The Saccharomyces cerevisiae COQ10 Gene Encodes a START Domain Protein Required for Function of Coenzyme Q in Respiration*

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Deletion of the Saccharomyces cerevisiae gene YOL008W, here referred to as COQ10, elicits a respiratory defect as a result of the inability of the mutant to oxidize NADH and succinate. Both activities are restored by exogenous coenzyme Q6. Respiration is also partially rescued by COQ2, COQ7, or COQ8/ABC1, when these genes are present in high copy. Unlike other coq mutants, all of which lack Q6, the coq10 mutant has near normal amounts of Q6 in mitochondria. Coq10p is widely distributed in bacteria and eukaryotes and is homologous to proteins of the “aromatic-rich protein family” Pfam03654 and to members of the START domain family (20), which is found in at least two steps (16, 17). In the present study we report a novel phenotype displayed by mutants with a deletion of reading frame YOL008W, which we have named COQ10. The coq10 mutant is similar to other coq mutants in which optimal oxidation of NADH and succinate by isolated mitochondria depends on addition of coenzyme Q6. Unlike the coq mutants, which lack Q6, the coq10 mutant reported here has nearly normal concentrations of Q6. The product of COQ10 is a hydrophobic protein located in the inner membrane of mitochondria. It is a member of the large Pfam03654 family of proteins (aromatic-rich protein family) (18). The recent NMR structure of a bacterial member of this family (19) has revealed a structural similarity to the START domain family (20), which includes the cholesterol-binding protein StarD4 (21). Based on the presence in Coq10p of this domain and the detection of Q6 in the purified protein, we propose that Coq10p is a Q6-binding protein, which functions in the delivery of Q6 to its proper location for electron transport.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Media—The strains of yeast used in this study are listed in TABLE ONE. Yeast were grown in YPD (2% glucose, 1% yeast extract, 2% peptone), YPGal (2% galactose, 1% yeast extract, 2% peptone), and minimal glucose (2% glucose, 0.67% nitrogen base without amino acids, supplemented with auxotrophic requirements).

Cloning of COQ2, COQ7, COQ8, and COQ10—Recombinant plasmids containing COQ2 (pG10/T1) and COQ7 (pG64/T2) and COQ8 (pG75/T2) were cloned by transformation of W303ΔCOQ10 with a yeast genomic library constructed from nuclear DNA partially digested with a combination of BamHI and BglIII and cloned in the yeast Escherichia coli shuttle plasmid YEp352 (22). Approximately 5 × 106 cells were transformed with 50 μg of the plasmid library by the method of Schiestl and Gietz (23). COQ10 was PCR amplified from yeast nuclear DNA with primers: 5′-caccagaaagcttagataagatg and 5′-ggcagactttcgctgatgctgatg. The 1.3-kb fragment containing the COQ10 reading frame plus 700 bp of 5′ and 50 bp of 3′-untranslated sequence was digested with HindIII and cloned in YEp352.

Disruption of COQ10—The COQ10 gene and ~500 bp of upstream and downstream sequence was amplified with primers: 5′-ggcagactttcctagctagctagatg and 5′-ggcagactttcctagctagctg. This fragment was digested with BamHI and cloned in pUC19 (24). This plasmid
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TABLE ONE

| Genotypes and sources of yeast strains | Strain | Genotype | Source |
|--------------------------------------|--------|----------|--------|
| W303-1A | MATa ade2-1 his3-1,15 leu2-3,112 trp1-1 1 ura3-1 | | |
| W303-1B | MATa ade2-1 his3-1,15 leu2-3,112 trp1-1 1 ura3-1 | | |
| BYΔCOQ10 | MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 coq10-1:KANMX4 | Invitrogen |
| BYΔCOQ10/R1 | MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 coq10-1:KANMX4 sup6Δ1101 | This study |
| W303ΔCOQ10 | MATa ade2-1 his3-1,15 leu2-3,112 trp1-1 1 ura3-1 coq10-1:HIS3 | This study |
| aW303ΔCOQ10 | MATa ade2-1 his3-1,15 leu2-3,112 trp1-1 1 ura3-1 coq10-1:HIS3 | This study |
| aW303ΔCOQ5 | MATa ade2-1 his3-1,15 leu2-3,112 trp1-1 1 ura3-1 coq5::HIS3 | Ref. 8 |
| aW303ΔCOQ9 | MATa ade2-1 his3-1,15 leu2-3,112 trp1-1 1 ura3-1 coq9::LIRA3 | Ref. 4 |

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was used to delete the COQ10 coding sequence with the two bidirectional primers 5’-ggcagatctcaggtatctttgccg and 5’-ggcagatctcgcggtaatccgataacaaagatc. The PCR product consisting of linear pUC19 plus the COQ10 flanking sequences was digested with BglII and ligated to a 1-kb BamHI fragment containing the yeast HIS3 gene (this fragment lacked the downstream PET56 gene). The linear BamHI fragment with the coq10::HIS3 allele was isolated from this plasmid and substituted for the wild type in W303-1A and W303-1B by the one-step gene replacement method (25).

Construction of Hybrid COQ10 Genes Expressing Coq10p with a Carboxyl-terminal Polyhistidine Extension and of a trpE Fusion Protein for Coq10p Antibody Production—The two PCR primers 5’-ggcagatctcaggtatctttgccg and 5’-ggcagatctcgcggtaatccgataacaaagatc were used to amplify COQ10 with a 18-nucleotide insertion between the last codon and the termination codon. The PCR amplified product was digested with a combination of BglII and HindIII and cloned into the Image clone 5299314 serving as the template. The 627-bp product was digested with a combination of SacI and BamHI and cloned into pMGL4, a plasmid. The insoluble protein was dissociated in SDS and was partially purified on a Bio-Gel A-0.5 column (26) and used to obtain a polyclonal antibody in rabbits.

Purification of the antibody was performed as described previously (5). Protein concentrations were determined by the method of Lowry et al. (29).

RESULTS

BYΔCOQ10 and W303ΔCOQ10 Phenotype—BYΔCOQ10 is a respiratory deficient mutant of the Genome Deletion Strain Collection. It was made by replacing reading frame YOL008W/COQ10 with the kanamycin-resistance cassette in strain BY4741. aW303ΔCOQ10 and W303ΔCOQ10 were obtained by replacing YOL008W with HIS3 in the wild type haploid W303-1A and W303-1B, respectively (see "Experimental Procedures"). The respiratory defect of both mutants is complemented by crosses to a 4’ tester indicating that null mutations in this gene are recessive. In rich glucose medium, an early stationary culture of BYΔCOQ10 and the other coq mutants to accumulate large deleterious structures. The generation time of W303ΔCOQ10 is reduced to 8.5 h in media supplemented with Q6 (TABLE TWO). Addition of Q6 to growth medium has been shown to promote growth of coq mutants on non-fermentable carbon sources (12). This is confirmed by the partial rescue of the coq5 and coq9 mutants in media supplemented with Q6. The generation time of aW303ΔCOQ10 is reduced to 8.5 h in media supplemented with Q6 (TABLE TWO).

Spectra of W303ΔCOQ10 mitochondria show decreased concentrations of "a" and "b" type cytochromes (Fig. 1A). Some of the decrease, however, is because of the large proportion of p5'-mutants in the culture. The tendency of nuclear pet mutants to accumulate large deletions in mitochondrial DNA is common when the mutations are in genes coding for components of the mitochondrial translation machinery (32). In vivo assays of mitochondrial protein synthesis in the coq10 null mutant revealed a normal pattern of mitochondrial translation...
products indicating that the excessive loss of the wild type mitochondrial genome was not caused by a defect in this translation system (Fig. 1B). The somewhat lower incorporation of [35S]methionine in the mutant is consistent with the percentage of \(^{35}S\) cells in the cultures used for the assays.

The reductions of cytochromes \(a, a_2, \) and \(b\) in the \(coq10\) null mutant is also evident in the lower NADH-cytochrome \(c\) reductase and cytochrome oxidase activities of mutant mitochondria (TABLE THREE). In both mutants examined, the NADH-cytochrome \(c\) reductase activity was \(\sim 10\%\) of that measured in wild type even though cytochrome oxidase was reduced by only \(40\%\). The lesion, therefore, was likely to be in a component of the respiratory chain preceding cytochrome oxidase. This activity was confirmed by the observation that the NADH-cytochrome \(c\) reductase activity of the \(coq10\) mutants could be restored to \(\sim 50\%\) of the wild type by addition of exogenous coenzyme \(Q_6\) to the assay (TABLE THREE). The \(Q_6\) dependent activity is commensurate with complete restoration of NADH-cytochrome \(c\) reductase because half of the culture used for the preparation of mitochondria consisted of \(^{35}S\) mutants and therefore lacked the \(bc_1\) complex. Coenzyme \(Q_6\) also restored NADH and succinate oxidation in mitochondria of the \(coq10\) mutant (Fig. 2). Measurements of NADH oxidase activity as a function of \(Q_6\) added to the assay indicated that the response of the \(coq10\) mutant was similar to another mutant (\(coq9\)) that lacks \(Q_6\) (Fig. 3).

**The coq10 Mutant Has Almost Normal Concentrations of Coenzyme \(Q_6\) in Mitochondria**—The biochemical phenotype of the \(coq10\) mutant, particularly the requirement of exogenous coenzyme \(Q_6\) for the NADH and succinate oxidase activities, are very similar to the properties of other \(coq\) mutants unable to synthesize this obligatory electron carrier (5). The concentration of \(Q_6\) in the mutant was determined by HPLC separation and quantification of mitochondrial lipid extracts on a reverse phase column with an electrochemical detector set for optimal detection of \(Q_6\). Samples were analyzed against authentic standards of known concentration. The HPLC-ECD elution profile indicated the presence of a redox-active compound with a retention time consistent with that of coenzyme \(Q_6\) (Fig. 4). The identity of this compound as \(Q_6\) was confirmed by mass spectrometry (Fig. 5), which revealed the presence in lipid extracts of the wild type and \(coq10\) null mutant of the correct molecular ion with a mass/charge ratio \((m/z)\) of \(Q_6\) at 591.4. This ion produced characteristic fragment ions including the predominant base peak and tropyllium ion at \(m/z\) 197, a definitive breakdown product of \(Q_6\) (35). The concentration of \(Q_6\) in the mutant was estimated to be \(3.32 \mu g/mg\) of mitochondrial protein (Fig. 3B). The value for the wild type parent was \(3.33 \mu g/mg\) protein.

**Suppressors of coq10 Mutants**—BY\(\Delta\)COQ10 and \(aw303\Delta\)COQ10 give rise to revertants when placed on a selective medium containing ethanol and glycerol as carbon sources. Diploid cells issued from back crosses of \(^{35}S\) derivatives of several revertants to the mutant had the revertant phenotype, indicating that the suppressor mutations were nuclear in origin. The nuclear DNA of a BY\(\Delta\)COQ10 revertant was used to construct a genomic plasmid library in the high-copy plasmid YEp352 (22). Transformation of \(aw303\Delta\)COQ10 with this library yielded a number of respiratory competent clones, which had plasmids containing COQ2, COQ7, or COQ8. Of the three genes the most effective in restoring respiration was COQ8, followed by COQ7, whereas the

![FIGURE 1. Mitochondrial spectra and translation in a coq10 null mutant. A, mitochondria were prepared from the respiratory competent parental strain W303-1B (upper trace) and W303\(\Delta\)COQ10 harboring a deletion in COQ10 (lower trace). The two strains were grown in 2% rich galactose medium (YPGal). Approximately 50% of the W303\(\Delta\)COQ10 culture consisted of secondary \(^{35}S\) mutants. The mitochondria were extracted at a protein concentration of 5 mg/ml in the presence of 1% potassium deoxycholate and 1 mM KCl (5). The difference spectrum of each extract oxidized with potassium ferricyanide and reduced with sodium dithionite was taken at room temperature. The absorption bands corresponding to cytochromes \(c, c_1, b, a, a_2, \) and \(a_3\) are indicated. B, mitochondrial gene products were labeled in vivo with [\(^{35}S\) methionine (30) and total cellular proteins from equivalent number of cells were separated by SDS-PAGE on a 17% polyacrylamide gel (31).](image-url)

| Strain             | Generation time (hs) | −Q_6 | +Q_6 | Cytochrome oxidase |
|--------------------|----------------------|------|------|-------------------|
| W303-1B            |                      | 3.1  | 3.4  | 15 μM             |
| aW303\(\Delta\)COQ5| >44                  | 14.3 | 10.5 |                   |
| aW303\(\Delta\)COQ9| >44                  | 15   | N.D. |                   |
| aW303\(\Delta\)COQ10| 11.8                | 8.6  | 8.5  |                   |

**TABLE THREE**

| Strain       | Micromoles of cytochrome \(c\) oxidized or reduced per min/mg of protein |
|--------------|-------------------------------------------------------------------------|
|              | NADH-cytochrome \(c\) reductase | +Coenzyme \(Q_6\) | Cytochrome oxidase |
|--------------|--------------------------------|------------------|------------------|
| W303-1B      | 2.27 ± 0.06                   | 2.29 ± 0.01      | 2.45 ± 0.13      |
| W303\(\Delta\)COQ10| 0.26 ± 0.00         | 1.16 ± 0.02      | 1.52 ± 0.01      |
| BY\(\Delta\)COQ10| 0.22 ± 0.02         | 1.36 ± 0.01      | 1.46 ± 0.02      |
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least effective was COQ2 (Fig. 6). The NADH oxidase activity measured in isolated mitochondria was also highest in the clone with the COQ8 (80% of wild type) and lowest in the clone with the COQ2-bearing plasmid (11% of wild type) (not shown).
Transformants harboring the different suppressors either on a multicopy plasmid or integrated into nuclear DNA did not grow on the non-fermentable carbon sources as well as the revertant (Fig. 6). This suggested that the three COQ genes did not have the suppressor mutation conferring respiratory competence to the revertant. Growth of the mutant on non-fermentable substrates was substantially improved when COQ2, COQ7, and COQ8 were introduced on a high-copy episomal plasmid rather than integrated into chromosomal DNA in single copy, suggesting that the rescue is because of a higher gene dosage allowing for more efficient synthesis of Q6 (Fig. 6). This is supported by the increased Q6 content (150–220% of wild type; Fig. 4) in mitochondria of the transformant with COQ8 on the high-copy plasmid. This strain also exhibited a greater stability of mitochondrial DNA, as stationary cultures of the coq10 null mutant transformed with COQ8 had very few secondary rho−/− clones.

**Complementation of the Yeast coq10 Mutant by a Human COQ10 Homologue**—A search of the current protein data base indicates that Coq10p homologues exist in diverse phylogenetic groups spanning bacteria and humans (Fig. 7A). Coq10p is a member of the large START superfamily (36) that is composed of polyketide cyclases/lipid transport proteins, aromatases, RNases, and other proteins of unknown function such as members of the ARPF/Pfam03654 family (“aromatic-rich protein family”). START proteins are defined as having a structurally similar, although not necessarily homologous, ligand binding pocket of the helix-grip type (36), formed from seven anti-parallel β strands apposed to a C-terminal helix. Many START proteins bind lipophilic compounds and some have been shown to be involved in lipid and sterol transport and delivery to membranes (37, 38).

To confirm that the human gene codes for a functional analogue of yeast Coq10p, the human cDNA was cloned in a yeast multicopy plasmid and was tested for complementation of the yeast coq10 null mutant. The functional identity of the yeast and human COQ10 (hCOQ10) was confirmed by rescue of growth of the yeast mutant on glycerol/ethanol by human cDNA (Fig. 7B).

**FIGURE 6.** Suppression of aW303ΔCOQ10 by COQ2, COQ7, and COQ8. Serial dilutions of the wild type W303-1A, the coq10 null mutant aW303ΔCOQ10, the revertant aW303ΔCOQ10/R1, and of the null mutant transformed with the COQ genes either in a multicopy plasmid (+COQ2, +COQ7, and +COQ8) or integrated into nuclear DNA (+ICOQ2, +ICOQ7, and +ICOQ8) were spotted on rich glucose medium (YPD) and on rich medium containing glycerol plus ethanol as carbon sources (YPEG). The photograph was taken after the plates had been incubated at 30 °C for 2 days.

**FIGURE 7.** Alignment of putative Coq10p homologues and complementation of the yeast coq10 mutant by the human COQ10 cDNA. A, the C. crescentus CC1736 protein is a member of the Pfam03654 family. Identical residues are highlighted in black. The six residues in CC1736 inferred from structural considerations to be important for ligand binding (19) are marked by asterisks. B, serial dilutions of the wild type W303-1A, the coq10 null mutant aW303ΔCOQ10, and the mutant transformed with the human COQ10 cDNA (hCOQ10) were spotted on rich glucose (YPD) and rich ethanol/glycerol (YPEG) plates and incubated at 30 °C for 48 h.

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Localization and Sizing of Coq10p—A polyclonal antibody against Coq10p was used to detect the protein in yeast. This antibody recognized a protein of ~23–25 kDa in wild type mitochondria. This protein was absent in the coq10 null mutant and was restituted in the mutant containing a copy of COQ10 integrated at the leu2 locus (Fig. 8A), thereby confirming the identity of the protein as Coq10p. Coq10p with a carboxyl-terminal polyhistidine tag was purified from yeast mitochondria (see below). The sequence of this protein starts with the phenylalanine at residue 31, indicating that the amino-terminal 30 residues of the primary translation product constitute a mitochondrial targeting sequence that is cleaved during transport.

No Coq10p was detected in the post-mitochondrial supernatant fractions consisting of cytosolic proteins (data not shown). Coq10p is a hydrophobic protein that is recovered in the membrane fraction following disruption of mitochondria by sonic irradiation (Fig. 8B), or by treatment with 0.2 M sodium carbonate (Fig. 8C) but is solubilized by dodecyl maltoside suggesting that it is an integral membrane protein (not shown). This is also supported by its hydrophobicity profile, which indicates at least four putative transmembrane domains.

Coq10p is an inner membrane protein facing the matrix compartment. This is evident from its lack of sensitivity to proteinase K in mitochondria and in mitoplasts, the latter lacking an intact outer membrane (Fig. 8D). Conversion of mitochondria to mitoplasts by hypotonic shock was evident from the substantial loss of the soluble intermembrane marker cytochrome b5. This is also supported by the decrease of Sco1p as a result of the proteinase K treatment of mitoplasts but not mitochondria. Sco1p is an inner membrane protein facing the intermembrane space (40). The matrix protein α-keto glutarate dehydrogenase was protected against proteinase K in mitochondria and mitoplasts confirming the intactness of the inner membrane in the mitoplasts.

Sucrose gradient sedimentation indicates that native Coq10p is considerably larger than the monomer. When extracted from mitochondria with 0.5% dodecyl maltoside the protein sediments slightly slower than lactate dehydrogenase, which has a molecular weight of 140,000 (Fig. 9A). At present we have no data to indicate whether Coq10p is a homodimer or hetero-oligomer. Coq10p does not co-sediment with either Coq3p or Coq5p but does overlap with Coq4p, which is broadly distributed indicating heterogeneity in size.

Steady-state Levels of Coenzyme Q Biosynthetic Enzymes in the coq10 Mutant—Mutations that block coenzyme Q biosynthesis lower the steady-state concentrations of a number of enzymes of this pathway (15, 41). To determine whether these proteins are also reduced in the coq10 mutant, total mitochondrial proteins were separated by SDS-PAGE, on a 12% polyacrylamide gel, and transferred to nitrocellulose. The Western blot was reacted with a rabbit polyclonal antibody against Coq10p. Following a secondary reaction with peroxidase-conjugated anti-rabbit IgG (Sigma), proteins were visualized with the SuperSignal chemiluminescent substrate kit (Pierce). B, mitochondria at a protein concentration of 10 mg/ml were sonically irradiated for 5 s with a Branson microtip sonic probe. The sample was centrifuged at 100,000 × g for 30 min and the clear part of the supernatant was collected. The packed part of the pellet was resuspended in the starting volume of 0.6 M sorbitol, 10 mM Hepes, pH 7.5. An intermediate oily layer consisting of very small membrane fragments was discarded. The mitochondria (Mit) representing 40 μg of protein and equivalent volumes of the supernatant (Sup) and pellet (SMP) were separated on a 12% polyacrylamide gel and processed as in A. C, wild type mitochondria were converted to submitochondrial particles as in B. The submitochondrial particles at a protein concentration of 10 mg/ml were mixed with an equal volume of 0.2 M sodium carbonate and incubated on ice for 30 min. The extracted membranes were separated from the solubilized proteins by centrifugation at 100,000 × g for 20 min. The mitochondrial (Mit) and equivalent volumes of the extracted membranes (C-pellet), and carbonate soluble proteins (C-sup) were separated on a 12% polyacrylamide gel. D, wild type mitochondria (Mit) were converted to mitoplasts (Mpl) by the method of Glick (39). Both mitochondria and mitoplasts were incubated on ice for 1 h in the absence or presence of 0.1 mg/ml proteinase K. Following addition of phenylmethylsulfonyl fluoride mitochondria were isolated by centrifugation for 10 min at 14,000 × g. Equivat amounts (40 μg) of mitochondria and mitoplasts were separated by SDS-PAGE and further processed as in panel A.

Steady-state Levels of Coenzyme Q Biosynthetic Enzymes in the coq10 Mutant—Mutations that block coenzyme Q biosynthesis lower the steady-state concentrations of a number of enzymes of this pathway (15, 41). To determine whether these proteins are also reduced in the coq10 mutant, total mitochondrial proteins were separated by SDS-PAGE, and Western blots were probed with antibodies against Coq3p, Coq4p, and Coq5p. The results indicated that the coq10 mutant has signifi-
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A

B

C

D

FIGURE 10. Purification of Coq10p-his and analysis of its Q6 content. A, extraction and affinity purification of Coq10p-his on Ni-NTA. Mitochondria from aW303ΔCOQ10/ST13 grown on rich 2% galactose medium (YPGal) were sonically irradiated for 30 s with a Branson sonifier. The submitochondrial particles obtained by centrifugation of the disrupted mitochondria at 100,000 g for 30 min were suspended in 0.6 M sorbitol, 20 mM Tris-HCl, pH 7.5, 0.5 mM EDTA and adjusted to 1% dodecyl maltoside and 1 M NaCl at a final protein concentration of 10 mg/ml. Most of the protein was adsorbed on Ni-NTA beads (Qiagen) at a ratio of 0.2 ml of beads per 10-ml extract, and mixed by rotation for 2 h at 4 °C. The slurry was poured into a column and the unadsorbed proteins followed by 5 column volumes of 10 mM imidazole was collected as a single fraction (flow-tru) by gravity flow. The beads were then washed with 5 volumes of 30 mM imidazole followed by 50 mM imidazole in 50 mM NaCl, 10 mM Tris-HCl, pH 7.5. The bulk of the protein was eluted in a final wash with 3 column volumes of 250 mM imidazole in 50 mM NaCl, 10 mM Tris-HCl, pH 7.5. This fraction was diluted with an equal volume of 10 mM Tris-HCl, pH 7.5, and applied to a 1-ml Pharmacia Mono S column. The column was developed sequentially with 10 ml of 50 mM NaCl, 5 ml of 300 mM NaCl, 1 ml NaCl in 10 mM Tris-HCl, pH 7.5. Most of the eluted protein was clarified as a trailing peak in the 300 mM NaCl fraction. Mock column fractions were obtained by replicating the Mono S column purification without protein. The submitochondrial particles (SMP), dodecyl maltoside (LM) extract, and the different imidazole washes of the Ni-NTA column were separated by SDS-PAGE and the Western blot was probed with the antibody against Coq10p as in the legend to Fig. 8A. Each fraction was adjusted for volume except that three times as much of the 250 mM imidazole eluate was applied to the gel. B, silver-stained gel of Coq10p-his eluted in the 300 mM NaCl fraction from the Mono S column. The two different preparations of the protein used for the Q6 analyses are shown. The faster migrating band seen in the gel of preparation 1 is detected by the Coq10p antibody and is probably a proteolytic product. C, the indicated amounts of purified Coq10p-his and mitochondrial proteins were separated by SDS-PAGE on a 12% polyacrylamide gel. The Western blot was processed as described in the legend to Fig. 8A. D, three separate lipid extractions and quantitative mass spectrometry measurements were performed on the lipid extract obtained from two preparations of Coq10p-his shown in B, mock column fractions and calibration curve samples. All extractions contained Q6 as an internal standard, and the amounts of Q6 standards ranged from 6 fmol/μl to 2 pmol/μl. The spectra shown are the relative intensities of the molecular ion for Q6 [M + H]+ = 591.4 and the internal standard Q4 [M + H]+ = 455.2 present in the lipids from the Coq10p-his fraction versus the mock column (offset trace) as obtained by parent ion scanning. Any compound detected that can produce the predominant tropylum fragment was demonstrated over the mass range 400–600 m/z. The inset spectra obtained from the MS/MS product analysis of the lipids from the Coq10p-his fraction show the familiar fragments of Q6 confirmed by direct comparison with authentic Q6 (Fig. 5.). See “Experimental Procedures” and Ref. 42 for details.

DISCUSSION

Deletion of reading frame YOL008W/COQ10 of S. cerevisiae chromosome XV confers a respiration defect. Here we show that mitochondrial NADH and succinate oxidase activities are decreased in coq10 mutants but that both activities are reconstituted by addition of Q6 to the assay. Q6-dependent respiration is a hallmark of mutants blocked in Q6 synthesis (5). Unlike other coq mutants, which are deficient in Q6, the coq10 null mutant reported in this study contains almost cantly reduced concentrations of Coq4p but not of Coq5p (Fig. 9B). Coq3p was also reduced in the mutant but not to the same degree as Coq4p. This pattern is very similar to that observed in mutants from the eight complementation groups previously shown to be Q6 deficient (15). In the transformant harboring COQ8 on a high copy, both Coq3p and Coq4p were restored to levels even higher than those observed in wild type. There was also a significant restoration of Coq3p and Coq4p in the strain in which COQ8 was integrated into the nuclear genome. Transformation of the mutant with COQ2 on a high copy plasmid, however, did not affect the level of either Coq3p or Coq4p (Fig. 9B).

Analysis of Q6 in Purified Coq10p-his—The presence of a START domain in Coq10p made it of interest to determine whether it is a Q6-binding protein. Coq10p was purified from mitochondria of a coq10 mutant transformed with a coq10 gene modified so as to express the protein with six histidine residues at the carboxyl terminus. This construct complemented the coq10 mutant when expressed from an episomal multicopy plasmid or integrated in single copy into chromosomal DNA. Complementation by the single copy integrated gene indicates that the presence of the histidine tag does not affect the activity of the protein. The histidine-tagged protein (Coq10p-his) was purified from the COQ10 overexpressing strain aW303ΔCOQ10/ST13 after extraction of mitochondria with 1% dodecyl maltoside in the presence of salt (Fig. 10A). Most of the Coq10p-his in the extract was adsorbed on Ni-NTA and was eluted at high concentration of imidazole. A highly purified preparation of Coq10p-his was obtained by chromatography of the Ni-NTA eluate on a Mono S column (Fig. 10B). A mock run was done with the same Mono S column developed under identical conditions but without prior application of protein. Two independent preparations of the protein (Fig. 10B) and analogous mock column fractions were analyzed for Q6, by three sensitive, quantitative mass spectrometric analyses. A robust Q6 signal was present in column fractions with Coq10p-his as compared with the corresponding fractions from a mock column (Fig. 10D). The amount of Q6 co-purified with Coq10p-his was calculated to be 0.032–0.034 nmol of Q6/mol of Coq10p-his.

A comparison of the Western signals of Coq10p in mitochondria to known amounts of purified Coq10p-his indicated that there is ~0.1 μg or 5 pmol of Coq10p/mg of mitochondrial protein (Fig. 10C). This value is some 3 orders of magnitude lower than the mitochondrial concentration of Q6. In aW303ΔCOQ10/ST13 the comparable value for Coq10p-his was 0.5 nmol/mg of mitochondrial protein. This corresponds to a 100-fold increase when the protein is expressed from the high copy plasmid (not shown).
normal concentrations of Q6 in mitochondria. This excludes Coq10p from being required for Q6 biosynthesis directly. Coq10p is a membrane protein located in the inner membrane. The 30 amino-terminal residues of the protein encoded by COQ10 are absent in the mature protein. This sequence is probably a mitochondrial targeting signal that is cleaved during transport. Coq10p is resistant to proteinase K in mitoplasts suggesting that it faces the matrix compartment. This localization is similar to all the other Coq polypeptides that function in Q6 biosynthesis in yeast (9–11, 15). Other features of coq mutants are also shared by the coq10 mutant. Coq3p (6), Coq4p (9), and Coq5p (8) have previously been shown to be required for Q6 synthesis. Coq3p and Coq5p function, respectively, as O- and C-methyltransf erases (see Ref. 11 for the biosynthetic pathway). Western analysis of these proteins indicates a large decrease of Coq4p and some reduction of Coq3p but not Coq5p in the coq10 mutant. This pattern is very similar to what has been reported for mutants in the eight coq genes (15). The pleiotropic effects of mutations in a single coq gene on the steady-state concentrations of other coq gene products provide genetic evidence that stability of these proteins may depend either on their association into complexes or on the presence of one or more biosynthetic intermediates of the pathway (15). Recent evidence suggests that Coq4p may be a structural component of a Q-biosynthetic polypeptide complex (42).

Results presented here suggest that Coq10p exists as a homo-oligomer or is complexed with other proteins. This is supported by the five times larger size of native Coq10p than the monomer. Coq10p sediments differently than Coq3p or Coq5p on sucrose gradients. Coq4p is broadly distributed in the gradient suggestive of several sedimenting species. The peak of Coq10p does coincide with that of Coq4p. Whether this is coincidental or indicative of an association of the two proteins cannot be concluded at present. The presence of normal amounts of Q6, despite the large reduction of Coq4p suggests that the mitochondrial concentration of the latter protein is substantially greater than what is needed for Q6 biosynthesis. COQ2, COQ7, and COQ8 are high copy suppressors of the coq10 mutant. COQ8, when present on a multicopy plasmid, is the most effective of the three. The efficiency of suppression, judged by growth on glycerol/ethanol, correlates with an increase in the mitochondrial concentrations of Coq4p and Coq3p and a greater stability of mitochondrial DNA. Overexpression of COQ8 also caused a 150–220% increase in the mitochondrial concentration of Q6. These observations indicate that the absence of Coq10p can be compensated to some extent by higher than normal concentrations of Q6, in mitochondria. When adjusted for the 50% of $p^{15} / p^{13}$ cells in cultures of the mutant, the NADH and succinate oxidation rates in the mutant correspond to 10% of wild type. This indicates that the absence of Coq10p does not totally abolish the ability of the mitochondria to oxidize and reduce endogenous Q6, and helps to explain the observed partial rescue of the mutant phenotype by supplement ation of growth media with Q6, or by the COQ8-induced higher mitochondrial concentration of Q6.

In addition to the three suppressors identified from transformations with the plasmid library, we also tested COQ1, COQ5, and COQ6. These genes did not improve growth of the coq10 mutant on glycerol/ethanol, suggesting that the encoded enzymes are not rate-limiting in Q6 biosynthesis. Coq10p is homologous to members of the Pfam03654 protein family, including the 18-kDa Caulobacter crescentus protein, C1736, whose solution structure was recently reported (19). This bacterial protein of unknown function has a structure similar to the mouse StarD4 cholesterol transport protein (21). The latter has a hydrophobic tunnel capable of accommodating one cholesterol molecule. A comparison of the CC1736 and the StarD4 structures has helped to identify amino acid residues likely to confer ligand specificity to the binding pocket (19, 21). It is striking that four of the six residues proposed to be important for ligand recognition are conserved in CC1736 and Coq10p (Fig. 7A). The presence of this putative binding site in Coq10p suggests that this protein and its homologues may bind coenzyme Q. This is supported by the presence of Q6 in purified preparations of Coq10p-his. The amount of Q6 detected, however, is not stoichiometric with the protein. Because the extraction and subsequent fractionation of Coq10p-his entailed the use of detergent, it is possible that most of the Q6 was lost during the purification. The substoichiometric concentration of Q6, associated with Coq10p-his could also be because of the fact that the protein was purified from a highly overexpressing strain of yeast.

Because Coq10p is not a subunit of the bc complex it is unlikely to function in the catalytic mechanism of this respiratory complex. The same argument applies to the succinate- and NADH-coenzyme Q reductase complexes of yeast, both of which have known subunit compositions. Coq10p may be a chaperone for Q6, within a newly identified high molecular weight complex containing other Coq polypeptides (42). Alternatively, Coq10p may function in transporting Q6, from its site of synthesis to the catalytic sites of the respiratory chain complexes where it is used. Another function of Coq10p could be to shuttle coenzyme Q from the dehydrogenase complexes to the bc complex during electron transport. This, however, seems highly unlikely in view of the mitochondrial Coq10p concentration, which we estimate to be 3 orders of magnitude lower than that of Q6, and at least 2 orders of magnitude lower than most components of the respiratory chain.

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