Coenzyme Q biochemistry and biosynthesis

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Abstract

Coenzyme Q (CoQ) is a remarkably hydrophobic, redox-active lipid that empowers diverse cellular processes. Although most known for shuttling electrons between mitochondrial electron transport chain (ETC) complexes, the roles for CoQ are far more wide-reaching and ever-expanding. CoQ serves as a conduit for electrons from myriad pathways to enter the ETC, acts as a cofactor for biosynthetic and catabolic reactions, detoxifies damaging lipid species, and engages in cellular signaling and oxygen sensing. Many open questions remain regarding the biosynthesis, transport, and metabolism of CoQ, which hinders our ability to treat human CoQ deficiency. Here, we recount progress in filling these knowledge gaps, highlight unanswered questions, and underscore the need for novel tools to enable discoveries and improve the treatment of CoQ-related diseases.

Keywords

Coenzyme Q; Ubiquinone; Complex Q; Oxidative Phosphorylation; Lipids; Mitochondria

Coenzyme Q in Biochemistry and Beyond

Coenzyme Q (**CoQ**, ubiquinone; see Glossary) is an essential redox-active lipid with extraordinary biochemical properties. Decades of research have linked CoQ to numerous cellular processes, human diseases, and therapeutic regimens, and recent efforts are revealing surprising new roles. Despite being discovered 65 years ago in Madison, WI, USA [1] and Liverpool, UK [2], many open questions regarding the biosynthesis, noncanonical function, and transport of CoQ continue to limit our ability to treat human diseases of

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None are declared by the authors

CoQ deficiency. We have previously provided an in-depth review of the biochemistry of CoQ synthesis [3]; here we will focus on an overview of the biosynthetic pathway, discuss the current state of knowledge on how CoQ is assimilated and distributed throughout the cell, and describe current efforts to overcome CoQ deficiency in disease, highlighting key findings in the past five years and underscoring aspects that remain unsolved.

Known Cellular Roles of Coenzyme Q

We begin by recounting established CoQ functions that highlight its diverse contributions to cellular homeostasis.

CoQ as a Mitochondrial Cofactor

CoQ performs its canonical role in the mitochondrial electron transport chain (ETC) by relaying electrons from oxidative phosphorylation (OxPhos) complexes I and II to complex III, helping to generate the proton motive force that drives ATP synthesis [3]. Although often overlooked, CoQ also accepts electrons as a cofactor for enzymes involved in pyrimidine biosynthesis, proline catabolism, glycolysis, fatty acid oxidation, and sulfide detoxification (Figure 1a), thereby providing diverse pathways for electrons to reach complex III [4]. In certain contexts, CoQ's cofactor function is paramount; for example, certain human cancer cells rely on complex III more for CoQ regeneration to drive **DHODH** activity and the TCA cycle than for assisting NAD+ regeneration or promoting ATP production [5]. Relatedly, a hypothesis surrounding CoQ segmentation has emerged, proposing that mitochondrial CoQ may exist in two functionally distinct pools: one population of CoQ that associates with respiratory super-complexes would receive their electrons from NADH via complex I, while the remaining freely diffusing IMM CoQ would receive electrons from FAD-dependent enzymes [6]. This topic is still highly debated, yet it is tempting to speculate that maintaining distinct CoQ pools might have important functional consequences, including modulating mitochondrial respiratory capacity and redox signaling [7].

Combatting Ferroptosis

Extramitochondrial CoQ has been implicated in combatting **ferroptosis**, a form of nonapoptotic cell death caused by iron-dependent phospholipid peroxidation [8]. Oxidative stress from environmental and metabolic stimuli can catalyze the formation of toxic phospholipid hydroperoxides (PLOOHs) from polyunsaturated fatty acids (PUFAs), resulting in a loss of membrane integrity, oxidative damage to macromolecules, and eventually cell death [9]. In mammals, GPX4, a phospholipid hydroperoxide glutathione peroxidase, provides a major form of defense against ferroptosis by neutralizing PLOOHs [10]. Recent studies identified a GPX4-independent mechanism of ferroptosis protection via FSP1, an oxidoreductase that reduces plasma membrane (PM) CoQ to the hydroquinone form (CoQH$_2$), which in turn suppresses PLOOHs [11,12].

Extramitochondrial Antioxidation

Before the discovery of FSP1, CoQ was long recognized as an important PM lipophilic antioxidant in both yeast and mammalian systems. The primary oxidoreductases responsible for maintaining the reduced PM CoQ pool are NAD(P)H:Quinone Oxidoreductase 1

(NQO1) and NADH-cytochrome b5 reductase (Cyb5Red) [13] (Figure 1b). NQO1 catalyzes the two-electron reduction of CoQ as part of the PM redox system, and can furthermore function as a superoxide reductase, generating NAD+ and modulating various signaling enzymes, including HIF1-α, p53, and sirtuins [14]. Cyb5Red, localized at both the PM and ER, is induced under stress conditions and catalyzes electron transfer from NADH to CoQ, thus contributing to the protective antioxidant pool of CoQ [15]. While this antioxidant function of Cyb5Red has been studied primarily at the PM [16], its presence in ER membranes suggests that it could likewise maintain the balance of $CoQ/CoQH_2$ in this organelle. Very little is known concerning how the redox state of CoQ is dictated in organellar membranes beyond mitochondria and the PM, nor the functional consequences of maintaining a precise $CoQ/CoQH_2$ ratio in these contexts. CoQ is also present in lysosomal membranes and can be reduced via a lysosomal ETC [17], although the proteins involved have yet to be identified. CoQ deficiency has been linked to aberrant lysosomal acidification [18], but how this relates to the lysosomal ETC is unknown.

Additional Understudied Roles of CoQ

Beyond its ETC, cofactor, and antioxidant activities, CoQ has been linked to mammalian energy regulation pathways, though these connections are often incompletely understood. Brown adipose tissue (BAT) can engage in facultative thermogenesis, whereby uncoupling proteins (UCPs) act to dissipate the mitochondrial proton gradient to release heat [19]. Early studies characterized CoQ as a requisite activator of proton transport by UCP1 [20] and UCP2/UCP3 [21] in reconstituted liposomes, though these findings have been disputed [22]. More recently, studies in BAT have demonstrated that the scavenger receptor (SR) protein CD36 is required for CoQ uptake and normal BAT function, with *Cd36–/–* mice exhibiting decreased CoQ content, defective non-shivering thermogenesis, and cold intolerance [23], yet the molecular mechanisms at play are still elusive. An emerging theme surrounding CoQ homeostasis is that the ratio of oxidized CoQ to reduced $CoQH_2$ can have important consequences in sensing and reflecting different metabolic states of the cell [24]. In mouse chemoreceptor cells, the $CoQH_2/CoQ$ ratio has been linked to acute oxygen sensing downstream of complex I-derived NADH and ROS [25]. Similarly, in states of hypoxia or impaired ETC in mice, high $CoQH_2/CoQ$ ratio can trigger reversal of complex II, resulting in the reduction of fumarate as an alternative electron acceptor [26]. Further work will be required to unravel if these may be mechanisms to link CoQ redox state to the regulation of nucleotide pools or downstream gene expression.

Coenzyme Q Biosynthesis and Regulation

CoQ is among the most hydrophobic molecules in nature, with a long polyisoprenoid lipid tail capped by a redox-active quinone head group (Figure 2a–b). These distinctive properties of CoQ present cells with significant challenges for its biosynthesis. Nonetheless, organisms across nearly all domains of life synthesize CoQ endogenously [27], with production in eukaryotes taking place primarily at the inner mitochondrial membrane (IMM) [28].

Recap of Biosynthetic Pathway and Updates

CoQ biosynthesis occurs in four stages: (i) head group production, (ii) isoprenoid tail production, (iii) attachment of the head group precursor to the tail, and (iv) head group modifications (Figure 2c). The universally conserved head group precursor, 4-hydroxybenzoate (4-HB), is derived from tyrosine in mammals, however yeast and bacteria can also produce it *de novo* via the shikimate pathway [3]. Though many of the steps in the tyrosine-to-4-HB pathway remain undefined, Hfd1p catalyzes the dehydrogenation of 4-hydroxybenzaldehyde (4-HBz) to 4-HB [29–31] in yeast. Efforts to elucidate missing enzymes in this pathway have encountered significant enzymatic redundancy (Box 1), with up to five aminotransferases capable of catalyzing the tyrosine transamination to 4-hydroxyphenylpyruvate (4-HPP) *in vivo* [31]. Recent work involving heavy O_2 tracing in human cells established 4-hydroxymandelate (4-HMA), produced from 4-HPP by hydroxyphenylpyruvate dioxygenase-like protein (HPLD), as an intermediate in this pathway [32], consistent with intermediates observed in yeast [31]. Work on the mandelate pathway in yeast suggests that there are multiple enzymes and additional steps required to convert 4-HPP to 4-HMA [33].

The isoprenoid tail, composed of isoprene repeats, is derived from isopentenyl pyrophosphate (IPP) or dimethylallyl pyrophosphate (DMAPP) subunits from the mevalonate pathway [3]. It is still unknown how the 4-HB and isoprene precursors are transported from the cytosol into the mitochondrial matrix. Upon precursor entry into the matrix, Coq1p/PDSS1/PDSS2 polymerizes isoprenes to form the full-length tail, whose length varies by species. **Coq2p/COQ2** then attaches the tail to 4-HB to yield polyprenyl-hydroxybenzoate (PPHB) [3].

The final stage of CoQ biosynthesis involves a series of modifications of the PPHB headgroup, including decarboxylation, hydroxylation, and methylation reactions carried out by Coq3-Coq9p/COQ3-COQ9 [3] (Figure 2C). The proposed order of these reactions is based on the accumulation of CoQ intermediates in mutant yeast [34] and bacterial strains [35]. However, given the accumulation of certain intermediates in both human and yeast *COQ6* mutant cells, there is some ambiguity concerning the order of reactions, namely, whether the decarboxylation and hydroxylation steps are executed before or after the Coq6-mediated hydroxylation reaction [36,37]. One outstanding question is what eukaryotic enzyme(s) catalyze the decarboxylation and hydroxylation steps, theoretically converting polyprenyl-vanillic acid (PPVA) to demethoxy-demethyl-coenzyme Q (DDMQ) (Box 1). Another active area of research seeks to elucidate the roles of auxiliary proteins in CoQ biosynthesis, including Coq4p/COQ4, Coq8p/COQ8A/COQ8B, Coq9p/COQ9, and Coq10/COQ10A/COQ10B. COQ8 is a member of the highly conserved UbiB family of kinase-like proteins with strong links to prenyl-lipid biosynthesis [38]. Coq8p has been hypothesized to be a protein kinase, and *coq8* yeast exhibit alterations in phosphorylation of Coq proteins [39]. However, mammalian COQ8A lacks protein kinase activity *in vitro*, and instead displays ATPase activity that can be modulated by lipids and small molecules [40]. How Coq8 functions as either a kinase or ATPase to support CoQ biosynthesis remains vague (Box 2). Although the lipid-binding protein Coq10p is not essential for CoQ biosynthesis, it is necessary for efficient CoQ synthesis and its utilization in OxPhos [41]. Further efforts are

required to elucidate how Coq10p and other auxiliary proteins supports CoQ biosynthesis in yeast and mammalian systems.

CoQ Biosynthetic Complex

The enzymes responsible for CoQ head-group modification form a complex at the matrix face of the IMM [42–45]. This complex, termed '**complex Q**' or the 'CoQ-synthome,' is a fascinating example of a 'metabolon' [46]—a dynamic enzyme complex carrying out the sequential steps of a metabolic pathway by **cluster channeling** [47]. Posited roles of metabolons include enhancing flux due to enzyme proximity and substrate channeling, and sequestering toxic intermediates. Indeed, early CoQ intermediates in yeast have demonstrated toxicity and their accumulation impairs trafficking of exogenously supplied CoQ [48]. CoQ exists almost entirely in the hydrophobic portion of the bilayer and its head-group structure is carefully designed to enable its precise redox processes. The CoQ metabolon could serve to access the early precursor PPHB, orchestrate sequential modifications, and prevent premature release of intermediates back into the bilayer.

Studies have identified Coq3-9p/COQ3-9 as core components of complex Q [43,45], with additional evidence to implicate the uncharacterized yeast proteins Coq11p [44] and Coq21p [49]. However, the complete composition and stoichiometry of the enzymes and auxiliary proteins in complex Q are not known. COQ8A and COQ9 also bind lipid intermediates [40,50], supporting the notion that complex Q is composed of proteinacious and non-proteinacious components. Studies in yeast have demonstrated that CoQ proteins exhibit interdependency for their stability [42], and have led to the hypothesis that CoQ or and/or its biosynthetic intermediates may also be required for complex Q formation and stability.

Due to the difficulties in purifying and reconstituting this complex *in vitro*, combined with the lack of detailed structural information on individual CoQ proteins, complex Q has evaded isolation and structural characterization. It is unclear whether complex Q is one uniform complex, or a varying collection of smaller sub-complexes. Recently, a cryo-electron microscopy structure of an octameric complex of human COQ7 and COQ9 was solved, providing structural insight into a complex of CoQ proteins at a lipid bilayer [51]. COQ9 is an isoprenoid lipid-binding protein and its disruption results in accumulation of the COQ7 substrate, DMQ, thus establishing the hypothesis that COQ9 may act as a lipid-presenting protein [50,52]. Consistently, this complex structure, along with molecular dynamics simulations, support a model in which a COQ7:COQ9 tetramer could bind and remodel membranes to extract CoQ lipid intermediates and help present them to other complex Q proteins for further modification [51]. Despite these advances, much remains obscure regarding the architecture and assembly of complex Q (Figure 3a and see Outstanding questions).

Regulation of CoQ Biosynthesis

As an essential metabolic pathway, CoQ production is regulated at the post-transcriptional, post-translational, and epigenetic levels, which have been reviewed in detail elsewhere [53]. Early studies placed CoQ biosynthetic genes under the control of the metabolic transcriptional regulators PPARα [54] and NF-κB [55] in mouse and human cell culture,

respectively. These factors may link regulation of CoQ biosynthesis with other branches of lipid metabolism, or drive biosynthesis as a response to oxidative stress, but further work is required to fully illustrate the contexts in which these mechanisms predominate. Studies in zebrafish embryos have identified CoQ biosynthesis genes as direct targets of the essential erythropoietic transcription factor TIFγ, with *tifγ* mutant embryos displaying decreased levels of CoQ [56]. The mammalian RNA-binding protein CLUH binds mRNA transcripts of many mitochondrial proteins, including COQ3 and COQ6, and has been implicated in the control of mitochondrial biogenesis [57]. More directly, CoQ abundance has been linked to the yeast RNA-binding protein Puf3p, which exerts post-transcriptional control by binding to 3'UTRs of mRNAs encoding proteins involved in mitochondrial biogenesis [58] and COQ5 [59].

Post-translation modifications (PTMs), including phosphorylation and acylation, have been observed for numerous COQ proteins [60], although these lack direct evidence of functional relevance. Additionally, post-translational processing of COQ proteins is emerging as a regulatory mechanism for proper stability and function. The yeast mitoprotease Oct1p directly processes Coq5p into its mature form [61]. Deletion of another mitochondrial protease, PARL, has been tied to decreased CoQ levels and defective complex III activity in mice [62]. On the converse, deletion of a yeast phosphatidylethanolamine N-methyltransferase gene, *cho2*, significantly increased cellular CoQ levels, potentially stemming from elevated mitochondrial methylation capacity due to increased SAM levels [63]. Finally, loss of the uncharacterized protein human PYURF was connected to a defect in both CoQ biosynthesis and complex I [64]. PYURF physically interacts with COQ5 and NDUFAF5, both SAM-dependent methyltransferases [64], nominating PYURF as a chaperone for concerted support of complex I and CoQ pathways. It is important to note that each example above describes a protein found either in yeast or mammals, but not yet in both. Further work is required to identify the functional orthologs that drive these processes.

CoQ Uptake, Distribution, and Transport

Remarkably, CoQ is found in nearly all cellular membranes [65] (Figure 1b). Despite its ubiquitous presence, key gaps in knowledge remain regarding the proteins and pathways that are responsible for distributing CoQ throughout the cell.

CoQ Domains

A recent study in yeast to uncover the *in vivo* spatial organization of CoQ biosynthetic proteins revealed that CoQ head-group modifying enzymes colocalize to multiple discrete foci, termed 'domains' [66]. The formation of CoQ domains is dependent on the presence of essential COQ proteins, and they exhibit the ability to assemble and disassemble in response to lipid substrate flux [66]. CoQ domains are positioned adjacent to ER-mitochondria contact sites, and defects in such contact sites disrupt CoQ domain formation and lead to the accumulation of CoQ biosynthetic intermediates [66]. CoQ domains have been found in proximity to the ER-mitochondria encounter structure (ERMES) complex, and consistently, deletion of ERMES subunits results in destabilization of domains [67]. These findings suggest that the proximity of CoQ domains to ER-mitochondrial contact sites may stem

from the need to coordinate metabolic pathways via the exchange of substrates and products, or the distribution of mature CoQ throughout the cell via the endomembrane system (Figure 3a).

Implication of Endomembrane System and Mitochondrial Contact Sites in CoQ Distribution

CoQ is extremely hydrophobic and embedded within lipid bilayers, although there is no clear consensus on its exact orientation within the membrane [68]. As such, it is highly unlikely that it is able to traverse the aqueous cellular milieu without dedicated protein machinery. CoQ species with six or more isoprene units are unable to freely exchange between synthetic liposomes, unlike the more hydrophilic artificial CoQ analogs with two or four isoprene units [48]. Despite this observation, the pathway required for CoQ transport has yet to be characterized. In a study to track cellular CoQ distribution, treatment of human cells with the headgroup precursor [^{14}C]4-HB resulted in accumulation of labeled CoQ first at mitochondria, subsequently in mitochondrial associated membranes, ER, and lastly at the PM [69]. Treatment with Brefeldin A, an inhibitor of ER-Golgi transport, disrupted incorporation of newly synthesized CoQ in the PM [69]. This work suggests a model in which CoQ is synthesized at the IMM, transported to the ER, then distributed throughout the cell via the endomembrane system, although specific proteins involved in bidirectional CoQ trafficking remain elusive (Figure 3b). As mitochondrial contact sites within and between organelles have been implicated in phospholipid trafficking throughout the cell [70,71], it is possible that they too play a role in CoQ distribution.

Studies on the Uptake of Exogenous CoQ

Considering the need for improved CoQ delivery strategies for the treatment of mitochondrial dysfunction arising from CoQ deficiencies, it is critical to understand the way in which exogenous CoQ is assimilated by the cell. In a *coq3* yeast strain dependent on exogenous CoQ for respiratory growth, deletion of four factors in the endocytic pathway (Erg2, Pep12, Tlg2, and Vps45) abrogated rescue by CoQ [72]. More recently, targeted screening in a *coq2* background revealed additional factors required for uptake of exogenous CoQ, including the previously identified genes and factors involved in clathrin-mediated endocytosis [48]. Many of these studies have been performed in yeast due to the well characterized pathways and ease of genetic manipulation, but studies in mammalian systems will be required to determine if these observations are conserved. In a human clinical trial involving long-term CoQ supplementation, polymorphisms in *ABCB1*, *CD36*, *CYP7A1*, and *NPC1L1* were found to be associated with higher levels of serum CoQ and beneficial effects of CoQ supplementation [73,74]. Given the established roles of *CD36* and *ABCB1* in fatty acid transport [75,76], and *NPC1L1* in cholesterol uptake [77], these genes are encouraging targets for further mechanistic investigations into CoQ assimilation. Future work will be required to appreciate the role of these targets, and others, in facilitating CoQ uptake.

Studies on the Inter-Organellar Distribution of CoQ

A recent forward genetics screen in yeast leveraged the role of extramitochondrial CoQ in combating oxidative stress to identify genes involved in cellular CoQ distribution. This screen, combined with biochemical fractionation, lipid profiling, and yeast genetics, linked

two IMM proteins, Cqd1p and Cqd2p, to CoQ distribution, and revealed that they work in a reciprocal fashion: deletion of Cqd1p results in low CoQ mitochondrial levels and higher PM CoQ levels, whereas loss of Cqd2p shows the opposite effects [78]. Cqd1p and Cqd2p are members of the UbiB family of proteins, and their conserved ATPase residues are required for their CoQ distribution functions [78]. This could suggest a model in which the ATPase activity of Cqd1p/Cqd2p is coupled to extraction of CoQ from the IMM to facilitate its transport. Notably, CoQ transport out of mitochondria still occurred in the absence of Cdq1/2p and was furthermore unaffected by deletion of genes involved in ERMES, MICOS, vCLAMP, or lipid homeostasis, which supports the possible redundancy or overlapping functions of these pathways in CoQ distribution [78]. This finding is consistent with previous studies that have demonstrated genetic interactions of Cdq2 with ERMES complex proteins [76] and genes involved in mitochondrial lipid metabolism [79], further underscoring the potential functional link between contact site proteins and those involved in CoQ transport.

CoQ Therapeutics and Tools

Stemming from its critical cellular roles, CoQ has wide-ranging impacts on human health and disease. Genetic defects in CoQ biosynthesis result in varied diseases, including nephropathies, ataxias, and myopathies [80]. Furthermore, secondary CoQ deficiency is observed in a broad array of mitochondrial disorders [81]. Conversely, genetic disruption of CoQ biosynthesis in *C. elegans* [82] and mice [83] has been shown to increase lifespan, though the mechanism is unknown. Due to poor bioavailability of CoQ [84] (Figure 4a), oral supplementation of exogenous CoQ is largely ineffective [85], posing a significant barrier to the treatment of CoQ deficiencies in the clinic (Figure 4b–c). To move towards more effective therapeutics, there is an urgent need to develop novel tools to improve cellular uptake, bypass dysfunctional steps, or manipulate the pathway. While various formulations and CoQ analogs have been reviewed extensively elsewhere [86,87], here we will focus on a handful of encouraging strategies to achieve these goals.

Novel Delivery Strategies

Various strategies have been explored to overcome the biophysical barriers associated with CoQ delivery, including mitochondrial targeting moieties (e.g., triphenylphosphonium (TPP)), encapsulation in liposomes, nanoparticles, micelles, and nanoemulsions [86] (Figure 4d). The most widely studied TPP compound, MitoQ [88], is a potent antioxidant with various clinical applications, but it is unable to rescue CoQ's ETC function, likely because the bulky TPP group prevents MitoQ's membrane incorporation [87]. Alternative tactics link TPP to the surface of CoQ-encapsulated nanoparticles [89], but the ability of such agents to restore respiration has yet to be examined. An emerging strategy is to encapsulate CoQ in mitochondrial-targeted liposomes, such as the MITO-Porter system, that are designed to fuse directly with the mitochondrial membrane [90,91]. Despite progress, these delivery modules remain highly lipophilic and subject to the biophysical barriers of delivery. A recent approach surmounts this problem via micellization of CoQ with the fungicide caspofungin (CF). This significantly improves the water solubility and cellular uptake of CoQ [92] resulting in increased tissue levels of CoQ and improved overall survival of a CoQ-deficient

mouse model; however, overcoming the need for many rounds of IV administration may present a challenge to therapeutic adoption [92] (Figure 4d). An alternative strategy to overcome the poor bioavailability of CoQ is to utilize water soluble CoQ head group analogs that can bypass specific enzymatic defects [93–95] (Figure 4d). Bypass compounds represent an appealing route because they are soluble, their incorporation into the native biosynthetic pathway results in proper cellular distribution of downstream CoQ, and they have demonstrated efficacy in restoring CoQ levels in human cell culture and extending lifespan in mouse models [37,95–97].

Small Molecule Modulators of CoQ Biosynthesis

A complementary approach to supplementation is the development of molecular tools that can modulate the CoQ pathway and help elucidate the molecular functions of poorly characterized CoQ-related proteins. In efforts to study the role of CoQ limitation and supplementation in aging-related disorders, various inhibitors of CoQ biosynthesis have been developed, including 4-nitrobenzoate (COQ2 inhibitor) [98], clioquinol (COQ7 inhibitor) [99], and zoledronic acid (farnesyl diphosphate synthase inhibitor) [100]. Recently, Coq8p and COQ8A have been prominent targets of chemical modulation. Though its ATPase activity is required for CoQ production [40,101], the precise molecular role of these COQ8 homologs remains obscure. A covalent inhibitor targeting an engineered Coq8p mutant has provided a probe to manipulate the CoQ biosynthetic pathway in yeast for mechanistic studies, though it is limited by lack of efficacy against the WT protein [102]. More recently, a selective COQ8A inhibitor has been developed and is able to robustly inhibit *de novo* CoQ production in a mammalian cellular context [103]. Although CoQ is a critical cofactor for energy generation and other metabolic processes, there are some potential benefits to inhibiting its biosynthesis. Studies in *C. elegans* [82] and mice [83] have demonstrated that CoQ limitation can increase lifespan; CoQ pathway inhibitors could assist in unraveling this surprising phenomenon. Additionally, there has been a surge in efforts to manipulate ferroptosis in pathological settings, specifically various cancers [104]. Limiting the amount of CoQ available for FSP1 reduction might therefore be an effective anticancer strategy. In contrast, the CoQ headgroup-like phenolic compound 2-propylphenol has been characterized as a direct activator of the ATPase activity of COQ8A [105]. Taken together, these novel probes expand the chemical toolkit for modulating the CoQ pathway in cells and provide a foundation for elucidating the role of auxiliary proteins in CoQ biosynthesis, with the potential to provide insights for future therapeutic development.

Concluding remarks

The remarkable biochemical properties of coenzyme Q are pivotal for cellular bioenergetics, biosynthesis, and homeostasis, and recent studies suggest further functions for this ubiquitous cofactor are likely to emerge. Given its key roles in mitochondria and beyond, it is imperative that we fully elucidate the biochemistry of CoQ synthesis and transport — processes that have remained partially or largely unclear for 65 years. Progress in these areas has been hampered by numerous challenges, including hydrophobicity barriers and enzyme redundancy, motivating a drive for new tools and experimental approaches. By bringing to light the varied, often overlooked cellular roles of CoQ, and by highlighting primary gaps

in knowledge, we hope that this review will help focus new efforts in CoQ biology and therapeutic development.

Acknowledgements

Glossary

Cluster channeling	a feature of multienzyme metabolons in which sequential steps of a metabolic pathway can be carried out by many copies of a given enzyme rather than depending on the enzyme in closest physical proximity.
Complex Q	Complex Q, also known as the CoQ-synthome, is the biosynthetic complex that performs he final stage of CoQ biosynthesis.
CoQ$_6$	Coenzyme Q (CoQ), also known as ubiquinone (UQ), is the redox-active lipid product of the biosynthetic pathway discussed here. The subscripted number specifies the number of isoprene units in the lipid tail (e.g. CoQ$_6$ indicates 6 isoprene units). The redox state of the lipid can also be specified: the fully reduced form (the hydroquinone) as 'CoQH$_2$' (ubiquinol, UQH$_2$), the singly dehydrogenated radical form (the semiquinone) as 'CoQH$^\bullet$' (ubisemiquinone, UQH$^\bullet$), and the oxidized form (the quinone) as 'CoQ' (ubiquinone, UQ).
COQ6	COQ6 is an example of a human enzyme require for CoQ biosynthesis.
Coq6p	Coq6p is an example of a yeast enzyme required for CoQ biosynthesis.
DHODH	dihydroorotate dehydrogenase, an enzyme that mediates the CoQ-dependent oxidation of dihydroorotate to orotate.
Ferroptosis	A type of programmed cell death characterized by iron-dependent phospholipid peroxidation.

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Outstanding Questions

- How are the precursors to CoQ biosynthesis transported into mitochondria?

- What enzyme carries out the decarboxylation and hydroxylation of PPVA to DDMQ? Is this transformation achieved by a single reaction mechanism or two distinct steps?

- What are the molecular functions of uncharacterized mitochondrial proteins related to CoQ biosynthesis?

- How is CoQ biosynthesis coordinated with mitochondrial biogenesis and OxPhos?

- What are the transcriptional, post-transcriptional, and post-translational mechanisms that regulate CoQ biosynthesis?

- How is CoQ degraded, and how is this turnover coordinated with biosynthesis?

- What is the complete composition of proteins, lipids, and metabolites of complex Q, and what is their stoichiometry?

- How is complex Q formed and maintained? Are there dedicated assembly factors?

- Is complex Q a uniform, stable complex or a dynamic assembly of sub-complexes?

- What is the function of complex Q? Does it enable substrate channeling or sequester potentially toxic intermediates?

- What is the functional difference between complex Q and CoQ domains?

- What role does the spatial positioning of complex Q at ER-mitochondrial contact sites serve?

- Can CoQ be synthesized in other organelles, such as the Golgi, and if so, what enzymes catalyze this process?

- How is exogenous CoQ assimilated by the cell?

- How is CoQ transported from mitochondria and distributed throughout the cell?

- What role do mitochondrial sites, such as ERMES and MICOS, as well as the endomembrane system play in CoQ distribution?

- Are there additional functions of CoQ in other organellar membranes?

- Can CoQ intermediates or CoQ analogs substitute for non-ETC functions of CoQ?

- Can CoQ inhibition be a beneficial therapeutic strategy in some contexts?

- Why do different tissues have different CoQ/CoQH$_2$ ratio? Could the ratio control the direction of enzymatic reactions in which CoQ participates as a cofactor?

Highlights

- Coenzyme Q (CoQ) plays major roles in cell metabolism beyond transferring electrons in the ETC, including acting as an antioxidant and enzyme cofactor. Its ubiquitous presence in cellular membranes suggests that more roles for CoQ are yet to be discovered.

- Recent studies have identified a role for plasma membrane CoQ in combating ferroptosis. These findings have reinvigorated efforts to understand how CoQ is transported throughout the cell.

- CoQ is synthesized in a metabolon-like complex that is proximal to mitochondrial contact sites, which may be important for facilitating CoQ transport out of mitochondria.

- The CoQ biosynthetic pathway remains incompletely characterized, with multiple enzymatic and transport steps lacking associated proteins. Discovery efforts have met challenges including enzyme redundancy, essentiality, and hydrophobic barriers associated with synthesizing CoQ.

Box 1.

Why is CoQ biosynthesis still not fully characterized?

The study of CoQ biology has been an active area of research for over 65 years, yet fundamental questions regarding its biosynthesis, transport, and functions remain unanswered. Why have such facets of CoQ biochemistry evaded discovery for decades, despite incredible advances in methodology? The challenges for the CoQ field are a microcosm of a grander challenge in the post-genomic age: to define functions for all proteins. Some CoQ-related proteins may have evaded detection due to redundancy, whereby one or more proteins can compensate for their loss. The issue of redundancy is illustrated in the Tyr-to-4-HB pathway in yeast, where multiple enzymes can transaminate tyrosine to 4-HPP under the tested conditions [31]. On the converse, one protein can perform multiple functions and participate in an array of cellular processes, making it difficult to delineate its function in CoQ biosynthesis. Gene essentiality poses an additional hurdle in genetic screening efforts because genes essential for survival will drop out in traditional negative-selection screening approaches. Alternatively, it is possible that select genes are not essential for CoQ biosynthesis under the standard laboratory conditions. These challenges are further compounded by the difficulties in purifying membrane-associated CoQ proteins and reconstituting their individual enzyme activity *in vitro*.

How, then, can we address such obstacles that have hindered our understanding of this critical cofactor for so long? Such issues necessitate the development of new tools and probes: to inhibit and activate the biosynthetic pathway, to map the movement of CoQ throughout the cell, and to track which proteins interact with CoQ. As many proteins involved in CoQ biosynthesis are understudied, many are lacking sufficient reagents, such as antibodies and cell lines that would empower their study. Addressing these issues will also require rethinking and updating traditional strategies to overcome prior limitations, such as by performing positive-selection, CRISPRi/a, or phenotypic genetic screening to home in on genes related to CoQ biosynthesis. Existing approaches could also be revisited in contexts that more closely resemble physiological conditions, for example performing genetic screens in human plasma-like medium [106]. Finally, integrative 'systems biochemistry' approaches [64,107] present a potential avenue to guide new hypothesis-driven studies to probe the molecular function of uncharacterized proteins in CoQ biosynthesis and associate new proteins with this process. We hope that in combination, these advancements in tools, strategies, and experimental frameworks will provide the keys to filling the open gaps in knowledge in CoQ biosynthesis.

Box 2.

The UbiB Protein Family

The UbiB family is an enigmatic group of kinase-like proteins strongly tied to the biosynthesis of CoQ and other prenyl lipids [38,108–110]. This protein family is conserved throughout all domains of life (Figure Ia), yet little functional characterization exists for its members. All five human homologs (COQ8A/B, ADCK1/2/5) have been associated with human diseases [80] (Figure Ib), underscoring the need to understand their precise molecular function. Although originally predicted to be protein kinases, the crystal structure of human COQ8A revealed conserved structural features that sterically occlude the active site [101] (Figure Ic). COQ8A exhibited low levels of autophosphorylation, but lacked any kinase activity *in trans*. Instead, COQ8A may leverage its ATPase activity to enable proper CoQ biosynthesis through an unknown mechanism [40]. Additional studies have demonstrated that Coq8p binds isoprenoid lipids that resemble CoQ intermediates, and that its ATPase activity is activated by cardiolipin-containing membranes [101,102], which further suggest an unorthodox kinase-like activity in maintaining CoQ biosynthesis. Overexpression of Coq8p in different Coq yeast deletions strains can stabilize Coq protein subcomplexes and enable the accumulation of late-stage CoQ intermediates [111]. Taken together, these data suggest a model in which Coq8p leverages its ATPase activity to support the formation of complex Q, potentially coupled with extraction of lipid intermediates from the IMM (Figure Id). Additional yeast UbiB family members, Cqd1/2p, have been implicated in CoQ distribution [78], pointing towards broader roles of the UbiB family proteins in CoQ homeostasis (Figure Ie). Although their precise mechanism of action remails elusive, the recent development of probes to target specific UbiB family proteins promises to help unravel their roles in CoQ biosynthesis, distribution, and more.
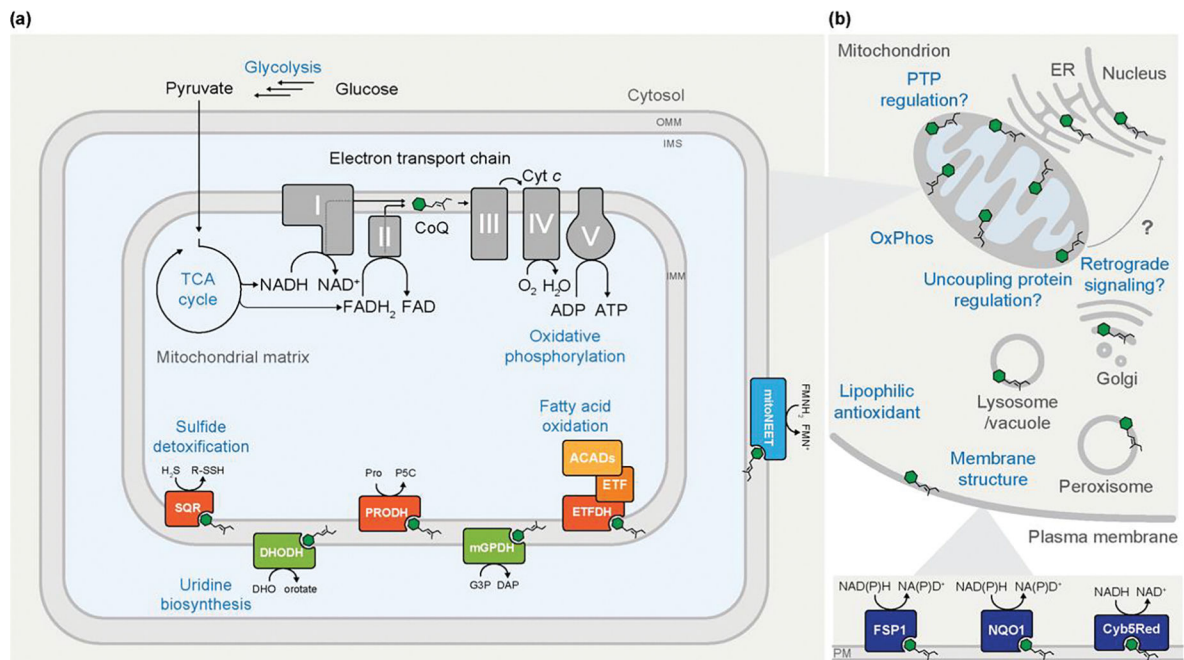
**Figure 1. Cellular Roles of Coenzyme Q.**

(A) Although coenzyme Q (CoQ) is typically regarded for its central role in the mitochondrial electron transport chain and oxidative phosphorylation (OxPhos), it additionally acts as a cofactor for multiple mitochondrial enzymes. As part of central energy metabolism, the tricarboxylic acid (TCA) cycle provides reducing equivalents that enable the reduction of CoQ at OxPhos complexes I and II. Reduction of CoQ by enzymes involved in uridine biosynthesis, fatty acid oxidation, and sulfide detoxification, among others, provides additional routes of electron entry to complex III of the ETC, which ultimately drives ATP synthesis. (B) An overview of the widespread cellular functions of CoQ. CoQ is present in nearly all cellular membranes. Some extramitochondrial roles of CoQ are depicted, including modulating membrane structure dynamics and regulating the uncoupling protein, though many of the roles are incompletely characterized. At the plasma membrane (PM), reduced $CoQH_2$ acts as lipophilic antioxidant where it can be reduced by various oxidoreductases to combat lipid peroxidation and oxidative damage.
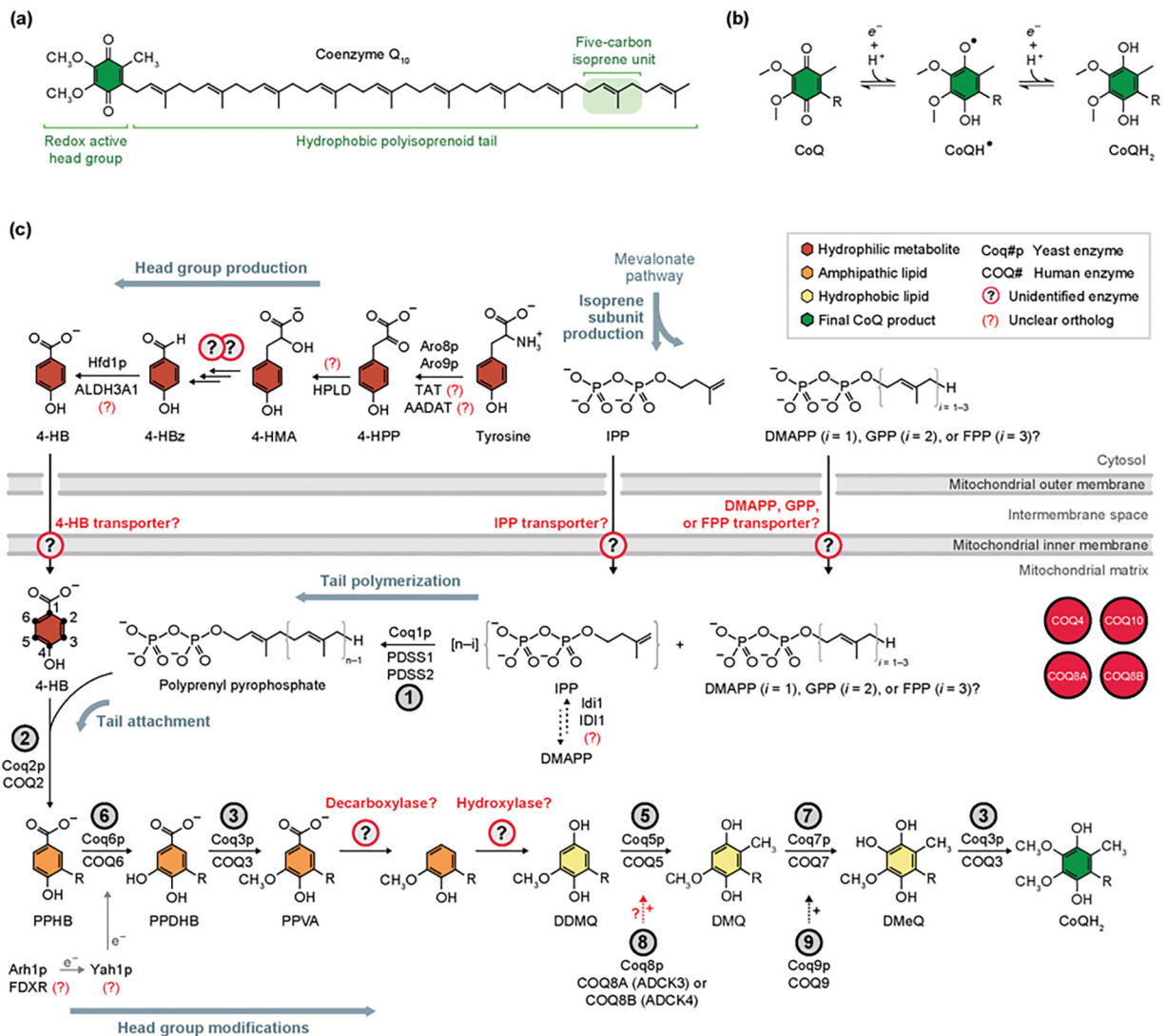
Figure 2. Coenzyme Q Structure and Eukaryotic Biosynthesis.

(A) The chemical structure of coenzyme Q_{10} (CoQ$_{10}$). (B) Single electron transfer redox reactions of the CoQ head group, whereby CoQ can cycle through the oxidized (CoQ), radical (CoQH$^{\bullet}$), and fully reduced (CoQH$_2$) forms. This redox activity allows CoQ function as a cofactor for numerous enzymes, relay electrons in the ETC, and act as an antioxidant. 'R' indicates the polyisoprenoid tail. (C) The primary eukaryotic pathway, conserved from *S. cerevisiae* to humans, of CoQ biosynthesis is depicted. CoQ production begins with the head group precursor 4-HB, derived from tyrosine, and tail subunit IPP, derived from the mevalonate pathway, which are both transported into the mitochondrial matrix by unknown means. Following tail polymerization and head group attachment, CoQ intermediates are processed through a series of head group modifications to yield mature CoQ. Biosynthetic enzymes that catalyze each reaction are denoted by the circled number above each arrow. Unidentified enzymes and transporters indicated by circled question marks. Auxiliary proteins with some unclear involvement in CoQ biosynthesis are depicted in red circles. '+' symbol near dashed arrows

designates a hypothesized supportive role for the indicated reaction. Abbreviations: 4-HBz, 4-hydroxybenzaldehyde; 4-HMA, 4-hydroxymandelate; 4-HPP, 4-hydroxyphenylpyruvate; AADAT, mitochondrial alpha-aminoadipate aminotransferase; ALDH3A1, aldehyde dehydrogenase 3A1; DDMQ, demethoxy-demethyl-coenzyme Q; DMQ, demethoxy-coenzyme Q; DMeQ, demethyl-coenzyme Q; DMAPP, dimethylallyl pyrophosphate; FDX2 (FDX1L), mitochondrial ferredoxin 2 (ferredoxin 1-like); FDXR, adrenodoxin reductase; FPP, farnesyl pyrophosphate; GPP, geranyl pyrophosphate; HPLD, hydroxyphenylpyruvate dioxygenase-like protein; IDI1, isopentenyl-diphosphate delta isomerase 1; IPP, isopentenyl pyrophosphate; PDSS1, prenyl (decaprenyl) diphosphate synthase subunit 1; PPDHB, polyprenyl-dihydroxybenzoate; PPHB, polyprenyl-hydroxybenzoate; PPVA, polyprenyl-vanillic acid; TAT, tyrosine aminotransferase.
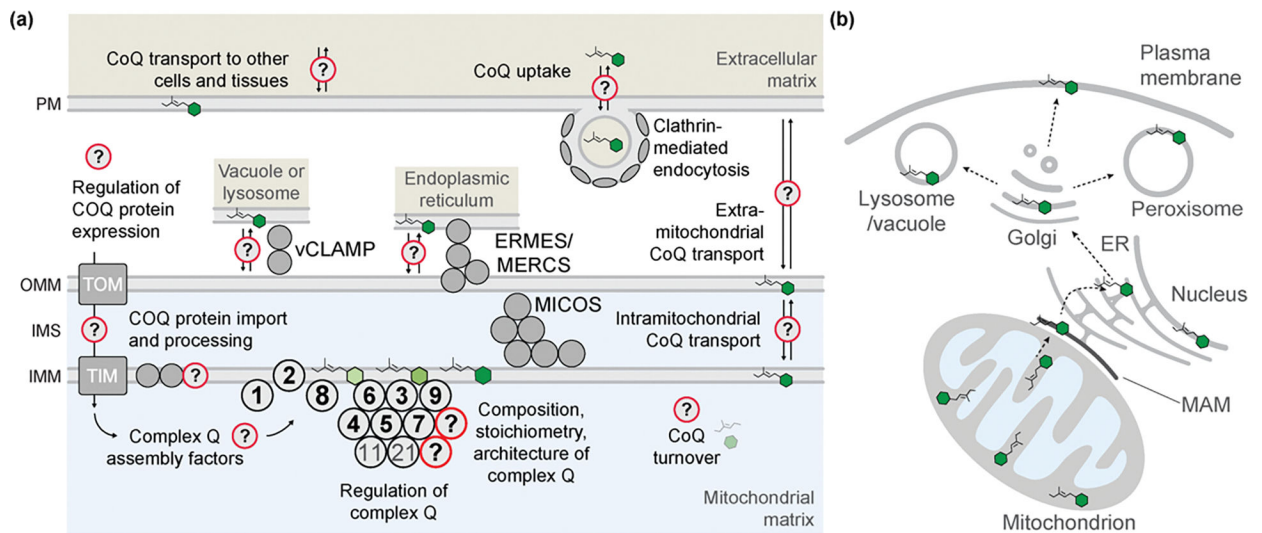
Figure 3. Models Framing Knowledge Gaps in Complex Q Organization and CoQ Distribution.
(A) A depiction of outstanding questions regarding complex Q composition and assembly, along with possible mechanisms contributing to CoQ transport and cellular distribution. Although there is much evidence to support the existence and identify core components of complex Q, its full composition, stoichiometry, and architecture are undefined, and how its assembly is regulated is unknown. All *COQ* genes are nuclearly encoded, but many questions exist regarding factors that regulate their gene expression and how they are processed and imported into mitochondria via TIM and TOM complexes. As complex Q has been observed in proximity to MICOS and ERMES, it is hypothesized that these complexes could facilitate import of CoQ precursors to mitochondria or export of mature CoQ to other organelles. Studies involving administration of exogenous CoQ has suggested that clathrin-mediated endocytosis machinery may be involved in CoQ assimilation. (B) Depiction of a putative transport pathway of CoQ throughout the cell. Following synthesis at the IMM, CoQ passes through mitochondrial-associated membranes (MAM) to the ER. There, CoQ encounters the endomembrane system and is packaged in the Golgi for widespread mobilization to different organelles and cellular membranes. Abbreviations: ERMES, ER-mitochondria encounter structure; MERCS, mitochondria-ER contact sites; MICOS, mitochondrial contact site and cristae organization system; TIM, translocase of the inner mitochondrial membrane; TOM, translocase of the outer mitochondrial membrane; vCLAMP, vacuolar and mitochondrial patch.
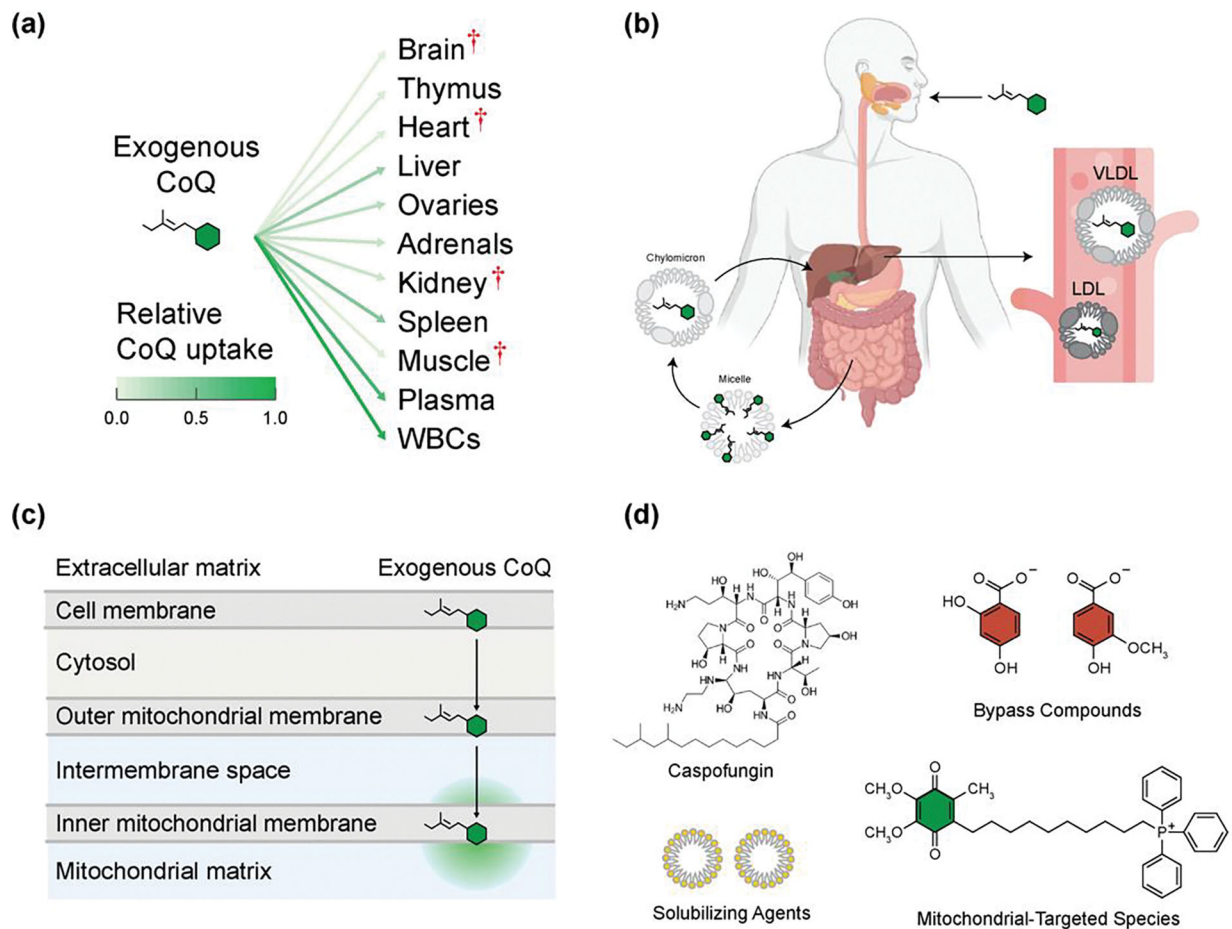
Figure 4. Challenges in Uptake of Exogenous CoQ and Strategies to Overcome Them to Treat CoQ Deficiency.

(A) Exogenous CoQ is taken up poorly by most rat tissues after intraperitoneal injection. Red cross denotes organs most commonly affected in CoQ deficiency. Data from [Bentinger *et al.*] [112]. (B) Following oral intake of exogenous CoQ, it is absorbed in the small intestine and packaged into chylomicrons, then taken up by the liver and repackaged into low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL), which can then enter blood circulation. Created with BioRender.com. (C) Once exogenous CoQ is in the bloodstream, it then must be transported across multiple lipid membranes and aqueous compartments to reach the mitochondrial inner membrane. (D) Depicted are examples of strategies to increase the solubility and uptake of exogenous CoQ by packaging with solubilizing agents like micelles, in novel formulations such as with caspofungin, or increasing organellar localization with the attachment of mitochondrial-targeting species like TPP. Alternative, CoQ deficiencies could by treated with bypass compounds that supersede the need for enzymes in the biosynthetic pathway that may be mutant or defective.
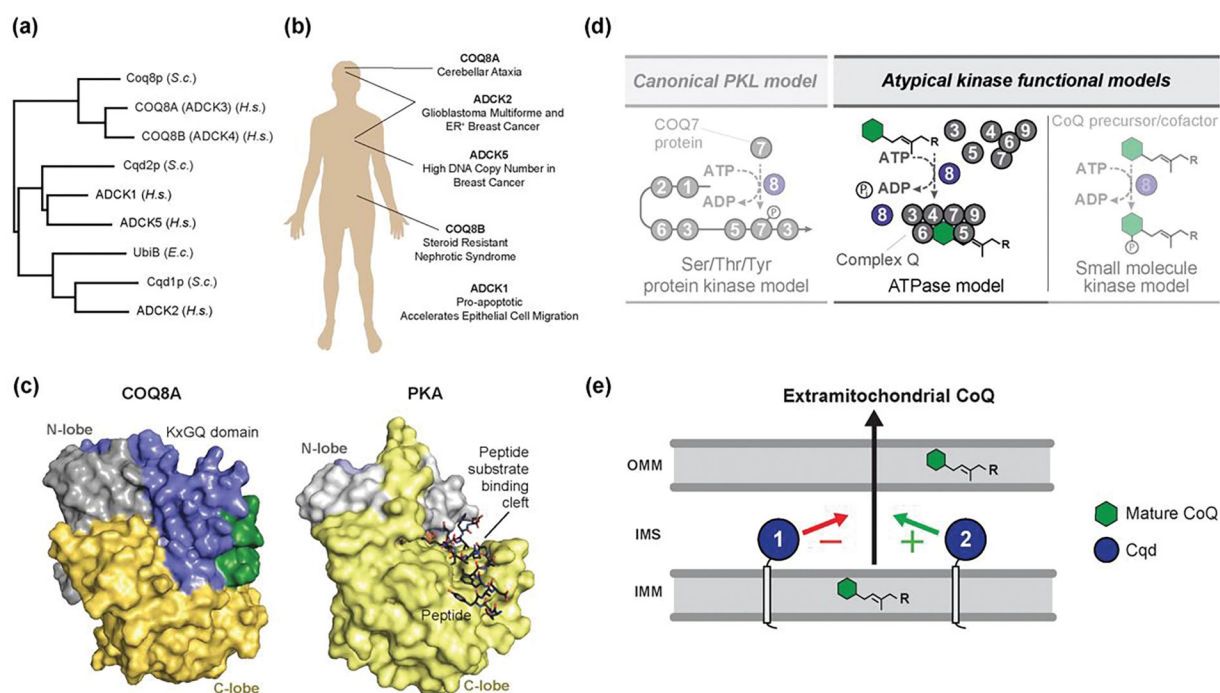
Figure I. UbiB Protein Structure and Function.

(A) UbiB proteins are conserved across all domains of life, from humans to bacteria. (B) Mutations in human UbiB family proteins have been linked to numerous diseases. (C) Surface representations of COQ8A$^{N\ 254}$ (PDB=4PED) and Protein Kinase A (PDB=1ATP) showing occlusion of the canonical peptide binding site by the highly conserved and UbiB-specific KxGQ domain (adapted from Stefely *et al.* [101]. (D) Potential models for the role of COQ8 in supporting CoQ biosynthesis. COQ8 could act as a canonical protein kinase to phosphorylate an enzyme in the biosynthetic pathway and modulate its activity, but this is not supported by current data. Alternatively, COQ8 could leverage its ATPase activity to extract hydrophobic substrates from the membrane and seed the formation of complex Q. Finally, COQ8 may act as a small-molecule kinase to support CoQ production, but no such substrate has been identified (adapted from Stefely *et al.* [40]. (E) Model of UbiB protein function in CoQ cellular distribution. Localized at the IMM, yeast Cqd1p and Cqd2p are proposed to have opposing roles in the extramitochondrial transport of mature CoQ, though the mechanism has yet to be defined. Abbreviations: *S.c.*, *Saccharomyces cerevisiae*; *H.s.*, *Homo sapiens*; *E.c.*, *Escherichia coli*.