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

Tanya Jonassen and Catherine F. Clarke‡

From the Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095

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-polyrenylbenzoic 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-polyrenyl 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 (QH2) forms. In eukaryotes, Q acts in the in the Q biosynthetic pathway. 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-QH2 (compound 3) to QH2 (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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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 JM45Acoq3 (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% dextrose, 0.14% NaH2PO4, 0.5% (NH4)2SO4, and complete amino acid supplement). All primers were synthesized with an Amersham Pharmacia Biotech LKB gene assembler using the phosphoramidite method. Sequences and restriction enzyme digests, hybridization overnight at 57 °C with approximately 1 ng of 32P-labeled probe/ml of hybridization buffer. The filters were washed two times for 5 min each at 50 °C with 1.5× SSC followed by 15 min (on ice 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.

**Fig. 1.** The proposed biosynthetic pathway for Q involves two O-methylation steps. The first O-methylation step requires the Coq3 polypeptide to convert 3,4-dihydroxy-5-polyphenylbenzoic acid (compound 1) to 3-methoxy-4-hydroxy-5-polyphenylbenzoic acid (compound 2). The second step converts 2-polyphenyl-3-methyl-4-hydroxy-6-methoxy-1,4-benzoquinol (compound 3) to ubiquinol (compound 4). In prokaryotes, the first O-methylation step requires the UbIG polypeptide and involves the O-methylation of 2-polyphenyl-6-hydroxyphenol (compound 5) to form 2-polyphenyl-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.

**Fig. 2.** The nucleotide and resultant amino acid sequences of the 1.3-kilobase pair insert of the phCOQ3 clone (GenBank™ accession number AF193016). The underlined amino acid sequences correspond to four conserved AdoMet-dependent methyltransferase regions.

**Table I** Descriptions and sources of clones

<table>
<thead>
<tr>
<th>Clone</th>
<th>Description</th>
<th>Source</th>
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<tbody>
<tr>
<td>PCCAB3–4</td>
<td>Rat Coq3 in pYES2S</td>
<td>This study</td>
</tr>
<tr>
<td>pCOQ3</td>
<td>Human Coq3 in pBluescript</td>
<td>This study</td>
</tr>
<tr>
<td>pscQ3–1</td>
<td>Human Coq3 in pAH01 (single copy)</td>
<td>This study</td>
</tr>
<tr>
<td>pmcQ3–1</td>
<td>Human Coq3 in pCH1 (multicycle)</td>
<td>This study</td>
</tr>
<tr>
<td>pRS12A-Sma</td>
<td>Yeast COQ3 in pRS316 (single copy)</td>
<td>Ref. 20</td>
</tr>
</tbody>
</table>
respectively. The yeast strain JM45Dcoq3 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$_6$H$_2$ were detected and quantified by a modification of the methods of Schultz and Clarke (29) and Finckh et al. (30). JM45Dcoq3 was grown to saturation in 50 ml of SDC media. JM45Dcoq3 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$_2$O, and transferred to 50-ml preweighed glass tubes. Cells were collected by centrifugation as before and resuspended in 12 ml of H$_2$O. 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$_2$O (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 x 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$_6$H$_2$, Q$_6$, and Q$_9$ were separated by reversed-phase high pressure liquid chromatography with a C18 column (Alltech Econosphere 5-µm, 4.6 x 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$_6$H$_2$, 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$_6$H$_2$ (extinction coefficients, EM$_{275}$ nm: Q$_6$, 14,900 M$^{-1}$ cm$^{-1}$; Q$_9$, 14,700 M$^{-1}$ cm$^{-1}$; EM$_{290}$ nm: Q$_6$H$_2$, 4921 M$^{-1}$ cm$^{-1}$ (31)). The Q$_6$H$_2$ standard was

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.
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 × 10⁶ plaque-forming units from a Lambda ZAP II human heart cDNA library were transferred to nitrocellulose filters. The filters were then hybridized with 32P-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 promoter CYC1 in single- and multicopy vectors. These constructs were each transformed into a coq3Δ 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 coq3Δ 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 coq3Δ mutant S. cerevisiae for growth on glycerol with human COQ3 cDNA.](image)

![Fig. 5. Amount of Q and QH₂ in JM45Δcoq3 harboring the vector pRS12A-Sma (yeast), pscQ3-1 (single copy human), or pmcQ3-1 (multicopy human) grown in both SD-Ura (dextrose (D)) and YPG (glycerol (G)).](image)
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. 5A). 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. 5B).

**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. 6A), demethyl-Q3 (compound 3; Fig. 6B), and 2-farnesyl-6-hydroxyphenol (compound 5; Fig. 6C). 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 methyltransfer 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-dihydroxyphenyl-epinephrine), and the biosynthetic precursors of dopamine (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, Mg2+, 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 UbiO 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.

Dinitrocatechol-based inhibitors of COMT (such as tolca-
pione) 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.

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**REFERENCES**


**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 (β₁, β₂, β₃, and β₄) and the important active site residues involved in the binding of ligands. a, AdoMet; m, magnesium; s, substrate (40).**