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Characterization of the *COQ5* Gene from *Saccharomyces cerevisiae*

EVIDENCE FOR A *C*-METHYLTRANSFERASE IN UBIQUINONE BIOSYNTHESIS*

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Ubiquinone (coenzyme Q or Q) is a lipophilic metabolite that functions in the electron transport chain in the plasma membrane of prokaryotes and in the inner mitochondrial membrane of eukaryotes. Q-deficient mutants of *Saccharomyces cerevisiae* fall into eight complementation groups (*coq1–coq8*). Yeast mutants from the *coq5* complementation group lack Q and as a result are respiration-defective and fail to grow on nonfermentable carbon sources. A nuclear gene, designated *COQ5* was isolated from a yeast genomic library based on its ability to restore growth of a representative *coq5* mutant on media containing glycerol as the sole carbon source. The DNA segment responsible for the complementation contained an open reading frame (GenBank™ accession number Z49210) with 44% sequence identity over 262 amino acids to UbiE, which is required for a *C*-methyltransferase step in the Q and menaquinone biosynthetic pathways in *Escherichia coli*. Both the *ubiE* and *COQ5* coding sequences contain sequence motifs common to a wide variety of *S*-adenosyl-L-methionine-dependent methyltransferases. A gene fusion expressing a biotinylated form of Coq5p retains function, as assayed by the complementation of the *coq5* mutant. This Coq5-biotinylated fusion protein is located in mitochondria.

The synthesis of two farnesylated analogs of intermediates in the ubiquinone biosynthetic pathway is reported. These reagents have been used to develop *in vitro C*-methylation assays with isolated yeast mitochondria. These studies show that Coq5p is required for the *C*-methyltransferase step that converts 2-methoxy-6-polyprenyl-1,4-benzoquinone to 2-methoxy-5-methyl-6-polyprenyl-1,4-benzoquinone.

Ubiquinone (coenzyme Q or Q)[1] is a lipid that consists of a quinone head group and a polyprenyl tail that varies in length depending on the organism. The primary function of Q is to transport electrons from Complex I or II to the cytochrome bc_1 complex in the inner mitochondrial membrane of eukaryotes and the plasma membrane of prokaryotes (1). This cycle is carried out by a series of reductions and oxidations of the head group of Q. This same redox chemistry also allows reduced Q (QH_2) to scavenge electrons and function as a lipid-soluble antioxidant. In this latter capacity, QH_2 may scavenge lipid peroxyl radicals directly in an analogous manner to vitamin E (2–4), or it may help regenerate α-tocopherol (5, 6). Recent evidence suggests that QH_2 plays an important role *in vivo* in protecting cells from autoxidation products of polyunsaturated fatty acids (7). Higher levels of QH_2 in low density lipoproteins usually correspond to an increased resistance to lipid peroxidation (8–11). It has been suggested that the level of antioxidants, such as QH_2, in low density lipoproteins may slow the development of atherosclerosis, since oxidatively modified low density lipoproteins are thought to play a role in the initiation of the disease (12, 13). This action of QH_2 as an antioxidant may also impact other age-related degenerative diseases and the aging process itself (14, 15).

Q is synthesized from *p*-hydroxybenzoic acid and polyisoprenediphosphate in both prokaryotes and eukaryotes (16). The proposed pathway for the biosynthesis of Q was elucidated from the characterization of Q intermediates that accumulated in Q-deficient strains of both *Saccharomyces cerevisiae* and *Escherichia coli* (16, 17). It was shown that the pathways in these organisms diverge after formation of 3-polyprenyl-4-hydroxybenzoate. After three additional steps, the Q biosynthetic pathways in *E. coli* and *S. cerevisiae* are thought to converge. The *ubi* mutants of *E. coli* are grouped into eight complementation groups (*ubiA–ubiH*) and fail to grow on media containing succinate as the sole carbon source (17, 18). The Q-deficient strains of *S. cerevisiae* have also been grouped into eight complementation groups (*coq1–coq8*) and are nonrespiring and therefore fail to grow on nonfermentable carbon sources (19). The Q deficiency in the *coq* complementation groups was based on the observation that *in vitro* assays of cytochrome *c* reductase activity could be returned to almost wild-type levels by the addition of Q (20). Yeast *coq* mutant strains (representative mutants from *coq3–coq8* groups) when grown in the presence of *p*-[U-^{14}C]hydroxybenzoic acid, a biosynthetic precursor of Q, fail to produce Q and accumulate an early intermediate that corresponds to 3-hexaprenyl-4-hydroxybenzoic acid (21). The presence of this intermediate is not necessarily diagnostic of the affected biosynthetic step, since it also accumulates in wild-type yeast (21, 22). This phenomenon is not observed in the *E. coli* Q-deficient *ubi* mutants, which tend to accumulate large amounts of each distinct Q intermediate (17).

The current study employs the strategy of comparing both sequence and function of the yeast *COQ* and *E. coli ubi* gene products to study the *C*-methylation step in yeast Q biosynthe-

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[1] The abbreviations used are: Q, ubiquinone; QH_2, ubiquinol; DDMQ, demethyl demethoxy ubiquinone or 2-polyprenyl-6-methoxy-1,4-benzoquinone; DMQ, demethoxy ubiquinone or 2-methyl-3-polyprenyl-5-methoxy-1,4-benzoquinone; kb, kilobase pair(s); HMPA, hexamethylphosphoric triamide; $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)palladium(0); HPLC, high performance liquid chromatography; EIMS, electron impact mass spectrometry; Mp, melting point; R_F, retention factor.

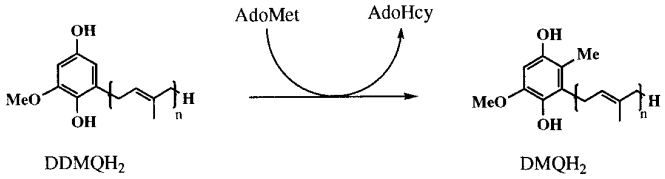

FIG. 1. **The *C*-methyltransferase reaction in Q biosynthesis.** The intermediates are 2-methoxy-6-polyprenyl-1,4-benzoquinol (*DDMQH$_2$*) and 2-methoxy-5-methyl-6-polyprenyl-1,4-benzoquinol (*DMQH$_2$*). In *S. cerevisiae*, $n = 6$; in *E. coli*, $n = 8$. *AdoMet*, *S*-adenosyl-L-methionine; *AdoHcy*, *S*-adenosyl-L-homocysteine.

sis (depicted in Fig. 1). This *C*-methyltransferase step was identified as being potentially defective in *E. coli ubiE* mutants, which accumulate the intermediate DDMQ (Fig. 1; Ref. 23). Searches of the literature have shown very few examples of *C*-methyltransferases, with most of the examples being in DNA/RNA and in steroid, corrin, and porphyrin biosynthesis (24). Recently, the *E. coli ubiE* gene has been identified and shown to harbor three sequence motifs common to a wide variety of *S*-adenosyl-L-methionine-dependent methyltransferase enzymes (25). The presence of an intact *ubiE* gene was required for production of Q *in vivo*, and disruption of the *ubiE* gene in a wild-type parental strain produced a mutant with growth defects on succinate and in both Q and menaquinone synthesis (25). The present study reports the isolation of a *ubiE* homolog from yeast, the *COQ5* gene, and provides evidence that it encodes a *C*-methyltransferase. To study the *C*-methylation step shown in Fig. 1, farnesylated analogs of the natural substrate and product were synthesized and used to develop an *in vitro* assay. The data presented indicate that the yeast Coq5 polypeptide is located in yeast mitochondria and is required for *C*-methylation in the synthesis of Q.

MATERIALS AND METHODS

Yeast and E. coli Strains and Growth Media—The genotypes and sources of the mutant and wild-type yeast strains used in this study are shown in Table I. Media for growth of yeast were prepared as described (29) and included YPD (1% yeast extract, 2% peptone, 2% dextrose), YPG (1% yeast extract, 2% peptone, 3% glycerol), YPGal (1% yeast extract, 2% peptone, 2% galactose), and YEPG (1% yeast extract, 2% peptone, 2% ethanol, 3% glycerol). The SD medium (0.18% yeast nitrogen base without amino acids, 2% dextrose, 0.14% NaH_2PO_4, 0.5% $(NH_4)_2SO_4$) was modified from the literature as described. Amounts of adenine, uracil, tryptophan, histidine HCl, and methionine were increased by a factor of 4; amounts of arginine HCl, isoleucine, and lysine HCl were doubled; phenylalanine and leucine were increased by 120%; tyrosine was increased by 130%; 80 mg/liter cysteine was added. SD-Leu and SD-Ura were SD media without leucine or uracil, respectively. 2% agar was added for solid media. Yeast were grown at 30 °C. The *E. coli* strain AN70 (*ubiE401*) harboring a mutation in the *ubiE* gene (23) was grown as described previously (25).

Cloning of the COQ5 Gene—C83/LH1 was transformed by the procedure of Beggs (30) with 5 μg of a yeast genomic library. The library was constructed by ligation to the *Bam*HI site of YEp13 of partial *Sau*3AI fragments (7–10 kb) of nuclear DNA isolated from the respiration-competent *S. cerevisiae* strain D273–10B. Two respiration-competent and leucine prototrophic clones were obtained from the transformation. Both phenotypes co-segregated, indicating their dependence on the presence of the autonomously replicating plasmid. The plasmid pG17/T1, isolated from one of the transformants, was used to characterize the complementing gene.

In a similar manner, CH83-B3 was transformed with the multiple-copy expression library prepared from yeast DNA in the vector YEp24 (31) containing the *URA3* gene as a selectable marker. Transformants were selected by plating onto SD-Ura medium and replica-plated after 2 days onto YPG medium to test for respiratory growth. One respiration-competent and uracil-prototrophic colony was obtained from the transformation, and the co-segregation of these phenotypes indicated the presence of a plasmid gene. Yeast plasmid DNA was recovered from the transformant and amplified in *E. coli* DH5α (Life Technologies, Inc.). Restriction enzyme mapping and Southern hybridization analysis of the recovered plasmid, pRB01, showed it to contain a segment of DNA that overlapped with the insert present in pG17/T1.

Subcloning and Disruption of the COQ5 Gene—pG17/ST1 was obtained by religation of pG17/T1 after removal of the 2-kb *Sph*I fragment. The 2-kb *Sph*I fragment containing one-half of the pG17/T1 insert and 200 base pairs of YEp13 sequence were subcloned into the vector YEp351 (32) to create pG17/ST2. Partial DNA sequence analysis of the 0.8-kb *Eco*RI fragment derived from pG17/ST1 showed the insert DNA to contain a segment of the chromosome XIII sequence reported by Skelton *et al.*[2] Based on the sequence of YEp13 and the reported open reading frame (accession number Z49210), the removal of the 2-kb *Sph*I fragment leads to the loss of the 28 amino-terminal residues and their substitution by the following sequence: MTQSAAGTCPTSCMIKKTV-ISAATIVMPRAHRKELTGLKALKGIGRRSPLCDSCIRKQPSSRLRPL-STAAARNG.

The disrupted *coq5* allele was constructed in pG17/T1 by substituting the 100-base pair *Bam*HI fragment internal to the gene with *HIS3* on a 1.7-kb *Bam*HI fragment (34). The resultant plasmid, pG17/ST3, was used to obtain a linear 3.5-kb *Hin*cII fragment with the disrupted gene. W303–1A and W303–1B were each transformed with 2 μg of DNA by the procedure of Schiestl *et al.* (35). Most of the histidine-independent clones issued from the transformations were respiration-defective and were complemented by ρ^0 but not by the *coq5* testers. Nuclear DNAs from two independent transformants, one with an "a" and the other with an α mating type were digested with *Eco*RI and separated on 1% agarose. Southern analysis of these independent transformants confirmed the presence of the *coq5::HIS3* allele in their chromosomal DNAs. The probe, an 800-base pair *Eco*RI fragment (Fig. 2) recognizes a 800-base pair fragment in the genomic DNA of the parental strain. The genomic DNA of the mutant strain, aW303ΔCOQ5, has a larger hybridizing species at approximately 2.5 kb, as expected for the disrupted allele.

Construction of Biotinylated Coq5p—The gene for biotinylated Coq5p was constructed by in-frame fusion of a polymerase chain reaction-amplified fragment containing the 5′-flanking and coding sequence to a bacterial sequence coding for the biotinylation site of transcarboxylase (36). The amplification was carried out with pG17/T1 as template, a forward primer (encoding the *Sac*I site) starting at nucleotide position −373, and a reverse primer starting at +907, in which the termination codon of *COQ5* was destroyed and replaced with a *Bgl*II site. The polymerase chain reaction product was digested with a combination of *Sac*I and *Bgl*II and ligated to the *Bam*HI and *Sac*I sites in the yeast/*E. coli* shuttle vector YEp352-Bio6 (33, 58). The fusion gene was introduced into W303ΔCOQ5 either on an autonomously replicating plasmid (pG17/ST5) or by integration of a single copy at the URA3 locus (pG17/ST6).

Localization of Biotinylated Coq5p—The wild-type strain W303–1B and the transformants W303ΔCOQ5/ST5 and aW303ΔCOQ5/ST5 were grown in YPGal (2% galactose, 1% yeast extract, 2% peptone) to early stationary phase. The cells were converted to spheroplasts by digestion with Zymolyase 20,000. Spheroplasts were lysed in 0.6 M sorbitol and debris, and unlysed cells were removed by centrifugation at 2,000 × *g* for 10 min. The mitochondria were sedimented at 12,000 × *g* for 15 min. Total mitochondrial and postmitochondrial supernatant proteins (20 μg) were loaded in each lane and separated in a 12% polyacrylamide gel prepared according to Laemmli (37). The proteins were transferred electrophoretically to nitrocellulose, and the Western blot was reacted first with avidin coupled to peroxidase. The blot was then stained with 4-chloro-1-naphthol in the presence of hydrogen peroxide. This procedure stains only proteins containing biotin.

Chemical Synthesis of Q Intermediates—All reagents for organic synthesis were purchased from Aldrich and used as received unless otherwise stated. Dichloromethane, hexamethylphosphoric triamide, and triethylamine were distilled from calcium hydride. Diethylether was distilled from sodium-benzophenone ketyl. Unless specified as dry, the solvents were of unpurified reagent grade. All air- or water-sensitive reactions were carried out under positive pressure of argon in oven-dried glassware. Reactions were followed by TLC using Whatman precoated plates of silica gel 60 with fluorescent indicator (0.25 mm). Reactions forming quinones were followed by leucomethylene blue stain. Flash chromatography was performed on Davisil Grade 643 silica gel (230–400 mesh). NMR spectra were measured on a Bruker AM360, ARX400, or ARX500 spectrometer and were recorded in ppm using the solvent signal as an internal standard. Mass spectra and high resolution mass spectra were recorded on a VG Autospec and are reported in units of mass to charge (*m/z*). High resolution mass spectra were re-

[2] J. Skelton, C. M. Churcher, B. Barrell, M. A. Rajandream, and S. V. Walsh, GenBank™ accession number Z49210.

TABLE I
Genotypes and sources of S. cerevisiae strains

Strain	Genotype	Source
D273–10B/A1	*MATα met6*	Ref. 26
LL20	*MATα his3 leu2–3,112*	Ref. 27
CB11	*MATα ade1*	Ref. 28
C83	*MATα met6 coq5–2*	Ref. 19
CH83-B3	*MATα coq5–2 ade2–1 his3–11 ura3–52*	C83 × W303–1A
B83	*MATα ade1 coq5–2*	C83 × CB11
C83/LH1	*MATα his3 leu2–3,112 coq5–2*	B83 × LL20
W303–1A	*MATα ade2–1 his3–1,15 leu2–3,112 trp1–1 ura3–1*	R. Rothstein[a]
W303–1B	*MATα ade2–1 his3–1,15 leu2–3,112 trp1–1 ura3–1*	R. Rothstein[a]
aW303ΔCOQ5	*MATα ade2–1 his3–1,15 leu2–3,112 trp1–1 ura3–1, coq5::HIS3*	This study
W303ΔCOQ5	*MATα ade2–1 his3–1,15 leu2–3,112 trp1–1 ura3–1, coq5::HIS3*	This study

[a] Dr. Rodney Rothstein, Department of Human Genetics, Columbia University.

corded with an EI source.

*2,5-Diacetoxy-3-methoxybromobenzene (Fig. 6, **2**)*—Vanillin (**1**) (2.00 g, 13.1 mmol) was dissolved in acetic acid (20 ml). Bromine (15.8 mmol, 1.0 M solution in CCl_4) was added slowly, and the reaction mixture was allowed to stir at room temperature overnight (12 h). Water was then added (20 ml), and the CCl_4 was removed *in vacuo*. The aqueous layer was adjusted to pH 5 with 1 M NaOH and extracted with ethyl acetate (2 × 20 ml). The combined organic extracts were washed with water (2 × 20 ml), 1.0 M $NaHCO_3$ (20 ml), and brine (20 ml), dried over $MgSO_4$, and filtered. After concentration, a combined mass of 2.61 g (86% yield) of orange crystalline 3-bromo-4-hydroxy-5-methoxybenzaldehyde was obtained. Mp: 157–159 °C. ^{1}H NMR ($CDCl_3$, 360 MHz) δ: 3.98 (s, 3H); 6.54 (br s, 1H); 7.36 (d, 1H, J = 1.7 Hz); 7.64 (d, 1H, J = 1.7 Hz); 9.78 (s, 1H). ^{13}C NMR ($CDCl_3$, 90 MHz) δ: 56.59; 107.95; 108.13; 129.98; 130.09; 148.85; 149.32; 189.70. IR (thin film on NaCl) (cm^{-1}): 3281; 1674; 1589; 1424; 1354; 1291; 1157; 1046; 681. HRMS calculated (Calcd) for $[C_8H_7BrO_3]^+$: 229.9579. Found: 229.9582.

The reaction vessel for the next step was soaked prior to reaction for 24 h in 0.1 M $NaOH/H_2O_2$ solution and rinsed with H_2O (38). 3-Bromo-4-hydroxy-5-methoxybenzaldehyde (1.00 g, 4.33 mmol) was dissolved in H_2O (20 ml) and treated with NaOH (381 mg, 9.53 mmol). Hydrogen peroxide (30% solution in H_2O; 1.08 ml, 9.53 mmol) was dissolved in H_2O (20 ml) and added dropwise to the reaction mixture. The reaction was stirred at room temperature for 90 min. The reaction mixture was then acidified to pH 3 with 10% HCl and extracted with ethyl acetate (2 × 40 ml). The combined extracts were washed with brine (40 ml) and dried over $MgSO_4$. Flash chromatography (R_F 0.3, 7:3 hexanes/ethyl acetate) was performed to give 557 mg (59% yield) of tan solid 2-bromo-4-hydroxy-6-methoxy phenol. ^{1}H NMR (Me_2SO-d_6, 360 MHz) δ: 3.69 (s, 3H); 6.43 (d, 1H, J = 2.4 Hz); 6.46 (d, 1H, J = 2.3 Hz); 8.57 (br s, 1H); 9.13 (br s, 1H). ^{13}C NMR (Me_2SO-d_6, 90 MHz) δ: 55.90; 99.96; 109.48; 109.62; 136.38; 149.20; 150.64. IR (thin film on NaCl) (cm^{-1}): 3258; 1615; 1470; 1429; 1294; 1190; 1134; 1035; 828. HRMS Calcd for $[C_7H_7BrO_3]^+$: 217.9579. Found: 217.9577.

2-Bromo-4-hydroxy-6-methoxy phenol (250 mg, 1.15 mmol) was suspended in CH_2Cl_2 (2.5 ml). Triethylamine (0.56 ml, 4.12 mmol) and acetic anhydride (0.33 ml, 3.44 mmol) were then added, followed by 4-(dimethylamino)pyridine (~15 mg). The reaction was allowed to proceed for 7 h at room temperature. The reaction was quenched with NH_4Cl, and the CH_2Cl_2 was removed *in vacuo*. The residue was extracted with ethyl acetate (2 × 5 ml), and the combined extracts were washed with brine (5 ml), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (R factor 0.3, 8:2 hexanes/ethyl acetate) was performed to obtain 330 mg (95% yield) colorless oil **2** in Fig. 6. ^{1}H NMR ($CDCl_3$, 360 MHz) δ: 2.25 (s, 3H); 2.22 (s, 3H); 3.77 (s, 3H); 6.68 (d, 1H, J = 1.8 Hz); 6.95 (d, 1H, J = 1.8 Hz). ^{13}C NMR ($CDCl_3$, 90 MHz) δ: 20.21; 20.82; 56.16; 105.65; 116.73; 117.19; 135.56; 148.66; 152.48; 167.65; 168.73. IR (thin film on NaCl) (cm^{-1}): 3020; 1771; 1601; 1482; 1412; 1369; 1204; 1175; 1134; 1044; 1015. HRMS Calcd for $[C_{11}H_{11}BrO_5]^+$: 301.9790. Found: 301.9792.

*2-Methoxy-6-farnesyl-1,4-benzoquinone (**3**)*—Tetrakis(triphenylphosphine)palladium (0) ($Pd(PPh_3)_4$; 23 mg, 0.020 mmol) was dissolved in hexamethylphosphoric triamide (HMPA; 1.0 ml), in a Schlenk tube under argon in a glove box. Compound **2** (305 mg, 1.00 mmol) was dissolved in HMPA (1.0 ml), and the resulting solution was added via syringe to the solution containing $Pd(PPh_3)_4$ (39). A solution of farnesyl tributylstannane (743 mg, 1.5 mmol) (40) dissolved in HMPA (1.5 ml) was then added to this reaction mixture. The reaction vessel was sealed under argon, removed from the glove box, and heated at 65 °C in a sand bath for 48 h. The reaction was then quenched with saturated NH_4Cl (3 ml) and extracted with ether (3 × 5 ml). The ethereal layers were washed with H_2O (5 ml) and brine (5 ml), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography was performed (R_F 0.5, 9:1 hexanes/ethyl acetate) to give 322 mg (75% yield) of pale yellow oil 1,4-diacetoxy-2-farnesyl-6-methoxybenzene. ^{1}H NMR ($CDCl_3$, 360 MHz) δ: 1.60 (s, 6H); 1.60 (s, 6H); 2.05 (m, 8H); 2.26 (s, 3H); 2.29 (s, 3H); 3.22 (d, 2H, J = 7.2 Hz); 3.77 (s, 3H); 5.09 (m, 2H); 5.21 (m, 1H); 6.55 (d, 1H, J = 2.6 Hz); 6.58 (d, 1H, J = 2.6 Hz). ^{13}C NMR ($CDCl_3$, 90 MHz) δ: 13.55; 15.93; 16.12; 17.17; 17.62; 20.39; 21.07; 25.61; 26.42; 26.69; 26.79; 27.79; 28.41; 39.58; 39.66; 56.00; 104.04; 113.79; 120.71; 123.97; 124.33; 131.21; 135.09; 135.49; 135.60; 137.32; 148.47; 151.49; 168.63; 169.32. IR (thin film on NaCl) (cm^{-1}): 2921; 1767; 1483; 1466; 1427; 1370; 1210; 1175; 1136; 1020. HRMS Calcd for $[C_{26}H_{36}O_5]^+$: 428.2563. Found: 428.2571.

$LiAlH_4$ (67 mg, 1.75 mmol) was suspended in ether (5 ml) and cooled to 0 °C. 1,4-Diacetoxy-2-farnesyl-6-methoxybenzene (150 mg, 0.350 mmol) was dissolved in ether (10 ml) and added dropwise to the suspension. The resulting reaction mixture was stirred for 1 h. The reaction was quenched with water (5 ml) and then 1 M NaOH (5 ml), followed by water (5 ml) and allowed to warm to room temperature. A colorless precipitate formed and was extracted with ether (2 × 15 ml), and the combined ethereal extracts were washed with brine (30 ml), dried over $MgSO_4$, filtered, and concentrated. The expected hydroquinone was oxidized during basic work-up, and a bright yellow oil (**3**) was obtained (110 mg, 92% yield). ^{1}H NMR ($CDCl_3$, 360 MHz) δ: 1.57 (s, 6H); 1.61 (s, 3H); 1.65 (s, 3H); 2.02 (m, 8H); 3.11 (d, 2H, J = 7.2 Hz); 3.79 (s, 3H); 5.06 (m, 2H); 5.13 (m, 1H); 5.85 (d, 1H, J = 2.4 Hz); 6.42 (d, 1H, J = 2.4 Hz). ^{13}C NMR ($CDCl_3$, 90 MHz) δ: 15.96; 16.08; 17.60; 25.63; 26.32; 26.61; 27.07; 39.56 (two signals); 56.21; 101.01; 117.62; 123.67; 124.27; 131.18; 132.73; 135.33; 140.05; 146.34; 158.80; 182.11; 187.64. IR (thin film on NaCl) (cm^{-1}): 3451; 2919; 2851; 1682; 1651; 1603; 1454; 1233; 1176; 1045; 911; 773. HRMS Calcd for $[C_{22}H_{30}O_3]^+$: 342.2195. Found: 342.2192. UV λ_{max} (nm): 264, 206.

*2-Methoxy-5-methyl-1,4-benzoquinone (**5**)*—To a solution of KH_2PO_4 (4 g, 29.4 mmol) in 400 ml of water was added freshly prepared potassium nitrosodisulfonate (6 g, 22.4 mmol) (41, 42). To this purple solution, 2-methoxy-5-methylaniline (**4**) (1.68 g, 12.2 mmol) in 50 ml of acetone was added. The reaction was stirred at 23 °C for 1.5 h, during which the color changed to reddish green. The reaction was extracted three times with 100 ml of chloroform. The chloroform was dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. The resulting solid was washed five times with cold ether (50-ml aliquots), leaving a yellowish green solid (**5**) (1.21 g, 7.95 mmol, 65%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 6.55 (d, 1H, J = 1.6 Hz), 5.92 (s, 1H), 3.18 (s, 3H), 2.06 (d, 3H, J = 1.6 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 187.66, 182.13, 158.71, 146.87, 131.26, 107.56, 56.22, 15.78; EIMS 152.05 [M^+], 137.03, 122.04; HRMS Calcd for $[C_8H_8O_3]^+$: 152.047344. Found: 152.047276.

*2-Methoxy-5-methyl-6-farnesyl-1,4-benzoquinone (**6**)*—To a solution of compound **5** (120 mg, 0.8 mmol) in 10 ml of dry 1,4-dioxane was added zinc powder (200 mg, 3 mmol) and 4 drops of concentrated HCl. The HCl was added dropwise followed by shaking until the solution turned nearly colorless. The zinc was precipitated by centrifugation, and the supernatant was transferred to a separate flask. To this flask was added BF_3-OEt_2 (0.89 g, 6.3 mmol) followed by *trans*,*trans*-farnesol (0.35 g, 1.6 mmol). The reaction was stirred at 23 °C for 16 h. The reaction was quenched by the addition of 20 ml of 1 M $NaHCO_3$. The reaction was extracted three times with ether, and the combined organic layers were dried over $MgSO_4$, filtered and concentrated by rotary evaporation. The remaining oil was taken up in 15 ml of benzene, and to it was added $FeCl_3$ (400 mg, 2.5 mmol) in 15 ml of water. This reaction was allowed to stir for 3 h, and then the organic layer was extracted, washed three times with water, dried over $MgSO_4$, filtered,

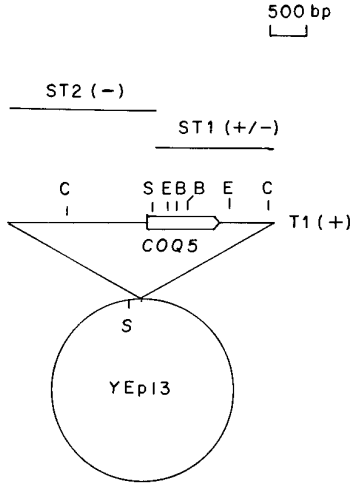

FIG. 2. **Restriction maps of pG17/T1 and derivative plasmids.** A partial restriction map of the pG17/T1 insert shows the locations of the sites for *Hin*cII (*C*), *Sph*I (*S*), *Eco*RI (*E*), and *Bam*HI (*B*). The subclones pG17/ST1 (*ST1*) and pG17/ST2 (*ST2*) are described under "Materials and Methods."

and concentrated by rotary evaporation. The crude product was purified by flash chromatography (9:1 hexanes:ethyl acetate) to afford 40 mg of both demethoxyubiquinone-3 and isodemethoxyubiquinone-3 (14.2% yield for each). TLC: R_F 0.33 (3:1 hexanes:ethyl acetate); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.87 (s, 1H), 5.06 (m, 2H), 4.94 (t, 1H, J = 1.2 Hz), 3.78 (s, 3H), 3.21 (d, 2H, J = 7 Hz), 2.04 (s, 3H), 1.93–2.10 (m, 8H), 1.74 (s, 3H), 1.67 (s, 3H), 1.58 (s, 3H), 1.56 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 187.81, 181.89, 158.34, 141.80, 141.34, 137.69, 135.2, 131.29, 124.33, 123.85, 118.80, 107.00, 56.11, 39.69, 29.24, 26.74, 26.43, 25.71, 25.27, 17.68, 16.34, 16.02, 12.19; EIMS 356.2 [M^+], 267.0, 245.1, 220.1, 205.1, 189.2, 167.1, 136.1, 121.1; HRMS Calcd for $[C_{23}H_{32}O_3]^+$: 356.235145. Found: 356.235365.

2-Methoxy-3-farnesyl-5-methyl-1,4-benzoquinone—TLC: R_F 0.31 (9:1 hexanes:ethyl acetate); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.42 (d, 1H, J = 1.6 Hz), 5.06 (m, 3H), 4.00 (s, 3H), 3.15 (d, 2H, J = 7.3 Hz), 2.03 (d, 3H, J = 1.6 Hz), 1.93–2.10 (m, 8H), 1.74 (s, 3H), 1.67 (s, 3H), 1.58 (s, 3H), 1.56 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 188.05, 184.08, 155.16, 145.65, 137.06, 135.05, 131.92, 131.44, 131.28, 124.32, 123.98, 119.94, 60.97, 39.69, 26.73, 26.46, 25.68, 22.55, 17.67, 16.15, 15.99, 15.82; EIMS 356.2 [M^+], 317.0, 267.0, 245.1, 220.1, 205.1, 189.2, 162.0, 136.1; HRMS Calcd for $[C_{23}H_{32}O_3]^+$: 356.235145. Found: 356.234814.

Preparation of Yeast Mitochondria and in Vitro Assays of C-Methyltransferase—Mitochondria were isolated by the method of Daum *et al.* (43) except that Oxalyticase (Enzogenetics) was substituted for Zymolyase. Mitochondria were either used immediately or stored for later use at −80 °C after being frozen in liquid nitrogen. Protein concentration was determined with the BCA method (Pierce). Each methylation reaction (250 μl) contained 1 mM $ZnSO_4$, 500 μM substrate (2-methoxy-6-farnesyl-1,4-benzoquinone) in methanol (5 μl total), 100 μl of yeast mitochondria (containing 1–4 mg of total protein), and 0.05 M sodium phosphate, pH 7.0. The concentration of NADH was 3.0 mM in reactions where it was required. The reaction was initiated by the addition of *S*-adenosyl-[*methyl*-^{3}H]L-methionine to a final concentration of 20 μM (DuPont NEN, 84.1 Ci/mmol; specific activity adjusted to 560 mCi/mmol with unlabeled *S*-adenosyl-L-methionine; ε 15,200 M^{-1} cm^{-1}, 256 nm, pH 1; Ref. 44). Following a 1-h incubation at 30 °C, the reaction was quenched by the addition of 2 μl of glacial acetic acid. The lipids were extracted with pentane (500 μl, twice), concentrated, and resuspended in methanol. The extracts were analyzed on a reverse-phase HPLC column (Alltech Lichrosorb C-18; 5 μm, 4.6 × 250 mm) with 9:1 methanol:water as a mobile phase at a rate of 1 ml/min (22). Each 1-ml fraction was analyzed for radioactivity by scintillation counting with Safety Solve (Research Products International Corp.) as a scintillation fluor.

E. coli Cell-free Extracts and C-Methyltransferase Assay—E. coli cell-free extracts were prepared as described previously (45). Each reaction was carried out the same way as described for the yeast mitochondrial *in vitro* assay with the following changes: 1) the DDMQ substrate concentration was reduced to 100 μM; and 2) following a 1-h incubation at 37 °C, lipids were extracted with hexane (0.5 ml, twice), concentrated, and resuspended in methanol.

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ATGGAAACTAGCTTCCGCATTTGACAAAAACTGAAAAAGTGGAAAAAAAAG -102
GATCACACAAGAAACTATAAGGATATCTGCCACCTTCACTCCTTATACAGC -51
TGCTGTTACAATTGCAAAGGGAATAGTACAGATCGCAGCAAGAAAGATATA -1
ATGTTGATTTCTTCACGGATCGTTCGAAGCTCGCTGGTAAATGTCCCGCTA 51
 M  L  I  S  S  R  I  V  R  S  S  L  V  N  V  P  L
AGATTATCTAGGTGTTTTACGCAAGCTCACAGAGCATGCAAAGAAGAAGAA 102
 R  L  S  R  C  F  T  Q  A  H  R  A  C  K  E  E  E
GTTAATAGTCCTTTATCATCCGCAGCTGAACAGCCAGAGCAGAAGTATACG 153
 V  N  S  P  L  S  S  A  A  E  Q  P  E  Q  K  Y  T
CATTTTGGTTCGAAGACTGTATTGAAGTCTACCAAGCAGAAGTTAGTTGGT 204
 H  F  G  S  K  T  V  L  K  S  T  K  Q  K  L  V  G
GATGTCTTTTCTTCCGTGGCCAATCGGTATGACTTGATGAATGATGTTATG 255
 D  V  F  S  S  V  A  N  R  Y  D  L  M  N  D  V  M
TCATTAGGAATTCATAGATTGTGGAAGGACCATTTTATCAATAAACTAGAT 306
 S  L  G  I  H  R  L  W  K  D  H  F  I  N  K  L  D
GCGGGAAAAAGGCCAAACTCTACGACTCCTTTGAACTTCATAGATGTGGCT 357
 A  G  K  R  P  N  S  T  T  P  L  N  F  I  D  V  A
GGGGGATCCGGTGATATTGCTTTCGGATTACTAGACCATGCTGAGTCGAAA 408
 G  G  S  G  D  I  A  F  G  L  L  D  H  A  E  S  K
TTTGGTGACACTGAGTCTACAATGGATATTGTAGATATCAACCCTGACATG 459
 F  G  D  T  E  S  T  M  D  I  V  D  I  N  P  D  M
CTTAAAGAAGGTGAGAAGAGAGCCATGGAACAAGGAAAATATTTCAAGGAT 510
 L  K  E  G  E  K  R  A  M  E  Q  G  K  Y  F  K  D
CCTCGTGTGAGATTTTTGGTTTCTAATGGTGAGAAACTAGAGGAGATTGAT 561
 P  R  V  R  F  L  V  S  N  G  E  K  L  E  E  I  D
TCTGATTCCAAGGACATCTACACAGTCTCCTTCGGTATCAGAAATTTCACC 612
 S  D  S  K  D  I  Y  T  V  S  F  G  I  R  N  F  T
GATATTCAAAAGGGTTTAAACACTGCTTATAGAGTTTTGAAACCGGGCGGT 663
 D  I  Q  K  G  L  N  T  A  Y  R  V  L  K  P  G  G
ATTTTTTATTGTCTAGAATTTTCCAAAATTGAGAATCCCCTAATGGACTTT 714
 I  F  Y  C  L  E  F  S  K  I  E  N  P  L  M  D  F
GCTTACCAACAGTGGGCTAAGGTCCTACCTGTAATGGGCTCGATGATTGCT 765
 A  Y  Q  Q  W  A  K  V  L  P  V  M  G  S  M  I  A
AATGACTACGACTCTTACCAGTATTTGGTGGAGTCTATCGAAAGATTTCCT 816
 N  D  Y  D  S  Y  Q  Y  L  V  E  S  I  E  R  F  P
GACCAAGAAACGTTCAAATCCATGATTGAGAAGGCAGGATTCAAATCTGCT 867
 D  Q  E  T  F  K  S  M  I  E  K  A  G  F  K  S  A
GGCTACGAAAGTTTAACTTTTGGTATATGTGCCATCCATTGGGGCATTAAA 918
 G  Y  E  S  L  T  F  G  I  C  A  I  H  W  G  I  K
GTTTAAAGTAAATAGAACAAATTTCCTTTTTTTTTTTGTCAAGAAAATCGAA 924
 V  .
GCTAGTAAAACATG
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FIG. 3. **Nucleotide sequence of *COQ5* and flanking regions.** The three methyltransferase motifs identified by Kagan and Clarke (24) are *underlined*.

RESULTS

Cloning and Sequencing of the COQ5 Gene—The *coq5* complementation group (G17) is composed of 20 independent isolates that contain recessive nuclear mutations resulting in the loss of respiration and Q synthesis (19). To learn more about the affected step in Q biosynthesis in the *coq5* mutant, the *COQ5* gene was cloned by transformation of either C83/LH1 or CH83-B3 with yeast genomic DNA as described under "Materials and Methods." Three respiration-competent transformants were obtained, one of which was used to characterize the complementing gene. This transformant contained a plasmid with a nuclear DNA insert of 3.8 kb. A partial restriction map is presented in Fig. 2. Subcloning analysis of the insert of pG17/T1 indicated that pG17/ST2 did not complement C83/LH1, while pG17/ST1 did complement, but the growth of these

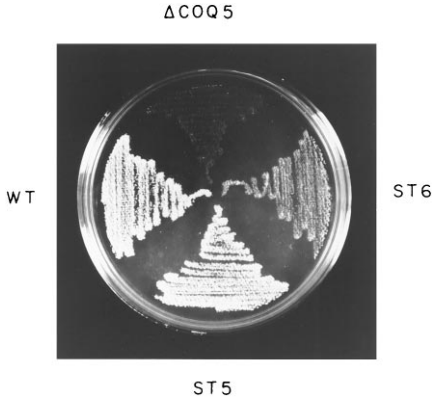

FIG. 4. **Complementation of a *coq5* mutant by biotinylated Coq5p.** The four strains of yeast were spread on rich glucose (YPD) and replicated on rich glycerol medium (YEPG) after overnight growth. The photograph of the plate was taken 24 h after replication. The strains used were W303–1B (*WT*), W303ΔCOQ5 (Δ*COQ5*), a transformant of W303ΔCOQ5 harboring the fusion gene on a multicopy plasmid (*ST5*), and the same mutant transformed with the fusion gene by homologous recombination at the *URA3* locus (*ST6*).

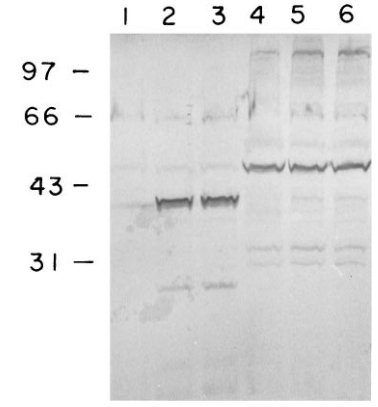

FIG. 5. **Expression of COQ5-BIO.** Biotinylated Coq5p migrates at 40–41 kDa. *Lane 1*, W303–1B mitochondria; *lane 2*, W303ΔCOQ5/ST5 mitochondria; *lane 3*, aW303ΔCOQ5/ST5 mitochondria; *lane 4*, W303–1B postmitochondrial supernatant; *lane 5*, W303ΔCOQ5/ST5 postmitochondrial supernatant; *lane 6*, aW303ΔCOQ5/ST5 postmitochondrial supernatant. The migrations of known size standards are marked on the *left*.

transformants on glycerol was slower than the ones containing pG17/T1. DNA sequence analysis of part of the 0.8-kb *Eco*RI fragment identified an open reading frame encoding a 34.7-kDa hypothetical protein (Fig. 3), located on chromosome XIII as reported by Skelton *et al.*[2] Based on this sequence, the construction of the pG17/ST1 clone leads to the loss of 28 amino-terminal residues from the coding region indicated in Fig. 3 and their substitution with a 74-residue amino-terminal extension derived from YEp13 DNA (see "Materials and Methods"). The expression of this modified version of Coq5p is presumably responsible for the slow growth phenotype observed when the CH83/LH1 mutant strain is transformed with pG17/ST1 multicopy plasmid. Analysis of the *COQ5* open reading frame identified three methyltransferase sequence motifs (Fig. 3) that are present in a large family of methyltransferases that use *S*-adenosyl-L-methionine as the methyl donor (24). The amino acid sequence of the Coq5 polypeptide is 44% identical over 262 amino acids with the *E. coli* UbiE polypeptide, which is required for the *C*-methylation reaction in Q biosynthesis (25). This degree of sequence identity and the presence of the methyltransferase motifs suggests that the Coq5 polypeptide is likely to function as a *C*-methyltransferase in eukaryotic Q biosynthesis.

In Situ Disruption of the COQ5 Gene—The one-step gene replacement procedure (34) was used to obtain the *coq5* null mutant strains aW303ΔCOQ5 and W303ΔCOQ5, as described under "Materials and Methods." These yeast strains are both histidine-prototrophic and respiration-deficient and are complemented by a ρ^0 strain but not by other G17 mutants. These results imply a genetic linkage of the *coq5::HIS3* null and the *coq5–1* mutant alleles. To verify this, C83/LH1 was transformed with a linear fragment of DNA containing the *coq5::HIS3* allele. Two histidine-prototrophic transformants obtained from the transformation were crossed to W303–1A. Diploid cells obtained from the crosses were sporulated on potassium acetate plates and subjected to tetrad analysis. Meiotic progeny from 11 complete tetrads derived from each cross were tested for respiration and histidine dependence. Both phenotypes segregated 2:2 in the 22 tetrads analyzed. In all cases, the respiration-deficient spores were histidine-independent while the respiration-competent spores were histidine auxotrophs, confirming the allelism between the cloned *COQ5* gene and the original *coq5–1* mutation.

Localization of a Biotinylated COQ5 Fusion Protein—The intracellular localization of Coq5p was studied in cells expressing the *COQ5* gene fused in-frame with a sequence coding for the biotinylation signal of a bacterial transcarboxylase. The fusion gene (*COQ5-BIO*) was expected to express a hybrid protein consisting of the native sequence of Coq5p, with a carboxyl-terminal extension of 78 residues containing covalently attached biotin. Transformation of the W303ΔCOQ5 null mutant with the fusion gene in the multicopy plasmid pG17/ST5 conferred wild-type growth properties on the nonfermentable substrate glycerol (Fig. 4). The fusion gene also complements when inserted into chromosomal DNA in single copy, although the latter transformants grow more slowly on glycerol. These results indicate the carboxyl-terminal addition of the bacterial sequence with covalently attached biotin only partially compromises the activity of the protein.

The distribution of biotinylated Coq5p was examined in transformants overexpressing the fusion protein from the multicopy plasmid pG17/ST5. Total mitochondrial and postmitochondrial proteins of the parental wild type and two independent transformants (W303ΔCOQ5/ST5 and aW303ΔCOQ5/ST5) were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose. Biotinylated proteins were detected by probing the Western blot with peroxidase coupled to avidin. These analyses indicated the presence of an abundant biotinylated protein in the transformants with an estimated mass of 40–41 kDa (Fig. 5). This protein is not detected in the postmitochondrial supernatant fraction, suggesting a mitochondrial localization. The size of the biotinylated Coq5p measured from its migration is in good agreement with a molecular weight of 42,302 calculated from the amino acid composition. Since the amino-terminal sequence has the characteristic features of mitochondrial import signals (46), it is likely that the primary translation product is proteolytically cleaved during transport into mitochondria, thus accounting for the slight difference in size.

C-Methylation Assays—To determine whether the Coq5 polypeptide is required for the *C*-methylation of DDMQ to form DMQ, farnesylated analogs of both were synthesized chemically (Fig. 6). The farnesylated analog of DDMQ (**3**) was tested as a substrate in an *in vitro* methylation assay (Fig. 7). Mitochondria isolated from the parental wild type (W303–1B) were incubated with **3** and the methyl donor *S*-[*methyl*-^{3}H]adenosyl-L-methionine, and the organic extract was analyzed by reverse-phase HPLC. The radioactive methylated product co-eluted with the corresponding chemically synthesized methylated product DMQ (Fig. 7*A*). *In vitro* assays performed with mitochondria isolated from the *coq5* null mutant (W303ΔCOQ5) produced no detectable methyltransferase activity over back-

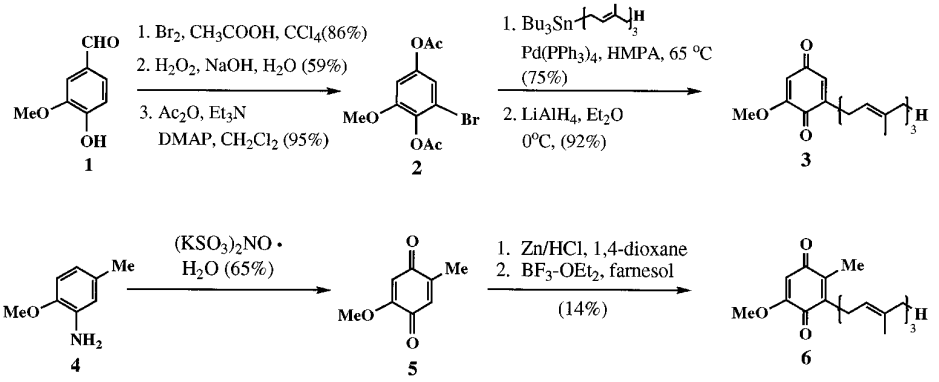

FIG. 6. **Scheme for the chemical synthesis of DDMQ and DMQ.** The farnesylated analogs of DDMQ and DMQ are designated as compounds **3** and **6**, respectively.

ground (Fig. 7*B*). Transformation of the *coq5* null mutant with the multicopy plasmid containing the yeast *COQ5* gene restored the ability to form the methylated product and in fact generated a significantly higher level of activity (0.38 pmol of product ·mg of protein^{-1}·h^{-1}) as compared with wild type (0.05 pmol of product·mg of protein^{-1}·h^{-1} (Fig. 7*C*). Assays in which either mitochondria or substrate (**3**) were omitted showed no methyltransferase activity. NADH was a necessary component of the assays, since in the absence of NADH the methyltransferase activity was reduced. The addition of NADH to the *in vitro* methylation reaction restored activity to normal levels (data not shown). A similar dependence of methylated product formation on the presence of the UbiE polypeptide was observed in *in vitro C*-methyltransferase assays with *E. coli* cell lysates. Cell-free extracts prepared from the *E. coli* strain AN70 harboring a mutation in the *ubiE* gene fail to generate detectable methylated product, while the same mutant rescued with a plasmid harboring the *ubiE* gene produces a methylated product that co-elutes with the DMQ standard (data not shown).

DISCUSSION

This study describes the isolation and characterization of the *COQ5* gene. The *COQ5* gene was recovered from a yeast genomic library on the basis of its ability to restore growth of a *coq5* mutant strain on glycerol, a nonfermentable carbon source. A segment of DNA sequence from the region responsible for complementation was identical to a previously reported *S. cerevisiae* open reading frame encoding a hypothetical 34.7-kDa protein.[2] This open reading frame contained 44% identity over 262 amino acid residues to UbiE, which is required for the *C*-methylation reactions in both Q and menaquinone biosynthesis in *E. coli* (23, 25, 47). Both the *E. coli* UbiE and yeast Coq5 amino acid sequences contain three structural motifs that appear to be conserved in most methyltransferases that use *S*-adenosyl-L-methionine as a methyl donor (24). Recent crystal structures suggest that these motifs either contact *S*-adenosyl-L-methionine or are used as structural scaffolding for other residues that contact it (48). Based on the presence of these motifs, and the degree of sequence identity between Coq5p and UbiE, it seems likely that *COQ5* and *ubiE* correspond to the structural genes encoding the *C*-methyltransferase enzyme in Q biosynthesis in yeast and *E. coli*, respectively.

The *in vitro* methyltransferase assays provide further evidence that Coq5p and UbiE catalyze a *C*-methyltransferase step in Q biosynthesis. These assays employed farnesylated analogs of both DDMQ and DMQ, and the syntheses presented for these two analogs yield large amounts of product from very inexpensive starting materials. The data presented indicate that both Coq5p and UbiE catalyze the step converting DDMQ to DMQ. These studies suggest that the length of the isoprene tail does not play a crucial role in substrate recognition, since normal substrates for this *C*-methylation contain eight and six

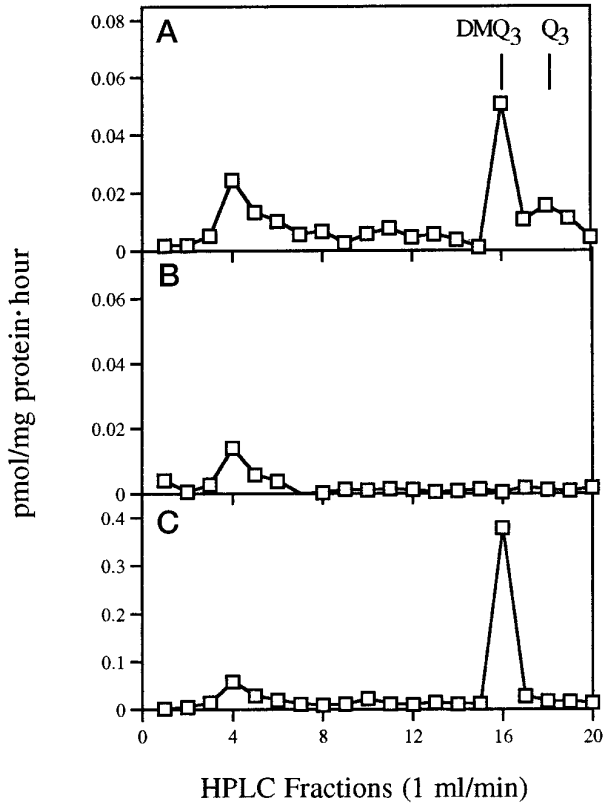

FIG. 7. ***In vitro* methylation of DDMQH$_2$ requires the presence of Coq5p.** Mitochondria were isolated from W303–1B (wild type, *panel A*), W303ΔCOQ5 harboring the vector YEp13 (*coq5* null mutant, *panel B*) or from W303ΔCOQ5 harboring pG17/T1 (rescued *coq5* mutant, *panel C*). *In vitro* methyltransferase assays were performed with the addition of DDMQ (**3** in Fig. 6) as the methyl acceptor substrate and *S*-adenosyl-[*methyl*-^{3}H]L-methionine. Radioactivity associated with the lipid extract of the *in vitro* assays was separated by reverse-phase HPLC. The standards corresponding to the methylated product, DMQ (**6** in Fig. 6), and Q$_3$ eluted in fractions 16 and 18, respectively.

isoprene units, in *E. coli* and *S. cerevisiae*, respectively. Several other studies also indicate the isoprenoid tail length does not play a crucial role in enzyme recognition (45, 49–51). In addition, a respiration-competent *S. cerevisiae* strain has been generated that produces only Q$_8$, indicating that a specific tail length of Q is not a crucial aspect of either the biosynthesis of Q or its respiratory function *in vivo* (52).

The *in vitro* assays with yeast mitochondria and *E. coli* lysates indicate that NADH is required for optimal activity. In assays with freshly prepared yeast mitochondria, the addition of NADH to 3 mM final concentration increases the rate of product formation by a factor of 2.3. However, in assays with mitochondria subjected to one freeze/thaw cycle, the addition of

NADH increased methylated product formation by a factor of 2.3–6.4. The addition of NADH to such freeze/thawed samples restored levels of methyltransferase activity to those observed with freshly prepared mitochondria (data not shown). The lower level of activity in the assays with the mitochondria subjected to a freeze/thaw cycle may be due to the oxidation or loss of endogenous NADH. The methylated products were recovered in quinone form due to autoxidation. A similar dependence on NADH has been reported for the *O*-methylation of demethyl-Q to form Q in isolated rat liver mitochondria (53). Reduction of the demethyl-Q substrate to the hydroquinone (demethyl-QH_2) eliminated the requirement for NADH in the *O*-methylation reaction. It is likely that the NADH is serving a similar function in the *C*-methylation assays and is acting to reduce the oxidized DDMQ substrate to the hydroquinone form ($DDMQH_2$), which presumably acts as the methyl acceptor.

To determine the localization of Coq5p, a fusion gene of *COQ5* and a sequence coding for the carboxyl-terminal biotinylation site of a bacterial transcarboxylase was constructed. This fusion gene complements the growth deficiency of the *coq5* mutant on glycerol, indicating that the fusion protein retains activity. The detection of the fusion protein in isolated mitochondria suggests that Coq5p is located in the mitochondria. In addition, the amino terminus of Coq5p is rich in positively charged and hydroxylated residues (11 of the first 30 residues are Ser, Thr, or Arg), a characteristic of leader sequences directing polypeptide import into mitochondria (46). The mitochondrial localization of Coq5p is significant because the localization of eukaryotic Q biosynthesis has been reported to occur in several different subcellular locations, including the endoplasmic reticulum, Golgi, and mitochondria (54–57).

The ability of *E. coli ubiE* to rescue a yeast mutant harboring the *coq5–2* allele was evaluated. Transformation of this strain with a single copy plasmid containing *E. coli ubiE* fused to an amino-terminal mitochondrial leader sequence and expressed from the yeast *CYC1* promoter restored growth on glycerol (data not shown). This same *CYC1*-mitochondrial leader-*ubiE* construct also rescued the *E. coli ubiE* mutant strain, AN70 (25). The functional complementation of the yeast *coq5* mutant by *ubiE* provides another line of evidence that these two gene products have the same function. Such interspecific functional complementation of the yeast *coq* mutants has been observed previously. For example, analogous constructs prepared with *ubiG* rescued the *coq3* yeast mutant (45), expression of the *E. coli* polyprenyl diphosphate synthase gene rescued the yeast *coq1* mutant (52), and expression of the rat *COQ3* and *COQ7* cDNAs rescued the corresponding yeast *coq* mutants (50, 51).

The availability of the yeast *coq* mutants provides the basis for the isolation and characterization of the *COQ* genes and their products in both yeast and mammals. The generation of synthetic analogs of Q intermediates provides reagents that serve as substrates for *in vitro* assays of enzyme activities. This approach as used in the current study shows that both *E. coli* UbiE and yeast Coq5 polypeptides are required for the *C*-methylation step in Q biosynthesis. The extent to which these gene products can independently act as *C*-methyltransferases is currently under investigation.

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