THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 271, No. 6, Issue of February 9, pp. 2995–3004, 1996

Printed in U.S.A.

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

(Received for publication, May 18, 1995, and in revised form, December 1, 1995)

B. Noelle Marbois and Catherine F. Clarke‡

From the Department of Chemistry and Biochemistry, Department of Biological Chemistry, School of Medicine, and the Molecular Biology Institute, University of California, Los Angeles, California 90095

Ubiquinone (coenzyme 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 predicts 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, demethoxyubiquinone 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)[1] is a lipid component of the electron transfer chain and functions in the transport of electrons from Complex I or II to the cytochrome bc_1 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 QH_2) and oxidation (to form Q). This same redox chemistry also allows QH_2 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). QH_2 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 QH_2 in low density lipoprotein particles with an increased resistance to lipid peroxidation (8, 9). Based on these observations, QH_2 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 Q_2 or Q_6 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 *UbiB 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 eukaryote. 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.

* This work has been supported by National Institutes of Health Grant GM45952. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Chemistry and Biochemistry, University of California, Los Angeles, 405 Hilgard Ave., Los Angeles, CA 90095-1569. Tel.: 310-825-0771; Fax: 310-206-5213; E-mail: cathy@ewald.mbi.ucla.edu.

[1] The abbreviations used are: Q, ubiquinone or coenzyme Q; DMQ, 2-hexaprenyl-3-methyl-6-methoxy-1,4-benzoquinone or 5-demethoxyubiquinone; EI, electron ionization; QH_2, ubiquinol; Q_n, ubiquinone containing *n* isoprene units; HPLC, high performance liquid chromatography; kb, kilobase pair(s); bp, base pair(s).

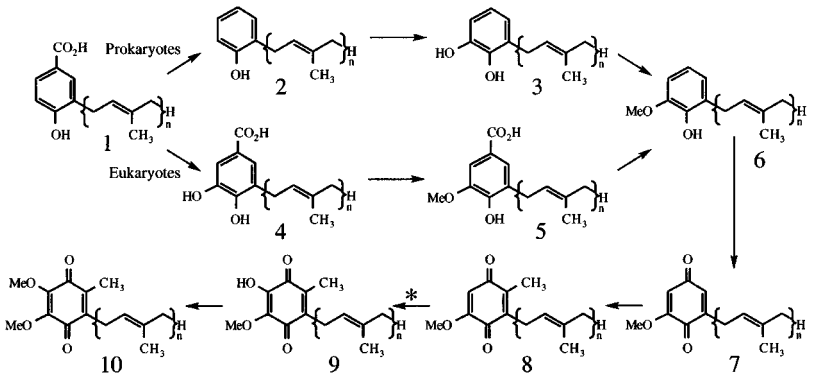

FIG. 1. **The pathway of Q biosynthesis.** The proposed biosynthetic pathway for Q in eukaryotes (including yeast) and in prokaryotes is thought to diverge after assembly of compound **1** (3-polyprenyl-4-hydroxybenzoate). The length of the isoprenoid chain (*n*) varies depending on the species and ranges 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-*n*). 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.

TABLE I
Genotypes and sources of S. cerevisiae strains

Strain	Genotype	Source or reference
DBY 1034	**a,** *ura* 3–52, *lys* 2–801, *his*	D. Botstein
D273–10B	α, *met* 6	12, 13
C97	α, *met* 6, *coq* 7–1	12, 13
JM6	**a**, *his* 4, ρ^0	55
JM43	α, *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* 1–1, *his* 3–11	13
FY250	α, *ade* 8, *leu* 2Δ1, *ura* 3–52, *trp* 1Δ63, *his* 3Δ200	Winston and Dollard (unpublished results)
NM101	**a**, *leu* 2–3, *leu* 2–112, *ura* 3–52, *coq* 7–1	This study
NM103	α, *ura* 3–52, *coq* 7–1	This study
JM43,*coq7*Δ-1	JM43,*coq7*Δ-1::*LEU2*	This study
JM43,*coq7*Δ-2	JM43,*coq7*Δ-2::*LEU2*	This study
W303,*coq7*Δ-1	W303,*coq7*Δ-1::*LEU2*	This study
FY250,*coq7*Δ-1	FY250,*coq7*Δ-1::*LEU2*	This study
NM101,*coq7*Δ-1	NM101,*coq7*Δ-1::*LEU2*	This study

MATERIALS AND METHODS

Strains and Media—Strains of *S. cerevisiae* are listed in Table I. NM101 and NM103 were generated as ascispores 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% dextrose; YPM, 1% yeast extract, 2% peptone, 2% maltose (Fluka BioChemika); YPG, 1% yeast extract, 2% peptone, 3% glycerol; SD, 0.67% yeast nitrogen base without amino acids, 2% dextrose. SD complete medium consisted of SD medium supplemented with amino acids adenine and uracil, or specific components were deleted as required for selection (25). Agar (2%) was added for solid media. Yeast were grown at 30 °C and shaken at 220 rpm when in liquid culture .

In Vivo Labeling of Q_6 and Q_6 Intermediates and Lipid Extraction—[*carboxyl*-^{14}C]*p*-hydroxybenzoic acid (50 Ci/mol) was from RPI Corp. (Mount Prospect, IL) and [U-^{14}C]*p*-hydroxybenzoic acid (365 Ci/mol) was synthesized from L-[U-^{14}C]tyrosine (469 Ci/mol, DuPont NEN) by alkali heat fusion as described by Clarke *et al.* (17). [U-^{14}C]*p*-Hydroxybenzoic acid was added to the SD complete medium yeast cultures before inoculation (0.65 μCi/liter). Yeast were harvested by centrifugation (1470 × *g*, 10 min) when the optical density (600 nm) of the culture was 8–11. Lipid extracts were prepared as described (26, 27), except that the initial volume of hexane:isopropanol (3:2) added was 8 ml/g wet weight cell pellet. Extracts were concentrated, transferred to graduated 10-ml conical borosilicate centrifuge tubes and the volume adjusted to 8 ml/liter of yeast culture extracted. All glassware was new or chromic-sulfuric acid washed. Extracts were stored under N_2 at −20 °C.

Analysis and Purification of Q and Q Intermediates by HPLC—Aliquots (1.5 or 2.0 ml) of ^{14}C-labeled yeast lipid extracts (containing Q and/or Q-intermediates) were dried under N_2 gas in new borosilicate tubes and subsequently resuspended in methanol (60 μl). A white precipitate (containing no significant radioactivity) was removed by centrifugation (1200 × *g*, 2 min), and the supernatant separated by a reverse phase HPLC system (26). A reverse-phase column (Econosphere C-18 5-μm, 4.6 mm × 250 mm, Alltech, Deerfield, IL) was equilibrated in solvent A (methanol:water, 9:1) at 1 ml/min and following sample injection (50 μl) the percentage of solvent B (methanol:isopropanol, 7:3, v:v) was increased linearly, starting at sample injection, over 20 min and held at 100% B for 10 min and returned to initial conditions by 40 min. Fractions 25 and 26 (1 ml each), corresponding to the retention time for standard Q (Sigma) or DMQ were collected following repeated injections of yeast total lipids. After collecting fractions 25 and 26 from successive injections, each like fraction was pooled, and then analyzed by normal phase HPLC on a cyanopropyl column (Zorbax®, 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 Q_6 (Sigma) using the integrated area units of identified peaks. Concentrations of Q_6 standards in ethanol were determined using $E_{275\,nm} = 15{,}300\ M^{-1}\ cm^{-1}$ (28). This method provides a reasonably accurate estimate of DMQ since the two compounds have similar spectral qualities; DMQ_9 in ethanol at 271 nm, $E = 14{,}500\ M^{-1}\ cm^{-1}$ (29).

Analytical HPLC of ^{14}C-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:water: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 ^{14}C window; the ^{14}C efficiency was 95%.

Analysis by Mass Spectrometry—HPLC fractions (1 ml) were dried under N_2 gas, resuspended in 5–20 μl of heptane, and transferred to glass capillary 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 ($M/\Delta M$]). A mass range of 50–700 was covered in the magnet 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-minus-uracil medium 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 during 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 *Bam*HI fragment of p7.8 was subcloned 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 the dideoxynucleotide chain termination method using the Sequenase version 1.0 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 had been submitted (33). Primers were constructed to allow the unidirectional verification of the reported sequence. Query of the BLAST program (NCBI) with the entire nucleotide sequence revealed the submission of the entire open reading frame of *COQ7* and an upstream open reading frame detailed in Fig. 7 (accession no. X82930, EMBL data base). This reported nucleic acid sequence was in complete agreement with our sequence. Restriction enzyme digestion of the 5′ region of pNMQ7 plasmid deleted a 414-bp region of the upstream open reading frame and created the rescuing plasmid pNMQ71. 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 *Hin*dIII to *Xho*I fragment from pNMQ7 was subcloned into the bacterial plasmid pT7–7 (34) to create plasmid pT1. This plasmid was subsequently digested with *Eco*RV and *Stu*I, liberating a 368-bp blunt-ended fragment within the coding region of the *COQ7* gene. This region of pT1 was replaced with one of two *LEU2* gene fragments (a 2.9-kb *Bgl*II or a 4.1-kb *Pst*I fragment of YEp13) to generate pYDQ71 and pYDQ72, respectively. Restriction enzyme digests of these clones with *Hin*dIII/*Bam*HI generated linear insert DNA, which was used in a one-step gene replacement (35). The resulting Leu^+ 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 *coq7*Δ strains obtained were not complemented for growth on glycerol by the *coq7–1* strains C97 or NM101, but were complemented by ρ° tester strains.

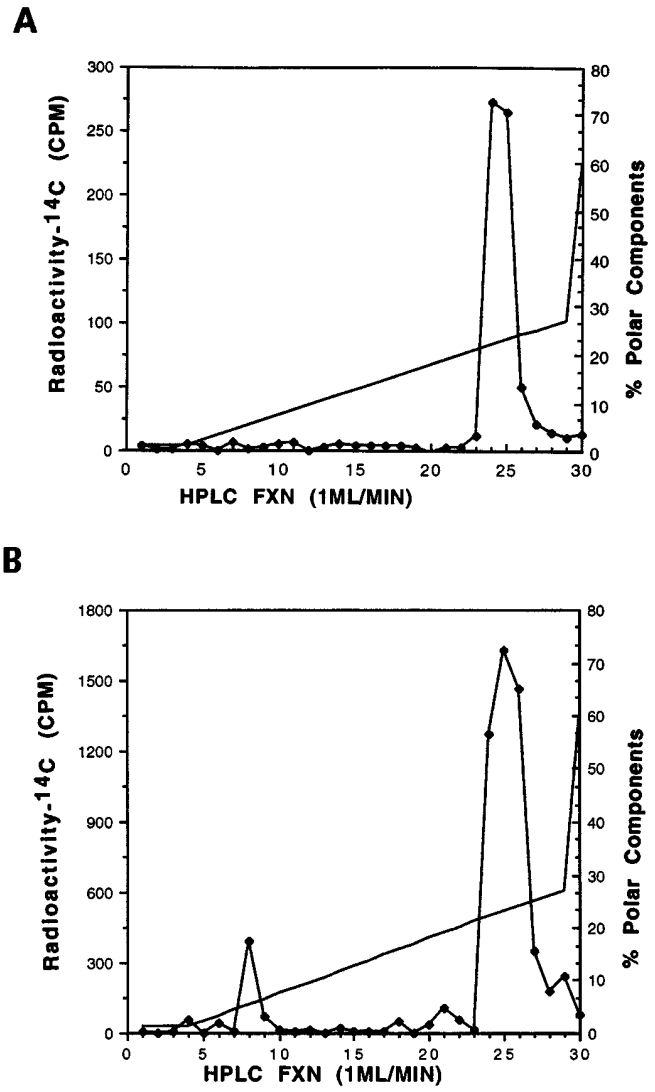

FIG. 2. **C97 (*coq7–1*) accumulates a quinone intermediate which is not Q.** *Panel A*, a lipid extract from the C97 yeast strain labeled with [*carboxyl*-^{14}C]*p*-hydroxybenzoic acid was separated by normal phase HPLC and 1-ml fractions were collected and ^{14}C radioactivity was determined by scintillation counting (◆). Values are plotted as the ^{14}C radioactivity in counts/min (minus background). The *superimposed line* shows the gradient profile of percent polar components (the B solvent). *Panel B*, a lipid extract of C97 was labeled with [U-^{14}C]*p*-hydroxybenzoic acid as described in *panel A*. The radioactivity detected in fraction 8 does not coincide with a ubiquinone standard, which would be present in fraction 6 (data not shown).

RNA 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 (0.50 M sodium phosphate, pH 7.0, 1.0% bovine serum albumin, 7% sodium dodecyl sulfate, 1 mM EDTA) (36). Probes were labeled with [α-^{32}P]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 ^{32}P-labeled DNA probes corresponding to either a segment of the yeast *COQ3* gene, a 0.7-kb *Bgl*II fragment isolated from pRS12A-2.5SB, (17), or a segment of DNA containing the *COQ7* gene (1.9-kb *Hin*dIII to *Xho*I restriction fragment from pNMQ7). This blot was subsequently re-probed with a clathrin heavy chain *CHC1* gene present in the plasmid pCHC101 (37) after expiration of 12 ^{32}P half-lives. Blots were

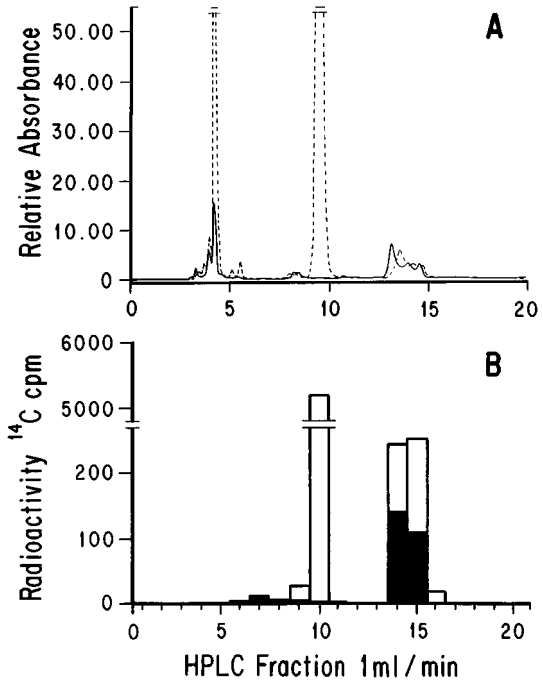

FIG. 3. **Purification and analysis of [U-^{14}C]*p*-hydroxybenzoic acid-labeled quinones.** Total lipids were extracted from 2 liters of the *coq7–1* strain (NM101) or from a 1-liter culture of JM43*coq7*Δ-1/pNMQ71. The total lipid extracts were first separated by reverse phase HPLC (not shown) as described under "Materials and Methods." This step removed the predominant ^{14}C-radiolabeled intermediate 3-hexaprenyl-4-hydroxybenzoate (compound **1**, Fig. 1) from the quinone-like material. Reverse phase fractions 25 and 26 contained the ^{14}C-labeled quinone material and were individually further purified by normal phase HPLC as shown in *panel A* (the isocratic solvent was 0.1% 2-propanol in heptane). The relative Absorbance units (266 nm) is shown as either a *solid line* (NM101, fraction 25), or a *dotted line* (JM43*coq7*Δ-1/pNMQ71, fraction 26). *Panel B*, radioactivity was monitored by scintillation counting of 10% of each 1-ml fraction of NM101 (*black bars*) or JM43*coq7*Δ-1/pNMQ71 (*open bars*).

hybridized for 18 h at 65 °C with 2 ng of ^{32}P-labeled probe per ml of hybridization buffer. Blots were washed three times, 30 min each with 0.2 × SSC, 0.05% SDS at 65 °C (1 × SSC is 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0). Washed blots were exposed to x-ray film (Kodak XAR-5) with two intensifying screens and placed at −80 °C.

RESULTS

A coq7–1 Mutant (C97) Lacks Q and Accumulates a "Quinone-like" Intermediate—To study the biochemical defects responsible for Q deficiency in the uncharacterized *coq* complementation groups, lipid extracts were prepared from 100-ml cultures of one representative strain from each *coq* complementation group (*coq4*–*coq8*) grown in the presence of either [U-^{14}C]*p*-hydroxybenzoic acid or [*carboxyl*-^{14}C]*p*-hydroxybenzoic acid. Lipid extracts were then fractionated by normal phase HPLC and monitored for ^{14}C radioactivity. Analysis of one of the mutant strains, C97 (containing the *coq7–1* allele; Ref. 14), is shown in Fig. 2. The phenotype exhibited by C97 is similar to other *coq* mutants, exhibiting a lack of growth on nonfermentable carbon sources or on maltose (38). The large peak of radioactivity in fractions 24–26 in *panels A* and *B* has been identified as 3-polyprenyl-4-hydroxybenzoate (compound **1**, Fig. 1). This intermediate is present at high amounts in yeast wild-type strains and in all of the *coq* mutant strains (Ref. 26 and data not shown). Because this Q intermediate retains the carboxyl group present in the [*carboxyl*-^{14}C]*p*-hydroxybenzoate precursor, it is identified in both chromotographic analyses in Fig. 1. However, the ^{14}C-radiolabeled compound present in *panel B* at fraction 8 is detected only after incubation with [U-^{14}C]*p*-hydroxybenzoate, indicating that this compound does not contain a carboxyl labeled carbon. The elution position at fraction 8 is 2 min later than a Q_6 standard and suggests that this intermediate is slightly more polar than Q_6. The retention times for this intermediate and for Q_6 by reverse phase HPLC are separated by less than 0.5 min (data not shown). These elution characteristics suggest that the [U-^{14}C]*p*-hydroxybenzoic acid-radiolabeled material eluting in fraction 8 may be a quinone intermediate in the Q biosynthetic pathway, slightly more polar than Q.

Purification of the ^{14}C-Radiolabeled Quinone-like Compound from coq7 Yeast Lipid Extracts—The [U-^{14}C]*p*-hydroxybenzoate radiolabeled compound was observed in lipid extracts prepared from a derived *coq7–1* yeast strain, NM101, and was purified by the sequential use of reverse- and normal-phase HPLC systems as described under "Materials and Methods." The ^{14}C-labeled quinone-like compound was first purified by reverse phase HPLC and collected as fractions 25 and 26 (data not shown). These fractions were then individually analyzed by normal phase HPLC. Fig. 3 shows the normal phase chromatogram (relative absorbance at 266 nm) resulting from injection of fraction 25 (*coq7–1* mutant; *panel A*, *solid line*) compared to a chromatogram of fraction 26 from a respiratory competent strain (JM43,*coq7–1*Δ/pNMQ71; *panel A*, *dotted line*). Normal phase HPLC analysis of fraction 26 from NM101 gave a very similar profile to that shown in Fig. 3 (data not shown). Both strains display a three-tiered peak of UV absorbance (fractions 13–15) that corresponds to a broad peak of radioactivity (*panel B*). However, in the lipids analyzed from respiratory competent yeast, an additional predominant UV and radioactive peak is observed at fractions 9 and 10 and co-elutes with a Q_6 standard. This material is greatly enriched in reverse phase fraction 26 compared to fraction 25 (data not shown). This peak is notably absent in the corresponding fractions of the *coq7–1* extract analyzed (Fig. 3 and data not shown). Thus the *coq7–1* mutant strain accumulates no detectable Q_6, but does accumulate a [U-^{14}C]*p*-hydroxybenzoate radiolabeled peak that is also present in smaller amounts in respiratory competent yeast.

Identification of ^{14}C-Radiolabeled Quinones as Q and 5-Demethoxy Q by Mass Spectroscopy—A portion of fraction 10 (Fig. 3, *panel B*) from the respiratory competent yeast strain was identified as Q_6 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_6, ($C_{39}H_{58}O_4$: 590.432022) 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 ($C_{39}H_{60}O_4$; 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-methoxy-1,4-benzoquinone, DMQ, compound **8**, Fig. 1; Refs. 41 and 42). As expected for a quinone-containing intermediate, both M+2 ($C_{38}H_{58}O_3$: 562.439117; observed mass 562.438596; PPM −0.9) and M ($C_{38}H_{56}O_3$: 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 C_{13} ions for M and M+2, which are also within ± 10 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*).

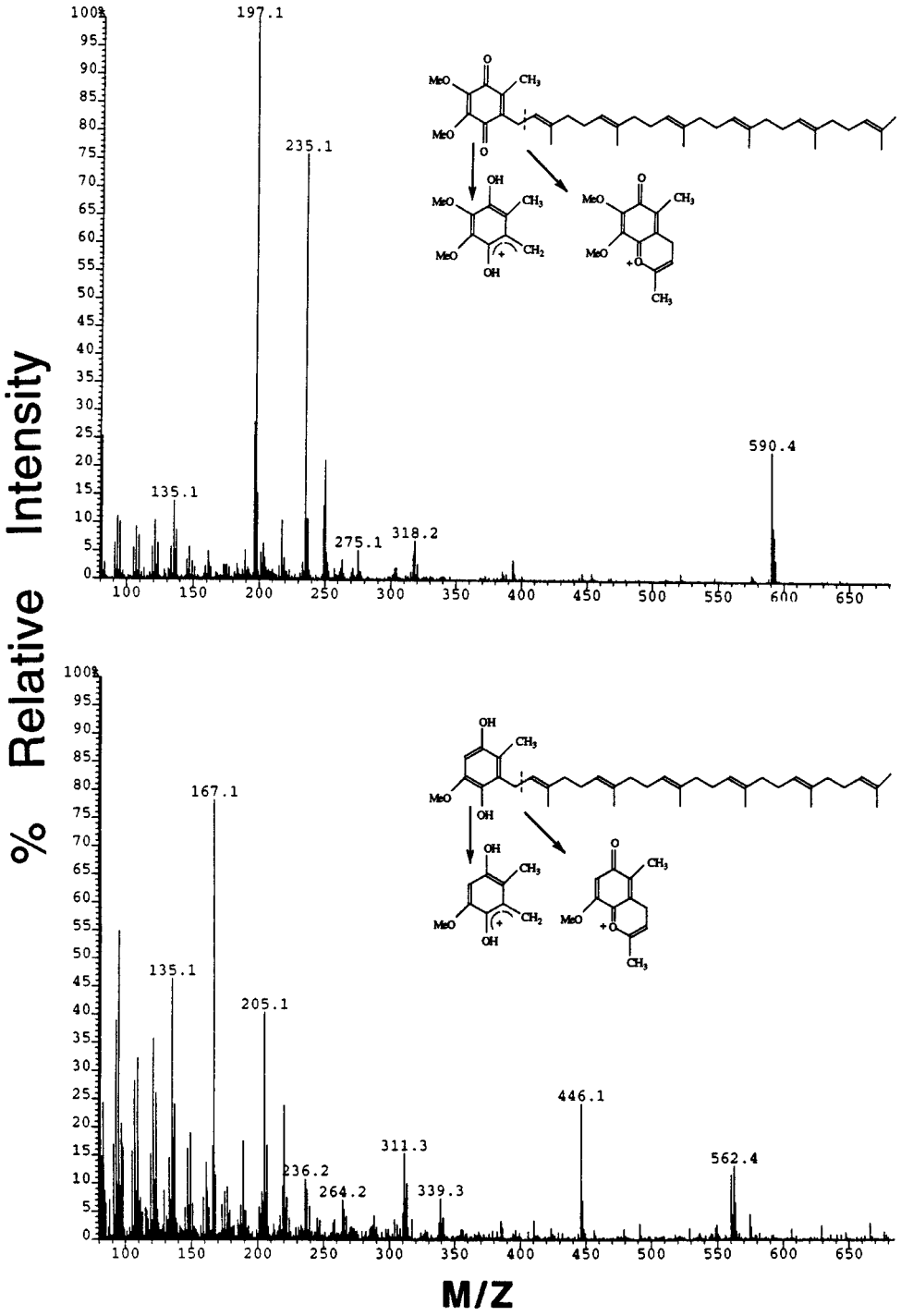

FIG. 4. **Identification of the accumulating quinones in NM101 and in JM43,*coq7*Δ1/pNMQ71.** The electron impact mass spectra for the purified radioactive compounds, purified as in Fig. 3, are shown. *Lower panel*, the fragments are arrayed by *m/z* along the *x* axis for fraction 14 purified from NM101. 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 JM43*coq7*Δ1/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.

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 Q_6 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 Q_6 (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 JM43,*coq7*Δ*-1*/pNMQ71). Thus the defect responsible for the absence of Q_6 in the coq7–1 strain does not cause DMQ to accumulate above levels of that found in respiratory competent yeast.

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 YEp24-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 *Bam*HI fragment contained within the insert DNA of p7.8 restored growth on glycerol when subcloned into the centromeric vector pRS316 to create the plasmid pNM782 (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 pNM782, and each smaller construct was tested for complementation of the glycerol growth defect (Fig. 5, *panel A*).

Initial determination of the DNA sequence of pNMQ7 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 pNMQ71 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 *coq7*Δ strains obtained were complemented for growth on glycerol by the *coq7–1* mutant strains C97 or NM101. The *coq7*Δ strains also failed to complement eight other independently derived *coq7* mutants. These results imply a genetic linkage of the *coq7*Δ*-1::LEU2* disrupted allele to the *coq7* mutation. To verify this, NM101 was transformed with the linear disruption construct *coq7*Δ*-1::LEU2* to generate NM101,*coq7*Δ*-1* (Table I) and the resulting disrupted strain was characterized as described under "Materials and Methods." Diploid cells obtained from the cross of NM101,*coq7*Δ*-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 investigators also reported (accession no. X82930) the presence of an upstream open reading frame fully encoded within the 1.9-kb sequence of the pNMQ7 plasmid (Fig. 5, *panel B*). This upstream open reading frame present was truncated to create pNMQ71. As detailed in Figs. 3 and 4, the presence of the *COQ7* open reading frame in pNMQ71 restores both respiration and synthesis of Q_6 in the *coq7*Δ strain, JM43,*coq7*Δ*-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 cz395 (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 coq7*Δ *Yeast Strains*—Yeast strains containing deletions of the *COQ7* gene were grown in the presence of [U-^{14}C]*p*-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. 6*A*). This amino acid change occurs within a highly conserved region of the *COQ7* gene as shown in Fig. 6*B*. Further testing of *coq7–1* and *coq7*Δ 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 clathrin heavy chain gene does not appear to be induced under the same conditions and, in this analysis, appears much more predominant in the YPD grown cells (Fig. 8, *panels B* and *D*).

DISCUSSION

This study describes the characterization of *coq7* 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

[2] M. Proft and K. D. Entian, personal communication.

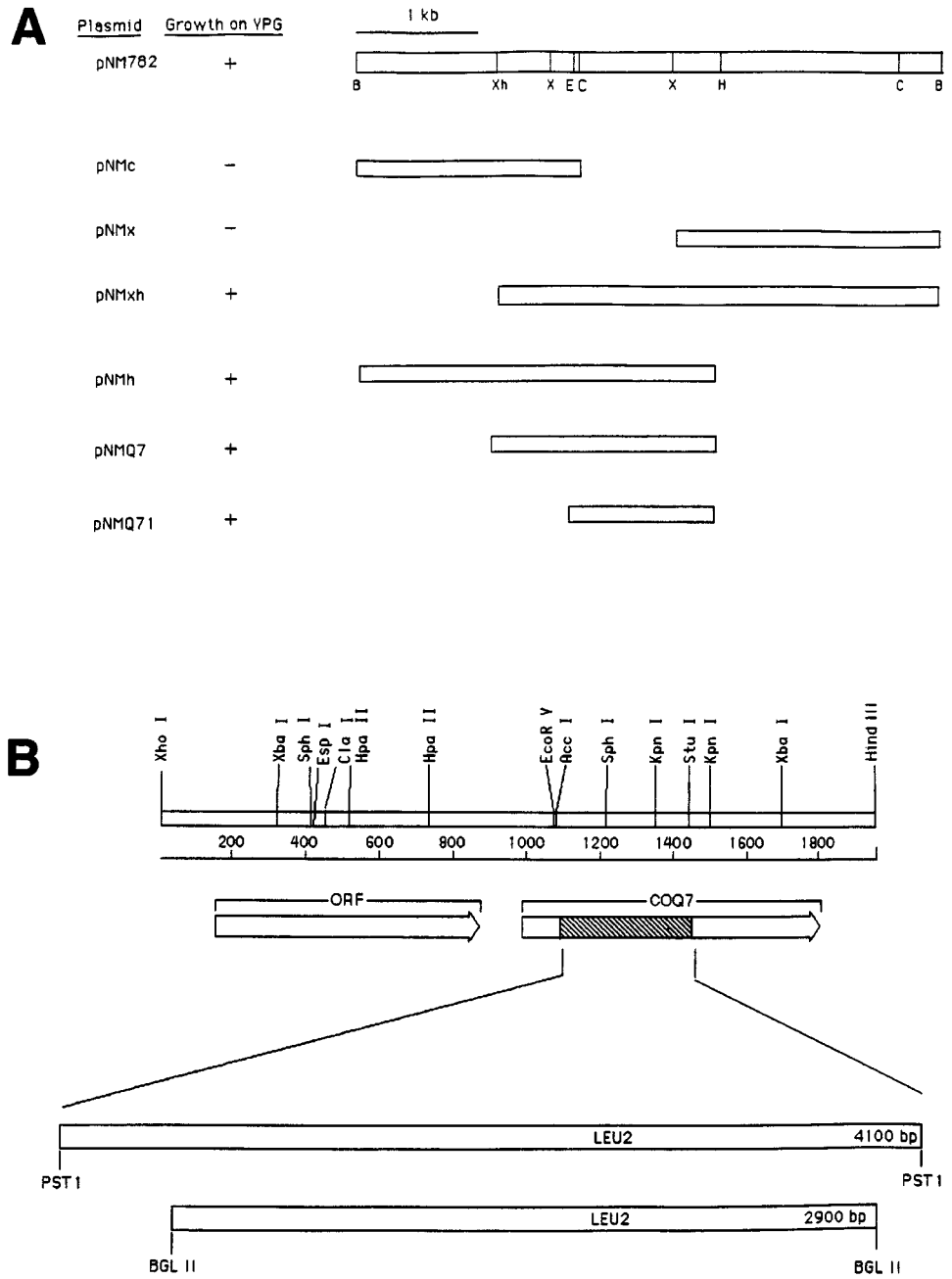

FIG. 5. **Manipulation of cloned sequences.** *Panel A*, restriction mapping and deletion analysis of the cloned genomic sequence rescuing the NM101 strain. A 4.8-kb *Bam*HI fragment of the p7.8 insert was subcloned into the single-copy yeast shuttle vector pRS316 to create pNM782. The 4.8-kb *Bam*HI fragment is represented by the *top bar* and shows relevant restriction sites. The remaining plasmids were created by the restriction digestion, deletion, and religation of pNM782. The plasmids were tested for functional complementation of the NM101 defect by transformation and growth on YPG plates. Those plasmids capable of restoring growth on YPG plates are shown at the *left* with a + sign, non-rescuing plasmids are shown with a − symbol. Restriction sites are symbolized with *single letters* in pNM782 and correspond to the following DNA restriction enzymes: *B*, *Bam*HI; *C*, *Cla*I; *E*, *Esp*I; *H*, *Hin*DIII; *X*, *Xba*I; *Xh*, *Xho*I. The *Esp*I site was mapped only within the pNMQ7 plasmid. *Panel B*, characterization of the 1.9-kb insert of pNMQ7 and the structure of *coq7* deletion constructs. A detailed map of restriction enzyme sites within the *COQ7* gene and the upstream open reading frame is shown. The upstream open reading frame was truncated by *Esp*I and *Xho*I digestion of pNMQ7 creating the rescuing plasmid pNMQ71 (*Panel A*). A deletion within the COQ7 open reading frame employed *Eco*RV and *Stu*I digestion to remove a 368-bp blunt-ended fragment, as shown by the *cross-hatched area*. This DNA segment was replaced with two different DNA fragments containing the *LEU2* gene. *LEU2* gene fragments are not drawn to scale and are contained on either *Bgl*II (2.9 kb) or *Pst*I (4.1 kb) restriction enzyme fragments of YEp13. No difference was seen between the integration of the larger disruption cassette, pYDQ72, *versus* the smaller disruption cassette, pYDQ71.

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

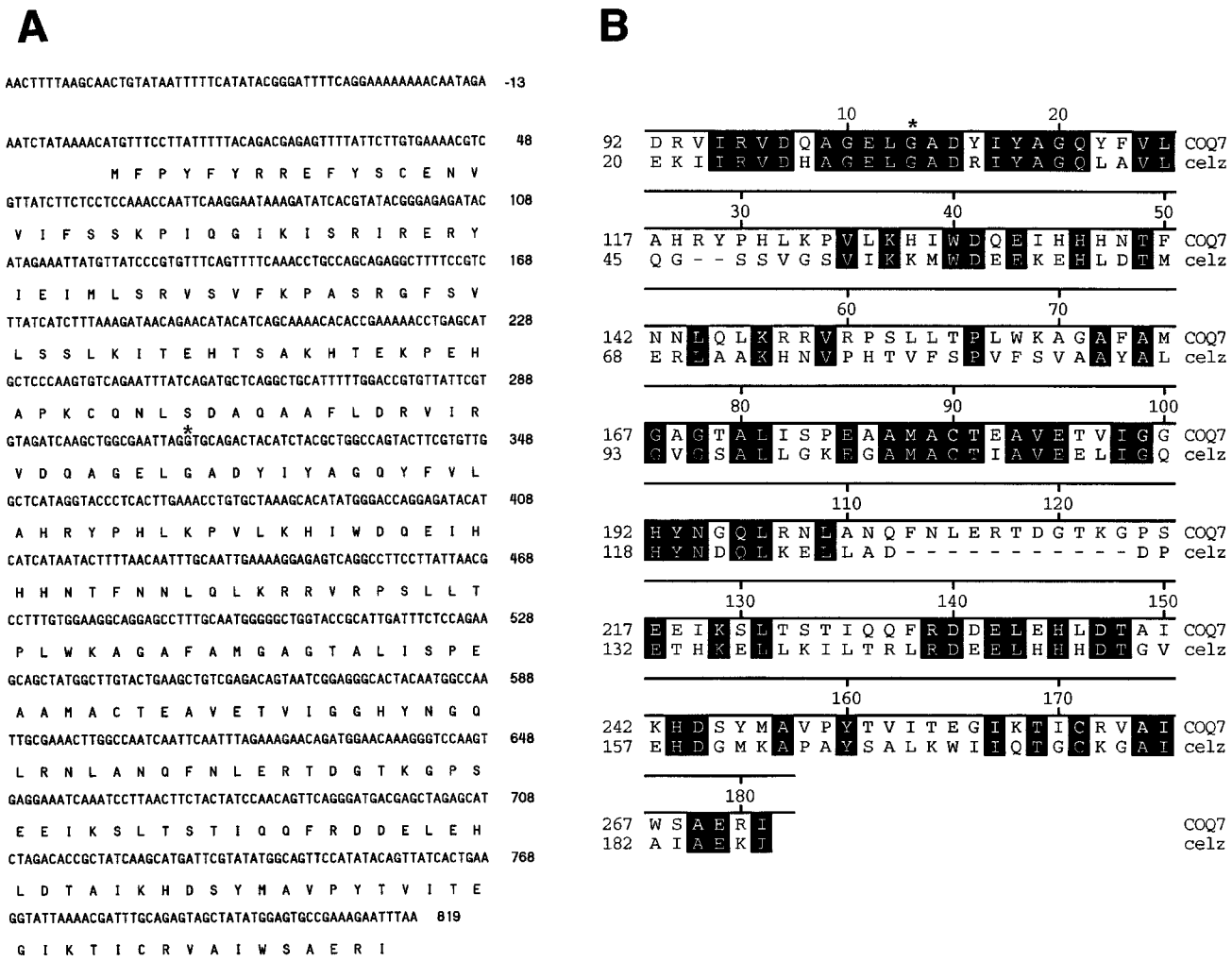

FIG. 6. ***COQ7* sequence analysis.** *Panel A*, the nucleotide sequence and deduced protein sequence is shown for the *COQ7* allele. Nucleotides are numbered from 5′ to 3′. Amino acid residues (*single-letter code*) are placed under the center of each codon, and the A of the first ATG codon is designated as +1. The *asterisk* at nucleotide 311 designates the single base pair change (A instead of G) present in the mutant *coq7–1* allele and predicts an Asp^{104} instead of Gly. *Panel B*, the truncated sequences of the yeast COQ7 protein and a probable *C. elegans* homologue (accession no. U13642, GenBank™ data base) are shown in an alignment generated from DNAstar™ MegAlign using the PAM 250 table and the Jotun Hein method for alignment. Identical residues are *shaded*. The sequences as shown span the region of highest homology, from amino acid 92 of Coq7p and amino acid 20 of the putative *C. elegans* homologue. These sequences were calculated to be 44% similar and are 42% identical. The *asterisk* designates the $Gly^{104} \rightarrow Asp$ change present in the Coq7–1p.

speculation must take into account the presence of DMQ in *coq7–1* mutants and its absence in *coq7*Δ 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 enzyme 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-

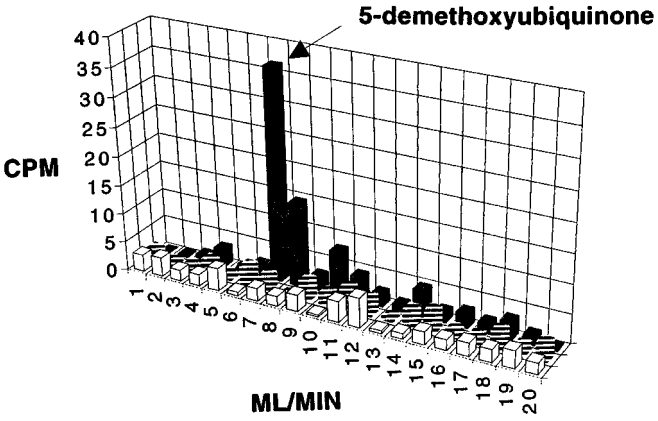

FIG. 7. **HPLC analysis of Q intermediates from NM101 yeast strain and *coq7 deletion strains shows that deletion of the gene abolishes the accumulation of DMQ.*** A three-dimensional profile of radioactivity present in normal phase HPLC fractions collected from the fractionation of 400 μl of total yeast lipids from two *coq7* deletion strains (FY250,*coq7*Δ-*1* and NM101,*coq7*Δ-*1*) and NM101 is shown; 400 μl represents one twentieth of the labeled lipid extract from 1 liter of yeast). Strains were labeled, extracted, and analyzed as detailed under "Materials and Methods." The *x* axis denotes the milliliters collected per minute, and the *y* axis represents ^{14}C radioactivity in 1-ml fractions (counts/min minus background). The identity of the lipid extract analyzed is shown in the *z* axis, with representative symbols in the legend. The largest peak present in the extract from the NM101 strain is labeled as DMQ. □, nm101D; ▤, fy250D; ■, NM101.

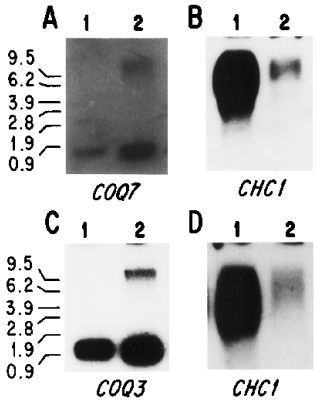

FIG. 8. **Northern analysis of the *COQ7* gene shows it is induced in nonfermentative growth conditions.** Two identical panels from the same Northern blot were each probed with ^{32}P-labeled DNA fragments corresponding to the *COQ7* gene (*A*) and the *COQ3* gene (*C*). These same panels were subsequently reprobed with ^{32}P-labeled plasmid DNA containing the clathrin heavy chain gene (*CHC1*, *panels B* and *D*) (37). *Lanes 1* and *2* indicate growth conditions of yeast from which mRNA was collected: *lane 1*, growth in YPD; *lane 2*, in YPG. Each RNA preparation (5 μg) was separated by electrophoresis on 1.2% agarose-formaldehyde gel. Subsequent Northern analysis was performed as described under "Materials and Methods" with the *COQ7* 1.9-kilobase cDNA insert of pNMQ7 (*A*) or with a 2.0-kilobase cDNA of *COQ3* (*B*). Probe specific activities were 3.0×10^9 and 2.8×10^9 cpm/μg, respectively. The blot in *panels A* and *C* was set aside >12 ^{32}P half-lives (6 months) and then hybridized with pCHC1001 (3.5×10^8 cpm/μg), as shown in *panels B* and *D*. Blots were hybridized at 65 °C and washed three times with 0.2 × SSC, 0.1% SDS at 55 °C. RNA size standards (Life Technologies, Inc.) are indicated. Autoradiographic exposure times were 12 days (*A* and *C*) and 5 days (*B* and *D*).

ity to any other class of protein, including any known monooxygenase or hydroxylase proteins. A probable *C. elegans* homologue was detected and was 42% identical within the sequences compared (Fig. 6). Recently other investigators have isolated the *COQ7* allele (*CAT5*) in a separate mutant screen[2] and have indicated that this protein is involved in glucose derepression. The *coq7* complementation group was originally isolated as a nuclear encoded *pet* yeast strain and was identified as Q-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 develope 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_4T (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.

Acknowledgments—We thank Alexander Tzagoloff for providing us with the *coq7* mutant strains and Markus Proft and Karl-Dieter Entian for providing us with their unpublished results. We thank Jon Lowenson and Greg Payne for their critical reading of our manuscript and the following individuals for their participation in useful discussions: Greg Payne, Alexander Van Der Bliek, Tomas Ganz, John Colicelli, Gerry Wienmaster, Ralf Landgraf, Han Phan, Phil Tan, Marybeth Mudgett, and Erika Valore. We thank the following individuals for substantial and altruistic material support: Steven Clarke, Kym Faull, David Sigman, Ron Kagan, Duncan MacLaren, Wayne Poon, Jeff Schultz, and Peter Thanth Lee.

REFERENCES

1. Brandt, U., and Trumpower, B. (1994) *Crit. Rev. Biochem. Mol. Biol.* **29,** 165–197
2. Trumpower, B. L. (1981) *J. Bioenerg. Biomembr.* **13,** 1–24
3. Ernster, L., and Forsmark-Andree, P. (1993) *Clin. Invest.* **71,** S60–S65
4. Kagan, V., Serbinova, E., and Packer, L. (1990) *Biochem. Biophys. Res. Commun.* **169,** 851–857
5. Bowry, V. W., Mohr, D., Cleary, J., and Stocker, R. (1995) *J. Biol. Chem.* **270,** 5756–5763
6. Aberg, F., Appelkvist, E.-L., Dallner, G., and Ernster, L. (1992) *Arch. Biochem. Biophys.* **295,** 230–234
7. Stocker, R., Bowry, V. W., and Frei, B. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 1646–1650
8. Mohr, D., Bowry, V. W., and Stocker, R. (1992) *Biochim. Biophys. Acta* **1126,** 247–254
9. Tribble, D. L., van den Berg, J. J. M., Motchnik, P. A., Ames, B. N., Lewis, D. M., Chait, A., and Krauss, R. M. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 1183–1187
10. Olson, R. E., and Rudney, H. (1983) *Vitam. Horm.* **40,** 1–43
11. Gibson, F. (1973) *Biochem. Soc. Trans.* **1,** 317–326
12. Tzagoloff, A., Akai, A., and Needleman, R. B. (1975) *J. Biol. Chem.* **250,** 8228–8235
13. Tzagoloff, A., Akai, A., and Needleman, R. B. (1975) *J. Bacteriol.* **122,** 826–831
14. Tzagoloff, A., and Dieckmann, C. L. (1990) *Microbiol. Rev.* **54,** 211–225
15. Ashby, M. N., and Edwards, P. A. (1990) *J. Biol. Chem.* **265,** 13157–13164
16. Ashby, M. N., Kutsunai, S. Y., Ackerman, S., Tzagoloff, A., and Edwards, P. A. (1992) *J. Biol. Chem.* **267,** 4128–4136
17. Clarke, C. F., Williams, W., and Teruya, J. H. (1991) *J. Biol. Chem.* **266,** 16636–16644
18. Cox, G. B., Young, I. G., McCann, L. M., and Gibson, F. (1969) *J. Bacteriol.* **99,** 450–458
19. Goewert, R. R., Sippel, C. J., and Olson, R. E. (1981) *Biochemistry* **20,** 4217–4223
20. Goewert, R. R., Sippel, C. J. Grimm, M. F., and Olson, R. E. (1981) *Biochemistry* **20,** 5611–5616
21. Gibson, F., and Young, I. G. (1978) *Methods Enzymol.* **53,** 600–609

22. Law, A., Threlfall, D. R., and Whistance, G. R. (1971) *Biochem. J.* **123,** 331–339
23. Marbois, B. N., Hsu, A., Pillai, R., Colicelli, J., and Clarke, C. F. (1994) *Gene* (*Amst.*) **138,** 213–217
24. Marbois, B. N., Xia, Y.-R., Lusis, A. J., and Clarke, C. F. (1994) *Arch. Biochem. Biophys.* **313,** 83–88
25. Sherman, F., Fink, G. R., and Hicks, J. B. (1986) *Laboratory Course Manual for Methods in Yeast Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
26. Poon, W. W., Marbois, B. N., Faull, K. F., and Clarke, C. F. (1995) *Arch. Biochem. Biophys.* **320,** 305–314
27. Radin, N. S. (1981) *Methods Enzymol.* **72,** 5–7
28. Barr, R., and Crane, F. L. (1985) in *Coenzyme Q: Biochemistry, Bioenergetics and Clinical Applications of Ubiquinone* (Lenaz, G., ed) pp. 58–59, John Wiley & Sons, New York
29. Trumpower, B. L., Opliger, C. E., and Olson, R. E (1974) *Chem. Phys. Lipids* **13,** 123–132
30. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., Struhl, K., Albright, L. M., Coen, D. M., Varki, A., and Janssen, K. (eds) (1994) *Current Protocols in Molecular Biology*, Greene Publishing Associates/Wiley-Interscience, New York
31. Carlson, M., and Botstein, D. (1982) *Cell* **28,** 145–154
32. Sikorski, R. S., and Hieter, P. (1989) *Genetics* **122,** 19–27
33. Baker, R. T., Tobias, J. W., and Varshavsky, A. (1992) *J. Biol. Chem.* **267,** 23364–23375
34. Tabor, S. (1990) in *Current Protocols in Molecular Biology* (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., Struhl, K., Albright, L. M., Coen, D. M., Varki, A., and Janssen, K., eds) pp. 16.2.1–16.2.11, Greene Publishing Associates/Wiley Interscience, New York
35. Rothstein, R. J. (1983) *Methods Enzymol.* **101,** 202–211
36. Church, G. M., and Gilbert, W. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 1991–1995
37. Payne, G. S., and Schekman, R. (1985) *Science* **230,** 1009–1014
38. Schamhart, D. H. J, Ten Berge, A. M., and Van De Poll, K. W. (1975) *J. Bacteriol.* **121,** 747–752
39. Muraca, R. F., Whittick, J. S., Daves, G. D., Friis, P., and Folkers, K. (1967) *J. Am. Chem. Soc.* **89,** 1505–1508
40. Elliott, W. H., and Waller, G. R. (1972) in *Biochemical Applications of Mass Spectrometry*, (Waller, G. R., ed) pp. 521–535, Wiley-Interscience, New York
41. Young, I. G., McCann, L. M., Stroobant, P., and Gibson, F. (1971) *J. Bacteriol.* **105,** 769–778
42. Trumpower, B. L., Aiyar, A. S., Opliger, C. E., and Olson, R. E. (1972) *J. Biol. Chem.* **247,** 2499–2511
43. Schneider, J. C., and Guarente, L. (1991) *Methods Enzymol.* **194,** 373–388
44. Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., Bonfield, J., Burton, J., Connell, M., Copsey, T., Cooper, J., Coulson, A., Craxton, M., Dear, S., Du, Z., Durbin, R., Favello, A., Fraser, A., Fulton, L., Gardner, A., Green, P., Hawkins, T., Hillier, L., Jier, M., Johnston, L., Jones, M., Kershaw, J., Kirsten, J., Laisster, N., Latreille, P., Lightning, J., Lloyd, C., Mortimore, B., O'Callaghan, M., Parsons, J., Percy, C., Rifken, L., Roopra, A., Saunders, D., Shownkeen, R., Sims, M., Smaldon, N., Smith, A., Smith, M., Sonnhammer, E., Staden, R., Sulston, J., Thierry-Mieg, J., Thomas, K., Vaudin, M., Vaughan, K., Waterston, R., Watson, A., Weinstock, L., Wilkinson- Sproat, J., and Wohldman, P. (1994) *Nature* **368,** 32–38
45. Koop, D. R. (1992) *FASEB J.* **6,** 724–730
46. Gatti, D. L., and Tzagoloff, A. (1990) *J. Biol. Chem.* **265,** 21468–21475
47. Tzagoloff, A., Yue, J., Jang, J., and Paul, M.-F. (1994) *J. Biol. Chem.* **269,** 26144- 26151
48. Hoogeveen, A. T., Verheijen, F. W., and Galjaard, H. (1983) *J. Biol. Chem.* **258,** 12143–12146
49. Alexander, K., and Young, I. G. (1978) *Biochemistry* **17,** 4745–4750
50. Werner-Washburne, M., Braun, E., Johnston, G. C., and Singer, R. A. (1993) *Microbiol. Rev.* **57,** 383–401
51. Gounalaki, N., and Thireos, G. (1994) *EMBO J.* **13,** 4036–4041
52. Stevens, B. (1981) in *The Molecular Biology of the Yeast Saccharomyces cerevisiae: Life Cycle and Inheritance* (Strathern, J. N., Jones, E. W., and Broach, J. R., eds) pp. 471–504, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
53. Craig, E. A. (1992) in *The Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression* (Jones, E. W., Pringle, J. R., and Broach, J. R., eds) Vol. 22, pp. 501–537, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
54. Kobayashi, N., and McEntee, K. (1993) *Mol. Cell. Biol.* **13,** 248–256
55. McEwen, J. E., Ko, C., Kloeckner-Gruissem, B., and Poyton, R. O. (1986) *J. Biol. Chem.* **261,** 11872–11879