Genetic evidence for a multi-subunit complex in the \( O \)-methyltransferase steps of coenzyme Q biosynthesis

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Abstract

\( \text{Coq3} \) \( O \)-methyltransferase carries out both \( O \)-methylation steps in coenzyme Q (ubiquinone) biosynthesis. The degree to which \( \text{Coq3} \) \( O \)-methyltransferase activity and expression are dependent on the other seven \( \text{COQ} \) gene products has been investigated. A panel of yeast mutant strains harboring null mutations in each of the genes required for coenzyme Q biosynthesis (\( \text{COQ1} - \text{COQ8} \)) have been prepared. Mitochondria have been isolated from each member of the yeast \( \text{coq} \) mutant collection, from the wild-type parental strains and from respiratory deficient mutants harboring deletions in \( \text{ATP2} \) or \( \text{COR1} \) genes. These latter strains constitute Q-replete, respiratory deficient controls. Each of these mitochondrial preparations has been analyzed for \( \text{COQ3} \)-encoded \( O \)-methyltransferase activity and steady state levels of Coq3 polypeptide.

The findings indicate that the presence of the other \( \text{COQ} \) gene products is required to observe normal levels of \( O \)-methyltransferase activity and the Coq3 polypeptide. However, \( \text{COQ3} \) steady state RNA levels are not decreased in any of the \( \text{coq} \) mutants, relative to either wild-type or respiratory deficient control strains, suggesting either a decreased rate of translation or a decreased stability of the Coq3 polypeptide. These data are consistent with the involvement of the Coq polypeptides (or the Q-intermediates formed by the Coq polypeptides) in a multi-subunit complex. It is our hypothesis that a deficiency in any one of the \( \text{COQ} \) gene products results in a defective complex in which the Coq3 polypeptide is rendered unstable. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Coenzyme Q; S-Adenosyl-L-methionine dependent methyltransferase; Ubiquinone; Yeast; \( \text{Saccharomyces cerevisiae} \)

1. Introduction

Coenzyme Q (ubiquinone or Q) is a prenylated benzoquinone lipid that is found in membranes throughout the cell, and functions in a wide variety of enzyme-mediated redox reactions. Q is best appreciated for its role in respiratory metabolism, where it functions in the inner mitochondrial membrane of eukaryotic cells as a transporter of electrons and protons in complexes II and III [1]. Q also functions in a \( \text{trans} \)-plasma membrane electron transport, where it enables cells to reduce extracellular compounds, such as ascorbyl radicals [2,3]. This plasma membrane redox function of Q appears to play an important role in cells with defects in mitochondrial respiration, where it participates in pathways that oxidize NADH, in order to regenerate NAD\(^{+}\) for glycolytic metabolism [4]. In the inner membrane of \( \text{Escherichia coli} \), Q functions in the \( \text{trans} \)-plasma
membrane electron transport system that plays a dual role in both respiration and in the introduction of disulfide bonds in periplasmic proteins mediated by the dsb system [5,6]. The redox chemistry of Q also enables it to function as a potent antioxidant, where it has the capacity to act directly as a chain terminating antioxidant or can act in concert with α-tocopherol by reducing α-tocopheroxyl radicals [7].

In general, cells rely on de novo biosynthesis for their supply of Q [8]. The yeast Saccharomyces cerevisiae provides a valuable model for characterizing the eukaryotic Q biosynthetic pathway. The availability of eight complementation groups of Q deficient mutants has greatly facilitated the recovery and characterization of the genes required for Q biosynthesis [9,10]. As shown in Fig. 1, Q is synthesized from the precursors 4-hydroxybenzoic acid and polyisoprene diphosphate [8]. The 4-HB:polyprenyldiphosphate transferase, encoded by the COQ2 gene [11], generates compound 5 in both the yeast and rat Q biosynthetic pathway. Further modifications of the aromatic ring involve a decarboxylation step, three hydroxylation steps and three methylation steps to produce the fully substituted benzoquinol ring, QH2. Both the C- and O-methyltransferases involved in Q biosynthesis have been identified [12–14]. The Coq3 polypeptide carries out both O-methylation steps in Q biosynthesis [15].

The Coq3 polypeptide is located within the matrix of the mitochondria, where it is peripherally associated with the inner membrane [15]. The other Coq polypeptides are also localized to mitochondria [10]. Attempts to solubilize Coq3p in an active form from yeast mitochondria have not yet been successful [15]. In addition, expression of yeast Coq3p in E. coli ubiG mutants (deficient in both O-methyltransferase steps of Q biosynthesis; [16]) does not restore Q biosynthesis, nor does it generate detectable O-methyltransferase activity with either the farnesylated analog of 6 (Fig. 1) or with the prokaryotic farnesylated catechol substrate (A.Y. Hsu, unpublished data). Together, these data indicate that Coq3p may require other yeast polypeptide components for its activity or stability.

Several lines of evidence argue for an interdependence of the COQ gene products: (1) a single early

![Fig. 1. The eukaryotic Q biosynthetic pathway. Dimethylallyl diphosphate (1) and isopentenyl diphosphate (2) provide the precursors for the assembly of all-trans polyprenyl diphosphate (3), as catalyzed by Coq1. The prenylation of 4-hydroxybenzoic acid (4) is catalyzed by Coq2 to form 3-polyprenyl-4-hydroxybenzoic acid (5). The other intermediates in the pathway are 3,4-dihydroxy-5-polyprenylbenzoic acid (6), 3-methoxy-4-hydroxy-5-polyprenylbenzoic acid (7), 2-polyprenyl-6-methoxy-phenol (8), 2-polyprenyl-6-methoxy-1,4-benzoquinol (9), 2-polyprenyl-3-methyl-6-methoxy-1,4-benzoquinol or 5-demethoxyubiquinol (10), 2-polyprenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinol or demethyl-QH2 (11), coenzyme QH2 (12). In S. cerevisiae, n = 6 and compound 5 is referred to as 3-hexaprenyl-4-hydroxybenzoate (HHB). S. cerevisiae gene products are identified as Coq.](https://example.com/fig1.png)
intermediate in the Q biosynthetic pathway (5) accumulates in strains harboring mutations in COQ3, COQ4, COQ5, COQ6, COQ7 or COQ8, suggesting that the absence of one COQ gene product prevents the function of the others [17]; (2) a yeast strain harboring a point mutation in the COQ7 gene (coq?-1; G104D) accumulates intermediate 10, while yeast strains harboring null alleles of coq7 produce only 5, the earlier intermediate [18]; and (3) yeast strains harboring certain point mutations in the COQ5 gene are rescued by expression of the E. coli Coq5 homologue, UbiE, however, the presence of E. coli UbiE does not rescue mutant strains harboring null alleles of coq5 [19]. These findings are compatible with the coordinate regulation of the COQ gene products, in a manner that is similar to the synthesis of flagella proteins [20]. Alternatively, the distinct phenotypes manifested by yeast strains harboring either null or point mutations are compatible with a multi-subunit complex of the COQ-encoded enzymes. Precedent for this second scenario is provided by the multi-subunit complexes of the respiratory electron transport chain. For example, yeast strains harboring mutations in one of the structural genes of complex IV, cytochrome oxidase, contain markedly decreased steady state levels of several of the other components of complex IV, due to their rapid proteolytic degradation [21]. Such rapid proteolytic degradation of the unassembled components is also observed in strains containing mutations in genes encoding subunits of complex III (QH2-cytochrome c reductase) [22], complex II (succinate dehydrogenase) [23] and in complex V (F1/F0 ATPase) [9].

The degree to which O-methyltransferase activity and expression are dependent on the other COQ gene products is the subject of this present study. A complete panel of yeast coq null mutants have been prepared, and the effect of the null mutations on the COQ3-encoded O-methyltransferase activity, polypeptide and RNA levels has been evaluated. The data presented here indicate that Coq3 O-methyltransferase activity and polypeptide levels depend on the presence of each of the other COQ gene products. At present, we favor the model that the low steady state level of the Coq3 polypeptide results from its enhanced degradation, resulting from either a defective or unassembled complex of Q biosynthetic enzymes.

2. Materials and methods

2.1. Yeast strains and growth media

The genotypes and sources of the mutant and wild-type yeast strains are shown in Table 1. Media for growth of yeast were prepared as described [12]. Yeast null mutant strains were generated by the one-step gene replacement procedure [28]. Segments of DNA containing either disruption or deletion mutations of the targeted genes were either generated from plasmids containing the null mutation or were PCR-amplified from the genomic DNA of designated null mutants. The deletion mutation in the COR1 gene was constructed by replacing a 0.25 kb KpnI/XbaI fragment internal to the COR1 open reading frame (ORF) (1.37 kb) with a 1.7 kb HIS3 cassette as described [25]. A deletion in the ATP2 gene was prepared by removal of the 0.84 kb BamHI/KpnI fragment internal to the ATP2 ORF and insertion of a 2.9 kb LEU2 cassette [26]. Disruption mutations were introduced in the COQ1 and COQ2 genes by the insertion of a 1.15 kb HIS3 into CvnI and PsrI sites of the respective ORFs. Deletion of either the COQ3, COQ5 or COQ7 gene was performed as described [14,12,18,27]. Disruption of the COQ4 gene was generated by the insertion of a 0.85 kb TRP1 cassette into the SphI site of COQ4 ORF (accession number AF005742, GenBank) (A.Y. Hsu, unpublished data). A 0.414 kb BglII fragment containing part of the COQ6 ORF (−146 to +268, accession number AF003698, GenBank) was replaced with a 1.7 kb HIS3 cassette to construct both W303AG63 and CENΔCOQ6 (A. Tzagoloff, unpublished data). W303ΔCOQ8 and CENΔCOQ8 were generated by replacement of a 1.1 kb AfIII/SalI fragment internal to the COQ8 ORF with a 1.15 kb HIS3 cassette as described (T.Q. Do and A.Y. Hsu, unpublished data). The presence of the gene disruption allele in each strain was indicated by the lack of growth on non-fermentable carbon sources, rho+ status and verification of the presence of the disrupted allele by either Southern blotting or by PCR analysis of genomic DNA.

2.2. Preparation of mitochondria

Yeast strains were grown at 30°C in YPGal (1%
yeast extract, 2% peptone and 2% galactose) to an OD\textsubscript{600} of 4.0, and mitochondria were isolated according to Yaffe [29]. The final mitochondrial pellets were resuspended in mitochondria isolation buffer (0.6 M mannitol, 20 mM HEPES-KOH, pH 7.4) with 0.5 mM PMSF and 10% glycerol (v/v) and stored in aliquots at −80°C. In selected preparations, protease inhibitors were included during cell lysis, mitochondrial isolation and storage at the following final concentrations: benzamidine, 1.0 mM; peptatin, 2 μg/ml; and leupeptin, chymostatin, aprotinin and antipain each at 1 μg/ml. The protein concentrations of the mitochondria samples were determined by the BCA method (Pierce), and the same concentration of glycerol was used in preparing a standard curve to correct for the glycerol interference with the BCA assay.

2.3. In vitro O-methyltransferase assay

Each reaction mixture (250 μl) contained 50 mM sodium phosphate, pH 7.0, 1.0 mM ZnSO\textsubscript{4}, 0.2 mM 3,4-dihydroxy-5-farnesylbenzoic acid (in 5 μl methanol), 100 μl of the mitochondrial suspension (containing from 0.70 to 1.00 mg protein) and 60 μM of radiolabeled S-adenosyl-L-[methyl-\textsuperscript{3}H]methionine (NEN-DuPont, specific activity was adjusted to 560 mCi/mmol with unlabeled S-adenosyl-L-methionine, Sigma). The reactions were carried out at 30°C for 60 min with mitochondria isolated from coq null mutant strains, and 30 min for reactions containing mitochondria isolated from wild-type, atp2 or cor1 mutants. These times were found to be within the linear range of the O-methyltransferase assay. To terminate the reaction, 2 μl of glacial acetic acid was added, and lipids were extracted twice with 0.5 ml of chloroform. The extract was then dried under a stream of N\textsubscript{2} gas, resuspended in methanol and analyzed by an isocratic reverse phase high performance liquid chromatography system with C18 column (Alltech LiChrosorb, 5 μM, 4.6×250 mm) and 9:1 methanol/water as mobile phase at a flow rate of 1 ml/min as described by Poon et al. [15,30]. One ml fractions were collected, mixed with 10 ml of fluor (Safety

Table 1
Genotypes and sources of S. cerevisiae strains

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<th>Strains</th>
<th>Relevant genotype</th>
<th>Source or reference</th>
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<td>R. Rothstein*</td>
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bDepartment of Biological Sciences, Columbia University, New York, USA.
Solve, RPI, Mount Prospect, IL, USA) and subjected to scintillation counting. The counting efficiency of $^3$H was 40.5%. Radioactivity present in fractions 7–9 (or in some analyses 8–10) represented material that co-eluted with the methylated product standard, 3-methoxy-4-hydroxy-5-farnesylbenzoic acid. The sum of the radioactivity in these fractions, minus the background radioactivity (defined as fractions 6, 11 and 12), was used to calculate the Coq3 O-methyltransferase activity (expressed as cpm/mg protein/h).

2.4. Western analysis of Coq3 polypeptide

Equal amounts of protein from isolated mitochondria were separated by electrophoresis on 12% polyacrylamide sodium dodecyl sulfate (SDS)–Tris–glycine gels and subsequently transferred to Hybond ECL nitrocellulose (Amersham Pharmacia Biotech). Western analysis with the ECL system was carried out as described by Amersham Pharmacia Biotech except that 10 mM Tris, pH 8.0, 154 mM NaCl, 0.1% Triton X-100 was used as the washing buffer. Affinity-purified antibodies to a GST-Coq3 fusion protein [15] were used at a final concentration of 1.0 μg/ml, and alkaline phosphate-conjugated goat anti-rabbit secondary antibodies (Amersham Pharmacia Biotech) were used at a 1:2000 dilution.

2.5. RNA isolation and Northern analysis

Yeast total RNA was isolated from the yeast strains prepared from the CEN.PK2-1C background as listed in Table 1. Yeast was grown in YPGal media, incubated at 30°C with shaking, harvested when cell density reached an OD$_{600}$ of 1.0 and RNA was isolated by the hot acidic phenol method as described [31]. RNA was analyzed by electrophoresis on formaldehyde gels [32], transferred to Bodion (ICN) nylon membranes and baked for 2 h at 80°C. A DNA segment containing the COQ3 gene was amplified by PCR with primers pBC3-1 (5′-TAAATTTCGCTAAGCTGCACCACGGTGATTTG-3′) and pBC3-2 (5′-CGCGGATCCATTCACTGTCTCTGAA-ATAGCCA-3′) and genomic DNA from the wild-type yeast strain D273-10B. The resulting PCR product, containing the entire COQ3 ORF and 650 bp 5′ non-coding sequence, was labeled by random priming with [α-32P]dCTP (3000 Ci/mmol, ICN Biochemicals) and the Oligolabeling kit (Amersham Pharmacia Biotech). Unincorporated nucleotides were eliminated with NucTrap push column (Stratagene). An actin specific probe was prepared similarly by PCR with forward primer pACT1 (5′-ATGTGTTAAAGCCGGTTTTGC-3′) and reverse primer pACT2 (5′-TTAGAAACACTTTGTTGGA-3′) to generate 1.083 kb yeast actin DNA product containing the entire ORF for labeling. Hybridization was performed at 57°C for 18 h as described [33]. Blots were washed twice with 2× SSC, 0.1% SDS at room temperature, and twice for 15 min each (55°C) with 0.2× SSC, 0.1% SDS, and analyzed with a phosphor-imager (Molecular Dynamics, version 4.0).

3. Results and discussion

3.1. Coq3 O-methyltransferase activity is decreased in the coq1, coq2, coq4, coq5, coq6, coq7 and coq8 null mutants

Yeast strains were constructed that harbored null mutations in one of the COQ genes, or in either the ATP2 or COR1 gene (Table 1). The latter two genes encode subunits of either complex III (the Cor1 subunit of the bc$_1$ complex) or complex V (the β-subunit of the F$_1$ ATPase), and provide respiratory deficient control strains [25,34]. Two different laboratory ‘wild-type’ yeast backgrounds were employed, in order to assess whether the genetic background might introduce variability in the assays of O-methyltransferase activity.

Mitochondria were prepared and assayed for the amount of O-methyltransferase activity with a farnesylated analog of compound 6 (Fig. 1) as substrate, as described in Section 2. As shown in Fig. 2A, 6-O-methyltransferase activity was greatly decreased in each of the coq null mutants. The 6-O-methyltransferase activity was also decreased in the atp2 and cor1 null mutant strains relative to wild-type, however, this decrease was not nearly as dramatic as that observed for the coq mutants. The second O-methylation step of Q biosynthesis was also assayed in the coq4 null mutant strain. As observed in assays of the first O-methyltransferase step, the coq4 null mutant strains were found to have only 4% of the wild-type level of 11-O-methyltransferase activity (data not
A similar degree of impairment in these two steps is not surprising, since the Coq3 O-methyltransferase is responsible for both O-methylation steps of Q biosynthesis [15].

Assays of O-methyltransferase activity were also performed on mitochondria isolated from mutants constructed in a different background (CEN.PK2-1C). As shown in Fig. 2B, there was a consistent trend among the mutant strains; atp2 and cor1 had the highest levels of O-methyltransferase activity, followed by coq6 and coq4. O-Methyltransferase activity levels in the coq1, coq2, coq7 and coq8 mutant strains were significantly decreased relative to the cor1 null mutant. However, considerable variation in the level of O-methyltransferase activity was observed in mitochondria isolated from both yeast strains, resulting in the large S.D.s shown. We then investigated the source of this variability. As shown in the inset of Fig. 2B, the variability cannot be attributed to the O-methyltransferase assay itself, since measurements of activity within a given preparation of mitochondria are reproducible. Purification of mitochondria from the coq6 mutant strain in the presence of various protease inhibitor cocktails was carried out to address the possibility that mitochondria isolated from the coq mutants exhibit an enhanced susceptibility to proteases. However, the inhibitors tested (see Section 2) had no effect on reducing the large variation observed in measurements of O-methyltransferase activity. The coq mutants are known to be sensitive to certain types of oxidative stress [26,17] and it seemed possible that the O-methyltransferase activity might be prone to oxidative damage. To test this idea, either 0.5 mM trolox (a water soluble analog of α-tocopherol) or 15 μM Q6 (a concentration which restores growth of each of the coq mutant strains on non-fermentable carbon sources) was added to the growth media. In addition, the effect of freezing and thawing on the samples was studied. However, none of these adjustments solved the problem of the inherent high variability experienced in assaying the amount of O-

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**Fig. 2.** Yeast mutants harboring deletions in any COQ gene have decreased levels of Coq3 O-methyltransferase activity. Yeast coq, atp2 and cor1 null mutants were generated in two yeast genetic backgrounds, W303 (A) and CEN.PK2-1C (B). The activity of the Coq3-mediated O-methyltransferase in mitochondrial preparations from these yeast strains was measured. The in vitro assays were performed with 3,4-dihydroxy-5-farnesy1benzoic acid (6) as the methyl acceptor substrate and S-adenosyl-L-[methyl-3H]methionine as described in Section 2. Following incubation, lipids were extracted and analyzed by reverse phase high performance liquid chromatography and the radioactivity was determined in each 1 ml fraction. The radioactivity co-eluting with the 3-methoxy-4-hydroxy-5-farnesylbenzoic acid standard, compound 7, provided a measure of O-methyltransferase activity (expressed as pmol methyl groups/h/mg protein). The O-methyltransferase activity from each strain is expressed as percentage of the wild-type activity. A and B represent the average values (±S.D.) from three sets of mitochondrial preparations. The average wild-type activity (100%) in A was 24.48 ± 4.53 pmol/h/mg protein and in B was 7.20 ± 2.37 pmol/h/mg protein. An asterisk (*) designates that activity is significantly different from the cor1Δ control strain; P = 0.05. The inset in B shows duplicate determinations of O-methyltransferase activity for each of two separate preparations of mitochondria from the coq4 null mutant strain CENΔCOQ4.
methyltransferase activity in the respiration defective yeast strains. Similar problems with variability in assays of NADH oxidase and NADH-cytochrome c reductase have been reported by others when studying respiration defective yeast mutants [35].

3.2. The steady state level of Coq3 polypeptide is decreased in coq mutant strains

The above results indicate that the presence of the other COQ genes is required to observe normal levels of O-methyltransferase activity. Such a requirement could reflect the involvement of the other COQ gene products in the expression or stability of the Coq3 polypeptide. In order to investigate this possibility, levels of the Coq3 polypeptide were evaluated by Western analysis with an antibody specific for yeast Coq3p [15]. Fig. 3 shows that decreased levels of the Coq3 polypeptide are present in mitochondria prepared from the coq null mutants. Within the coq mutant panel, Coq3p levels are present at highest levels in the coq4 and coq6 mutants. When this analysis was performed with two other sets of independently isolated mitochondria from the CEN.PK2-1C background, the same trend was observed. Coq3p levels were dramatically decreased in the coq mutant panel relative to wild-type, cor1 or atp2 mutants, but were highest in the coq4 and coq6 null mutants (Fig. 4). However, there does not appear to be a strict correlation between the amount of Coq3 polypeptide and the level of O-methyltransferase activity. This is apparent from the values presented in Fig. 3, legend: when the O-methyltransferase activity was normalized to the amount of Coq3p, the O-methyltransferase activity is still greatly reduced. Figs. 2–4 show that the atp2Δ and cor1Δ respiratory deficient control mutant strains appear to have decreased O-methyltransferase activity but not a decreased level of Coq3p. Perhaps in the absence of respiration there may be a decreased need for coenzyme Q, and the activity of enzymes in the Q biosynthetic pathway is subject to repression. In summary, two classes of coq null mutants can be identified: (1) those with very low or absent Coq3p and O-methyltransferase activity (e.g. coq1, coq2, coq5, coq7 and coq8); and (2) those with relatively high Coq3p and detectable O-methyltransferase activity (coq4 and coq6).

3.3. COQ3 RNA levels are not decreased in the panel of coq mutants

To examine the possibility that a lack of one of the COQ gene products results in a lower level of COQ3...
RNA, Northern blot analysis was performed. As shown in Fig. 5, COQ3 steady state RNA levels are not decreased in any of the coq mutants, relative to either wild-type or respiratory deficient control strains. In fact, the level of the COQ3 mRNA is significantly increased in the coq deletion mutant panel (coq1, 2, 4–8) and even more so in the atp2 and cor1 deletion mutant strains. These results are quite striking and are in the opposite direction as might be predicted from the activity and protein levels of Coq3p. The finding of elevated levels of COQ3 RNA suggests that either the translation of the Coq3 polypeptide is decreased or that Coq3 polypeptide is produced at normal levels but is less stable.

3.4. Is a multi-subunit complex involved in Q biosynthesis?

The data presented here show that Coq3p O-methyltransferase activity is greatly decreased in coq1, coq2, coq4, coq5, coq6, coq7 or coq8 null mutant strains. The coq null strains also have greatly decreased levels of the Coq3 polypeptide, yet levels of COQ3 RNA are not decreased. The data are consistent with two models: (1) an involvement of the COQ gene products in regulating the Coq3 polypeptide at the level of translation; and (2) an involvement of the Coq polypeptides (or their lipid products) in a multi-subunit complex. In the latter case, a deficiency in any one of the COQ gene products would result in a defective complex in which the Coq3 polypeptide is rendered unstable. Analysis of the rates of Coq3 polypeptide synthesis and degradation should distinguish between these two models. Our attempts to solubilize Coq3 activity and determine the native molecular weight by sonication or detergent treatment have not yet met with success [15].

We have previously postulated the involvement of a multi-subunit complex in converting 5 to Q [18]. This model is consistent with the accumulation of 5 as the sole predominant intermediate detected in each of the coq null mutants (coq3–coq8) [17]. This model is also consistent with the observation that the activity of the COQ1 and COQ2 encoded enzymes is independent of the other COQ gene products [36]. However, it is notable that both the coq1 and coq2 null mutants each have profoundly decreased levels of O-methyltransferase activity and Coq3 polypeptide (Fig. 2–4). Are these polypeptides also part of the postulated multi-subunit complex? While possible, this scenario does not seem likely, because COQ1 homologues from a diverse array of species have been shown to restore Q synthesis in yeast coq1 null mutants [37,38]. For example, the Haemophilus influenzae ispB gene (GenBank accession no. U32770; 28% amino acid identity with S. cerevisiae Coq1) encodes a heptaprenyl diphosphate synthase in demethyl-menauquinone biosynthesis but does not participate in Q biosynthesis because H. influenzae does not produce Q [39]. Similarly, the ddsA gene from Gluconobacter suboxydans encodes a decaprenyl diphosphate synthase with relatively low sequence identity to yeast Coq1 (DDBJ accession no. AB006850; 26% amino acid identity with S. cerevisiae Coq1) [40]. Expression of either the H. influenzae ispB gene or the G. suboxydans ddsA gene in a coq1 null mutant strain of S. cerevisiae restores respiration and the synthesis of either Q7 or Q10, respectively [38]. These and other experiments by Okada et al. [37,38] show that the Q isoform produced (Q5 to Q10) depends solely on the type of COQ1 homologue being expressed. It seems unlikely that each of these distinct Coq1 homologues would function to reconstitute a multi-subunit complex. An alternate, more probable model is that the lipid product formed by Coq1 and Coq2 (e.g. compound 5) may provide the component required for the assembly or stability of
the complex. In this model, the low level of Coq3p and O-methyltransferase activity detected in the coq1 and coq2 yeast mutant strains may result from the absence of 5, as opposed to the absence of the Coq1 or Coq2 polypeptides.

Compound 5 is readily detected in wild-type yeast [30]. In fact, when cells are cultured in glucose containing media in the presence of [U-14C]-4-hydroxybenzoic acid and harvested at log phase, there is 4-fold more radioactivity recovered in 5 than in Q [30]. Compound 5 is also the predominant Q-intermediate in rat heart mitochondria [41]. While the accumulation of this intermediate may serve as a stockpile of a biosynthetic intermediate committed to Q, it may also act as an important component of the Q biosynthetic complex and aid in either the assembly or stability of such a complex. Precedent for this idea derives from the work by Knoell [42,43] in the study of Q biosynthesis in E. coli. Although the ubi mutants of E. coli each accumulate distinct intermediates that are diagnostic of the blocked step [44], there is evidence that the Ubi polypeptides form a complex together with a pool of the prokaryotic Q biosynthetic precursor, 2-octaprenyl phenol [42,43].

The genetic analysis of the different types of coq yeast mutants indicates that some components may play relatively peripheral roles in complex assembly or stability. For example, the yeast COQ3 gene can be functionally replaced by human, rat, Arabidopsis or E. coli COQ3 homologues [45–47,16]. In each case, the rescue is less optimal than with the wild-type yeast gene; for example, rescue with the human homologue requires its presence on a multi-copy plasmid, yet growth is much slower and levels of Q are decreased about 70% [45]. Similarly, the COQ7 gene can be functionally replaced by human, rat and Caenorhabditis elegans homologues, when present on multi-copy plasmids [48–50]. These studies suggest that certain polypeptide components are readily replaced by homologues from different species, and that their function in the complex assembly or stability may not be critical, or may be indirect (as postulated for Coq1p above). While the rescue of each of the other coq mutants is still under investigation, it is clear that the yeast COQ5 gene cannot be replaced by its E. coli homologue, ubiE. In fact, from the analysis of specific point mutant alleles, it is clear that the yeast COQ5 gene product plays a second function in stabilizing yeast Coq3p [19]. Thus, some of the components may provide crucial roles in the assembly or stability of the putative complex, while others may require the complex for their activity but are themselves only peripheral components.

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References


