The COQ7 Gene Encodes a Protein in Saccharomyces cerevisiae Necessary for Ubiquinone Biosynthesis

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Ubiquinone (coenzyme Q, or Q) is a lipid that transports electrons in the respiratory chains of both prokaryotes and eukaryotes. Mutants of Saccharomyces cerevisiae deficient in ubiquinone biosynthesis fail to grow on nonfermentable carbon sources and have been classified into eight complementation groups (coq1–coq8; Tzagoloff, A., and Dieckmann, C. L. (1990) Microbiol. Rev. 54, 211–225). In this study we show that although yeast coq7 mutants lack detectable ubiquinone, the coq7-1 mutant does synthesize demethoxyubiquinone (2-hexaprenyl-3-methyl-6-methoxy-1,4-benzoquinone), a ubiquinone biosynthetic intermediate. The corresponding wild-type COQ7 gene was isolated, sequenced, and found to restore growth on nonfermentable carbon sources and the synthesis of ubiquinone. The sequence of a polypeptide of 272 amino acids which is 40% identical to a previously reported Caenorhabditis elegans open reading frame. Deletion of the chromosomal COQ7 gene generates respiration defective yeast mutants deficient in ubiquinone. Analysis of several coq7 deletion strains indicates that, unlike the coq7-1 mutant, demethoxy-ubiquinone is not produced. Both coq7-1 and coq7 deletion mutants, like other coq mutants, accumulate an early intermediate in the ubiquinone biosynthetic pathway, 3-hexaprenyl-4-hydroxybenzoate. The data suggest that the yeast COQ7 gene may encode a protein involved in one or more monoxygenase or hydroxylase steps of ubiquinone biosynthesis.

Ubiquinone (coenzyme Q, or Q) is a lipid component of the electron transfer chain and functions in the transport of electrons from Complex I or II to the cytochrome bc₁ complex found in the inner mitochondrial membrane of eukaryotes, and in the plasma membrane of prokaryotes (1, 2). Q carries out this function via cycles of reduction (to form the hydroquinone, ubiquinol, or QH₂) and oxidation (to form Q). This same redox chemistry also allows QH₂ to function as a lipid soluble antioxidant, directly scavenging lipid peroxyl radicals in a capacity similar to vitamin E (3), and/or by its ability to reduce tocopherol radicals and hence regenerate vitamin E (4, 5). QH₂ is found in a variety of eukaryotic intracellular membranes and is present in lipoproteins, where it may serve a primary function as an antioxidant (6, 7). Supplementation of diets with Q results in increased levels of QH₂ in low density lipoprotein particles with an increased resistance to lipid peroxidation (8, 9). Based on these observations, QH₂ may play an important role in the protection of lipids in cellular membranes and in lipoprotein particles and, hence, function to prevent or slow atherosclerosis and possibly other disease processes related to oxidative stress.

Q is synthesized from the precursors p-hydroxybenzoic acid and isoprene diphosphate in both eukaryotes and prokaryotes (10). The proposed pathway for the biosynthesis of Q (Fig. 1) derives from the characterization of accumulating Q biosynthetic intermediates in Q-deficient mutant strains of Escherichia coli and Saccharomyces cerevisiae (10, 11). Q mutant strains of S. cerevisiae are non-respiring or petite mutants (12, 13) and have been classified into eight complementation groups, coq1–coq8 (14). Addition of Q₂ or Q₆ to mitochondrial extracts prepared from each coq mutant restored NADH-cytochrome c reductase activity to levels near that of the wild-type parental strain (12). Three of the complementation groups (coq1–coq3) have been characterized. In S. cerevisiae synthesis of compound 1 is carried out by enzymes encoded by the COQ1 and COQ2 genes (15, 16). The COQ3 gene encodes an O-methyltransferase thought to catalyze the synthesis of compound 5 (17). Evidence for the branched pathways between prokaryotes and eukaryotes derives from the isolation of compound 2 (Fig. 1) in E. coli mutants (18), compound 4 in coq3 mutants of S. cerevisiae (19) and compound 5 in another S. cerevisiae mutant (20). Gibson and Young (21) analyzed other E. coli mutants and characterized UbiH, UbiE, UbiF, and UbiG mutants as accumulating compounds 6, 7, 8, and 9, respectively. Corresponding yeast mutants to these steps have not been described, although intermediate 8 has been detected in wild-type yeast (22). Given the divergence of the early steps in the pathway, it is important to fully characterize Q biosynthesis in a yeast. Recent evidence suggests that the Q biosynthetic pathway in higher eukaryotes mirrors that of S. cerevisiae, since a rat cDNA homologue to the yeast COQ3 gene was isolated based on its ability to restore synthesis of Q in a coq3 mutant (23, 24).

In this work a yeast mutant from the coq7 complementation group has been shown to lack detectable Q, but produces 2-hexaprenyl-3-methyl-6-methoxy-1,4-benzoquinone (5-demethoxyubiquinone or DMQ, compound 8, Fig. 1). We demonstrate here that the COQ7 gene encodes a protein of 272 amino acids, which is necessary for growth on nonfermentable carbon sources and which restores Q biosynthesis in the coq7-1 mutant. Curiously, deletion of the COQ7 gene generates mutant strains that do not accumulate DMQ, but accumulate large amounts of 3-hexaprenyl-4-hydroxybenzoic acid (compound 1). This anomaly is discussed.
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![Diagram of Q biosynthesis]

**FIG. 1. The pathway of Q biosynthesis.** The proposed biosynthetic pathway for Q in eukaryotes (including yeast) and in prokaryotes is thought to diverge after assembly of compound 1 (3-polyprenyl-4-hydroxybenzoate). The length of the isoprenoid chain (n) varies depending on the species and ranges from n = 6 (S. cerevisiae) to n = 10 (Homo sapiens). The other intermediates in the pathway are 2 (2-polyprenylphenol), 3 (2-polyprenyl-6-hydroxyphenol), 4 (3,4-dihydroxy-5-polyprenylbenzoate), 5 (3-methoxy-4-hydroxy-5-polyprenylbenzoate), 6 (2-polyprenyl-6-methoxyphenol), 7 (2-polyprenyl-6-methoxy-1,4-benzoquinone), 8 (2-polyprenyl-3-methyl-6-methoxy-1,4-benzoquinone or DMQ), 9 (2-polyprenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinone), and 10 (ubiquinone-3). Intermediates 6, 7 and 9 are hypothetical in S. cerevisiae, as is intermediate 3 in E. coli. The asterisk designates the reaction catalyzed by DMQ monoxygenase.

### MATERIALS AND METHODS

Strains and Media—Strains of *S. cerevisiae* are listed in Table I. NM101 and NM103 were generated as ascospores from the mating of DBY 1034 and C97, to incorporate auxotrophic markers for selection; sporulation and tetrad analysis was done as described (25). Media components were obtained from Difco; other chemicals were from Sigma, Fisher Scientific, or as specified. Strains were grown in liquid and solid media in standard use: YPD, 1% yeast extract, 2% peptone, 2% maltose (Fluka and solid media in standard use: YPD, 1% yeast extract, 2% peptone, 2% dextrose; YPM, 1% yeast extract, 2% peptone, 2% maltose (Fluka). 

| Strain | Genotype | Source or reference |
|--------|----------|---------------------|
| DBY 1034 | a, ura 3-52, lys 2-801, his | D. Botstein |
| D273-108 | a, met 6 | 12, 13 |
| C97 | a, mel 6, coq 7-1 | 12, 13 |
| JM6 | a, his 4, p| 55 |
| JM43 | a, leu 2-3, leu 2-112, ura 3-52, trp 1-289, his 4-580 | 55 |
| JM45 | a, leu 2-3, leu 2-112, ura 3-52, trp 1-289, his 4-580 | 55 |
| W303 | a, ade 2-1, leu 2-3,112, ura 3-1, trp 1-3, his 3-11 | 13 |
| FY250 | a, ade 8, leu 231, ura 3-52, trp 1-363, his 3-2000 | This study |
| NM101 | a, leu 2-3, leu 2-112, ura 3-52, coq 7-1 | This study |
| NM103 | a, ura 3-52, coq 7-1 | This study |
| JM43,coq7a-1 | JM43,coq7a-1::LEU2 | This study |
| JM43,coq7a-2 | JM43,coq7a-2::LEU2 | This study |
| W303,coq7a-1 | W303,coq7a-1::LEU2 | This study |
| FY250,coq7a-1 | FY250,coq7a-1::LEU2 | This study |
| NM101,coq7a-1 | NM101,coq7a-1::LEU2 | This study |

**TABLE I**

| Strain | Genotypes and sources of S. cerevisiae strains | Source or reference |
|--------|------------------------------------------------|---------------------|
| DBY 1034 | a, ura 3–52, lys 2–801, his | D. Botstein |
| D273-108 | a, met 6 | 12, 13 |
| C97 | a, mel 6, coq 7-1 | 12, 13 |
| JM6 | a, his 4, p| 55 |
| JM43 | a, leu 2-3, leu 2-112, ura 3-52, trp 1-289, his 4-580 | 55 |
| JM45 | a, leu 2-3, leu 2-112, ura 3-52, trp 1-289, his 4-580 | 55 |
| W303 | a, ade 2-1, leu 2-3,112, ura 3-1, trp 1-3, his 3-11 | 13 |
| FY250 | a, ade 8, leu 231, ura 3-52, trp 1-363, his 3-2000 | This study |
| NM101 | a, leu 2-3, leu 2-112, ura 3-52, coq 7-1 | This study |
| NM103 | a, ura 3-52, coq 7-1 | This study |
| JM43,coq7a-1 | JM43,coq7a-1::LEU2 | This study |
| JM43,coq7a-2 | JM43,coq7a-2::LEU2 | This study |
| W303,coq7a-1 | W303,coq7a-1::LEU2 | This study |
| FY250,coq7a-1 | FY250,coq7a-1::LEU2 | This study |
| NM101,coq7a-1 | NM101,coq7a-1::LEU2 | This study |

**In Vivo Labeling of Q₆ and Q₆ Intermediates and Lipid Extraction**

- **Analytical HPLC of ¹⁴C-labeled yeast total lipid extracts employed a cyanopropyl column (Zorbax® C8, 5 μm, 4.6 mm × 250 mm, MacMod Analytical, Chadds Ford, PA) in isocratic conditions (0.1% isopropanol in heptane). Aliquots (100 μl) of each 1-ml fraction were analyzed in a scintillation counter. Fractions containing radioactivity or corresponding to apparent peaks at 266 nm were individually analyzed by mass spectrometry.**

- **Quantitation of Q and DMQ was by external standard injection of known quantities of Q₆ (Sigma) using the integrated area units of identified peaks. Concentrations of Q₆ standards in ethanol were determined using E₇₅₅,₅₅ = 15,300 M⁻¹ cm⁻¹ (28). This method provides a reasonably accurate estimate of DMQ since the two compounds have similar spectral qualities; DMQ₆ in ethanol at 271 nm, ε = 14,500 M⁻¹ cm⁻¹ (29).**

- **Analytical HPLC of ¹³C-labeled yeast total lipid extracts employed a cyanopropyl column equilibrated for at least 10 min in 98% solvent A (hexane) and 2% solvent B (isopropanol:hexane:methylene chloride, 52:41:5:2) at a flow rate of 1 ml/min. Ten minutes after sample injection (10–50 μl), the percentage of solvent B increased linearly at 1.75 percent/min, a linear gradient was used for 20 min to a ratio of 63:37 (solvent A:B). At 35 min buffer B reached 45%, and by 45 min it was 100% B. Base-line conditions were restored within 55 min.**

- **Scintillation Counting—Fractions collected from HPLC separation were added to plastic vials containing 5–10 ml of BiOsafe nonaqueous.
scintillation mixture (Research Products International). The average counts/min present in each fraction was determined in a Beckman model LS-3133P scintillation counter using the full 14C window; the 14C efficiency was 95%.

Analysis by Mass Spectrometry—HPLC fractions (1 ml) were dried under N2 gas, resuspended in 5–20 μl of heptane, and transferred to glass microcentrifuge tubes for direct inlet introduction to the mass spectrometer. Electron ionization (EI) mass spectra (70 eV ionization energy) were recorded on a VG Autospec (Manchester, United Kingdom) using a conventional solid probe for sample introduction (ramped from 50 to 350°C at 100 °C/min) at a nominal mass resolution of 10,000 (M/ΔM). A mass range of 50–700 was covered in the mass scan mode. Assignment of m/z values to ions of interest was made by reference to the signals obtained from the continuously introduced calibrant (PFK-H, PCR Inc., Gainesville, FL). For the purpose of illustrations (Fig. 5), the calibrant signals were subtracted from the mass spectra using the data system supplied with the instrument.

General Molecular Biological Methods—Preparation and propagation of bacterial plasmid DNA, yeast genomic and plasmid DNA, restriction enzyme digestions, agarose gel electrophoresis, and generation of DNA by polymerase chain reaction were done according to standard methods (30).

Cloning and Delimitation of the COQ7 Gene—NM101 yeast were transformed (23) 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 selective media and replica plated after 2 days to YPG medium to test for respiratory growth. Of approximately 28,800 Ura+ transformants, 34 colonies grew on the glycerol-containing plates. The 34 colonies were then tested for co-segregation of Ura+ and Coq+ phenotypes following plasmid loss due to vegetative growth in rich media. In many transformants such co-segregation was observed, indicating that the growth on glycerol was due to a plasmid gene. Yeast plasmid DNA was recovered from two transformants (p7.8 and p8.2) and amplified in DH5α E. coli (Life Technologies, Inc.). Restriction mapping indicated the two plasmids contained overlapping segments of DNA. Clone p7.8 was found to contain a 9.7-kb insert. A 4.8-kb BamHI fragment of p7.8 was cloned into the single copy vector pRS316 (32). The resulting subclone, pNM783, rescued the Coq+ phenotype. The region of the insert responsible for restoring growth on glycerol was delimited by deletions that made use of restriction enzyme sites in the polylinker of pNM783 and convenient restriction sites in the insert.

Sequence Analysis—DNA sequence analysis was determined by a dyeoxyxynucleotide chain termination method using the Sequenase version 2.0 kit (U.S. Biochemical Corp.) and primers to either the vector sequences of pRS316 or to cloned insert DNAs (Fig. 4). Oligonucleotide primers were synthesized by the phosphoramidite method on a Gene Assembler II instrument (Pharmacia Biotech, Inc.). Query of the GenBank data base revealed the partial sequencing of this open reading frame detailed in Fig. 7 (accession no. X82930, EMBL data base). This reported nucleic acid sequence was incomplete agreement with our sequence. Restriction enzyme digestion of the 5′ region of pNM7 plasmid deleted a 434-bp region of the upstream open reading frame and created the rescuing plasmid pNM7Q1. DNA sequence analysis of the coq7-1 allele was performed directly on the polymerase chain reaction product amplified (Vent DNA polymerase, New England Biolabs) from NM101 genomic DNA. The entire open reading frame and 76 bp of the 5′ noncoding region was sequenced unidirectionally, and greater than 60% of the amplified segment was sequenced bidirectionally (Fig. 6).

Disruption of the COQ7 Gene—the 1.9-kb HindIII to XhoI fragment from pNM7Q was subcloned into the bacterial plasmid pT7-7-7 (34) to create plasmid pT7. This plasmid was subsequently digested with EcoRV and StuI, liberating a 368-bp blunt-ended fragment within the coding region of the COQ7 gene. This region with pT7 was replaced with one of two LEU2 gene fragments (a 2.9-kb BglII or a 4.3-kb PstI fragment of YEp13) to generate pYDQ71 and pYDQ72, respectively. Restriction enzyme digests of these clones with BglI generated linear insert DNA, which was used in a one-step gene replacement (35). The resulting LEU2 transformants were tested for respiratory competence by replica-plating onto YPG plates. Genomic DNA was isolated from the strains listed in Table I (respiratory deficient, Leu−), and disruption of the locus was verified by Southern analysis using standard techniques (30). The coq7Δ strains obtained were not complemented for growth on glycerol by the coq7-1 strains C97 or NM101, but were complemented by pRD tester strains.

DNA Isolation and Northern Analysis—Yeast poly(A)+ RNA was isolated as described (30) from D273–10B cells grown in YPD or in YPG to optical densities (600 nm) of 3.24 or 0.4, respectively. Poly(A)+ RNA (5 μg) was separated by electrophoresis on 1.2% agarose gels containing 2.2 M formaldehyde (30) and transferred to GeneScreen membranes (DuPont NEN) as described by the manufacturer. Northern blots were prehybridized for about 30 min at 65°C in hybridization buffer (0.5 M sodium phosphate, pH 7.0, 1.0% bovine serum albumin, 7% sodium dodecyl sulfate, 1 mM EDTA) (36). Probes were labeled with [α-32P]dCTP (3000 Ci/mmol, ICN Biomedicals, Inc.) with an oligolabeling kit (Pharmacia) and unincorporated nucleotides were removed with NucTrap® push columns (Stratagene). Northern blots were hybridized with 32P-labeled DNA probes corresponding to either a segment of the yeast COQ3 gene, a 0.7-kb BglII fragment isolated from prS12A-2.5SB, (17), or a segment of DNA containing the COQ7 gene (1.9-kb HindIII to XhoI restriction fragment from pNM7Q). This blot was subsequently re-probed with a cDNA fragment upstream of the COQ7 gene (37) after digestion of 12 32P half-lives. Blots were
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Identification of 14C-Radiolabeled Quinones as Q and 5-DeoxyQ by Mass Spectroscopy—A portion of fraction 10 (Fig. 3, panel B) from the respiratory competent yeast strain was identified as Q by solid probe EI mass spectrometry (Fig. 4, upper panel). Mass spectral analyses of Q have identified a predictable fragmentation pattern for the EI spectra of this compound (39, 40). The two base peaks at 197 and 235 correspond to tropylium and pyrylium ions, respectively. The theoretical mass of Q (C39H58O4; 592.432022) corresponds to the observed mass of the molecular ion (590.433511, PPM 2.5). Also present is the reduced form of the quinone M+2 (C39H60O4; 592.453064; observed mass was 592.449161; PPM ~6.6).

The lower panel of Fig. 4 shows the EI mass spectra observed for fraction 14 (Fig. 3) from the coq7–1 strain NM101. The spectra obtained show a fragmentation pattern consistent with that for demethoxyubiquinone (2-hexaprenyl-3-methyl-6-deoxy-1,4-benzoquione, DMQ, compound 8, Fig. 1; Refs. 41 and 42). As expected for a quinone-containing intermediate, both M+2 (C39H60O4; 562.439117; observed mass 562.438596; PPM ~0.9) and M (C39H58O4; 560.421753; observed mass 560.422946; PPM ~2.1) ions were present. Further confirmation of the M and M+2 ions for this compound is found in the presence of C13 ions for M and M+2, which are also within +0.1 PPM error (data not shown). Characteristic base peaks at 167 and 205 correspond to the tropylium and pyrylium ions, respectively, as detailed above. The peak at 446.1 corresponds to a known contaminant. Similar spectra were observed for fraction 15 from NM101 (Fig. 3, panel B) and for fractions 14 and 15 from the respiratory competent yeast strain (Fig. 3, panel B).
Thus DMQ is detected in both the coq7–1 mutant strain and in a respiratory competent yeast strain. Other fractions were also analyzed by EI mass spectrometry, but produced no evidence for the presence of either Q or Q intermediates.

The amount of DMQ found under the UV peaks at 14–15 min in the chromatograms shown in Fig. 3 was estimated by comparing the integrated areas to the area corresponding to a known amount of Q₆ chromatographed under the same conditions. This method provides a reasonably accurate estimate of the amount of DMQ since it has similar UV spectral qualities to Q₆ (see "Materials and Methods"). The amount of DMQ accumulating in the coq7–1 mutant (109 ng of DMQ/g wet weight NM101 yeast) was found to be similar to the amount of DMQ present in the respiratory competent strain (159 ng of DMQ/g wet weight J M43,coq7Δ1/pNMQ71). Thus the defect responsible for the absence of Q₆ in the coq7–1 strain does not cause DMQ to accumulate above levels of that found in respiratory competent yeast.

**FIG. 4. Identification of the accumulating quinones in NM101 and in J M43,coq7Δ1/pNMQ71.** The electron impact mass spectra for the purified radioactive compounds, purified as in Fig. 3, are shown. Lower panel, the fragments are arrayed by m/z along the x axis for fraction 14 purified from NM101. The chemical structure of the intermediate and the likely origin of the fragment ions are shown. The peak at 446.1 corresponds to a known contaminant. Upper panel, the fragmentation pattern for fraction 10 purified from J M43,coq7Δ1/pNMQ71. Molecular structure of Q and the structure of the base peak ions are shown. The y axis for both panels is the percentage of relative intensity collected for the represented ions in each spectrum.
Isolation and DNA Sequence of the Yeast COQ7 Gene—The strain NM101 was derived as detailed (Table I) to incorporate useful auxotrophic markers for subcloning of the COQ7 gene. Tetrad dissection of the progenitor diploid strain gave a 2:2 segregation of the glycerol growth minus phenotype in five tetrads analyzed. Diploids from the mating of NM101 to a ρ− tester strain were able to grow on glycerol and on maltose, indicating that the inability to respire did not result from a defect in mitochondrial DNA. Testing for development of ρ− phenotype was performed when strains were revived from frozen stocks, but this phenotype was not observed. The NM101 strain was transformed with the YEEp24-based yeast genomic DNA library (31) and analysis of yeast transformants was as described (see “Materials and Methods”). One clone, p7.8, containing an insert of 9.7 kb, was characterized in detail. A 4.8-kb BamHI fragment contained within the insert DNA of p7.8 restored growth on glycerol when subcloned into the centromeric vector pRS316 to create the plasmid pN7M782 (Fig. 5, panel A). This plasmid is maintained at one or two copies/cell (43) and indicates that the presence of relatively low amounts of Coq7p will rescue the respiration deficient phenotype. The rescuing sequence was further delimited in pN7M782, and each smaller construct was tested for complementation of the glycerol growth defect (Fig. 5, panel A).

Initial determination of the DNA sequence of pN7M782 made use of oligonucleotide primers derived from the vector sequence of pRS316. Submission of this partial sequence to GenBank revealed complete identity with the nucleic acid sequence of a truncated open reading frame (ORF C), 357 bp upstream of the UBP2 gene (Ref. 33; accession no. M94916). The sequence corresponding to this entire open reading frame was then determined and is shown in Fig. 6. The DNA sequence predicts a polypeptide of 272 amino acids with a predicted molecular mass of 30,924.5 daltons.

In Situ Disruption of the COQ7 Gene—To establish that the open reading frame present in pN7M782 corresponds to COQ7, the chromosomal copy of this gene was disrupted. The disruption constructs were prepared as shown in Fig. 5 (panel B). Three haploid respiratory competent yeast strains were transformed with linear DNA fragments containing the disrupted allele. The resulting disrupted strains (Table I) were characterized as described under “Materials and Methods.” None of the coq7Δ strains obtained were complemented for growth on glycerol by the coq7−1 mutant strains C97 or NM101. The coq7Δ strains also failed to complement eight other independently derived coq7 mutants. These results imply a genetic linkage of the coq7Δ-1::LEU2 disrupted allele to the coq7 mutation. To verify this, NM101 was transformed with the linear disruption construct coq7Δ-1::LEU2 to generate NM101,coq7Δ-1 (Table I) and the resulting disrupted strain was characterized as described under “Materials and Methods.” Diploid cells obtained from the cross of NM101, coq7Δ-1 and FY250 were sporulated and subjected to tetrad analysis as described (25). Meiotic progeny from 15 complete tetrads derived from each cross were tested for respiration and leucine dependence. Both phenotypes segregated 2:2, and in each case the respiratory deficient spores were leucine-independent while the respiratory competent spores were leucine auxotrophs, confirming the allelism between the cloned COQ7 gene and the original coq7 mutation.

Recently other investigators made use of an independent screen and isolated and sequenced a yeast gene CAT5 (accession no. X82930) which has complete sequence identity with COQ7 and may be involved in glucose derepression.2 These investigations also reported (accession no. X82930) the presence of an upstream open reading frame fully encoded within the 1.9-kb sequence of the pN7M782 plasmid (Fig. 5, panel B). This upstream open reading frame present was truncated to create pN7M71. As detailed in Figs. 3 and 4, the presence of the COQ7 open reading frame in pN7M71 restores both respiration and synthesis of Q in the coq7Δ strain, JM43,coq7Δ-1, indicating that the 272 amino acid polypeptide encoded by the COQ7 gene restores Q production.

The amino acid sequence encoded by the COQ7 (CAT5) gene has no remarkable similarity to any known protein (PAM 120 or PAM 250 matrices and the available protein data bases) other than a putative Caenorhabditis elegans homologue present in the cosmid sequence c239S (44). The yeast Coq7p and the C. elegans predicted protein sequence are 42% identical (Fig. 6, panel B).

Comparative Analyses of Q Intermediates—Accumulating in coq7−1 and coq7Δ Yeast Strains—Yeasts containing deletions of the COQ7 gene were grown in the presence of [U-14C]hydroxybenzoate and the lipid extracts analyzed for the presence of the previously identified DMQ compound. As shown in Fig. 7, deletion of 368 bp within the COQ7 open reading frame abolished the accumulation of DMQ. Disruption of the COQ7 gene in two other wild-type backgrounds gave the same result (data not shown). Additionally this same deletion in the NM101 strain also abolished the accumulation of DMQ (Fig. 7), indicating that the accumulation of DMQ in NM101 was due to the coq7−1 allele itself. To determine the nature of the defect in the coq7−1 allele, a segment of NM101 genomic DNA encompassing the COQ7 coding region plus 118 bp of 5′-flanking sequence was amplified by polymerase chain reaction and the DNA sequence of the product was determined as described under “Materials and Methods.” The sequence analysis revealed a single base change of G to A at position 311, resulting in a change from Gly to Asp at amino acid 104 (Fig. 6A). This amino acid change occurs within a highly conserved region of the COQ7 gene as shown in Fig. 6B. Further testing of coq7−1 and coq7Δ mutants revealed no discernible growth differences on YPD or on nonfermentable carbon sources.

Characterization of COQ7 mRNA—Analysis of the induction of the message for the COQ7 gene shows that it is regulated in a manner similar to that of the COQ3 gene (17). Growth of the wild-type strain D273–10B in YPG induces the production of both genes, as would be expected for genes influencing the function of the respiratory chain (Fig. 8, panels A and C). In contrast, the amount of the mRNA for the daftherin heavy chain gene does not appear to be induced under the same conditions and, in this analysis, appears much more predominant in theYPD grown cells (Fig. 8, panels B and D).

DISCUSSION

This study describes the characterization of coq7−1 mutants and the isolation of the corresponding COQ7 gene affecting the production of Q in S. cerevisiae. The coq7−1 mutant lacks detectable Q, but does synthesize 3-hexaprenyl-4-hydroxybenzoate and DMQ (compounds 1 and 8, respectively, Fig. 1). The accumulation of 3-hexaprenyl-4-hydroxybenzoate is observed in wild-type yeast and in coq3− coq8 mutants (Ref. 26 and data not shown). The yeast COQ7 gene restores both respiration and the synthesis of Q in the coq7−1 mutant. As expected, coq7 deletion mutants fail to respire and are Q-deficient, but curiously, such mutants fail to produce any detectable DMQ intermediate. Unlike other Q biosynthetic intermediates, which are extremely air- and light-sensitive and difficult to purify (19, 26), DMQ is fairly stable. In fact DMQ can be readily recovered from wild-type yeast (22) and has been found as an impurity in some commercial sources of Q (42). Thus it is unlikely that our
failure to detect DMQ in the coq7 deletion mutant results from the instability of DMQ. It is also unlikely that DMQ is the product of an unproductive or "side reaction" of Q synthesis that might predominate in the coq7–1 mutant, since Law et al. (22) have shown a precursor-product relationship between DMQ and Q in *S. cerevisiae*. Finally, it is notable that the levels of DMQ present in the coq7–1 mutant do not accumulate to the extent that Q accumulates in wild-type yeast, and in fact the amount of DMQ in the coq7–1 strain is about two-thirds the amount of DMQ of the rescued strain.

Based on the presence of DMQ and the absence of Q in the coq7–1 yeast mutant, it is tempting to speculate that the COQ7 gene encodes a polypeptide involved in a monoxygenase or hydroxylase step with DMQ as a substrate. However, such
speculation must take into account the presence of DMQ in coq7–1 mutants and its absence in coq7 D mutants. Two models are consistent with the above observations; (i) Coq7p serves a dual function in both the first and last monoxygenase/hydroxylase steps, and (ii) Coq7p provides a component essential for the formation of an enzymatic complex that converts intermediate (1) to Q (Fig. 1). In model i, the nature of the mutation in the coq7–1 allele might generate a partially functional Coq7–1p, which although blocked in the conversion of 8 to 9, nonetheless allows the conversion of 1 to 4 to some extent, resulting in the production of DMQ. Precedent for model i is provided by examples of P450 oxidoreductases, some of which catalyze the oxidation of both related and unrelated substrates (45). Alternatively, in model ii Coq7–1p would provide a defective polypeptide creating a defective multi-enzyme complex that produces a small amount of DMQ, but is unable to produce Q. Deletion mutants in either model would accumulate only compound 1 because they would be devoid of any monoxygenase/hydroxylase activity (model i) or would fail to provide the polypeptide component required for the Q-biosynthetic enzyme complex (model ii). Precedent for model ii is provided by the eukaryotic multi-subunit respiratory complexes (46, 47). A further example is found in the lysosomal storage disease galactosialidosis, where the loss of a protective protein results in a loss of the multimeric form of β-galactosidase (48). In these examples, a single “missing” or mutant component results in a characteristic drastic phenotype in which many related components are either missing, unstable, or inactive. It is important to note that the one base pair mutation identified in the coq7–1 allele is consistent with either of the above models and predicts the formation of an intact polypeptide (Coq7–1p) in which glycine 104 is replaced by aspartate (Fig. 6). Testing of these models will require the availability of chemical amounts of the Q-intermediates to use as substrates for in vitro assays and antibodies to enzymes of the Q biosynthetic pathway.

Studies of ubiquinone synthesis in E. coli have shown that the three hydroxylation reactions involved in the aerobic synthesis of the quinone ring from p-hydroxybenzoate utilize molecular oxygen and hence are catalyzed by monoxygenases (49). The DMQ intermediate has been observed in Q-deficient UbiF mutants of E. coli (41) and the UbiF gene in E. coli may correspond to a DMQ monoxygenase. The E. coli UbiF gene has not yet been sequenced, and homology searches with the amino acid sequence of Coq7p revealed no highly significant similar-
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deficient because in vitro assays of cytochrome c reductase showed that levels of activity could be returned to almost wild type by addition of Q. From these results, it is possible that the Coq7p functions as a regulator of glucose derepression and of Q biosynthesis.

COQ7 mRNA is induced by heat shock (33). Our results demonstrate an induction of the COQ7 mRNA when the cells are grown in conditions demanding respiratory competence. This is intriguing because of recent evidence which suggests that heat shock, diauxic shift, and oxidative stress may be related phenomena through the coordinate control of genes induced by these stresses (50, 51). Mitochondria and mitochondrial structures of the cell do not fully form until the cell reaches stationary phase (52), when the cell has exhausted fermentable carbon sources and is forced to fully develop the electron transport chain. As cells growing in glucose-based medium pass through the diauxic shift to respiratory metabolism, they become thermoresistant (53) and a subset of heat shock genes are known to be induced (50). Two consensus heat shock elements are present in the 5′ region of the COQ7 genomic sequence at −261 to −243 and at −34 to −15. Each of these sequences lies in the middle of a stretch of nucleotides forming an imperfect inverted repeat. The sequence found at −261 to −243 is CACTTTTCCGGAAAAGGG, the 5′ sequence at −43 to −15, is TTTTCAGGAAAA. The heat shock elements are underlined. In addition a novel heat shock response element, C•T (54), is present in the upstream region of the COQ7 gene (−541). The observed induction of the COQ7 mRNA by heat shock and by shift to a nonfermentable carbon source are intriguing and deserve further investigation.

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