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Biochemistry of Mitochondrial Coenzyme Q Biosynthesis

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Abstract

Coenzyme Q (CoQ, ubiquinone) is a redox active lipid produced across all domains of life that functions in electron transport and oxidative phosphorylation and whose deficiency causes human diseases. Yet, CoQ biosynthesis has not been fully defined in any organism. Several proteins with unclear molecular functions facilitate CoQ biosynthesis through unknown means, and multiple steps in the pathway are catalyzed by currently unidentified enzymes. Here we highlight recent progress toward filling these knowledge gaps through both traditional biochemistry and cutting-edge “omics” approaches. To help fill the remaining gaps, we present questions framed by the recently discovered CoQ biosynthetic complex and by putative biophysical barriers. Mapping CoQ biosynthesis, metabolism, and transport pathways has great potential to enhance treatment of numerous human diseases.

Keywords

Coenzyme Q; Ubiquinone; Biosynthesis; Metabolon; Protein Complex; complex Q; CoQ-synthome; Mitochondria; Lipids; Oxidative Phosphorylation; Mitochondrial Disease

Functions of Coenzyme Q and an Overview of its Biosynthesis

Coenzyme Q (CoQ; see **Glossary** and **Box 1**) plays a key role in an increasing number of biological processes, human diseases, and therapeutic regimens. While CoQ was discovered 60 years ago in Madison, Wisconsin [1] and Cambridge, England [2], gaps in our knowledge of CoQ biosynthesis, metabolism, and transport continue to limit our understanding of CoQ’s role in human disease and our ability to treat these diseases. Spurred by an influx of new experimental technologies merged with traditional biochemical approaches, such gaps in knowledge are now being filled rapidly, and these efforts have opened promising new therapeutic avenues.

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Box 1**Nomenclature of CoQ Biosynthesis**

CoQ₈ vs COQ8? The nomenclature of CoQ biochemistry involves subtle, yet meaningful, differences in capitalization.

CoQ

Coenzyme Q (CoQ), also known as ubiquinone (UQ), is the redox active lipid shown in Figure 1A. The names “CoQ” and “UQ” derive from its quinone head group (CoQ and ubiquinone), its function as a coenzyme (CoQ), and its nearly ubiquitous presence across all domains of life (ubiquinone). “CoQ” is the general abbreviation. To specify the number of isoprene subunits in the lipid tail, a subscripted number is included (e.g., “CoQ₁₀”, indicating 10 isoprene subunits). CoQ tail length varies by organism: humans predominantly produce CoQ₁₀; mice, CoQ₉; *E. coli*, CoQ₈; and *S. cerevisiae*, CoQ₆. The redox state of the lipid can also be specified: the fully reduced form (the hydroquinone) as “CoQH₂” (ubiquinol, UQH₂), the singly dehydrogenated radical form (the semiquinone) as “CoQH” (ubisemiquinone, UQH[•]), and the oxidized form (the quinone) as “CoQ” (ubiquinone, UQ) (Figure 1B). “Q” can also be found in the literature as an abbreviation for CoQ, but “CoQ” is favored to avoid confusion with the standard abbreviation for the amino acid glutamine (Q).

COQ8

COQ8 is an example of a protein required for CoQ biosynthesis. Eukaryotic genes and proteins required for CoQ biosynthesis are typically given “COQ” or “Coq” names, while their prokaryotic homologs typically have “Ubi” names (for ubiquinone). “COQ” or “Coq” proteins with the same numbers are typically homologs (e.g., human COQ5 and yeast Coq5p are homologs). Capitalization follows the standard format for each species (e.g., human proteins COQ8A and COQ8B, human genes *COQ8A* and *COQ8B*, yeast protein Coq8p, yeast gene *coq8*, *E. coli* protein UbiB, and *E. coli* gene *ubiB*).

Complex Q

The biosynthetic complex that produces CoQ is termed “complex Q” (a name analogous to that of the mitochondrial oxidative phosphorylation complexes I–V) or the “CoQ-synthome”. Complex Q is thought to contain proteins required for the terminal stage of CoQ biosynthesis (COQ3–COQ9), lipids (phospholipids and isoprenoid lipids), small molecule co-factors, and metal ions. However, a complete catalogue of the proteins, lipids, and metabolites that comprise complex Q remains to be determined.

CoQ is a lipid with exceptional biochemical properties (Figure 1). The chemical structure of CoQ includes an extraordinarily long polyisoprenoid lipid tail, which makes it one of the most hydrophobic molecules in life (Figure 1A). This tail is capped by a quinone head group, which is reduction-oxidation (redox) active (Figure 1B) [3, 4]. The redox activity of CoQ allows it to function as a cofactor for numerous enzymes, including those of the mitochondrial electron transport chain (ETC) (Figure 1C). In the ETC, CoQ relays electrons from oxidative phosphorylation (OxPhos) complexes I and II to complex III. The redox

chemistry of CoQ also involves protons (Figure 1B), the movement of which is part of the “Q-cycle” [5] that helps generate the proton motive force that drives ATP production via OxPhos.

In addition to CoQ’s fundamental role in OxPhos, which it performs across all domains of life [6], broader cellular functions for CoQ are increasingly recognized (Figure 1D). Within mitochondria, CoQ is a critical cofactor for uridine biosynthesis [7], fatty acid oxidation [8], and mitochondrial uncoupling proteins [9], and it is thought to regulate the permeability transition pore [10]. Moreover, an increasing number of extra-mitochondrial functions are coming to light for CoQ, which is found in nearly all eukaryotic cellular membranes [11]. For example, CoQ functions as a lipophilic antioxidant in yeast and in human plasma lipoproteins [12–14] and as a membrane stabilizer [15]. Furthermore, plastoquinone, a plant CoQ analog, can act as a retrograde chloroplast-to-nucleus signal [16], suggesting that mitochondrial CoQ may play an analogous role.

Given its widespread cellular functions, CoQ has powerful and complex effects on health and disease. On one hand, genetic defects in CoQ biosynthesis can cause diseases such as myopathies and ataxias (Box 2), and CoQ abundance decreases with age [17]. On the other hand, genetic disruption of CoQ biosynthesis or dietary depletion of CoQ in *C. elegans* [18–21] or mice [22] can increase lifespan. The complex mechanisms driving these contrasting effects remain largely undefined [23], in part because of numerous gaps in our understanding of CoQ biochemistry.

Box 2

CoQ Deficiency in the Clinic

Primary CoQ deficiencies—genetic diseases due to mutations in genes encoding CoQ biosynthesis proteins—are known to be caused by nuclear DNA mutations in *COQ2* [145–148], *COQ4* [149, 150], *COQ6* [151, 152], *COQ7* [153], *COQ8A* (*ADCK3*) [136, 154–158], *COQ8B* (*ADCK4*) [118, 159], *COQ9* [160, 161], *PDSS1* [162], or *PDSS2* [163, 164] (Figure 1A). These diseases variably disrupt multiple organ systems.

Commonly affected organs include the brain (e.g., encephalopathy, seizures, or cerebellar ataxia), the heart (e.g., hypertrophic cardiomyopathy), the kidneys (e.g., nephrotic syndrome), and skeletal muscles (e.g., myopathy)—organs with high demand for mitochondrial oxidative phosphorylation.

Mutations in some *COQ* genes (e.g., *COQ2*) can affect nearly all organ systems. In contrast, other *COQ* gene mutations have more selective effects. For example, mutations in *COQ8A* (*ADCK3*) primarily disrupt brain function, with cerebellar ataxia as the predominant presentation, while mutations in *COQ8B* (*ADCK4*) primarily disrupt kidney function, with steroid resistant nephrotic syndrome (SNRS) as the primary feature. Why mutations in different *COQ* genes have such distinct effects remains unclear. Tissue-specific differences in gene expression or metabolism-regulated protein-protein interactions may play a role [87, 117].

Different mutations within an individual *COQ* gene can also have widespread clinical presentations with highly variable age of onset—ranging from the day of birth to late

adulthood—and differentially affected organ systems. Even the same *COQ* point mutation can present variably across different patients, potentially due to differences in heteroplasmic mitochondrial DNA (mtDNA) pools, which can affect mitochondrial-nuclear genome interactions. Together, these factors create an extremely complicated scenario for affected families, clinicians, and scientists.

CoQ deficiency can also occur secondary to mutations in genes not directly linked to CoQ biosynthesis such as *ETFDH* [165], *MUT* [166], *APTX* [167], and *BRAF* [168] (Figure IB), but the underlying mechanisms are unclear. Some drugs, such as statins, may also cause secondary CoQ deficiency [169], and gene-drug interactions have been suggested by case reports [170].

Over half of the reported clinical cases of CoQ deficiency lack a molecular diagnosis (an identified causative gene mutation) [171]. Recent biochemical work in model systems nominates additional genes as candidates for currently undefined primary CoQ deficiency syndromes (Figure IC), providing an important foundation for ongoing investigations. Further basic science studies are needed to complete the list of candidate *COQ* disease genes.

The outstanding knowledge gaps and highly variable gene-phenotype relationships of CoQ deficiencies are an important microcosm of similar multifactorial complexity observed in essentially all mitochondrial diseases [172]. Ongoing research has great potential to help guide treatment strategies (Box 3) and genetic counseling.

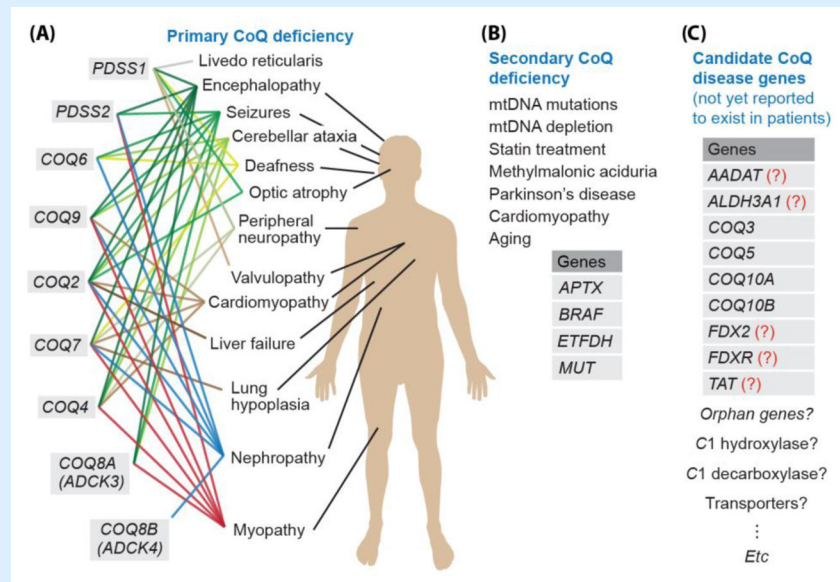


Figure I. CoQ Deficiency in Human Health and Disease

(A) Network of phenotypes and genes associated with primary CoQ deficiency. Lines indicate phenotypes reported to be caused by mutations in the indicate gene(s). (B) Diseases, conditions, drugs, and genes linked to secondary CoQ deficiency. (C) Candidate disease genes for potential primary CoQ deficiencies that have not yet been reported to exist in patients. “(?)” indicates an unproven ortholog relationship to a yeast gene that has been linked to CoQ biosynthesis. Additional uncharacterized (orphan)

genes may also be linked to CoQ biosynthesis (e.g., the gene that encodes the unidentified *C1* hydroxylase).

CoQ is synthesized endogenously by organisms across all domains of life [6]. Moreover, nearly every mammalian cell produces CoQ, likely because CoQ is poorly absorbed into cells and tissues [24]. The poor absorption of CoQ hinders treatment of CoQ deficiencies in the clinic, but new strategies to bypass this problem are under development (Box 3). Most eukaryotic CoQ is generated at the inner mitochondrial membrane [25], but some CoQ may be produced outside of mitochondria [26–28]. Here, we focus on eukaryotic mitochondrial CoQ biosynthesis, with an emphasis on CoQ production in mammals and in the yeast *Saccharomyces cerevisiae*, which has been a workhorse model organism for studying CoQ. However, many of the principles discussed also apply to prokaryotic [29, 30] and extra-mitochondrial CoQ production.

Box 3

Therapeutic Strategies for Treating CoQ Deficiency

Early diagnosis of primary CoQ deficiencies is critical because they are among the very few mitochondrial diseases that can be treatable. Therapy should be initiated as soon as possible to limit irreversible tissue damage [173]. The urgency is such that prior to diagnostic tests, newborn siblings of affected individuals have been treated [148].

The first-line therapy for CoQ deficiency is oral supplementation with high dose exogenous CoQ₁₀. Oral CoQ₁₀ treatment has been at least partially successful in a number of cases of primary CoQ deficiency including those caused by some mutations in *COQ2* [145, 173], *COQ4* [149], *COQ6* [151], *COQ7* [153], and *COQ8B* [118, 159]. However, clinical response to CoQ₁₀ is highly variable, and treatment failure is unfortunately common.

Delivering CoQ to specific cells in need, such as cerebellar Purkinje cells [87], is a major challenge. Exogenous CoQ is efficiently taken up by liver [174, 175], ovarian [176], and brown adipose tissues [177]. However, tissues commonly disrupted by CoQ deficiency, such as the brain and muscle (Box 2), poorly uptake CoQ [178] (Figure IA). Moreover, exogenous CoQ must traverse multiple subcellular compartments to reach the inner mitochondrial membrane (Figure IB). Work in yeast [79, 80, 179] and mouse [174, 175] model systems demonstrates that such CoQ transport is possible, with potential involvement of the endomembrane system [131, 132], but the precise mechanisms are unclear. A recent study showed improved delivery of reduced CoQ (CoQH₂) compared to oxidized CoQ [180]. However, uptake is still considered to be inefficient, and new strategies for enhancing CoQ delivery are needed.

Recently, an alternative treatment strategy has emerged that uses CoQ “bypass” precursors to sidestep enzymatic defects. This strategy leverages water soluble CoQ head group precursors, which are predicted to be more bioavailable than CoQ. Mice with defective *COQ7* were treated with 2,4-dihydroxybenzoate (2,4-DHB), an unnatural CoQ head group precursor, which was able to bypass the *COQ7*-catalyzed *C6*-hydroxylation,

elevate CoQ levels, and extend lifespan [181] (Figure IC). 2,4-DHB treatment was similarly effective in human fibroblasts with COQ7 mutations [153] and mice with COQ9 mutations [182]. Building on pioneering work in yeast [89], a similar strategy using vanillic acid or 3,4-dihydroxybenzoate can likewise bypass defects in COQ6 [152]. Importantly, this bypass strategy appears to be limited to *COQ* mutations that disrupt enzyme activity but maintain complex Q stability, and to select unnatural head group precursors [111].

New strategies for boosting CoQ levels could have widespread impact beyond treatment of primary CoQ deficiencies. CoQ₁₀ supplementation is ubiquitous in the treatment of diverse mitochondrial diseases [183], often as part of a “mitochondrial cocktail” [184], and CoQ₁₀ has been proposed as a therapy for numerous common diseases including Alzheimer’s, Parkinson’s, and Huntington’s diseases [185, 186].

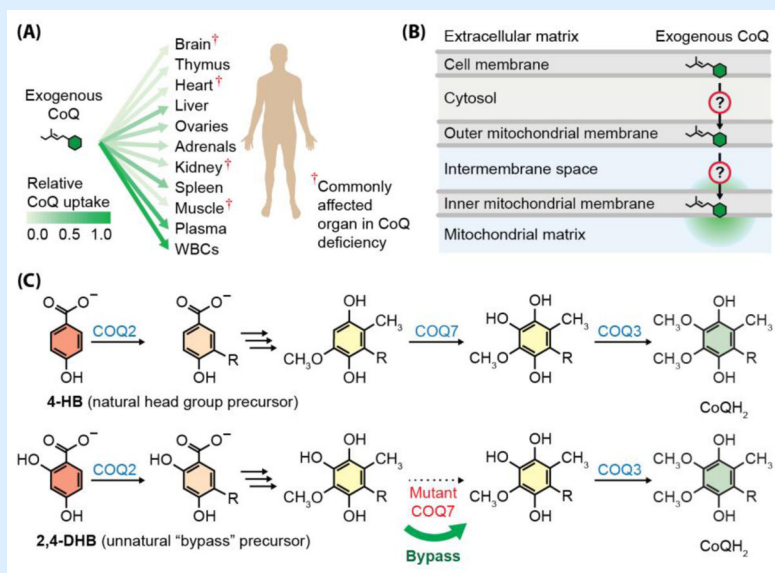


Figure 1. Strategies for Treatment of CoQ Deficiency

(A) Efficient uptake of exogenous CoQ into rat tissues after intraperitoneal injection is limited to white blood cells, liver, and spleen. Lesser uptake occurs in other tissues. Data in figure from Bentinger et al [178]. (B) Exogenous CoQ must be transported across multiple lipid membranes and multiple aqueous compartments to reach the mitochondrial inner membrane, but the mechanisms facilitating this CoQ transport are unclear. (C) An example of the “CoQ intermediate bypass strategy” for treating select CoQ deficiencies. 4-hydroxybenzoate (4-HB) is the natural CoQ head group precursor. 2,4-dihydroxybenzoate (2,4-DHB) be used as an alternative head group precursor to bypass a defect in COQ7 [181].

CoQ biosynthesis requires: (i) head group production, (ii) tail production—including isoprene biosynthesis and tail polymerization, (iii) attachment of the tail to the head group, and (iv) a series of head group modifications (Figure 2, Key Figure, and Table 1). In general, CoQ biosynthesis intermediates become increasingly lipophilic as they progress through the

pathway, which spans multiple cellular compartments and numerous “omic planes” (e.g., metabolome, lipidome), making it well-suited for investigation with emerging multi-omic technologies (Box 4). Many questions about CoQ biosynthesis remain unanswered, as framed here in the context of existing knowledge about each phase of the pathway.

Box 4

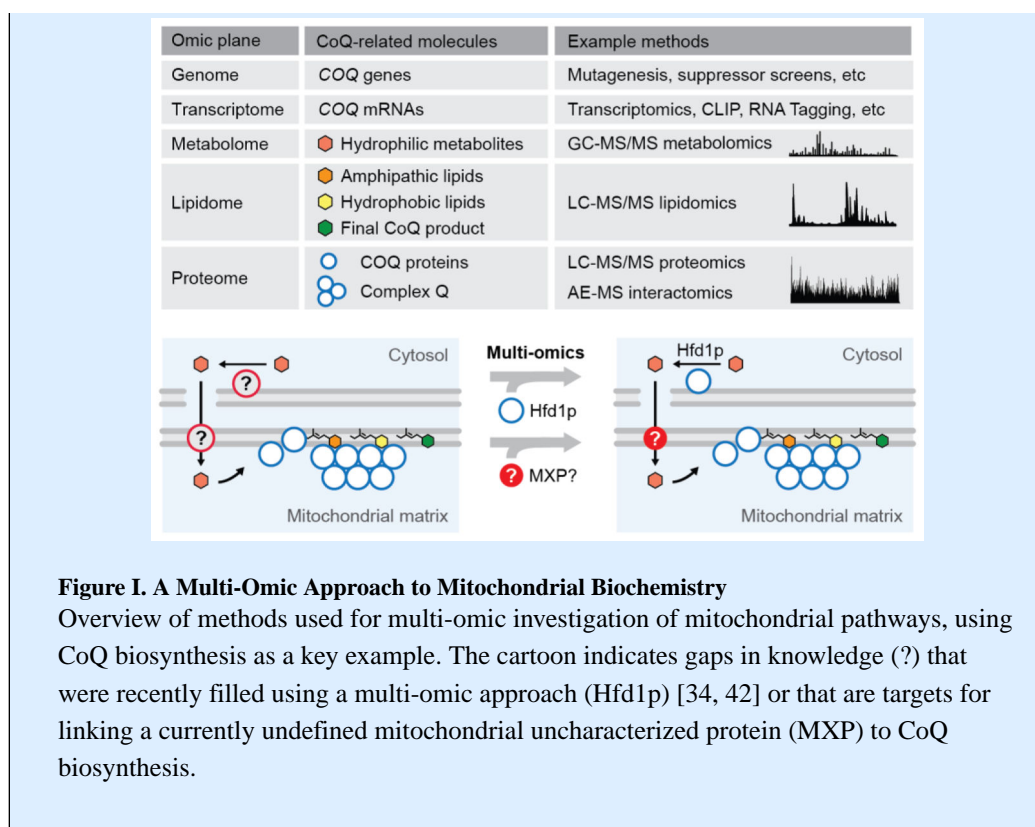
Multi-omic Investigation of Mitochondrial Pathways

A grand challenge in mitochondrial biology is to completely characterize the functions of all ~1,200 mammalian mitochondrial proteins, ~20% of which are currently “mitochondrial uncharacterized (x) proteins” (MXPs) [117]. Accomplishing this goal would enhance understanding of how mitochondria impact human health and disease. Proven approaches for characterizing MXPs include both traditional biochemical methods and cutting-edge “omics” methods (Figure I).

As complex organelles composed of dynamic and intertwined populations of proteins, lipids, and soluble metabolites, mitochondria are also prime targets for emerging “multi-omic” (“transomic”) approaches. Multi-omic investigations integrate data across multiple “omic planes” (Figure I), which can provide deeper and more confident insight than that of an individual omic method. Large-scale multi-omic approaches were recently used to discover previously unknown facets of mitochondrial biochemistry in mice [187] and yeast [42].

The CoQ biosynthesis pathway provides a salient example of a multi-omic mitochondrial process. CoQ-related molecules span numerous omic planes (Figure I). Early CoQ precursors are small soluble metabolites, the terminal CoQ intermediates are lipids, and COQ proteins (e.g., the enzymes in the pathway) form a protein complex (complex Q) and are encoded by *COQ* mRNAs and *COQ* genes. As such, the outstanding gaps in knowledge about CoQ biosynthesis are aptly positioned for study using multi-omics.

A large-scale multi-omic project in yeast recently uncovered a role for the enzyme Hfd1p in producing the CoQ head group precursor 4-hydroxybenzoate (4-HB) (Figure I) [42]. Notably, this function of Hfd1p was independently validated and co-discovered via an orthogonal genetic approach [34]. The discovery via multi-omics hinged on integration of both metabolomics, which uncovered diagnostic changes in the metabolites 4-HB and 4-hydroxybenzaldehyde, and lipidomics, which revealed telltale changes in lipid CoQ intermediates. The large-scale nature of this work also helped highlight the biological importance of a statistically significant, but small in magnitude, decrease in 4-HB observed in yeast that lack Hfd1p because this change was unique across all strains in the study. We hypothesize that similar multi-omic approaches will be central to filling the remaining knowledge gaps in CoQ biosynthesis and, more broadly, key for completely mapping mitochondrial biology.



CoQ Head Group Biosynthesis

In mammals, the CoQ head group is derived from the essential amino acid phenylalanine, which is converted into tyrosine (Tyr) and, subsequently, 4-hydroxybenzoate (4-HB) [31–33], but the responsible enzymes are unidentified (Figure 2). A Tyr-to-4-HB pathway also operates in yeast [34]. However, unlike mammals, bacteria and yeast can produce 4-HB *de novo* through the shikimate pathway [30, 35–38]. Yeast can also use *p*-aminobenzoate (pABA) as an alternative head group precursor [39, 40]. Because typical yeast culture media contain either 4-HB (in rich media) or pABA (in synthetic media), the Tyr-to-4-HB pathway is often not required for growth under laboratory conditions. It is likely for this reason that the responsible genes have been bypassed in many genetic screens. Recognizing this technical barrier and using distinct methods, two independent studies recently identified the first and last enzymes in the yeast Tyr-to-4-HB pathway [34, 41, 42].

Tyrosine Transamination by Aro8p or Aro9p

The yeast aromatic amino acid transaminases Aro8p and Aro9p were recently linked to the first step of the Tyr-to-4-HB pathway, initially through either hypothesis-driven heavy atom tracing studies [34] or unbiased global protein co-expression studies [42] (Figure 2). The analogous mammalian enzymes remain unidentified, but the Aro8p/Aro9p homolog AADAT and the tyrosine aminotransferase TAT are candidates.

4-Hydroxybenzaldehyde Dehydrogenation by Hfd1p

While steps after the tyrosine transamination remain unclear, Hfd1p was recently discovered to catalyze the final step in the Tyr-to-4-HB pathway—dehydrogenation of 4-hydroxybenzaldehyde (4-HBz) to generate 4-HB (Figure 2) [34, 42]. One of four human co-orthologs of Hfd1p, ALDH3A1, was also shown to catalyze 4-HB production *in vitro* and to rescue the growth defect of yeast that lack Hfd1p. However, whether ALDH3A1 participates in endogenous CoQ biosynthesis in mammalian cells remains unknown. The independent and complementary approaches used to discover this activity for Hfd1p and ALDH3A1 are summarized in Box 4.

Because Hfd1p is localized to the mitochondrial outer membrane [43] and exogenous 4-HB can rescue CoQ production in yeast lacking Hfd1p, we predict that 4-HB is the head group precursor that is transported across the inner mitochondrial membrane into the mitochondrial matrix, but this has not yet been demonstrated. Moreover, the putative mitochondrial membrane transporter for the head group precursor remains unidentified (Figure 2).

Biosynthesis and Attachment of the Polyisoprenoid Tail

In mammals, the isoprene subunits for CoQ biosynthesis are generated through the mevalonate pathway [32, 44–46], stitched together by head-to-tail polymerization [47, 48], and attached to the head group via an electrophilic aromatic substitution [49] (Figure 2). The responsible prenyltransferases, Coq1p (PDSS1 and PDSS2) and Coq2p (COQ2) (see **Glossary** and Box 1 for “COQ” nomenclature) are localized to the inner mitochondrial membrane [25, 50–59], a key point marking the switch from cytosolic precursor generation to mitochondrial CoQ biosynthesis. Coq1p, which independently determines the CoQ tail length [60], is peripherally associated with the matrix face of the inner mitochondrial membrane [56], while Coq2p is an integral membrane protein [59, 61].

The mechanism by which the highly polar isopentenyl pyrophosphate (IPP) molecule is transported from the cytosol to the mitochondrial matrix is unknown. We hypothesize that an IPP transporter exists in the inner mitochondrial membrane (Figure 2), but it remains unidentified. An allylic pyrophosphate is also needed for the isoprene polymerization reactions. *In vitro* evidence suggests that Coq1p can use dimethylallyl pyrophosphate (DMAPP), geranyl pyrophosphate (GPP), or farnesyl pyrophosphate (FPP) [51], but which is used *in vivo* is unclear. DMAPP could potentially be formed by IPP isomerase activity, which has been inferred to exist in mitochondria [25] (Figure 2), but the presence and identity of this enzyme is unclear. If an IPP isomerase is not active in the mitochondrial matrix, then a transporter for the allylic pyrophosphate is also likely to exist.

The Terminal Stage of CoQ Biosynthesis – Head Group Modifications

In the terminal stage of CoQ biosynthesis, the head group is modified by a decarboxylation and a series of hydroxylations and methylations (Figure 2). In bacteria and in yeast, the added methyl groups and hydroxyl groups derive from *S*-adenosyl methionine (SAM) [62] and O₂ [63, 64], respectively, and the same is predicted to be true in mammals. The

proposed order of these reactions is based on isolation of CoQ intermediates from various strains of bacteria [65–69] and yeast [52, 53, 70–72].

Hypothesized Chemical Logic

The proposed sequence of CoQ head group modifications (Figure 2) follows the chemical logic of electrophilic aromatic substitution (EAS) reactions. First, the *C*4-hydroxyl of 4-HB acts as an *ortho*-directing group to enhance EAS at *C*3 and *C*5. Second, the *C*4-hydroxyl acts as a *para*-directing group to enhance EAS at *C*1. Third, the newly installed *C*1-hydroxyl group acts as an *ortho*-directing group to enhance EAS at *C*2 and *C*6. This EAS chemistry, combined with substrate orientation in enzyme active sites, likely affords the regioselectivity needed to produce CoQ, yet these hypotheses are untested *in vitro*. A precise understanding of this chemistry could assist design of new CoQ intermediate “bypass precursors” for treating primary CoQ deficiencies (Box 3).

Sub-cellular Location of COQ Proteins

The proteins required for the terminal phase of CoQ production localize to the mitochondrial matrix. Mammalian COQ3–COQ9 were localized to mitochondria in the “MitoCarta” study [57], and COQ3, COQ5, COQ6, COQ8A/B, and COQ9 were localized to the mitochondrial matrix via an “APEX” study [58]. Biochemical fractionation studies localized yeast Coq3p–Coq9p to the mitochondrial matrix and, importantly, showed that they are peripherally associated with the matrix face of the inner membrane [59, 73–84]. An exception to this peripheral association is COQ8A, which has a transmembrane helix [85–87], although its yeast ortholog Coq8p may be peripherally associated [59, 81].

COQ enzymes might also co-localize to extra-mitochondrial locations. A nuclear form of COQ7 was suggested to function in mitochondrial-nuclear retrograde signaling [88], but this remains debated [21]. Additionally, the COQ2 homolog UBIAD1 localizes to the Golgi membrane, where it supports Golgi CoQ production [28]. Whether the downstream CoQ biosynthesis proteins (COQ3–9) also localize to the Golgi is unclear, but, interestingly, Golgi dysmorphology was observed in mice that lack COQ8A [87].

C5-Hydroxylation by COQ6

The *C*5-hydroxylation is catalyzed by COQ6 in eukaryotes [89] (Figure 2) and UbiI in prokaryotes [90]. Coq6p is a predicted flavin-dependent monooxygenase that uses flavin adenine dinucleotide (FAD) as a cofactor and NAD(P)H as a coenzyme for reduction of FAD [89]. A structure of truncated UbiI revealed a Rossmann-like fold—a widespread structural fold that can bind nucleotide cofactors—with a FAD binding site [90], but no *in vitro* enzymology was reported.

In yeast, the *C*5-hydroxylation requires the ferredoxin Yah1p—an essential iron-sulfur protein that mediates electron transfers in numerous pathways—and the ferredoxin reductase Arh1p [40], which are predicted to provide electrons to Coq6p (Figure 2). FDXR and FDX2 are the mammalian orthologs of Arh1p and Yah1p, respectively, but whether they function in mammalian CoQ biosynthesis is unknown.

O-Methylation by COQ3

The *O*-methylations are catalyzed by COQ3 in eukaryotes [70, 91, 92] and UbiG in prokaryotes [73, 93–95]. Purified UbiG or mitochondrial extracts containing COQ3 can catalyze both CoQ head group *O*-methylations *in vitro* in a reaction that depends on SAM and a divalent cation (e.g., Zn²⁺ or Co²⁺) [73, 92, 94, 95]. The molecular basis for the regioselectivity of COQ3 for each of its reactions (e.g., selective methylation of the DMeQ C6-hydroxyl over the C1-hydroxyl, see Figure 2) is unknown.

An Unidentified Decarboxylase

UbiD catalyzes the decarboxylation in prokaryotes [96], but the eukaryotic decarboxylase remains unidentified. Evidence for the mammalian decarboxylation was demonstrated by lack of radioactivity in CoQ when [carbonyl-C¹⁴]-4-HBz was used as a precursor [97]. Polyprenyl-vanillic acid (Figure 2) has been isolated from a mutant yeast strain [71], suggesting that C5-methoxy group installation occurs before the decarboxylation. A second prokaryotic enzyme, UbiX, was recently shown to support the decarboxylation by generating a previously unknown prenylated-FMN (prFMN) cofactor used by UbiD [98–100]. Whether prFMN functions similarly in eukaryotes is unclear [101]. No sequence homolog of UbiD or UbiX exists in humans and, thus, the decarboxylation appears to be a mechanistic deviation between prokaryotes and eukaryotes. A possibility to consider is that the eukaryotic C1-hydroxylation could be part of the decarboxylation mechanism.

An Unidentified Hydroxylase

UbiH catalyzes the C1-hydroxylation in *Escherichia coli* [69], but the eukaryotic enzyme has not been identified. Unlike the C5-hydroxylation described above, the eukaryotic C1-hydroxylation does not require Arh1p and Yah1p and may not require FAD [40], suggesting a different enzyme mechanism. In contrast, prokaryotic UbiH is likely a flavin-dependent monooxygenase, as it shares 30% sequence identity with UbiI, the C5-hydroxylase described above [90].

C2-Methylation by COQ5

The C2-methylation is catalyzed by COQ5 in eukaryotes [75–77, 102] and UbiE in prokaryotes [103, 104]. Lysates containing Coq5p or UbiE can catalyze demethoxy-demethyl-CoQ (DDMQ) C2-methylation *in vitro* in a reaction that depends on SAM and NADH [75]. Why NADH enhances this reaction is unclear, but we hypothesize that NADH helps keep the head group in the reduced state, which would enhance electrophilic aromatic substitution. Recently, X-ray structures of Coq5p revealed a Rossmann-like fold with a SAM-dependent methyltransferase domain [105], but no *in vitro* activity was reported.

C6-Hydroxylation by COQ7

The C6-hydroxylation is catalyzed by COQ7 in eukaryotes [19, 79, 106, 107] and UbiF in prokaryotes [103]. COQ7 is a carboxylate-bridged diiron hydroxylase [107], and its mechanism involves NADH passing electrons through demethoxy-CoQ (DMQ) to the diiron center [108, 109]. In contrast, *E. coli* UbiF is predicted to be a flavin-dependent monooxygenase similar to UbiI and UbiH, the C5- and C1- hydroxylases described above

[90]. Why prokaryotes use highly similar enzymes for all three CoQ head group hydroxylations [110], but eukaryotes use different enzyme chemistries, is unknown.

The CoQ Biosynthetic Complex

The enzymes in the terminal phase of CoQ biosynthesis form a biosynthetic complex termed **complex Q** (or the “CoQ-synthome”), which is located on the matrix face of the inner mitochondrial membrane.

The presence of a yeast CoQ biosynthetic complex was initially suggested by genetic studies that showed interdependence between Coq proteins: deleting any one of the genes *coq3–9* caused depletion of numerous Coq proteins and accumulation of the same CoQ precursor, PPHB [72, 111, 112] (Figure 2). Subsequently, physical evidence for complex Q was uncovered through protein-protein interaction studies and native gel electrophoresis experiments [59, 84, 113–115]. These studies also demonstrated that lipids, including CoQ and CoQ intermediates, are part of complex Q [115].

Recently, evidence for a mammalian complex Q has also been revealed. Global mass spectrometry proteomics studies demonstrated that deletion of *Coq8a* or *Coq9* in mice causes selective and significant depletion of numerous COQ proteins [87, 116], not just that encoded by the deleted gene. Furthermore, a mitochondria-focused affinity enrichment mass spectrometry (AE-MS) study revealed a dynamic network of protein-protein interactions between COQ3–COQ9 [117]. Numerous complex Q interactions were also confirmed by cell-free protein expression and purification studies [117], and by immunoprecipitation-immunoblot studies of COQ5 [77], COQ8A [87], and COQ8B [118]. Additionally, native gel electrophoresis studies tracking COQ5 suggested the presence of a high molecular weight complex Q in human cells [102]. Select lipids and small molecules have also been shown to bind to individual complex Q proteins, such as COQ9 and COQ8A [87, 116], suggesting that mammalian complex Q contains nonproteinaceous components. Together, these studies provide robust support for complex Q in mammals.

Similar “metabolons”, defined as “supramolecular complexes of sequential metabolic enzymes and cellular structural elements” [119], have been observed in other systems, such as the tricarboxylic acid cycle [120], the purinosome [121], dhurrin biosynthesis [122], and steroid biosynthesis [123]. Identifying the functions of such metabolon organization remains an active area of research with multiple hypotheses. First, metabolons are thought to enhance flux via enzyme proximity, local concentration of substrates, or substrate channeling. Second, metabolons may sequester potentially toxic biosynthetic intermediates (e.g., redox active CoQ intermediates) or help keep potentially poisonous enzymes (e.g., the hydroxylase COQ7) from escaping and promiscuously disrupting other processes. Third, metabolons may be key for regulation via allosteric enzyme activation, post-translational modifications, or organizing assembly in time and space. Finally, metabolons could also help solve biophysical problems, such as the barriers to CoQ biosynthesis and transport discussed in the next section.

While numerous exciting questions about complex Q components, structure, assembly, regulation, and activity remain unanswered (Figure 3 and Outstanding Questions), the discovery of this CoQ metabolon has dramatically altered the way that we think about each individual COQ protein and the biosynthetic process as a whole.

Outstanding Questions Box

- What enzymes catalyze the undefined steps in the Tyr-to-4-HB pathway in yeast and in mammals?
- How are the CoQ biosynthesis precursors—4-HB and isoprenoid subunits—transported into mitochondria?
- What eukaryotic enzymes catalyze the *C1* decarboxylation and the *C1* hydroxylation of CoQ biosynthesis? In eukaryotes, does the *C1* hydroxylation occur before, after, or in concert with the *C1* decarboxylation?
- What are the precise biochemical functions of the MXP CoQs COQ4, COQ8, COQ9, COQ10, and Coq11p in CoQ biosynthesis?
- How are lipophilic CoQ intermediates extracted from the membrane to be acted upon by COQ enzymes?
- What proteins, lipids, and metabolites constitute complex Q? What is their stoichiometry?
- What is the three-dimensional structure of complex Q?
- How is complex Q anchored to the mitochondrial membrane?
- Can a functional complex Q be reconstituted *in vitro*?
- Does complex Q activity require its endogenous context on the inner mitochondrial membrane?
- What is the complex Q assembly pathway? Are assembly factors required? How is complex Q assembly seeded?
- What benefit does organizing the COQ proteins in a biosynthetic complex provide? Does complex Q enable substrate channeling? Does complex Q sequester CoQ intermediates or promiscuous COQ enzymes?
- Does complex Q localize to a specific region of the inner mitochondrial membrane?
- Does complex Q interact with any of the oxidative phosphorylation complexes?
- How is complex Q activity regulated and coordinated with other mitochondrial processes, such as mitochondrial biogenesis and OxPhos?
- How is CoQ abundance regulated through biosynthesis and turnover?
- How are COQ proteins regulated transcriptionally, post-transcriptionally, and post-translationally?

- Do canonical COQ proteins function in extra-mitochondrial CoQ biosynthesis?
- How is CoQ transported from the inner mitochondrial membrane to the outer mitochondrial membrane?
- How is CoQ transported from mitochondria to other organelles throughout the cell?

Biophysical Barriers in CoQ Biosynthesis

Transmembrane Transport of Polar Precursors and Proteins

The precursors for building CoQ are negatively charged small molecules that cannot passively cross the inner mitochondrial membrane (Figure 2). How these polar CoQ precursors are transported from their site of production outside of mitochondria into the mitochondrial matrix is unclear.

The COQ proteins, which are encoded by nuclear DNA, must also be transported into the mitochondrial matrix. Mitochondrial protein import and processing is a complex process that can help coordinate and regulate biochemical pathways [124]. Although some transcriptional regulators [125] and post-transcriptional coordinators [126] of CoQ biosynthesis have been identified, the specific mitochondrial import and processing pathways of COQ proteins are incompletely defined (Figure 3A). As leads for future work, the import of yeast Coq2p is mediated by the Tim9p-Tim10p complex [127], the import of mouse COQ7 requires a mitochondrial targeting sequence and ATP, but not the mitochondrial membrane potential [128], and a recent large-scale study linked the mitochondrial intermediate peptidase Oct1p to CoQ biosynthesis [42].

Lipophilic Quinone Inaccessibility

CoQ resides primarily in the midplane of the lipid bilayer [129, 130], between the distal ends of the phospholipid tails (Figure 3B). This creates a biophysical problem for its transport to membranes outside of the mitochondria, where it is known to be present [11]. We predict that a series of CoQ lipid transport pathways exist (Figure 3A), likely involving both specific lipid transport proteins and the endomembrane system [131, 132]. These CoQ transport pathways are also likely to function in the reverse process—transport of extracellular CoQ into mitochondria—which has important implications for treatment of CoQ deficiency via delivery of exogenous CoQ (see Box 3 for further discussion of this point and evidence for CoQ transport).

We predict that the hydrophobic quinone-containing CoQ intermediates (Figure 2) are also localized to the lipid bilayer midplane (Figure 3B). In contrast, CoQ biosynthesis enzymes are primarily peripheral membrane proteins, located at the membrane surface. How these peripheral membrane enzymes access substrates located deep in the midplane of the lipid bilayer is unclear (Figure 3B). One or more of the biochemically uncharacterized COQ proteins could potentially help solve these biophysical problems.

Uncharacterized Proteins in CoQ Biosynthesis

A number of genes that support CoQ biosynthesis encode proteins of unknown molecular function. These CoQ-related mitochondrial uncharacterized (x) proteins (**MXP**s) include COQ4, COQ8A (ADCK3), COQ8B (ADCK4), COQ9, COQ10A, COQ10B, and Coq11p.

COQ4 – Complex Q Scaffold?

Coq4p stabilizes complex Q and is required for efficient CoQ biosynthesis [113, 133], but the underlying mechanism is undefined. Coq4p has homologs in some proteobacteria, which share a motif predicted to bind a divalent cation [133], but the specific biochemical functions of Coq4p remain unclear. The current model is that COQ4 is a complex Q scaffold protein that binds both proteins and lipids, as suggested by an X-ray structure of a COQ4 homolog that co-crystallized with geranylgeranyl monophosphate (PDB **3KB4**).

COQ8 – ATPase or Kinase?

The ancient UbiB family of enzymes [134], which includes COQ8, is found across all domains of life. *E. coli* UbiB [96, 135], yeast Coq8p [80], and mammalian COQ8A (ADCK3) [87, 136] and COQ8B (ADCK4) [118] each enhance CoQ biosynthesis, but their precise biochemical activities remain obscure. Coq8p is required for complex Q integrity, and Coq8p overexpression has a remarkable ability to rescue complex Q stability in various mutant yeast strains that would otherwise be complex Q deficient [59, 111]. Yet, how Coq8p stabilizes complex Q is unknown. A physical and functional interaction between COQ8 and COQ5 may be particularly important, as suggested by robust physical interactions between COQ8A and COQ5 [87, 117], and the common occurrence of prokaryotic operons containing both *ubiB* and *ubiE* [30], the *COQ8* and *COQ5* orthologs, respectively.

Coq8p and its UbiB family members fit within the protein kinase-like (PKL) superfamily [137], which includes protein kinases, lipid kinases, and ATPases. Initially, Coq8p was hypothesized to be a protein kinase. Indeed, phosphorylation of Coq3p, Coq5p, and Coq7p is altered in *coq8* yeast [81, 82], but whether this effect is caused directly by the absence of Coq8p is unclear. More recently, an X-ray structure of COQ8A revealed a PKL fold with unique features that inhibit protein kinase activity and afford an unusual preference for binding ADP over ATP [86]. Furthermore, COQ8A was shown to lack canonical protein kinase activity *in trans*, and to instead bind lipid CoQ intermediates and have ATPase activity that is enhanced by binding to cardiolipin-containing liposomes [87, 138]. Based on these findings, new models for COQ8A function were presented [87], including the possibility that it acts primarily as an ATPase, or that its ATPase activity is indicative of small molecule or lipid kinase activity against an undiscovered substrate. Yet, precisely how COQ8's ATPase activity (or kinase activity) and lipid binding function might stabilize complex Q to enhance CoQ biosynthesis remains an open question.

COQ9 – Lipid Sensor or Precursor Presenter?

Coq9p and COQ9 stabilize complex Q through an unclear mechanism [83, 84, 116, 139]. Recently, an X-ray structure of COQ9 revealed a lipid-binding pocket that is important for function *in vivo* [116], but the endogenous lipid ligands remain undefined. Coq9p interacts

physically and functionally with Coq7p to enhance the C6-hydroxylation reaction [116]. Coq9p also appears to enhance Coq6p activity and the deamination of CoQ intermediates derived from pABA [64, 140]. Our current model is that COQ9 helps extract hydrophobic CoQ intermediates from the membrane and presents them to other COQ enzymes, such as COQ7. An alternative model is that COQ9 is a lipid sensor that allosterically regulates complex Q upon ligand binding.

COQ10 – Lipid Transporter?

Coq10p, COQ10A, and COQ10B localize to mitochondria [57, 141]. Coq10p contains a conserved lipid-binding domain that binds CoQ and CoQ intermediates *in vitro* [141, 142]. CoQ biosynthesis is less efficient in yeast with *coq10* mutations, and the CoQ produced is used less efficiently for OxPhos [142], but the molecular basis for these effects is unclear. Neither Coq10p nor Coq9p has a known homolog in *E. coli*. However, the recently discovered *E. coli* CoQ biosynthesis proteins UbiJ and UbiK, which physically bind each other and a lipid [143, 144], could be functional homologs of Coq10p or Coq9p.

Coq11p – Dehydrogenase or Reductase?

Yeast Coq11p (YLR290c) was recently linked to CoQ biosynthesis via immunoprecipitation studies of complex Q [115]. Unlike most *coq* gene deletion yeast strains, yeast lacking Coq11p retain respiratory growth and are not completely CoQ deficient [42, 115]. The primary sequence of Coq11p places it in the short chain dehydrogenase/reductase (SDR) superfamily [115], but its biochemical activity is unclear.

We hope that the specific questions and knowledge gaps highlighted by this review, especially those related to complex Q function and biophysical barriers (Figure 3), will help guide ongoing research to definitively characterize the molecular functions of these CoQ-related MXPs.

Concluding Remarks and Future Perspectives

In recent years, the known functions of CoQ in human health and disease have expanded, rekindling the drive to fully define its biosynthesis. CoQ now has numerous defined functions outside of its primary role in mitochondrial OxPhos, including regulation of mitochondrial and possibly extra-mitochondrial functions (Figure 1). Likewise, human diseases with links to CoQ biosynthesis have expanded to include common diseases such as Parkinson's disease, rare diseases such as a cerebellar ataxia, and even the aging process itself (Box 2), elevating the potential impact of therapies that boost CoQ levels (Box 3).

Given the importance of CoQ in human biology and the fact that it was discovered 60 years ago [1, 2], it may be surprising that we still do not fully understand its biosynthesis. This apparent disconnect highlights the challenges associated with studying CoQ biosynthesis, which include unstable membrane-bound proteins, fleeting biosynthetic intermediates, highly hydrophobic lipids, and numerous undefined biochemical components. Fortunately, advances in protein and lipid biochemistry, X-ray crystallography, mass spectrometry, and multi-omic systems biology (Box 4) are rising to meet the challenge. Yet, numerous knowledge gaps still exist (Figures 2 and 3 and Outstanding Questions). We hope that this

review—by defining gaps in knowledge about CoQ biosynthesis at the biochemical level—will catalyze further progress toward understanding CoQ in human health and disease.

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Glossary

CoQ

Coenzyme Q (CoQ) is the redox-active lipid product of the biosynthetic pathway discussed here. See Box 1 for further explanations of CoQ nomenclature.

COQ5

COQ5 is an example of a human enzyme required for CoQ biosynthesis.

Coq5p

Coq5p is an example of a yeast enzyme required for CoQ biosynthesis.

Complex Q (CoQ-synthome)

Complex Q, also known as the CoQ-synthome, is the biosynthetic complex that produces CoQ.

MXP

A mitochondrial uncharacterized (x) protein (MXP) is a protein that localizes to mitochondria but lacks a well-defined molecular or cellular function in mitochondria.

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Trends Box

- A biosynthetic complex for producing coenzyme Q (CoQ) was recently revealed in yeast and mammals. This complex likely contains proteins, lipids, and polar small molecules, but its precise composition, structure, and activities remain largely unknown.
- Multiple mitochondrial uncharacterized proteins (MXPs) have been linked to CoQ biosynthesis, and multiple steps in the pathway are enabled by currently unidentified proteins.
- Recent progress was made toward understanding the biochemistry of a dehydrogenase, a deaminase, a lipid-binding protein, and a protein kinase-like enzyme in the CoQ pathway.
- CoQ biosynthesis spans multiple “omic” planes—the metabolome, the lipidome, and the proteome—and thus has proven to be a prime target for investigation with emerging multi-omic technologies.
- Mapping CoQ biochemistry recently spurred new therapeutic strategies.

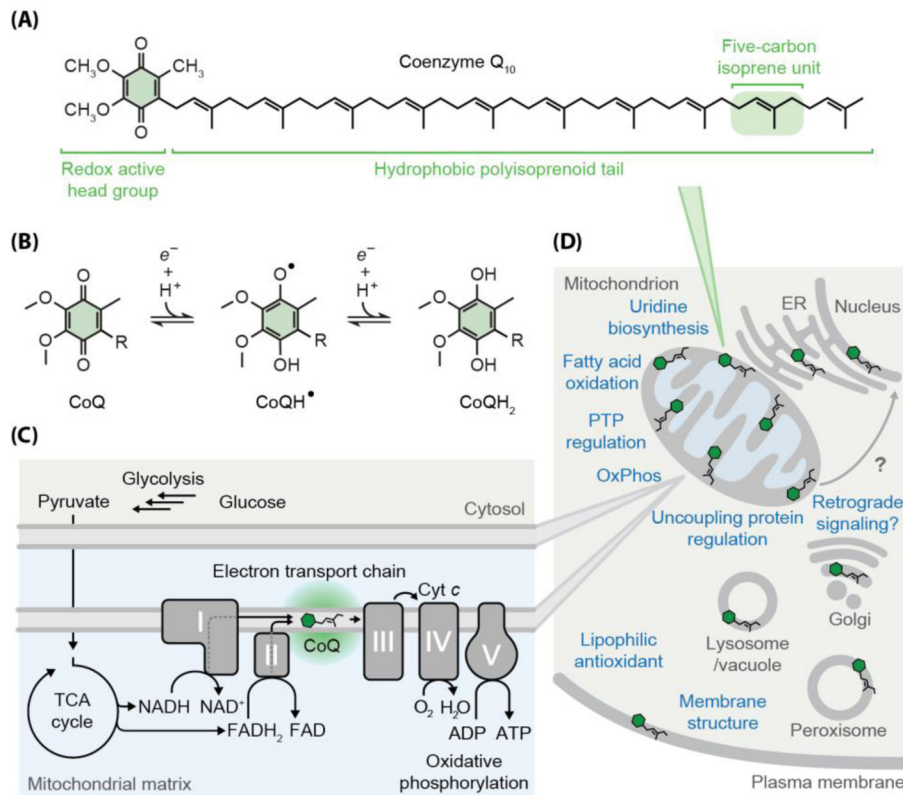


Figure 1. Chemical and Biological Functions of Coenzyme Q

(A) Chemical structure of coenzyme Q₁₀ (CoQ₁₀). (B) Reduction-oxidation reactions of the CoQ head group. "R" indicates the polyisoprenoid tail. (C) Cartoon indicating the central role of CoQ in the electron transport chain and mitochondrial oxidative phosphorylation (OxPhos). I–V, OxPhos complexes I–V; Cyt *c*, cytochrome *c*; TCA cycle, tricarboxylic acid cycle. (D) Overview of the widespread cellular functions of CoQ. Within mitochondria, CoQ supports and/or regulates the mitochondrial permeability transition pore (PTP) [10], mitochondrial uncoupling proteins [9], uridine biosynthesis [7], fatty acid oxidation [8], and OxPhos. More broadly, CoQ functions as a lipophilic antioxidant [12–14] and a membrane stabilizer [15]. In plants, plastoquinone functions as a retrograde signal from chloroplasts to the nucleus [16], suggesting that mitochondrial CoQ could play an analogous role.

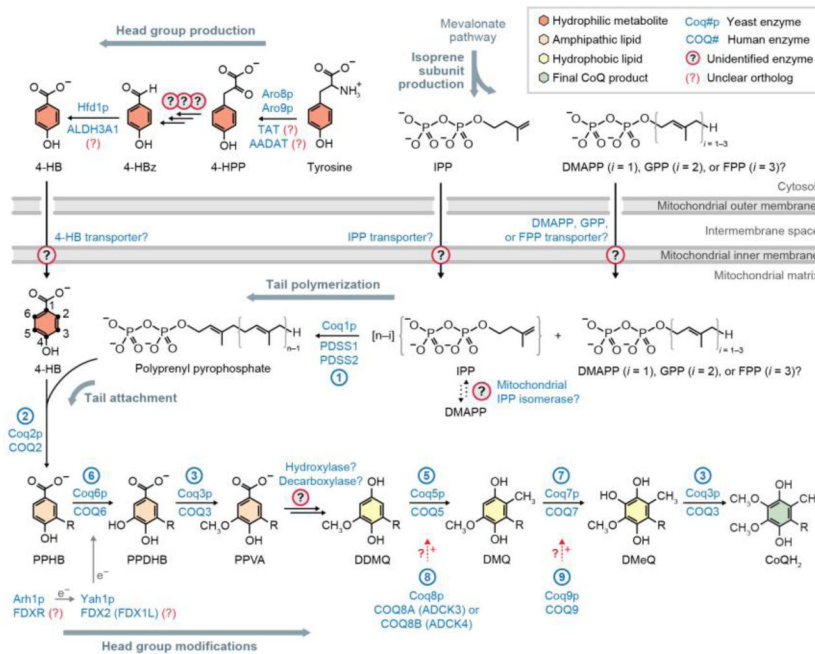


Figure 2. Current Model for the Eukaryotic CoQ Biosynthesis Pathway

Scheme of eukaryotic CoQ biosynthesis with currently unidentified enzymes indicated by question marks. The primary CoQ pathway, which is conserved from yeast to humans, is depicted. A secondary CoQ pathway in yeast uses *para*-aminobenzoate (pABA) as the head group precursor instead of 4-hydroxybenzoate (4-HB) [39, 40], and Coq6p and Coq9p subsequently support C4-deamination via C4-hydroxylation [64, 140]. ‘R’ indicates the polyisoprenoid tail (Figure 1A), which would likely be anchored in the mitochondrial inner membrane. “+” symbol by the arrow from COQ8 and COQ9 indicates a hypothesized supportive role for the indicated reaction. AADAT, mitochondrial alpha-aminoadipate aminotransferase; ALDH3A1, aldehyde dehydrogenase 3A1; FDXR, adrenodoxin reductase; FDX2 (FDX1L), mitochondrial ferredoxin 2 (ferredoxin 1-like); PDSS1, prenyl (decaprenyl) diphosphate synthase subunit 1; TAT, tyrosine aminotransferase; DMAPP, dimethylallyl pyrophosphate; GPP, geranyl pyrophosphate; FPP, farnesyl pyrophosphate; IPP, isopentenyl pyrophosphate; 4-HPP, 4-hydroxyphenylpyruvate; 4-HBz, 4-hydroxybenzaldehyde; PPHB, polyprenyl-hydroxybenzoate; PPDHB, polyprenyl-dihydroxybenzoate; PPVA, polyprenyl-vanillic acid; DDMQ, demethoxy-demethyl-coenzyme Q; DMQ, demethoxy-coenzyme Q; DMeQ, demethyl-coenzyme Q.

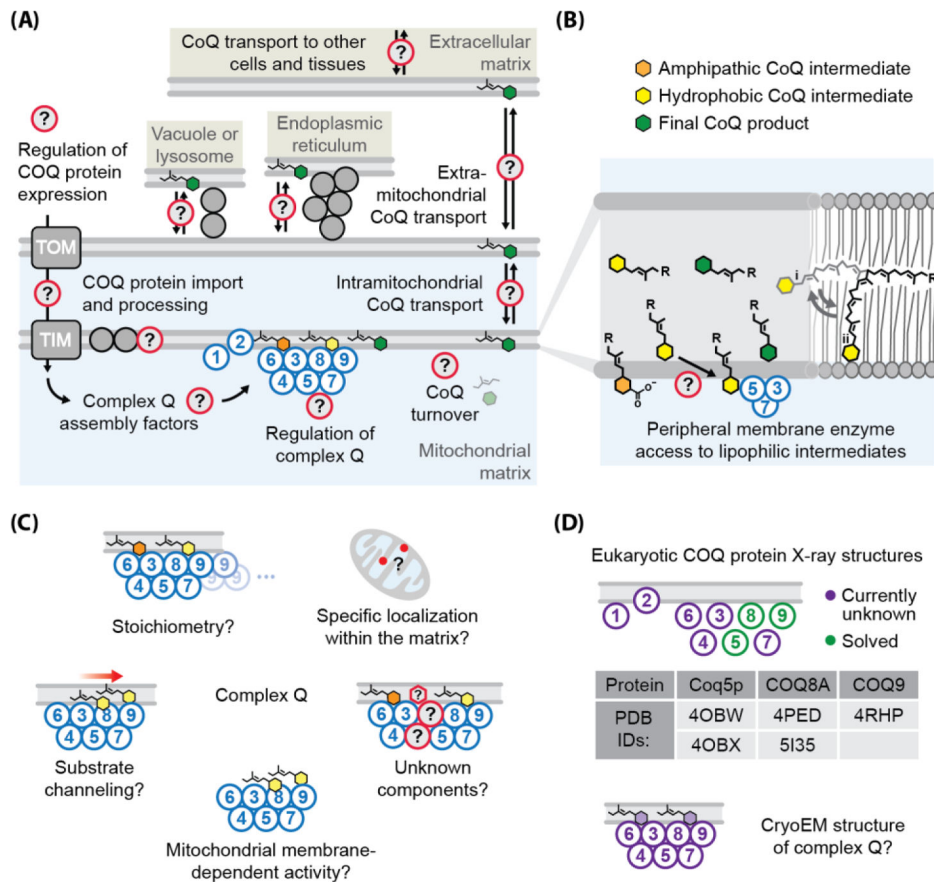


Figure 3. Models Framing Outstanding Questions and Knowledge Gaps in CoQ Biochemistry

(A) Model framing questions about complex Q assembly, regulation of CoQ biosynthesis, and mechanisms for CoQ transport. (B) Model for a biophysical barrier to accessing lipophilic CoQ intermediates, highlighting the currently unclear mechanism for solving this problem. CoQ and hydrophobic CoQ intermediates are predicted to reside near the lipid bilayer midplane (i) with some movement (gray arrows) toward the bilayer surface (ii), but not beyond the glycerol backbones [129, 130]. Biochemical mechanisms for moving CoQ intermediates past the layer of glycerol backbones and polar lipid head groups are unclear. (C) Models framing unanswered questions about complex Q biochemistry. (D) Summary of the currently incomplete state of complex Q structural biology. X-ray structures have been reported for Coq5p (PDB IDs **4OBW** and **4OBX**) [105], COQ8A (ADCK3) (PDB IDs **4PED** and **5I35**) [86] [87], and COQ9 (PDB ID **4RHP**) [116].

Table 1

Reactions and Enzymes of CoQ Biosynthesis

Reaction	Eukaryotic Reaction Diagram ^a	Cofactors in Eukaryotes	Mammalian Enzyme(s) ^b	Yeast Enzyme	Prokaryotic Enzyme(s) ^c	Refs
4-HBz dehydrogenation		NAD ⁺	ALDH3A1 (?)	Hfd1p	Unidentified	[34,42]
Isoprene polymerization		Mg ²⁺	PDSS1 and PDSS2	Coq1p	IspA and IspB	[54,163]
C1 - Hydroxylation		Unidentified	Unidentified	Unidentified	UbiH	[69,110]
C1 - Decarboxylation		Unidentified	Unidentified	Unidentified ^d	UbiD and UbiX	[96,99]
C2-Methylation		SAM, Mg ²⁺	COQ5	Coq5p	UbiE	[75,76,103]
C3- Prenylation		Mg ²⁺	COQ2	Coq2p	UbiA	[55,145,188]
C4- Deamination		FAD and NAD(P)H	N.A.	Coq6p	N.A.	[64]

Reaction	Eukaryotic Reaction Diagram ^a	Cofactors in Eukaryotes	Mammalian Enzyme(s) ^b	Yeast Enzyme	Prokaryotic Enzyme(s) ^c	Refs
C5- Hydroxylation		FAD and NAD(P)H	COQ6	Coq6p	UbiI	[89,90]
C6- Hydroxylation		Diiron center, NADH	COQ7	Coq7p	UbiF	[103,106,108]
O-Methylations		SAM, Zn ²⁺	COQ3	Coq3p	UbiG	[91-93]
ATP Hydrolysis or Phosphorylation?	 	Mg ²⁺	COQ8A (ADCCK3) and COQ8B (ADCCK4)	Coq8p	UbiB	[80,82,86,87,96,135]
Lipid Binding?		N.A.	COQ9	Coq9p	UbiI?	[83,116,139,144]
Lipid Transport?		N.A.	COQ10A and COQ10B	Coq10p	UbiI?	[141, 142, 144]
Electron Transfers		Fe-S cluster	FDX2 (FDX1L)	Yah1p	Unidentified	[40]
Electron Transfers		NADPH	FDXR	Arh1p	Unidentified	[40]
Head group Reduction?		Unidentified	Unidentified	Coq11p (?)	Unidentified	[115]
Head group Oxidation?		Unidentified	Unidentified	Coq11p (?)	Unidentified	[115]

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^g“R” indicates polyisoprenoid tail. 4-HB, 4-hydroxybenzoate; 4-HBz, 4-hydroxybenzaldehyde; PPHB, polyprenyl-hydroxybenzoate; PPDHB, polyprenyl-dihydroxybenzoate; PPVA, polyprenyl-vanillic acid; DDMQ, demethoxy-demethyl-coenzyme Q; DMQ, demethoxy-coenzyme Q; DMeQ, demethyl-coenzyme Q.

^hALDH3A1, aldehyde dehydrogenase 3A1; FDXR, adrenodoxin reductase; FDX2 (FDX1L), mitochondrial ferredoxin 2 (ferredoxin 1-like); PDSS1, prenyl (decaprenyl) diphosphate synthase subunit 1.

^cSome, but not all, of these prokaryotic enzymes are sequence homologs of the eukaryotic enzymes.

^dThe *Saccharomyces cerevisiae* genome, but not the human genome, encodes sequence homologs of *E. coli* UbiD and UbiX: Fdc1p and Pad1p, respectively. However, whether yeast Pad1p or Fdc1p functions in CoQ biosynthesis remains unclear [101]. Interestingly, a recent large-scale screen showed a significant increase in CoQ in yeast lacking Fdc1p when cultured under respiration conditions (lipidome data available at y3kproject.org) [42].