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Isolation and Functional Expression of Human *COQ3*, a Gene Encoding a Methyltransferase Required for Ubiquinone Biosynthesis*

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The *COQ3* gene in *Saccharomyces cerevisiae* encodes an *O*-methyltransferase required for two steps in the biosynthetic pathway of ubiquinone (coenzyme Q, or Q). This enzyme methylates an early Q intermediate, 3,4-dihydroxy-5-polyprenylbenzoic acid, as well as the final intermediate in the pathway, converting demethyl-Q to Q. This enzyme is also capable of methylating the distinct prokaryotic early intermediate 2-hydroxy-6-polyprenyl phenol. A full-length cDNA encoding the human homologue of *COQ3* was isolated from a human heart cDNA library by sequence homology to rat *Coq3*. The clone contained a 933-base pair open reading frame that encoded a polypeptide with a great deal of sequence identity to a variety of eukaryotic and prokaryotic Coq3 homologues. In the region between amino acids 89 and 255 in the human sequence, the rat and human homologues are 87% identical, whereas human and yeast are 35% identical. When expressed in multicopy, the human construct rescued the growth of a yeast *coq3* null mutant on a nonfermentable carbon source and restored coenzyme Q biosynthesis, although at lower levels than that of wild type yeast. *In vitro* methyltransferase assays using farnesylated analogues of intermediates in the coenzyme Q biosynthetic pathway as substrates showed that the human enzyme is active with all three substrates tested.

Ubiquinone, also known as coenzyme Q (Q),[1] is a critical component of the electron transport pathways of both eukaryotes and prokaryotes (1). This lipid consists of a hydrophobic isoprenoid tail and a quinone head group. The tail varies in length depending on the organism studied, but its purpose is to anchor Q to the membrane. The quinone head group is responsible for the activity of Q in the respiratory chain. It undergoes reversible redox cycling between the quinone (Q), semiquinone, and hydroquinone (QH_2) forms. In eukaryotes, Q acts in the inner mitochondrial membrane to shuttle electrons from Complex I (NADH dehydrogenase) or Complex II (succinate dehydrogenase) to Complex III (the cytochrome bc_1 complex). It is thought that extramitochondrial ubiquinone acts as a lipid-soluble antioxidant, capable of scavenging lipid peroxyl radicals directly (2) or indirectly by reducing α-tocopherol radicals (3–5). Q in the plasma membrane functions in trans-plasma membrane electron transport to scavenge extracellular ascorbate free radicals (6, 7). The antioxidant function of Q is important *in vivo*, as demonstrated by the hypersensitivity of Q-deficient mutants of *Saccharomyces cerevisiae* and *Saccharomyces pombe* to treatment with autoxidized polyunsaturated fatty acids and hydrogen peroxide, respectively (8–10).

Q is used widely as a dietary supplement and in various clinical therapies, although the mechanisms of its uptake, distribution to tissues, and modes of action remain obscure. In young rats, Q supplementation increases levels of Q in only the blood and liver but does not produce elevated levels in other tissues (11). However, there is a growing body of evidence that Q supplementation in aged rats and mice increases Q levels in mitochondria of other tissues as well (12, 13). Such Q supplementation of aged rats enhances tolerance of myocardium to aerobic stress (14), exerts neuroprotective effects in experimental models of neurodegenerative disease (12), and improves endothelium-dependent vasodilation (15). Q is the only lipid-soluble antioxidant that can be synthesized by mammalian cells, and it plays critical roles in the defense mechanisms of the cell. Given the diverse beneficial effects of Q, it is important to identify and functionally characterize human genes involved in the Q biosynthetic pathway.

The proposed biosynthetic pathway of Q was elucidated by the characterization of accumulating Q biosynthetic intermediates in *Escherichia coli* and *S. cerevisiae* Q-deficient mutants (16, 17). In yeast, the Q-deficient mutant strains have been placed into eight complementation groups, *coq1–coq8* (18, 19). Such mutants are respiratory-deficient and are therefore unable to grow on nonfermentable carbon sources such as glycerol. The *COQ3* gene product was originally identified as the *O*-methyltransferase responsible for converting 3,4-dihydroxy-5-hexaprenylbenzoic acid (Fig. 1, compound **1**) to 3-methoxy-4-hydroxy-5-hexaprenylbenzoic acid (compound **2**) (20). This was confirmed both by amino acid sequence analysis, which showed that Coq3p contains four conserved motifs identified for the family of AdoMet-dependent methyltransferases (21), and *in vitro* methyltransferase assays (22), in which the farnesylated analogue of compound **1** required functional Coq3p to be methylated to form farnesylated compound **2**. Recent evidence indicates that Coq3p is a promiscuous methyltransferase; it is responsible not only for the first *O*-methyltransferase step, but the final one as well, converting demethyl-QH_2 (compound **3**) to QH_2 (compound **4**) (23). This is analogous to the *E. coli* system in which the UbiGp is required for both the first and last *O*-methylation steps (24). Similar *in vitro* studies show that this *O*-methyltransferase activity is conserved between rat and

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF193016.

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[1] The abbreviations used are: Q, coenzyme Q (ubiquinone); AdoMet, *S*-adenosylmethionine; COMT, catechol *O*-methyltransferase; L-DOPA, 3,4-dihydroxyphenyl-L-alanine; QH_2, ubiquinol.

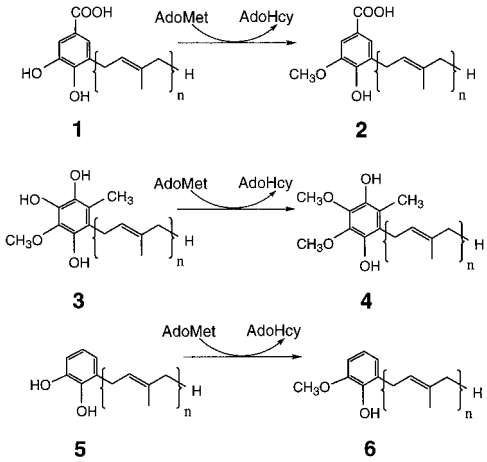

FIG. 1. **The proposed biosynthetic pathway for Q involves two *O*-methylation steps.** In eukaryotes, the first *O*-methylation step requires the Coq3 polypeptide to convert 3,4-dihydroxy-5-polyprenylbenzoic acid (*compound **1***) to 3-methoxy-4-hydroxy-5-polyprenylbenzoic acid (*compound **2***). The second step converts 2-polyprenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinol (*compound **3***) to ubiquinol-*n* (*compound **4***). In prokaryotes, the first *O*-methylation step requires the UbiG polypeptide and involves the *O*-methylation of 2-polyprenyl-6-hydroxyphenol (*compound **5***) to form 2-polyprenyl-6-methoxyphenol (*compound **6***). The length of the polyisoprenoid tail is designated by *n*; *S. cerevisiae*, $n = 6$; *E. coli*, $n = 8$; human, $n = 10$.

yeast. Yeast mitochondria isolated from yeast *coq3* null mutants rescued with the rat *Coq3* cDNA are capable of methylating these farnesylated analogues as well (23).

This study describes the isolation and characterization of the human homologue of *COQ3*. This homologue was obtained by screening a human heart cDNA library with the rat *Coq3* gene. The ability of this human homologue to restore Q synthesis in a yeast *coq3* null mutant is described in terms of growth on glycerol, Q levels, and methyltransferase activity.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Media—The *S. cerevisiae* strain used in this study was JM45Δcoq3 (MATa, *leu2–3, -112, ura3–52, trp1–289, his4–580, coq3::LEU2*) (20). Growth media for yeast were prepared as described (25) and included YPG (1% yeast extract, 2% peptone, 3% glycerol), SDC (0.18% yeast nitrogen base without amino acids, 2% dextrose, 0.14% NaH_2PO_4, 0.5% $(NH_4)_2SO_4$, and complete amino acid supplement), and SD-Ura (SDC, but the amino acid supplement lacks uracil). The supplement was modified so that the final concentrations of the components were as follows: 80 mg/liter adenine sulfate, uracil, tryptophan, histidine, methionine, and cysteine; 40 mg/liter arginine and tyrosine; 120 mg/liter leucine; 60 mg/liter isoleucine, lysine, and phenylalanine; 100 mg/liter glutamic acid and aspartic acid; 150 mg/liter valine; 200 mg/liter threonine; and 400 mg/liter serine. Yeast were grown at 30 °C with shaking.

Screening by Sequence Homology—The rat *Coq3* homologue was used to screen a Lambda ZAP II human heart cDNA library (Stratagene, La Jolla, CA). A DNA segment containing part of the rat *Coq3* cDNA was amplified by polymerase chain reaction with primers pCC-1 (5′-GGAAGCTTCGCTTTCTCAGGGAACCAGCTCAC-3′) and pCC-2 (5′-CCCTCGAGCCCCCTTTCAAGCAGACTCAGCAG-3′) with pAB-3 (26) as template DNA. The resulting polymerase chain reaction product, containing 70 nucleotides of 5′ untranslated sequence and extending through amino acid 257 of the rat *Coq3* coding region, was digested with *Hin*DIII and *Xho*I and subcloned into the *Hin*DIII and *Xho*I sites of pYES2 (Invitrogen) to create pCCAB3–4. The rat *Coq3* probe was prepared by digesting pCCAB3–4 (Table I) with *Hin*DIII and *Xho*I to release the 937-base pair insert. The insert was then size selected on an agarose gel and labeled with [α^{32}-P]dCTP (specific activity, 3000 Ci/mmol, ICN Biochemicals, Costa Mesa, CA) with an Amersham Pharmacia Biotech oligolabeling kit. A Stratagene NucTrap push column was used to separate unincorporated nucleotides from the radiolabeled rat *Coq3* probe. The primary library screen was done on approximately 1×10^6 plaque-forming units grown on XL1-Blue MRF′ (5×10^4 plaque-forming units/plate, 20 plates). The nitrocellulose filters were

TABLE I
Descriptions and sources of clones

Clone	Description	Source
PCCAB3–4	Rat *Coq3* in pYES2	This study
phCOQ3	Human *Coq3* in pBluescript	This study
pscQ3–1	Human *Coq3* in pAH01 (single copy)	This study
pmcQ3–1	Human *Coq3* in pCH1 (multicopy)	This study
pRS12A2-Sma	Yeast *COQ3* in pRS316 (single copy)	Ref. 20

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                                                CGGGGGTTGGTTTTTAA  -125
AGTCTGGGGCCTGGAGCTGTAATACAAAGCTGCGCGTCCCTTAATTTCCT   -75
CGGCGGTTTATGTGAAGAACCAGCTCAGTGGACTCTACAGATTAACCAGG   -25
GGTTTTCAATGAATACAGAACCATATGTTCAAATCCTACAGGACGATCTT    26
                                M  F  K  S  Y  R  T  I  F     9
TTCCTGTTTGAACAGAATAAAGAGTTTCAGGTACCCTTGGGCGAGACTGT    76
  S  C  L  N  R  I  K  S  F  R  Y  P  W  A  R  L      25
ACAGTACTTCCCAAACCACTGTCGACAGCGGTGAGGTAAAAACCTTCTTG   126
Y  S  T  S  Q  T  T  V  D  S  G  E  V  K  T  F  L     42
GCCCTGGCTCACAAATGGTGGGATGAACAAGGAGTATATGCACCTCTTCA   176
 A  L  A  H  K  W  W  D  E  Q  G  V  Y  A  P  L  H    59
TTCCATGAATGACCTGAGGGTGCCATTTATTAGGGACAATCTTCTGAAAA   226
  S  M  N  D  L  R  V  P  F  I  R  D  N  L  L  K      75
CAATTCCTAATCACCAGCCAGGAAAACCTTTGTTGGGGATGAAGATTCTT   276
T  I  P  N  H  Q  P  G  K  P  L  L  G  M  K  I  L     92
GACGTTGGCTGTGGTGGTGGGCTGTTAACTGAACCTCTAGGGCGGCTTGG   326
 D  V  G  C  G  G  G  L  L  T  E  P  L  G  R  L  G    109
GGCTTCAGTTATTGGAATCGACCCTGTGGATGAGAACATTAAAACAGCAC   376
  A  S  V  I  G  I  D  P  V  D  E  N  I  K  T  A      125
AATGCCATAAATCATTTGATCCAGTCCTGGATAAGAGAATAGAGTACAGA   426
Q  C  H  K  S  F  D  P  V  L  D  K  R  I  E  Y  R    142
GTGTGTTCCCTGGAAGAGATTGTGGAAGAGACTGCAGAAACATTTGATGC   476
 V  C  S  L  E  E  I  V  E  E  T  A  E  T  F  D  A    159
TGTTGTAGCTTCTGAAGTTGTAGAACATGTGATTGATCTAGAAACATTTT   526
  V  V  A  S  E  V  V  E  H  V  I  D  L  E  T  F      175
TACAGTGCTGCTGTCAAGTGTTAAAACCCGGTGGTTCTTTATTCATTACT   576
L  Q  C  C  C  Q  V  L  K  P  G  G  S  L  F  I  T    192
ACAATCAACAAAACACAACTTTCCTATGCCTTGGGAATTGTTTTTTCAGA   626
 T  I  N  K  T  Q  L  S  Y  A  L  G  I  V  F  S  E    209
GCAAATTGCAGGTATTGTACCAAAAGGTACTCATACATGGGAGAAGTTTG   676
  Q  I  A  G  I  V  P  K  G  T  H  T  W  E  K  F      225
TTTCACCTGAAACACTAGAGAGCATTCTGGAATCAAATGGTCTGTCAGTT   726
V  S  P  E  T  L  E  S  I  L  E  S  N  G  L  S  V    242
CAAACAGTGGTAGGAATGCTCTATAACCCCTTCTCAGGTTACTGGCATTG   776
 Q  T  V  V  G  M  L  Y  N  P  F  S  G  Y  W  H  W    259
GAGTGAAAATACCAGCCTTAACTATGCAGCTCATGCTGTGAAATCCAGGG   826
  S  E  N  T  S  L  N  Y  A  A  H  A  V  K  S  R      275
TCCAGGAACACCCAGCCTCTGCTGAGTTTGTTTTAAAGGGAGAAACAGAA   876
V  Q  E  H  P  A  S  A  E  F  V  L  K  G  E  T  E    292
GAGCTCCAAGCTAATGCCTGCACCAATCCAGCGTGCATTGAAAAGCTGAA   926
 E  L  Q  A  N  A  C  T  N  P  A  C  I  E  K  L  K    309
GAAATGAATTGTTTCTGAGAACTATAGTAATATGGCTTGGATATCTGATG   976
  K  .                                                311
TTTTCAAATACAAGAAATGTACAATTTATCCTTTGAGAGAGAATCATGAA  1026
GAAAAGAAGGGGCCCCTCAGAATGATATTTGGCCTCACGGGAGGACATAG  1076
CCCCTGGAGGTGCTGTTGCTATAGTTGCAAGCAGGAGGATAATGCCGGAA  1126
TTCCAAAAAAAAAAAAAAAAAA                              1148
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FIG. 2. **The nucleotide and resultant amino acid sequences of the 1.3-kilobase insert of the phCOQ3 clone (GenBank™ accession number AF193016).** The *underlined* amino acid sequences correspond to four conserved AdoMet-dependent methyltransferase regions.

hybridized overnight at 57 °C with approximately 1 ng of ^{32}P-labeled probe/ml of hybridization buffer. The filters were washed two times for 30 min each at 57 °C with 2× SSC-0.1% SDS and two times in 0.5× SSC-0.1% SDS at 50 °C (once for 1.5 h and once for 30 min). 1× SSC is 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0. The filters were placed under x-ray film (Eastman Kodak Co.) with double intensifying screens and placed at −80 °C. The primary screen resulted in only one potential positive. The phagemid, phCOQ3, was recovered with the ExAssist/SOLR system (Stratagene), and restriction enzyme digests with *Sst*I and *Kpn*I revealed an approximately 1.3-kilobase pair insert.

Sequence Determination and Analysis—The 1.3-kilobase pair cDNA insert of the phCOQ3 clone was sequenced in both directions with a Sequenase kit (U. S. Biochemical Corp., Cleveland, OH) and [α-^{35}S]dATP (specific activity, 1270 Ci/mmol; NEN Life Science Products). All primers were synthesized with an Amersham Pharmacia Biotech LKB gene assembler using the phosphoramidite method. Sequence analysis was done with DNASTAR/Geneman software and the BLAST (27) sequence analysis program.

Complementation of a S. cerevisiae coq3 Mutant—The human *COQ3* cDNA was excised with *Bam*HI and *Cla*I from phCOQ3 and blunt-end ligated into the *Hin*DIII site of pAH01 and the *Bam*HI site of pCH1. pAH01 and pCH1 have been described (24). The resultant constructs pscQ3–1 and pmcQ3–1 express the human *COQ3* cDNA behind the constitutive yeast *CYC1* promoter in single- and multicopy-plasmids,

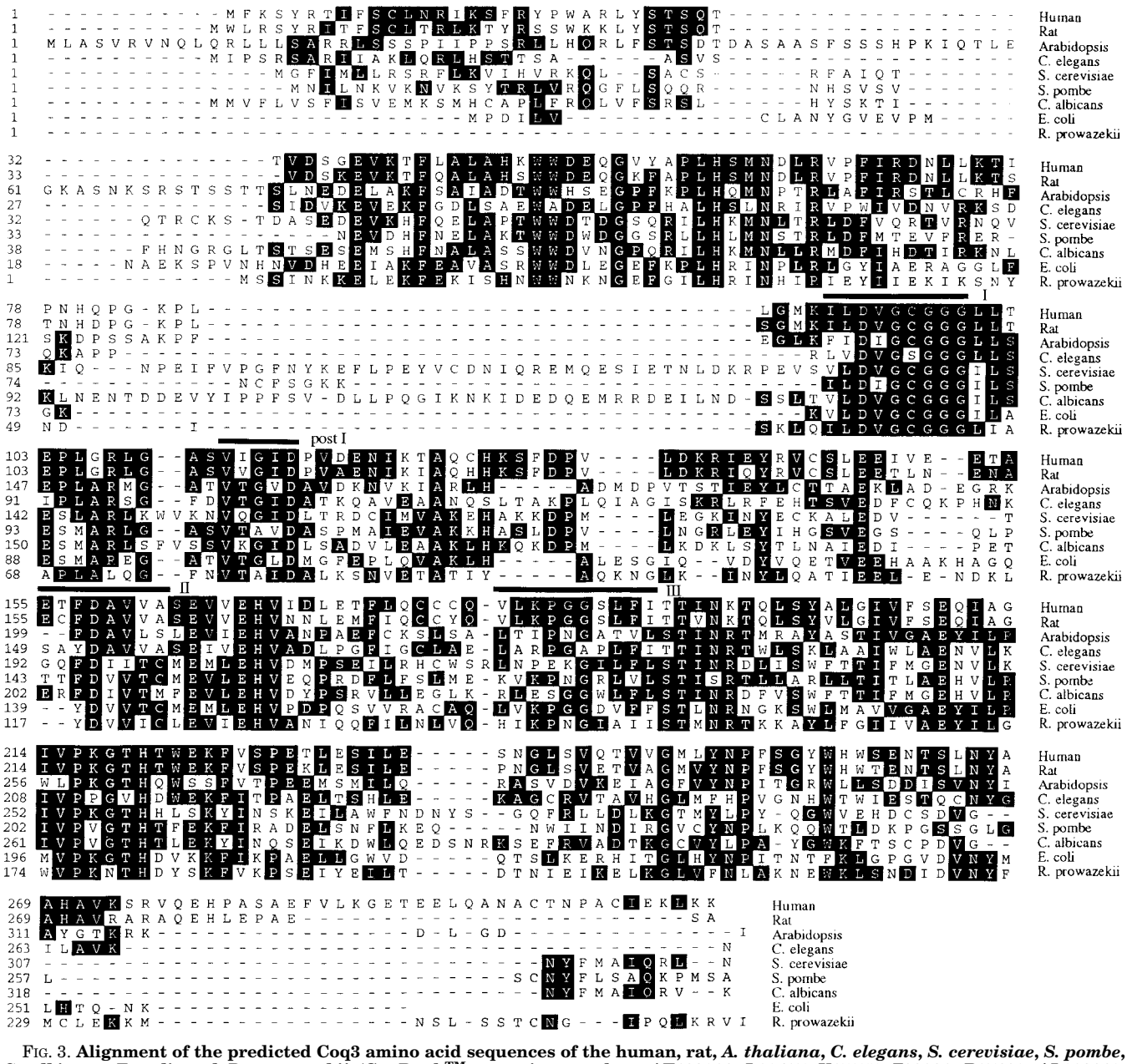

FIG. 3. **Alignment of the predicted Coq3 amino acid sequences of the human, rat, *A. thaliana*, *C. elegans*, *S. cerevisiae*, *S. pombe*, *C. albicans*, *E. coli*, and *R. prowazekii* (GenBank™ accession numbers AF193016, L20427, Y15055, Z99281, P27680, AL023860, AL033391, P17993, and AJ235272, respectively).** This alignment was done using the Clustal method in the Megalign/DNASTAR program. Shared amino acid identities are *shaded,* and introduced gaps are designated with *dashes*.

respectively. The yeast strain JM45Δcoq3 was transformed (28) with a single copy vector containing the yeast *COQ3* gene (20) or with one of the following plasmids: pscQ3–1, pmcQ3–1, pYEP24, pAH01, or pCH1. Transformants were selected on SD-Ura plates. Ura^+ colonies were replica-plated onto YPG plates.

Quantitation of Ubiquinone Levels—Levels of Q_6 and Q_6H_2 were detected and quantified by a modification of the methods of Schultz and Clarke (29) and Finckh *et al.* (30). JM45Δcoq3 was grown to saturation in 50 ml of SDC media. JM45Δcoq3 harboring either pRS12A2-Sma, pscQ3–1, or pmcQ3–1 was grown to saturation in SD-Ura. Those strains capable of growing on YPG, namely those harboring pRS12A2-Sma or pmcQ3–1, were also cultured in YPG. Cells were collected by centrifugation (10 min at 624 × *g* and 4 °C), resuspended in 10 ml of H_2O, and transferred to 50-ml preweighed glass tubes. Cells were collected by centrifugation as before and resuspended in 12 ml of H_2O. Three 1-ml aliquots were taken of each sample, and the cell pellet wet weights were determined. The cell pellets were dried for 48 h at 37 °C, and the dry cell weight determined. The ratio of dry to wet weight of the 1-ml aliquots was used to calculate the dry weight of the remaining sample. The remaining cell suspensions (9 ml) were again centrifuged, and the total wet weight of the cell pellet was determined. Glass beads (10 times the cell pellet wet weight) and H_2O (3.5 times the pellet wet weight) were added, and 4 μl of Q_9 (1.38 μg/μl) was also added as an internal standard. The tubes were then flooded with argon, capped, covered with foil, and kept on ice. Cells were lysed by vortexing for 2 min. Lipids were extracted by adding 9 ml of methanol and 6 ml of petroleum ether and vortexing for 30 s. The phases were separated by centrifugation (10 min at 624 × *g* and 4 °C). The upper petroleum ether layer was transferred to a 10-ml glass tube. 4 ml of petroleum ether was added to the glass bead-aqueous phase, and the samples were vortexed for 30 s. The petroleum ether layers from a total of three extractions were pooled and dried under nitrogen. The lipids were resuspended in 1 ml of 9:1 MeOH/EtOH. Q_6H_2, Q_6, and Q_9 were separated by reversed-phase high pressure liquid chromatography with a C18 column (Alltech Econosphere 5-μm, 4.6 × 250-mm column) and quantitated with an ESA Coulochem II electrochemical detector (E1, −450 mV; E2, 500 mV). The mobile phase was used at a flow rate of 1 ml/min and consisted of methanol/ethanol/2-propanol (88/24/10) and 13.4 mM lithium perchlorate. Q_6H_2, Q_6, and Q_9 were quantitated directly from the electrical chemical defector results using external standards of Q_6 and Q_9, and Q_6H_2 (extinction coefficients, $E^M_{275\ nm}$: Q_6, 14,900 M^{-1} cm^{-1}; Q_9, 14,700 M^{-1} cm^{-1}; $E^M_{290\ nm}$: Q_6H_2, 4921 M^{-1} cm^{-1} (31)). The Q_6H_2 standard was

generated by reduction with dithionite (32) and stored in acidic ethanol.

Methyltransferase Activities—Three substrate analogues were used for *O*-methyltransferase activity assays. The synthesis of 3,4-dihydroxy-5-farnesylbenzoic acid (compound **1**), demethyl-ubiquinone-3 (compound **3**), and 2-farnesyl-6-hydroxyphenol (compound **5**) have been described previously (22–24). Crude mitochondrial preparations and enzyme assays were as described in Ref. 23, except that the concentration of the 2-farnesyl-6-hydroxyphenol substrate (compound **5**) was 250 μM, and each reaction contained 0.1–0.3 mg of crude mitochondrial protein. The background level of radioactivity was determined by averaging the counts found in the fractions representing the expected product peak for the vector control. This value was considered background and was subtracted from all samples.

RESULTS AND DISCUSSION

Screening of a Human Heart cDNA Library by Sequence Homology to Rat Coq3—Approximately 1×10^6 plaque-forming units from a Lambda ZAP II human heart cDNA library were transferred to nitrocellulose filters. The filters were then hybridized with ^{32}P-labeled rat *Coq3* cDNA as described under "Experimental Procedures." One potential positive was discovered in this primary screen. Two subsequent screenings of this candidate verified that it in fact had homology to the rat *Coq3* cDNA. The phagemid was isolated and designated phCOQ3.

Isolation and Characterization of a Human Coq3 cDNA—Fig. 2 shows the nucleotide and the resulting amino acid sequences of the 1.3-kilobase pair insert of phCOQ3. The cDNA contained a 933-nucleotide open reading frame encoding a 311-amino acid polypeptide. The amino-terminal region contains many basic and hydroxylated residues; there are no acidic amino acids until position 34. These features are consistent with a classic mitochondrial targeting sequence (33). In fact, the yeast Coq3p has been shown to reside within the mitochondrial matrix (23). The predicted amino acid sequence also contains four regions that are conserved among methyltransferase enzymes that utilize *S*-adenosylmethionine as a methyl donor (21). These regions are underlined in Fig. 2. Regions I, post-I, II, and III are amino acids 91–99, 112–116, 155–162, and 182–191, respectively. Fig. 3 displays an alignment between the *COQ3* homologues in human, rat, *Arabidopsis thaliana*, *Caenorhabditis elegans*, *S. cerevisiae*, *S. pombe*, *Candida albicans*, *E. coli*, and *Rickettsia prowazekii*. As can be seen, there is a great deal of sequence homology across the species, from human to the intracellular parasitic bacterium *R. prowazekii*. Each homologue contains the four motifs found in *S*-adenosylmethionine-dependent methyltransferases, as designated by the *bars* in Fig. 3.

Human Coq3 Can Rescue coq3Δ Yeast Growth on a Nonfermentable Carbon Source—Yeast *coq3* deletion mutants cannot produce Q and are therefore unable to grow on nonfermentable carbon sources. The isolated human *Coq3* cDNA was placed behind the constitutive yeast promotor *CYC1* in single- and multicopy vectors. These constructs were each transformed into a *coq3*Δ strain (JM45Δcoq3). The transformants were tested for their ability to grow on liquid medium containing glycerol as the sole carbon source (Fig. 4). The data indicate that human *Coq3*, when expressed from a multicopy plasmid, can rescue the yeast *coq3* mutant, albeit growth occurs at a slower rate than the yeast homologue. However, it cannot rescue growth on YPG when expressed from a single copy plasmid. Such rescue of a *coq3*Δ mutant has also been seen with the rat and *Arabidopsis* homologues (26, 34). Together these results show that human *Coq3* serves the same function as the yeast 3,4-dihydroxy-5-hexaprenylbenzoic acid methyltransferase. The ability of higher eukaryotic polypeptides to function in the synthesis of coenzyme Q in yeast is not uncom-

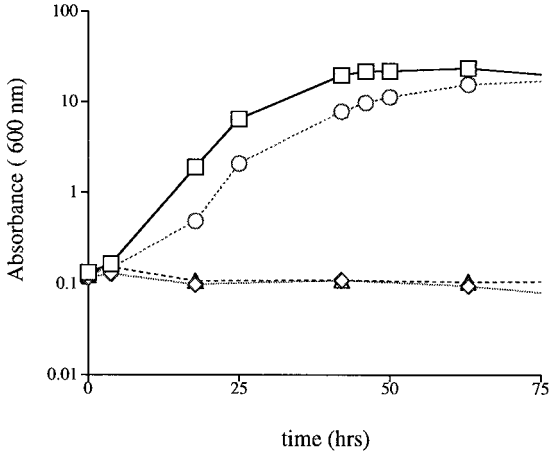

FIG. 4. **Rescue of *coq3*Δ mutant *S. cerevisiae* for growth on glycerol with human *COQ3* cDNA.** Yeast strains JM45Δcoq3 (△) harboring the vectors containing yeast (□) or human *COQ3* (single copy, ◇; multicopy, ○) were grown overnight in 5 ml of SD-Ura. The cultures were then diluted into 25 ml of YPG (A_{600} = 0.1). Growth was monitored by A_{600} measurements.

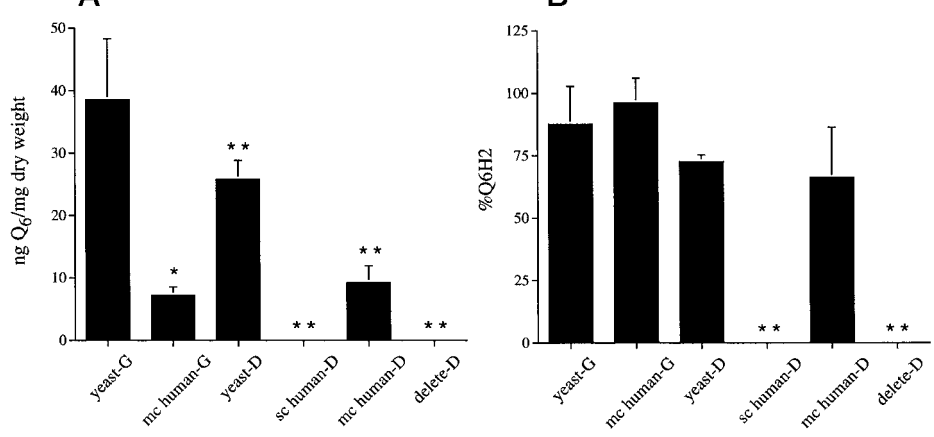

FIG. 5. **Amount of Q_6 and Q_6H_2 in JM45Δcoq3 harboring the vector pRS12A2-Sma (yeast), pscQ3–1 (single copy human), or pmcQ3–1 (multicopy human) grown in both SD-Ura (dextrose (*D*)) and YPG (glycerol (*G*)).** *A,* ng of Q_{6+} Q_6H_2 per mg of dry weight for each sample was determined via high pressure liquid chromatography coupled with electrochemical detection. These levels have been adjusted to account for the percentage of recovery as determined by a Q_9 internal standard. *B,* percentage of the total Q in the reduced state (Q_6H_2). *Error bars* represent the S.D. for three high pressure liquid chromatography injections. Statistical significance was determined using Student's *t* test. Paired differences were considered significant if $p < 0.05$. * denotes a significant difference between the glycerol samples. ** denotes a significant difference from the wild type yeast sample grown in dextrose.

mon. The human, rat, and *C. elegans* homologues of Coq7p are all able to restore Q biosynthesis in *coq7* null mutants of *S. cerevisiae* (35–37).

Coq3Δ Yeast Expressing Human Coq3 Can Produce Coenzyme Q—The actual Q levels produced in each of the strains were determined using a reversed-phase high pressure liquid chromatography system coupled to electrochemical detection. Strains grown on both glucose and glycerol are compared (Fig. 5*A*). Neither the strains harboring the single-copy human construct nor the delete control show any detectable Q. In contrast, yeast harboring human *Coq3* on a multicopy-plasmid produce significant amounts of Q, although the levels are lower than those of the wild type yeast. When grown in glycerol, yeast containing the yeast *COQ3* produce approximately 38 ng of Q per mg of dry weight, whereas yeast with the multicopy human plasmid make only about 7 ng of Q per mg of dry weight. In dextrose, the levels are about 26 and 9 ng of Q per mg for the yeast and human forms, respectively. In all cases, the Q that is produced is mostly in the reduced state, ranging from about 67 to 97% reduced Q (Fig. 5*B*).

Human Coq3p Methylates Q Intermediates in Vitro—Three substrate analogues were used to directly test *O*-methyltransferase activity, namely 3,4-dihydroxy-5-farnesylbenzoic acid (compound **1**; Fig. 6*A*), demethyl-Q_3 (compound **3**; Fig. 6*B*), and 2-farnesyl-6-hydroxyphenol (compound **5**; Fig. 6*C*). Essentially no activity was detected with mitochondria isolated from yeast harboring the single copy human construct using any of the three substrates; however, mitochondrial extracts from the yeast bearing the multicopy human construct were capable of methylating all three substrates, albeit at lower levels than that of the yeast form. This activity is not detected in mitochondria from a *coq3* null mutant. The human enzyme showed similar activities in methylating substrates **1** and **3,** indicating that methylation occurs at sites both *meta* and *para* relative to the polyisoprenoid tail substituent. It is interesting to note that yeast Coq3p shows an approximately 30-fold higher activity with the substrate **3** compared with substrate **1,** suggesting that the favored methylation site for the yeast enzyme is the hydroxyl group *para* to the polyisoprenoid tail. Both enzymes show a lower methylating activity with **5,** the prokaryotic substrate. Compounds that are methylated *ortho* to the tail have not been observed (24). It is clear that the Coq3 *O*-methyltransferase has a wide substrate specificity; the *E. coli*, yeast, rat, and human enzymes each methylate both eukaryotic substrates and the distinct prokaryotic substrate. The results presented here indicate that yeast provides an excellent model for characterizing Q biosynthesis in higher eukaryotes.

Relationship of Human Coq3 to Catechol O-Methyltransferase (COMT)—This type of promiscuous substrate recognition and the ability to *O*-methylate at both *meta* and *para* ring positions are features of COMT. COMT has numerous relevant physiological substrates, including catecholic steroids, the catecholamine neurotransmitters (dopamine, norepinephrine, and epinephrine), and the biosynthetic precursors of dopamine (3,4-dihydroxyphenyl-L-alanine (L-DOPA) and 3,4-dihydroxyphenylacetic acid) (38, 39). The determination of the structure of the rat COMT has allowed the amino acid residues making contacts with AdoMet, Mg^{2+}, and the catechol substrate to be identified (40). These residues are located within or are immediately adjacent to four conserved motifs found in a large family of AdoMet-dependent methyltransferases, including COMT, eukaryotic Coq3, and the *E. coli* UbiG polypeptides (Fig. 7) (21). Based on the conservation of amino acids within these motifs and the similarity of the catechol-containing substrates, it seems likely that the *O*-methylation steps in Q biosynthesis may be similar to the mechanism described for COMT (41). The comparison of the amino acid sequences outside of these motifs fails to reveal other regions of homology.

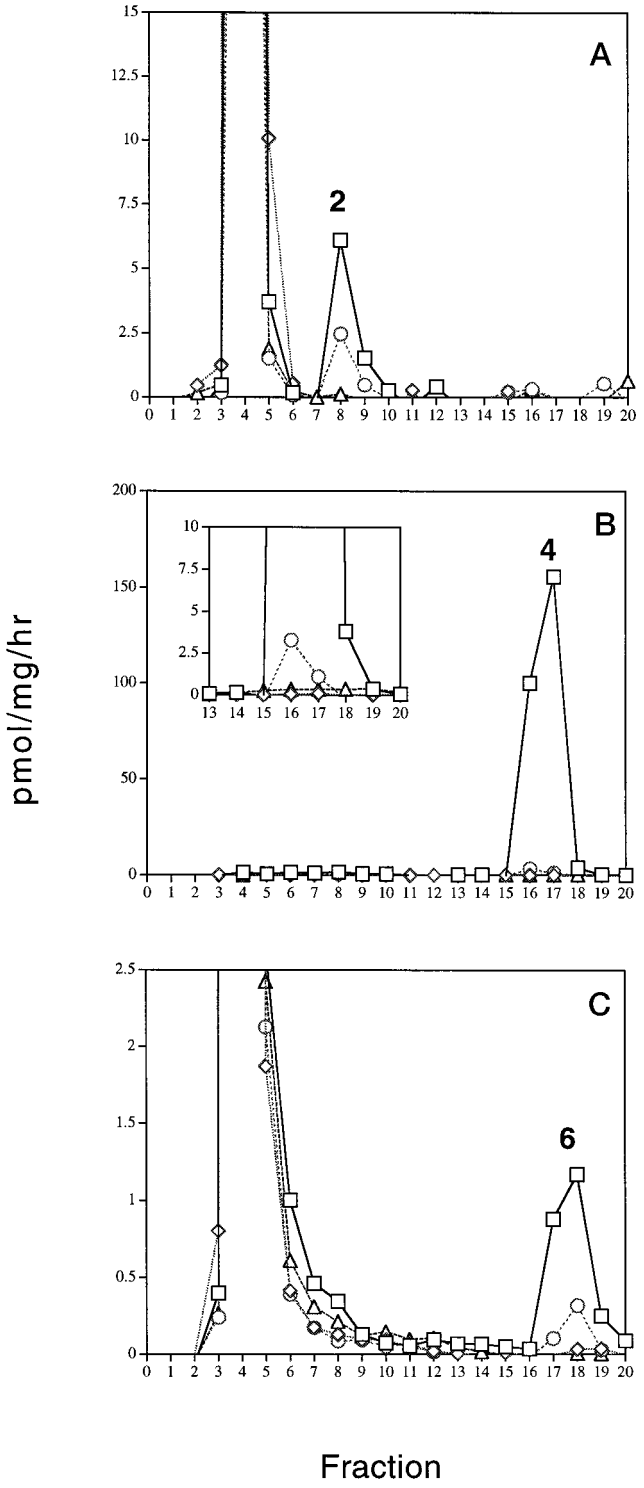

FIG. 6. ***O*-Methyltransferase assays were completed utilizing three different analogues of Q-intermediates as substrates: 3,4-dihydroxy-5-farnesylbenzoic acid (compound *1; A*), demethyl-ubiquinone-3 (compound *3; B*), and 2-farnesyl-6-hydroxyphenol (compound *5; C*).** The respective radiolabeled products will elute at fraction 8–9 (compound ***2,*** *A*), fractions 16–17 (compound ***4,*** *B*), or 17–18 (compound ***6,*** *C*). Crude mitochondria from JM45Δcoq3 harboring the vector pRS12A2-Sma (yeast; □), pscQ3–1 (single copy human; ◇), pmcQ3–1 (multicopy human; ○), or pAH01 (vector control; △) were used for the assays. The peak heights were determined as pmol of methyl groups/mg of mitochondria/h.

Dinitrocatechol-based inhibitors of COMT (such as tolca-

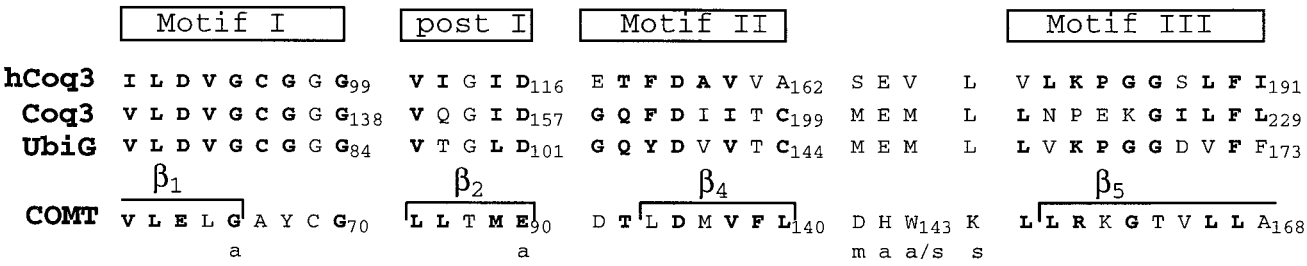

FIG. 7. **Alignment of the human Coq3, *S. cerevisiae* Coq3, *E. coli* UbiG, and rat COMT amino acid sequences across methyltransferase motifs I, post-I, II, and III.** The four motifs of sequence similarity present in AdoMet-dependent methyltransferases are shown. *Boldface* residues designate a match with the consensus amino acids identified by Kagan and Clarke (21). The carboxyl-terminal residue of each motif is numbered and indicates the position of the motifs relative to the linear amino acid sequence of the respective polypeptide chains. Also indicated are the secondary structure elements in the crystal structure of the rat soluble COMT (β_1, β_2, β_4, and β_5) and the important active site residues involved in the binding of ligands. *a,* AdoMet; *m,* magnesium; *s,* substrate (40).

pone) are being used in combination with L-DOPA as a therapy for Parkinson's disease (42). The rationale behind such therapy is that provision of L-DOPA replenishes stores of dopamine, whereas inhibition of COMT slows down the metabolism of both dopamine and L-DOPA. The extent to which these drugs might inhibit the human Coq3 *O*-methyltransferase (and the synthesis of Q) has not been investigated. This is a potentially important point because levels of Q are reported to be lower in blood platelets of Parkinson's disease patients (43), and supplementation with Q appears to have neuroprotective effects in mice treated with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, an animal model of Parkinson's disease (44). Indeed, a clinical trial testing the therapeutic efficacy of Q supplementation in human Parkinson's disease patients is currently under way (45). The extent to which the human *O*-methyltransferase steps of Q biosynthesis are affected by drug therapies of Parkinson's disease warrants investigation. The ability to use a well defined yeast model system in these types of studies is very valuable and should enable the effects of potential drugs on the activity of human Coq3p to be assessed.

Human COQ3 Is a Candidate Gene for Q-deficiency Diseases—In humans, Q_{10} deficiency has been documented in several individuals. Severe Q_{10} deficiencies (3–5% of normal) were noted in two sisters who displayed muscle weakness, fatigability, learning disabilities, decreased stature, and central nervous system dysfunction (46). A young boy with muscle weakness, seizures, and elevated levels of lactate in the cerebrospinal fluid contained Q_{10} levels in muscle mitochondria that were 6% of normal (47). A less severe Q_{10} deficiency (25% of normal) was described in an adult female with seizures, muscle weakness, and premature exertional fatigue (48). Such Q depletion was tissue-specific because normal levels of Q were observed in cultured fibroblasts or in lymphoblastoid cell lines derived from the patients (46, 47). Dietary supplementation with Q_{10} produced significant improvement in physical performance of each of these patients (46, 47). The studies of these Q-deficient human patients indicate that there may be tissue-specific expression of one or more Q biosynthetic enzymes. The human *COQ3* homologue identified here and the other *COQ* human homologues identified so far (35, 49) are important candidate genes for studying human Q deficiencies.

Potential Link between Q and Aging—In studies of aging, there are thought to be two general classes of genes affecting longevity: those that affect repair processes and those that affect metabolism (50). It is intriguing that Q functionally bridges both categories, through its action as a crucial primary and secondary antioxidant and as an essential component in oxidative energy metabolism. One *COQ* gene has already been implicated in life span determination in the nematode *C. elegans*, namely *COQ7*/*Clk-1* (37). Specific mutations in the *C. elegans clk-1* gene result in delayed embryonic and postembryonic development, a slowing of adult behaviors, increased resistance to UV- and heat-induced stress, and an extended life span (37, 51–53). It seems likely that the mutations in *clk-1* result in a change in Q levels and/or a relocation of Q within the cells (19, 54). The function of *Clk-1*/*COQ7* in Q biosynthesis is conserved from yeast to humans (35). The recent identification of a gene regulating both stress response and life span in mammals ($p66^{shc}$) supports the idea that genes affecting longevity function in pathways that are conserved across species (55). It will be very important to assess the potential role of *COQ*7, *COQ3,* and other *COQ* genes in the aging process. The studies presented here show that the yeast system can be exploited to answer fundamental questions concerning human *COQ* gene function.

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