CellPress
REVIEWS

Review

The Complexity of Making Ubiquinone

Ying Wang[1] and Siegfried Hekimi[1,*]

Ubiquinone (UQ, coenzyme Q) is an essential electron transfer lipid in the mitochondrial respiratory chain. It is a main source of mitochondrial reactive oxygen species (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 (UQ), 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: ten subunits in human (UQ_{10}), a mix of UQ_9 and UQ_{10} in rodents, UQ_6 in *Saccharomyces cerevisiae*, and UQ_8 in *Escherichia 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 reoxidized 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

Highlights

Eukaryotic UQ biosynthesis depends on the formation of a multisubunit 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 submitochondrial 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.

[1]Department of Biology, McGill University, Montreal, Canada

*Correspondence:
siegfried.hekimi@mcgill.ca

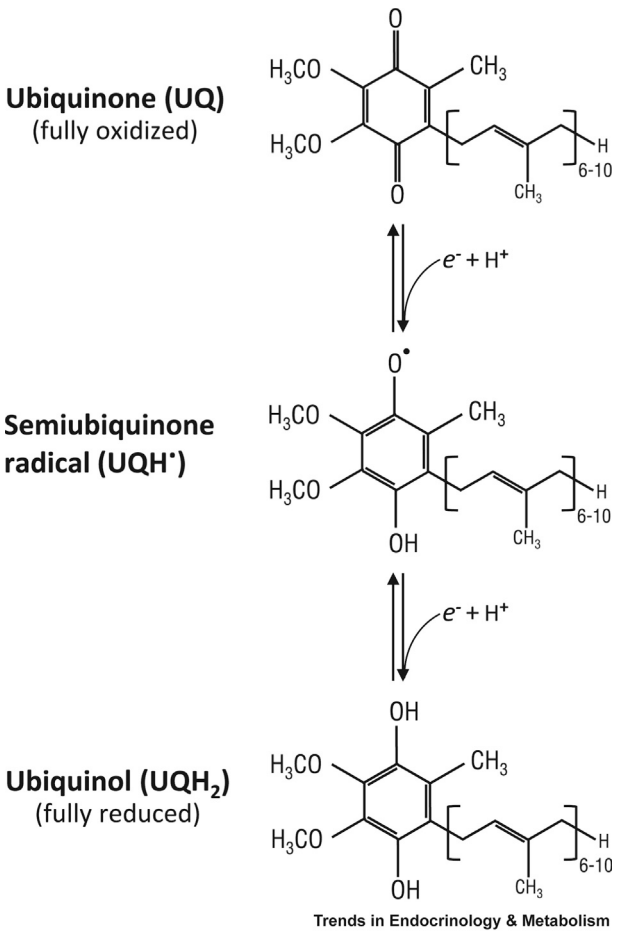


Figure 1. Chemical Structure and Three Redox Forms of Ubiquinone (UQ).
UQ is a prenylated benzoquinone that can exist in three oxidation states: oxidized, as a partially reduced intermediate, and fully reduced. The length of its hydrophobic polyisoprenoid side-chain varies among species, ranging from six to ten isoprene units.

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 other hand, in its reduced form, UQ has antioxidant properties [6]; yet, 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 associated 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 re-establishment of UQ levels, as we observed in a *Coq7* knockout mouse model of UQ deficiency [11,24].

Normal and Defective UQ 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

Glossary

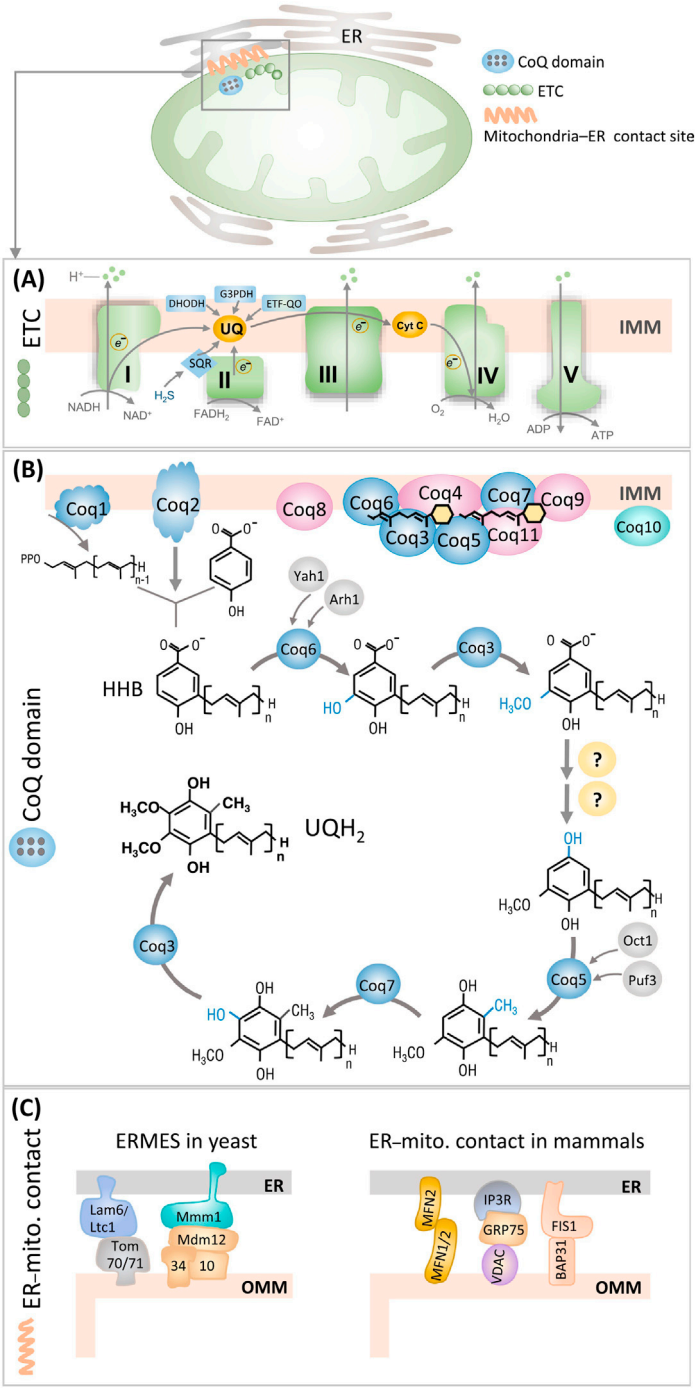
***COQ* genes**: the eukaryotic genes in the ubiquinone (UQ) biosynthetic pathway are named *COQ* genes (for Coenzyme Q). They are highly conserved across species. In the budding yeast *S. cerevisiae* and humans, they are written *COQ* in capital letters except for the human *PDSS1* and *PDSS2* genes, which encode two different subunits of decaprenyl diphosphate synthase. The yeast homolog responsible for the production of the hexaprenyl side-chain is encoded by a single gene, *COQ1*. For mouse homologs, 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 (Box 2).

CoQ synthome: a large multisubunit 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 results 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.

Ubiquinone (UQ, Coenzyme Q, or CoQ): 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.

Key Figure

Schematic of Mitochondrial Ubiquinone (UQ) Biosynthesis



Trends in Endocrinology & Metabolism

(See figure legend at the bottom of the next page.)

deficiency (SUD). PUD is characterized by a severe and heterogeneous clinical spectrum and often presents as a multisystems 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. The most common precursor for the benzoquinone ring is 4-hydroxybenzoic acid (4-HB), derived from tyrosine [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 homologs 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.

Figure 2. (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. (B) Diagram of current model of UQ biosynthetic pathway in yeast. In the inner mitochondrial membrane (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 endoplasmic reticulum (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. (C) Schematic representation of ER–mitochondria contact sites. In yeast, close contacts between the ER and mitochondria is referred to as ER–mitochondria encounter structure (ERMES), which comprises four core subunits: Mmm1, Mdm10, Mdm12 (shown as '10'), and Mdm34 (shown as '34'). The ER protein Ltc1/Lam6 binds the outer mitochondrial membrane (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. The molecular composition of ER–mitochondria contacts is not yet completely defined. Shown are several of the relatively better understood components. Abbreviations: DHODH, dihydroorotate dehydrogenase; ETF-QO, electron transfer flavoprotein oxidoreductase; G3PDH, glycerol-3-phosphate dehydrogenase; HHB, 3-hexaprenyl-4-hydroxybenzoic acid; SQR, sulfide:quinone oxidoreductase.

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) [26,100]. 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 [101,102]. Their exact roles, however, remain to be characterized.

A recent study demonstrated that Ubi proteins are organized into a mega-complex in order to synthesize UQ. And, strikingly, the Ubi complex appears to function as a soluble and stable metabolon [103]. More specifically, it was shown that the last six reactions of UQ biosynthesis rely on an obligate multiprotein complex of seven Ubi proteins [five Ubi enzymes (UbiI, G, H, E, and F) and two accessory factors (UbiJ and UbiK)] that is found in the soluble cytoplasmic fractions instead of membrane extracts [103]. 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 [103]. 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 [103].

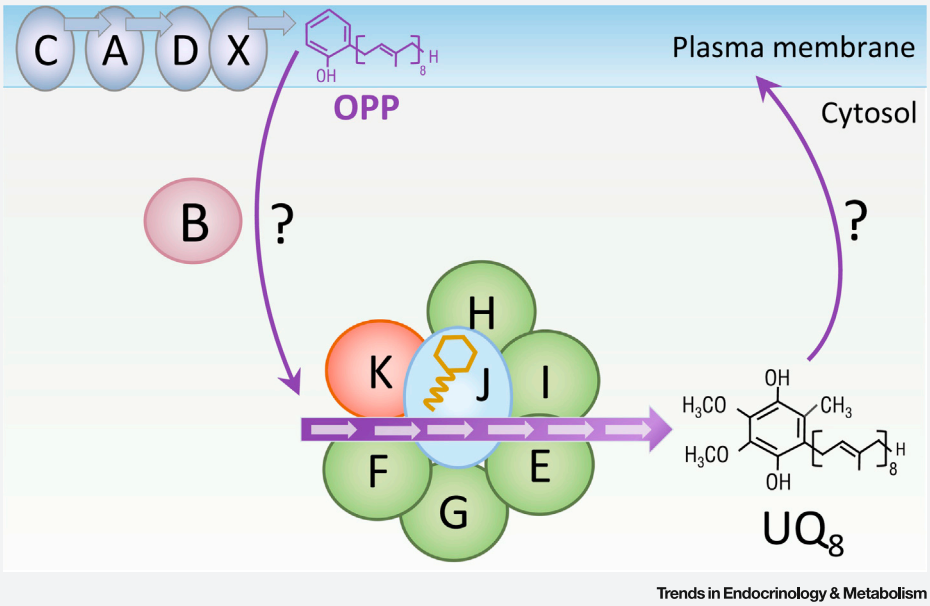


Figure I. Schematic Illustration of UQ Biosynthesis in *Escherichia coli*.

Abbreviations: OPP, 3-Octaprenylphenol; UQ, ubiquinone.

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

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 [104]. 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 [105]. Mitochondrial membrane-associated ferredoxin reductase (*FDXR*) is the ortholog of the yeast gene *ARH1*. Human mutations have been identified for *FDX2* and *FDXR* [106,107]. 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 eight amino acids off the N termini of selected proteins following their initial processing by the mitochondrial processing peptidase [108]. 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 [109]. However, several results with Ptc7 are somewhat contradictory, preventing a clear view of its functions [110,111]. For example, the two splicing forms of Ptc7 were shown to have opposing effects on UQ level [111]. There is a mammalian ortholog of *PTC7* (*Pptc*) that also produces two isoforms through differential splicing. *Pptc* knockout mice exhibit global mitochondrial defects and severe metabolic phenotypes and die within one day of birth [112]. But no changes in UQ levels were observed in the mitochondria of these mice [112].

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 [113].

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] (Figure 2).

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]. The *coq3-coq9* single

Box 3. A Different Kind of *COQ* Gene: *COQ10*

Coq10 is not indispensable for UQ biosynthesis as *coq10* null mutants produce near normal levels of UQ_6 in stationary growth phase. However, they display low UQ biosynthesis and accumulate early biosynthetic intermediates during log growth phase [114–116]. However, like other *coq* mutants, the *coq10* mutants still result in a respiratory defect that improves after UQ supplementation, thus the name *COQ10* [114–116]. 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 [116]. 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 [115,117].

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 [115]. This could explain the deleterious effects on respiration of overproducing Coq10 (binding of UQ to an excess of Coq10 could limit the UQ pool available to function in the ETC) [118]. 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 with 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 [117]. The mutant mitochondria, however, were not responsive to the 'proximal' Q_o site inhibitor myxothiazol, which is believed to prevent electron entry into CIII [117]. 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 [118]. 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 [119].

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 in the handling of 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 homologs 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 homolog 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 nonenzymatic 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, 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 bait, 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 copurified 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 abundance 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 colocalize 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

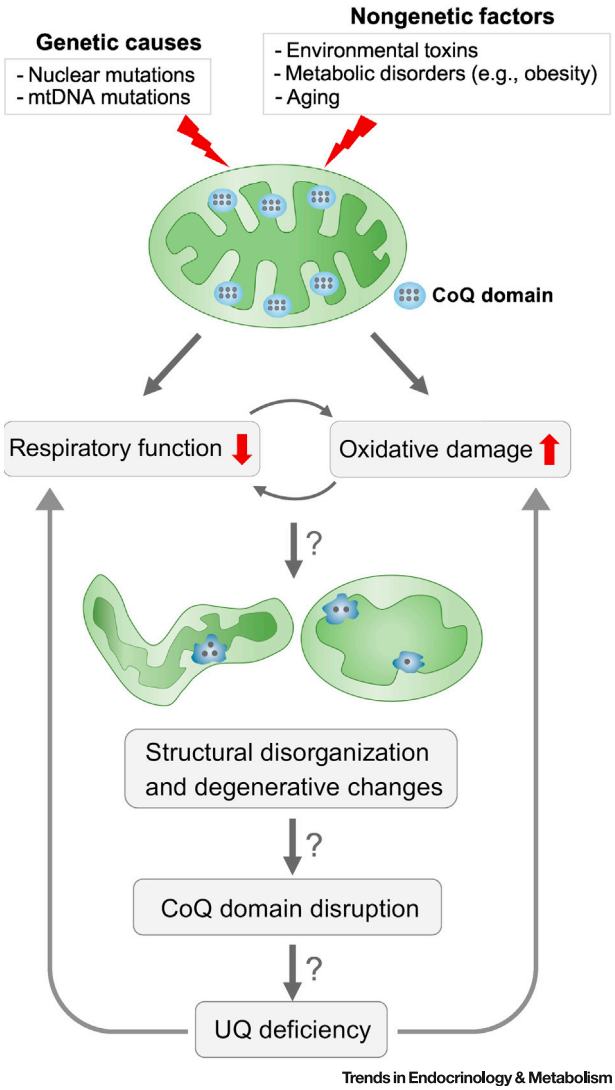


Figure 3. A Conceptual Model of Secondary Ubiquinone (UQ) Deficiency in Mitochondrial Disease.
Genetic mutations, environmental effects, or the aging process all can inflict damage on mitochondria. Severe mitochondrial dysfunction induces structural disorganization and degenerative changes of the mitochondrial membranes (especially the inner mitochondrial membrane). 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.

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 endoplasmic reticulum (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 nonvesicular 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 the 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 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 CIII 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 downregulation 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 localized 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% of 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 are starting 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 (see Outstanding Questions). For example, for some genes and some mutations, it was shown that it might be possible to use precursor analogs to regain better UQ levels [11,40,41,75,99]. 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. Yet, for patients with severe mitochondrial defects, including ultrastructural defects, effective supplementation of exogenous UQ might be the only possible treatment option.

Outstanding Questions

What exactly are the nonenzymatic 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?

Acknowledgments

Research in the laboratory of S.H. is funded by a Foundation grant from the Canadian Institutes of Health Research: FDN-159916, as well as by McGill University. S.H. is Campbell Chair of Developmental Biology.

References

1. Sun, I.L., *et al.* (1992) Requirement for coenzyme Q in plasma membrane electron transport. *Proc. Natl. Acad. Sci. U. S. A.* 89, 11126–11130
2. Turunen, M., *et al.* (2004) Metabolism and function of coenzyme Q. *Biochim. Biophys. Acta* 1660, 171–199
3. Crane, F.L. (2007) Discovery of ubiquinone (coenzyme Q) and an overview of function. *Mitochondrion* 7, S2–S7
4. Gille, L., and Nohl, H. (2000) The existence of a lysosomal redox chain and the role of ubiquinone. *Arch. Biochem. Biophys.* 375, 347–354
5. Wang, Y., and Hekimi, S. (2016) Understanding ubiquinone. *Trends Cell Biol.* 26, 367–378
6. Bentinger, M., *et al.* (2007) The antioxidant role of coenzyme Q. *Mitochondrion* 7, S41–S50
7. Hernandez-Camacho, J.D., *et al.* (2018) Coenzyme Q10 supplementation in aging and disease. *Front. Physiol.* 9, 44
8. Hargreaves, I.P. (2014) Coenzyme Q10 as a therapy for mitochondrial disease. *Int. J. Biochem. Cell Biol.* 49, 105–111
9. Milenkovic, D., *et al.* (2017) The enigma of the respiratory chain supercomplex. *Cell Metab.* 25, 765–776
10. Wang, Y., and Hekimi, S. (2013) Molecular genetics of ubiquinone biosynthesis in animals. *Crit. Rev. Biochem. Mol. Biol.* 48, 69–88
11. Wang, Y., *et al.* (2015) Mitochondrial function and lifespan of mice with controlled ubiquinone biosynthesis. *Nat. Commun.* 6, 6393
12. Wang, Y., and Hekimi, S. (2013) Mitochondrial respiration without ubiquinone biosynthesis. *Hum. Mol. Genet.* 22, 4768–4783
13. Garcia-Corzo, L., *et al.* (2013) Dysfunctional Coq9 protein causes predominant encephalomyopathy associated with CoQ deficiency. *Hum. Mol. Genet.* 22, 1233–1248
14. Fedor, J.G., and Hirst, J. (2018) Mitochondrial supercomplexes do not enhance catalysis by quinone channeling. *Cell Metab.* 28, 525–531
15. Diaz, F., *et al.* (2012) Cells lacking Rieske iron-sulfur protein have a reactive oxygen species-associated decrease in respiratory complexes I and IV. *Mol. Cell. Biol.* 32, 415–429
16. Stanley, I.A., *et al.* (2014) Changing appetites: the adaptive advantages of fuel choice. *Trends Cell Biol.* 24, 118–127
17. Acin-Perez, R., *et al.* (2014) ROS-triggered phosphorylation of complex II by Fgr kinase regulates cellular adaptation to fuel use. *Cell Metab.* 19, 1020–1033
18. Guaras, A., *et al.* (2016) The CoQH2/CoQ ratio serves as a sensor of respiratory chain efficiency. *Cell Rep.* 15, 197–209
19. Desbats, M.A., *et al.* (2015) Genetic bases and clinical manifestations of coenzyme Q10 (CoQ 10) deficiency. *J. Inherit. Metab. Dis.* 38, 145–156
20. Quinzii, C.M., *et al.* (2017) The role of sulfide oxidation impairment in the pathogenesis of primary CoQ deficiency. *Front. Physiol.* 8, 525
21. Cape, J.L., *et al.* (2007) A semiquinone intermediate generated at the Qo site of the cytochrome bc1 complex: importance for the Q-cycle and superoxide production. *Proc. Natl. Acad. Sci. U. S. A.* 104, 7887–7892
22. Quinzii, C.M., *et al.* (2008) Respiratory chain dysfunction and oxidative stress correlate with severity of primary CoQ10 deficiency. *FASEB J.* 22, 1874–1885
23. Quinzii, C.M., *et al.* (2012) Effects of inhibiting CoQ10 biosynthesis with 4-nitrobenzoate in human fibroblasts. *PLoS One* 7, e30606

24. Wang, Y., and Hekimi, S. (2015) Mitochondrial dysfunction and longevity in animals: untangling the knot. *Science* 350, 1204–1207
25. Tran, U.C., and Clarke, C.F. (2007) Endogenous synthesis of coenzyme Q in eukaryotes. *Mitochondrion* 7, S62–S71
26. Meganathan, R. (2001) Ubiquinone biosynthesis in microorganisms. *FEMS Microbiol. Lett.* 203, 131–139
27. Quinzii, C.M., and Hirano, M. (2010) Coenzyme Q and mitochondrial disease. *Dev. Disabil. Res. Rev.* 16, 183–188
28. Emmanuele, V., *et al.* (2012) Heterogeneity of coenzyme Q10 deficiency: patient study and literature review. *Arch. Neurol.* 69, 978–983
29. Hughes, B.G., *et al.* (2017) Estimating the occurrence of primary ubiquinone deficiency by analysis of large-scale sequencing data. *Sci. Rep.* 7, 17744
30. Yubero, D., *et al.* (2016) Secondary coenzyme Q10 deficiencies in oxidative phosphorylation (OXPHOS) and non-OXPHOS disorders. *Mitochondrion* 30, 51–58
31. Montero, R., *et al.* (2013) Coenzyme Q(1)(0) deficiency in mitochondrial DNA depletion syndromes. *Mitochondrion* 13, 337–341
32. Fazakerley, D.J., *et al.* (2018) Mitochondrial CoQ deficiency is a common driver of mitochondrial oxidants and insulin resistance. *Elife* 7, e32111
33. Bentinger, M., *et al.* (2010) Coenzyme Q–biosynthesis and functions. *Biochem. Biophys. Res. Commun.* 396, 74–79
34. Stefely, J.A., *et al.* (2016) Mitochondrial protein functions elucidated by multi-omic mass spectrometry profiling. *Nat. Biotechnol.* 34, 1191–1197
35. Payet, L.A., *et al.* (2016) Mechanistic details of early steps in coenzyme Q biosynthesis pathway in yeast. *Cell Chem. Biol.* 23, 1241–1250
36. Marbois, B., *et al.* (2010) para-Aminobenzoic acid is a precursor in coenzyme Q6 biosynthesis in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 285, 27827–27838
37. Pierrel, F., *et al.* (2010) Involvement of mitochondrial ferredoxin and para-aminobenzoic acid in yeast coenzyme Q biosynthesis. *Chem. Biol.* 17, 449–459
38. Xie, L.X., *et al.* (2015) Resveratrol and para-coumarate serve as ring precursors for coenzyme Q biosynthesis. *J. Lipid Res.* 56, 909–919
39. Xie, L.X., *et al.* (2012) Overexpression of the Coq8 kinase in *Saccharomyces cerevisiae* coq null mutants allows for accumulation of diagnostic intermediates of the coenzyme Q6 biosynthetic pathway. *J. Biol. Chem.* 287, 23571–23581
40. Freyer, C., *et al.* (2015) Rescue of primary ubiquinone deficiency due to a novel COQ7 defect using 2,4-dihydroxybensoic acid. *J. Med. Genet.* 52, 779–783
41. Doimo, M., *et al.* (2014) Effect of vanillic acid on COQ6 mutants identified in patients with coenzyme Q10 deficiency. *Biochim. Biophys. Acta* 1842, 1–6
42. Widmeier, E., *et al.* (2019) Treatment with 2,4-dihydroxybenzoic acid prevents FSGS progression and renal fibrosis in podocyte-specific Coq6 knockout mice. *J. Am. Soc. Nephrol.* Published online February 8, 2019. https://doi.org/10.1681/ASN.2018060625
43. Johnson, A., *et al.* (2005) COQ9, a new gene required for the biosynthesis of coenzyme Q in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 280, 31397–31404
44. Clarke, C.F. (2011) Coq6 hydroxylase: unmasked and bypassed. *Chem. Biol.* 18, 1069–1070
45. Allan, C.M., *et al.* (2015) Identification of Coq11, a new coenzyme Q biosynthetic protein in the CoQ-synthome in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 290, 7517–7534
46. Lapointe, C.P., *et al.* (2018) Multi-omics reveal specific targets of the RNA-binding protein Puf3p and its orchestration of mitochondrial biogenesis. *Cell Syst.* 6, 125–135
47. Veling, M.T., *et al.* (2017) Multi-omic mitoprotease profiling defines a role for Oct1p in coenzyme Q production. *Mol. Cell* 68, 970–977
48. Xie, L.X., *et al.* (2011) Expression of the human atypical kinase ADCK3 rescues coenzyme Q biosynthesis and phosphorylation of Coq polypeptides in yeast coq8 mutants. *Biochim. Biophys. Acta* 1811, 348–360
49. Nguyen, T.P., *et al.* (2014) Molecular characterization of the human COQ5 C-methyltransferase in coenzyme Q10 biosynthesis. *Biochim. Biophys. Acta* 1841, 1628–1638
50. Heeringa, S.F., *et al.* (2011) COQ6 mutations in human patients produce nephrotic syndrome with sensorineural deafness. *J. Clin. Invest.* 121, 2013–2024
51. Ozeir, M., *et al.* (2011) Coenzyme Q biosynthesis: Coq6 is required for the C5-hydroxylation reaction and substrate analogs rescue Coq6 deficiency. *Chem. Biol.* 18, 1134–1142
52. Barkovich, R.J., *et al.* (1997) Characterization of the COQ5 gene from *Saccharomyces cerevisiae*. Evidence for a C-methyltransferase in ubiquinone biosynthesis. *J. Biol. Chem.* 272, 9182–9188
53. Marbois, B., *et al.* (2009) The yeast Coq4 polypeptide organizes a mitochondrial protein complex essential for coenzyme Q biosynthesis. *Biochim. Biophys. Acta* 1791, 69–75
54. Do, T.Q., *et al.* (2001) A defect in coenzyme Q biosynthesis is responsible for the respiratory deficiency in *Saccharomyces cerevisiae* abc1 mutants. *J. Biol. Chem.* 276, 18161–18168
55. Gonzalez-Mariscal, I., *et al.* (2014) The regulation of coenzyme q biosynthesis in eukaryotic cells: all that yeast can tell us. *Mol. Syndromol.* 5, 107–118
56. Stefely, J.A., and Pagliarini, D.J. (2017) Biochemistry of mitochondrial coenzyme Q biosynthesis. *Trends Biochem. Sci.* 42, 824–843
57. Floyd, B.J., *et al.* (2016) Mitochondrial protein interaction mapping identifies regulators of respiratory chain function. *Mol. Cell* 63, 621–632
58. Tsui, H.S., and Clarke, C.F. (2019) Ubiquinone biosynthetic complexes in prokaryotes and eukaryotes. *Cell Chem. Biol.* 26, 465–467
59. He, C.H., *et al.* (2014) Coenzyme Q supplementation or over-expression of the yeast Coq8 putative kinase stabilizes multi-subunit Coq polypeptide complexes in yeast coq null mutants. *Biochim. Biophys. Acta* 1841, 630–644
60. Marbois, B., *et al.* (2005) Coq3 and Coq4 define a polypeptide complex in yeast mitochondria for the biosynthesis of coenzyme Q. *J. Biol. Chem.* 280, 20231–20238
61. Hsieh, E.J., *et al.* (2007) *Saccharomyces cerevisiae* Coq9 polypeptide is a subunit of the mitochondrial coenzyme Q biosynthetic complex. *Arch. Biochem. Biophys.* 463, 19–26
62. Leonard, C.J., *et al.* (1998) Novel families of putative protein kinases in bacteria and archaea: evolution of the "eukaryotic" protein kinase superfamily. *Genome Res.* 8, 1038–1047
63. Stefely, J.A., *et al.* (2016) Cerebellar ataxia and coenzyme Q deficiency through loss of unorthodox kinase activity. *Mol. Cell* 63, 608–620
64. Reidenbach, A.G., *et al.* (2018) Conserved lipid and small-molecule modulation of COQ8 reveals regulation of the ancient kinase-like UbiB family. *Cell Chem. Biol.* 25, 154–165

65. Kuhl, I., *et al.* (2017) Transcriptomic and proteomic landscape of mitochondrial dysfunction reveals secondary coenzyme Q deficiency in mammals. *Elife* 6, e30952
66. He, C.H., *et al.* (2015) Yeast Coq9 controls deamination of coenzyme Q intermediates that derive from para-aminobenzoic acid. *Biochim. Biophys. Acta* 1851, 1227–1239
67. Ozeir, M., *et al.* (2015) Coq6 is responsible for the C4-deamination reaction in coenzyme Q biosynthesis in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 290, 24140–24151
68. Duncan, A.J., *et al.* (2009) A nonsense mutation in COQ9 causes autosomal-recessive neonatal-onset primary coenzyme Q10 deficiency: a potentially treatable form of mitochondrial disease. *Am. J. Hum. Genet.* 84, 558–566
69. Danhauser, K., *et al.* (2016) Fatal neonatal encephalopathy and lactic acidosis caused by a homozygous loss-of-function variant in COQ9. *Eur. J. Hum. Genet.* 24, 450–454
70. Smith, A.C., *et al.* (2018) A family segregating lethal neonatal coenzyme Q10 deficiency caused by mutations in COQ9. *J. Inherit. Metab. Dis.* 41, 719–729
71. Herebian, D., *et al.* (2017) Detection of 6-demethoxyubiquinone in CoQ10 deficiency disorders: insights into enzyme interactions and identification of potential therapeutics. *Mol. Genet. Metab.* 121, 216–223
72. Lohman, D.C., *et al.* (2019) An isoprene lipid-binding protein promotes eukaryotic coenzyme Q biosynthesis. *Mol. Cell* 73, 763–774
73. Lohman, D.C., *et al.* (2014) Mitochondrial COQ9 is a lipid-binding protein that associates with COQ7 to enable coenzyme Q biosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* 111, E4697–E4705
74. Ashraf, S., *et al.* (2013) ADCK4 mutations promote steroid-resistant nephrotic syndrome through CoQ10 biosynthesis disruption. *J. Clin. Invest.* 123, 5179–5189
75. Wang, Y., *et al.* (2017) Pathogenicity of two COQ7 mutations and responses to 2,4-dihydroxybenzoate bypass treatment. *J. Cell. Mol. Med.* 21, 2329–2343
76. Jiang, N., *et al.* (2001) Mouse CLK-1 is imported into mitochondria by an unusual process that requires a leader sequence but no membrane potential. *J. Biol. Chem.* 276, 29218–29225
77. Miyadera, H., *et al.* (2001) Altered quinone biosynthesis in the long-lived clk-1 mutants of *Caenorhabditis elegans*. *J. Biol. Chem.* 276, 7713–7716
78. Burgess, J., *et al.* (2003) Molecular mechanism of maternal rescue in the clk-1 mutants of *Caenorhabditis elegans*. *J. Biol. Chem.* 278, 49555–49562
79. Lagier-Tourenne, C., *et al.* (2008) ADCK3, an ancestral kinase, is mutated in a form of recessive ataxia associated with coenzyme Q10 deficiency. *Am. J. Hum. Genet.* 82, 661–672
80. Subramanian, K., *et al.* (2019) Coenzyme Q biosynthetic proteins assemble in a substrate-dependent manner into domains at ER-mitochondria contacts. *J. Cell Biol.* 218, 1353–1369
81. Eisenberg-Bord, M., *et al.* (2019) The endoplasmic reticulum-mitochondria encounter structure complex coordinates coenzyme Q biosynthesis. *Contact (Thousand Oaks)* 2, 2515256418825409.
82. Phillips, M.J., and Voeltz, G.K. (2016) Structure and function of ER membrane contact sites with other organelles. *Nat. Rev. Mol. Cell Biol.* 17, 69–82
83. Hirano, M., *et al.* (2012) CoQ(10) deficiencies and MNGIE: two treatable mitochondrial disorders. *Biochim. Biophys. Acta* 1820, 625–631
84. Cotan, D., *et al.* (2011) Secondary coenzyme Q10 deficiency triggers mitochondria degradation by mitophagy in MELAS fibroblasts. *FASEB J.* 25, 2669–2687
85. Kuhlbrandt, W. (2015) Structure and function of mitochondrial membrane protein complexes. *BMC Biol.* 13, 89
86. Mourier, A., *et al.* (2015) Mitofusin 2 is required to maintain mitochondrial coenzyme Q levels. *J. Cell Biol.* 208, 429–442
87. Ruzzenente, B., *et al.* (2012) LRPPRC is necessary for polyadenylation and coordination of translation of mitochondrial mRNAs. *EMBO J.* 31, 443–456
88. Kuhl, I., *et al.* (2016) POLRMT regulates the switch between replication primer formation and gene expression of mammalian mtDNA. *Sci. Adv.* 2, e1600963
89. Spinazzi, M., *et al.* (2019) PARL deficiency in mouse causes Complex III defects, coenzyme Q depletion, and Leigh-like syndrome. *Proc. Natl. Acad. Sci. U. S. A.* 116, 277–286
90. Oka, T., *et al.* (2008) Identification of a novel protein MICS1 that is involved in maintenance of mitochondrial morphology and apoptotic release of cytochrome c. *Mol. Biol. Cell* 19, 2597–2608
91. Merkwirth, C., and Langer, T. (2008) Mitofusin 2 builds a bridge between ER and mitochondria. *Cell* 135, 1165–1167
92. Chen, H., *et al.* (2003) Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J. Cell Biol.* 160, 189–200
93. Smethurst, D.G.J., and Cooper, K.F. (2017) ER fatalities – the role of ER-mitochondrial contact sites in yeast life and death decisions. *Mech. Ageing Dev.* 161, 225–233
94. Rizzuto, R., *et al.* (2009) Ca(2+) transfer from the ER to mitochondria: when, how and why. *Biochim. Biophys. Acta* 1787, 1342–1351
95. Lapointe, J., *et al.* (2012) The submitochondrial distribution of ubiquinone affects respiration in long-lived Mclk1+/- mice. *J. Cell Biol.* 199, 215–224
96. Wang, J., *et al.* (1999) Dilated cardiomyopathy and atrioventricular conduction blocks induced by heart-specific inactivation of mitochondrial DNA gene expression. *Nat. Genet.* 21, 133–137
97. Camara, Y., *et al.* (2011) MTERF4 regulates translation by targeting the methyltransferase NSUN4 to the mammalian mitochondrial ribosome. *Cell Metab.* 13, 527–539
98. Milenkovic, D., *et al.* (2013) TWINKLE is an essential mitochondrial helicase required for synthesis of nascent D-loop strands and complete mtDNA replication. *Hum. Mol. Genet.* 22, 1983–1993
99. Hidalgo-Gutierrez, A., *et al.* (2019) beta-RA reduces DMQ/CoQ ratio and rescues the encephalopathic phenotype in Coq9 (R239X) mice. *EMBO Mol. Med.* 11, e9466
100. Aussel, L., *et al.* (2014) Biosynthesis and physiology of coenzyme Q in bacteria. *Biochim. Biophys. Acta* 1837, 1004–1011
101. Loiseau, L., *et al.* (2017) The UbiK protein is an accessory factor necessary for bacterial ubiquinone (UQ) biosynthesis and forms a complex with the UQ biogenesis factor UbiJ. *J. Biol. Chem.* 292, 11937–11950
102. Aussel, L., *et al.* (2014) UbiJ, a new gene required for aerobic growth and proliferation in macrophage, is involved in coenzyme Q biosynthesis in *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. *J. Bacteriol.* 196, 70–79
103. Hajj Chehade, M., *et al.* (2018) A soluble metabolon synthesizes the isoprenoid lipid ubiquinone. *Cell Chem. Biol.* 26, 482–492

104. Li, J., *et al.* (2001) Adrenodoxin reductase homolog (Arh1p) of yeast mitochondria required for iron homeostasis. *J. Biol. Chem.* 276, 1503–1509
105. Sheftel, A.D., *et al.* (2010) Humans possess two mitochondrial ferredoxins, Fdx1 and Fdx2, with distinct roles in steroidogenesis, heme, and Fe/S cluster biosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* 107, 11775–11780
106. Spiegel, R., *et al.* (2014) Deleterious mutation in FDX1L gene is associated with a novel mitochondrial muscle myopathy. *Eur. J. Hum. Genet.* 22, 902–906
107. Gurgel-Giannetti, J., *et al.* (2018) A novel complex neurological phenotype due to a homozygous mutation in FDX2. *Brain* 141, 2289–2298
108. Vogtle, F.N., *et al.* (2011) Mitochondrial protein turnover: role of the precursor intermediate peptidase Oct1 in protein stabilization. *Mol. Biol. Cell* 22, 2135–2143
109. Martin-Montalvo, A., *et al.* (2013) The phosphatase Ptc7 induces coenzyme Q biosynthesis by activating the hydroxylase Coq7 in yeast. *J. Biol. Chem.* 288, 28126–28137
110. Guo, X., *et al.* (2017) Ptc7p dephosphorylates select mitochondrial proteins to enhance metabolic function. *Cell Rep.* 18, 307–313
111. Awad, A.M., *et al.* (2017) Chromatin-remodeling SWI/SNF complex regulates coenzyme Q6 synthesis and a metabolic shift to respiration in yeast. *J. Biol. Chem.* 292, 14851–14866
112. Gonzalez-Mariscal, I., *et al.* (2018) The mitochondrial phosphatase PPTC7 orchestrates mitochondrial metabolism regulating coenzyme Q10 biosynthesis. *Biochim. Biophys. Acta Bioenerg.* 1859, 1235–1248
113. Zahedi, R.P., *et al.* (2006) Proteomic analysis of the yeast mitochondrial outer membrane reveals accumulation of a subclass of preproteins. *Mol. Biol. Cell* 17, 1436–1450
114. Cui, T.Z., and Kawamukai, M. (2009) Coq10, a mitochondrial coenzyme Q binding protein, is required for proper respiration in *Schizosaccharomyces pombe*. *FEBS J.* 276, 748–759
115. Barros, M.H., *et al.* (2005) The *Saccharomyces cerevisiae* COQ10 gene encodes a START domain protein required for function of coenzyme Q in respiration. *J. Biol. Chem.* 280, 42627–42635
116. Allan, C.M., *et al.* (2013) A conserved START domain coenzyme Q-binding polypeptide is required for efficient Q biosynthesis, respiratory electron transport, and antioxidant function in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* 1831, 776–791
117. Busso, C., *et al.* (2010) *Saccharomyces cerevisiae* coq10 null mutants are responsive to antimycin A. *FEBS J.* 277, 4530–4538
118. Zampol, M.A., *et al.* (2010) Over-expression of COQ10 in *Saccharomyces cerevisiae* inhibits mitochondrial respiration. *Biochem. Biophys. Res. Commun.* 402, 82–87
119. Tsui, H.S., *et al.* (2019) Human COQ10A and COQ10B are distinct lipid-binding START domain proteins required for coenzyme Q function. *J. Lipid Res.* 60, 1293–1310