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Biosynthesis of coenzyme Q in eukaryotes

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Abbreviations: CoQ, coenzyme Q; DMQ, demethoxyubiquinone; pABA, *para*-amino benzoic acid; PHB, *para*-hydroxybenzoate; SAM, *S*-adenosyl methionine.

Abstract

Coenzyme Q (CoQ) is a component of the electron transport chain that participates in aerobic cellular respiration to produce ATP. In addition, CoQ acts as an electron acceptor in several enzymatic reactions involving oxidation-reduction. Biosynthesis of CoQ has been investigated mainly in *Escherichia coli* and *Saccharomyces cerevisiae*, and the findings have been extended to various higher organisms, including plants and humans. Analyses in yeast have contributed greatly to current understanding of human diseases related to CoQ biosynthesis. To date, human genetic disorders related to mutations in eight *COQ* biosynthetic genes have been reported. In addition, the crystal structures of a number of proteins involved in CoQ synthesis have been solved, including those of IspB, UbiA, UbiD, UbiX, UbiI, Alr8543 (Coq4 homolog), Coq5, ADCK3, and COQ9. Over the last decade, knowledge of CoQ biosynthesis has accumulated and striking advances in related human genetic disorders and the crystal structure of proteins required for CoQ synthesis have been made. This review focuses on the biosynthesis of CoQ in eukaryotes, with some comparisons to the process in prokaryotes.

Introduction

Coenzyme Q (CoQ, also known as ubiquinone or 2,3-dimethoxy-5-methyl-6-polyprenyl-1, 4-benzoquinone) is a well-known component of the electron transport chain that participates in aerobic cellular respiration within the mitochondria of eukaryotes and the plasma membrane of prokaryotes. CoQ exists in both reduced and oxidized forms;[1)] conversion between these states allows it to transfer electrons to substrates and act as a cofactor of enzymatic reactions (Fig. 1). CoQ plays the role of the electron donor during disulfide bond formation in *Escherichia coli*,[2)] and its reduction is coupled to sulfide oxidation by sulfide quinone oxidoreductase in *Schizosaccharomyces pombe* and mammals.[3)] CoQ also couples with electron transfer by glycerol-3-phosphate dehydrogenase and electron-transferring flavoprotein dehydrogenase, the latter of which is involved in the beta-oxidation of fatty acids in mammals.[4)] Moreover, CoQ is required for the *de novo* synthesis of UMP as a cofactor of dihydroorotate dehydrogenase in many eukaryotes (Fig. 1).[5, 6)]

CoQ synthesis is divided into two parts: the synthesis of isoprenoid (Fig. 2) and the modification of quinone (Fig. 3). The synthesis of isoprenoids has been studied extensively; however, the mechanism by which quinone is synthesized in eukaryotes requires clarification. Living organisms possess a number of species of CoQ with differing isoprenoid side chain lengths. For example, the human and *S. pombe* CoQ contain ten isoprene units, whereas CoQ from *Arabidopsis thaliana*, *E. coli*, and *Saccharomyces cerevisiae* contains nine, eight, and six units, respectively. The length of the CoQ side chain is defined by the product generated by polyprenyl diphosphate synthases. [7, 8)]

In prokaryotes, the biosynthetic pathway that converts *para*-hydroxybenzoate (PHB) into CoQ consists of at least eight steps and the order of reactions is believed to be different in eukaryotes (Fig. 3). These steps include the condensation and transfer of the isoprenoid side chain to PHB, followed by hydroxylation, methylation, and decarboxylation. To date, this pathway has been studied most extensively in *E. coli* and

S. cerevisiae using genetic and biochemical analyses. At least 11 genes (*ubiA*, *B*, *C*, *D*, *E*, *F*, *G*, *H*, *I*, *J*, and *X*) are involved in CoQ biosynthesis in *E. coli*, and 11 genes (*YAH1*, *ARH1*, and *COQ1-COQ9*) are required for this process in *S. cerevisiae*. Involvement of *para*-amino benzoic acid (pABA) in CoQ biosynthesis is only shown in *S. cerevisiae.*

Following on from my previous reviews published in 2002[9] and 2009,[1] the current review provides an overall picture of the biosynthetic pathway of CoQ, with a special focus on recent advances in structually-based insights and the relationship between CoQ and human genetic disorders. Because bacterial biosynthesis of CoQ has been comprehensively reviewed by Aussell *et al.*,[10] this review focuses on the process in eukaryotes.

Genes responsible for CoQ biosynthesis

Genes involved in the CoQ biosynthetic pathway have been studied most extensively in two model organisms, namely, *E. coli* and *S. cerevisiae*, which are representative of prokaryotes and eukaryotes, respectively.[1, 9] *E. coli ubi* (*ubiA–J* and *ubiX*) mutants and *S. cerevisiae coq* (*coq1-coq9*) mutants, which are unable to synthesize CoQ, were used to define the biosynthetic pathway of CoQ.[11-14] Until recently, all corresponding genes for *E. coli ubi* mutants and *S. cerevisiae coq* mutants have been identified. While the functions of all *E. coli ubi* genes except *ubiB* have been completely assigned, the functions of three *S. cerevisiae COQ* genes (*COQ4*, *COQ8*, and *COQ9*) remain elusive.[1, 12] Table 1 shows a comparison of the CoQ biosynthetic genes from seven species, namely, *Homo sapiens*, *Mus musculus*, *Caenorhabditis elegans*, *A. thaliana*, *S. cerevisiae*, *S. pombe*, and *E. coli*. The order of the first three steps of the CoQ biosynthetic pathway differs in *E. coli* and *S. cerevisiae* (Fig. 3). The decarboxylation stage of the ring modification procedure precedes the hydroxylation and methylation stages in *E. coli*, but the order of these reactions is thought to be different in *S. cerevisiae.* The species-specific phenotypes of all *coq* gene knockout *S. pombe*[3, 15] and *C. elegans*[16] strains have been reported. It is generally considered that

the CoQ biosynthesis process is common in eukaryotes (Table 1); however, some differences have been reported: (i) polyprenyl diphosphate synthase forms a homodimeric or homotetrameric structure in *S. cerevisiae*,[17] but the homologous enzyme forms a heterotetramer structure composed of two subunits in *S. pombe*, mouse, and human[18, 19]; (ii) orthologs of Coq7 are missing in plant genomic databases; and (iii) Coq9 is missing in *C. elegans*.

Recent studies of human inherited diseases led to the identification of genetic disorders related to CoQ biosynthesis (Table 2). The following sections describe the genes involved in CoQ biosynthesis in *S. cerevisiae* in detail.

Coq1/IspB/PDSS1+PDSS2/polyprenyl diphosphate synthase

Polyprenyl diphosphate synthase (Coq1 in *S. cerevisiae*, IspB in *E. coli*, and a heteromer of PDSS1 and PDSS2 in *H. sapiens*), the enzyme that synthesizes the isoprenoid chain of CoQ, is classified into two types, namely, the homomer and heteromer types. *S. cerevisiae* Coq1 (hexaprenyl diphosphate synthase) catalyzes the condensation of isopentenyl diphosphate and farnesyl diphosphate or geranylgeranyl diphosphate to produce six units of prenyl diphosphate, which are used to produce CoQ6 (Fig. 3).[17] Long-chain *trans*-polyprenyl diphosphate synthases such as Coq1 have been studied in many species ranging from bacteria to humans. These enzymes possess seven conserved regions, including two DDXXD motifs that involve binding of substrates in association with Mg^{2+}.[20] Bacterial polyprenyl diphosphate synthases, *S. cerevisiae* Coq1, and *A. thaliana* solanesyl diphosphate synthase are homomeric enzymes.[21-24] Meanwhile, decaprenyl diphosphate synthases from *S. pombe* and human are heterotetramers consisting of dimer of two proteins, namely, Dps1/Dlp1 and PDSS1/PDSS2, respectively.[18, 19, 25] PDSS2/Dlp1 lacks the aspartate-rich motifs and is bound to PDSS1/Dps1 to form a heteromeric enzyme.[18, 19] Recent studies generated functional artificial heteromeric polyprenyl diphosphate synthases comprising *S. cerevisiae* Coq1 and *S. pombe* Dps1, or *E. coli* IspB and *S. pombe* Dps1, the latter of

which (Dps1) is non-functional by itself, suggesting that it may support the activities of polyprenyl diphosphate synthases by partnering with a related protein.[17, 26] The crystal structures of octaprenyl diphosphate synthase (IspB) from *Thermotoga maritime* and *E. coli* have been solved,[27, 28] and analyses of these enzymes in complex with farnesyl diphosphate and isopentenyl diphosphate clearly defined the substrate-binding sites (Fig. 4). Mice carrying mutations in the *Pdss2* gene display focal segmental glomerulopathy-like kidney disease that is rescued by CoQ10 supplementation.[29] In addition, mutations in the human *PDSS1* and *PDSS2* genes, such as those associated with Leigh disease and nephropathy, result in poor mitochondrial functionality (Table 2).[30, 31]

Coq2/UbiA/COQ2/PHB-polyprenyl diphosphate transferase

PHB-polyprenyl diphosphate transferase (Coq2 in *S. cerevisiae*, UbiA in *E. coli*, and COQ2 in *H. sapiens*) catalyzes the condensation of PHB with the isoprenoid chain,[32, 33] and genetic and biochemical analyses have revealed that this enzyme has broad substrate specificity.[34] The yeast *coq2* mutant and the *E. coli ubiA* mutant, which display a complete loss of PHB-polyprenyl diphosphate transferase activity, are complemented by the human and *S. cerevisiae COQ2* genes, respectively.[32] The analysis of a T-DNA insertion mutant revealed that the *A. thaliana PPT1* gene (a homolog of *S. cerevisiae COQ2*) is essential for embryo development.[35] Alongside hydroxy benzoic acid, the novel precursor pABA can also be used as a substrate for Coq2 in *S. cerevisiae*.[36, 37] but presumably not in humans. Analysis of the crystal structure of UbiA from *Aeropyrum pernix* (Fig. 4) revealed that it comprises nine transmembrane domains and enabled visualization of the potential entry site of the substrate (PHB).[38] Mutations in the human *COQ2* gene cause encephalomyopathy, cerebellar ataxia, neurological distress, and other disorders (Table 2).[31, 39, 40]

Coq3/UbiG/COQ3/O-methyltransferase

Dihydroxypolyprenylbenzoate methyltransferase (*O*-methyltransferase) is encoded by the *ubiG* gene in *E. coli*, and the *COQ3* gene in *S. cerevisiae* and *H. sapiens*.[41, 42] During CoQ biosynthesis, this enzyme catalyzes two *O*-methylation steps at positions 5 and 6 of the ring structure after hydroxylation by Coq6 and Coq7. The amino acid sequences of the proteins encoded by *COQ3* homologs contain four regions that are conserved in a large family of methyl transferase enzymes that utilize *S*-adenosyl methionine (SAM) as a methyl donor. *E. coli* UbiG complements the function of *S. cerevisiae* Coq1, indicating the functional similarity of these proteins.[43] *S. pombe* mutants lacking *coq3* display the common phenotypes found in other *coq* mutants.[44] Homozygous *C. elegans coq3* mutants that lack methyltransferase activity display delayed development and a sterile phenotype, and these mutants are lethal at the embryonic stage in the next generation.[45]

Coq4/COQ4

Coq4 is absolutely required for the biosynthesis of CoQ in *S. cerevisiae*, *S. pombe*, and *C. elegans*. Homologs of Coq4 are also found in cyanobacteria; however, the molecular function of the Coq4 protein has not been elucidated in any organism.[15, 46] The amino acid sequence of the Coq4 protein does not share any significant homology with protein domains or motifs with known enzymatic activity. The Coq4 protein is peripherally associated with the matrix side of the inner mitochondrial membrane and may play a structural role in the putative polypeptide CoQ biosynthetic complex.[46] Lack of *COQ4* in *S. cerevisiae* causes the instability of several Coq proteins, such as Coq7 and Coq3. Haploinsufficiency of *COQ4* leads to encephalomyopathy in humans.[47]

Coq5/UbiE/COQ5/C-methyltransferase

S. cerevisiae Coq5, *E. coli* UbiE, and *H. sapiens* COQ5 catalyze the *C*-methylation step in the CoQ biosynthetic pathway. These enzymes contain four

196 sequence motifs that are present in a large family of SAM-dependent methyltransferase
197 enzymes. Demonstration of the enzymatic activity of Coq5 methyltransferase using
198 2-methoxy-6-polypreny-1,4-benzoquinone revealed its exact role in CoQ synthesis.[48] *E.*
199 *coli* UbiE complements the function of Coq5 in *S. cerevisiae*,[48] and human COQ5
200 associates with COQ4. Analysis of the crystal structure of *S. cerevisiae* Coq5 in both the
201 apo and SAM-bound forms revealed that it forms a dimer and contains a typical SAM
202 domain (Fig. 4).[49]
203
204 *Coq6/UbiI/COQ6/monooxygenase*
205 *S. cerevisiae* Coq6, *E. coli* UbiI, and *H. sapiens* COQ6 are putative
206 flavin-dependent monooxygenases that are responsible for introducing a hydroxy group
207 to 4-hydroxy-3-polyprenyl benzoic acid. The Coq6 protein contains three conserved
208 regions: an ADP-binding motif, a FAD/NADH-binding motif, and a consensus sequence
209 that binds to the ribityl moiety of FAD.[50] A recent analysis demonstrated that Coq6 is
210 involved in C-5 oxidation during the early stage of CoQ synthesis. The involvement of
211 mitochondrial ferredoxin (YAH1) and its reductase (ARH1) in CoQ synthesis has also
212 been proposed.[51] Recently, *E. coli* UbiI was shown to be a counterpart of Coq6.[14]
213 Mutations in the human *COQ6* gene cause nephrotic syndrome (Table 2).[52]
214
215 *Coq7/UbiF/COQ7/monooxygenase*
216 *S. cerevisiae* Coq7, *E. coli* UbiF, and *H. sapiens* COQ7 proteins are putative
217 flavin-dependent enzymes that catalyze mono-oxygenation in the penultimate step of
218 CoQ biosynthesis. The Coq7 protein belongs to a family of di-iron-binding oxidases
219 that contain a conserved EXXH motif. The *clk-1/coq7* mutant of *C. elegans*, which
220 accumulates the CoQ precursor demethoxyubiquinone (DMQ), shows a prolonged
221 life-span, developmental delay, and low egg production.[53-55] DMQ is also accumulated
222 in the *S. pombe coq7* mutant; however, no apparent role of DMQ has been observed in
223 this species.[44] *E. coli* UbiF also catalyzes the same step of CoQ biosynthesis as Coq7

and *C. elegans* Clk-1. The Coq7, Clk-1, and UbiF proteins are highly conserved among different kingdoms, but analyses of DNA sequences revealed no apparent ortholog in plants. PKA-mediated phosphorylation and Ptc7-mediated dephosphorylation of Coq7 and other Coq proteins have been reported in *S. cerevisiae*.[56)]

Coq8/UbiB/ADCK3 (ADCK4)/protein kinase

S. cerevisiae Coq8 and *H. sapiens* ADCK3 (ADCK4) are protein kinases involved in CoQ synthesis. In *S. cerevisiae*, *COQ8* has now been approved as an official gene name because the previously used name (*ABC1*), which was based on a chaperone of the bc1 complex, has been questioned.[57)] The *S. pombe coq8* gene is also essential for CoQ biosynthesis.[58)] Coq8 has been classified as a putative protein kinase based on the presence of conserved kinase motifs in its primary structure. A recent study demonstrated that Coq8 is involved in the phosphorylation of Coq3, either directly or indirectly, and now it is considered to be a regulator of the Coq enzyme complex.[59)] The structure of human ADCK3 has been solved.[60)] There are five Coq8-like kinases (ADCK1–5) in humans; ADCK3 is involved in CoQ synthesis and mutations in the *ADCK4* gene are related to human CoQ10 deficiency.[61)] In fact, mutations in the *ADCK3* and *ADCK4* genes have been linked with a number of human genetic diseases (Table 2);[62)] however, is unclear whether the other ADCK proteins are also involved in CoQ biosynthesis.

Coq9/COQ9

The *COQ9* gene is absolutely required for CoQ biosynthesis in *S. cerevisiae*[63)] and *S. pombe*;[15)] however, the function of the encoded protein is still unknown. Although the Coq9 protein is conserved in eukaryotes, it has no primary sequence homology with known proteins. Furthermore, there is no apparent ortholog of *COQ9* in *E. coli*. The Coq9 protein is a component of the multi-subunit CoQ biosynthetic complex[64)] and is required for removal of the nitrogen substituent from pABA-derived Q.[65)] Analysis of

252 the crystal structure of human COQ9 (Fig. 4)[66] revealed that it contains a lipid-binding
253 site and is similar to the bacterial TetR family of transcriptional regulators. Coq9
254 associates with Coq7 in humans. Notably, the relationship between a human genetic
255 disorder and a mutation in the human *COQ9* gene suggests its involvement in the ring
256 modification of CoQ (Table 2).[67]
257
258 *Coq10/COQ10A and COQ10B/a CoQ binding proteins*
259 Although Coq10 is not involved in its biosynthesis directly, it is a unique binding
260 partner of CoQ. The Coq10 protein is localized to the mitochondria but does not belong
261 to the respiration complex in *S. cerevisiae*.[68] The existence of a CoQ-binding protein in
262 mitochondria challenges the current model that CoQ is a free lipid molecule in
263 membranes. Recently, Coq10 from *S. pombe* was also characterized as a mitochondrial
264 CoQ-binding protein that is required for proper respiration.[69] Further characterization
265 of Coq10 suggested that it is essential for proper functioning of the electron transfer
266 system, possibly by assisting in the transfer of CoQ from one site to another in the
267 mitochondrial membranes of eukaryotes.[70] A photo-affinity labeling experiment
268 revealed that a FVPFCQK sequence in Coq10 is responsible for binding to CoQ.[71]
269
270 *Coq11*
271 Very recently, Coq11 was identified as a protein associated with the Coq
272 biosynthetic complex.[72] The *COQ11* gene is not absolutely required for CoQ synthesis
273 but its deletion reduces the CoQ level in *S. cerevisiae*.
274
275 **Three-dimensional structures of proteins involved in CoQ synthesis**
276 The three-dimensional structures of nine proteins responsible for CoQ synthesis
277 (IspB, UbiA, UbiC, UbiD, UbiX, UbiI, *S. cerevisiae* Coq5, *H. sapiens* ADCK3, and *H.*
278 *sapiens* COQ9), as well as a protein that is homologous to Coq4, have been determined.
279 The structures of the eukaryotic proteins or their homologs found in other organisms are

shown in Figure 4. *E. coli* IspB, which is a homolog of Coq1, consists of 14 α-helices.[28] The recent co-crystallization of IspB with its substrates (farnesyl diphosphate and isopentenyl diphosphate) revealed that the substrate-binding regions are located around aspartate-rich motifs and identified a pocket that determines the acceptable chain length. Analysis of the crystal structure of *A. pernix* UbiA, a homolog of Coq2, showed that it comprises nine transmembrane structures and identified the proposed substrate (PHB) binding and entry sites.[38] Investigation of a truncated form of *E. coli* UbiI, which is a homolog of Coq6, revealed that it forms a tetramer that resembles the structure of typical flavin-dependent monooxygenases.[14] The crystal structure of Coq5 from *S. cerevisiae* resembles that of a typical class I SAM-dependent methyltransferase,[49] and the residues involved in the interaction of Coq5 with SAM are located on four loops. Examination of the crystal structure of a truncated form of ADCK3, a member of the UbiB family of protein kinases, revealed an atypical protein kinase with multiple UbiB-specific features positioned to inhibit protein kinase activity.[60] These inhibitory regions include an N-terminal domain that occupies the typical substrate-binding pocket, and a unique A-rich loop that limits ATP binding.[60] This structure would explain why *in vitro* kinase activity was not detected for UbiB. *H. sapiens* COQ9 displays a striking structural homology to members of the TetR family of regulators and contains a lipid-binding pocket. The protein consists of nine α-helices and forms a dimer, and the deduced crystal structure included a phospholipid in its hydrophobic interface.[66] In addition to the structures of five proteins that are apparently involved in CoQ synthesis, the crystal structure of the cyanobacteria Alr8543 protein (*Nostoc sp.* PCC7120), which is homologous to Coq4, has also been solved.[73] The direct involvement of the Alr8543 protein in CoQ synthesis has not been proven, but its co-crystallization with geranylgeranyl monophosphate supports a role as a substrate holder during CoQ synthesis.[73]

In addition to the three-dimensional structures of Coq proteins and their homologs, the crystal structures of the bacterial decarboxylases UbiD (PDB ID: 4IP2)[74] and UbiX

(PDB ID: 1SBZ)[75] have also been solved. A UbiX homolog named Pad1 exists in yeast, but it is not thought to be involved in CoQ synthesis.[76] The crystal structure of the bacterial chorismate lyase UbiC (PDB ID: 1TT8) has also been solved, but its counterpart is not found in eukaryotes.[77]

Complex formation

In *S. cerevisiae*, the enzymes involved in CoQ synthesis are thought to form a large complex termed the CoQ synthome (Fig. 5). In this complex, Coq2 spans the inner membrane and the other enzymes are peripherally associated with this membrane on the matrix side. Native polyacrylamide gel electrophoresis analyses identified a 700 kDa band containing Coq3, Coq5, Coq6, Coq9, and Coq4, as well as a larger 1,300 kDa band containing Coq7 also. Coq4 plays a central role in the function of the CoQ synthome and associates with Coq3, Coq5, Coq6, and Coq7. Coq9 associates weakly with the complex, and Coq2 and Coq8 function separately.[78] Coq8 is a kinase that is involved in complex formation through the phosphorylation of Coq3, Coq5, and Coq7; however, it is unclear whether Coq8 phosphorylates these proteins directly or indirectly. Several lines of evidence indicate that the human CoQ biosynthetic enzymes also form a complex;[66, 79] this topic has been comprehensively reviewed by Gonzalez-Mariscal *et al*.[80]

Diseases caused by CoQ deficiency

The diseases caused by a deficiency in CoQ have been reviewed previously.[81, 82] The first mutations related to human CoQ deficiency were identified in *COQ2*; since then, mutations in seven other CoQ biosynthetic genes (*PDSS1*, *PDSS2*, *COQ4*, *COQ6*, *ADCK3*, *ADCK4*, and *COQ9*) have been shown to cause various diseases (summarized in Table 2). Encephalomyopathy, nephropathy, cerebellar ataxia, and seizures are common features of CoQ deficiency; these symptoms are associated with mitochondrial disorders. The level of CoQ10 differs between patients and depends on the specific

mutations, and some disease symptoms are eased by supplementation with CoQ10. There are also reports that secondary CoQ deficiency can be caused by mutations in genes that are not directly involved in CoQ synthesis, such as an oncogene *B-RAF* and aprataxin (*APTX*). [81, 82)]

Sites of CoQ synthesis

In eukaryotes, CoQ biosynthesis occurs mainly in the mitochondria; indeed, all *S. cerevisiae* and *S. pombe* proteins involved in the process localize to this cellular compartment.[15)] Human COQ proteins also localize to the mitochondria, but the Golgi-localized enzyme UBIAD1 (prenyl transferase), which is homologous to *E. coli* UbiA, can also participate in CoQ.[83)] Loss of UBIAD1 apparently reduces the cytosolic pool of CoQ10. As most of other enzymes involves CoQ localize to mitochondria, the mechanism by which UBIAD1 assists with CoQ production in the Golgi is still unclear.

Regulation of the expression of *COQ* genes and genes regulated by CoQ

Little is currently known about the regulation of *COQ* gene expression. The expression of *S. cerevisiae COQ5* is reportedly regulated by Mig1, Rtg3, and Hap2.[84)] A microarray analysis revealed that a deficiency of endogenous CoQ in *C. elegans clk1* mutants down-regulates a cluster of genes that are important for growth and up-regulates oxidation reactions and protein interactions.[85)] In addition, a microarray analysis demonstrated the induction of specific genes related to cell survival in CoQ-deficient human cells.[86)]

Bioproduction of CoQ10 in microorganisms and plants

Because CoQ10 is a commercially sold food supplement, its efficient production by microorganisms has been explored.[87)] *E. coli*, *Agrobacterium*, and photosynthetic bacteria such as *Rhodobacter spheroides* and *Rhodobacter capsulatus* have been used to produce CoQ10. *E. coli* produces CoQ8 naturally, but expressing decaprenyl

diphosphate synthase in cells that lack the endogenous *ispB* gene enables them to produce CoQ10.[21] A previous study generated an engineered *E. coli* strain that expressed the *Agrobacterium tumefaciens* decaprenyl diphosphate synthase gene (*ddsA*) and had a strengthened mevalonate pathway; this strain was capable of producing substantial amounts of CoQ10.[88] By optimizing the growth medium and conditions, photosynthetic *Rhodobacter* capable of producing CoQ10 at concentrations up to 8.70 mg/mg dry cell weight was generated.[89] In addition, *Agrobacterium* can produce CoQ10 at concentrations up to 11.84 mg/mg dry cell weight.[90] The fission yeast *S. pombe* is a good candidate for CoQ10 production, and the yield can be doubled by genetic engineering, although it is not as high as that obtained from other microorganisms.[91] In addition to microbial production, researchers have attempted to produce CoQ10 using other systems; the attempts to produce CoQ10 in rice are worth noting.[92, 93]

Concluding remarks

Recent studies of the mechanisms of CoQ synthesis have been very fruitful. In particular, current understanding of the correlation between defective genes involved in CoQ synthesis and human diseases is surprisingly advanced. The three-dimensional structures of proteins involved in CoQ synthesis have also been determined recently. Despite this accumulating knowledge of CoQ biosynthesis, we still do not have a clear picture of the whole biosynthetic pathway in eukaryotes. For example, Coq4 and Coq9 are absolutely required for CoQ biosynthesis in eukaryotes, but their specific functions are unclear. Furthermore, additional factors, such as Coq10 and Coq11, also appear to be involved in CoQ biosynthesis. Besides the electron transport system, reactions involving sulfide quinone oxidoreductase, glycerol-3-phosphate dehydrogenase, dihydroorotate dehydrogenase, and electron-transferring flavoprotein dehydrogenase require CoQ as a cofactor. Overall, additional work is required to understand the complete pathway of CoQ biosynthesis.

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772 **Figure Legends**

773 **Figure 1. The electron transfer system and enzymes involved in CoQ biosynthesis.**
774 The position of CoQ in the electron transfer system is shown. Complexes I and II
775 transfer electrons to CoQ from NADH and $FADH_2$, respectively. In yeast, NADH
776 dehydrogenase replaces Complex I in the first reaction. Electrons are transferred to
777 Complex III from $CoQH_2$, a reduced form of CoQ, and then further transferred to
778 Complex IV through cytochrome *c* (Cyt*c*). Protons are transferred to the intermembrane
779 space and this proton gradient drives ATP production through Complex V. A number of
780 different enzymes are coupled with CoQ in oxidation-reduction reactions: DHODH,
781 dihydroorotate dehydrogenase; SQR, sulfide quinone reductase; ETFDH, electron
782 transfer flavoprotein dehydrogenase; and GPDH, glycerol-3-phosphate dehydrogenase.
783
784 **Figure 2. Biosynthetic pathway of the isoprenoid tail of CoQ.**
785 Isopentenyl diphosphate (IPP) is synthesized via the mevalonate (MVA) pathway in
786 eukaryotes and the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway in prokaryotes
787 and plants. In each organism, *trans*-polyprenyl diphosphate of a certain length is
788 synthesized by polyprenyl diphosphate synthase. *S. cerevisiae* Coq1 synthesizes six
789 isoprene units, *E. coli* IspB synthesizes eight isoprene units, and *H. sapiens* or *S. pombe*
790 decaprenyl diphosphate (DPP; a heteromer of PDSS1 and PDSS2 or Dps1 and Dlp1,
791 respectively) synthesize ten isoprene units. *S. cerevisiae* Coq2, *E. coli* UbiA, and *H.*
792 *sapiens* COQ2 or *S. pombe* Ppt1(Coq2) condense PHB with *trans*-polyprenyl
793 diphosphate to form CoQ6, CoQ8, and CoQ10, respectively. DMAPP, dimethylallyl
794 diphosphate; GPP, geranly diphosphate; FPP, farnesyl diphosphate; IPP, isopentenyl
795 diphosphate; HexPP, Hexaprenyl diphosphate; OPP, Octaprenyl diphosphate.
796
797 **Figure 3. Overview of the proposed CoQ biosynthetic pathway.**
798 The CoQ biosynthetic pathways of prokaryotes (represented by *E. coli*) and eukaryotes
799 (represented by *S. cerevisiae*). In general, the gene names *ubi** and *COQ*(*coq*)* are used

800 in prokaryotes and eukaryotes, respectively; however, the nomenclature of genes can
801 differ among species. After condensation of PHB with *trans*-polyprenyl diphosphate,
802 the ring structure is modifed. In *E. coli*, decarboxylation by UbiD or UbiX is followed
803 by hydroxyation by UbiI, *O*-methylation by UbiG, hydroxyation by UbiH,
804 *C*-methylation by UbiE, a final hydroxylation by UbiF, and then *O*-methylation by
805 UbiG. At least eight genes are responsible for CoQ biosynthesis in *E. coli*. In *S.*
806 *cerevisiae*, pABA is also used as a substrate in addition to PHB. In this species, the first
807 ring is modifed by hydroxyation by Coq6, followed by *O*-methylation by Coq3;
808 however, the subsequent decarboxylation and hydroxylation steps are unclear. The ring
809 is then modified further by *C*-methylation by Coq5, a final hydroxylation by Coq7, and
810 *O*-methylation by Coq3.
811
812 **Figure 4. Crystal structures of the proteins involved in CoQ synthesis.**
813 The structures of seven enzymes involved in CoQ synthesis. UbiI forms a tetramer;
814 IspB, Alr8543, Coq5, and COQ9 form dimers; and UbiA and ADCK3 form monomers.
815 The PHB substrate is included in the UbiA structure and lipid is included in the Coq9
816 structure. (A) IspB (Coq1 homolog) from *E. coli* (PDB ID:3WJO). (B) UbiA (Coq2
817 homolog) from *A. pernix* (PDB ID: 4OD5). (C) Alr8543 (Coq4 homolog) from *Nostoc*
818 sp. PCC7120 (PDB ID: 3KB4). (D) Coq5 from *S. cerevisiae* (PDB ID: 4OBW). (E)
819 UbiI (Coq6 homolog) from *E. coli* (PDB ID: 4K22). (F) ADCK3 (Coq8 homolog) from
820 *H. sapiens* (PDBID:4PED). (G) COQ9 from *H. sapiens* (PDB ID: 4RHP).
821
822 **Figure 5. Structure of the CoQ biosynthetic enzyme complex.**
823 The enzymes involved in CoQ synthesis form a complex in *S. cerevisiae*.[78] There is
824 also some evidence that this complex exists in humans. This figure is modified from the
825 figure reported by Allan et al.[72] Proteins in the figure are not proportional to the actual
826 molecular sizes. The structure of *S. cerevisiae* Coq5 has been solved; for the other
827 enzymes, the structures of homologs from other species are indicated. *E. coli* IspB is

828 shown as a Coq1 homolog, *A. pernix* UbiA is shown as a Coq2 homolog, Alr8543 from
829 *Nostoc* sp. PCC7120 is shown as a Coq4 homolog, *E. coli* UbiI is shown as a Coq6
830 homolog, human ADCK3 is shown as a Coq8 homolog, and human COQ9 is shown as
831 a Coq9 homolog. Coq1 is separated from the complex. Coq2 spans the inner membrane.
832 The positions of the other proteins have not been defined experimentally, although Coq4
833 seems to be in the center, and Coq7 and Coq8 seem to be located at the edge of the
834 complex.

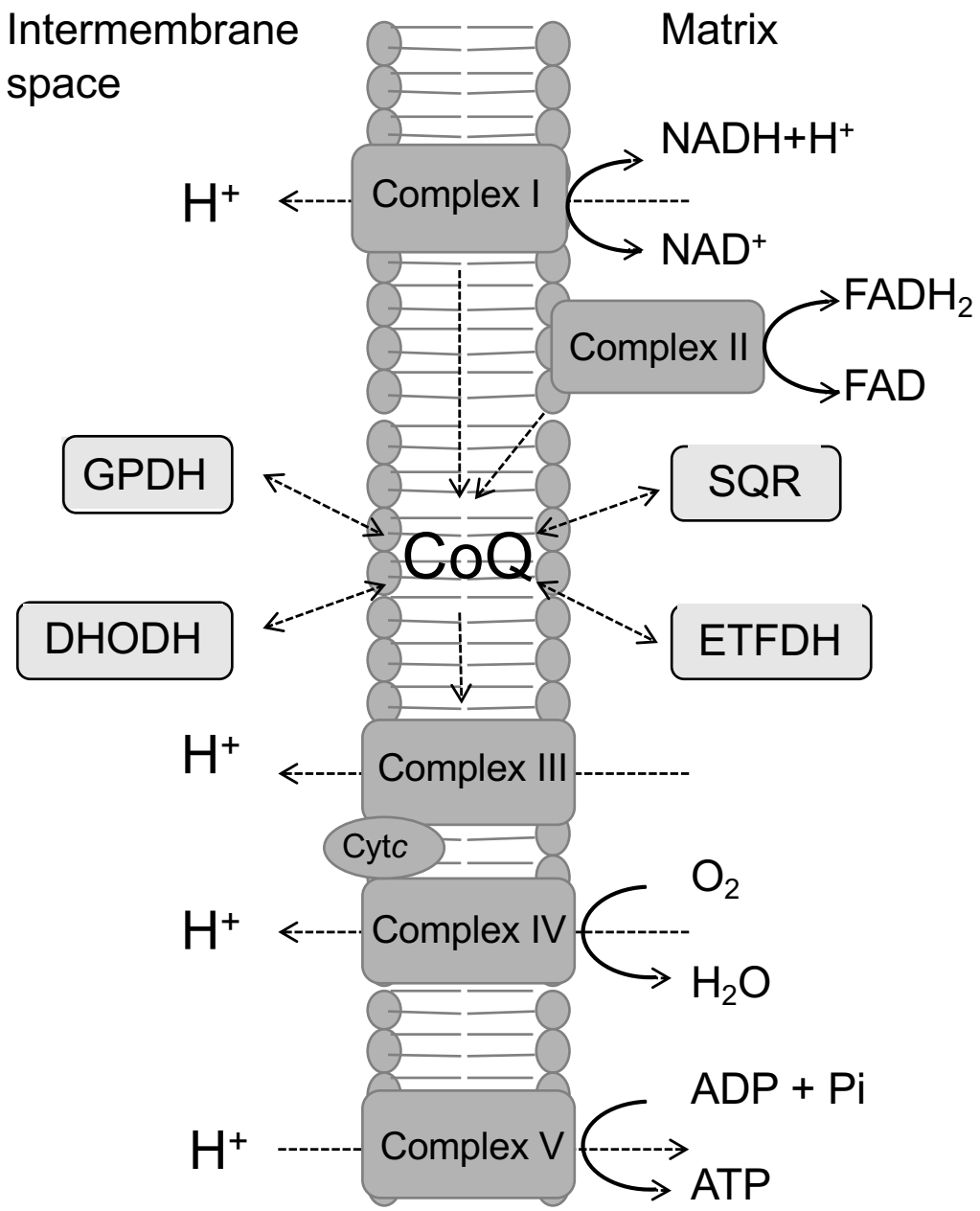

Intermembrane space
Matrix
NADH+H+
H+
Complex I
NAD+
FADH2
Complex II
FAD
GPDH
SQR
CoQ
DHODH
ETFDH
H+
Complex III
Cytc
O2
H+
Complex IV
H2O
ADP + Pi
H+
Complex V
ATP

Fig. 1

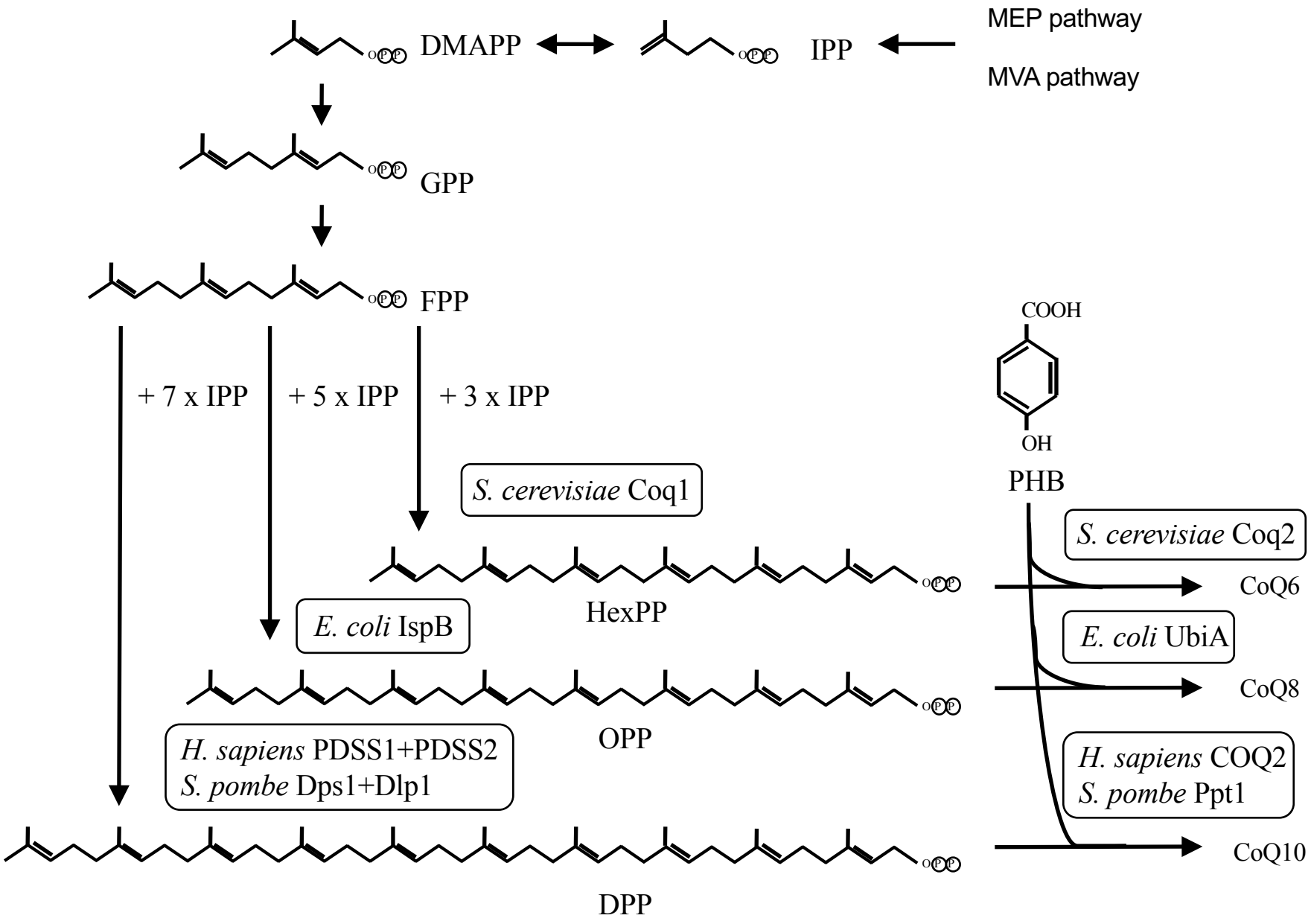

DMAPP
IPP
MEP pathway
MVA pathway
GPP
FPP
+ 7 x IPP
+ 5 x IPP
+ 3 x IPP
S. cerevisiae Coq1
HexPP
E. coli IspB
OPP
H. sapiens PDSS1+PDSS2
S. pombe Dps1+Dlp1
DPP
COOH
OH
PHB
S. cerevisiae Coq2
CoQ6
E. coli UbiA
CoQ8
H. sapiens COQ2
S. pombe Ppt1
CoQ10

Fig. 2

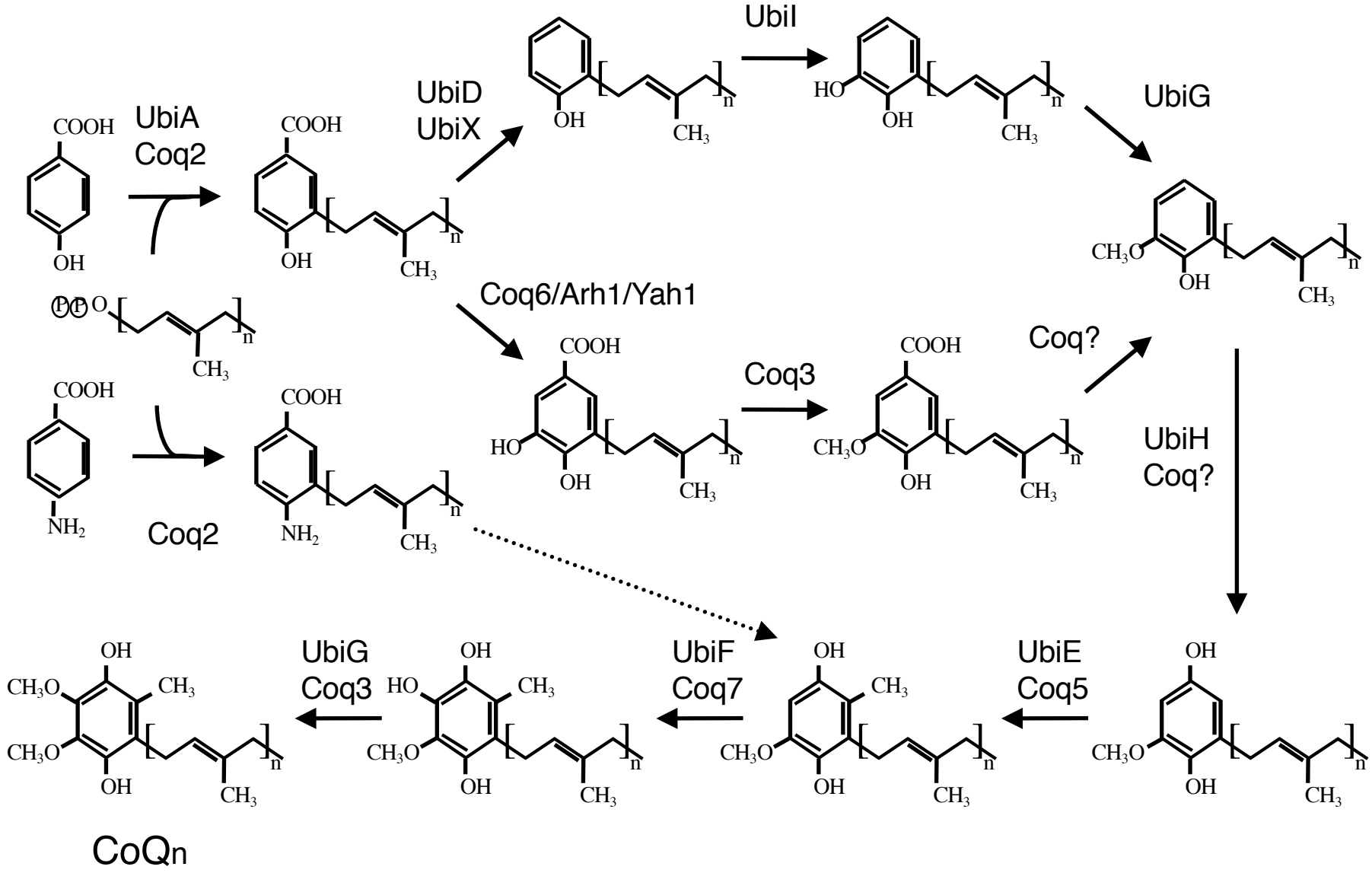
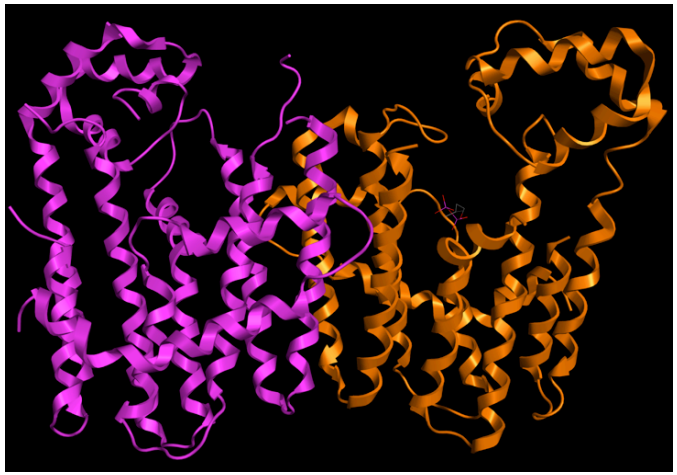
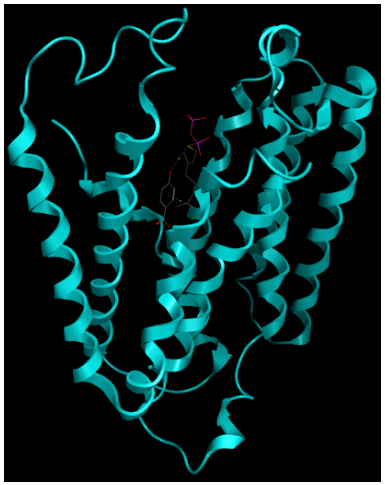

UbiA
Coq2
UbiD
UbiX
UbiI
UbiG
Coq6/Arh1/Yah1
Coq3
Coq?
UbiH
Coq?
Coq2
UbiE
Coq5
UbiF
Coq7
UbiG
Coq3
CoQn

Fig. 3

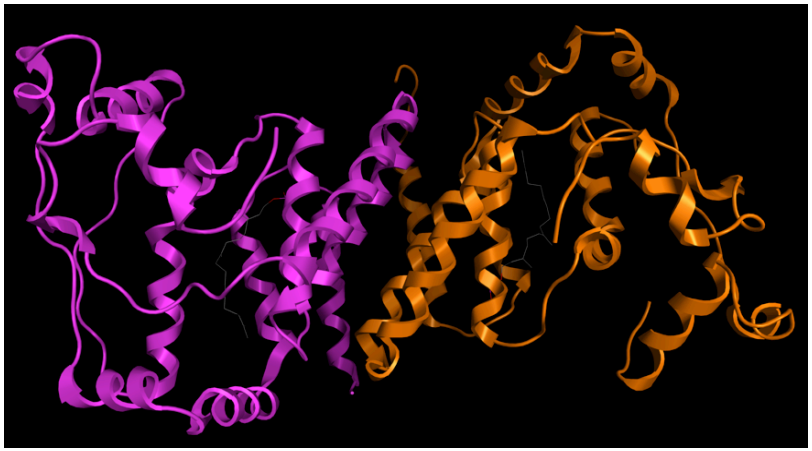
A) IspB (Coq1)



B) UbiA (Coq2)

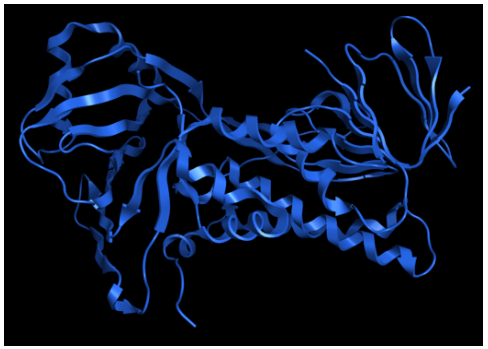


C) Alr8543 (Coq4)



D) Coq5

E) UbiI (Coq6)



F) ADCK3 (Coq8)

G) hCOQ9

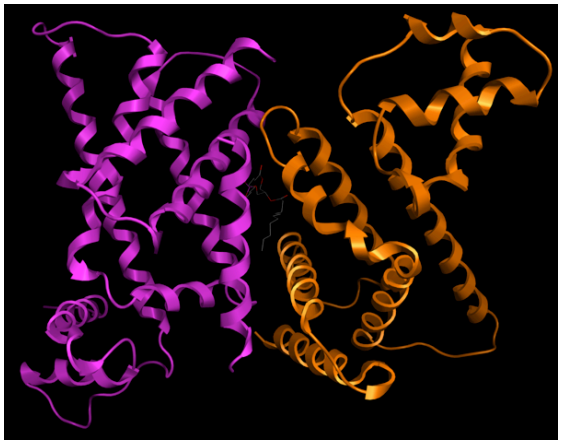


Fig. 4

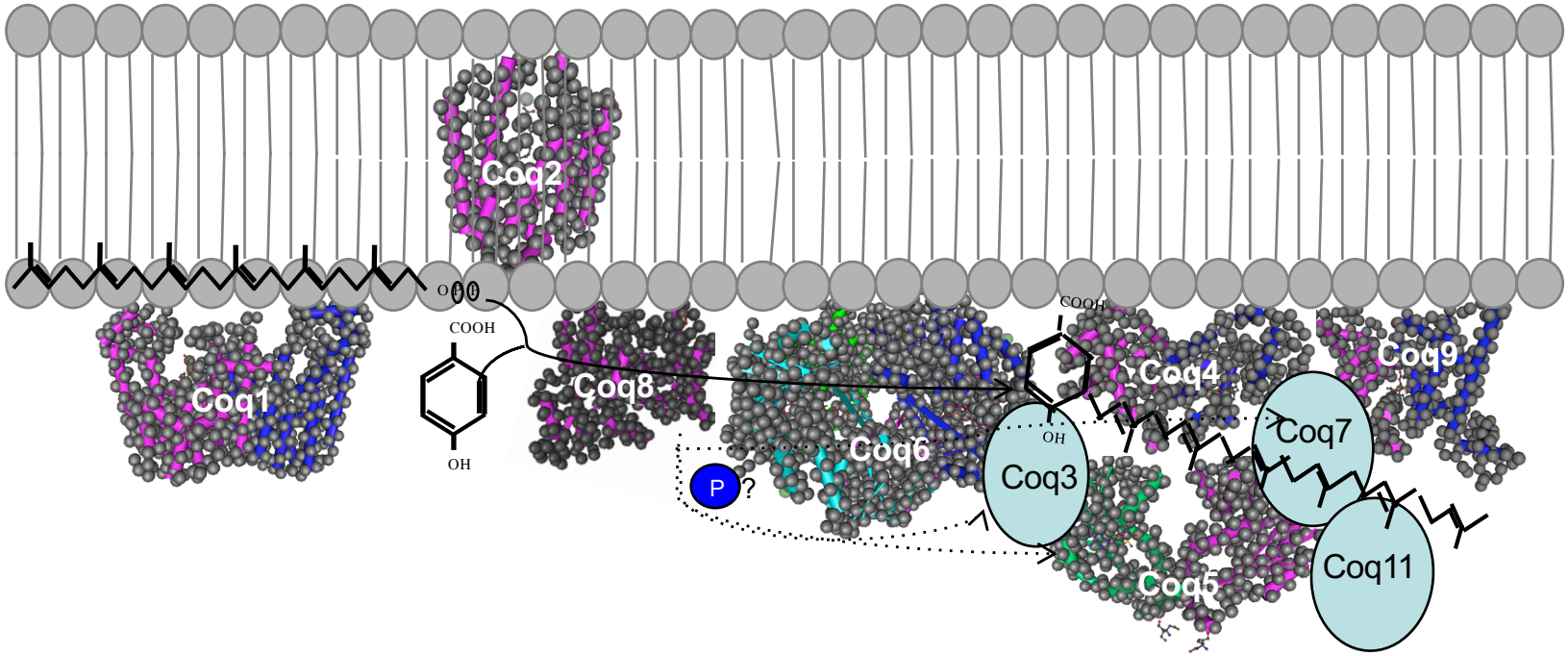


Fig. 5

Table 1. CoQ biosynthetic genes from various species.

Function	*H. sapiens*	*M. musculus*	*C. elegans*	*A. thaliana*	*S. cerevisiae*	*S. pombe*	*E. coli*
Polyprenyl diphosphate synthase	*PDSS1+PDSS2*	*Pdss1+Pdss2*	*COQ1*	*SPS3*	*COQ1*	*dps1+dlp1*	*ispB*
PHB-polyprenyl diphosphate transferase	*COQ2*	*Coq2*	*COQ2*	*PPT1*	*COQ2*	*coq2* (*ppt1*)	*ubiA*
O-methyl transferase	*COQ3*	*Coq3*	*COQ3*	*COQ3*	*COQ3*	*coq3*	*ubiG*
Unknown	*COQ4*	*Coq4*	*COQ4*	*COQ4*	*COQ4*	*coq4*	-
C-methyl transferase	*COQ5*	*Coq5*	*COQ5*	*COQ5*	*COQ5*	*coq5*	*ubiE*
Monooxygenase	*COQ6*	*Coq6*	*COQ6*	*COQ6*	*COQ6*	*coq6*	*ubiI*
Monooxygenase	*COQ7*	*Coq7* (*Clk1*)	*COQ7*	-	*COQ7*	*coq7*	*ubiF*
Protein kinase	*ADCK3, ADCK4*	*Adck3, Adck4*	*COQ8*	*COQ8* (*ABC1*)	*COQ8* (*ABC1*)	*coq8*	*(ubiB)*
Unknown	*COQ9*	*Coq9*	-	*COQ9*	*COQ9*	*coq9*	-

Table 2. Genotype-phenotype correlations in inherited deficiencies of CoQ10 biosynthesis in humans.

Gene	Amino acid change caused by mutation	CoQ10 level	Clinical features	Reference
PDSS1	D308E	3%**	Deafness, Bulimia, Obesity, Optic atrophy, Valvulopathy, Macrocephathy, Peripheral neuropathy	[31]
PDSS2	Q322X & S382L	2%*, 13%**	Steroid-resistant nephrotic syndrome, Leigh syndrome	[30]
COQ2	Y297C	37%*, 18%**	Encephalomyopathy, Nephropathy	[39]
COQ2	R197H & N228S S146N	36%* 2%*	Steroid-resistant nephrotic syndrome Acute renal failure, Epileptic encephalopathy	[40]
COQ2	N401fsX415	24%**	Neurological distress, Liver failure, Nephrotic syndrome, Anemia, Pancytopenia, Diabetes, Cytolysis, Seizures	[31]
COQ2	R387Q & V343A	63%***	Multiple-system atrophy	[94]
COQ4	Del &WT	44%**	Mental retardation, Encephalomyopathy, Dysmorphic features	[47]
COQ6	G255R A353D	- -	Nephrotic syndrome	[52]
CABC1/ADCK3	E551K R213W & G272V G272D & fs (1812insG)	8%* 29%* <5%*	Cerebellar ataxia, Strabismus, Seizures Seizures, Cerebellar ataxia, Ptosis, Cerebellar atrophy	[95]
CABC1/ADCK3	Q167LfsX36 Y514C & T584X	63%** 51%**	Cerebellar ataxia, Cerebellar atrophy Cerebellar ataxia, Cerebellar atrophy	[62]

COQ8*/*CABC1*/*ADCK3	R348X, L379X	-	Cerebellar ataxia, Atrophy	[96]
ADCK4	R178W	9.7%***	Steroid-resistant nephrotic syndrome	[61]
COQ9	R244X	15%*	Neonatal lactic acidosis, Renal tubular dysfunction, Seizures, Hypertrophic cardiomyopathy, Global development delay	[67]

*muscle; ** fibroblast; ***lymphoblast; fs, frame shift; del, deletion.