Coenzyme Q (ubiquinone or Q) is a prenylated benzoquinone found in cell membranes that functions in redox chemistry as both an oxidant (Q) and reductant (QH2) (1). Q is most commonly associated with its role in the respiratory chain where it transports electrons from either Complex I or Complex II to Complex III (2); however, Q serves multiple functions. Q has been demonstrated to play a role in stabilizing the bc1 complex (3). Additionally, Q functions in the electron transport chains of lysosomal and plasma membranes (4, 5), and QH2 acts as a chain breaking antioxidant of lipid peroxyl radicals (6). In Escherichia coli, a high QH2:Q ratio is sensed by ArcB, a transmembrane sensor kinase, that phosphorylates ArcA, activating operons involved in fermentation and repressing those involved in respiration (7). In Caenorhabditis elegans, dietary Q produces a shortened life span (8). This phenomenon has been attributed to the generation of superoxide by Q7 (the Q semiquinone radical) generated during respiratory electron transport (9). In humans, Q supplementation has been shown to be effective in treating patients with specific respiratory chain defects (10) and to slow the progression of Parkinson’s disease symptoms (11).

Cells normally acquire Q through de novo synthesis, and the length of the prenyl tail varies among different organisms (12). Saccharomyces cerevisiae produce Q6, which has six isoprene units, whereas E. coli tail varies among different organisms (12). Saccharomyces cerevisiae produce Q8, which has six isoprene units, whereas E. coli tail varies among different organisms (12). Eight COQ genes have been identified to be required for Q synthesis in S. cerevisiae (13, 14). Fig. 1 shows the pathway for Q biosynthesis in both prokaryotes and eukaryotes. In yeast, mutation in any of the eight COQ genes results in cells that cannot synthesize Q and fail to grow on nonfermentable carbon sources. Yeast coq3–coq8 mutants each accumulate the same early intermediate in Q biosynthesis, 3-hexaprenyl-4-hydroxybenzoic acid (Fig. 1, HHB or compound 1) (15).

In this work the yeast COQ6 gene has been isolated by functional complementation of a mutant from the G63 (coq6) complementation group. Transformation of coq6 mutant strains with a plasmid bearing wild type COQ6 restores Q biosynthesis and growth on nonfermentable carbon sources. In contrast to a previous report (16), we find COQ6 to be a non-essential gene. Here we show the Coq6 polypeptide is imported into mitochondria and is peripherally associated with the inner mitochondrial membrane. Based on sequence homology to known proteins, we suggest that COQ6 encodes a flavin-dependent monooxygenase required for one or more steps in Q biosynthesis.

**EXPERIMENTAL PROCEDURES**

*Strains and Growth Media—* The strains used in this study are listed in Table I. Growth media for yeast were prepared as described (17) and included YPD (1% yeast extract, 2% peptone, 2% dextrose), YPG (1% yeast extract, 2% peptone, 3% glycerol), YPGal (1% yeast extract, 2% peptone, 2% galactose, 0.1% dextrose), SDC (0.18% yeast nitrogen base without amino acids, 2% dextrose, 0.14% NaH2PO4, 0.5% (NH4)2SO4, and complete supplement of amino acids), SD–Leu (SDC minus leucine), and SD–Ura (SDC minus uracil). The complete supplement was modified as described (18). Semisynthetic lactate medium was prepared as described (19). Media for sporulation and tetrad analysis were prepared as described (17). All components of growth medium were purchased from Difeo, Fisher, or Sigma. 2% agar was added for solid medium.

**Cloning of the COQ6 Gene—*The haploid strain SR128-3C containing the coq6-1 allele was obtained from mating C128 and FY250 (Table I). SR128-3C yeast were grown to early-log phase in YPD medium and transformed (20) with the Ycpl50 centromeric plasmid library of S. cerevisiae genomic DNA (21) containing the URA3 gene as a selectable marker. Transformants were selected on SD–Ura plates and after a 3-day incubation at 30 °C were replica-plated onto YPG plates to test for respiratory growth. Putative Q prototrophic transformants, able to grow on medium containing a nonfermentable carbon source, were further purified and tested for co-segregation of uracil prototrophy and
respiration competency following vegetative growth in rich medium. Such co-segregation was observed in two transformants, indicating that these traits were plasmid-linked. Yeast plasmid DNA was recovered from one transformant (494SR) and was amplified in DH5α/H9251 E. coli (Invitrogen). The plasmid p494SR contained an insert of 4.1-kb and transformation of SR128-3C with p494SR restored growth on YPG medium. A similar cloning procedure was also performed with a multicopy expression library prepared from yeast genomic DNA in the vector YEp24 (22) and resulted in the isolation of pG63/T1, which was found to contain a 2.8-kb segment of DNA that overlapped with the insert present in p494SR (Fig. 2). Seven other rescuing yeast genomic DNA clones were similarly isolated from a recombinant pUV1-based plasmid library (generous gift of Junichi Nikawa and Michael Wigler, Cold Spring Harbor Laboratory). Southern analysis showed that all of the clones contained overlapping DNA fragments (data not shown).

Subcloning and Disruption of the COQ6 Gene
—
A 3.8-kb HindIII fragment was isolated from p494SR and was ligated to the HindIII site of pRSS16 (23) to generate pSR1-1. The insert of pSR1-1 was sequenced and found to contain two ORFs located on a segment of chromosome VII. Only one ORF, YGR255C, was also present on pG63/T1, and was designated COQ6 (deposited in GenBank with accession number AF003698).

To construct a disruption allele of COQ6, a 2.9-kb HindIII fragment,
containing about 350 bp of the YEp24 sequence and 2.55 kb of yeast genomic DNA, was excised from pG63/T1 and inserted into the HindIII site of YEp352 to generate the plasmid pG66/ST3. As shown in Fig. 2, the coq6 deletion plasmid was constructed by replacing a 414-bp BglII fragment in pG66/ST3 with a 1.7-kb BamHI fragment containing HIS3 (24). The resulting plasmid, pG66/ST3, was used to obtain a 4.0-kb EcoRI fragment with the disrupted gene. W303-1A, W303-1B, and SR128-3C were each transformed with 1 μg of the plasmid. After selection on SD-Leu plates at 30°C for 3 days, 750 ng of Q 9 was added as an internal standard to a yeast cell pellet (100 mg of wet weight), and the cells were lysed by vortexing with 1 g of glass beads in 0.35 ml of water.

Mitochondrial Import Assay—An in vitro transcription template plasmid was constructed by inserting the COQ6 ORF into the pRS426 vector downstream of the T7 promoter (32). The resulting plasmid was constructed by inserting the COQ6 ORF with EcoRI and NotI links in the 3' ends, respectively, was PCR-amplified with the template pRS426-T7 (pRS426-T7). The resulting mRNA was then translated with Premega Flexi rabbit reticulocyte lysate system in the presence of [35S]methionine from Amersham Biosciences (1000 Ci/mmol, at a final concentration of 0.75 μCi). Both the mRNA and the 35S-labeled polyadenylates were stored at −80°C.

**FIG. 2.** Restriction map of isolated yeast genomic DNA containing the COQ6 gene and strategy of COQ6 deletion. The gray bars indicate the positions of the GND2 and COQ6 genes present on p494SR. Each of the plasmids depicted was the able to restore growth of coq6 yeast mutant strains on YPG plate medium as identified by the + sign. A deletion construct was prepared by replacing a 414-bp BglII fragment (black bar) with the HIS3 gene on a BamHI fragment, as described under "Experimental Procedures." Restriction sites are symbolized by either one or two letters and correspond to the following DNA restriction enzymes: B, BglII; H, HindIII; R, EcoRI; RV, EcoRV.
Mitochondrial Localization of Coq6p—Yeast cultures (W303-1A) were grown in YPGal to an OD600 nm of 1.0 and were harvested by centrifugation. The cell pellets were resuspended in PBS and sonicated with a tight fitting pestle as described (19). Mitochondria were isolated and washed once as described (33). Proteinase K treatment after 30-min incubation at 30 °C was used to generate antiserum in rabbits (Cocalico). The mitochondrial fractions of cells were analyzed by immunoblot.

Immunoblot Analysis—The yeast cells were grown on a magnetic stirrer plate and suspended in five volumes of 20 mM HEPES-KOH, pH 7.4, and incubated on ice for 20 min. The mixture was then sedimented for 40 min at 150,000 g to separate the mitochondrial and cytosolic fractions (supernatant) from the mitochondrial (pellet). The mitochondrial and cytosolic fractions were then lysed in SDS-PAGE sample buffer and separated by SDS-PAGE. The proteins were transferred to a nitrocellulose membrane and probed with anti-Coq6p, 1:5000; anti-Coq3p, 1:1000; anti-Coq4p, 1:1000; anti-cytochrome b_6, 1:1000; anti-Hsp60p, 1:10,000; anti-β subunit of F_{1}-ATPase, 1:10,000; anti-cytochrome c_1, 1:1000; anti-OM45p, 1:1000; and anti-Mas2p, 1:1000. Goat anti-rabbit secondary antibodies conjugated to horseradish peroxidase (Calbiochem) were used at a 1:10,000 dilution.

Isolation and in Situ Disruption of the COQ6 Gene—The original mutant in the coq6 complementation group of yeast mutants (G63) was identified as a Q-deficient nuclear petite strain (13). The respiratory deficiency was ascribed to a defect in Q because addition of exogenous Q_{2} or Q_{6} to isolated mitochondria rescued NADH-cytochrome c reductase activity (39). SR128-3C, one of the strains resulting from the cross between C128 (coq6-1) and FY251, had a very low reversion rate and provided the biological tool for cloning the COQ6 gene by screening yeast genomic DNA libraries as described under “Experimental Procedures.” Nine reversion-competent transformants were obtained, and the representative plasmid p494SR was studied in detail. A 3.8-kb HindIII fragment was isolated from p494SR and ligated to the HindIII site of pRS316, a centromeric vector, to form pSR1-1 (Fig. 2). Transformation of SR128-3C with pSR1-1 restored growth on media containing glycerol. DNA sequence analysis revealed the insert to contain two complete ORFs, identified as YGR256W (the GND2 gene) and YGR255C. The plasmids pG63/T1 and pG63/ST2 containing the complete YGR255C ORF and only a portion of the GND2 gene, each restored the ability of SR128-3C to grow on YPG (Fig. 2). Expression of the YGR255C ORF as a carboxyterminal HA-tagged fusion protein from the alcohol dehydrogenase promoter also restored YPG growth of SR128-3C.

Isolation and in Situ Disruption of the COQ6 Gene—The one-step gene replacement procedure (24) was used to obtain strains harboring disrupted alleles of the COQ6 gene (W3033OCOQ6-1, dW3033OCOQ6-1, and SR3ACOQ6-1). Analysis of these strains (see “Experimental Procedures”) confirmed the allelism between the coq6-null mutants and the original coq6-1 mutant. These data identify the YGR255C ORF as COQ6.

COQ6 Is Not an Essential Gene—It has been reported that a complete disruption of the COQ6 ORF results in lethality because heterozygous knockouts failed to produce viable spores containing a coq6 disruption (16). However, all of the coq6 mutant strains used in this study were viable. A complete ORF disruption was created to address this apparent discrepancy. Three haploid strains (W303-1A, W3031B, and CEN.PK2-1C) were transformed with a PCR-generated disruption cassette, and all produced viable disruptants (W3033OCOQ6-2, W3033OCOQ6-2, and CENACOQ6-2) as confirmed by PCR and restriction digest (data not shown). Similarly, a W303-1AB diploid was created by mating W303-1A with W303-1B and was transformed using the same cassette. The heterozygous COQ6/ coq6 diploid strain was subjected to sporulation and tetrad

---

**FIG. 3.** Yeast coq6 mutants lack Q and accumulate a polar intermediate that corresponds to IHIB. Lipid extracts were prepared from SR128-3C (coq6-1 allele) (a) or from W3033OCOQ6-1 (coq6 deletion mutant) (b) and separated by normal phase HPLC as described under “Experimental Procedures.” The fractions were collected (1 ml), and 14C radioactivity was determined by scintillation counting. A Q_{6} standard eluted in fraction 6.
dissection. Each of the 10 tetrads analyzed produced four viable spores on YPD plate medium. The spores from each tetrad showed a 2:2 segregation for respiratory competence and leucine auxotrophy. These results indicate that the COQ6 gene is not essential for viability but is required for growth on nonfermentable carbon sources.

Yeast coq6-null Mutants Lack Q 6 and Accumulate HHB—Growth of coq6-1 mutants in the presence of 4-[U-14C]hydroxybenzoic acid, the ring precursor in Q biosynthesis, showed this mutant lacked Q6 and accumulated HHB (15). HHB is an early intermediate in the Q biosynthetic pathway and accumulates in mutant yeast strains harboring deletions or disruptions in any one of the COQ3, COQ4, COQ5, COQ7, or COQ8/ABC1 genes (15, 29, 31, 40, 41). To characterize the defect in Q biosynthesis in a coq6-null mutant, both SR128-3C (coq6-1) and W303/H9004 COQ6-1 (coq6/H9004) were grown in the presence of 4-[U-14C]hydroxybenzoic acid, and lipid extracts were analyzed by normal phase HPLC as described under "Experimental Procedures." Both strains lacked Q6 and accumulate a radioactive intermediate that co-migrated with HHB in fraction 26 (Fig. 3). Neither SR128-3C nor W303/H9004 COQ6-1 was observed to produce Q6 as analyzed by electrochemical detection, a method that can detect as little as 2 pmol of Q6/mg of wet weight of yeast. Q6 levels of W303-1A and W303/H9004 COQ6-1:pSR1-1 were 187 and 148 pmol/mg of wet weight.
of yeast, respectively, when grown to log phase, and were 189 and 169 pmol/mg of wet weight of yeast, respectively, when grown to the stationary phase (data not shown).

Coq6p May Act as a Flavin-dependent Monooxygenase to Catalyze Quinone Formation—The predicted amino acid sequence of the \( \text{COQ6} \) ORF revealed 21 and 24% sequence identity with the respective \( \text{E. coli} \) \( \text{UbiH} \) and \( \text{UbiF} \) polypeptides (Fig. 4). \( \text{E. coli} \) \( \text{ubiH} \) mutants lack Q8 and accumulate compound 6, indicating that \( \text{UbiH} \) is required for the monooxygenase step that catalyzes quinone formation (Fig. 1 and Ref. 42). \( \text{UbiH} \) gene product was identified as a flavin-dependent monooxygenase (43). The \( \text{E. coli} \) \( \text{ubiF} \) gene product has also been identified as a flavin-dependent monooxygenase, with 31% amino acid sequence identity to \( \text{UbiH} \) (44). \( \text{E. coli} \) \( \text{ubiF} \) mutants lack Q8, accumulate compound 8, and both \( \text{ubiH} \) and \( \text{ubiF} \) mutants fail to grow on media containing succinate (Fig. 1 and Ref. 45). Coq6p, \( \text{UbiH} \), \( \text{UbiF} \), and other eukaryotic homologs of Coq6 each contain three regions with amino acid sequence identities that are present in a large family of flavin-dependent monooxygenases (Fig. 5 and Ref. 46). Region 1 contains an ADP-binding fingerprint (47), Region 2 is implicated in the recognition of NADH or NADPH and is also involved indirectly in binding the pyrophosphate moiety of FAD (48). Region 3 contains a consensus sequence for binding to the ribityl moiety of FAD (49). Based on the homology with \( \text{UbiH} \), \( \text{UbiF} \) and the presence of the conserved motifs found in other aromatic flavin-dependent monooxygenases, it seems likely that Coq6p functions in one or more hydroxylation steps in Q biosynthesis.

Mitochondrial Import of Coq6p—The amino-terminal sequence predicted for Coq6p showed characteristics of mitochondrial targeting sequences (14). The first 28 amino acid residues are abundant in positively charged amino acid residues and devoid of acidic residues. Arrangement of these 28 residues in a helical wheel shows the positively charged residues are located along one side of the \( \alpha \)-helix. The amino-terminal region
contains the sequence motif common in many mitochondrial matrix proteins that are proteolytically cleaved twice once imported into matrix, as characterized by an arginine at −10, a hydrophobic residue (phenylalanine, leucine, or isoleucine) at −8, and serine, threonine, or glycine at −5 relative to the amino-terminal residue of the proteolytically processed protein. To determine whether Coq6p is a mitochondrial protein, an in vitro mitochondrial import assay of the Coq6 polypeptide was performed. Upon incubation with mitochondria prepared from wild type yeast, the in vitro translated Coq6p was cleaved, and the resulting mature form of the protein was observed to have a mass of about 51 kDa, consistent with lysine 21 as the putative cleavage site (Fig. 6). This mature protein was protected and resistant to exogenous proteinase K treatment (Fig. 6, lane 3). Disruption of mitochondrial membrane with detergent exposed the imported and processed Coq6p, which became accessible to the proteinase K (Fig. 6, lane 4). The import of Coq6p also required a mitochondrial membrane potential, because the addition of valinomycin inhibited import of Coq6p (Fig. 6, lanes 5 and 6).

**Mitochondrial Localization of Coq6p**—Our initial investigation of the subcellular localization of Coq6p made use of a carboxyl-terminal fusion protein between Coq6p and the HA epitope peptide (see “Experimental Procedures”). The Coq6-HA epitope fusion protein retained activity as assayed by the ability of either the single- or multi-copy plasmid construct to rescue coq6-null mutant yeast strains for growth on medium containing a nonfermentable carbon source (YPG plates; data not shown). However, subsequent subcellular and submitochondrial fractionation analysis revealed that the Coq6-HA tagged construct was present in mitochondria as an insoluble aggregate because it remained in the pellet following treatment with 1% Triton X-100 but including a post-import proteinase K treatment. Lanes 5 and 6, in vitro translation product was incubated with mitochondria in the presence of the uncoupler valinomycin (final concentration, 10 μg/ml). After the incubation, the reisolated mitochondria were either treated with proteinase K as in lane 5 or were directly analyzed as in lane 6. The samples were mixed with sample buffer and heated at 100 °C for 5 min prior to analysis by SDS-polyacrylamide electrophoresis (12% polyacrylamide) and autoradiography.

![Fig. 7. Coq6p is peripherally associated with the inner membrane of mitochondria.](#)

### a) In vitro mitochondrial import and proteolytic processing of Coq6. Lane 1, 10% of the radiolabeled in vitro translation product used in each import reaction. Lane 2, in vitro translation product incubated with isolated mitochondria. Lane 3, same as lane 2 but including a post-import proteinase K treatment. Lane 4, same as lane 3 except Triton X-100 was added to disrupt the mitochondria before proteinase K treatment. Lanes 5 and 6, in vitro translation product was incubated with mitochondria in the presence of the uncoupler valinomycin (final concentration, 10 μg/ml). After the incubation, the reisolated mitochondria were either treated with proteinase K as in lane 5 or were directly analyzed as in lane 6. The samples were mixed with sample buffer and heated at 100 °C for 5 min prior to analysis by SDS-polyacrylamide electrophoresis (12% polyacrylamide) and autoradiography. P and M indicate the positions of the precursor form and the mature form of Coq6p, respectively.

### Mitochondrial Localization of Coq6p

| Treatment            | Lane 1 | Lane 2 | Lane 3 | Lane 4 | Lane 5 | Lane 6 |
|----------------------|--------|--------|--------|--------|--------|--------|
| Mitochondria         | +      | +      | +      | +      | +      | +      |
| Proteinase K         | -      | -      | +      | +      | +      | +      |
| Triton X-100         | -      | -      | -      | +      | +      | +      |
| Valinomycin          | -      | -      | -      | +      | +      | +      |

---

**Mitochondrial Localization of Coq6p**—Our initial investigation of the subcellular localization of Coq6p made use of a carboxyl-terminal fusion protein between Coq6p and the HA epitope peptide (see “Experimental Procedures”). The Coq6-HA epitope fusion protein retained activity as assayed by the ability of either the single- or multi-copy plasmid construct to rescue coq6-null mutant yeast strains for growth on medium containing a nonfermentable carbon source (YPG plates; data not shown). However, subsequent subcellular and submitochondrial fractionation analysis revealed that the Coq6-HA tagged construct was present in mitochondria as an insoluble aggregate because it remained in the pellet following treatment with 1% Triton X-100 but including a post-import proteinase K treatment. Lanes 5 and 6, in vitro translation product was incubated with mitochondria in the presence of the uncoupler valinomycin (final concentration, 10 μg/ml). After the incubation, the reisolated mitochondria were either treated with proteinase K as in lane 5 or were directly analyzed as in lane 6. The samples were mixed with sample buffer and heated at 100 °C for 5 min prior to analysis by SDS-polyacrylamide electrophoresis (12% polyacrylamide) and autoradiography. P and M indicate the positions of the precursor form and the mature form of Coq6p, respectively.

**Mitochondrial Localization of Coq6p**—Our initial investigation of the subcellular localization of Coq6p made use of a carboxyl-terminal fusion protein between Coq6p and the HA epitope peptide (see “Experimental Procedures”). The Coq6-HA epitope fusion protein retained activity as assayed by the ability of either the single- or multi-copy plasmid construct to rescue coq6-null mutant yeast strains for growth on medium containing a nonfermentable carbon source (YPG plates; data not shown). However, subsequent subcellular and submitochondrial fractionation analysis revealed that the Coq6-HA tagged construct was present in mitochondria as an insoluble aggregate because it remained in the pellet following treatment with 1% Triton X-100 but including a post-import proteinase K treatment. Lanes 5 and 6, in vitro translation product was incubated with mitochondria in the presence of the uncoupler valinomycin (final concentration, 10 μg/ml). After the incubation, the reisolated mitochondria were either treated with proteinase K as in lane 5 or were directly analyzed as in lane 6. The samples were mixed with sample buffer and heated at 100 °C for 5 min prior to analysis by SDS-polyacrylamide electrophoresis (12% polyacrylamide) and autoradiography. P and M indicate the positions of the precursor form and the mature form of Coq6p, respectively.

**Mitochondrial Localization of Coq6p**—Our initial investigation of the subcellular localization of Coq6p made use of a carboxyl-terminal fusion protein between Coq6p and the HA epitope peptide (see “Experimental Procedures”). The Coq6-HA epitope fusion protein retained activity as assayed by the ability of either the single- or multi-copy plasmid construct to rescue coq6-null mutant yeast strains for growth on medium containing a nonfermentable carbon source (YPG plates; data not shown). However, subsequent subcellular and submitochondrial fractionation analysis revealed that the Coq6-HA tagged construct was present in mitochondria as an insoluble aggregate because it remained in the pellet following treatment with 1% Triton X-100 but including a post-import proteinase K treatment. Lanes 5 and 6, in vitro translation product was incubated with mitochondria in the presence of the uncoupler valinomycin (final concentration, 10 μg/ml). After the incubation, the reisolated mitochondria were either treated with proteinase K as in lane 5 or were directly analyzed as in lane 6. The samples were mixed with sample buffer and heated at 100 °C for 5 min prior to analysis by SDS-polyacrylamide electrophoresis (12% polyacrylamide) and autoradiography. P and M indicate the positions of the precursor form and the mature form of Coq6p, respectively.
Coq6 Is a Flavin-dependent Monooxygenase in Q Biosynthesis

DISCUSSION

This work characterizes yeast coq6 mutants and the isolation of the COQ6 gene. COQ6 is necessary for Q biosynthesis. In the original coq6 mutant and in both coq6 partial and complete deletion mutants, Q₆ is undetectable, and as a result, these cells are rendered respiratory incompetent. Based on the data presented here, COQ6 encodes a mitochondrial protein necessary for Q biosynthesis. Similar to Coq2p, Coq3p, Coq4p, Coq5p, Coq7p, and Coq6p/Ace1p, the Coq6 polypeptide is imported into mitochondria (14). In agreement with submitochondrial localization studies of Coq1p, Coq3p, Coq4p, Coq5p, and Coq7p, Coq6p is localized to the matrix side of the inner membrane (18, 40, 50).

S. cerevisiae is a facultative anaerobe capable of fermentation and aerobic respiration, and none of the other COQ genes have been identified as being essential. Complete deletions of the COQ6 gene were introduced into both haploid and diploid strains of W303 and also into CEN.PK2-1C, a haploid strain similar in genetic background to the diploid used by Fiori et al. (16). In contrast to their results, all of the yeast strains were viable when Coq6 was deleted, indicating that none of the eight COQ genes is essential. Q itself is nonessential for growth in this organism.

The function of Coq6p is still unknown, as are the functions of Coq4p and Coq8p. Based on sequence homology to known flavin dependent monoxygenases, it is likely that Coq6p acts similarly. Although Coq6p and UbiF share 24% sequence identity, it seems unlikely that Coq6p functions like UbiF in the hydroxylation of compound 8 in yeast, because previous studies have shown that yeast Coq7p is required for this hydroxylation step (29). The Coq7 polypeptide has been identified as a di-iron protein, and the hydroxylation substrate for Coq7p may be responsible for either or both of these steps. Coq6p has been identified as a di-iron protein, and the hydroxylation substrate for Coq7p may be responsible for either or both of these steps.

The function of Coq6p is still unknown, as are the functions of Coq4p and Coq8p. Based on sequence homology to known flavin dependent monoxygenases, it is likely that Coq6p acts similarly. Although Coq6p and UbiF share 24% sequence identity, it seems unlikely that Coq6p functions like UbiF in the hydroxylation of compound 8 in yeast, because previous studies have shown that yeast Coq7p is required for this hydroxylation step (29). The Coq7 polypeptide has been identified as a di-iron protein, and the hydroxylation substrate for Coq7p may be responsible for either or both of these steps. Coq6p has been identified as a di-iron protein, and the hydroxylation substrate for Coq7p may be responsible for either or both of these steps.

REFERENCES

1. Brandt, U., and Trumpower, B. (1994) Crit. Rev. Biochem. Mol. Biol. 29, 165–197
2. Dutton, P. L., Ohnishi, T., Darrouzet, E., Leonard, M. A., Sharp, R. E., Gibney, B. R., Daidal, P., and Mass, C. (2000) in Coenzyme Q: Molecular Mechanisms in Health and Disease (Kagan, V. E., and Quinn, P. J., eds) pp. 65–82, CRC Press, Boca Raton, FL
3. Santos-Ocana, C., Do, T. Q., Padilla, S., Navas, and Clarke, C. F. (2002) J. Biol. Chem. 277, 19773–19781
4. Gille, L., and Nohl, H. (2000) Arch. Biochem. Biophys. 375, 347–354
5. Santos-Ocana, C., Cordoba, F., Crane, F. L., Clarke, C. F., and Navas, P. (1998) J. Biol. Chem. 273, 30999–31005
6. Kagan, V. K., Nohl, H., and Quinn, P. J. (1996) in Handbook of Antioxidants (Cadenas, E., and Packer, L., eds) pp. 157–201, Dekker, New York
7. Georgellis, D., Kwon, O., and Lin, E. C. (2001) Science 292, 2314–2316
8. Larson, P. L., and Clarke, C. F. (2002) Science 295, 120–123
9. Jonassen, T., Marbois, B. N., Faull, K. F., Clarke, C. F., and Larsen, P. L. (2002) J. Biol. Chem. 277, 45020–45027
10. Gerenel, V., Durin, N., Christen, D., Benit, P., DeLonlay, P., Rotig, A., Munnich, A., and Rustin, P. (2002) Mol. Genet. Metab. 77, 21–30
11. Shults, C. W., Oakes, D., Kieburtz, K., Beal, M. F., Haas, R., Flahm, S., Juncos, J. L., Nutt, J., Shoulson, I., Carter, J., Kompfli, K., Pertumuter, J. S., Reich, S., Stern, M., Watts, R. L., Kurlan, R., Mohlo, E., Harrison, M., and Lew, M. (2002) Arch. Neurol. 59, 1541–1550
12. Mecynathran, R. (2001) PEM Microbiol. Lett. 203, 131–139
13. Tragoloff, A., and Dieckmann, C. L. (1999) Microbiol. Rev. 54, 221–225
14. Jonassen, T., and Clarke, C. F. (2001) in Coenzyme Q: Molecular Mechanisms in Health and Disease (Kagan, V. E., and Quinn, P. J., eds) pp. 185–208, CRC Press, Boca Raton, FL
15. Poon, W. W., Do, T. Q., Marbois, B. N., and Clarke, C. F. (1997) Mol. Aspects Med. 18, 1211–1217
16. Fiori, A., Bianchi, M. M., Faull, L., Falcione, C., Francisci, S., Paliocchi, D., Solimando, N., Uccelletti, D., and Frontali, L. (2000) Yeast 16, 377–386
17. Adams, A., Göttsching, D. E., Kaiser, C. A., and Starna, T. (1997) Methods in Yeast Genetics, pp. 145–154, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Poon, W. W., Burkhovj, R. J., Hsu, A. Y., Frankel, A., Lee, P. T., Shepherd, J. N., Myles, D. C., and Clarke, C. F. (1999) J. Biol. Chem. 274, 21665–21672
19. Glick, B. S., and Lus, L. A. (1995) Methods Enzymol. 260, 213–223
20. Marbois, B. N., Hsu, A., Pillai, R., Colleumet, J., and Clarke, C. F. (1994) Gene (Amst.) 138, 213–217
21. Rose, M. D., Novick, P., Thomas, J. H., Botstein, D., and Fink, G. R. (1987) Science 239, 281–287
22. Carlson, M., and Botstein, D. (1982) Cell 28, 145–154
23. Sikorska, R. S., and Hieter, P. (1989) Genetics 122, 19–27
24. Rothstein, R. S. (1983) Methods Enzymol. 101, 202–211
25. Epperson, R. A. (1992) BioTechniques 14, 18–20
26. Baudin, A., Ozer-Kalergopoulou, O., Denoel, A., Lacroix, F., and Cullin, C. (1995) Nucleic Acids Res. 23, 3329–3330
27. Garrett, S., Schiestl, R., Wilkins, A., and Woods, R. (1995) Yeast 11, 355–360
28. Clarke, C. F., Williams, W., and Teruya, J. H. (1991) J. Biol. Chem. 266, 16636–16644
29. Marbois, B. N., and Clarke, C. F. (1996) J. Biol. Chem. 271, 2995–3004

Footnotes:

2. P. Gin and C. F. Clarke, unpublished data.

3. E. Hsieh and C. F. Clarke, unpublished data.
30. Poon, W. W., Marbois, B. N., Faull, K. F., and Clarke, C. F. (1995) Arch. Biochem. Biophys. 320, 305–314
31. Jonassen, T., and Clarke, C. F. (2000) J. Biol. Chem. 275, 12381–12387
32. Christianson, T. W., Sikorski, R. S., Dante, M., Shero, J. H., and Hieter, P. (1992) Gene (Amst.) 110, 119–122
33. Yaffe, M. P. (1991) Methods Enzymol. 194, 627–643
34. Hsu, A. Y., Poon, W. W., Shepherd, J. A., Myles, D. A., and Clarke, C. F. (1996) Biochemistry 35, 9797–9806
35. Niman, H. L., Houghten, R. A., Walker, L. E., Reisfeld, R. A., Wilson, I. A., Hogle, J. M., and Lerner, R. A. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 4849–4853
36. Spain, B. H., Koo, D., Ramakrishnan, M., Drudzor, B., and Colicelli, J. (1995) J. Biol. Chem. 270, 25435–25444
37. Jarosh, E., Tuller, G., Daum, G., Waldherr, M., Voskova, A., and Schweyen, R. J. (1996) J. Biol. Chem. 271, 17219–17225
38. Glick, B. S., Brandt, A., Cunningham, K., Muller, S., Hallberg, R. L., and Schatz, G. (1992) Cell 69, 809–822
39. Tzagoloff, A., Akai, A., and Needleman, R. B. (1975) J. Biol. Chem. 250, 8228–8235
40. Belogrudov, G. I., Lee, P. T., Jonassen, T., Hsu, A. Y., Gin, P., and Clarke, C. F. (2001) Arch. Biochem. Biophys. 392, 48–58
41. Do, T. Q., Hsu, A. Y., Jonassen, T., Lee, P. T., and Clarke, C. F. (2001) J. Biol. Chem. 276, 18161–18168
42. Young, I. G., Stroobant, P., MacDonald, C. G., and Gibson, F. (1973) J. Bacteriol. 114, 42–52
43. Nakahigashi, K., Miyamoto, K., Nishimura, K., and Inoue, H. (1992) J. Bacteriol. 174, 7352–7359
44. Kwon, O., Kotsakis, A., and Meganathan, R. (2000) FEMS Microbiol. Lett. 186, 157–161
45. Young, I. G., McCann, L. M., Stroobant, P., and Gibson, F. (1971) J. Bacteriol. 105, 769–778
46. Palley, B. A., Ballou, D. P., and Massey, V. (1995) in Active Oxygen in Biochemistry (Valentine, J. S., Foote, C. S., Greenberg, A., and Liebman, J. P., eds) pp. 37–83, Blackie Academic & Professional Press, Glasgow, UK
47. Wierenga, R. K., Terpstra, P., and Hol, W. G. J. (1986) J. Mol. Biol. 187, 101–107
48. Eggink, M. H. M., Schreuder, H. A., and Van Berkel, W. J. H. (1997) Protein Sci. 6, 2454–2458
49. Eggink, G., Engel, H., Vriend, G., Terpstra, P., and Witholt, B. (1990) J. Mol. Biol. 212, 135–142
50. Jonassen, T., Proft, M., Randez-Gil, F., Schultz, J. R., Marbois, B. N., Entian, K. D., and Clarke, C. F. (1998) J. Biol. Chem. 273, 3351–3357
51. Stenmark, P., Gruner, J., Mattsson, J., Sindelar, P. J., Nordlund, P., and Berthold, D. A. (2001) J. Biol. Chem. 276, 33297–33300
52. Eggink, M. H. M., Boeren, S. A., Vervoort, J., and Van Berkel, W. J. H. (1997) J. Bacteriol. 179, 6680–6687
53. Katagiri, M., Takemori, S., Suzuki, K., and Yasuda, H. (1966) J. Biol. Chem. 241, 5675–5677
54. Goevert, R. R., Sippel, C. J., Grimm, M. F., and Olson, R. E. (1978) FEBS Lett. 87, 219–221
55. Tzagoloff, A., Akai, A., and Foury, F. (1976) FEBS Lett. 65, 391–395
56. Proft, M., Ketter, P., Hedges, D., Bojunga, N., and Entian, K. D. (1995) EMBO J. 14, 6116–6126
57. McEwen, J. E., Ko, C., Kloekner-Gruissem, B., and Poyton, R. O. (1986) J. Biol. Chem. 261, 11872–11879
58. Wang, C. M., Dilworth, M. J., and Glenn, A. R. (1994) Microbiology 140, 2775–2786
59. Kalin, M., Neujahr, H. Y., Weismahr, R. N., Sejlitza, T., Johl, R., Fiechter, A., and Reiser, J. (1992) J. Bacteriol. 174, 7112–7120
60. Suzuki, K., Mizuguchi, M., Ohnishi, K., and Itagaki, E. (1996) Biochim. Biophys. Acta 1275, 154–156
61. Orser, C. S., Lange, C. C., Xun, L., Zahrt, T. C., and Schneider, B. J. (1993) J. Bacteriol. 175, 411–416
62. Weijer, W. J., Hofsteenge, J., Beintema, J. J., Wierenga, R. K., and Drenth, J. (2001) Eur. J. Biochem. 278, 187, 1007–118
63. DeMarco, A. A., Averhoff, B. A., Kin, E. E., and Ornston, L. N. (1993) Gene (Amst.) 125, 5–33
64. Filioppi, S., Solinas, M. M., Breene, U., Schurer, M. B., Gasboni, D., Ciavonetti, G., Colombi, A. L., and Gardano, L. (1996) Microbiology 141, 1007–1016
65. Nork, A., Kasak, L., and Kwasaar, M. (1991) Gene (Amst.) 102, 13–18
The Saccharomyces cerevisiae COQ6 Gene Encodes a Mitochondrial Flavin-dependent Monooxygenase Required for Coenzyme Q Biosynthesis

Peter Gin, Adam Y. Hsu, Steven C. Rothman, Tanya Jonassen, Peter T. Lee, Alexander Tzagoloff and Catherine F. Clarke

J. Biol. Chem. 2003, 278:25308-25316.
doi: 10.1074/jbc.M303234200 originally published online April 29, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M303234200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 60 references, 26 of which can be accessed free at http://www.jbc.org/content/278/28/25308.full.html#ref-list-1