Understanding Ubiquinone

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Abstract

Ubiquinone (Coenzyme Q) is a mobile component of the mitochondrial electron transport chain, where it acts as a pro-oxidant in its ubisemiquinone state. Despite this, ubiquinone is also believed to be a membrane antioxidant. These properties place ubiquinone at the center of hotly debated questions about how mitochondria and ROS impact aging and disease. New studies using transgenic mouse models have provided unexpected insights into whether, and how, ubiquinone is required in various processes, cell types, and sub-cellular locations. These studies have shed light on the role of mitochondria and ROS in the aging process, but also question the mechanisms of action by which ubiquinone might function as a therapeutic agent.

Highlights:

- Ubiquinone is necessary for electron transport, yet mouse lifespan can be normal despite partial ubiquinone deficiency.
- Ubiquinone is a pro-oxidant.
- Ubiquinone might not be an antioxidant in vivo.
- Theories of aging can be tested by manipulating ubiquinone levels.

Ubiquinone biosynthesis and distribution

Ubiquinone (UQ), also known as coenzyme Q (CoQ), is a redox-active, lipophilic molecule present in all eukaryotic species, and probably every cell. Its redox-active benzoquinone head group is conjugated to a polyisoprenoid side-chain of species-specific length (6-10 subunits), which confines the molecule to lipid-rich structures (Figure 1a). UQ is found in the plasma membrane and in several endomembrane systems. Most importantly, it plays a central role in mitochondrial energy generation and the production of reactive oxygen species (ROS). Reduced UQ (ubiquinol, UQH_2) is a potential antioxidant and UQ is therefore intensely marketed as a nutritional anti-aging supplement, as well as for patients with mitochondrial conditions. Here we review past and recent studies of the functions of UQ that shed light on broader questions about the role of mitochondria and ROS in cellular function and the aging process. The function of UQ in other endomembranes will not be discussed due to space constrains.

It is well established that UQ is synthesized in all cell types, and its abundance does not dependent on dietary supply. Many details of UQ biosynthesis have been worked out, including the final biosynthetic steps associated with the inner mitochondrial membrane (IMM) [1, 2](Figure 1b). The possibility of extramitochondrial biosynthesis of UQ has also been suggested [3]. Several new observations begin to elucidate how its synthesis in mitochondria and its distribution are regulated. Mitofusin 1 and 2 (Mfn1/2) are important players in the constant fusion/fission events that are necessary for the viability, normal distribution and function of mitochondria [4]. UQ concentration was found to be severely reduced in the mitochondria of *Mfn2* knockout hearts, but not from *Mfn1* knockouts [5]. Interestingly, Mfn2, but not Mfn1, is required for tethering the ER to

mitochondria [6], and this could be required for UQ export, as newly synthesized [^{14}C]-labeled UQ_{10} appears in mitochondria-associated membranes (MAM) and the endoplasmic reticulum (ER) immediately after its synthesis in mitochondria [7]. Thus, the lack of Mfn2 might prevent normal export of UQ out of mitochondria and thus inhibit UQ synthesis.

In eukaryotes, the biosynthetic precursor for the benzoquinone ring of UQ is 4-hydroxybenzoate (4-HB). Interestingly, analogues of 4-HB (such as 2,4-diHB or 3,4-diHB; Figure 1b) were found to be able to serve as unnatural, alternative, precursors in UQ synthesis in yeast as well as in mammalian cells [8, 9]. Providing these analogues by-passes the need for the hydroxylation steps catalyzed by MCLK1 (a.k.a. COQ7) or COQ6, respectively, and could benefit patients with corresponding primary UQ deficiency [9-12]. Most strikingly, it was observed that feeding *Mclk1* KO mice with 2,4-diHB resulted in a partial restoration of UQ biosynthesis and dramatic phenotypic rescue. In fact the treated *Mclk1* KO mice appeared barely different from control animals. A normal lifespan could even be restored when mutants were treated only after severe mitochondrial dysfunction from UQ loss had already produced a near-lethal phenotype [9]. These observations have been used to challenge the notion that mitochondrial dysfunction is causal to the aging process [13].

Naturally occurring UQs are large molecules that do not easily penetrate cells. Their long side chains are necessary for their function in the membrane and make them highly lipophilic and very difficult to absorb. The poor bioavailability of exogenous UQ likely accounts for its unsatisfactory efficacy in human patients [14, 15]. In mice, exogenous UQ_{10} can nonetheless be efficiently taken up by the liver, ovary and brown

fat, but not other tissues tested (kidney, heart and skeletal muscle) [9, 16-18]. What determines uptake is not known, except for brown adipose tissue, where uptake and the maintenance of sufficient levels is mediated by the scavenger receptor CD36 [18]. These findings suggest that UQ re-distribution across organs might have important functional consequences and participate in metabolic integration.

UQ in mitochondrial bioenergetics

UQ as a mobile electron carrier in the respiratory chain

UQ can exist in three different redox states: fully oxidized (UQ), partially reduced (ubisemiquinone, $UQ^{\cdot-}$), and fully reduced (UQH_2). The ability of UQ to undergo reversible redox cycling between the three states is the basis of the function of UQ as an electron carrier in the mitochondrial respiratory chain. In the IMM UQ transfers electrons from complex I and II to complex III. Chemical extraction of UQ from mitochondrial membranes results in loss of the NADH oxidase and succinate oxidase activities (a measure of complex I and II activity, respectively), which are restored when UQ is reincorporated into the membranes [19].

The long side-chain of UQ is embedded in the central hydrophobic portion of the membrane with the benzoquinone head sticking out into the hydrophilic regions [20]. In the random collision model of mitochondrial electron transport, all redox components diffuse laterally and randomly in the IMM and the transfer of electrons occurs upon random encounters between complexes and the two mobile electron carriers (UQ and cytochrome *c*). Addition of phospholipids to the IMM to dilute its components results in decreased rates of electron transfer from complex I or II to complex III, while subsequent

addition of UQ restores electron transfer substantially [21]. This suggests a direct influence of UQ concentration on electron transfer. Being much smaller, UQ should diffuse faster than the much bulkier complexes. However, it remains unclear whether UQ diffusion is in fact rate-limiting for electron transport.

In recent years the existence of mitochondrial respiratory supercomplexes has been convincingly demonstrated [22, 23]. The I-III-IV supercomplex is the most intriguing as it contains all the redox enzymes required for electron flow from NADH to the final electron acceptor, oxygen [24]. In such supercomplexes electron transfer should not depend on the random encounter of ETC components [25]. In fact, I-III-IV supercomplexes isolated from mammalian cells contain both mobile electron carriers and are capable of respiration [24]. Single or few UQ molecules might be integral to such supercomplexes, where electron transport might be mediated by the microdiffusion of UQ or direct inter-protein quantum tunnelling [26]. It is interesting to note that within supercomplexes containing complex I and III, UQ binding sites were found to be located in sufficiently close proximity for efficient electron transfer, that is, at a distance of ~ 13 nm, which is much shorter than the minimum distance that UQ is expected to need to diffuse for electron transfer by random collision (~ 37.9 nm) [27]. In contrast to complex I, complex II does not seem to participate in any supercomplex structures [23]. In addition, there are other activities that feed electrons into the respiratory chain via UQ, such as electron-transferring flavoprotein ubiquinone oxidoreductase (ETFQOR), glycerol 3-phosphate dehydrogenase (G3PDH), dihydroorotate dehydrogenase (DHODH), choline dehydrogenase (CHDH), proline dehydrogenase (PRODH), and sulfide:quinone oxidoreductase (SQOR). One working hypothesis is therefore that UQ is not limiting for

electron transfer from complex I to III, which is achieved through respiratory supercomplexes that include integral UQ molecules, whereas all other electron transfer activities, including complex II, reduce a common UQ pool and might compete for mobile UQ [22, 28-30]. However, it has also been proposed that the size of the mobile UQ pool dictates the amount of UQ bound within supercomplexes and thus that the UQ in the IMM essentially behaves as a single functional pool [26, 31]. Of note, UQ deficiency due to a *Coq9* mutation (*Coq9* encodes a regulatory component of UQ biosynthesis; Figure 1b) appears to be unfavorable for supercomplex formation in the brain [32].

Relationship between mitochondrial UQ content and respiratory capacity

Human patients and model organisms with defects in UQ synthesis are being increasingly reported (for mouse models, see Table 1) [2, 15, 33]. Most studies find that loss of UQ results in a significant reduction in UQ-dependent respiratory chain activities [32, 34-39]. UQ level in those mutants is often drastically low, explaining why all UQ functions are affected. However, one much milder model yielded additional information. *Mclk1*$^{+/-}$ heterozygous mice have low levels of the hydroxylase but roughly normal levels of overall mitochondrial UQ. Despite this, these mutants display a variety of phenotypes, including an extended lifespan [40]. Detailed examination revealed that UQ concentration in the IMM of hepatocytes was ~ 20% lower than in controls, and this was sufficient to impair electron transport activity between complexes I and III as well as between complexes II and III [17, 34]. This suggests that, despite supercomplexes, all UQ functions are fairly sensitive to UQ availability. At the other end of the spectrum, homozygous liver-specific loss of either *Mclk1* or *Pdss2* (coding for another UQ biosynthetic enzyme; Figure 1b)

results in a dramatic loss of UQ (>80%) but produces only a mild impairment of respiratory chain function, consistent with a low prevalence of liver dysfunction in UQ deficiency patients [41, 42]. Furthermore, no gross abnormalities were detected in the KO livers, and *Mclk1* liver KO mice show normal lifespan. Taken together, these results suggest a nonlinear dependence of mitochondrial respiratory capacity on UQ content in hepatocytes, with a small deficit (~20%) producing the same effect as a much larger deficit (~80%). Unlike the liver, a severe loss of UQ (~ 90%) due to loss of *Mclk1* in the kidney and heart mitochondria produced a dramatic reduction in the maximum capacity of the respiratory chain (~ 50%). Interestingly, a partial restoration of UQ content to ~ 35% of the normal level by 2,4-diHB treatment is sufficient to restore mitochondrial respiration fully in the kidney, but not in the heart [9]. This substantial difference in UQ reserve levels between kidney and heart is intriguing, as both tissues are considered highly metabolic. Furthermore, hearts with ~10% normal UQ are still able to function sufficiently well to support life despite severely impaired mitochondrial function [9].

UQ as a source of mitochondrial ROS (mtROS)

Mitochondria are a source of ROS, which are potentially harmful but also important signaling molecules. Superoxide ($O_2^{\bullet -}$) generation results from single-electron premature reduction of oxygen by electrons moving through the ETC [43](Figure 2). In complex I electrons flow from NADH to flavin mononucleotide (FMN), and are then transferred to a series of Fe-S clusters. The terminal Fe-S cluster was shown to interact with ubisemiquinone, and is therefore thought to be the electron donor to UQ [44]. Various studies have suggested that superoxide production by complex I originates mainly from

the FMN [45-48]. However, reduced FMN transfers its electrons to UQ across the Fe-S centers in two one-electron reduction steps: transfer of the first electron results in transient formation of ubisemiquinone, and transfer of the second electron reduces the semi-reduced form of UQ to the fully reduced UQH_2. As ubisemiquinone can react with oxygen to form superoxide this is a likely additional site of superoxide production at complex I, as supported by a number of experimental observations [49, 50].

Reverse Electron Transport (RET) refers to uphill electron flow from succinate through complex II to UQ and then complex I and its flavin group, which finally reduces matrix NAD^+ (Figure 2). Succinate, as well as lipid metabolism and other metabolic pathways that reduce the UQ pool can induce RET in mitochondrial preparations. Its importance *in vivo*, however, is still debated. The rate of ROS production by RET seems to be the highest that can occur in mitochondria, although it is not understood why this is the case [43, 51]. Blocking the UQ-reduction site of complex I with inhibitors markedly diminishes superoxide production by RET, confirming that during RET electrons enter into complex I through the UQ-binding site [51].

In contrast to complex I, the mechanism of superoxide production by complex III is well established. At complex III, electrons are transferred from UQH_2 to cytochrome *c* in the process called the Q-cycle. Briefly, UQH_2 binds to the Q_o site near the outer side of the IMM and transfers the first electron to the Rieske iron-sulfur protein (RISP). The unstable remaining ubisemiquinone donates the second electron to the low-potential heme (b_L) of cytochrome *b* and is then conveyed to the high-potential heme (b_H) near the "in" side (the matrix side) of the membrane. From b_H, it passes to a UQ at the second UQ-binding site Q_i, leading to the formation of a stable ubisemiquinone (Figure 2). In the

second part of the Q cycle, with oxidation of a second UQH_2 at the Q_o site all steps are repeated and the net result of one complete cycle is oxidation of two UQH_2 molecules at the Q_o site, generation of one UQH_2 at the Q_i site, reduction of two cytochrome *c* molecules, and deposit of four protons into the intermembrane space. The Q_o site has long been regarded as the principle site of superoxide production from complex III [52]. The most cited experimental evidence is from studies that involved combining inhibitors specific for the two distinct UQ binding sites of complex III. In the presence of antimycin A, which interrupts the transfer of the second electron to the Q_i site, thereby increasing steady-state levels of ubisemiquinone at the Q_o site, mitochondria produce superoxide at high rates [52, 53]. In contrast, Q_o site inhibitors (e.g., myxothiazol and stigmatellin) prevent ubisemiquinone formation, and supress superoxide production by antimycin A-inhibited complex III [54-56]. Thus, by inference, ubisemiquinone at the Q_o site is the most plausible source of mtROS at complex III.

Note that several non-respiratory chain dehydrogenase, such as ETFQOR, G3PDH and DHODH, produce mtROS while feeding electrons into the UQ pool [57]. Most likely, the relative contributions of different substrates or specific ROS production sites vary widely between cell types and tissues, and also can change according to physiological and pathological conditions [58]. For example, brown adipose tissue heavily expresses G3PDH, which appears to account for a large fraction of mitochondrial ROS production in this tissue [57] .

mtROS production and UQ content

The consequences of severe loss of UQ in mice have been most extensively studied in a model where MCLK1 expression is lost by induced excision of the corresponding gene in

young adults (adult-onset global *Mclk1* KO mice, aog*Mclk1* KO)[9]. Several months after loss of UQ synthesis, heart mitochondria from these mice contained only ~ 10% of the normal UQ content and had lower mtROS production rates from all known sites of mtROS production, including forward electron transport in the presence of antimycin A and RET from succinate oxidation. The mtROS measurements were conducted under conditions in which the level of respiration in the deficient and control mitochondria were equal. Thus the lower mtROS generation by the mutant mitochondria was not simply the result of low electron transport. Furthermore, these intact UQ-depleted mitochondria were isolated from living tissues and not produced by chemical extraction of UQ from isolated membranes. This confirms that UQ is indeed a pro-oxidant *in vivo*. However, it is unclear how low levels of UQ lower mtROS formation. One possibility is that low UQ induces the formation of supercomplexes. Indeed, one important role envisioned for supercomplexes is an enhancement of electron-transport efficiency from one redox component to the next, thus minimizing electron leakage and mtROS production [26, 59]. Based on work with cultured fibroblasts from human patients with UQ deficiency, it has been suggested that mtROS production might increase during intermediate UQ deficiency (50%-70%) as a result of enhanced ubisemiquinone generation from increased redox cycling of the limited UQ pool, while more severe loss of UQ (>85%) is not accompanied by significant ROS production [60]. Thus, additional studies in intact organisms will be necessary to further elucidate the relationship between the level of UQ and mtROS production.

Modulation of mtROS signaling

Although oxidative stress from ROS overproduction or insufficient ROS defenses can be harmful, there is a growing appreciation for the role of ROS as signaling molecules. ROS have emerged as critical signaling intermediates in a multitude of basic cellular processes such as proliferation, differentiation, stress responses, inflammation, metabolism and cell survival [61]. It is currently believed that superoxide produced from complex I is exclusively released into the mitochondrial matrix, while the Q_o site of complex III releases superoxide into the intermembrane space [62]. Superoxide cannot cross membranes and is quickly transformed into hydrogen peroxide (H_2O_2) by the action of superoxide dismutase (SOD). H_2O_2 is substantially more stable than superoxide and can pass membranes and diffuse over a relatively long distance from its site of generation. Thus, H_2O_2 generated in the intermembrane space, but possibly also in the matrix, can readily diffuse into the cytosol.

We mentioned earlier that the long-lived *Mclk1*$^{+/-}$ heterozygous mouse mutants have a mild UQ deficiency in the IMM associated with increased mtROS [34]. Interestingly, *Mclk1*$^{+/-}$ mutants exhibited increased expression of HIF-1α (liver and macrophages) in association with elevated expression of inflammatory cytokines, and enhanced immune reaction against grafted cancer cells [63, 64]. Furthermore, both spontaneous and grafted tumor development was delayed in the mutants [64]. HIF-1α is an immune stimulator that is known to be stabilized by mtROS from complex III [65]. In aog*Mclk1* KO mice, a much more severe UQ deficiency was obtained, leading to reduced production of ROS from mitochondria. Interestingly, the steady-state expression levels of SOD1 and catalase (both cytosolic) were lower in some KO tissues [9], possibly because of active, compensatory, down-regulation to maintain ROS levels required for signaling.

Is UQ an endogenous antioxidant?

The antioxidant properties of UQ have attracted a lot of attention and it is the rationale for its use as a health supplement. Early studies using sub-mitochondrial particles showed that chemically-induced lipid peroxidation of mitochondrial membranes could be inhibited by the addition of succinate or high concentration of NADH. This was interpreted in terms of the ability of NADH and succinate to reduce UQ via the ETC and of UQH_2 acting as antioxidant [66, 67]. In UQ-depleted membranes this protective effect was abolished, and reincorporation of UQ restored the ability of NADH or succinate to inhibit peroxidation [67, 68]. Furthermore, it was shown that ETC inhibitors that can bind to UQ-sites and prevent the reduction of UQ, abolished the inhibitory effect of respiratory chain substrates on lipid peroxidation [69]. Similar experiments were conducted to demonstrate a protective role of UQH_2 against protein carbonylation and oxidative damage to DNA [70, 71].

At the plasma membrane, UQ acts as an intermediate electron carrier involved in the transfer of electrons across the membrane [72]. The physiological importance of UQ-dependent electron transfer in plasma membrane is not yet fully understood. One proposed function is protection of membrane lipids from peroxidation [72, 73]. Experiments using liposomes showed a protective effect of UQH_2 on membrane lipid peroxidation, with similar efficiency as α-tocopherol [74]. Furthermore, another study reported that following exposure to peroxyl radicals, the lipid peroxidation rate of human low-density lipoprotein (LDL) was low as long as UQH_2 was present but increased rapidly after its consumption [75]. This finding and later UQ supplementation studies have been taken as evidence that UQ is the most active antioxidant in LDL [75, 76]. It is also established that UQH_2 can regenerate other powerful antioxidants such as α-tocopherol

and ascorbate via electron donation and recycles them back to their active reduced forms, thereby enhancing the activity of other antioxidant defenses [77].

All the aforementioned studies demonstrated the potential of UQH_2 in protecting biomolecules from oxidative damage. However, most observations were made in artificial systems and only provided correlations. Whether UQ carries out an indispensable antioxidant role *in vivo* remains unclear. A more direct evidence would be a correlation between UQ level and oxidative damage. Yeast strains lacking UQ biosynthesis were not found to have become hypersensitive to most forms of oxidative stress that include treatments with 100% oxygen, paraquat, peroxides, menadione and metals [78]. *C. elegans* mutants of *clk-1* (homologue to *Mclk1*) were found to have decreased levels of oxidative damage, as indicated by lower protein oxidation and decreased accumulation of oxidized lipids and lipoproteins [79, 80]. They are more sensitive to an acute exposure to oxidative stress but resistant to chronic oxidative stress [81]. No evidence of increased oxidative stress was observed in *Pdss2* conditional knockout livers, while increased levels of some oxidative damage markers were reported for the kidneys (but not other tissues) of *Pdss2*$^{kd/kd}$ missense mutant mice [82, 83]. Mouse mutants with a partial-loss of function mutation in *Coq9* exhibit severe UQ deficiency (<=20%) in all tissues tested [32]. Immunochemistry detected increased number of brain cells positive for 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of DNA oxidation, but no increase in stains for the lipid peroxidation marker 4-Hydroxynonenal (4-HNE) [32]. In a recent study, it was shown that a severe loss of UQ in most tissues of aog*Mclk1* KO mice was not associated with elevation of measures of oxidative damage [9]. Studies on skin fibroblasts from primary UQ deficiency patients showed that the cells with less than 20% residual UQ_{10} were not

associated with significant ROS production and did not suffer from oxidative stress [60, 84]. Assessments of glutathione redox status and the mitochondrial SOD activity (SOD2) suggested no elevation of other antioxidant systems in those severely UQ-depleted human cells [84]. Considered broadly, severe UQ deficit does not cause an elevated level of oxidative stress, and thus UQ is unlikely to be a crucial antioxidant *in vivo*. In this regard, we believe it is necessary to re-examine potential health effects of exogenous UQ and to make a more rational use of UQ as a supplement.

Concluding remarks

UQ is present in all cells and membranes and is essential to life. In particular, it is necessary for mitochondrial energy production, is a prominent source of ROS, and has antioxidant properties in its reduced form. In the past, information about the functions of UQ was mainly obtained by biochemical studies examining the effects of chemical extraction and re-supply of UQ in isolated membrane systems. However, more recent studies of models with deficient endogenous UQ synthesis, especially several mouse models, have shed new light on the properties and functions of this essential molecule. Furthermore, because of UQ's central importance for mitochondrial function, these models have also shed light on how mitochondrial function and dysfunction may affect tissue and organism physiology. Importantly, a better understanding of UQ will be paramount to the development of truly effective treatments for UQ deficiency diseases. Generally, with better tools and more dedicated studies, we can look forward to a better understanding of how much UQ is in fact required for mitochondrial function and health. For example, why are metabolically active tissues such as the heart able to function sufficiently well to support life with almost no UQ? How are UQ production and distribution

regulated within cells? What is the significance of exogenous UQ uptake by some tissues but not by others? Finally, by manipulating UQ levels, and thus controlling mitochondrial function at will, we should be able to determine whether the gradual loss of mitochondrial function observed during aging might in fact be adaptive (Outstanding question box).

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Table 1. Mouse mutants with genetic defects in ubiquinone biosynthesis.

Gene name	Type of mutant	Strain Genotype	UQ level	UQ-dependent mitochondrial function	Oxidative stress	Phenotype	Ref.
Pdss2	*kd* (V117M) missense mutation, homozygous	*Pdss2*$^{kd/kd}$	↓ in kidney (≥72%), brain (≥72%), liver (≥32%) and muscle (≥65%)	↓ CI- and CII-dependent RC capacity in liver and muscle, ↓ CI+CIII or CII+CIII activity in kidney, liver, and brain	↑ ROS production in kidney and muscle, ↑ oxidative damage in kidney, no change in ACON and MnSO2 activities in kidney Mito.	nephrotic syndrome (albuminuria, dilated tubules, interstitial infiltration, glomerulopathy), myopathy, impairment of motor coordination and locomotor activity, ↓ TH-positive neurons in SN, ↑ serum triglycerides, hypercholesterolemia	[42, 82, 83, 85, 86]
	conditional KO targeted to renal glomerular podocytes	*Podocin-cre, Pdss2*$^{loxP/loxP}$	no change in total liver or kidney homogenates	no change in CI+III and CII+CIII activities in kidney Mito.	ND	nephrotic syndrome (albuminuria, dilated tubules, interstitial infiltration, glomerulopathy), slight hypercholesterolemia	[42]
	conditional KO targeted to renal proximal tubule	*PEPCK-cre, Pdss2*$^{loxP/loxP}$	no change in total liver or kidney homogenates	no change in CI+III and CII+CIII activities in kidney Mito.	ND	no albuminuria	[42]
	conditional KO targeted to myeloid cell lineage	*LysM-cre, Pdss2*$^{loxP/loxP}$	ND	ND	ND	no albuminuria	[42]
	conditional KO targeted to hepatocytes	*Alb-cre, Pdss2*$^{loxP/loxP}$	severe ↓ (>80%) in the liver	↓ CI- and CII-dependent RC capacity, and ↓ CI+CIII activity in liver Mito.	no oxidative stress (no change of ACON and MnSO2 activities in liver Mito.)	grossly healthy, hypercholesterolemia	[42, 82]
	conditional KO targeted to cerebellum	*Pax2-cre, Pdss2*$^{loxP/-}$	ND	ND	ND	neonatal death, cerebellum hypoplasia, cleft palate, micrognathia	[87]
	conditional KO in cerebellum Purkinje cells after birth	*Pcp2-cre, Pdss2*$^{loxP/-}$	ND	ND	ND	progressive loss of cerebellum Purkinje cells, cerebellar neuronal apoptosis, gradual development of motor incoordination and ataxia	[87]
	conditional KO targeted to dopaminergic neurons	*DAT/cre, Pdss2*$^{loxP/loxP}$	ND	ND	ND	impairment of motor coordination and locomotor activity, ↓ TH-positive neurons in SN	[86]
Coq3	germline KO mutation, heterozygous	*Coq3*$^{+/-}$	no change	no change in RC capacity in liver Mito.	no oxidative stress (no change of ACON activity in liver Mito.)	grossly healthy, normal lifespan	[17]
Mclk1/ Coq7	germline KO mutation, heterozygous	*Mclk1*$^{+/-}$	↓ in IMM ↑ in OMM	↓ CI- and CII-dependent RC capacity, ↓ CI+CIII activity and ↓ CII+CIII activity, ↓ ATP levels	↑ Mito. oxidative stress (↑ ACON activity, ↑ protein carbonylation, ↑ GPx and MnSOD activities), ↓systemic oxidative stress biomarkers	grossly healthy, ↑ expression of HIF-1α and inflammatory cytokines, enhanced immune response, resistance to cerebral ischemia-reperfusion, increased lifespan	[17, 34, 63, 64, 88]
	conditional KO targeted to hepatocytes	*Alb-cre, Mclk1*$^{loxP/-}$	severe ↓ (85%) in liver	mild ↓ CI- and CII-dependent RC capacity (<20%) and ↓ CII+CIII activity in liver Mito.	no oxidative stress (no change of ACON activity in liver Mito.)	grossly healthy, normal lifespan	[41]
	conditional inducible whole-body KO	*CAG-CreER*^{T2}, *Mclk1*$^{loxP/loxP}$	severe ↓ (>85% in most affected tissues)	↓ CI- and CII-dependent RC capacity (50%), ↓ CI+CIII and CII+CIII activities	↓ Mito. ROS production in heart, no increase of oxidative damage	growth retardation, hair loss, early death, ↑ blood lactase, ↓ glucose. ↓ triglycerides	[9]
Coq9	R239X (missense mutation), homozygous	*Coq9*^{R239X}	severe ↓ (~ 80%)	↓ CI- and CII-dependent RC capacity, ↓ CI+CIII activity, ↓ ATP level with the brain being most affected	↑ staining for DNA oxidative damage in brain sections (no increase in stains for lipid peroxidation)	growth delay, encephalomyopathy, early death	[12, 32]
	Q95X, homozygous	*Coq9*^{Q95X}	moderate ↓ (50-70%)	↓ RC capacity, ↓ CI+CIII activity	ND	mild myopathic phenotype	[12]

Abbreviations: KO, knockout; RC, respiratory chain; CI, complex I; CII, complex II; CIII, complex III; ACON, aconitase; MnSOD, manganese-dependent superoxide dismutase; GPx, Glutathione peroxidase; Mito, mitochondria; TH, tyrosine hydroxylase; SN, substantia nigra; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; ND, not determined. The numbers in brackets show the percentages of reduction.

Figure Legends

Figure 1. Eukaryotic ubiquinone biosynthesis. (a) The ubiquinone (UQ) benzoquinone ring can exist in three redox states. Partial reduction of the oxidized form by one electron creates an ubisemiquinone that is either protonated or an unstable ubisemiquinone radical upon deprotonation. The addition of a second electron and proton generates the fully reduced form. “n” indicates the number of isoprenoid units in the side chain (n = 9 in mice and n = 10 in humans). **(b)** The final steps of UQ biosynthesis are associated with the inner mitochondrial membrane. 4-hydroxybenzoate (4-HB) derived from tyrosine is the precursor of the UQ quinone ring. The polyisoprenoid side chain is made from farnesyl pyrophosphate (FPP) and several isopentenyl pyrophosphate (IPP) molecules, which all originate from the mevalonate pathway. The UQ biosynthetic pathway starts with the assembly of the side chain followed by its subsequent attachment to 4-HB. Enzymes in red are those whose mutations have been found in human patients with primary UQ deficiency. The dashed arrows indicate multiple enzymatic steps, and the question mark means “unknown”. UQ might be present in all cellular membranes, but how it exits mitochondria and is loaded into other membranes is presently unknown. One possibility

for which there is some evidence is that UQ is transported into the endoplasmic reticulum (ER) via the mitochondria-associated membranes (MAM) and that other endomembranes subsequently receive their constitutive UQ from the ER-Golgi system [7]. 3,4-dihydroxybenzoate (3,4-diHB) and 2,4-diHB are close analogs of 4-HB and their use for UQ biosynthesis allows the cell to bypass the need for COQ6 and MCLK1/COQ7, respectively.

Figure 2. Functions of ubiquinone in the mitochondrial respiratory chain (RC). In normal forward electron transfer, UQ accepts electrons from complex I and II and passes them singly to complex III. At complex III the "Q cycle", which allows pumping of protons from the matrix into the intermembrane space, involves two distinct UQ binding sites. UQH_2 is reduced at the Q_o site passing one electron to cytochrome c (cyt *c*) and the other down to the Q_i site where the electron is given to a bound UQ during the first cycle, forming $UQ^{\cdot-}$, or to a bound $UQ^{\cdot-}$ generated during the first cycle. Oxidized UQ formed at the Q_o site and UQH_2 formed at the Q_i site after completion of the "Q-cycle" are free to diffuse out into the UQ pool. As electrons are transported, they may leak to oxygen, forming superoxide ($O_2^{\cdot-}$). Red stars indicate potential sources of $O_2^{\cdot-}$ production. Superoxide dismutase (SOD) converts $O_2^{\cdot-}$ to hydrogen peroxide (H_2O_2) that is reduced to water by glutathione peroxidase (GPX). Both $O_2^{\cdot-}$ and H_2O_2 have been implied in modulating the function of signal transduction pathways ("other reactions" in the figure). UQ also accepts electrons from several non-RC dehydrogenases, including the mitochondrial glycerol-3-phosphate dehydrogenase (G3PDH), dihydroorotate dehydrogenase (DHODH) and electron transfer flavoprotein oxidoreductase (ETFQOQ) (see main text for other

dehydrogenases not shown in the figure). Uphill electron transfer from UQH_2 to NAD^+ through complex I is known as reverse electron transport. Individual RC complexes assemble into supercomplexes. The I–III–IV supercomplex, which is the most active supramolecular form, is schematically shown on the left of the figure.

Figure 1

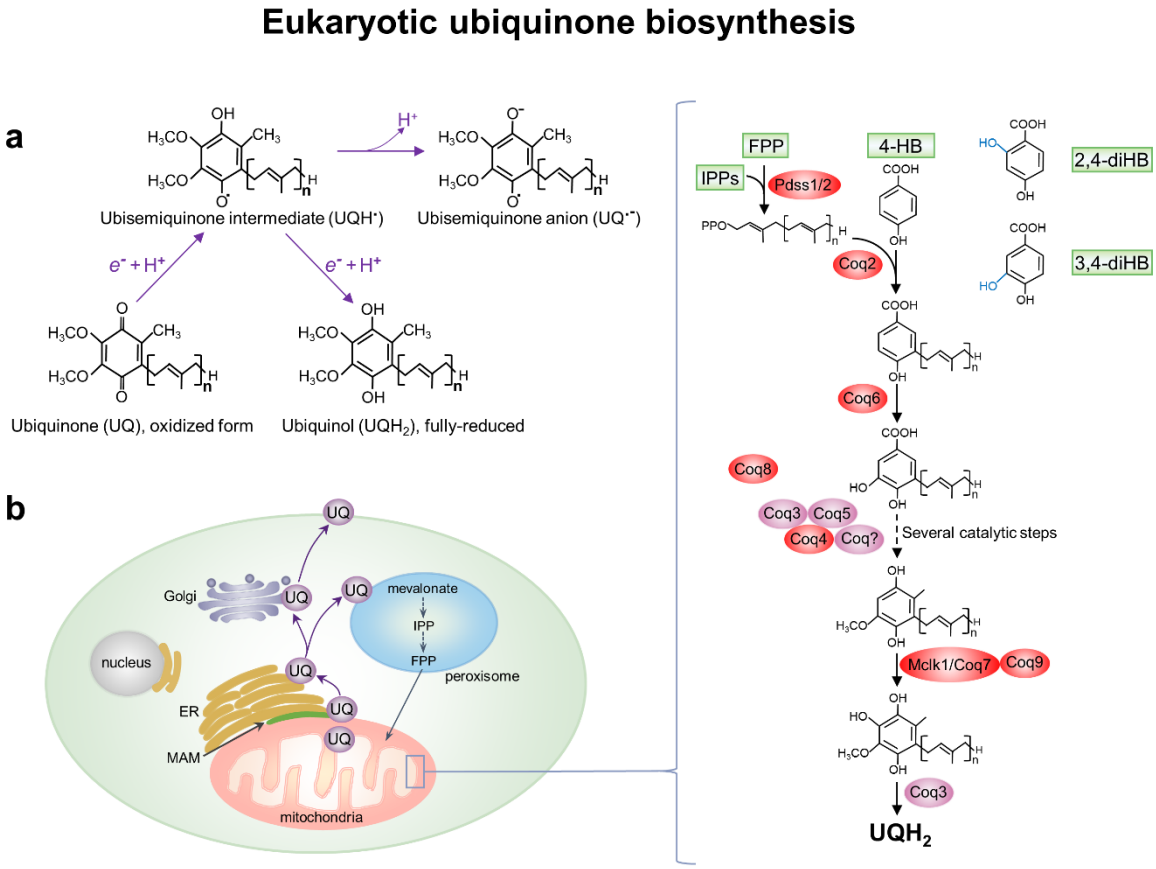
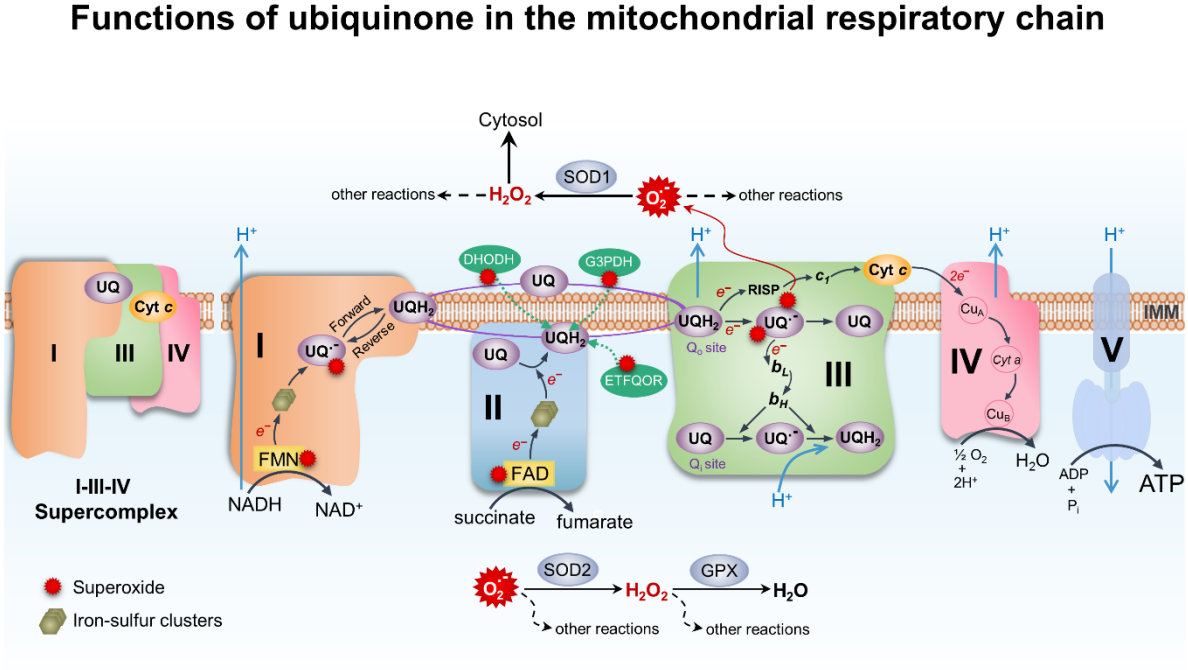

Eukaryotic ubiquinone biosynthesis
a
Ubisemiquinone intermediate (UQH•)
Ubisemiquinone anion (UQ•⁻)
Ubiquinone (UQ), oxidized form
Ubiquinol (UQH2), fully-reduced
b
Golgi
nucleus
ER
MAM
mitochondria
peroxisome
mevalonate
IPP
FPP
UQ
FPP
IPPs
Pdss1/2
4-HB
2,4-diHB
3,4-diHB
Coq2
Coq6
Coq8
Coq3
Coq5
Coq4
Coq?
Several catalytic steps
Mclk1/Coq7
Coq9
Coq3
UQH2

Figure 2


Functions of ubiquinone in the mitochondrial respiratory chain
Cytosol
other reactions
H_2O_2
SOD1
$O_2^{\cdot-}$
other reactions
H^+
UQ
Cyt c
I
III
IV
I-III-IV
Supercomplex
I
Forward
Reverse
UQH_2
$UQ^{\cdot-}$
e^-
FMN
NADH
NAD^+
DHODH
G3PDH
UQ
UQH_2
II
FAD
succinate
fumarate
ETFQOR
RISP
c_1
Cyt c
Q_o site
Q_i site
b_L
b_H
III
IV
Cu_A
Cyt a
Cu_B
$2e^-$
$½ O_2$ + $2H^+$
H_2O
V
IMM
ADP + P_i
ATP
Superoxide
Iron-sulfur clusters
SOD2
GPX
H_2O
other reactions
other reactions