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Endogenous Synthesis of Coenzyme Q in Eukaryotes

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Abstract

Coenzyme Q (Q) functions in the mitochondrial respiratory chain and serves as a lipophilic antioxidant. There is increasing interest in the use of Q as a nutritional supplement. Although the physiological significance of Q is extensively investigated in eukaryotes, ranging from yeast to human, the eukaryotic Q biosynthesis pathway is best characterized in the budding yeast *Saccharomyces cerevisiae*. At least ten genes (*COQ1-COQ10*) have been shown to be required for Q biosynthesis and function in respiration. This review highlights recent knowledge about the endogenous synthesis of Q in eukaryotes, with emphasis on *S. cerevisiae* as a model system.

Keywords

Coenzyme Q; Mitochondria; Eukaryotes

1. Overview of Coenzyme Q Biosynthesis

Cells generally rely on *de novo* synthesis for their supply of Q. Current knowledge about the Q biosynthetic pathway in eukaryotes is mostly derived from characterization of accumulating intermediates in Q-deficient mutant strains of *Saccharomyces cerevisiae*, reviewed by (Jonassen and Clarke, 2001,Meganathan, 1996,Turunen et al., 2004). Q biosynthesis starts with formation of a hydroxybenzoic acid head group and a lipophilic polyisoprenoid tail (Olson and Rudney, 1983,Pennock and Threlfall, 1983). The aromatic precursor of the benzoquinone ring is 4-hydroxybenzoic acid (4-HB) derived from tyrosine, an essential amino acid in mammals. In yeast, 4-HB can also be synthesized from chorismate via the shikimate pathway (Goewert, 1980). The building blocks for the synthesis of the polyisoprenyl chain are provided by dimethylallyl diphosphate and isoprenyl diphosphate. In yeast and mammals, these five-carbon precursors are derived from acetyl-coenzyme A via the mevalonate pathway (Grunler et al., 1994).

The putative eukaryotic Q biosynthetic pathway is shown in Figure 1. First, the polyisoprenoid tail is assembled by polyprenyl diphosphate synthase, which is responsible for determining the number of isoprene units (designated as *n*). Next, polyprenyl diphosphate: 4-HB transferase catalyzes the formation of covalent linkage between the benzoquinone head group and the tail, producing the 4-hydroxy-3-polyprenyl benzoic acid intermediate. The order of subsequent reactions presented in Figure 1 is speculative, as only a few of the diagnostic intermediates of the blocked steps have been recovered in yeast mutant strains. Proposed modifications of the

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aromatic ring start with hydroxylation, followed by *O*-methylation, and decarboxylation to form the 6-methoxy-2-polyprenyl phenol intermediate. Afterward, two additional hydroxylations, one *C*-methylation, and one *O*-methylation step are necessary to generate the fully substituted hydroquinone.

So far, nine complementation groups of Q-deficient yeast mutants (*COQ1* through *COQ9*) have been identified (Tzagoloff and Dieckmann, 1990,Johnson et al., 2005). Mammalian homologues of the yeast *COQ* genes have been identified via sequence homology. Human homologues of Coq2, Coq3, and Coq7 proteins were demonstrated to functionally complement the corresponding yeast null mutants (Forsgren et al., 2004,Jonassen and Clarke, 2000,Vajo, 1999), further indicating that the yeast Q biosynthesis pathway is conserved in humans. The yeast *coq* mutants are non-respiring (unable to grow on non-fermentable carbon sources such as ethanol and glycerol) and petite (forming smaller colonies than wild-type cells when grown on glucose, a fermentable sugar) (Tzagoloff et al., 1975a,Tzagoloff et al., 1975b). The hallmark feature of these mutants is that defective NADH-cytochrome *c* reductase and succinate-cytochrome *c* reductase activities in isolated mitochondria of each *coq* mutant strain can be restored to near wild-type level by addition of Q_2 (Tzagoloff et al., 1975b,Johnson et al., 2005). Addition of exogenous Q_6 to *coq* mutants cultured in liquid media with vigorous aeration also restores respiration (Jonassen et al., 1998,Do et al., 2001). Recently, a novel yeast *coq* mutant with defects in respiration and Q-dependent oxidation of NADH and succinate has been identified (Barros et al., 2005). However, unlike the other Q-deficient *coq* mutants (*coq1-coq9*), the *coq10* mutant has nearly normal levels of Q_6, indicating that this protein is not required for Q biosynthesis. Instead, the Coq10 polypeptide may function as a Q-binding chaperone, required for the proper function of Q in respiratory electron transport. The evidence for this proposal is discussed in section three.

While Coq1, Coq2, Coq3, Coq5, Coq6, and Coq7 proteins have known or proposed enzymatic functions in Q biosynthesis (Jonassen and Clarke, 2001,Gin et al., 2003) (Figure 1), it is not clear whether the other Coq proteins also possess enzymatic activities. Coq1 through Coq9 polypeptides localize to the mitochondria (Belogrudov et al., 2001,Gin and Clarke, 2005,Gin et al., 2003,Hsu et al., 1996,Leuenberger et al., 1999,Jonassen et al., 1998,Do et al., 2001,Johnson et al., 2005,Dibrov et al., 1997). *In vitro* mitochondria import were investigated for seven of the yeast Coq polypeptides and demonstrated to be dependent on a mitochondrial membrane potential (Jonassen and Clarke, 2001). Following is a brief discussion about function and submitochondrial localization of the nine Coq proteins, required for Q biosynthesis in eukaryotes (summarized in Table 1). A model incorporating genetic and physical evidence for a yeast Q biosynthetic multi-subunit complex is shown in Figure 2.

Coq1

Formation of the *trans*-polyprenyl diphosphate synthase tail in *S. cerevisiae* is catalyzed by the polypeptide encoded by the *COQ1* gene (Ashby and Edwards, 1990), which is responsible for determining the species-specific tail length of Q (Okada et al., 1996). The amino acid sequences of Coq1 protein and related isoprenyl diphosphate synthases from different eukaryotes contain seven highly conserved motifs (Wang and Ohnuma, 2000). Interestingly, expression of Coq1 homologues from a variety of organisms can restore Q biosynthesis and respiration in yeast *coq1* null mutants via production of Q isoforms with distinct number of isoprene units (Okada et al., 1998,Okada et al., 1997). The Coq1 ortholog from the fission yeast *Schizosaccharomyces pombe* (Dps1) fails to complement the *S. cerevisiae coq1* null mutant (Suzuki et al., 1997). However, polyprenyl diphosphate synthases of fission yeast, mouse, and human are each heterotetramers of two protein subunits, PDSS1 and PDSS2 (Saiki et al., 2005,Saiki et al., 2003), while Coq1 from *S. cerevisiae* and the plant *Arabidopsis thaliana* (Jun et al., 2004) function as homo-oligomers. Expression of both subunits of the trans-

polyprenyl diphosphate synthase of *S. pombe*, mouse or human restores production of the polyisoprene diphosphate and production of Q in complementation assays (Saiki et al., 2005,Saiki et al., 2003). Submitochondrial fractionation studies demonstrated that the *S. cerevisiae* Coq1 protein is peripherally associated with the inner mitochondrial membrane on the matrix side (Gin and Clarke, 2005).

Coq2

The 4-HB polyprenyltransferase is a key enzyme catalyzing the attachment of the polyisoprenoid side chain to the 4-HB ring, generating the first membrane bound Q intermediate, 4-hydroxy-3-polyprenylbenzoic acid. The *S. cerevisiae* and *Homo sapiens* genes encoding this enzyme are called *COQ2* (Ashby et al., 1992,Forsgren et al., 2004). Ortholog/homologues of Coq2 protein have also been isolated and characterized in other eukaryotes including *S. pombe* (Uchida et al., 2000), *A. thaliana* (Okada et al., 2004), and rice (Ohara et al., 2006). *In vitro* assays in isolated rat liver demonstrated that the polyprenyl diphosphate:4-HB activity is present mainly in mitochondria (Momose and Rudney, 1972). Polyprenyltransferases involved in Q biosynthesis generally display a lack of specificity for the chain length of the isoprenyl diphosphate substrate (Meganathan, 2001,Gin and Clarke, 2005,Ashby et al., 1992,Okada et al., 2004); however, the specificity was shown to be influenced by Mg^{2+} concentration in whole yeast extracts (Ashby et al., 1992).

Analysis of the predicted amino acid sequence of the *S. cerevisiae* Coq2 protein revealed two conserved putative substrate binding domains found in a family of polyprenyltransferases, six potential membrane spanning domains, and a typical mitochondrial targeting sequence (Ashby et al., 1992). *In vitro* import studies demonstrated that the polypeptide is imported and fully processed within the mitochondria (Leuenberger et al., 1999). Recently, submitochondrial fractionation analysis for the Coq2 protein has been carried out (Tran, U.C., Gulmezian, M., Santos-Ocaña, C., Saiki, R., Navas, P., and Clarke, C. F., manuscript in preparation). Coq2 protein behaves as an integral membrane protein associated to the inner mitochondrial membrane, facing the matrix side.

Coq3

Two *O*-methylation steps in the Q biosynthetic pathway are apparently catalyzed by the same enzyme encoded by *COQ3* gene, as demonstrated by *in vitro* assays with synthetic farnesylated analogs (Poon et al., 1999,Shepherd et al., 1996). The *COQ3* gene was originally identified in *S. cerevisiae* (Clarke et al., 1991) by its ability to restore Q biosynthesis, and hence respiration, in a *coq3* mutant named C39 (Tzagoloff et al., 1975a,Sippel et al., 1983). Homologues of the *COQ3* gene in rat, *A. thaliana*, and human were subsequently isolated via functional complementation of yeast *coq3* null mutants (Marbois et al., 1994,Avelange-Macherel and Joyard, 1998,Jonassen and Clarke, 2000). The amino acid sequences of the proteins encoded by these *COQ3* homologues all contain four regions that are conserved in a large family of methyltransferase enzymes utilizing *S*-adenosylmethionine (SAM or AdoMet) as the methyl donor (Kagan and Clarke, 1994,Niewmierzycka and Clarke, 1999,Katz et al., 2003) and required a divalent cation (Turunen et al., 2004,Jonassen and Clarke, 2001).

Like most of the other Coq polypeptides, the yeast Coq3 protein also contains a typical mitochondrial targeting sequence at the N-terminus. *In vitro* assays (Hsu et al., 1996) and subcellular localization (Poon et al., 1999) studies showed that the Coq3 preprotein was imported and processed to the mature form in the mitochondria, in a membrane-potential-dependent manner. Further submitochondrial fractionation demonstrated that it is a peripheral protein associated to the matrix side of the inner mitochondrial membrane (Poon et al., 1999). As indicated by the functional complementation and *in vitro* assays mentioned earlier, it is apparent that the Coq3 *O*- methyltransferase has broad substrate specificity. This type of

promiscuous substrate recognition is a characteristic shared with catechol-*O*-methyltransferase (COMT), which has numerous physiological substrates including the biosynthetic precursors of dopamine and certain steroids and neurotransmitters (Vidgren et al., 1999). However, the amino acid sequence of COMT fails to show any homology with the yeast Coq3 polypeptide, outside of the conserved methyltransferase motifs (Turunen et al., 2004,Jonassen and Clarke, 2001).

Coq4

Similar to the Coq3 protein encoding gene, *S. cerevisiae COQ4* gene was cloned via a functional complementation of a Q-deficient *coq4* mutant harboring the E266K point mutation (C9-E1 or *coq4-1*) (Belogrudov et al., 2001). Growth on a non-fermentable carbon source (which requires respiration) caused up-regulation of *COQ4* mRNA steady state levels, consistent with its role in Q biosynthesis. However, the enzymatic function of Coq4 protein, a peripheral protein associated with the inner mitochondrial membrane on the matrix side (Belogrudov et al., 2001) has been a mystery. While it is appealing to speculate that Coq4 protein may serve as a hydroxylase or a carboxylase in the yet-to-be-characterized steps (designated “Coq?” in Figure 1), the amino acid sequence of Coq4 does not share significant homology with protein domains or motifs with known enzymatic activity. Interestingly, steady state levels of Coq3 and Coq7 proteins, which are diminished in *coq4* null mutants, are at wild-type levels in the *coq4-1* point mutant (Belogrudov et al., 2001). This result, taken together with recent work demonstrating that the native Coq4 polypeptide co-migrates with Coq3, Coq6, and Coq7 proteins as a high molecular mass complex (Marbois et al., 2005,Tran et al., 2006), indicates that the Coq4 protein has a structural role in the putative polypeptide Q biosynthetic complex (further discussed below).

Coq5

2-methoxy-6-polyprenyl-1,4-benzoquinone methyltransferase catalyzes the only *C*-methylation step in the Q biosynthetic pathway, generating the 2-methoxy-5-methyl-6-polyprenyl-1,4-benzoquinone intermediate. In *S. cerevisiae*, the gene encoding this *C*-methyl transferase is designated *COQ5*. The *COQ5* gene was isolated from a yeast genomic DNA library based on its ability to restore respiratory proficiency in two different *coq5* point mutants, *coq5-1* (Dibrov et al., 1997) and *coq5-2* (Barkovich et al., 1997). Analysis of the *COQ5* promoter region identified consensus binding sites for Gcr1, Mig1, Rtg1/2/3, and Hap2/3/4 transcription factors (Hagerman et al., 2002,Hagerman and Willis, 2002), which regulate gene expression in response to energy sources. Results of Northern blot and Western blot analyses clearly demonstrated *COQ5* expression is up-regulated with glycerol and oleic acid treatments, compared to dextrose, with the highest induction observed during growth on oleic acid (Hagerman et al., 2002,Hagerman and Willis, 2002). The *COQ5* open reading frame harbors four sequence motifs present in a large family of AdoMet-dependent methyltransferase enzymes (Katz et al., 2003). *In vitro C*-methyltransferase assays with the farnesylated analogs of the corresponding intermediates confirmed that Coq5 polypeptide is required for conversion of 2-methoxy-6-polyprenyl-1,4-benzoquinone to 2-methoxy-5-methyl-6-polyprenyl-1,4-benzoquinone in Q biosynthesis (Baba et al., 2004,Barkovich et al., 1997). These enzyme assays further demonstrated that the length of the polyisoprenoid tail does not play a crucial role in substrate recognition of Coq5 protein. Inclusion of NADH is essential for optimal enzymatic activity and is most likely required to convert the quinone to the hydroquinone, generating a nucleophile for the *C*-methyl transfer.

Submitochondrial fractionation analyes demonstrated the Coq5 protein is peripherally associated with the inner mitochondria membrane on the matrix side (Baba et al., 2004). Interestingly, the *coq5-2* and *coq5-5* point mutants maintained normal levels of Coq3, Coq4, and Coq5 polypeptides, while levels of these proteins were greatly diminished in each of the

other *coq5* mutants (Baba et al., 2004). These point mutants are the only *coq5* mutants rescued by expression of *Escherichia coli ubiE*, a homolog of *COQ5* (Lee et al., 1997). Taken together, these results indicate that Coq5 protein is essential for the stability and activity of at least two other Coq polypeptides, and provide genetic evidence for a complex of Coq polypeptides in yeast Q biosynthesis.

Coq6

Functional complementation of a yeast mutant from the G63 (*coq6-1*) complementation group (Tzagoloff and Dieckmann, 1990) resulted in the isolation of the *COQ6* gene (Gin et al., 2003). In contrast to an earlier report (Fiori et al., 2000), *COQ6* is a non-essential gene for viability but is required for growth on non-fermentable carbon sources (Gin et al., 2003). The Coq6 protein is a mitochondrial protein, which is imported in a membrane-potential-dependent manner and peripherally associated with the matrix side of the inner membrane (Gin et al., 2003). *S. cerevisiae* Coq6 protein and its homologues in *H. sapiens*, mouse, and *C. elegans* each contains three conserved regions (Gin et al., 2003): an ADP-binding fingerprint (Wierenga et al., 1986), a motif with a putative dual function in FAD/NAD(P)H binding (Eppink et al., 1997), and a consensus sequence that binds to the ribityl moiety of FAD (Eggink et al., 1990). These conserved regions are common features of a large family of FAD-binding-aromatic hydroxylases (Palfey et al., 1995). Consequently, Coq6 protein has been considered as a putative flavin-dependent monooxygenase responsible for adding the hydroxy group to 4-hydroxy-3-polyprenyl benzoic acid and/or 6-methoxy-2-polyprenyl phenol, two uncharacterized hydroxylation steps in Q biosynthesis.

Coq7

Yeast *COQ7/CAT5* gene was independently isolated and characterized as required for Q synthesis (Marbois and Clarke, 1996,Tzagoloff and Dieckmann, 1990) and involved in carbon catabolite repression/de-repression (Proft et al., 1995). Catabolite repression/de-repression is a global system that regulates transcription of gluconeogenic genes, alternative sugar metabolism, and respiration (Gancedo, 1998). However, the catabolite-regulation defect in *coq7* mutants was later demonstrated to be a secondary effect of respiration deficiency and could be rescued by the addition of exogenous Q_6 (Jonassen et al., 1998), implicating direct involvement of *COQ7* in Q biosynthesis. Moreover, expression of *COQ7* homologues from *C. elegans* (Ewbank et al., 1997), rat (Jonassen et al., 1996), or human (Vajo, 1999) were shown to rescue the yeast *coq7* null mutant for growth on non-fermentable carbon sources, indicating functional conservation across species.

Coq7 protein was shown to be required for the hydroxylation of 5-demethoxyubiquinol ($DMQH_2$) (Marbois and Clarke, 1996). Interestingly, G65D *coq7* point mutant was found to accumulate DMQ_6, as well as the earlier intermediate 3-hexaprenyl-4-hydroxybenzoic acid (HHB), though the *coq7* null mutant produced only HHB (Marbois and Clarke, 1996). Similarly, yeast mutants expressing Coq7 protein with the missense mutation (E194K) produced DMQ_6, while DMQ_6 was not detected in strains harboring a *coq7*-nonsense mutation, where the carboxyl-terminal half of the protein is missing (Padilla et al., 2004). These results suggest that Coq7 protein is either involved in one or more mono-oxygenase steps or serves as an essential component of the putative multi-subunit enzyme complex. Biochemical function of Coq7 protein as a hydroxylase was further supported by the determination that it belongs to a family of di-iron binding oxidases containing a conserved sequence motif for the iron ligands, EXXH (Stenmark et al., 2001). Coq7 homologues from *Pseudomonas aeroginosa*, *Thiobacillus ferroxidans*, *C. elegans* restored Q biosynthesis in an *E.coli ubiF* mutant (Adachi et al., 2003,Stenmark et al., 2001). *E. coli* UbiF, a flavin-dependent DMQ hydroxylase that shares no homology to Coq7 protein, has been shown to functionally complement both the *coq7* null mutant and the DMQ_6-producing *coq7E194K* mutant, with better efficiency in the

latter (Tran et al., 2006). Collectively, these findings indicate that yeast Coq7 protein functions in the hydroxylation of DMQ. Moreover, steady state levels of the Coq3, Coq4, and Coq6 polypeptides were higher in the *coq7E194K* mutant than in the null mutant, suggesting that Coq7 protein and DMQ_6 serve to stabilize other Coq polypeptides.

Recent submitochondria fractionation studies (Tran, U.C., Gulmezian, M., Santos-Ocaña, C., Saiki, R., Navas, P., and Clarke, C. F., manuscript in preparation) demonstrate that yeast Coq7 protein, like its homologues in mice (Jiang et al., 2001), is peripherally associated to the inner membrane on the matrix side. However, earlier studies have modeled Coq7 as an interfacial inner mitochondrial membrane protein (Stenmark et al., 2001,Berthold and Stenmark, 2003). Interfacial membrane proteins, such as prostaglandin synthase (Picot et al., 1994) and squalene cyclase (Wendt et al., 1999), are embedded in the membrane via interaction with only one leaflet of the bilayer. Unlike the Coq7 polypeptide, proteins classified as interfacial (based on X-ray crystal structures), including prostaglandin synthase, squalene cyclase, fatty acid amide hydrolase, and microsomal cytochrome P450, each behaved as integral proteins in biochemical assays (Bracey et al., 2004). The true nature of the Coq7 protein-membrane association awaits a structure determination for yeast Coq7p or one of its homologues.

Coq8

The *COQ8* gene was initially identified as *ABC1* (**A**ctivity of $\boldsymbol{bc_1}$ complex) for its ability to partially suppress, in multicopy, the cytochrome *b* translation defect due to the *cbs2-223* mutation in the *CBS2* gene (Bousquet et al., 1991). *CBS2* is a yeast nuclear gene encoding a translational activator of cytochrome *b* (Rodel, 1986). It was observed that inactivation of *ABC1* resulted in respiratory defect and absence of NADH-cytochrome *c* reductase activity (Bousquet et al., 1991); a phenotype similar to that of Q-deficient strains (Tzagoloff and Dieckmann, 1990). It was subsequently shown that the respiratory complexes II, III, and IV of the *abc1* null mutant were thermo-sensitive and addition of exogenous Q could partially compensate for the respiratory deficiency (Brasseur et al., 1997). These results led to a hypothesis that the *ABC1* gene product may function as a chaperone that is essential for the proper conformation and activity of the bc_1 and its neighboring complexes (Brasseur et al., 1997). However, Do et al (Do et al., 2001) demonstrated that the *COQ8* gene, required for Q biosynthesis (Poon et al., 1997), is the same as the *ABC1* gene and provided data indicating that Q-deficiency is exclusively responsible for the pleiotropic defects of *abc1/coq8* mutants. Moreover, a neighboring $tRNA^{TRP}$ gene located downstream of *COQ8/ABC1* gene was demonstrated to account for the suppression of the *cbs2-223*, a UGA nonsense mutation (Hsieh et al., 2004). Although its biochemical function in Q biosynthesis is currently unknown, Coq8/Abc1 protein has been classified as putative protein kinase based on the identification of four kinase conserved motifs in its amino acid sequence (Leonard et al., 1998).

Coq9

The *COQ9* gene was recently identified and characterized as a new gene that, when mutated, results in a Q-deficient phenotype, in a similar manner to other *COQ* genes (Johnson et al., 2005). However, the function of Coq9 protein in Q biosynthesis is not yet known. The amino acid sequence of Coq9 protein contains a distinct conserved domain present in the COG5590 protein family (Marchler-Bauer et al., 2005). While *COQ9* homologues are well-represented in genomes of eukaryotes and alpha-proteobacteria, Coq9 protein has no homology to proteins with known function. Intriguingly, multicopy expression of the *COQ8* gene was shown to restore respiration in a specific *coq9* point mutant (*coq9-1*; E151STOP nonsense mutation). Although a small amount of Coq9 polypeptide was detected in the *coq9-1* nonsense mutant strain, levels were not elevated by the multicopy suppression mediated by the *COQ8* gene (Johnson et al., 2005,Hsieh et al., 2007). Consequently, the mechanism responsible for the multi-copy *COQ8* suppression of *coq9-1* is unknown.

Based on the mobility in the SDS-PAGE, the molecular mass of Coq9 protein is about 25 kDa (Hsieh et al., 2007), slightly smaller than the predicted precursor (29.9 kDa) (Johnson et al., 2005), and is consistent with the removal of a putative mitochondrial targeting sequence. However, the native size of Coq9 protein estimated from its sedimentation on sucrose gradients is about three times larger, indicating that the protein is either a homo-oligomer or in a complex with other proteins (Johnson et al., 2005). Potential partners in such a complex are Coq3 and Coq5 polypeptides, which were shown to co-sediment with the Coq9 protein. Recently, submitochondrial localization analysis has demonstrated that Coq9 protein is a peripheral membrane protein associated with the matrix side of the mitochondrial inner membrane (Hsieh et al., 2007).

2. *S. cerevisiae* Q Biosynthesis Requires a Multiple-enzyme Complex or Complexes

There are many well-characterized mitochondrial respiratory protein complexes in yeast, for example, cytochrome oxidase, ATP synthase, and the cytochrome bc_1 complexes. In these systems, the absence or mutation in one component results in proteolytic degradation, instability, or inactivation of the remaining subunits (Glerum et al., 1997,Tzagoloff et al., 1994). Multi-subunit enzyme complexes allow channeling of labile/reactive intermediates, enhance catalytic efficiency, and provide a mechanism for coordinative regulation of components. This seems to be the case in Q biosynthesis as well.

Previous studies have provided numerous lines of genetic evidence for a Q biosynthetic complex and for interdependent relationship among Coq polypeptides. Each of the null *coq3* to *coq9* mutants predominantly accumulates the same earlier intermediate HHB, the product of Coq2p, instead of the corresponding diagnostic intermediate (Poon et al., 1995,Poon et al., 1997,Johnson et al., 2005). Steady state levels of Coq3, Coq4, Coq6, Coq7, and Coq9 polypeptides are significantly decreased in mitochondria isolated from any of the other *coq* null mutants (Hsu et al., 2000,Baba et al., 2004,Belogrudov et al., 2001,Gin and Clarke, 2005,Tran et al., 2006,Hsieh et al., 2007). In addition, a null mutation in any of the *COQ* genes led to decreased Coq3 *O*-methyl transferase activity, although *COQ3* RNA levels were not affected (Hsu et al., 2000). These phenotypes were not due to a lack of respiration, because other mutants with defects in the respiratory complexes such as *atp2* and *cor1* null mutants retained wild-type levels of *O*-methyl transferase activity. Although Coq1 polypeptide levels remain unchanged in any of the other *coq* null mutants, the protein itself and/or its lipid product appears to be essential for stabilization of Coq3, Coq4, Coq6, Coq7, and Coq9 proteins (Gin and Clarke, 2005,Hsieh et al., 2007). It has been demonstrated that phenotypes of certain *coq* point mutants dramatically differ from the respective null mutants. For example, the *coq7E194K* point mutant but not the *coq7* null mutant was rescued by low copy expression of *E. coli ubiF* (Tran et al., 2006). Similarly, the *coq5-2* and *coq5-5* mutants, which have normal levels of Coq3, Coq4, and Coq5 polypeptides, are the only two *coq5* mutants significantly rescued by expression of *E. coli ubiE*, a homolog of *COQ5* gene (Baba et al., 2004). Moreover, the *coq4-1* (E226K) mutant maintained wild-type levels of Coq3 and Coq7 polypeptides, which were greatly diminished in the null *coq4* mutants (Belogrudov et al., 2001). This data renders support for the proposed structural/regulatory role of Coq4 protein in a multi-protein complex in Q biosynthesis. In such a model, the complete absence of Coq4p results in instability of several of the other Coq polypeptides, while certain amino acid substitution mutations in the Coq4 protein serve to stabilize the Coq polypeptides. Taken together, these results are consistent with the involvement of the Coq polypeptides and/or the Q-intermediates formed by these proteins in a multi-subunit complex or complexes. Postulated lipid components of the Q biosynthetic complex may also include the final product Q_6 because the addition of exogenous Q was shown to stabilize steady state levels of Coq3 and Coq4 polypeptides in the null *coq7* mutant (Tran et al., 2006).

Recent biochemical analyses provide physical evidence for the model of Q biosynthetic complex. Size exclusion chromatography (gel filtration) coupled with *O*-methyltransferase assays of the supernatant from digitonin-solubilized mitochondria demonstrated that Coq3, Coq4, Coq6, and Coq7 polypeptides co-elute as a high molecular mass complex with the Coq3 *O*-methyltransferase activity (Marbois et al., 2005,Tran et al., 2006). Further analysis of the representative gel-filtration fractions with mass spectrometry indicated that the DMQ_6 intermediate, the substrate of Coq7 protein, is also associated with the complex (Marbois et al., 2005). Recent gel filtration analysis using newly generated specific antibodies showed that Coq2 protein co-elutes with Coq4 and Coq7 polypeptides (Tran, U.C., Gulmezian, M., Santos-Ocaña, C., Saiki, R., Navas, P., and Clarke, C. F., manuscript in preparation), and Coq9 polypeptide co-elutes with Coq3, Coq4, Coq5, and Coq8 proteins (Hsieh, E. J. and Clarke, C. F., unpublished data). Additionally, two dimensional Blue native gel electrophoresis (BN-PAGE/ SDS-PAGE) analyses of the supernatant from digitonin-solubilized mitochondria yield data indicating that Coq2, Coq3, Coq4, Coq7, and Coq9 polypeptides co-migrate as a high molecular mass complex or complexes (Tran et al., 2006) (Tran, U.C., Gulmezian, M., Santos-Ocaña, C., Saiki, R., Navas, P., and Clarke, C. F., manuscript in preparation) (Hsieh et al., 2007). Co- precipitation of biotinylated-Coq3 protein with Coq4 identified a physical interaction between Coq3 and Coq4 polypeptides (Marbois et al., 2005). Moreover, Coq9-HA (hemagglutinin antigen) fusion protein was recently demonstrated to physically interact with Coq4, Coq5, Coq6, and Coq7 polypeptide via co- precipitation. All together, these results support the existence of a multi-subunit-Q-biosynthetic complex or complexes consisting of the Coq polypeptides and some of the corresponding lipid Q-intermediates.

A proposed model for the Q biosynthetic complex in which Coq2 serves as an anchor to the inner mitochondrial membrane is depicted in Figure 2. In this figure, peripheral protein components of the complex are modeled in association with Coq2. However, the complex could also be anchored to the membrane via other uncharacterized polypeptides and/or lipid components of the inner membrane. Further studies are needed to determine the stoichiometry of the polypeptide and lipid components of the complex, elucidate the nature of the membrane association, identify other potential constituents, and examine the effects of *coq* mutations on the dynamic of the complex.

3. Regulation of Q Function and Biosynthesis in *S. cerevisiae*

3.1. A potential Q_6 binding polypeptide-Coq10 protein

Complementation of a partially respiratory deficient mutant from the Genome Deletion Strain Collection identified *COQ10*, a new gene required for Q function in respiration (Barros et al., 2005). Similar to previously characterized *coq* mutants (*coq1-coq9*), the yeast *coq10* mutant exhibits defective NADH-cytochrome *c* reductase and succinate-cytochrome *c* reductase activities, which can be restored to near wild-type level by addition of Q_2. Unlike the other *coq* mutants, however, the *coq10* mutant grows slowly on medium containing non-fermentable carbon sources (ethanol and glycerol) and produces near wild-type levels of Q_6. Interestingly, the slow-growing phenotype on of the *coq10* mutant on medium containing ethanol and glycerol was partially rescued by exogenous Q_6 supplementation or multi-copy expression of the *COQ2*, *COQ7*, or *COQ8* genes. This suggests that endogenous Q_6 produced by the mutant is not as “functional” as that synthesized in the corresponding wild-type strain. Sequence analysis of yeast Coq10 protein, as well as its homologues in *Caulobacter crescentus* and other eukaryotes, identifies it as a member of the protein superfamily containing the START domain (Shen et al., 2005). The solution structure of the *C. crescentus* homolog of Coq10 identified a hydrophobic tunnel which in other START family members functions in binding cholesterol, polyketides, or phospholipids (Miller, 2007). Because polypeptides belonging to this superfamily have been shown to be involved in lipid binding and trafficking, it is likely that

Coq10 protein may function in transport and/or directing newly synthesized Q to its correct location in the mitochondrial electron transport chain.

3.2. Effects of carbon catabolites on Q biosynthesis

In *S. cerevisiae*, Q levels are directly correlated to mitochondrial development and oxygen availability (Pennock and Threlfall, 1983). Biosynthesis of Q was found to be highest in aerobically grown cells and barely detectable in anaerobic cells (Lester and Crane, 1959). When yeasts were grown in aerobic batch cultures, the amount of Q varied depending on carbon catabolites (Gordon and Stewart, 1969). High glucose concentration inhibited Q biosynthesis to a higher degree than similar concentration of galactose, a non-repressing fermentable carbon source. As expected, Q production was greatly increased in media containing non-fermentable carbon source, when catabolite repression is at the minimum. Interestingly, supplementation with cAMP alleviates the inhibitory effect of glucose on Q biosynthesis at the enzymatic level (Sippel et al., 1983). Previous studies have demonstrated that mRNA levels of *COQ3*, *COQ4*, *COQ5*, and *COQ7* genes were higher in yeasts grown in glycerol containing media than in cultures containing fermentable dextrose (Marbois and Clarke, 1996,Hagerman et al., 2002,Clarke et al., 1991,Belogrudov et al., 2001). The amount of Coq7 polypeptide was significantly increased by growth on media containing ethanol (Jonassen et al., 1998). To further understand the mechanism that underlies this carbon-catabolite regulatory control of Q biosynthesis, it is necessary to examine how growth in media containing different carbon sources affects Q_6 content, steady state levels of Coq proteins, and the dynamic of the multi-subunit-Q-biosynthetic complex.

4. Perspectives

Coenzyme Q (ubiquinone or Q) is a prenylated benzoquinone lipid that is found in membranes throughout eukaryotic cells. The reversible redox chemistry of Q is responsible for its function in the respiratory electron transport chain of inner mitochondrial membranes and as a lipophilic antioxidant. Q is widely used as a dietary supplement and in a variety of clinical therapies, including treatment of several neuro-degenerative diseases (Ferrante et al., 2002,Grundman et al., 2002,Muller et al., 2003,Beal, 2004,Shults, 2005) and certain respiratory chain defects (Geromel et al., 2002).The studies reviewed in this article employed a combination of genetics, molecular biology, and biochemistry to delinate the eukaryotic biosynthetic pathway of Q, with *S. cerevisiae* as model system. Considering the nutritional and therapeutic aspects of Q, it is likely that characterization of Q biosynthesis and regulation will promote our understanding Q metabolism and its recent use in clinical therapies.

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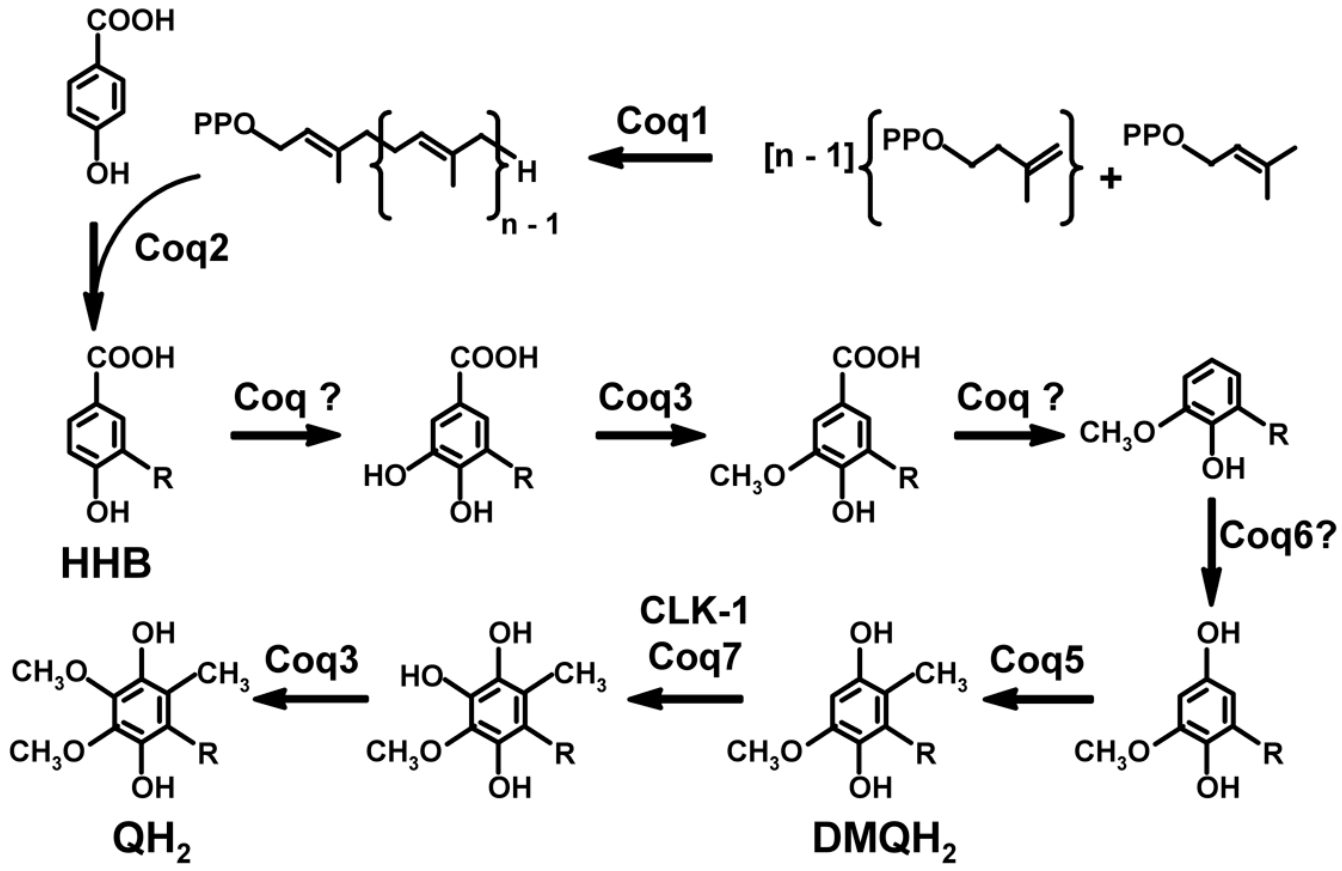

Fig 1. Proposed Q biosynthetic pathway in eukaryotes

The length of the polyisoprenoid chain of Q, designated by *n*, varies depending on the species; n = 6 in *S. cerevisiae*, 9 in *C. elegans*, and 10 in *H. sapiens*. In *S. cerevisiae*, there are nine identified Coq proteins necessary for the synthesis of QH_2 from dimethylallyl diphosphate and isopentenyl diphosphate precursors. The enzymatic functions of Coq4, Coq6, Coq8 and Coq9 polypeptides have yet to be characterized. Molecular oxygen and AdoMet are proposed donors for the hydroxy and methyl group, respectively (Olson and Rudney, 1983). CLK-1 is the *C. elegans* Coq7 homologue.

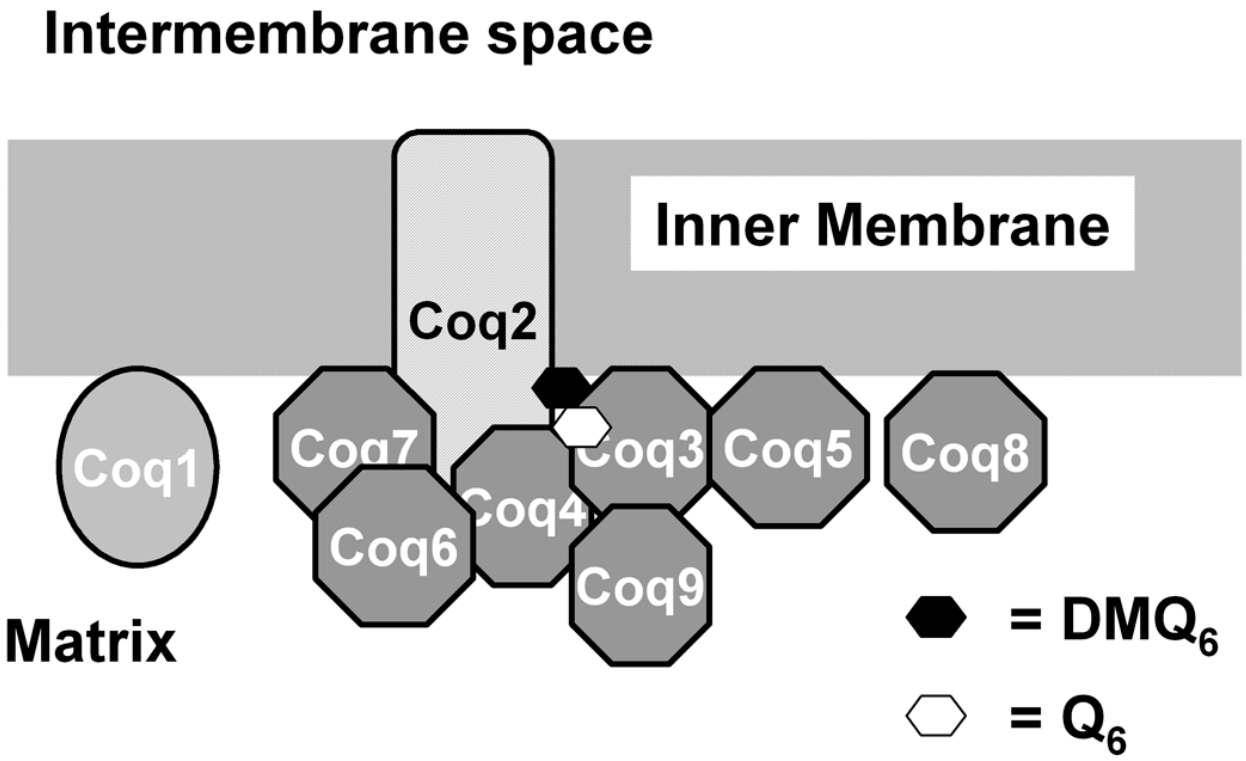

Fig 2. A model of the mitochondrial Q biosynthetic protein complex in *S. cerevisiae*

The putative complex contains six Coq polypeptides which are peripherally associated with the inner mitochondrial membrane (*dark grey octagons*) and a spanning integral membrane Coq protein (*hatched rectangle*). Proposed lipid components of the multi-subunit complexes include DMQ_6 (*black hexagon*) and the final product Q_6 (*white hexagon*). The stoichiometry of the components has yet to be determined.

Table 1

Characteristics of the nine *S. cerevisiae* Coq proteins required for Q biosynthesis

Yeast protein	Human homolog	mature M.W (kDa)[a]	Localized *in vitro* import	to mitochondria by: submitochondrial fractionation	Component of Q biosynthetic complex	Complementation of yeast mutants by human homologs
Coq1	hDPS1/hDLP1*	53	Unknown	peripheral i.m. matrix side	?	?
Coq2	Coq2	30	+	integral i.m. matrix side	yes	yes
Coq3	Coq3	33	+	peripheral i.m. matrix side	yes	yes
Coq4	NP_057119**	36	+	peripheral i.m. matrix side	yes	?
Coq5	CAI46073***	31	+	peripheral i.m. matrix side	yes	?
Coq6	NP_872282***	51	+	peripheral i.m. matrix side	yes	?
Coq7/Cat5	Clk-1/Coq7	23	+	peripheral i.m. matrix side	yes	yes
Coq8/Abc1	Adck-1—Adck5	53	+	peripheral i.m. matrix side	?	?
Coq9	AAH54340#	25	Unknown	peripheral i.m. matrix side	yes	?

[a] via SDS-PAGE migration.

* Also known as PDSS1/PDSS2.

** NCBI Blastp score > 150.

*** Blastp score > 200.

Blastp score > 50