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# The complexity of making ubiquinone

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## ABSTRACT

Ubiquinone (coenzyme Q) is an essential electron transfer lipid in the mitochondrial respiratory chain. It is a main source of mitochondrial ROS but also has antioxidant properties. This mix of characteristics is why ubiquinone supplementation is considered a potential therapy for many diseases involving mitochondrial dysfunction. Mutations in the ubiquinone biosynthetic pathway are increasingly being identified in patients. Furthermore, secondary ubiquinone deficiency is a common finding associated with mitochondrial disorders and might exacerbate these conditions. Recent developments have suggested that ubiquinone biosynthesis occurs in discrete domains of the mitochondrial inner membrane close to ER-mitochondria contact sites. This spatial requirement for ubiquinone biosynthesis could be the link between secondary ubiquinone deficiency and mitochondrial dysfunction, which commonly results in loss of mitochondrial structural integrity.

## At the center of mitochondrial energy metabolism

Ubiquinone (**UQ, Coenzyme Q or CoQ**) (See Glossary) is composed of a benzoquinone ring connected to a polyisoprenoid side-chain. The ring structure of UQ is its functional moiety and exists in three different redox states: oxidized (ubiquinone), reduced (ubiquinol) and partially reduced (ubisemiquinone) (**Figure 1**). The side-chain is extremely lipophilic and UQ is believed to localize mostly to the hydrophobic core of lipid bilayers. Only the length of the side-chain varies between species: 10 subunits in human ($UQ_{10}$), a mix of $UQ_9$ and $UQ_{10}$ in rodents, $UQ_6$ in *Saccharomyces cerevisiae* (*S. cerevisiae*), and $UQ_8$ in *Escherichia coli* (*E. coli*). The functional meaning of the length variations in the side-chain is not understood. UQ is made in all or most cells and found in all or most cellular membranes, but it is particularly abundant in mitochondria, where it functions as a mobile electron carrier in the electron transport chain (ETC) [1-5]. It mediates the electron transfer from complex I (CI) and II (CII), and several other enzymes, to complex III (CIII) where reduced UQ is re-oxidized and electrons are passed further down the chain (**Figure 2, Key Figure**). UQ is potentially a strong endogenous membrane antioxidant and it is known to protect from lipid oxidation in circulating lipoproteins [2, 6, 7]. As its two primary functions are in mitochondrial electron transport and redox control, it is extensively studied as a potential therapeutic agent for many diseases with a component of mitochondrial dysfunction [8].

Most UQ is free in the membrane bilayer. In the inner membrane of mitochondria (IMM) it comes in contact with respiratory chain complexes by random collision [3]. Some UQ might be associated with respiratory supercomplexes formed by the dynamic association of ETC complexes [9]. The primary pathophysiology of low UQ levels is the loss of adequate **mitochondrial bioenergetics**. As such, the clinical features of UQ deficiency are very similar to those of other mitochondrial diseases that impair ETC function [10]. In mammalian mitochondria, 85%–100% of CI is found in supercomplexes, while CII mostly exists in free-floating form [9]. Yet, CI- and CII-dependent respiration are affected to a similar degree by UQ deficiency [11-13]. This is consistent with a model in which all functions of UQ in the ETC are carried out by a shared, freely exchanging, UQ pool in the IMM [14]. The organization of the ETC into supercomplexes is believed to help the adaptation to metabolic changes. For example, it has been reported that CI is degraded in cells with CIII or CIV defects or by the activation of CII [15-17]. UQ is essential to this responsive ETC configuration: accumulation of reduced UQ triggers reverse electron transport which oxidizes

critical CI proteins, inducing their degradation [18]. Release of CIII from supercomplexes as a result of CI degradation increases the availability of CIII for $FADH_2$-derived electrons and is therefore believed to be involved in the accommodation of the substrate switch from glucose to fatty acids [16, 18].

The function of UQ in the ETC is also crucial for other pathways, including sulfide ($H_2S$) oxidation, pyrimidine synthesis, fatty acid β-oxidation and branched-chain amino acid oxidation [19, 20]. Complete reduction of UQ requires two electrons and two protons and occurs in two one-step transfers of one electron each. This is important because it is in the ubisemiquinone state that electrons can leak from UQ to dioxygen and generate superoxide [21]. On the order hand, in its reduced form UQ has antioxidant properties [6]. However, in our view, it remains an open question as to whether oxidative stress contributes to the pathogenesis of UQ deficiency. Although increased oxidative stress was reported in cells with modest loss of UQ and in some mouse models of defective UQ biosynthesis, severe UQ deficiency (>85%) was not found to be associate with elevated mitochondrial ROS production [11, 13, 22, 23]. Furthermore, in contrast to other mitochondrial disorders, which can induce permanent oxidative damage, the effects of severe UQ deficiency were reversible after successful partial reestablishment of UQ levels, as we observed in a *Coq7* knockout mouse model of UQ deficiency [11, 24].

## Normal and defective ubiquinone biosynthesis

The fundamentals of our current understanding of UQ biosynthesis come from studies of yeast and bacterial mutants [25, 26]. Here, we focus mostly on crucial new findings in eukaryotes (but see **Box 1** for important new insights from *E. coli*). Mutations in human UQ biosynthetic genes leads to **primary UQ deficiency** (PUD), and pathophysiological perturbations of UQ synthesis leads to **secondary UQ deficiency** (SUD). PUD is characterized by a severe and heterogeneous clinical spectrum and often presents as a multi-systems disorder [10, 19, 27-29]. SUD is particularly associated with mitochondrial disorders [30, 31]. However, conditions that unexpectedly lead to SUD are increasingly described. For example, mitochondrial UQ was reported to be lower in insulin-resistant cell models and it was also found to be selectively decreased in the adipose and muscle tissues of high-fat, high-sugar diet fed mice [32]. Unfortunately, UQ has very poor bioavailability, which hinders efficient replacement therapy for PUD and SUD. A better

understanding of all aspects of UQ biology is needed to identify ways to prevent the loss of UQ in SUD, provide it efficiently, or boost residual UQ synthesis in PUD.

The conserved biochemical pathway of UQ synthesis starts with the synthesis of the polyisoprenoid tail and its attachment to the aromatic ring precursor of UQ. The ring structure is then modified in successive steps to yield UQ (**Figure 2**). In eukaryotes, the isoprene carbon units for making the UQ side-chain are derived from the mevalonate pathway. 4-hydroxybenzoic acid (4-HB), derived from tyrosine, is the most common precursor for the benzoquinone ring [33]. Recent studies have started to reveal steps necessary for the synthesis of 4-HB from tyrosine which include the activities of the transaminases Aro8p and Aro9p and the aldehyde dehydrogenase Hfd1 [34, 35] (See more details in **Box 2**). The budding yeast *S. cerevisiae* can also use *para*-aminobenzoic acid (pABA), a well-known precursor of folate [36, 37]. However, human and *E. coli* cells do not utilize pABA for UQ synthesis [38]. Interestingly, resveratrol and coumarate can be used as head group precursor of UQ across species [38]. On a related note, studies have demonstrated that cells can also use unnatural precursors of UQ biosynthesis, namely 2,4-dihydroxybenzoate, 3,4-dihydroxybenzoate and vanillic acid [11, 39-42]. When provided, they enter the UQ biosynthetic pathway and compete with the natural precursor 4-HB and natural intermediates for the pathway enzymes [40, 41].

Both the tail and ring precursors are made in the cytosol but all the next steps, starting with the attachment of the tail to the ring precursor, occur in association with the matrix side of the IMM [25]. To date, very little is known about the mechanism of import of the precursors into mitochondria. In yeast, at least **14** gene products, Coq1-Coq9, Coq11, Arh1, Yah1, Oct1 and Puf3 participate in UQ biosynthesis (**Figure 2**)[25, 39, 43-47]. Among these, mammalian homologues of *COQ1* to *COQ9* have been identified [10, 48-50]. In the next section, we describe those ***COQ* genes** that are essential for UQ biosynthesis, focusing on the more recent developments and on those whose functions are not completely elucidated. Consult **Box 2** for other relevant players

## *COQ* genes that are required for UQ biosynthesis

### *Enzymatic pathway components*

In yeast, Coq1 is the polyprenyl diphosphate synthase that makes the isoprenoid tail of UQ, while in mice and humans it is a heteromeric complex of PDSS1 and PDSS2 [10, 25]. Coq2/COQ2 catalyzes the attachment of the isoprenoid side-chain to the aromatic ring precursors. Subsequent ring modification steps include two hydroxylations at positions 5 and 6 of the ring structure that are catalyzed by Coq6/COQ6 and Coq7/COQ7 respectively, each followed by an O-methylation step catalyzed by Coq3/COQ3 [10, 25, 44, 51]. Coq5/COQ5 catalyzes the only C-methylation step in the UQ biosynthetic pathway [49, 52] (**Figure2**).

***Activities that are necessary but do not act enzymatically on UQ intermediates***

Besides the COQ enzymes that catalyze the chemical reactions of the biosynthetic pathway, other proteins are also essential for efficient UQ production. In eukaryotes, they include COQ4, COQ8, COQ9 and COQ11. Deletion of any one of the *COQ1-COQ9* genes in yeast leads to total loss of $UQ_6$ production [43, 45, 53, 54]. The currently well-accepted model of UQ biosynthesis is that UQ is produced in the IMM by a large complex formed by a cohort of *COQ* gene products [55-57]. The Coq protein complex has been termed the **CoQ synthome** or **Complex Q** [45, 58]. The complex is necessary for the stability and function of its individual constituents [39, 59]. *coq3-coq9* single null mutants all have decreased steady-state levels of several of the other Coq polypeptides and can only produce the earliest $UQ_6$ biosynthetic intermediates, 3-hexaprenyl-4-hydroxybenzoic acid (HHB) or 3-hexaprenyl-4-aminobenzoic acid (HAB) (**Figure 2)**, consistent with destabilization of the synthome [36, 37, 39, 59]. Those components that are not enzymes are likely needed to form and stabilize the synthome, and to help handling the very hydrophobic intermediates.

Yeast **Coq4**, whose only identifiable feature is a putative zinc binding domain [53], was shown to physically interact with Coq3, Coq5, Coq6, Coq7 and Coq9 [60, 61]. Interestingly, *coq4* null mutants overexpressing *COQ8,* as well as a *coq4* point mutant, were shown to lack UQ despite high levels of other Coq proteins [39, 53]. Thus, Coq4 appears to play an essential structural role in the correct assembly of the CoQ synthome.

*E. coli* UbiB, yeast **Coq8**, and its mammalian homologues COQ8A/ADCK3 and COQ8B /ADCK4 are members of an atypical kinase family that lacks canonical protein kinase activity but instead displays an ATPase activity that is strongly stimulated by binding to phenolic compounds that

mimic UQ pathway intermediates or when binding to cardiolipin-containing liposomes, as shown for human COQ8A and yeast Coq8 [48, 62-64]. The phospholipid cardiolipin is an essential constituent of the IMM, where it is intimately involved in numerous mitochondrial functions. Thus, COQ8 might act as a chaperone that facilitates extraction of the lipophilic UQ intermediates out of the mitochondrial membrane and into the aqueous matrix environment, where they can be modified by other COQ enzymes [64]. Intriguingly, in mice, the expressions of the *Coq8A* and *Coq8B* genes respond in opposite ways to OXPHOS dysfunction induced by mtDNA defects, suggesting very different regulatory roles in UQ biosynthesis [65].

Yeast **Coq9** is also an integral member of the CoQ synthome and is required for its assembly [61]. In addition, Coq9 is specifically required for the hydroxylation steps catalyzed by Coq6 and Coq7 [39]. Furthermore, loss of Coq9 was shown to impair the deamination step catalyzed by Coq6 when pABA is used as the UQ ring precursor[39, 66, 67]. In all species, dysfunction of COQ9 leads to a dramatic reduction in COQ7 level and in the accumulation of demethoxyubiquinone (DMQ), the substrate of COQ7, but some COQ7 activity remains (some UQ is still made) [13, 39, 68-71]. The crystal structure of the human COQ9 was solved at 2.4-Å resolution, revealing that COQ9 shares structural homology to members of the ancient TetR family of transcriptional regulators (TFRs) with an amphipathic C-terminal α-helix [72]. The TetR fold in COQ9 enables binding of aromatic isoprene lipids (UQ and UQ intermediates), and the amphipathic terminal α-helix, which drives the interaction with membranes, was shown to be sensitive to cardiolipin content [72]. Furthermore, a physical interaction of COQ7 and COQ9 has been demonstrated *in vitro* [72, 73]. Remarkably, the COQ9 hydrophobic surface for isoprene binding is close to the active site of COQ7 when the two proteins are physically bound [72]. Thus, the key function of COQ9 might be to extract hydrophobic DMQ from the membrane and present it to COQ7 [72, 73].

***COQ11*** is the most recently identified yeast UQ biosynthetic gene, with no functional homologue so far experimentally demonstrated to exist in animal genomes [45]. Loss of Coq11 lowers UQ levels but does not affect the stability of other Coq polypeptides [45]. Its function is still unknown, but co-purification and proteomic analysis suggest that it exists in physical association with other yeast Coq proteins, indicating that it may be a constituent of the CoQ synthome [45]. See **Box 3** for key findings on *COQ10*, a gene that is crucial for UQ function but not for its biosynthesis.

## Structural organization of the UQ biosynthetic pathway

### *The CoQ synthome*

Eukaryotic UQ biosynthesis occurs in the mitochondrial membrane and UQ intermediates are very hydrophobic. The CoQ synthome might therefore play a role in confining the COQ enzymes and pathway intermediates in a constrained space and thus facilitating the passage of the intermediates through successive reactions. Indeed, the generation of UQ intermediates is required for synthome formation. Findings with some of the non-enzymatic gene products that are present in the synthome, or needed for its assembly, are suggestive of lipid chaperone activities necessary to bring the enzymes in contact with their substrates [56]. For example, COQ9 specifically stimulates COQ7 activity by bringing the enzyme and substrate into close proximity [72]. On the other hand, COQ8, which might not be physically part of the synthome, is also proposed to function in UQ intermediate extraction from the IMM and thus promoting the enzymatic reactions catalyzed by other COQ proteins [64]. A recent study revealed that Ubi proteins in *E. coli* are also organized into a multiprotein complex with unexpected features (**Box 1**).

It is tempting to think that the CoQ synthome also exists in higher organisms, including humans. Suggestive data was recently provided by the use of an affinity enrichment mass spectrometry (AE-MS) approach to connect uncharacterized proteins to known pathways. Using individual COQ proteins as baits, the AE-MS data shows high interconnectivity among human COQ proteins [57]. The same study demonstrated that nearly all COQ proteins were unstable or insoluble in isolation, but are stabilized when expressed in pairs and that several COQ proteins (COQ3, 4, 5, 6, 7, and 9) can be co-purified as a group using either COQ7 or COQ9 as bait [57]. Other evidence suggesting the existence of a vertebrate CoQ synthome are also being reported [63, 73, 74]. For example, proteome analysis revealed a decrease in abundances of multiple COQ proteins in *Coq9*$^{Q239X}$ and *Coq8a*-absent mouse tissues [63, 73]. By and large, the composition of the CoQ synthome appears to be evolutionally conserved, with COQ3 to COQ7 and COQ9 being the core constituents.

Despite the similarities between yeast and vertebrates, some obvious differences remain. For example, yeast *coq7* null mutants show extremely low steady-state levels of some of the other Coq proteins (e.g., Coq8 and Coq9) and the mutants accumulate early pathway intermediates instead of DMQ, the immediate substrate of the enzyme, suggesting complete inhibition of complex

formation [39]. In animal cells, however, complete loss of COQ7 does not affect COQ9 levels and results in high accumulation of DMQ, indicating that there is only a minimal effect on the UQ biosynthetic complex [75-78]. Furthermore, unlike yeast *coq8* null mutants that are completely deficient in UQ, the knockout of *Coq8a/ADCK3* in mice shows only a mild, tissue-specific, UQ deficiency [63]. There are several ADCK proteins in vertebrates, and the two vertebrate orthologs of yeast ABC1/Coq8, which are COQ8A/ADCK3 and COQ8B/ADCK4, are very similar [79]. The mild UQ deficit in *Coq8a/Adck3*$^{-/-}$ tissues could therefore be due to functional compensation by other ADCK proteins.

***Focal localization of COQ proteins at the IMM: a potential mechanism for SUD?***

A recent study in yeast, using in vivo visualizing of tagged Coq proteins, suggests that most Coq proteins co-localize into discrete foci associated with the IMM [80]. The sites corresponding to the foci were named **mitochondrial CoQ domains** [80]. Coq3, Coq5, Coq4, Coq6, Coq7, Coq9 and Coq11 all localize at these foci in the mitochondrial membrane, while the other Coq proteins, Coq1, Coq2, Coq8 and Coq10, were not found there [80]. With the exception of Coq8, the essential components of the CoQ domain (Coq3, Coq4, Coq5, Coq6, Coq7 and Coq9) correspond to the proposed integral parts of the CoQ synthome [80]. Loss of Coq1, Coq2, Coq8 or Coq10, also impairs CoQ domain formation [80]. Moreover, it was shown that overexpression of Coq8 in *coq5*, *coq6*, and *coq7* null mutants (which partially rescues CoQ synthome formation by an unknown mechanism) also restores formation of CoQ domains. However, overexpression of Coq8 failed to suppress the domain assembly defect in cells lacking Coq1 or Coq2. Presumably, because such cells lack polyisoprenylated ring intermediates [80]. Interestingly, short-time inhibition of UQ biosynthesis by inhibiting an analog sensitive-version of Coq8 (which does not significantly decrease total UQ content) was found to lead to a decrease of CoQ domain copy number, further pointing to the importance of UQ intermediates in CoQ domain formation [80]. These findings are fully consistent with the biochemical data that led to the prediction of a CoQ synthome and that it requires the presence of prenylated UQ intermediates [25, 59].

The foci corresponding to CoQ domains are not randomly distributed but are found in close proximity to the ER-mitochondrial contact sites [80, 81]. In yeast, the ER-mitochondria encounter structure (ERMES) is a molecular tether between the ER and the outer mitochondrial membrane

(OMM) (**Figure 2**). It primarily functions in non-vesicular transfer of lipids between the ER and mitochondria [82]. Eisenberg-Bord, *et al.* showed that genetic manipulations of ERMES (most effects were seen with deletion of *mdm-10*, *mdm-12* or *mdm-34*) resulted in increased overall cellular UQ and an aberrant accumulation of early and late UQ biosynthetic intermediates, except in mitochondria, where the contents of UQ and of some late-stage intermediates were significantly reduced [81]. In addition, accumulation of early UQ intermediates was reported by Subramanian, *et al.* for a *mmm1-1* temperature-sensitive mutant and *mmm1-1Δltc1* cells along with CoQ domain changes (decrease in abundance and increase in intensity) [80]. However, no significant changes in UQ levels were found [80]. These findings suggest a possible importance of the ER-mitochondria contacts for UQ production and distribution following its synthesis in the mitochondria [80, 81].

We can imagine that for a pathway composed of multiple components and several consecutive enzymatic reactions spatial restriction of steady state components would be advantageous as it would allow for local enrichment of key pathway components as well as facilitate efficient substrate accessibility. It is reasonable to assume that the CoQ synthome lives in the CoQ domains. One important potential significance of these findings is that they provide a first hint as to why mitochondrial disorders are often associated with UQ deficiency (which might in turn exacerbate the disease pathophysiology). SUD has been described in patients with various mitochondrial respiratory chain (MRC) defects, especially those involving mtDNA mutations or depletion [30, 31, 83, 84]. If the final steps of UQ biosynthesis need to be carried out in the CoQ domain, then disturbance of the IMM structure, which is commonly observed in dysfunctional or aged mitochondria [85-88], could impair CoQ domain formation and thus UQ production.

## SUD in mice

We discuss the findings in three mouse models of SUD in terms of their implications for our understanding of UQ biosynthesis.

### *Parl*

Presenilin-Associated Rhomboid-Like (PARL) is an intramembrane serine protease localized to the IMM. *Parl*$^{-/-}$ mice show ~ 50% reduction of brain but not muscle UQ, defective complex III

activity and a lethal phenotype presenting like a Leigh encephalomyopathy [89]. In addition to some expected effects of known PARL substrates, a change of expression levels of several other mitochondrial proteins was also noticed. Those include decreased expression of several COQ proteins, a marked down-regulation of the CIII-regulating protein TTC19, a significant decrease of the sulfide-CoQ oxidoreductase, and reduced protein expression of mitochondrial morphology and cristae structure 1 (*MICS1*)/growth hormone-inducible transmembrane protein (*GHITM*) [89]. MICS1/GHITM is an inner mitochondrial membrane protein that is required for normal mitochondrial morphology, as its name implies [90]. The brain of *Parl*$^{-/-}$ mice showed loss of CIII activity, altered mitochondrial calcium metabolism, and severe progressive mitochondrial ultrastructural abnormalities [89]. We speculate that the severe perturbation in mitochondrial morphology in the *Parl*$^{-/-}$ neurons leads to UQ deficiency by disrupting the proper assembly of the CoQ domains.

***Mfn2***

Mitochondria are dynamic organelles that divide and fuse continuously to alter their size, morphology and turnover. Mammalian cells have two mitofusins, MFN1 and MFN2, which are mitochondrial outer membrane proteins that mediate outer membrane fusion [91]. In a heart conditional knockout model, ablation of *Mfn2* was shown to severely inhibit UQ biosynthesis [86]. A similar effect was also observed in *Mfn2* knockout mouse embryonic fibroblasts (MEFs) [86]. In contrast, inactivation of *Mfn1* had no effect on UQ levels in the heart or MEFs [86]. Strikingly, cardiomyocytes isolated from *Mfn2* knockout hearts showed mitochondrial morphological heterogeneity with the appearance of enlarged mitochondria, while no aberrant morphology at all was observed with loss of heart *Mfn1* [86]. In MEFs loss of *Mfn1* or *Mfn2* resulted both in significant mitochondrial fragmentation with very short mitochondrial tubules and small spheres of nearly uniform size for *Mfn1*, and spheres of widely varying size for *Mfn2* knockout mitochondria [92]. In both *Mfn1* and *Mfn2* knockout MEFs, proteomic analysis detected downregulation of the isoprenoid synthesis pathway, and metabolomics analysis identified a more severe reduction in some of the mevalonate pathway metabolites in the *Mfn2* knockout heart [86]. This suggested the possibility that the UQ deficiency in *Mfn2* knockout cells resulted from a deficiency in the ability of the mevalonate pathway to synthesize the side-chain [86]. The gross overall morphology of the mitochondrial network has not been shown to be well correlated with

UQ content in *Mfn2* mutant cells [86]. However, in light of the findings of the CoQ domain structure in the IMM, it remains an interesting possibility that decreased UQ biosynthesis in *Mfn2* knockout cells is caused by structural or functional alterations of the IMM that lead to CoQ domain destabilization.

Earlier in this section, we mentioned that although in yeast the mitochondrial CoQ domains are positioned close to ERMES, disruption of ERMES does not impair UQ biosynthesis, although it affects UQ distribution. So far, no clear ERMES homolog has been identified in mammals. In higher eukaryotes, the regions of close contact between the ER and mitochondria are known as mitochondrial associated ER membranes or MAMS (**Figure 2**) [93, 94]. It remains to be demonstrated whether mammalian CoQ domains are also localised closely to the ER-mitochondria contact sites. UQ is made in the IMM. Its transport out of the site of synthesis appears to be a regulated process. Mitochondria from *Mclk1/Coq7*$^{+/-}$ livers were shown to have lower UQ levels in the IMM, but higher levels in the OMM [95]. The positioning of the UQ synthesis site close to where the OMM interacts with the ER membrane might be necessary for UQ export from the mitochondria, and MFN2 function might be crucial to this process.

The possibility that CoQ domain formation is vulnerable to the pathophysiological changes in IMM organization provides a mechanism for why mitochondrial dysfunction is often associated with secondary UQ deficiency (**Figure 3**). For example, it is reported that 75% patients with mtDNA depletion syndrome presented with a decreased level of muscle UQ [31]. In mice, a systematic comparative analysis of five heart conditional knockout models targeting key genes that regulate mtDNA gene expression (*Twnk*, *Tfam*, *Polrmt*, *Lrpprc* or *Mterf4*) revealed, remarkably, that one of the very few features shared by these mice is lower levels of UQ in the heart (a ~25 to ~50% decrease) [65]. Aberrant heart mitochondrial morphology was described for all five cardiac-specific models [87, 88, 96-98].

## Concluding remarks

UQ is extremely hydrophobic and its biosynthesis is associated with the IMM. This creates challenges for its biosynthesis. In particular, how to allow COQ enzymes to gain access to specific

hydrophobic intermediates and how the end product gets exported out of the site of synthesis to other intracellular locations. Recent findings start to shed lights on these questions. For example, COQ8 and COQ9 are able to bind to lipids and most likely function in substrate extraction and substrate presentation to the enzymes. The need for mitochondrial CoQ biosynthesis domains might also be linked to the special needs of UQ biosynthesis. Beyond this, the association of UQ biosynthesis with the ER-mitochondria contact sites points to an extra layer of factors that influence the synthesis and distribution of UQ.

Mitochondrial ultrastructure is responsive to the bioenergetic state of the mitochondria. To probe the key factors that determine the formation and/or localization of the CoQ domains, we need to examine how their numbers and location are linked to ETC function, to the lipid and protein composition of the IMM, and to the overall shape and ultrastructure of mitochondria. For example, how UQ production is affected by depletion of cardiolipin, by severe ETC deficiency, and by abnormal IMM organization (e.g., disrupted cristae structures). A better understanding of UQ biosynthesis is crucial for the development of effective treatments for UQ deficiency (**Outstanding Questions Box**). For example, for some genes and some mutations it was shown that it might be possible to use precursor analogues to regain better UQ levels [11, 40, 41, 99, 100]. It is very unlikely that targeting a single component of the biosynthetic machinery could be sufficient to boost UQ production. However, understanding the structure of, and need for CoQ domains could lead to new ideas about possible treatments. On the other hand, for patients with severe mitochondrial defects, including ultrastructural defects, effective supplementation of exogenous UQ might be the only possible treatment option.

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**Box 1**

**Soluble Ubi complex in *E. coli***

UQ biosynthesis in *E. coli* requires at least a dozen proteins. The first committed step is the formation of 4-hydroxybenzoate (4-HB) from chorismate by UbiC. UbiA, which is membrane-bound, adds the octaprenyl tail to the 4-HB ring, generating the first membrane-bound UQ intermediate 3-octaprenyl-4-hydroxybenzoate (OHB), which is subsequently decarboxylated to 3-octaprenylphenol (OPP) by UbiD and UbiX. Additional ring modifications are catalyzed by UbiI, UbiG, UbiH, UbiE and UbiF generating the final product $UQ_8$ (**Figure I**) [26, 101]. Moreover, UQ biosynthesis in *E. coli* requires additional factors (UbiB, UbiK and UbiJ) that are not directly involved in the chemical modifications of the aromatic ring [102, 103]. Their exact roles, however, remain to be characterized.

A recent study demonstrated that Ubi proteins are organized into a mega-complex to synthesis UQ. And, strikingly, the Ubi complex appears to function as a soluble and stable metabolon [104]. More specifically, it was shown that the last 6 reactions of UQ biosynthesis rely on an obligate multi-protein complex of 7 Ubi proteins (5 Ubi enzymes (UbiI, G, H, E, and F) and 2 accessory factors (UbiJ and UbiK)) that is found in the soluble cytoplasmic fractions instead of membrane extracts [104]. Based on these findings, a new model has been proposed (**Figure I**) according to which the conversion from octaprenylphenol to UQ is catalyzed by the mega Ubi complex in the cytosol. UbiJ is able to bind UQ and the UQ biosynthetic intermediates. it might therefore be that activity that allows for substrate accessibility for the Ubi enzymes in the complex [104]. However, how octaprenylphenol moves out of the membrane and binds to UbiJ in the soluble Ubi complex and how the final product is delivered to the membrane remain open questions. For example, UbiB, the homolog of COQ8, may assist in the extraction of early octaprenyl intermediates (either OHB or OPP) out of the membrane [104].

**Box 2**

**Other gene products that support UQ biosynthesis**

***Ferredoxins***

In yeast, the only known ferredoxin, Yah1, is a mitochondrial matrix protein that contains a [2Fe-2S] cluster. Working in concert with its reductase Arh1, it plays an essential role in Fe/S cluster biogenesis [105]. Loss of these gene activities in yeast strongly limits the activity of Coq6 [37]. Two ferredoxins (FDX1 and FDX2) are present in mammals and expressed in the matrix of

mitochondria [106]. Mitochondrial membrane-associated ferredoxin reductase (*FDXR*) is the ortholog of the yeast gene *ARH1*. Human mutations have been identified for *FDX2* and *FDXR* [107, 108]. Whether they have an effect on UQ levels is not known.

***Oct1 and Puf3***

Two mechanisms of post-transcriptional regulation were recently described for yeast Coq5. First, the mitochondrial peptidase Oct1 was found to be required for proteolytic processing of Coq5 [47]. Disrupted Oct1 processing leads to a reduction in the stability of Coq5 and thus a marked depletion of $UQ_6$ levels [47]. Oct1 cleaves 8 amino acids off the N-termini of selected proteins following their initial processing by the mitochondrial processing peptidase [109]. In addition, the translation of the Coq5 mRNA has been reported to be subject to regulation by the RNA binding protein Puf3 [46].

***Ptc7***

Yeast *PTC7* encodes two splicing isoforms of a type 2C serine/threonine protein phosphatase. Deletion of *PTC7* leads to a deficiency in $UQ_6$ levels and compromised mitochondrial respiration, and it was proposed that Ptc7 regulates $UQ_6$ biosynthesis through the dephosphorylation of Coq7 [110]. However, several results with Ptc7 are somewhat contradictory, preventing a clear view of its functions [111, 112]. For example, the 2 splicing forms of Ptc7 were shown to have opposing effects on UQ level [112]. There is a mammalian ortholog of *PTC7*(*Pptc*) that also produces 2 isoforms through differential splicing. *Pptc* knockout mice exhibit global mitochondrial defects and severe metabolic phenotypes, and die within one day of birth [113]. But no changes in UQ levels were observed in the mitochondria of these mice [113].

***Genes that are required for 4-HB production from tyrosine***

It had remained elusive how 4-HB, a ring precursor of UQ, is produced from tyrosine in eukaryotes. However, more recently, steps of this process are beginning to be uncovered. Payet et al. showed that Aro8 and Aro9, which are two aminotransferases in the shikimate pathway catalyzing the last reaction of the biosynthesis of tyrosine, can also catalyze the reverse reaction: the deamination of tyrosine to 4-hydroxyphenylpyruvate (4-HPP) [35]. They discovered that 4-HPP, originating from the shikimate pathway and from the deamination of tyrosine, is a precursor of 4-HB in the synthesis of $UQ_6$ [35]. Steps producing 4-HB from 4-HPP are not yet elucidated, but the last reaction is

dehydrogenation of 4-hydroxybenzaldehyde (Hbz) which is catalyzed by the aldehyde dehydrogenase Hfd1[35]. By means of a multi-omics data analysis approach, Stefely et al. also linked Aro9 and Hfd1 to 4-HB production in yeast [34]. Moreover, they predicted and validated additional factors necessary for the synthesis of 4-HB, namely MXP Aim18p and Aro10 [34]. Furthermore, both studies demonstrated that Hfd1p human homologs ALDH3A1 can catalyze the same Hbz oxidation reaction, and therefore its expression was able to rescue the UQ production defect of *Δhfd1* yeast, suggesting conservation of the pathway from tyrosine to 4-HB from yeast to humans [34, 35]. Hfd1 is located primarily in the outer mitochondrial membrane [114].

**Box3**

**A different kind of *COQ* gene: *COQ10***

Coq10 is not indispensable for UQ biosynthesis as *coq10* null mutants produce near normal level of $UQ_6$ in stationary growth phase. However, they display low UQ biosynthesis and accumulate early biosynthetic intermediates during log growth phase [115-117]. However, like other *coq* mutants, the *coq10* mutants still result in a respiratory defect that improves after UQ supplementation, thus the name *COQ10* [115-117]. In mitochondria isolated from the *coq10* null mutants, steady state levels of several of the other Coq polypeptides (Coq4, Coq6, Coq7, and Coq9) are significantly decreased and *COQ8* overexpression, which can help stabilize the CoQ synthome, was found to rescue the inefficient UQ production during log growth phase [117]. More importantly, the findings with *coq10* null mutants suggest that for proper UQ function it is not enough to make UQ: additional activities are needed to make it available to carry out its function in the ETC. However, lack of Coq10 does not make UQ completely inactive in the ETC, as the adverse effect on respiration of lacking Coq10 can be compensated to some extent by higher than normal concentrations of UQ in mitochondria [116, 118].

Based on the fact that Coq10 contains a putative steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain, and that there are detectable amounts of $UQ_6$ bound to Coq10, it has been suggested that COQ10 serves as a chaperone facilitating the delivery of UQ into the sites where it is used for the mitochondrial respiration [116]. This could explain the deleterious effects on respiration of over-producing Coq10 (binding of UQ to an excess of Coq10 could limit the UQ pool available to function in the ETC) [119]. However, the chaperone

hypothesis does not easily account for some of the other effects of loss of Coq10. For example, mitochondria from *coq10* null mutants were reported to produce more $H_2O_2$ compared to the wild-type, and exposure of purified *coq10* mitochondria to the Qi site inhibitor antimycin A resulted in even more $H_2O_2$ production, indicating an active Q cycle in the *coq10* mutants [118]. The mutant mitochondria, however, were not responsive to the 'proximal' $Q_o$ site inhibitor myxothiazol which is believed to prevents electron entry into CIII [118]. There are even more questions: for example, exogenously added UQ fails to rescue a *coq2/coq10* double mutant, but rescues each of the single mutants [119]. These phenomena are not understood. Humans have two homologs of yeast *COQ10*, namely *COQ10A* and *COQ10B*. Expression of either of them can rescue yeast *coq10* mutant phenotypes [120].

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## Figure legends

**Figure 1. Chemical structure and three redox forms of UQ.** UQ is a prenylated benzoquinone that can exist in three oxidation states: oxidized, as a partially reduced intermediate, and fully reduced. The length of its hydrophobic polyisoprenoid side-chain varies among species ranging from 6 to 10 isoprene units.

**Figure 2. Schematic of mitochondrial UQ biosynthesis. (a),** Functions of UQ in the mitochondrial electron transport chain (ETC). It is a pivotal component acting as the electron

carrier to pass the electrons from complex I, complex II and several other dehydrogenases to complex III. IMM: inner mitochondrial membrane, G3PDH: glycerol-3-phosphate dehydrogenase, DHODH: dihydroorotate dehydrogenase, ETF-QO: electron transfer flavoprotein oxidoreductase, SQR: Sulfide:quinone oxidoreductase. (**b)**, Diagram of current model of UQ biosynthetic pathway in yeast. In the IMM, a cohort of Coq proteins (Coq3-9 and Coq11) are assembled into a supramolecular complex, termed the CoQ synthome. The CoQ synthome is further sequestered into discrete foci, called mitochondrial CoQ domains, which are positioned close to the ER-mitochondrial contact site. In addition to the protein constituents, generation of polyisoprenylated UQ intermediates is also required for the formation and stabilization of the CoQ synthome and the CoQ domains. Coq proteins with known enzymatic functions are shown in blue. There are two enzymatic steps in the pathway that are not characterized. HHB: 3-hexaprenyl-4-hydroxybenzoic acid. (**c)**. Schematic representation of ER–mitochondria contact sites. In yeast, close contacts between the ER and mitochondria is referred to as ERMES which comprises four core subunits: Mmm1, Mdm10, Mdm12 (shown as '10') and Mdm34 (shown as '34'). The ER protein Ltc1/Lam6 binds the OMM proteins Tom70/71, constituting an additional tether. The ER-mitochondria contact sites in mammalian cells are known as mitochondria-associated endoplasmic reticulum membranes (MAMs). Several MAM tethers or tethering complexes connecting the two organelles were identified, including Mfn1/2, Fis1-Bap31, and the IP3R-grp75-VDAC1 tripartite complex. OMM: outer mitochondrial membrane. The molecular composition of ER–mitochondria contacts is not yet completely defined. Shown are several of the relatively better understood components.

**Figure 3. A conceptual model of secondary UQ deficiency in mitochondrial disease.** Genetic mutations, environmental effects, or the aging process all can inflict damage on mitochondria. Severe mitochondrial dysfunction induces structural disorganization and degenerative changes of the mitochondrial membranes (especially the IMM). This could impair the structure and/or stability of the mitochondria CoQ domain, thus leading to inefficient UQ biosynthesis. Because of its essential functions in mitochondrial respiration and redox control, insufficient production of UQ could exacerbate the mitochondrial respiratory deficit and cause further oxidative stress, creating a vicious cycle.

## Glossary

**COQ genes and proteins:** The eukaryotic genes in the ubiquinone (UQ) biosynthetic pathway are named *COQ* genes (for Coenzyme Q). They are highly conserved across species. In the budding yeast *S. cerevisiae* and humans, they are written *COQ* in capital letter except for the human *PDSS1* and *PDSS2* genes which encode two different subunits of decaprenyl diphosphate synthase. The yeast homologue responsible for the production of the hexaprenyl side-chain is encoded by a single gene, *COQ1*. For mouse homologues, the gene names are written with the first letter capitalized (e.g., *Pdss1*, *Pdss2*, *Coq2*-*Coq10*). In bacteria these genes are called *ubi* genes. The corresponding protein symbols are in uppercase letters, except for yeast proteins, which are written as the gene name (but not in italics) with only the first letter capitalized, for example, Coq3. In addition to the COQ proteins, several other gene products have been identified to be involved in UQ biosynthesis (see **Box 2**).

**UQ, CoQ:** Ubiquinone (abbreviated UQ) was first isolated from the mitochondria of the beef heart about 60 years ago. The name ubiquinone was given to mean "ubiquitous quinone", because it has been found almost universally in living cells and its chemical structure contains a quinone ring. It is also called Coenzyme Q (abbreviated CoQ or Q), because it is an essential cofactor in mitochondrial respiration. The designation for UQ is $UQ_n$ or $CoQ_n$, where n is the number of the isoprenoid units in the side-chain.

**The CoQ synthome:** A large multi-subunit protein complex made of several *COQ* gene products that produces UQ. It is also called the UQ or CoQ biosynthetic complex or complex Q. Data are also presented indicating that some result also suggest that UQ and certain polyisoprenylated UQ-intermediates might also be associated with the complex. However, the exact composition and all structural details of the CoQ synthome are not yet fully defined.

## Highlights

- Eukaryotic UQ biosynthesis depends on the formation of a multi-subunit complex of COQ proteins (the CoQ synthome or complex Q), likely because UQ and UQ biosynthetic intermediates are highly hydrophobic. Gene products that are required for synthome formation and stabilization have been identified, and recent studies suggest that some of them function by extracting UQ intermediates from the membrane lipid bilayer for substrate presentation to enzymatic components of the complex.

- COQ enzymes in yeast are shown to localize into sub-mitochondrial domains associated with mitochondria-ER contact sites. Disruption of the focal localization leads to UQ biosynthetic defects. This provides a possible mechanism for the frequent association of secondary ubiquinone deficiency with mitochondrial disorders.

- In bacteria, multiple steps of UQ biosynthesis are carried out in the cytoplasmic fraction. Seven Ubi proteins form a stable metabolon. Whether a similar mechanism occurs in eukaryotes is not known.

## Outstanding Questions

- What exactly are the non-enzymatic activities of COQ4, COQ8, COQ9, COQ10, and Coq11 in UQ biosynthesis?

- What processes control and trigger the formation of the CoQ synthome and CoQ domains? Which polyisoprenylated ring intermediate(s) stimulates their formation and how?

- Does the regulation of UQ biosynthesis occur by altering the stability or configuration of the UQ biosynthetic complex or CoQ domain, or does it involve regulating the activity or expression level of particular COQ proteins (such as COQ7)?

- What features of the IMM architecture are crucial for the formation and localization of the UQ biosynthetic complex in CoQ domains?

- How is UQ transported out of the mitochondria and distributed to other subcellular compartments?

- Is there a spatial and functional relationship between the UQ biosynthetic complex (or CoQ domains) and mitochondrial respiratory complexes and supercomplexes?

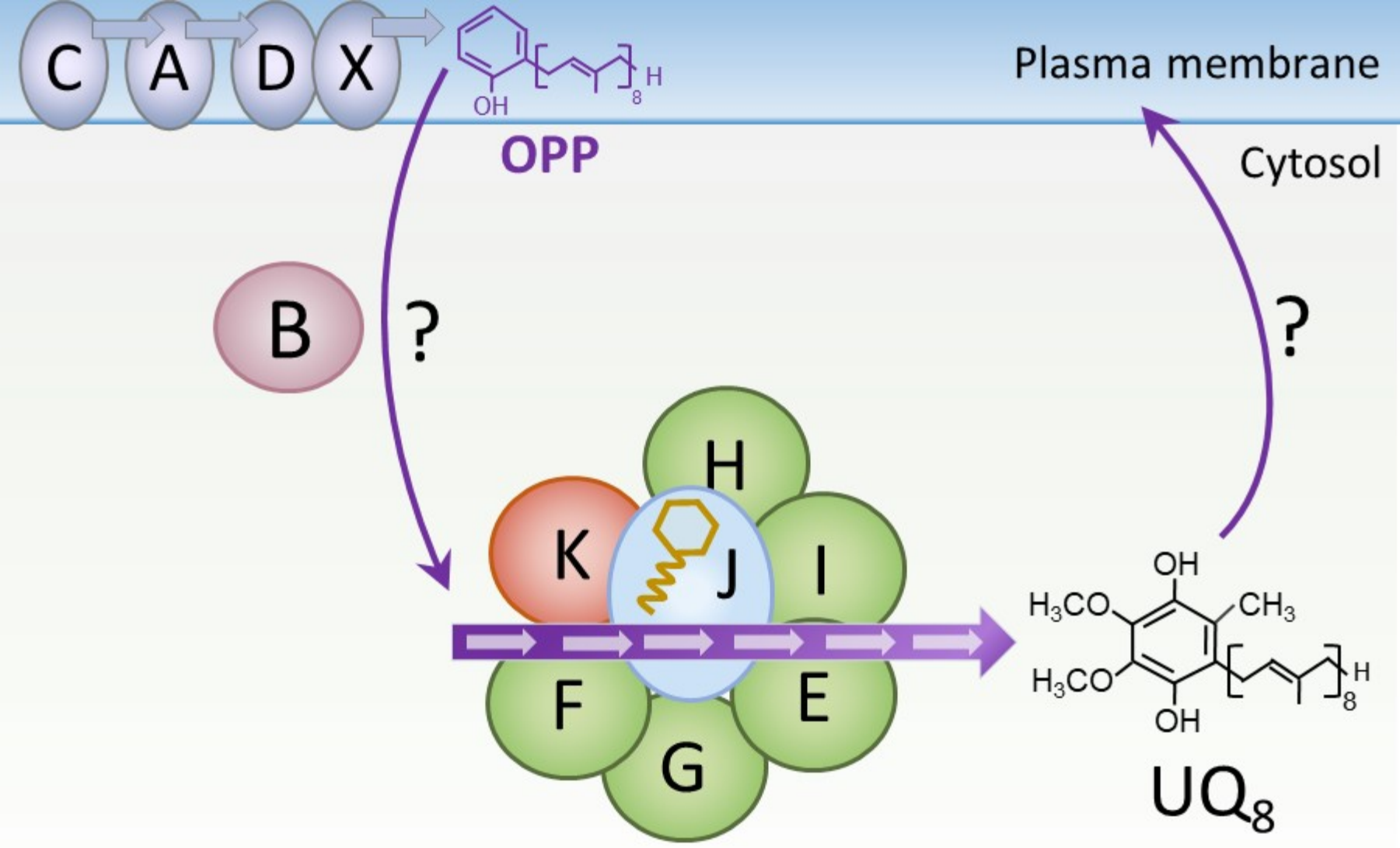
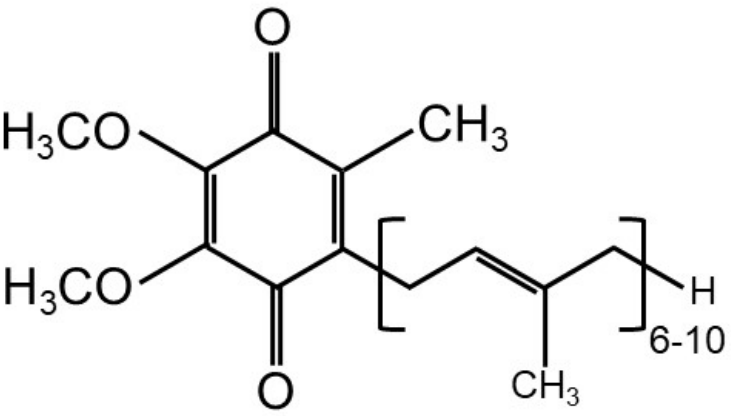
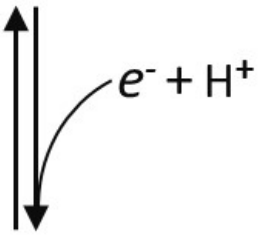


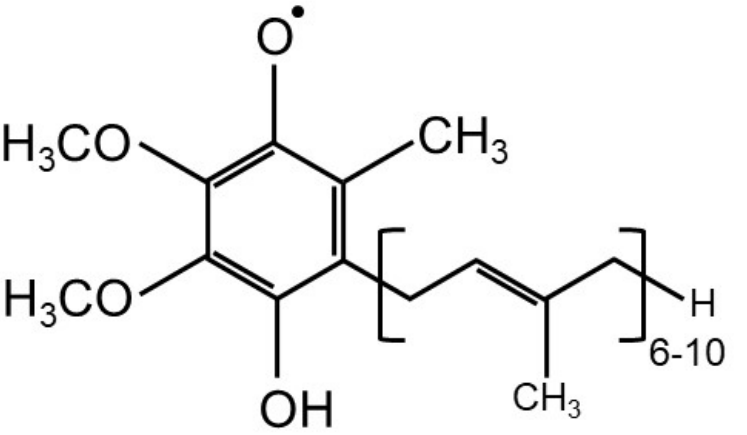

**Figure I. Schematic illustration of UQ biosynthesis in *E. coli*.**

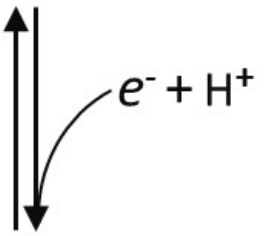
**Ubiquinone (UQ)**
(fully oxidized)



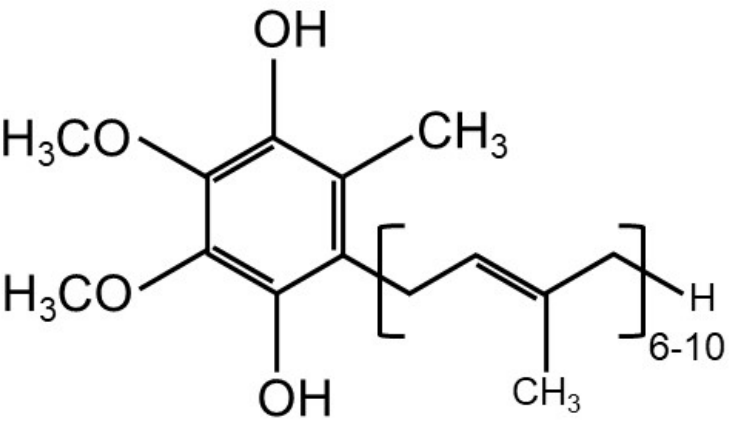



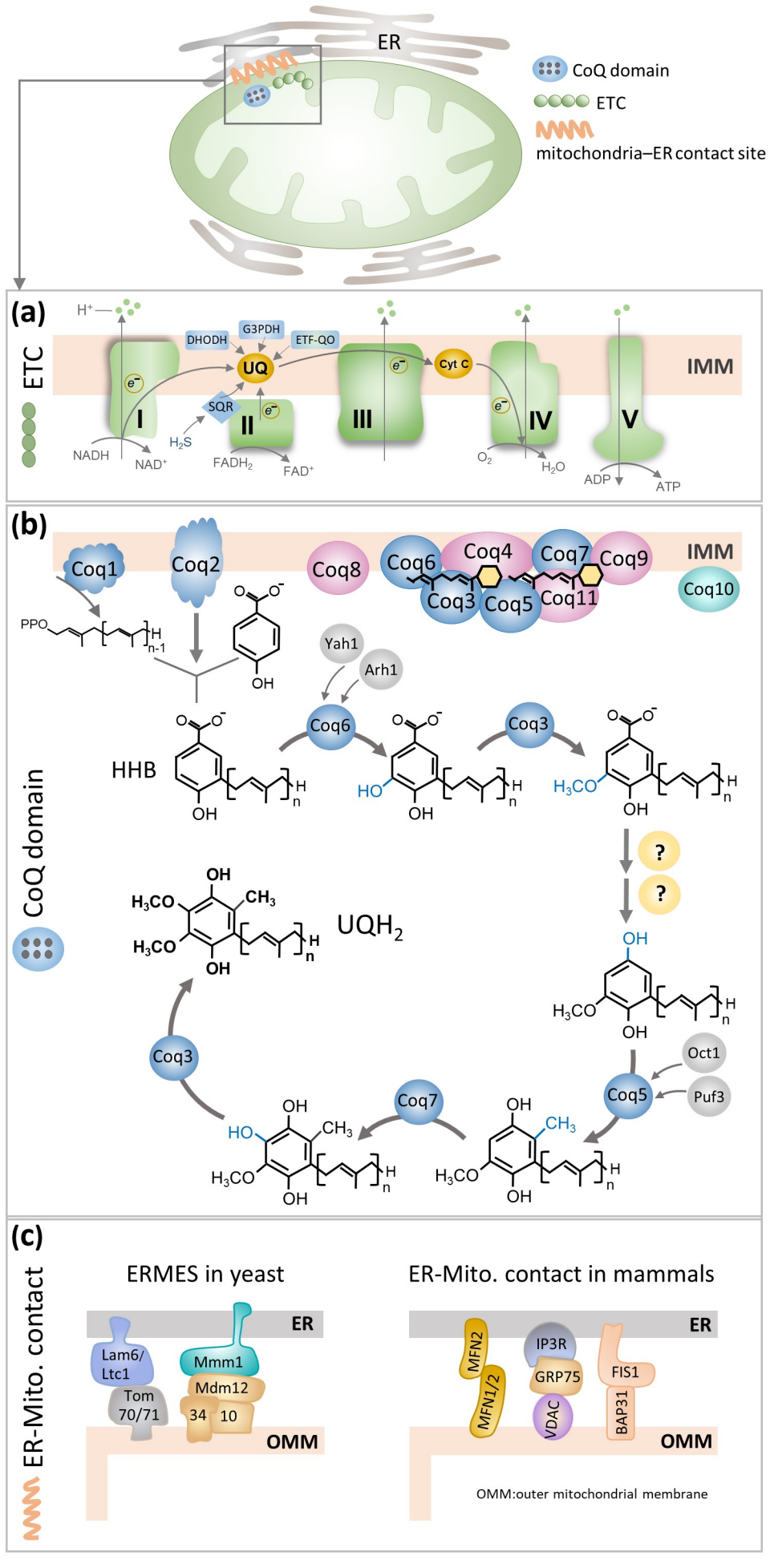

**Semiubiquinone radical ($UQH^{\bullet}$)**

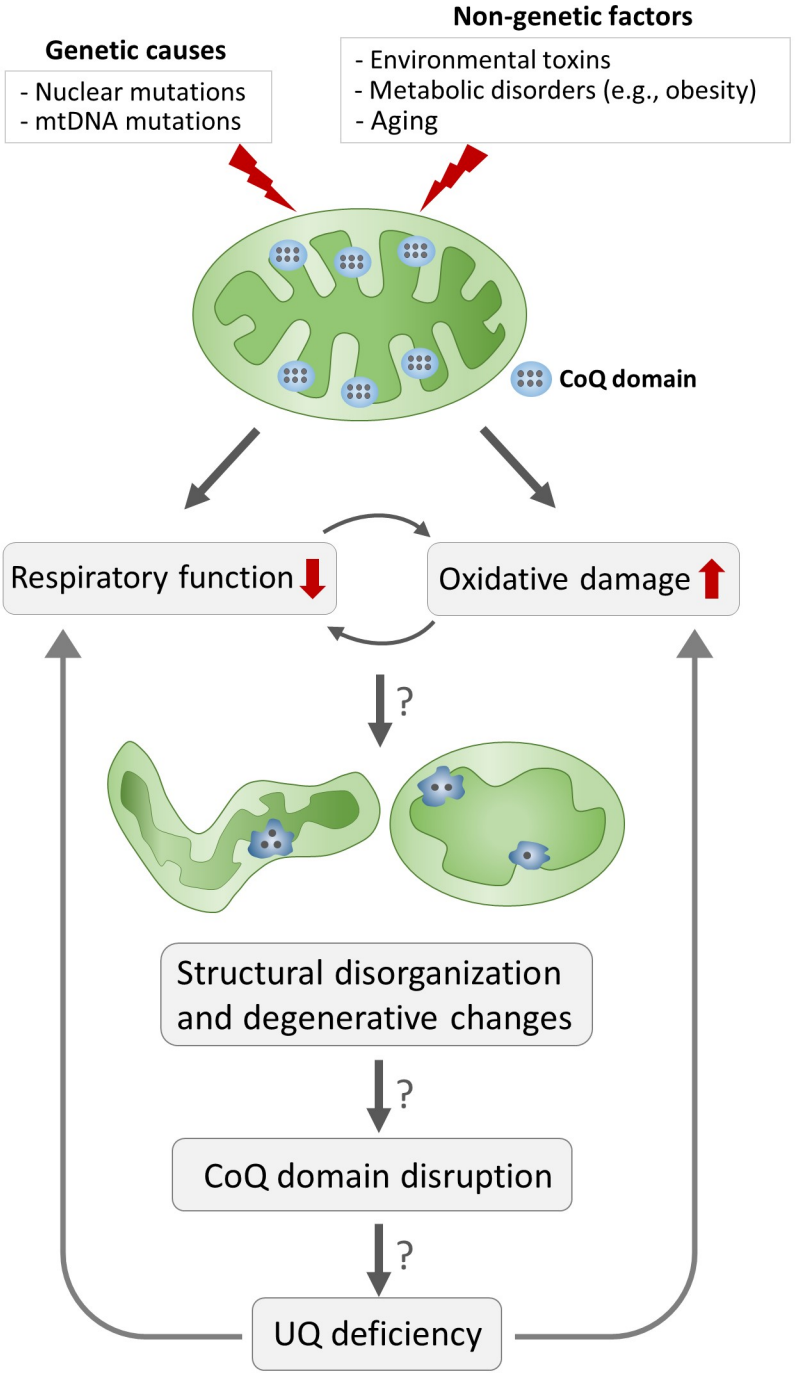



**Ubiquinol ($UQH_2$)**
(fully reduced)




ER
CoQ domain
ETC
mitochondria–ER contact site
(a)
ETC
IMM
DHODH
G3PDH
ETF-QO
UQ
Cyt C
SQR
I
II
III
IV
V
NADH
NAD+
H2S
FADH2
FAD+
O2
H2O
ADP
ATP
(b)
CoQ domain
IMM
Coq1
Coq2
Coq8
Coq6
Coq4
Coq7
Coq9
Coq3
Coq5
Coq11
Coq10
PPO
Yah1
Arh1
HHB
Coq6
Coq3
?
?
UQH2
Oct1
Puf3
Coq5
Coq7
Coq3
(c)
ER-Mito. contact
ERMES in yeast
ER
Lam6/
Ltc1
Mmm1
Mdm12
Tom
70/71
34
10
OMM
ER-Mito. contact in mammals
ER
MFN2
MFN1/2
IP3R
GRP75
VDAC
FIS1
BAP31
OMM
OMM:outer mitochondrial membrane


Genetic causes
- Nuclear mutations
- mtDNA mutations
Non-genetic factors
- Environmental toxins
- Metabolic disorders (e.g., obesity)
- Aging
CoQ domain
Respiratory function
Oxidative damage
?
Structural disorganization and degenerative changes
?
CoQ domain disruption
?
UQ deficiency