 synthesis in yeast mitochondria
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The mitochondrial complexes of the electron transport chain associate into large macromolecular assemblies called supercomplexes (SC), which are believed to facilitate efficient electron flow. To gain better insight into the assembly of SC, mitochondria were isolated from 143B cells and mutant cybrids lacking complex IV, and subsequently analyzed by blue-native polyacrylamide gel electrophoresis (BN-PAGE). The composition of the bands corresponding to SC was determined by using a high-throughput proteomics-based approach. This analysis identified, among others, a new OXPHOS-related protein with unknown function, which we termed Supercomplex Protein 2 (SCP2). Western-blot analyses in 2D-BN/SDS-PAGE gels showed colocalization between the signals corresponding to SCP2 and free complexes III and IV, the intermediate SC (I + II2 + III2 + IV) and the respirasome (or SC I + III2 + IV). Functional RNAi studies showed decreased levels of the SC together with an accumulation of complex I assembly intermediates in both the wild-type and COX mutant cells, without free complexes III and IV being affected by the reduced levels of SCP2. Our results therefore suggest that SCP2 could play a central role in the assembly and physiology of the mitochondrial supercomplexes.

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S5.P5

Proteomics for the identification of new proteins related with the mitochondrial respiratory chain complexes assembly
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The mitochondrial complexes of the electron transport chain associate into large macromolecular assemblies called supercomplexes (SC), which are believed to facilitate efficient electron flow. To gain better insight into the assembly of SC, mitochondria were isolated from 143B cells and mutant cybrids lacking complex IV, and subsequently analyzed by blue-native polyacrylamide gel electrophoresis (BN-PAGE). The composition of the bands corresponding to SC was determined by using a high-throughput proteomics-based approach. This analysis identified, among others, a new OXPHOS-related protein with unknown function, which we termed Supercomplex Protein 2 (SCP2). Western-blot analyses in 2D-BN/SDS-PAGE gels showed colocalization between the signals corresponding to SCP2 and free complexes III and IV, the intermediate SC (I + II2 + III2 + IV) and the respirasome (or SC I + III2 + IV). Functional RNAi studies showed decreased levels of the SC together with an accumulation of complex I assembly intermediates in both the wild-type and COX mutant cells, without free complexes III and IV being affected by the reduced levels of SCP2. Our results therefore suggest that SCP2 could play a central role in the assembly and physiology of the mitochondrial supercomplexes.

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S5.P6

Escherichia coli RIC participates in the formation of iron-sulfur centres through iron donation
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Repair of Iron Centres proteins (RIC) are a newly identified family of diiron four-helix bundle proteins widespread in bacteria, protozoa and fungi, involved in the repair of Fe–S centres [1]. RIC was first noted in the transcriptome of Escherichia coli cells upon exposure to NO due to a...