MINIREVIEW

Biosynthesis and bioproduction of coenzyme Q_{10} by yeasts and other organisms

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CoQ (coenzyme Q), an isoprenylated benzoquinone, is a well-known component of the electron-transfer system in eukaryotes. The main role of CoQ is to transfer electrons from NADH dehydrogenase and succinate dehydrogenase to CoQ:cytochrome *c* reductase in the respiratory chain. However, recent evidence indicates that an involvement in respiration is not the only role of CoQ. The second apparent role of CoQ is its anti-oxidation property, and other novel roles for CoQ, such as in disulfide-bond formation, sulfide oxidation and pyrimidine metabolism, have been reported. CoQ_{10}, having ten isoprene units in the isoprenoid side chain, has been used as a medicine and is now commercially popular as a food supplement. Two yeast species, namely the budding yeast *Saccharomyces cerevisiae*, which produces CoQ_6, and the fission yeast *Schizosaccharomyces pombe*, which produces CoQ_{10}, are the main subjects of the present minireview because they have greatly contributed to our basic knowledge of CoQ biosynthesis among eukaryotes. The biosynthetic pathway that converts *p*-hydroxybenzoate into CoQ consists of eight steps in yeasts. The five enzymes involved in the biosynthetic pathway have been identified in both yeasts, yet the functions of three proteins were still not known. Analyses of the biosynthetic pathway in yeasts also contribute to the understanding of human genetic diseases related to CoQ deficiency. In the present minireview I focus on the biochemical and commercial aspects of CoQ in yeasts and in other organisms for comparison.

Introduction

CoQ (coenzyme Q), also known as ubiquinone, is a natural lipid widely distributed in almost all living organisms. CoQ is composed of a benzoquinone moiety and an isoprenoid side chain (Figure 1). CoQ primarily functions as an electron transporter in aerobic respiration and oxidative phosphorylation in the respiratory chain located in the mitochondrial inner membrane of eukaryotes [1–3]. CoQ serves as the electron transporter of NADH dehydrogenase and succinate dehydrogenase to CoQ:cytochrome *c* reductase and thus contributes essentially to the bioenergetic activity of ATP synthesis (Figure 2). The two predominant forms of CoQ are the oxidized CoQ and the reduced $CoQH_2$. Besides these two stable forms, other short-lived redox and protonation states, such as a semiquinone type, which behaves as a pro-oxidant, exist in mitochondria during electron transfer.

The roles of CoQ, other than as a component of the respiratory chain, have been extensively studied. Many reports suggest that CoQ also functions as a lipid-soluble antioxidant in cellular biomembranes that scavenges reactive oxygen species [4]. Much of the CoQ in cell membranes is in the reduced form and thus acts as an effective antioxidant. Indeed, studies on several mutants, such as those of the bacterium *Escherichia coli*, yeasts and the nematode worm *Caenorhabditis elegans*, that do not produce CoQ suggest that an *in vivo* function of CoQ is to protect against oxidants [5–7]. Besides these two main functions of CoQ, namely respiration and antioxidation, other roles, in such mechanisms as disulfide-bond formation, sulfide oxidation and pyrimidine metabolism, have also been reported [3,8,9].

Living organisms possess different species of CoQ depending on the length of the isoprenoid side chain. For example, among yeasts, *Schizosaccharomyces pombe* produces CoQ_{10}, *Candida albicans* produces CoQ_9, *Candida utilis* produces CoQ_7, and *Saccharomyces cerevisiae* produces CoQ_6 [1]. One type of CoQ is dominant in each organism,

Key words: antioxidant, coenzyme Q (CoQ; ubiquinone), respiration, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, yeast.

Abbreviations used: AdoMet, *S*-adenosylmethionine; *At* (in gene names), *Arabidopsis thaliana*; CoQ, coenzyme Q; Dlp1, D-less polyprenyl diphosphate synthase; DMAPP, dimethylallyl diphosphate; DMQ, demethoxyubiquinone; Dps1, decaprenyl diphosphate synthase; DsbA and DsbB, disulfide-bond-formation facilitators A and B; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; HHB, 3-hexaprenyl-4-hydroxybenzoic acid; IPP, isopentenyl diphosphate; IspB, octaprenyl diphosphate synthase; MEP, 2-*C*-methyl-D-erythritol 4-phosphate; MK, menaquinone; PDS, prenyl diphosphate synthase; PHB, *p*-hydroxybenzoate; ppt1, PHB:polyprenyl diphosphate transferase; PQ, plastoquinone; RQ, rhodoquinone.

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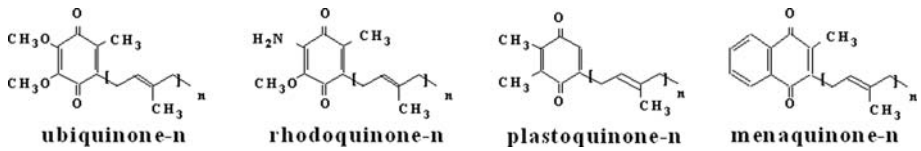


Figure 1 Structures of quinones

Among the natural quinones found in living organisms, CoQ, RQ and PQ are derived from structurally similar types of benzoquinones, whereas MK is derived from a naphthoquinone. -n (or $_n$ on the structure), number of isoprene units.

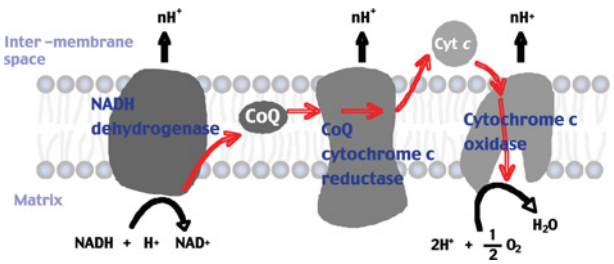


Figure 2 Electron-transfer system in yeasts

Respiration in yeasts requires an electron-transfer system that comprises NADH dehydrogenase, CoQ:cytochrome *c* reductase and cytochrome *c* oxidase. Succinate dehydrogenase is omitted from this diagram for simplicity. nH^+, protons.

but (a) minor type(s) of CoQ is also occasionally detected. The length of the side chain of CoQ is precisely defined by *trans*-polyprenyl diphosphate synthases rather than by the PHB (*p*-hydroxybenzoate):polyprenyl diphosphate transferases that catalyse the condensation of PHB and polyprenyl diphosphate [10–13].

Among various CoQ species, CoQ_{10} (a human type) has been used as a medicine [3] and, more recently, as a nutritional supplement. A growing body of evidence suggests that the oral administration of CoQ_{10} is beneficial in the treatment of human disorders such as cardiomyopathy, diabetes, Parkinson's and Alzheimer's diseases, and can also reduce the risks of myopathy associated with the use of statin drugs [14,15]. Human CoQ_{10} deficiency, caused either by mutations in CoQ biosynthetic enzymes or by mutations indirectly leading to low CoQ_{10} levels, has been associated with cases of encephalomyopathy, cerebellar ataxia and Leigh syndrome (subacute necrotizing encephalomyelopathy) [16,17]. The use of CoQ_{10} is also gaining popularity in the cosmetic industry, owing to its antioxidant properties. CoQ_{10} is the only lipid antioxidant which humans can biosynthesize by themselves, and therefore the side effects of CoQ_{10} supplementation are few.

In the present minireview I focus on the biosynthesis of CoQ mainly in yeasts, because these organisms are the best studied eukaryotes in respect of the CoQ biosynthetic pathway.

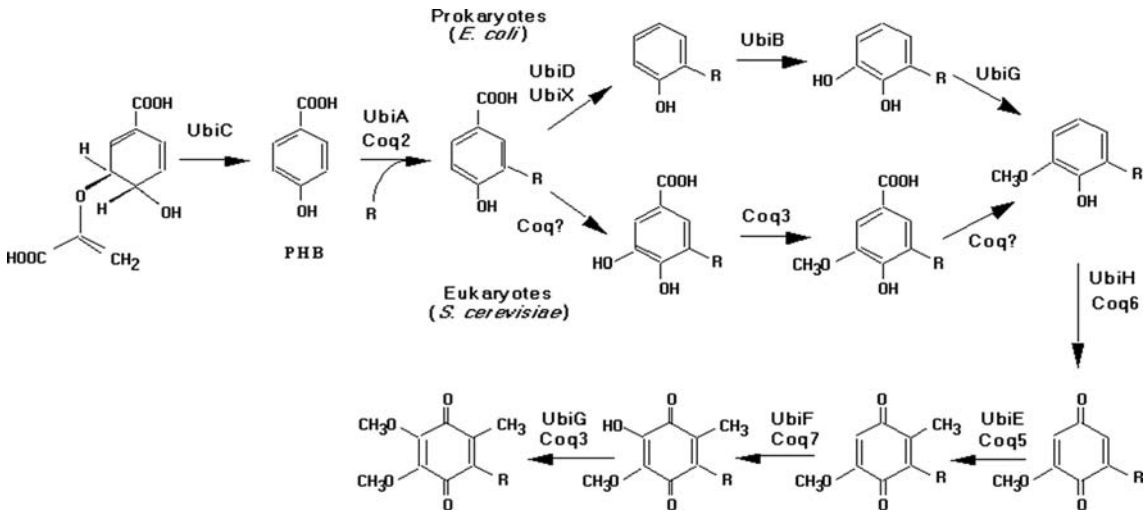
CoQ is one of the natural quinones in living organisms.

Living organisms possess different types of isoprenoid quinones such as CoQ (also known as ubiquinone), PQ (plastoquinone), MK (menaquinone) and RQ (rhodoquinone) (Figure 1). CoQ, PQ and RQ are made from structurally similar types of benzoquinones, whereas MK is synthesized from a naphthoquinone. Whereas CoQ participates in aerobic respiration, MK and DMK (demethylmenaquinone) play roles in anaerobic respiration.

All types of quinones can be found in prokaryotes and eukaryotes, whereas MK is found only in prokaryotes. Some organisms contain two types of quinones, i.e. *E. coli* possesses MK and CoQ, *C. elegans* possesses CoQ and RQ, and plants possess CoQ and PQ. But yeasts and higher animals exclusively contain CoQ. All those quinones basically function as electron-transfer carriers in respiration or photosynthesis. But there are also other roles for MK and CoQ in bacteria. For example, in *E. coli* MK and CoQ provide the electron for disulfide-bond formation in proteins [8]. The DsbA–DsbB system operates in this protein-disulfide-bond formation and the disulfide bond formation facilitator DsbA is reduced when the disulfide bond is formed. The electron is transferred from DsbA to DsbB, then DsbA is re-oxidized by DsbB. Under anaerobic conditions, MK is used, and under aerobic conditions, CoQ is used, to oxidize DsbB. This role of CoQ as an electron acceptor in disulfide-bond formation is not observed in eukaryotes.

Biosynthesis of CoQ

The biosynthetic pathway that converts PHB into CoQ consists of eight steps in yeasts. Starting from the synthesis of PHB, seven enzymes are thought to be involved in CoQ biosynthesis (Scheme 1). These steps include the condensation and transfer of the isoprenoid side chain to PHB, followed by methylations, decarboxylation and hydroxylations [1,18]. The elucidation of this pathway in eukaryotes has mostly come from genetic and biochemical studies using CoQ-deficient mutants of *Sa. cerevisiae* [1,19]. Nine *Sa. cerevisiae coq* mutants were used to define the biosynthesis of CoQ [18,20]. Until recently, although the genes



Scheme 1 Pathway of CoQ biosynthesis in yeasts and bacteria

The pathways of CoQ biosynthesis in *E. coli* and *Sa. cerevisiae* are shown. All *E. coli ubi* genes involved in the CoQ synthesis are assigned in the pathway, whereas two steps in the pathway and three *COQ* genes, namely *COQ4*, *COQ8* and *COQ9*, are not functionally defined in *Sa. cerevisiae*.

for all *coq* mutants have been identified, not all functions of these *COQ* genes were determined in *Sa. cerevisiae*. PHB:polyprenyl diphosphate transferase represented by *Sa. cerevisiae* Coq2 catalyses the condensation of PHB with the isoprenoid chain. Both genetic and biochemical analyses indicate that the PHB:polyprenyl diphosphate transferase has a broad substrate specificity [10]. *COQ3* encodes dihydroxy-polyprenylbenzoate methyltransferase and *COQ5* encodes *C*-methyltransferase. Both *COQ6* and *COQ7* genes encode mono-oxygenases [21,22], but catalyse at different points in CoQ biosynthesis. Recently, a novel gene named *COQ9* was shown to be involved in biosynthesis of CoQ, but its function remains unknown [23]. The functions of the *COQ4* and *COQ8* genes also have not yet been identified. The novel gene called *COQ10* has now been shown to be a binding protein of CoQ, but it is not involved in the CoQ biosynthesis [24,25]. All Coq proteins from *Sa. cerevisiae* and *Sc. pombe* were shown to localize in mitochondria. The analysis in *Sa. cerevisiae* suggested that Coq proteins form a large complex in the mitochondrial inner membrane [26]. Those biochemical aspects of CoQ will be discussed below in detail.

There are some differences between the CoQ-biosynthetic pathways of *Sc. pombe* and *Sa. cerevisiae*. First, polyisoprenyl diphosphate synthase in *Sc. pombe* is a hetero-tetramer composed of two subunits, Dps1 and Dlp1 [27,28], similar to the situation in mice and humans [29], but different from the homodimeric (or homotetrameric) structure seen in the *Sa. cerevisiae* enzyme [30]. Secondly, the disruption of certain CoQ-synthetic genes in *Sc. pombe* results in the production of different intermediates (M. Kawamukai, unpublished work) from those found in *Sa. cerevisiae*, in which the specific early intermediate HHB (3-hexaprenyl-4-hydroxybenzoic acid) accumulates [31]. Thirdly, the role of CoQ in some biological processes differs between *Sc. pombe* and *Sa. cerevisiae*. For example, CoQ reduction is coupled to sulfide oxidation in *Sc. pombe* [9,32], but not in *Sa. cerevisiae*.

Several genes for the biosynthesis of CoQ in animals and plants have been reported, but basically the genes are similar to those from *Sa. cerevisiae*, except for the *trans*-polyprenyl diphosphate synthase that synthesizes the isoprenoid side chain (Table 1). In this enzyme, two types, one with a monomeric structure and one with a heteromeric structure, have been found in eukaryotes. Although there are some differences in enzymes, it is generally considered that the eukaryotic type of CoQ biosynthetic pathway is common.

Synthesis of PHB

The aromatic precursor of the benzoquinone ring of CoQ is PHB. The synthesis of PHB, which is catalysed by chorismate lyase (UbiC) in *E. coli*, does not occur in *Sa. cerevisiae* [20]. In *Sa. cerevisiae,* two possible pathways to synthesize PHB are considered. One is a pathway derived from chorismate and the other from tyrosine. Exact biochemical reactions have not been defined for either pathway.

Biosynthesis of the isoprenoid side chain of CoQ

The isoprenoid side chain of CoQ is synthesized through the mevalonate pathway in eukaryotes, but is synthesized through the MEP (2-*C*-methyl-D-erythritol 4-phosphate) (or non-mevalonate) pathway in prokaryotes

Table 1 Comparison of CoQ biosynthetic genes among yeasts, *C. elegans*, *Homo sapiens* (human), *Arabidopsis* and *E. coli*

The genes and enzymes for CoQ biosynthesis have been best characterized in *Sa. cerevisiae* and *E. coli*. The genes which are functionally or structurally homologous with each other appear on the same row. The genes experimentally examined are italicized, but others are indicated by their (Roman) database name. In *E. coli* it has been shown that chorismate lyase is encoded by *ubiC*, another mono-oxygenase is encoded by *ubiB* and decarboxylase is encoded by *ubiD* or *ubiX*. These genes are omitted from the list.

Gene						
Sa. cerevisiae	*Sc. pombe*	*C. elegans*	*H. sapiens*	*Arabidopsis*	*E. coli*	Enzyme function
COQ1	*dps1–dlp1*	*COQ1*	*hDPS1-hDLP1*	*AtSPS1, AtSPS2*	*ispB*	Polyprenyl diphosphate synthase
COQ2	*ppt1*	*COQ2*	*hPPT1/COQ2*	*AtPPT1*	*ubiA*	PHB:polyprenyl diphosphate transferase
COQ3	*coq3*	*COQ3*	*COQ3*	*AtCOQ3*	*ubiG*	O-Methyltransferase
COQ4	*coq4*	*COQ4*	*COQ4*	At2g03690	–	Unknown
COQ5	*coq5*	*COQ5*	MGC4767	At5g57300	*ubiE*	C-Methyltransferase
COQ6	*coq6*	*COQ6*	MGC23201	At3g24200	*ubiH*	Mono-oxygenase
COQ7	*coq7*	*COQ7/clk-1*	*COQ7*	–	*ubiF*	Mono-oxygenase
COQ8/ABC1	*coq8/abc1*	*COQ8*	*Adck1–Adck5*	*AtABC1*	–	Unknown
COQ9	*coq9*	*COQ9*	AAH13114	At1g19140	–	Unknown

and in plant chloroplasts [20]. DMAPP (dimethylallyl diphosphate), the isomer of IPP (isopentenyl diphosphate) is a primer for the condensation of one unit of IPP to construct isoprenoid with a certain length (Scheme 2) [33]. A side chain longer than C_{25} is general for CoQ and a side chain shorter than C_{20} is rare in natural CoQs.

Coq1/prenyl diphosphate synthase

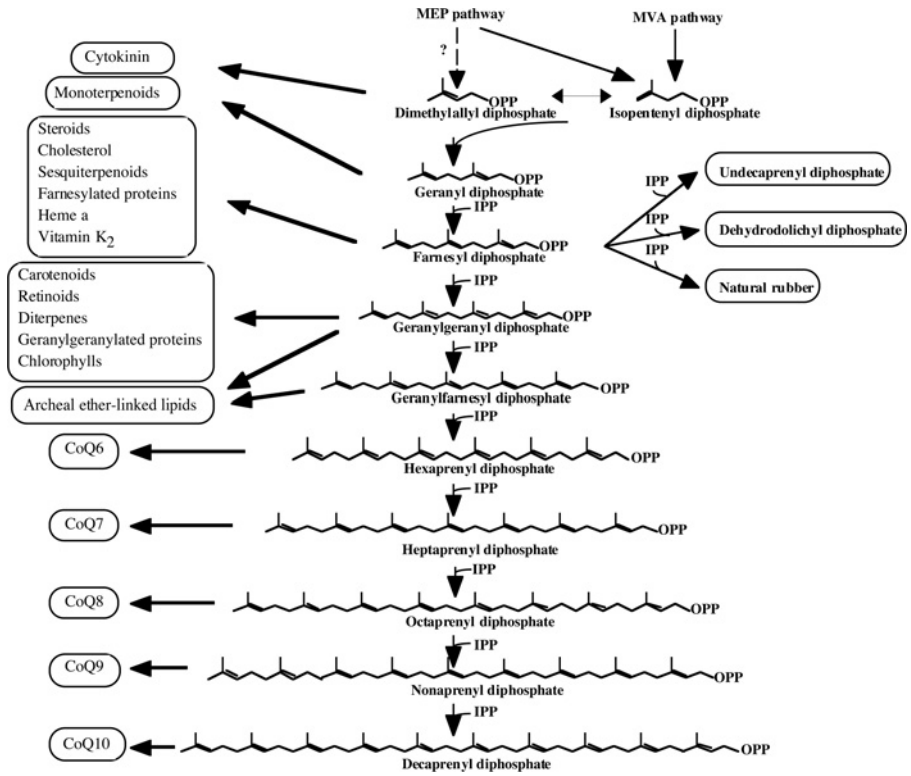
Long-chain *trans*-polyprenyl diphosphate (C_{30}–C_{50}) synthases, represented by Coq1 in *Sa. cerevisiae*, catalyse the condensation of FPP (farnesyl diphosphate) or GGPP (geranylgeranyl diphosphate), which acts as a primer, and IPP to produce the various prenyl diphosphates bearing chains of various lengths. Although short-chain polyprenyl diphosphate (C_{15}, C_{20}) synthases, such as FPP synthase and GGPP synthase, have been identified in many organisms ranging from bacteria through to plants and mammals [27,34–36], analyses of the long-chain *trans*-polyprenyl diphosphate synthases have been relatively limited to those in several bacteria, yeasts, trypanosomes, plants and mammals [28,29,37–41].

These enzymes possess seven conserved regions, including two DDXXD motifs that are binding sites for the substrates in association with Mg^{2+} [42]. Various mutants of DdsA (decaprenyl diphosphate synthase) from *Gluconobacter suboxydans* [43] and IspB (octaprenyl diphosphate synthase) from *E. coli* [37] were constructed. There are two aspartate-rich regions in the enzymes. The most significant site for the determination of the length was the fifth amino acid position from the first aspartate-rich region, as proposed from the analysis of FPP synthase and GGPP synthase [43]. It was also found that the formation of an IspB dimer is essential for the determination of the length [37]. The structure of IspB in *Thermotoga maritima* was recently solved and was found to be very similar to that of FPP synthase [44].

Analyses of Coq1 (hexaprenyl diphosphate synthase) from *Sa. cerevisiae* and the solanesyl diphosphate synthase from the flowering plant *Arabidopsis thaliana* (thale cress) suggest that the long-chain *trans*-polyprenyl diphosphate synthases that synthesize the CoQ side chain tend to be homomeric enzymes [30,38,45]. However, the decaprenyl diphosphate synthase from *Sc. pombe* is a heterotetramer of two proteins, Dps1 (decaprenyl diphosphate synthase) and Dlp1 [D(aspartate)-less polyprenyl diphosphate synthase] [28]. Dlp1 lacks the aspartate-rich motifs and is bound to Dps1 to constitute the heteromeric enzyme [28,29]. This heterotetramer composition of polyprenyl diphosphate synthases was also found in human and mouse [29]. In an animal model, namely the mouse, mutations in *dlp1* were found to be the cause of renal disease [46]. Human genetic disorders in the *DPS1* (*PDSS1*) and *DLP1* (*PDSS2*) were also reported to be a cause of Leigh disease [47,48].

Coq2/Ppt1 (PHB:polyprenyl diphosphate transferase)

PHB:polyprenyl diphosphate transferase, represented by *Sa. cerevisiae* Coq2, catalyses the condensation of PHB with the isoprenoid chain. Both genetic and biochemical analyses indicate that the PHB:polyprenyl diphosphate transferase (UbiA/Coq2) has broad substrate specificity [10]. Biochemically, the purified UbiA protein accepts isoprenoid tail lengths ranging from $n = 2$ to $n = 9$. This is supported by observations showing that purified PHB:polyprenyl diphosphate transferases from *Pseudomonas putida* and *E. coli* [49] have fairly wide substrate specificities in terms of polyisoprenols. *E. coli ubiA* and *Sa. cerevisiae coq2* mutants, both of which are defective in PHB:polyprenyl diphosphate transferase activity, can only grow on a medium containing



Scheme 2 Pathway of polyisoprenoid chain synthesis

The synthesis of various isoprenoid compounds either via the mevalonate (MVA) pathway or the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway are shown. DMAPP is a C_5 unit compound that serves as the precursor to condense multiple units of IPP. Isoprenoids with more than C_{25} units are generally used for the synthesis of the side chain of CoQ.

a fermentable carbon source, because these CoQ-deficient mutants are respiration-incapable [10,50]. Genetically, human *COQ2* complements yeast *coq2* mutant [51], and yeast *COQ2* complements the *E. coli ubiA* mutant [10], supporting the notion that PHB:polyprenyl diphosphate transferase is highly conserved. From mutagenesis analysis, it was found that *E. coli* UbiA and also other related enzymes have, for some reason, two active sites (M. Kawamukai, unpublished work).

An *Arabidopsis* T-DNA insertion mutant in the *AtPPT1* (*Arabidopsis* ppt1) gene that had completely lost PHB:polyprenyl diphosphate transferase activity was selected and analysed. *Arabidopsis AtPPT1* was shown to be essential for embryo development in the plants [12], similar to the situation in mammals where CoQ is also required for development [52].

Coq3/O-methyltransferase

Sa. cerevisiae COQ3 encodes dihydroxypolyprenylbenzoate methyltransferase. This enzyme apparently catalyses the two O-methylation steps in the CoQ biosynthetic pathway. The amino acid sequences of the proteins encoded by *COQ3* homologues all contain four regions that are conserved in a large family of methyltransferase enzymes utilizing *S*-adenosylmethionine (AdoMet) as the methyl donor. *Sc. pombe* mutants lacking *coq3* displayed the common phenotypes found in other *coq* mutants [22]. The *C. elegans coq3* mutant that lacks methyltransferase has been generated as the CoQ-null mutant. This mutant displays delayed development and a sterile phenotype at the first homozygote, and these are lethal at the embryonic stage in the next generation [53].

Coq4

The Coq4 protein is absolutely required for the biosynthesis, but its enzymatic function has not been elucidated. The amino acid sequence of the Coq4 protein does not share significant homology with protein domains or motifs with known enzymatic activity. The Coq4 protein is peripherally associated with the mitochondrial inner membrane on the matrix side and may have a structural role in the putative polypeptide CoQ biosynthetic complex [54].

Coq5/C-methyltransferase

The Coq5 protein catalyses the only C-methylation step in the CoQ biosynthetic pathway. It contains four sequence motifs present in a large family of AdoMet-dependent methyltransferase enzymes. The enzymatic activity of methyltransferase using 2-methoxy-6-polypreny-1,4-benzoquinone was successful, demonstrating the exact role of Coq5 as *C*-methyltransferase [55]. Submitochondrial fractionation analysis demonstrated the Coq5 protein is peripherally associated with the mitochondrial inner membrane on the matrix side.

Coq6/mono-oxygenase

The Coq6 protein is also a peripherally associated mitochondrial membrane protein that is imported in a membrane-potential-dependent manner. The Coq6 protein contains three conserved regions: an ADP-binding region, a motif with an FAD/NADH-binding activity and a consensus sequence that binds to the ribityl moiety of FAD. Coq6 has been considered a putative flavin-dependent monooxygenase responsible for adding the hydroxy group to 4-hydroxy-3-polyprenylbenzoic acid in the CoQ biosynthetic pathway [56].

Coq7/mono-oxygenase

The *COQ7* gene encodes a mono-oxygenase that is involved in the penultimate step of CoQ biosynthesis. The Coq7 protein belongs to a family of di-iron-binding oxidases containing a conserved motif, FXXH, for the iron ligands. A homologue of Coq7 in *C. elegans,* Clk-1, was found to be involved in lifespan extension. The *clk-1* mutant of *C. elegans*, which accumulated the precursor DMQ (demethoxyubiquinone) owing to lack of the penultimate step of CoQ biosynthesis, shows a prolonged lifespan, developmental delay and low egg production [57–59]. The *clk-1* gene in *C. elegans* is an functional orthologue of *COQ7*, the function of which was identified as DMQ mono-oxygenase in *Sa. cerevisiae* [21]. *E. coli* UbiF also catalyses the same step as does Coq7 and Clk-1, as typically demonstrated by rescuing CoQ biosynthesis in an *E. coli ubiF* mutant by expression of *clk-1* [60]. Thus Coq7, Clk-1 and UbiF are highly conserved proteins among different Kingdoms, but, intriguingly, there is no apparent orthologue in plants, as judged from DNA sequences.

The long lifespan of the *C. elegans clk-1* mutant has been attributed to the presence of DMQ_9, because it is believed to have fewer pro-oxidant properties than CoQ and is shown to function in the respiratory chain to some extent [61]. However, as alternative factors, CoQ_8 from *E. coli* and endogenous RQ_9 were also shown to influence the life-extension phenotype in the *clk-1* mutant [62,63]. Thus several different type of quinones need to be considered to explain the long lifespan of the *C. elegans clk-1* mutant, but, because of the complexity, it was not clear which quinone is most responsible for the phenomenon. *Sc. pombe* appeared to be an excellent system with which to judge whether DMQ has any specific biological role, because DMQ accumulated in the *Sc. pombe coq7* mutant [22]. However, no apparent role of DMQ was in fact observed [22].

Coq8/Abc1

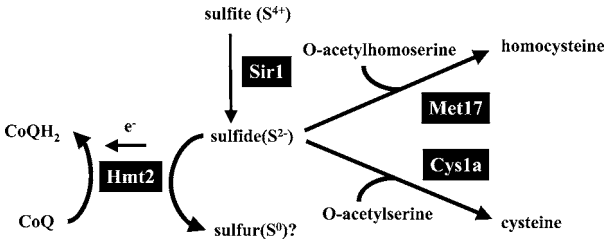
The *COQ8* gene in *Sa. cerevisiae* was found to be identical with the *ABC1* gene, which was isolated in the screening to find the factor involved in the translational regulation of cytochrome *b* [64,65]. *Sc. pombe coq8* was also shown to be essential for CoQ biosynthesis. Coq8 has been classified as a putative protein kinase on the basis of the presence of conserved kinase motifs in its primary structure. Recent results in fact demonstrate that Coq8 phosphorylates Coq3, and for that reason it is considered to be a regulator of Coq enzymes [66].

Coq9

The *COQ9* gene was identified and characterized as a new gene that is required for CoQ biosynthesis in *Sa. cerevisiae* [23]. However, the function of the Coq9 protein is not yet known. The Coq9 protein is conserved in eukaryotes, but has no homology with known proteins. The Coq9 protein has also been shown to be within a multisubunit complex [67].

Coq10/a CoQ-binding protein

The name of this Coq10 protein suggests that this protein is involved in the biosynthesis of CoQ, but in fact the Coq10 protein is a CoQ-binding protein. The Coq10 protein is located in the mitochondria, but does not belong to the succinate:CoQ/NADH:CoQ reductase nor the bc_1 complex in *Sa. cerevisiae* [24]. The existence of a CoQ-binding protein in mitochondria not only extends our knowledge of the regulation of CoQ, but also challenges the current model, that has existed for a long time, that CoQ is a free lipid molecule in membranes. Recently, Coq10 from *Sc. pombe* was characterized as a mitochondrial CoQ-binding protein that is required for proper respiration [25]. Thus Coq10 is a conserved CoQ-binding protein that is essential for the proper function of the electron-transfer system, possibly by assisting in the transfer of CoQ from one site to another in the mitochondrial membranes of eukaryotes.



Scheme 3 Sulfide metabolism and CoQ in fission yeast

In fission yeast, sulfide is synthesized from sulfite through sulfite reductase (Sir1). Sulfide is used for the synthesis of cysteine by cysteine synthase (Cys1a) from *O*-acetylserine [69] and also for the synthesis of homocysteine by homocysteine synthase (Met17) from *O*-acetylhomoserine [70]. Sulfide is oxidized by sulfide:quinone oxidoreductase (Hmt2).

A complex theory

It has been proposed that the CoQ enzymes form a complex in *Sa. cerevisiae* [18,26]. Each of the null *coq3–coq9* mutants predominantly accumulates the earlier intermediate HHB, the product of Coq2. 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 [68]. Size-exclusion chromatography demonstrated that Coq3, Coq4, Coq6 and Coq7 polypeptides were co-eluted as a high-molecular-mass complex with the Coq3 O-methyltransferase activity, supporting the notion of complex formation. Coq2 is an integral membrane protein, whereas other Coq proteins are peripherally associated membrane proteins. A current model for the CoQ biosynthetic complex suggests that Coq2 serves as an anchor to the mitochondrial inner membrane. A multisubunit enzyme complex like this Coq complex would allow channelling of reactive intermediates, enhance catalytic efficiency and provide a mechanism for co-ordinated regulation of components [18].

Sulfur metabolism and CoQ in fission yeast

In the fission yeast *Sc. pombe*, sulfide is synthesized from sulfite through sulfite reductase encoded by *sir1* [32]. Sulfide is required for the biosynthesis of both cysteine and methionine in this yeast (Scheme 3). Cysteine synthase encoded by *cys1a* synthesizes cysteine from *O*-acetylserine and sulfide [69]. Homocysteine synthase encoded by *met17* synthesizes homocysteine from *O*-acetylhomoserine and sulfide [70]. Sulfide is oxidized by sulfide:quinone oxidoreductase encoded by *hmt2,* which was originally identified in a mutant highly sensitive to Cd^{2+} [71]. Because sulfide is a toxic compound for cells, the amount of sulfide needs to be controlled tightly. Sulfide:quinone oxidoreductase is a necessary enzyme for detoxifying high production of sulfide. All *Sc. pombe* CoQ-deficient mutants, as well as respiration-deficient mutants, accumulated sulfide [22], indicating that CoQ is required to detoxify sulfide. There are orthologues of sulfide:quinone oxidoreductase in many other eukayotes, including nematode worms and humans, but not in *Sa. cerevisiae* [9].

Phenotypes of CoQ-null yeast mutants and other organisms

In *Sa. cerevisiae* and *Sc. pombe*, CoQ is not essential for growth. The CoQ-deficient *Sa. cerevisiae* only grows on a medium containing a fermentable carbon source because of respiration deficiency. CoQ-deficient *Sc. pombe* displays a much more severe phenotype than CoQ-deficient *Sa. cerevisiae*. It grows very slowly on minimum medium, is sensitive to oxidative stress, does not survive in the stationary phase and produces a large amount of sulfide [9,11,28,65]. Because *E. coli* contains both MK and CoQ, the function of CoQ can be replaced by MK. Therefore *E. coli* can survive without CoQ, but cannot survive without both quinones [72]. In *C. elegans*, CoQ is essential for development. Although *C. elegans* cannot survive most *COQ* gene knockouts, intriguingly, *coq7* mutants have a long lifespan [57,73,74]. In mouse, *coq7* has also been shown to be essential for embryogenesis. However, cell lines of a mouse *coq7* knockout could be maintained, indicating that CoQ is only required for development [52].

Human CoQ deficiency

CoQ has been used therapeutically for the treatment of heart failure, but is now widely used as a nutritional supplement. Since humans synthesize CoQ, it is no doubt a 'natural' compound. The association of a lack of CoQ_{10} with disease has also been reported. A patient who possessed only 10% of the normal amount of CoQ_{10} suffered from familial mitochondrial myopathy [75,76]. This patient possessed very little CoQ in muscle, but normal levels in other tissues such as fibroblasts and serum. CoQ_{10} supplementation was very effective in this instance, suggesting that the patient had some problems in the metabolic pathway of CoQ. Recently, examples were identified in Leigh disease that are caused by the alteration of the genes involved in the biosynthesis of CoQ. One is the mutation in *COQ2,* the second one is in *DPS1*(*PDSS1*) and the third one is in *DLP1*(*PDSS2*), and the fourth one is in *COQ8* [16,17,47,48,77]. These findings, which have correlated a genetic disorder in the biosynthetic enzymes of CoQ with a mitochondrially located disease, have contributed to human health, as have related biochemical and genetic studies on yeasts.

Expression of genes from higher eukaryotes in yeasts

In mammals and plants, the biosynthetic pathway of CoQ appears to be similar to that in yeasts. However, not so much was known about the CoQ biosynthetic pathway in higher eukaryotes. It is important to determine the nature of the CoQ_{10} biosynthetic pathway in humans, because CoQ_{10} is essential for energy production and is the only endogenously synthesized lipid-soluble antioxidant in humans. It is also important clinically to clarify the pathway, since a disease (familial mitochondrial cytopathy) related to human muscle CoQ_{10} deficiency has been reported [75]. Despite their importance, only a few mammalian CoQ-biosynthesis-related genes, namely *hCOQ2, hCOQ3, hCOQ4*, *hCOQ7* and *hDPS1-hDLP1*, have been experimentally analysed [29,51,78–80].

Only three genes have been experimentally characterized as CoQ biosynthetic genes in plant species. One is the *AtCOQ3* gene encoding dihydroxypolyprenylbenzoate methyltransferase [81]. The other gene is *AtSPS1* and the *AtSPS*2 genes encoding solanesyl diphosphate synthase, which produces the C_{45} isoprenoid tail and can be used for CoQ9 or plastoquinone-9 biosynthesis [38]. The third one is *AtPPT1* [12]. However, and intriguingly, no apparent *COQ7* orthologue has been found in *Arabidopsis*.

As almost all human and *Arabidopsis* orthologous genes can complement fission-yeast *coq* mutants (M. Kawamukai, unpublished work; [29]), and many human genes have been shown to be functional in *Sa. cerevisiae* [78], it is thus crucial, first, to elucidate the function of all Coq proteins in yeasts.

Bioproduction of CoQ in yeasts and other organisms

Industrial-scale bioproduction of CoQ by micro-organisms has been established by several Japanese companies. Micro-organisms that produce CoQ_{10}, including photosynthetic bacteria and yeasts, were selected and used for the fermentation production of CoQ_{10} [82,83]. This industrial-scale fermentation production of CoQ_{10} has become more widespread than chemical synthesis. Up until now, successful approaches for the commercial production of CoQ_{10} have relied predominantly on bacterial and yeast mutants selected for their high CoQ_{10} content. Recently, some developments in the fermentative production of CoQ_{10} by micro-organisms have been reported [84]. A maximum production of CoQ_{10} of 458 mg/l under industrial-plant-scale conditions has been achieved using *Agrobacterium* [83].

The application of genetic engineering to the production of CoQ was first attempted in *E. coli* as a pilot experiment [85]. Because the side chain of CoQ was essentially determined by the supplied isoprenoid, the heterologous expression in *E. coli* and *Sa. cerevisiae* of *trans*-polyprenyl diphosphate synthase genes from other organisms generated the same type of CoQ as is expressed in the donor organisms [41,72,86]. CoQ_5–CoQ_{10} have been successfully produced in *Sa. cerevisiae* and CoQ_7–CoQ_{10} in *E. coli* [72,86].

Subsequently several groups attempted CoQ_{10} production in *E. coli* using genetic-engineering techniques [87,88]. In both cases, decaprenyl diphosphate synthase from other micro-organisms was expressed. A trial to express the *ppt1* gene was reported to be effective for CoQ_{10} production in fission yeast [89], although I have failed to achieve this (M. Kawamakai, unpublished work). It was also reported that CoQ_{10} can be successfully produced in rice (*Oryza sativa*), which naturally produces CoQ_9, by genetic engineering [90]. This success may provide a way of ingesting CoQ_{10} through a daily meal.

Future perspectives

CoQ is a prenylated benzoquinone lipid that is found in membranes throughout eukaryotic cells. The reversible redox chemistry of CoQ is responsible for its function in the respiratory electron-transport chain of the mitochondrial inner membrane and as a lipophilic antioxidant. CoQ is widely used as a dietary supplement and in a variety of clinical therapies, including treatment of cardiovascular and neurodegenerative diseases.

All the genes responsible for CoQ biosynthesis were first discovered in *Sa. cerevisiae*, and, subsequently, their orthologues were identified in other eukaryotes. Despite all the efforts to elucidate the biochemical reactions involved in the synthesis of CoQ, there are still several unsolved reactions. Especially the roles of Coq4, Coq8 and Coq9 are presently unknown and need to be elucidated. Such CoQ biosynthesis studies will also assist in the production of CoQ in yeasts. In this respect, more work will be necessary to achieve efficient production in *Schizosaccharomyces*, because this fission yeast naturally produces CoQ_{10}. So far, the isolation of strains by mutagenesis and selection on inhibitors has proved to be the most successful strategy to increase yields of CoQ_{10}.

Commercially, CoQ_{10} has now become popular as a food supplement and, concomitantly the demand for a decrease in its price is increasing. Improvements in the production of CoQ_{10} in yeasts are anticipated and such efforts need to be continued.

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