The COQ5 Gene Encodes a Yeast Mitochondrial Protein Necessary for Ubiquinone Biosynthesis and the Assembly of the Respiratory Chain*

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Saccharomyces cerevisiae is a facultative anaerobe capable of meeting its energy requirements by fermentation and is thus an ideal system for studying the biogenesis of respiring mitochondria. We have isolated a respiration-deficient mutant exhibiting a pleiotropic loss of the mitochondrial electron transport chain. The corresponding wild-type gene, COQ5, was cloned, sequenced, and able to restore respiratory growth. Deletion of the chromosomal COQ5 gene results in a respiration deficiency and reduced levels of respiratory protein components. Exogenously added decylubiquinone can partially restore electron transport chain function to mitochondrial membranes from the deletion mutant. The COQ5 nucleotide sequence predicts a polypeptide of 307 amino acids containing a mitochondrial targeting signal. COQ5p is 43% identical to the polypeptide predicted by the Escherichia coli open reading frame, o2511. The COQ5 gene, when introduced into E. coli, complements the respiratory deficiency of an ubiE mutant that maps near o251, suggesting that it is the yeast homolog of the ubiE gene product. We conclude that the COQ5 gene encodes the mitochondria-localized 2-hexaprenyl-6-methoxy-1,4-benzoquinone methyltransferase of the yeast ubiquinone biosynthetic pathway.

The yeast, Saccharomyces cerevisiae, is the ideal organism for investigating the processes necessary for the production and maintenance of a functional electron transport chain in the mitochondria because it is able to make sufficient ATP for growth by fermentation. Respiration-deficient mutants form smaller colonies than wild-type cells when grown on glucose-containing media, and are therefore called petite. Such mutants can arise in two ways, by mutation of the nuclear genome or by mutation of the mitochondrial genome giving rise to nuclear petites or by mutation of the mitochondrial genome giving rise to cytoplasmic petites. Thus, the biogenesis of the mitochondrial electron transport chain is a complex process involving the coordinate expression of both nuclear and mitochondrial genes.

Ubiquinone is a lipid component of the mitochondrial electron transport chain that serves to transport electrons from the NADH or succinate dehydrogenase complexes to the cytochrome bc₁ complex, where it functions in a Q₁ cycle to generate a proton motive force across the inner membrane (2). In addition to its mitochondrial location, ubiquinone is found in a variety of intracellular membranes and in lipoproteins, where it may serve as an antioxidant (3). Ubiquinone is also an electron carrier in the plasma membrane respiratory chains of prokaryotes.

In yeast, ubiquinone-deficient mutants have been assigned to eight complementation groups, coq1-coq8 (4). The proposed pathway for ubiquinone biosynthesis is derived from the accumulation of intermediates in mutant strains of Escherichia coli and S. cerevisiae (Fig. 5; Refs. 5–9). Yeast mutants in the COQ1, COQ2, COQ3, and COQ7 genes have been characterized (7, 8, 10, 11).

We have been interested in the biogenesis of the succinate dehydrogenase complex of the electron transport chain and the tricarboxylic acid. This enzyme is composed of four nuclear encoded protein products, a covalent FAD cofactor, iron-sulfur clusters, and heme and donates the electrons derived from succinate to ubiquinol (12). To better understand the biogenesis of this enzyme, we have isolated nuclear petite mutants that are defective in succinate dehydrogenase. Mutations affecting the biogenesis of more than one enzyme will have pleiotropic effects on mitochondrial function. A large number of pleiotropic mutants have been isolated, but few have been studied (4). We have studied one such mutant, TCM7, that has lost several respiratory chain activities including succinate oxidase activity.

The TCM7 mutant was complemented to respiration proficiency by transformation with a genomic yeast DNA library. The complementing gene, identified as COQ5, has been cloned, and its nucleotide sequence has been determined. It corresponds to a hypothetical protein encoded on chromosome XIII, YMS339.09c, whose sequence is retrievable under the GenBank™ accession number P49017. COQ5 encodes a protein of 34.7 kDa (307 amino acids), which has an amino terminus that resembles a mitochondrial targeting sequence. The COQ5 gene probably encodes a methyltransferase in the ubiquinone biosynthetic pathway, since its expression in E. coli can complement a ubiE mutant, which lacks the 2-octaprenyl-6-methoxy-1,4-benzoquinone methyltransferase. Furthermore, the succinate oxidase activities of cytoplasmic membranes from an E. coli ubiE mutant or of yeast mitochondrial membranes from a coq5 mutant can be partially restored in vitro by the addition of quinone analogs.

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EXPERIMENTAL PROCEDURES

Strains and Media—The yeast strains used in this study are listed in Table I. Yeast media have been described (13, 14). Yeast transformation was with lithium acetate (15). The E. coli strain, AN70 (Hfr, metB, StrR, L, LeuR, TrpR, AprR, KanR, LeuR, TcrR, RvR, 0-1000), was kindly provided by Dr. I. G. Young (16).

Cloning and Identification of the COQ5 Gene—The mutant, TCM7, was transformed with a yeast genomic DNA library (17) and transformants were selected for uracil prototrophy on SD (0.67% yeast nitrogen base without amino acids, 3% glycerol) plates to test for complementation to petite mutants are unable to grow on glycerol as a sole carbon source (19) with the oligonucleotides 5′-CTAACAGTAATCTCGCAGTT-3′ and 5′-TGTGGAAGGACCA-3′](http://www.jbc.org/). The latter oligonucleotide also incorporates two glycine residues between the COQ5 and the c-myc sequences, a stop codon, and a SalI restriction site for cloning. The carboxyl-terminal 280 base pairs of COQ5 were amplified, digested with XbaI and SalI, and cloned into likewise digested pSF63 to generate pSF63myc encoding the tagged protein. The amplified region was sequenced and verified the presence of the disruption allele, coq5Δ::TRP1, in the transformants.

Construction and Expression of a Tagged COQ5 Protein—A polymerase chain reaction with the following oligonucleotides 5′-CATGAT- TGTGGAAGGACCA-3′ and 5′-CTAACAGTAATCTCGCAGTT-3′ was performed to add the 10-residue c-myc protooncogene sequence recognized by the monoclonal antibody, 9E10, to the carboxyl terminus of COQ5. The latter oligonucleotide also incorporates two glycine residues between the COQ5 and the c-myc sequences, a stop codon, and a SalI restriction site for cloning. The carboxy-terminal 280 base pairs of COQ5, now tagged with the myc sequence were amplified, digested with XbaI and SalI, and cloned into likewise digested pSF63 to generate pSF63myc encoding the tagged protein. The amplified region was sequenced and confirmed the tagged construction. For overexpression of the tagged COQ5p, the 1.0-kb Mun1 to Sall fragment encoding the entire gene was cloned into the vector, pUC119, under control of the copper-inducible CUP1 promoter (20) to generate pUC119myc. This plasmid was introduced into MH125, the transformants were grown on lactate medium (21) and inoculated into lactate medium supplemented with 20 μg/ml histidine, leucine, and tryptophan containing 0.5 mM CuSO4. Samples for Western blot analysis were prepared and transferred to nitrocellulose as described (13) and detected with the monoclonal antibody, 9E10 (Chemicon International Inc., Temecula, CA), goat anti-mouse secondary antibody (Bio-Rad Laboratories Ltd., Hercules, CA), and the ECL detection system (Amersham Canada Ltd., Oakville, Ontario, Canada).

Cloning of Nested Deletions—Cloning and expression of nested deletions were performed as described by the supplier (Promega Corp., Madison, WI) using TranS3-L-Label (ICN Biomedicals, St. Laurent, Quebec). Import reactions were performed as described (22).

Miscellaneous Methods—Standard procedures were used for plasmid isolation from E. coli (23). Yeast mitochondrial membranes and enzymatic activities were measured as described (13). Everted bacterial vesicles were prepared (24), and respiratory activities were measured in a Clark-type electrode (Rank Brothers, Cambridge, UK). The ubiquinone analogs, decylubiquinone (Q0), and 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q2) (Sigma) were added as ethanolic solutions. Immunofluorescence localization of COQ5p was performed as described (25) using fluorescein isothiocyanate-coupled goat anti-mouse as secondary antibody. Ubiquinone and its intermediates were isolated from E. coli by extraction with ethanol/ethyl ether (3:1) as described (26).

RESULTS

TCM7 Is a Nuclear petite Mutant—To better understand the mechanisms by which complex membrane proteins are assembled into the mitochondrial inner membrane, we have screened for mutants that are defective in the activity of our model protein, succinate dehydrogenase. Succinate dehydrogenase mutants are unable to grow on glycerol as a sole carbon source but still possess a functional electron transport chain linking NADH or glycrryl-1-phosphate oxidation to the reduction of oxygen (13, 27). In our screen, we took advantage of the observation that strains unable to grow on glycerol form small or petite colonies on YPDG (1% yeast extract, 2% peptone, 0.1% dextrose, 3% glycerol) plates (28). ρ0 or ρ− strains that are completely lacking an electron transport chain due to defects in their mitochondrial genomes were identified by their inability to reduce tetrazolium dye and eliminate. The secretion of acid, as monitored by the indicator dye, bromcresol purple, is taken as a preliminary indication of a succinate dehydrogenase activity.

Table I. Yeast media have been described (13, 14). Yeast transformation was with lithium acetate (15). The E. coli strain, AN70 (Hfr, metB, StrR, L, LeuR, TrpR, AprR, KanR, LeuR, TcrR, RvR, 0-1000), was kindly provided by Dr. I. G. Young (16).

### Table I

| Strain   | Genotype       | Parent/Source          |
|----------|----------------|------------------------|
| MH125    | a trp1 ura3–52 leu2–3,112 his 3 his 4 rme | Laboratory collection |
| MH123    | a trp1 ura3–52 leu2–3,112 his 3 his 4 rme | Laboratory collection |
| DAUL1    | a ade2 ura3 Δ lys2 | Laboratory collection |
| TCM7     | MH125, coq5–1 | This work              |
| TCM7–2A5A| a his ade2 Δ lys2 ura3 LEU2 TRP1 coq5–1 | TCM7 and DAUL1        |
| ED7      | MH125, coq5–1:1-TRP1 | This work              |
| CH83-B1  | ade2-1 his3–11 leu2–3,112 trp1–1 ura3–1 coq5 | Dr. C. Clarke         |
| CH83-B3  | ade2-1 his3–11 ura3–1 coq5 | Dr. C. Clarke         |
| a4rho5   | a ade1 lys2 [rho0] | Laboratory collection |
| MS10     | a kar1–1 leu2–3 canR [rho0] | Laboratory collection |
with a pleiotropic loss of most respiratory chain proteins, including the cytochrome bc₁ complex and succinate dehydrogenase. It defines one of four complementation groups isolated. When the mutant is mated and the resulting diploid is sporulated, the respiration deficiency in TCM7 segregates 2:2 in over 20 tetrads as expected of a single-gene, nuclear mutation.

Construction of a TCM7 Null Allele—To more fully characterize the function of the TCM7 gene in a nonmutagenized background, we decided to clone the wild-type gene (see “Experimental Procedures”) and to create a null allele by a one-step gene disruption (Fig. 1). The plasmid, pSK7-TRP, was transformed into the haploid, MH125, and the diploid, MH123, and tryptophan prototrophs were selected. Verification of the presence of the tcm7A-1::TRP1 allele in both haploid and diploid transformants was shown by polymerase chain reactions using genomic DNA as template (Fig. 2, lanes 2 and 4, respectively). The successful isolation of disruption mutants in the haploid, MH125, indicates that TCM7 is not essential for viability (lane 4). The disruption mutant, ED7, like TCM7, is respiration-deficient; it does not grow on YPG and has a low growth yield on a medium containing low levels of glucose (not shown). When mated to the tcm7–1 mutant, TCM7–2A5A, ED7 was not complemented, indicating the tcm7A-1::TRP1 allele belongs to the same complementation group as the tcm7–1 mutation in TCM7–2A5A and TCM7. ED7 is also rescued to respiration proficiency by the plasmid, pTS6. When a diploid carrying a single copy of the tcm7A-1::TRP1 disruption is sporulated, respiration deficiency and tryptophan prototrophy cosegregated in six dissected tetrads.

TCM7 Is a coq5 Mutant—As discussed below, we discovered that the cloned TCM7 gene could complement an E. coli ubiquinone biosynthetic mutant. This raised the possibility that the TCM7 gene encodes an enzyme of the yeast ubiquinone biosynthetic pathway. When TCM7 or ED7 are mated to the known coq5 mutant, CH83-B3, the diploids remain respiration-deficient, demonstrating that the mutations are allelic. As a control, we ensured that all haploids were protected location (lane 3) that is made accessible by the addition of detergent (lane 6). Import is accompanied by a proteolytic cleavage event to a faster migrating species (lane 5) and the intermembrane space (lane 3), but these levels reflect cross-contamination of fractions as judged by the membrane (porin), matrix (hisp60), and intermembrane space (cytochrome b₅₆₇₅) marker proteins. Our mitochondrial fractions contain contaminating organelles that co-purify, and hence we cannot exclude the possibility that COQ5p is located in other organelles beside mitochondria.

To independently confirm that COQ5p is a mitochondrial protein, in vitro synthesized precursor protein was imported into isolated yeast mitochondria (Fig. 4). COQ5p bound to mitochondria (lanes 2 and 4) and could be imported in an energy-dependent manner (lanes 4 and 5) to a protease-protected location (lane 3) that is made accessible by the addition of detergent (lane 6). Import is accompanied by a proteolytic cleavage event to a faster migrating species (lanes 2 and 3). The COQ5p precursor sequence contains the sequence RC↓FTQA-HRAC↓ which resembles the consensus sequence for twice cleaved precursor proteins, RX↓(F/L/X/T)RXX↓ (32). This motif is found in many proteins required for respiratory function (32) and would predict that lysine 31 is the mature amino terminus. We have not detected the presence of an intermediate species in the maturation of COQ5p in our experiments.

The Yeast COQ5 Complements an E. coli ubiE Mutant—The sequence of COQ5p is 42.8% identical to the polypeptide encoded by o251, an open reading frame located at 86 min on the...
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...plasmid p765. COQ5 is able to complement AN70 and restore growth on succinate. Furthermore, when the quinone pools are extracted from AN70, quinones are not detectable by thin layer chromatography in either chloroform/benzene (1:1) or in chloroform/methanol (95:5) solvent systems (not shown). In contrast, the quinone pool from AN70 expressing COQ5 contained compounds that comigrate with a Q0 standard in both solvent systems used (Rf (relative mobilities) = 0.2 and 0.85, respectively). This result suggests strongly that COQ5 complements AN70 by restoring ubiquinone biosynthesis.

Membrane vesicles prepared from AN70 have no detectable succinate oxidase activity (Fig. 6). The addition of increasing concentrations of Q0 restored succinate oxidase activity. Membrane vesicles from AN70 transformed with p765 displayed a high level of succinate oxidase activity in the absence of added quinone; this level of oxidase activity is similar to that found in a related wild-type strain of E. coli (34). The addition of 200 μm coenzyme Q0 to COQ5-complemented bacterial membranes only slightly increased respiratory activity, indicating that the yeast COQ5p had restored adequate quinone levels.

Exogenous Quinone Stimulates Respiration in ED7 Mitochondrial Membranes—Submitochondrial membranes were prepared from the yeast disruption mutant, ED7, and its wild-type parent, MH125, and analyzed for respiratory chain activities (Table II). ED7 membranes showed a dramatic loss of NADH oxidase and NADH-cytochrome c reductase activities. Even more dramatically, succinate oxidase and succinate-cytochrome c reductase activities were undetectable, although a small amount of succinate-dependent phenazine methosulfate-mediated reduction of the artificial electron acceptor, dichlorophenol indophenol, a ubiquinone-independent activity, remained. The addition of 200 μm decylubiquinone, Q0, led to the severalfold stimulation of some of the respiratory activities, with NADH oxidase activity being most affected. Higher concentrations of quinone could not further increase activity levels. The ED7 culture used in this experiment had remained at least 75% p−; the less than full recovery of respiratory chain activities with added quinone cannot be accounted for by mitochondrial DNA mutations.

The COQ5 Mutant Has Lowered Levels of Electron Transport Chain Components—The failure of added quinone to fully restore respiratory function to ED7 mitochondrial membranes might be due to lower levels of assembled respiratory chain components. We examined mitochondrial membranes for the presence of respiratory chain components by Western blot analysis and normalized mutant and wild-type samples by loading equivalent amounts of the outer membrane protein, porin (Fig. 7A). We reasoned that respiratory deficiency should not affect the import of mitochondrial outer membrane proteins, since their insertion is energy-independent (35). We detected low levels (less than 10%) of succinate dehydrogenase with an antibody against the iron-sulfur subunit (panel B), low levels of ATP synthase with antibodies against the β-subunit (panel C), and low levels of cytochrome c oxidase with antibodies against subunit IV (panel D), consistent with the reduced enzymatic activities reported in Table II. Therefore, a ubiquinone deficiency as present in the COQ5 null mutant leads to lowered steady state levels of electron transport chain components.

DISCUSSION

In this study, we characterize a coq5 mutant, isolate the corresponding wild-type COQ5 gene, and provide evidence that COQ5p is involved in ubiquinone biosynthesis in S. cerevisiae. Loss of COQ5 function and the resultant loss of ubiquinone synthesis should result in a respiration-deficient phenotype that is manifested as an inability to grow on nonfermentable carbon sources. The original mutant, TCM7, however, is at...
FIG. 5. The ubiquinone biosynthetic pathway. The proposed ubiquinone biosynthetic pathways of eukaryotes and prokaryotes is thought to diverge after the synthesis of intermediate I. The length of the isoprenoid chain (R) varies depending on the species; in yeast it is 6 units long, and in E. coli it is 8 units long. The intermediates are as follows: I, 3-polypropyl-4-hydroxybenzoate; II, 2-polypropenylphenol; III, 2-polypropenyl-6-hydroxyphenyl; IV, 3,4-dihydroxy-5-polypropenylbenzoate; V, 3-methoxy-4-hydroxy-5-polypropenylbenzoate; VI, 2-polypropenyl-6-methoxyphenol; VII, 2-polypropenyl-6-methoxy-1,4-benzoquinone; VIII, 2-polypropenyl-3-methyl-6-methoxy-1,4-benzoquinone; IX, 2-polypropenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinone; X, ubiquinone. Relevant E. coli and yeast mutations are listed above and below the affected biosynthetic reactions, respectively. This figure is modified from one presented by Marbois and Clarke (8).

FIG. 6. Succinate oxidase activities can be stimulated by added quinone. AN70 (solid bars) or AN70 with the plasmid, pT7S6 (hatched bars), was grown on Luria Broth with 20 mM glucose and harvested, and everted membrane vesicles prepared as described (24). To a 1.5-ml chamber filled with buffer (50 mM Tricine, pH 7.5, 10 mM MgSO4, 150 mM KCl) was added 3 mg of bacterial membrane protein. Oxidase measurements were begun with the addition of the desired amount of Q0, followed by the addition of 10 mM sodium succinate, pH 7.4.

TABLE II
Respiratory chain activities of mitochondrial membranes

| Activity                  | MH125 | ED7 | ED7 with 200 μM Q2 |
|---------------------------|-------|-----|------------------|
| Succinate oxidase         | 59    | ND* | ND               |
| NADH oxidase              | 241   | 5   | 14               |
| Succinate-cytochrome c reductase | 29 | ND | 1 |
| NADH-cytochrome c reductase | 315 | 13 | 27 |
| Succinate-dichlorophenol indophenol reductase | 172 | 4 | —* |

*Activities are expressed as nanoatoms of oxygen min⁻¹ mg⁻¹.

†ND, not detectable.

‡Activities are expressed as nmol of cytochrome c reduced min⁻¹ mg⁻¹.

§Activities are expressed as nmol of dichlorophenol indophenol reduced min⁻¹ mg⁻¹.

#Not done.

least partially respiration-proficient in that it is able to reduce the tetrazolium dye used in our mutant screen, suggesting that the coq5–1 allele is leaky. Mutations that completely block ubiquinone biosynthesis might not have been detected by our screening procedure. The secretion of acid by TCM7 and the low succinate dehydrogenase activity in the COQ5 disruption mutant (Table II) suggest that the tricarboxylic acid cycle is also impaired. We have not measured the levels of other tricarboxylic acid cycle enzymes.

The E. coli ubiE mutant, AN70, is characterized by an inability to grow on nonfermentable carbon sources such as succinate and by low oxidase activities (33). Expression of the yeast COQ5 gene restores the bacterium’s ability to grow with succinate as the carbon source and the succinate oxidase activity of isolated bacterial membranes. The COQ5 gene also rescues the ubiquinone synthesis deficiency of the ubiE mutation; although we did not characterize the exact nature of the products, the COQ5 gene results in the production of quinone compounds with a mobility by thin layer chromatography similar to Q0 of yeast in a chloroform/methanol solvent system.

The ubiE mutant accumulates intermediate VII or 2-octaprenyl-6-methoxy-1,4-benzoquinone (Fig. 5; Ref. 16). Restoration of ubiquinone biosynthesis in AN70 by the yeast COQ5 strongly suggests that COQ5p is the methyltransferase that modifies position 3 of the benzoquinone ring. We have not attempted to show methyltransferase activity in vitro nor have we attempted to isolate the intermediates that accumulate in ED7. There are two other methyltransferases, both O-methyltransferases, in the ubiquinone biosynthetic pathway. The yeast COQ3 gene encodes the 3,4-dihydroxy-5-hexaprenylbenzoate methyltransferase, which converts intermediate IV to V. It has been cloned, sequenced, and characterized and is distinct from the COQ5 gene (7). The second O-methyltransferase catalyzes the final step in the pathway, converting intermediate IX to ubiquinone (X) and has not been characterized in yeast (9). In E. coli, the second O-methyltransferase is encoded by the E. coli ubiG gene. The ubiG gene has recently been shown to complement a yeast coq3 mutant, strongly suggesting that the ubiG protein catalyzes both O-methyltransferase reactions in E. coli and leading to speculation that a bifunctional O-methyltransferase may also exist in yeast (9). The ubiG gene has
been sequenced and is not related in sequence to the yeast COQ5 gene except in the proposed S-adenosylmethionine binding domains (Fig. 8; Ref. 6). The simplest explanation for our results is that COQ5 encodes the yeast 2-hexaprenyl-6-methoxy-1,4-benzoquinone methyltransferase, which can functionally complement a mutation in the E. coli homolog encoded by the ubiE gene.

The COQ5p sequence is weakly related to other methyltransferases; it does contain a closely related version of a consensus amino acid sequence common to methyltransferase enzymes that use S-adenosylmethionine as a donor (Fig. 8; Refs. 6 and 36). As in the human glycine methyltransferase sequence, the first of the conserved glycines in the motif is replaced by an alanine in COQ5p, but the remainder of the adjacent COQ5p sequence conforms to the consensus motif (Fig. 8A). There is a second region of sequence similarity found in a smaller number of methyltransferases that is also found in COQ5p (Fig. 8B; Ref. 36). These sequence similarities are consistent with the use of S-adenosylmethionine as the donor for all the methyl groups in the ubiquinone biosynthetic pathway (37).

We localized COQ5p to the mitochondrial matrix by Western blot analysis of a tagged, overexpressed protein (Fig. 3) and by in vitro import into isolated mitochondria (Fig. 4). This localization is consistent with the proposed role for COQ5p in ubiquinone biosynthesis, which is generally believed to be compartmentalized within mitochondria (7). Complementation of a yeast coq3 mutant by the E. coli ubiG gene required the addition of a mitochondrial targeting signal to the ubiG gene, indicating a need for organellar targeting of some proteins in the ubiquinone biosynthetic pathway (9).

The COQ5p sequence is weakly related to other methyltransferases; it does contain a closely related version of a consensus amino acid sequence common to methyltransferase enzymes that use S-adenosylmethionine as a donor (Fig. 8; Refs. 6 and 36). As in the human glycine methyltransferase sequence, the first of the conserved glycines in the motif is replaced by an alanine in COQ5p, but the remainder of the adjacent COQ5p sequence conforms to the consensus motif (Fig. 8A). There is a second region of sequence similarity found in a smaller number of methyltransferases that is also found in COQ5p (Fig. 8B; Ref. 36). These sequence similarities are consistent with the use of S-adenosylmethionine as the donor for all the methyl groups in the ubiquinone biosynthetic pathway (37).

The predicted protein sequence does not contain any putative membrane-spanning domains, so COQ5p was expected to be soluble. In E. coli, the enzymes of the ubiquinol biosynthetic pathway that can convert 2-octaprenylphenol (intermediate II) to ubiquinone-8 can be released from membranes as a complex without detergent despite the hydrophobic nature of their substrates (37). Ubiquinone’s primary role is in electron transfer to the cytochrome c reductase complex in the mitochondrial respiratory chain, consistent with a mitochondrial site of synthesis, but it is also found in other eukaryotic organelles where its function is less well defined.

The addition of an ubiquinone analogue to ED7 ubiquinone-deficient membranes only partially restores respiratory activities, particularly succinate oxidase activity. In contrast, the ubiE mutant, AN70, which is similarly blocked in ubiquinone synthesis, shows a more marked recovery of succinate oxidase activity with added Q0 (Fig. 6). We have shown that the partial recovery of respiratory activities is likely attributable to the substantially lower levels of respiratory chain components in ED7 membranes (Fig. 7 and Table II). Why does the absence of COQ5p lead to low levels of respiratory chain components in yeast, whereas some other nuclear petite mutations do not? For example, sdh1 or sdh4 mutants have normal levels of NADH oxidase activities in their mitochondrial membranes, despite their respiration deficiencies due to losses of succinate dehydrogenase subunits (13, 27). A yeast coq3 mutant also has normal levels of succinate or NADH oxidase activities if a quinone analog is added to the assays (30).

We do not believe ubiquinone deficiency is affecting the translocation of proteins into the organelle. Even cytoplasmic petite mutants that are devoid of respiratory activity are still able to import and assemble normal F1-ATP synthase (38). One possibility is that a lack of ubiquinone destabilizes the respiratory chain complexes and leads to their rapid degradation and low steady state levels. Destabilization could be manifested by a change in the structural properties of the bilayer or of the proteins themselves. Alternatively, the low levels of respiratory components may arise from the failure of the cells to adapt to respiratory conditions and activate the expression of mitochondrial proteins upon the depletion of available glucose. The production of respiratory chain components is normally accelerated in stationary phase cells that we used for our studies (39). We have not been able to test whether the failure to recover from catabolite repression is responsible for the low levels of respiratory components because our coq5 mutants do not grow under nonrepressing conditions with galactose as a carbon source. An explanation for this phenomenon awaits further investigations.

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