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Molecular characterization of the human COQ5 *C*-methyltransferase in coenzyme Q_{10} biosynthesis ☆

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ABSTRACT

Coq5 catalyzes the only *C*-methylation involved in the biosynthesis of coenzyme Q (Q or ubiquinone) in humans and yeast *Saccharomyces cerevisiae*. As one of eleven polypeptides required for Q production in yeast, Coq5 has also been shown to assemble with the multi-subunit complex termed the CoQ-synthome. In humans, mutations in several *COQ* genes cause primary Q deficiency, and a decrease in Q biosynthesis is associated with mitochondrial, cardiovascular, kidney and neurodegenerative diseases. In this study, we characterize the human COQ5 polypeptide and examine its complementation of yeast *coq5* point and null mutants. We show that human *COQ5* RNA is expressed in all tissues and that the COQ5 polypeptide is associated with the mitochondrial inner membrane on the matrix side. Previous work in yeast has shown that point mutations within or adjacent to conserved *COQ5* methyltransferase motifs result in a loss of Coq5 function but not Coq5 steady state levels. Here, we show that stabilization of the CoQ-synthome within *coq5* point mutants or by over-expression of *COQ8* in *coq5* null mutants permits the human *COQ5* homolog to partially restore *coq5* mutant growth on respiratory media and Q_6 content. Immunoblotting against the human COQ5 polypeptide in isolated yeast mitochondria shows that the human Coq5 polypeptide migrates in two-dimensional blue-native/SDS-PAGE at the same high molecular mass as other yeast Coq proteins. The results presented suggest that human and *Escherichia coli* Coq5 homologs expressed in yeast retain *C*-methyltransferase activity but are capable of rescuing the *coq5* yeast mutants only when the CoQ-synthome is assembled.

1. Introduction

Coenzyme Q (ubiquinone or Q) is an essential lipophilic electron carrier found within the mitochondrial inner membrane of eukaryotes and the plasma membrane of many prokaryotes. Q is composed of a polyisoprenoid "tail" that anchors it to the lipid membrane, and a benzoquinone "head" that confers the abilities to shuttle electrons from Complexes I and II to Complex III [1,2]. Q also acts as a cofactor of uncoupling proteins and several mitochondrial dehydrogenases, and in its reduced or hydroquinone form can quench lipid radical species as an antioxidant [3,4].

The biosynthesis of the isoprenoid tail derives from either the mevalonate or 1-deoxy-D-xylulose-5-phosphate pathways [5], with the number of isoprene units varying in different species: six in *Saccharomyces cerevisiae*, eight in *Escherichia coli*, and ten in humans. In *S. cerevisiae*, the hexaprenyl diphosphate tail is attached to either 4-hydroxybenzoic acid (4HB) or para-aminobenzoic acid (pABA), both of which have been shown to serve as aromatic ring precursors in *S. cerevisiae* Q_6 biosynthesis [6,7].

Abbreviations: 2D-BN-SDS/PAGE, two-dimensional blue-native-sodium dodecyl sulfate/polyacrylamide gel electrophoresis; DDMQ, demethyl-demethoxy-Q; $DDMQH_2$, demethyl-demethoxy-QH_2; DMQ, demethoxy-Q; DOD, drop-out growth medium with dextrose; 4HB, 4-hydroxybenzoic acid; HPLC, high performance liquid chromatography; IDMQ, 4-imino-demethoxy-Q; MRM, multiple reaction monitoring; MTase, methyltransferase; pABA, para-aminobenzoic acid; PK, proteinase K; Q, coenzyme Q or ubiquinone; QH_2, coenzyme QH_2, ubiquinol, or ubihydroquinone; SD, minimal synthetic media with dextrose; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TCA, trichloroacetic acid; YPD, rich growth medium with dextrose; YPG, rich growth medium with glycerol; YPGal, rich growth medium with galactose

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Eleven *S. cerevisiae* genes (*COQ1-9*, *ARH1*, *YAH1*) are required for Q_6 biosynthesis, several of which encode proteins associated with the mitochondrial inner membrane in a multi-subunit complex termed the CoQ-synthome [8]. In the absence or deficiency of any one of these genes, Q_6 is not made and growth on medium containing a non-fermentable carbon source is not possible; the presence of each Coq polypeptide is essential for the assembly of the Coq polypeptide complex and for the proper function of each individual enzyme [9]. The over-expression of *COQ8*, which encodes a putative regulatory kinase, has been shown to restore steady state levels of several Coq polypeptides [10] and the assembly of the high molecular mass CoQ-synthome [8].

In humans, decreased levels of Q_{10} are associated with mitochondrial, cardiovascular, kidney and neurodegenerative diseases [3,11]. Mutations in *COQ* genes cause primary Q_{10} deficiency (OMIM #607426), one of the few treatable mitochondrial disorders; in fact some affected patients respond well to oral Q_{10} supplementation [12]. *COQ* genes are highly conserved throughout evolution, and several human *COQ* genes have complemented the corresponding yeast *coq* null mutant [11,13]. Previously, we have shown that expression of human ADCK3 (a yeast Coq8 ortholog) fused with an N-terminal yeast mitochondrial leader sequence rescued the growth of yeast *coq8* null mutants and restored de novo Q biosynthesis [14].

In this study, we report the cloning and functional characterization of the human ortholog of yeast *COQ5* and test its ability to complement yeast *coq5* point and null mutants. Coq5 catalyzes the only *C*-methylation involved in the synthesis of Q_6 in yeast [15]. Previous work in yeast has shown that certain point mutations within or adjacent to conserved *COQ5* methyltransferase motifs result in a loss of Coq5 methyltransferase function, but mutants harboring these alleles (*coq5-2*, *coq5-5*) still retain steady state levels of Coq5 protein [15,16]. Complementation of these yeast point mutants with *ubiE*, an *E. coli COQ5* homolog, restored respiration and *C*-methyltransferase activity [16]. Here, we examine the function of human COQ5, and show that expression of human COQ5 in yeast mutants identifies the functional conservation of the yeast and human Q biosynthetic pathways, with implications for the diagnosis and treatment of Q_{10} deficiencies in patients.

2. Material and methods

2.1. Yeast strains and growth media

S. cerevisiae strains used in this study are listed in Table 1. Media were prepared as described [17], and included: YPD (2% glucose, 1% yeast extract, 2% peptone), YPGal (2% galactose, 1% yeast extract, 2% peptone, 0.1% glucose), and YPG (3% glycerol, 1% yeast extract, 2% peptone). Synthetic dextrose/minimal medium (SD, SD–Ura, and SD–Ura–Leu) consisted of 0.18% yeast nitrogen base without amino acids, 2% dextrose, 0.14% NaH_2PO_4, and 0.5% $(NH_4)_2SO_4$, and amino acids (minus uracil or leucine for selective media) were added at final concentrations as described in [15]. Drop-out media with dextrose (DOD) lacking folate and para-amino benzoic acid and with proper amino acid selection were prepared as described in [18]. Plate media contained 2% bacto agar.

Table 1
Genotype and source of yeast strains.

Strain	Genotype	Source or reference
W303-1A	MAT **a** *ade2-1* his3-*1,15 leu2-3,112 trp1-1 ura3-1*	R. Rothstein[a]
W303ΔCOQ5	MAT **a** *ade2-1* his3-*1,15 leu2-3,112 trp1-1 ura3-1 coq5::HIS3*	[15]
CH83-B3	MAT α *ade2-1 coq5-2 his3-1,15 ura3-1*	[16]
CH316-6B	MAT α *coq5-5 trp1-1 ura3-1*	[16]

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2.2. Identification of human COQ5

The human *COQ5* gene (*hCOQ5*) cDNA sequence was identified by screening the Expressed Sequence Tag (EST) database with the tBLASTn algorithm (www.ncbi.nlm.nih.gov/blast) and the yeast protein sequence as bait [19].

2.3. Plasmid construction

Plasmids used in this study are listed in Table 2. Human *COQ5* was amplified from cDNA obtained from human skin fibroblasts [20] using primers COQ5F and COQ5-1077R. PCR products were cloned in pCRII TOPO (Invitrogen), and the high copy pYES2.1V5His yeast expression vectors (Invitrogen). The *COQ5* insert from pCRIITOPO-COQ5 was then cloned into the centromeric pCM189 vector (EUROSCARF) [21]. Yeast *COQ5* (yCOQ5) was amplified from genomic DNA obtained from a wild-type BY4741 strain using standard protocols and cloned into the same vectors. Amplification primers and conditions for all reactions are shown in Table S1. The hybrid yeast–human *COQ5* (yhCOQ5) gene was obtained by amplifying a 5′ segment of yCOQ5, corresponding to the mitochondrial targeting region (encoding aa 1–54; with primers yCOQ5F and hybridCOQ5R) and the 3′ of human *COQ5* (encoding aa 56–327; with primers hybridCOQ5F and COQ5-1037R). The two PCR products were joined with a sequential PCR protocol [22].

To generate the *COQ5-GFP* fusion gene, the human *COQ5* coding region (devoid of the termination codon) was amplified from pCRIITOPO-COQ5 with primers containing HindIII and PstI restriction sites, digested with HindIII and Pst1, and cloned into the pEGFP-N1 vector (Clontech) digested with the same enzymes. Finally a myc-tag was added to the C-terminus of hCOQ5 by PCR with primers COQ5F and COQ5mycR (Table S1) and cloned into the pCDNA3.1V5HisTOPO vector (Invitrogen) to yield pCOQ5-myc. All plasmid-constructs were sequenced to confirm the presence of the inserted DNA segments and to ascertain they were free of errors.

2.4. Northern blot and RACE

The probe for Northern blot analysis was obtained by EcoRI digestion of pCRII TOPO-COQ5 and was labeled and purified as described in [19] and hybridized to a commercial membrane (FirstChoice Human Blot 1 membrane–Ambion) containing 2 μg/lane of poly(A) + RNA from ten human tissues, previously used as described [23] and stripped. Radioactivity was detected with a Storm PhosphoImager (Molecular Dynamics, Sunnyvale, CA, USA) after an overnight exposure. The 5′ and 3′ termini of *COQ5* transcripts were characterized by RACE (Rapid Amplification of cDNA Ends), a protocol that has been detailed elsewhere [19]. RNA was extracted from cultured skin fibroblasts and from HeLa cells. Primers are reported in Table S1.

2.5. Localization of human COQ5 polypeptide in HeLa and HEK cells

The COQ5-GFP construct was used to transfect HeLa cells stably expressing mitochondrial Red Florescent Protein (mtRFP) as previously reported [20]. Procedures for mitochondrial isolation, proteinase K protection assays, and carbonate extraction were performed as described [24]. Antibodies used are described in Table 3. Tagged versions of COQ5 were employed since our anti-COQ5 antibody detected only very faint signals in cultured cells.

2.6. Immunoprecipitation of hCOQ5 from cultured cells

HEK 293 cells were co-transfected with the pCOQ5-myc and pCOQ4-V5, and were harvested after 48 h and mitochondria were isolated as above. About 3–4 mg of mitochondria were resuspended in 1 ml of lysis buffer (150 mM NaCl; 10 mM Tris/HCl pH 7,4; 1 mM EDTA; 0.5% Triton X-100; protease and phosphatase inhibitors) and incubated