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Review

Mitochondrial Coenzyme Q Redox Homeostasis and Reactive Oxygen Species Production

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Abstract

Mitochondrial coenzyme Q (mtQ) of the inner mitochondrial membrane is a redox active mobile carrier in the respiratory chain that transfers electrons between reducing dehydrogenases and oxidizing pathway(s). mtQ is also involved in mitochondrial reactive oxygen species (mtROS) formation through the mitochondrial respiratory chain. Some mtQ-binding sites related to the respiratory chain can directly form the superoxide anion from semiubiquinone radicals. On the other hand, reduced mtQ (ubiquinol, $mtQH_2$) recycles other antioxidants and directly acts on free radicals, preventing oxidative modifications. The redox state of the mtQ pool is a central bioenergetic patameter that alters in response to changes in mitochondrial function. It reflects mitochondrial bioenergetic activity and mtROS formation level, and thus the oxidative stress associated with the mitochondria. Surprisingly, there are few studies describing a direct relationship between the mtQ redox state and mtROS production under physiological and pathological conditions. Here, we provide a first overview of what is known about the factors affecting mtQ redox homeostasis and its relationship to mtROS production. We have proposed that the level of reduction (the endogenous redox state) of mtQ may be a useful indirect marker to assess total mtROS formation. A higher mtQ reduction level ($mtQH_2/mtQ_{total}$) indicates greater mtROS formation. The mtQ reduction level, and thus the mtROS formation, depends on the size of the mtQ pool and the activity of the mtQ-reducing and $mtQH_2$-oxidizing pathway(s) of respiratory chain. We focus on a number of physiological and pathophysiological factors affecting the amount of mtQ and thus its redox homeostasis and mtROS production level.

Keywords: mitochondrial coenzyme Q (mtQ); mtQ redox state; mtQ pool size; mtQ homeostasis; mtQ-reducing and QH_2-oxidizing pathways; reactive oxygen species formation

1. Introduction

Mitochondria are multifunctional organelles crucial for cellular energy production and the formation of reactive oxygen species (ROS) [1]. In addition, mitochondria are involved in many other processes critical to cell function and dysfunction, including maintaining metabolic and ionic homeostasis, calcium signaling, cell differentiation and growth, cell cycle control, and cell death.

Mitochondria generate a significant amount of energy in the form of the adenosine 5′-triphosphate (ATP) molecule needed to power the cell's biochemical reactions [2]. The tricarboxylic acid cycle (the TCA cycle) and oxidative phosphorylation are the mitochondrial stages of cellular respiration leading to the oxidation of reduced carbon compounds. The four electron transport complexes of the respiratory chain (complexes I–IV) and ATP synthase (complex V), which directly catalyzes the formation of ATP, form the oxidative phosphorylation system located in the inner mitochondrial membrane (Fig. 1). The mitochondrial coenzyme Q (mtQ)-reducing pathway of the respiratory chain consists of complex I (NADH:mtQ oxidoreductase) and complex II (succinate oxidoreductase:mtQ), which transfer electrons from reduced nucleotides, nicotinamide adenine dinucleotide (NADH) and dinucleotide adenine ($FADH_2$), respectively. Complex III ($mtQH_2$:cytochrome *c* oxidoreductase, bc_1 complex), cytochrome *c*, and finally complex IV constitute the $mtQH_2$-oxidizing pathway in the respiratory chain. Electron transport in the respiratory chain and ATP production are coupled by a proton electrochemical gradient formed by proton pumps (complexes I, III and IV) across the inner mitochondrial membrane. In addition to ATP synthesis by ADP phosphorylation, the proton electrochemical gradient can drive proton leak pathways, which do not generate ATP but may protect mitochondria from oxidative damage by decreasing mitochondrial ROS (mtROS) formation [3]. Moreover, the protonmotive force is coupled directly to the transport of several metabolites and calcium ions across the inner mitochondrial membrane and to the nicotinamide nucleotide transhydrogenase that regulates mitochondrial NADPH levels and mitochondrial redox balance.

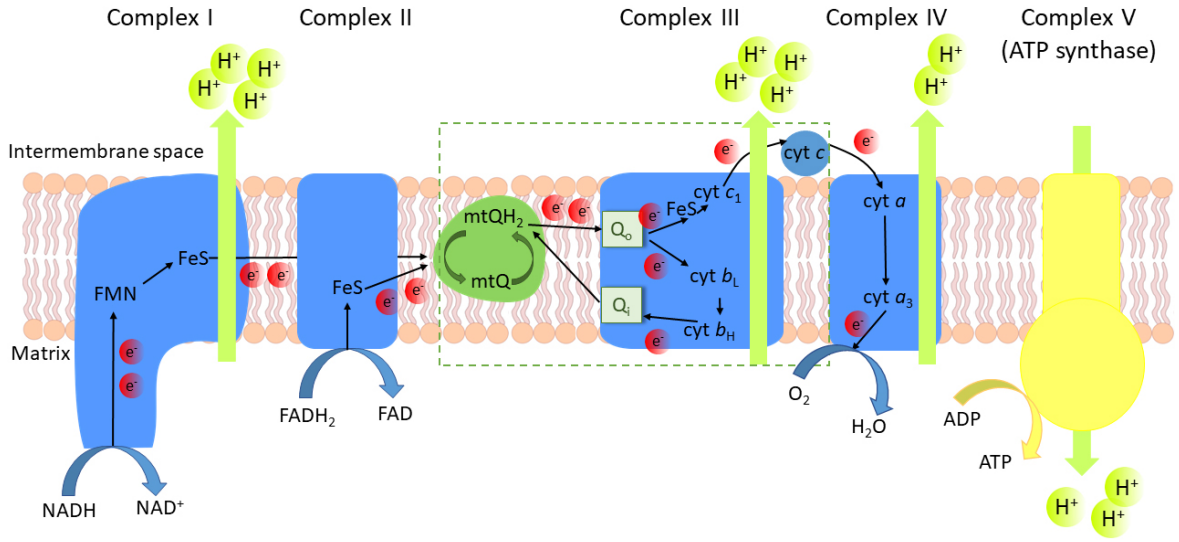

Fig. 1. The oxidative phosphorylation system in the inner mitochondrial membrane. Electron (e^-) transfer cofactors: FMN, flavin mononucleotide; FeS, iron-sulfur cluster; heme b_H and heme b_L of cytochrome *b*; heme c_1 of cytochrome c_1; heme *c* of cytochrome *c*; mtQ, oxidized mitochondrial coenzyme Q; $mtQH_2$, reduced mtQ, ubiquinol. Q_o, $mtQH_2$ binding site of complex III; Q_i, mtQ binding site of complex III. The mtQ cycle is marked in the box.

1.1 Structure and Redox States of Q

Coenzyme Q (Q, ubiquinone or 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone) is a ubiquitous fat-soluble vitamin-like compound distributed in all cell membrane systems, including the plasma membrane and all intracellular membranes, primarily the inner mitochondrial membrane [4–6]. Q consists of a redox active benzoquinone ring with two methoxy groups, one methyl group, and an isoprenoid side chain with a species-specific length (6 to 10 units) (Fig. 2). For example, ten isoprenoid units (Q10) have been described as the most commonly found Q in humans and nine units (Q9) in rodents (Q6) [7]. Some species have more than one Q isoform, e.g., rodent tissues contain different proportions of Q9 and Q10. In cells, Q is present in three different redox states, i.e., fully oxidized (ubiquinone, Q), intermediate semioxidized (semiubiquinone radical, $QH^{\bullet}$), and fully reduced (ubiquinol, QH_2) (Fig. 2) [8–10]. Thus, Q has the ability to accept and transfer one or two electrons, which is crucial for its role as a one-electron carrier in the mitochondrial electron transport chain because of the iron-sulfur (FeS) clusters, which can only accept one electron at a time [11], and for its role as a powerful antioxidant scavenging free radicals [12,13].

1.2 Q Biosynthesis

Unlike other lipophilic antioxidants such as vitamin A, carotenoids, and vitamin E, which are usually obtained from dietary sources, Q can also be produced endogenously [14]. The synthesis of Q takes place in two main steps, one of which provides the quinone ring, while the other produces the isoprenoid tail (for reviews see [15–20]). The 4-benzoquinone ring is synthesized by converting tyrosine (or phenylalanine) to 4-hydroxybenzoate, while the polyprenyl tail is provided via the mevalonate pathway, which also synthesizes cholesterol and dolichols, i.e., large isoprenoid chains comprised of multiple isoprene units. The synthesis of Q precursors (4-hydroxybenzoate and polyprenylpyrophosphate) takes place in the cytosol, and the last stages of Q biosynthesis take place on the matrix side of the inner mitochondrial membrane [21,22]. The quinone head is attached to a polyprenylated tail by a prenyl transferase (COQ2) which, together with other enzymes, forms a multiprotein Q biosynthetic complex on the matrix side of the inner mitochondrial membrane [7,21]. After multiple modifications of the aromatic ring, including methylation, decarboxylation, hydroxylation and deamination (catalyzed by COQ3, COQ5, COQ6, and COQ7, respectively), the final Q molecule is formed. Defects in Q synthesis, including the highly regulated mitochondrial biosynthetic complex, can lead to a reduction in mtQ levels and thus to disturbance of mtQ redox homeostasis and oxidative stress [23].

Despite the vast number of publications on both cellular and mitochondrial Q, its antioxidant and bioenergetic functions, role in health and disease, the literature is surprisingly limited regarding the relationship between Q redox state and ROS production in mitochondria under physiological and pathological conditions. In addition, the role of the mtQ redox state (mQH_2/mtQ ratio) is poorly understood because determining its value is technically difficult. In this review, we focus on factors affecting mtQ redox homeostasis and its relationship to mtROS production. We first describe the mtQ-reducing and mtQ-oxidizing path-

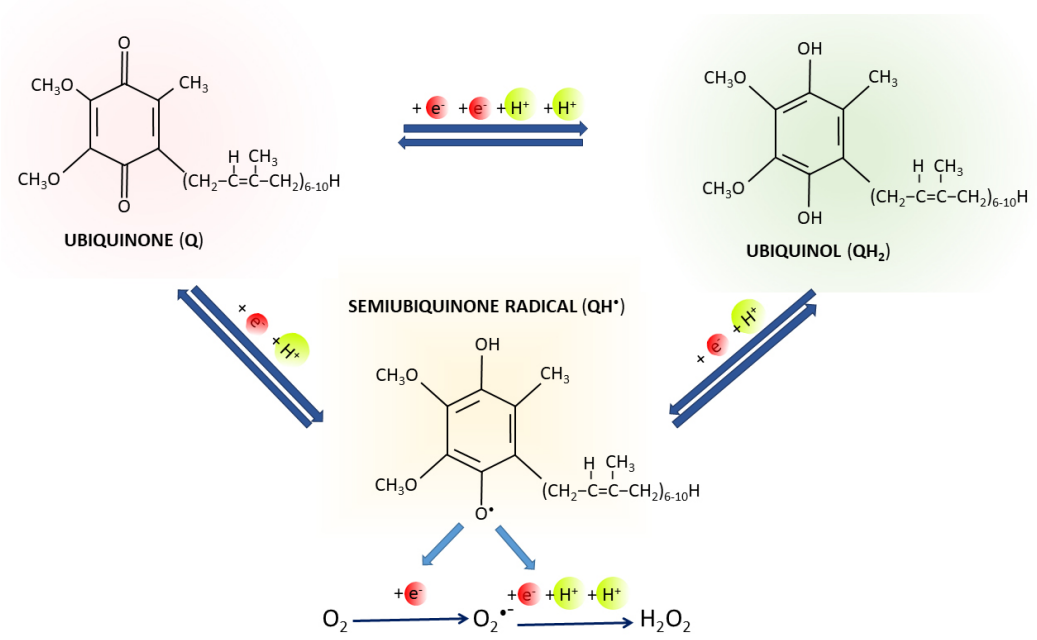

Fig. 2. Q redox states. Oxidized form of Q (ubiquinone) can be reduced to ubiquinol (QH_2) by two one-electron reactions through semiubiquinone ($QH^{\bullet}$) intermediate, or by one reaction of two electrons. Superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) can be produced by the leakage of electrons from the redox group of $QH^{\bullet}$ with unpaired electron.

ways of the mitochondrial electron transport chain and the factors influencing the redox state or reduction level of the mtQ pool. We then discuss the relationship between mtQ and mtROS formation, with an emphasis on the mtQ-biding sites of mtROS production, the dependence of mtROS formation on mtQ reduction level, and the relationship between mtQ content and mtROS production. Finally, we provided an introduction to the role of reduced mtQ as an antioxidant. In conclusions, we describe the technical progress in determining the redox state of Q in cells, tissues and isolated mitochondria.

2. mtQ as an Electron Carrier in the Mitochondrial Respiratory Chain

mtQ forms the mtQ pool in the lipid phase of the inner mitochondrial membrane. mtQ plays a central role as an electron carrier in the mitochondrial respiratory chain [24–26]. Dehydrogenases that oxidize respiratory substrates reduce mtQ to $mtQH_2$, and mQH_2-oxidizing pathway(s) convert $mtQH_2$ to mtQ (Fig. 1). mtQ regulates the flow of electrons in the respiratory chain and the building of a proton electrochemical gradient (including the mitochondrial membrane potential, $mt\Delta\Psi$) across the inner mitochondrial membrane [3,18,27,28]. Therefore, mtQ is the only mobile nonprotein electron carrier in the respiratory chain involved in the formation of the protonmotive force and, consequently, the oxidative phosphorylation process leading to ATP synthesis.

2.1 mtQ-Reducing Pathways

mtQ is reduced to $mtQH_2$ by several dehydrogenases that oxidize reducing equivalents derived from various food fuels, i.e., glucose, fats and amino acids [29]. Thus, mtQ is a common reservoir of electrons that contribute to oxidative oxidative phosphorylation, regardless of source.

Complex I and complex II of the mitochondrial respiratory chain reduce mtQ pool with electrons from the reduced nucleotides (NADH and $FADH_2$, respectively) derived mainly from the TCA cycle and thus from the oxidation of major food molecules. In the rotenone-sensitive complex I, two electrons are transferred from NADH to the flavin mononucleotide (FMN) and then, through the iron-sulfur centers to mtQ, which is reduced to $mtQH_2$ [30–32]. The malonate-sensitive complex II is an enzyme involved in the TCA cycle and oxidizes succinate to fumarate. Complex II transfers two electrons from $FADH_2$ through the iron-sulfur centers to mtQ, thereby also introducing two electrons into the mitochondrial respiratory chain [33–35].

In addition to the basic dehydrogenase complexes of the respiratory chain, i.e., complex I and complex II, mtQ receives electrons from a number of electron transporting flavoproteins that supply electrons to the mtQ pool [36–38]. mtQ is reduced by electron transfer flavoprotein (ETF):mtQ oxidoreductase (ETF:mtQ oxidoreductase) that oxidizes $FADH_2$ derived from β-oxidation of fatty acids in the mitochondrial matrix [39,40]. Electrons are also delivered to the mtQ pool from the oxidation of dihydroorotate to orotate by dihydroorotate dehydrogenase, a key mitochon-

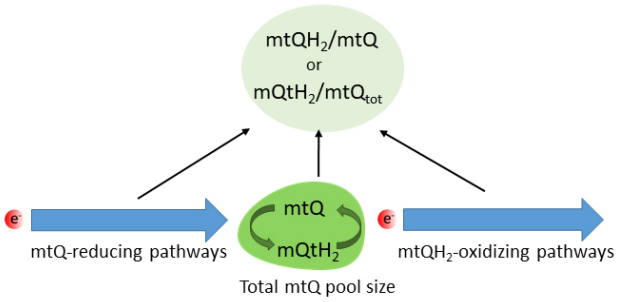

Fig. 3. Factors influencing the mtQ reduction level. mtQ, oxidized mitochondrial coenzyme Q; $mtQH_2$, reduced mtQ, quinol; mtQ_{tot}, total mtQ (mtQ + $mtQH_2$) in the inner mitochondrial membrane.

drial enzyme in pyrimidine nucleotide biosynthesis [41,42]. Another mtQ-reducing dehydrogenase located on the outer surface of the inner mitochondrial membrane is FAD-linked glycerol-3-phosphate dehydrogenase, which catalyzes the conversion of glycerol-3-phosphate to dihydroxyacetone [43,44]. The oxidation of glycerol-3-phosphate by mitochondrial glycerol 3-phosphate dehydrogenase is a major pathway for the transfer of cytosolic reducing equivalents from lipid and carbohydrate metabolism to the mitochondrial electron transport chain. In addition, mtQ accepts electrons from FAD-linked proline dehydrogenase, which is involved in the metabolism of amino acids [45,46]. In the branched mitochondrial respiratory chain of plants, fungi, and some protists, mtQ receives electrons from alternative rotenone-resistant NAD(P)H dehydrogenases located on the outer and inner surfaces of the inner mitochondrial membrane [47,48]. Among the mtQ-reducing dehydrogenases, only complex I of mitochondrial respiratory chain pumps protons and therefore participates in the generation of the proton electrochemical gradient.

2.2 $mtQH_2$-Oxidizing Pathway(s)

mtQ metabolism depends on $mtQH_2$ reoxidation by the mitochondrial $mtQH_2$-oxidizing pathway(s). In mammalian mitochondria, the cytochrome pathway (complex III, cytochrome *c*, and finally complex IV) is the only $mtQH_2$-oxidizing pathway in the respiratory chain [2,27]. The antimycin A- and myxothiazol-sensitive complex III and the cyanide-sensitive complex IV pump protons through the inner mitochondrial membrane to the intermembrane space and therefore participates in the generation of the protonmotive force. In the branched mitochondrial respiratory chain of plants, fungi, and some protists, there is an additional antimycin A- and cyanide-resistant alternative quinol oxidase (AOX) that oxidizes mtQ, bypassing the cytochrome pathway [49,50]. Transfer of electrons in AOX is not accompanied by proton pumping and thus mitochondrial ATP production.

Complex III ($mtQH_2$:cytochrome *c* oxidoreductase, cytochrome bc_1 complex) of the cytochrome pathway oxidizes the $mtQH_2$ pool directly, catalyzing a series of electron transfer reactions from $mtQH_2$ to cytochrome *c*, called the mtQ cycle (Fig. 1, marked box) [51–53]. Thus, complex III connects two mobile electron carriers of the respiratory chain, mtQ and cytochrome *c*. The reducing force of the $mtQH_2$ pool and the oxidizing force of the cytochrome *c* pool influence the redox state of the four metal redox centers in complex III: hemes b_H and b_L in cytochrome *b*, and heme c_1 and the Rieske FeS center in cytochrome c_1. mtQ reduction and $mtQH_2$ oxidation reactions take place at two separate catalytic centers. The myxothiazol-sensitive Q_o site is the site of binding and oxidation of QH_2 located toward the intermembrane space, while the antimycin A-sensitive Q_i site is the site of binding and reduction of mtQ located on the matrix side.

The mtQ cycle of complex III involves all three forms of Q (fully oxidized, semioxidized, and fully reduced) (Fig. 3) and participates in the generation of the protonmotive force as during $mtQH_2$ oxidation protons are released into intermembrane space [51–54]. The mtQ cycle involves a series of one-electron redox reactions as a result of bifurcation of electrons at the Q_o site (Fig. 1, marked box). At this site, one electron from $mtQH_2$ is transferred to the Rieske protein, then to cytochrome c_1, and finally to cytochrome *c*; the second electron is translocated through heme b_H and heme b_L to the catalytic site Q_i, where mtQ is reduced to a semiubiquinone radical ($QH^{\bullet}$) [51–55]. The oxidation of two $mtQH_2$ molecules involves the reduction of two cytochrome *c* molecules and completes the reduction of semiubiquinone to $mtQH_2$ at the Q_i site. Thus, a complete enzymatic cycle of complex III gives the oxidation of one $mtQH_2$ with the reduction of two molecules of cytochrome *c* and the return of half of the electrons within the mtQ pool.

Although one of the main functions of the mtQ-reducing and $mtQH_2$-oxidizing pathways is to maintain the mtQ redox homeostasis, there are few studies describing a direct relationship between their activity and the level of mtQ reduction (or mtQ redox state) and mtROS production under physiological and pathological conditions.

3. Redox State or Reduction Level of the mtQ Pool

mtQ levels, its redox state and its interaction with mtQ-dependent enzymes may play a key role in modulating mitochondrial homeostasis. One of the main properties of the mtQ pool in the inner membrane is its redox state or mtQ reduction level, defined as the $mtQH_2$/Q ratio or the ratio of $mtQH_2$ to the total mtQ content in the membrane ($mtQH_2/mtQ_{total}$ ratio), respectively. The redox state of mtQ depends on the several factors, including the activity of mtQ-reducing pathways (respiratory substrate-oxidizing dehydrogenases), the activity of $mtQH_2$-oxidizing pathway(s) (the cytochrome pathway and AOX), and the size of

the mtQ pool of the inner mitochondrial membrane (Fig. 3). The $mtQH_2$/mtQ ratio is sensitive to an imbalance between the supply of electrons from reducing equivalents and the need for ATP. It changes rapidly and reversibly in response to changes in the function of mitochondria. Interestingly, it has recently been proposed that succinate evolved as a signaling modality because its concentration reflects the mtQ redox state [56]. By balancing with the mtQ pool, succinate enables the transfer of the mtQ redox state from the mitochondria to the rest of the cell, to the circulation and other cells.

The redox state of the mtQ pool reflects the bioenergetic mitochondrial activity of cells and is considered to be one of the markers of oxidative stress [29,52,57–61]. It represents the level of mtROS formation, and thus mitochondrial oxidative stress, and the need for protective antioxidants in mitochondria. It should be noted that mitochondrial reactive nitrogen species are also key contributors to oxidative stress [62,63]. Nitric oxide (NO) can react with ubiquinol (QH_2) to form nitric oxide radical ($NO^{\bullet}$), which can react with O_2 to form peroxynitrite ($ONOO^{\bullet-}$), and semiubiquinone ($QH^{\bullet}$), which can react with O_2 to form superoxide ($O_2^{\bullet-}$) [64].

The size of the mtQ pool together with cellular Q may be modified by various factors, including endurance training [65–67], statin-induced mtQ synthesis impairment [68], cell age and oxygen level [69–71] or certain diseases, including cardiovascular and neurodegenerative disease or cancer [22,60,72,73]. However, the size of the mtQ pool is rarely determined. Similarly, alteration in the redox state of the mtQ pool has so far been determined for only a few physiological and pathophysiological conditions, including endurance training [65–67] and statin treatment [68]. Interestingly, the size of the reduced nonoxidizable pools of mtQ may reflect the cell's need for Q as an antioxidant [74]. Namely, we have recently shown that in the tissues of rats with different energy requirements (heart, brain, liver and lungs), the production of ROS at the tissue and mitochondrial levels was strongly related to the available reduced nonoxidizable Q and mtQ pools, i.e., the cellular QH_2 and $mtQH_2$ content measured in the absence or depletion of oxidative metabolism substrates [74].

The mtQ reduction level adapts dynamically to variable metabolic conditions in the cell. Therefore, another possible function of the mtQ reduction level is related to the action of mitochondrial uncoupling proteins (UCPs), which mediate regulated proton leakage across the inner mitochondrial membrane, leading to the dissipation of energy stored in the proton electrochemical gradient [75,76]. First, it was observed that mtQ is an obligatory cofactor for UCP function [77], and the regulation of uncoupling activity by mtQ depends on its reduction level [78]. Moreover, we have reported that $mtQH_2$ but not mtQ acts as a negative modulator for the UCP inhibition by purine nucleotides [79].

4. mtQ and mtROS Formation

Mitochondria are an important source of ROS (mtROS) in eukaryotic cells. Superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) are produced by the leakage of electrons from the redox centers of the mitochondrial respiratory chain and substrate oxidation-associated enzymes, leading to a one-electron or two-electron reduction of oxygen [36,80,81]. mtQ participates in the generation of $O_2^{\bullet-}$ from semiubiquinone radicals by the leakage of electrons from its redox group with unpaired electron (Fig. 2).

mtROS are formed as a by-product of normal aerobic metabolism or under conditions of metabolic stress leading to oxidative stress. Under physiological conditions, mtROS can act as beneficial signaling molecules sent from the mitochondria to other parts of the cell that is essential for cellular adaptive responses [36,82–84]. Dysfunction of the mitochondrial respiratory chain leads to decreased energy production and mtROS overproduction. Under oxidative stress conditions, excessive mtROS production can lead to a range of oxidative damage, including oxidative modification of cellular macromolecules such as lipids, DNA and proteins, which is the cause of aging and many diseases. In addition, damaged mitochondria release apoptotic factors that act as signals inducing cell death.

In mammalian mitochondria, at least eleven sites can generate mtROS ($O_2^{\bullet-}$ and/or H_2O_2) in the electron transport chain (seven sites) and related enzymes (four sites) involved in the oxidation of the reduced substrates, including the TCA cycle and β-fatty acid oxidation enzymes (Fig. 4) [80,85]. The sites of $O_2^{\bullet-}/H_2O_2$ production are present in respiratory chain complexes (complexes I, II and III) and dehydrogenases (mitochondrial glycerol-3-phosphate dehydrogenase, dihydroorotate dehydrogenase, and ETF:mtQ oxidoreductase), the redox cofactor of which is mtQ. mtQ is directly involved in the formation of $O_2^{\bullet-}/H_2O_2$ at four mtQ-binding sites, i.e., the mtQ-reducing sites of complex I (I_Q) [86], mitochondrial glycerol-3-phosphate dehydrogenase (G_Q) [87], dihydroorotate dehydrogenase (D_Q) [88], and the $mtQH_2$-oxidizing site of complex III (III_{Qo}) [89]. Other dehydrogenases linked to mtQ, such as proline dehydrogenase, feed electrons into the mtQ pool and then reduce other sites that produce $O_2^{\bullet-}/H_2O_2$ [90]. However, it is possible that these dehydrogenases also produce $O_2^{\bullet-}/H_2O_2$ at mtQ-reducing sites, but this production is too small to be detected [80]. Changes in the bioenergetic states of mitochondria, as a consequence of alterations of mitochondrial substrate availability and the reduction states of mitochondrial redox centers, including mtQ, affect the rate of $O_2^{\bullet-}/H_2O_2$ production in various mitochondrial sites [80,85]. There are seven characterized $O_2^{\bullet-}/H_2O_2$ production sites that are not directly related to mtQ reduction but contain redox-active flavin (FMN or FAD): I_F of complex I [86], II_F of complex II [91], E_F of ETF/ETF:mtQ system [92], and O_F, B_F, A_F, and P_F sites of 2-oxoacid dehydrogenase complexes, i.e., 2-oxoglutarate dehydrogenase (the

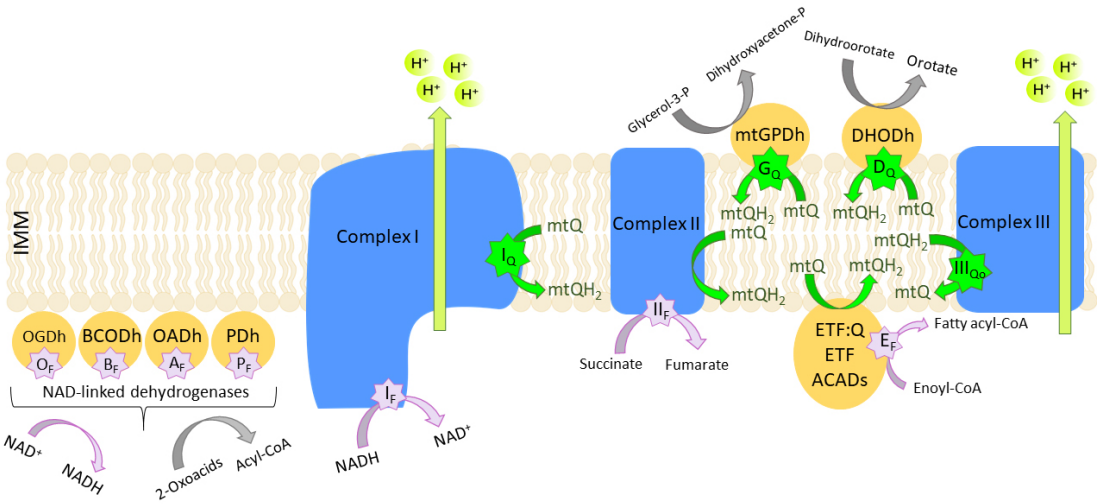

Fig. 4. Eleven mitochondrial sites of $O_2^{\bullet-}/H_2O_2$ formation. mtQ, ubiquinone; $mtQH_2$, ubiquinol; ETF, electron transfer flavoprotein; ETF:Q, ETF:Q oxidoreductase; ACADS, acetyl-CoA dehydrogenase; IMM, inner mitochondrial membrane. Flavin-dependent (F, purple stars) or mtQ-binding sites (Q, green stars) of the 2-oxoglutarate dehydrogenase (OGDh) (O_F), branched chain 2-oxoacid dehydrogenase (BCODh) (B_F), 2-oxoadipate dehydrogenase (OADh) (A_F), and pyruvate dehydrogenase (PDh) (P_F) complexes, of complex I (I_F, I_Q), complex II (II_F), the outer mtQ site of complex III (III_{Qo}), mitochondrial glycerol-3-phosphate dehydrogenase (mtGPDh) (G_Q), the ETF/ETF:mtQ oxidoreductase system (ETF/ETF:mtQ) (E_F), and dihydroorotate dehydrogenase (DHODh) (D_Q). Based on [85].

TCA cycle enzyme), branched-chain 2-oxoacid dehydrogenase, 2-oxoadipate dehydrogenase, and pyruvate dehydrogenase complexes, respectively [93]. The 2-oxoacid dehydrogenase complexes contain a dihydrolipoamide dehydrogenase subunit with redox-active FAD, which acts as an $O_2^{\bullet-}/H_2O_2$ production site. They feed electrons to NAD^+. Other NAD-linked dehydrogenases, such as malate dehydrogenase, isocitrate dehydrogenase (TCA cycle enzymes), and glutamate dehydrogenase reduce other sites that produce $O_2^{\bullet-}/H_2O_2$ [80]. Electrons from NADH formed in all NAD-linked dehydrogenases are transferred to the flavin (FMN) in the NADH-oxidizing site of complex I (I_F) and then to the mtQ-reducing site of complex I (I_Q).

Site III_{Qo} of complex III and site G_Q of glycerol-3-phosphate dehydrogenase generate $O_2^{\bullet-}$ in both the mitochondrial matrix and intermembrane space [80,87,89]. Other mtROS formation sites produce $O_2^{\bullet-}/H_2O_2$ in the mitochondrial matrix. These different $O_2^{\bullet-}/H_2O_2$ formation topologies are important for intracellular signaling using mtROS as signaling molecules.

4.1 mtQ-Biding Sites of mtROS Production

In the inner mitochondrial membrane, mtQ is a molecule directly involved in the production of mtROS due to the formation of $O_2^{\bullet-}/H_2O_2$ by the leakage of electrons from the semiubiquinone radical. All four mtQ-binding sites (I_Q,III_{Qo}, G_Q, D_Q) produce $O_2^{\bullet-}$ and only one of them (D_Q) is likely to produce H_2O_2 as well [80,85].

At I_Q of complex I, $O_2^{\bullet-}$ is mainly generated during reverse electron transport (RET), i.e., a transfer of electrons against the redox potential gradient supported by a high proton electrochemical gradient (a high protonmotive force) across the inner mitochondrial membrane and a high mtQ redox state (highly reduced mtQ pool) [86]. Under these conditions, $mtQH_2$ is oxidized to mtQ at the mtQ-binding site (I_Q) to drive the reduction of NAD^+ to NADH at the FMN site of complex I (I_F). Inhibition of mtQ-binding with rotenone inhibits RET and lowers mtROS production.

At the outer mtQ-binding site of complex III (III_{Qo}), $O_2^{\bullet-}$ production is low in the absence of Q_i site inhibitors [94]. In the presence of antimycin A, which binds to the Q_i site and blocks the oxidation of cytochrome b_L heme, the mtQ cycle is disrupted. The resulting accumulation of reduced cytochrome b_L heme limits semiubiquinone oxidation at site Q_o. Semiubiquinone interacts with oxygen to form $O_2^{\bullet-}$ on both sides of the inner mitochondrial membrane. In the absence of antimycin A, a high protonmotive force and highly reduced mtQ pool decreases the electron transfer from the cytochrome b_L heme to the cytochrome b_H heme, leading to more reduced b_L heme and thus increased $O_2^{\bullet-}$ production [95].

Site G_Q of mitochondrial glycerol-3-phosphate dehydrogenase produces $O_2^{\bullet-}$ in approximately equal amounts on each side of the mitochondrial inner membrane, suggesting that the mtQ-binding site of the enzyme is the major site of $O_2^{\bullet-}$ formation during glycerol-3-phosphate oxidation [87]. Dihydroorotate dehydrogenase can generate $O_2^{\bullet-}$ and/or H_2O_2 directly at its mtQ-binding site (D_Q) at low rates [88]. The enzyme is also capable of indirect production at higher rates from other sites through its ability to reduce the mtQ pool.

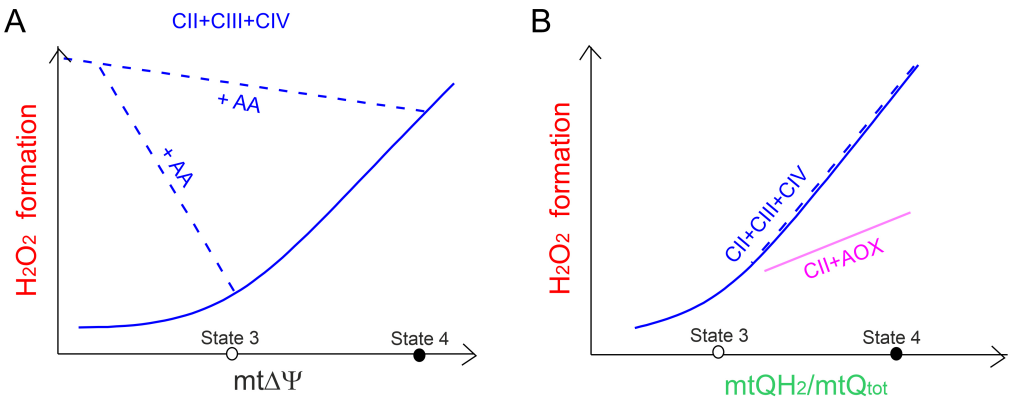

Fig. 5. The relationship between mtROS formation versus mtΔΨ and the mtQ reduction level. The relationship between H_2O_2 formation versus mtΔΨ (A) for the cytochrome pathway (CII + CIII + CIV) fueled by complex II (CII) and the relationship between H_2O_2 formation versus the mtQ reduction level ($mtQH_2/mtQ_{tot}$) (B) for the CII + CIII + CIV pathway (blue curves and lines) and the CII-fueled alternative oxidase (AOX) (CII + AOX) (purple line). Based on results described in *A. castellanii* mitochondria [59]. The mtΔΨ level (A) and mtQ reduction level (B) of the CII + CIII + CIV pathway was varied with the ATP synthesis and CII inhibitors, with the uncoupler (blue solid curves), and with the inhibitor of CIII (antimycin A, AA) (blue dashed lines) when AOX inhibitor was present. Measurements were carried out under nonphosphorylating (state 4) and phosphorylating (state 3) conditions. (B) For the CII + AOX pathway, the mtQ reduction level was titrated with an activator of AOX (GMP) and an inhibitor of CII (malonate) in the presence of AA to block the cytochrome pathway.

In a cell, the net $O_2^{\bullet-}/H_2O_2$ formation rate at mitochondrial mtQ-binding sites depends on the substrates being oxidized and the antioxidant systems acting on both sides of the inner mitochondrial membrane. Among the mtQ-binding sites, III_{Qo} and I_Q, which is mainly active during RET, dominate in $O_2^{\bullet-}$ production and play an important role in redox signaling and the induction of oxidative damage [80].

4.2 mtQ Reduction Level and mtROS Formation

As a mobile component of the mitochondrial electron transport chain, mtQ acts as a pro-oxidant in the semiubiquinone state. It is generally accepted that the production of mtROS depends on the level of reduction of the mtQ pool, which determines the formation of $O_2^{\bullet-}$ from semiubiquinone. However, there are only a few data relating the mtQ redox state, i.e., $mtQH_2/Q$ ratio [96] and $mtQH_2/mtQ_{total}$ ratio [59,66,68,95], with mtROS generation. The formation of mtROS related to the activity of the respiratory chain, and thus the mtQ redox state, depends on the activity of all mtQ-reducing dehydrogenases and $mtQH_2$-oxidazing pathway(s), and on the activity of the oxidative phosphorylation process in which ATP is formed [59]. We have shown that under ADP phosphorylating conditions (state 3), a low mtQ reduction level and a low mΔΨ are accompanied by decreased mtROS production [59]. In a nonphosphorylating state (state 4), a highly reduced mtQ pool (a high mtQ reduction level) and a high mΔΨ are accompanied by increased mtROS production. Thus, the $mtQH_2/mtQ$ ratio is sensitive to changes in electron supply from reducing equivalents and to ATP demand. The activity of uncoupling proteins (UCPs), which mediate proton leakage, can lower mΔΨ and the mtQ reduction level, leading to a decrease in mtROS production [76,84].

The relationship between mtROS production and mtΔΨ was investigated more often than the interplay between mtROS formation and the mtQ reduction level. For the first time, the dependence of mtROS formation on mtΔΨ was described in rat heart mitochondria [97]. Namely, a very steep dependence of H_2O_2 formation on mtΔΨ was observed in nonphosphorylating rat heart mitochondria after exceeding a threshold value near mtΔΨ of ADP phosphorylating mitochondria. However, not under all conditions lowering mtΔΨ leads to a decrease in mtROS production (e.g., in the presence of inhibitors of complexes III and IV), indicating that mtROS is not a direct function of the mtΔΨ level (Fig. 5A) [59]. For example, we have shown that in the mitochondria of the amoeba *Acanthamoeba castellanii*, with inhibition of the Q_i site of complex III by antimycin A or complex IV by cyanide, mΔΨ decreases while the mtQ reduction and H_2O_2 production increase [59]. Marphy's group has shown that the formation of $O_2^{\bullet-}$ at the Q_o site is associated with the accumulation of reduced cytochrome b_L when the Q_i site is blocked by antimycin A, and this phenomenon is not dependent on mtΔΨ [95].

We have kinetically described a direct dependence of mtROS production on the mtQ reduction level in *A. castellanii* mitochondria [59]. For both $mtQH_2$-oxidizing pathways (the cytochrome and AOX pathways), during the oxidation of succinate fueling the respiratory chain at the level of complex II, a higher level of mtQ reduction leads to

greater mtROS formation (Fig. 5B) [59]. This relationship is also observed for the cytochrome pathway in the presence of antimycin A, which by inhibiting the Q_i site lowers $m\Delta\Psi$ and increases mtQ reduction level. In the case of the cytochrome pathway, H_2O_2 generation depends nonlinearly on the reduction level of mtQ pool. At levels of mtQ reduction higher than that of the phosphorylating state (above 35%) there is a steep relationship between H_2O_2 generation and the mtQ reduction level. In contrast, AOX is active only when the mtQ reduction level is high (above 40%) and the dependence between H_2O_2 generation and mtQ reduction level is approximately linear (Fig. 5B). We have proposed that the mtQ pool reduction level (the endogenous redox state of mtQ) may be a useful endogenous marker that allows for the assessment of total mitochondrial mtROS production [59].

High levels of mtROS are generated in response to stress via RET through complex I [61,98]. RET occurs when the mtQ pool it is strongly reduced by electrons from the respiratory complex II (succinate dehydrogenase) [95,98]. In various cell lines, it has been observed how the respiratory chain is optimized for better oxidation of various reducing respiratory fuels by the redox state of mtQ ($mtQH_2$/Q ratio) acting as a metabolic detector and mtROS formed by complex I (via RET) acting as an executor [96]. In the rat heart mitochondria, the formation of mtROS by RET at complex I has been shown to be dependent on the mtQ reduction level and $m\Delta\Psi$ that indicates the sensitivity of $O_2^{\bullet-}$ formation by the mtQ-binding site of complex I to these two metabolic variables [95].

4.3 mtQ Content and mtROS Production

It seems that the production of mtROS does not directly depend on the amount of mtQ but rather on the reduction level of the mtQ pool ($mtQH_2/mtQ_{total}$ ratio) as a function of the activity of mtQ-reducing pathways (mtQ-reducing dehydrogenases) and the $mtQH_2$-oxidizing cytochrome pathway(s). In the heart mitochondria of MCLK1 (CoQ7) mutant mice, which contain only ~10% of normal Q content, lower rates of mtROS production from all known mtROS production sites were detected at respiration levels similar to those in control mitochondria [99]. One possible explanation for how low mtQ levels reduce mtROS formation is that low mtQ induces the formation of supercomplexes that have been proposed to increase electron transport efficiency from one redox component to another, thus minimizing electron leakage and mtROS production [100,101]. In cultured fibroblasts from Q-deficient patients, increased mtROS production was observed during intermediate Q deficiency (50–70%) possibly as a result of increased semiubiquinone generation from increased redox cycling of the restricted mtQ pool, while a more severe loss of Q (>85%) was not accompanied by significant mtROS production [102]. Interestingly, we have shown that in lung mitochondria of endurance-trained rats, reduced mtQ content (mtQ9 by ~16% and mtQ10 by ~42%) and decreased activity of complex I as well as increased activity of cytochrome pathway (complex III plus complex IV) may account for the observed diminished mtQ reduction level during succinate and/or malate oxidation, resulting in a general decrease of mtROS formation by mitochondria [66]. Moreover, endurance training downregulates complex I in supercomplexes and upregulates complex III in the $CIII_2$ + CIV supercomplex in the inner membrane of lung mitochondria. In addition, comparison of the effects of endurance training on mtQ amount and mtROS generation in rat tissues with high energy demand, i.e., in the brain, liver and heart, indicates that endurance training may induce various mitochondrial and tissue responses related to mtQ acting as an electron carrier in the respiratory chain and as an antioxidant [67]. Changes in the formation of mtROS observed in the mitochondria of individual rat tissues may result from changes in the size of the pool of oxidized mtQ, which acts as an electron carrier, as well as the amount and activity of individual complexes of the oxidative phosphorylation system and its molecular organization.

A decrease in the mtQ pool size and mtQ reduction level may results from drug treatment. We have shown that in cultured endothelial cells, chronic exposure to atorvastatin, an inhibitor of HMG-CoA reductase led to a significant decrease in mtQ10 content (~23%) [68]. The activity of complex III and its amount in a supercomplex with complex IV was also diminished. The statin-induced changes at the respiratory chain level of the endothelial mitochondria led to a decrease in succinate and/or malate oxidation accompanied by a decreased $m\Delta\Psi$, as well as to an increase in the mtQ10 reduction level and consequently increased mtROS production.

Thus, mtQ redox homeostasis is a key factor in modulating mtROS production. As mentioned above, depending on the activity of mtQ-reducing pathways and $mtQH_2$-oxidizing pathways, a decreased amount of mtQ can lead to either an increased reduction level of mtQ (a higher $mtQH_2/mtQ_{total}$ ratio) and thus to an increased production of mtROS or to a decreased reduction level of mtQ (a lower $mtQH_2/mtQ_{total}$ ratio) and hence decreased production of mtROS. Superphysiological mtQ levels could possibly have the same effect by altering the level of mtQ reduction. Thus, changing the size of the mtQ pool and its redox state is an important physiological adaptation. The metabolic response to the altered mtQ pool appears to depend on whether it is more important to maintain an efficient oxidative phosphorylation process or whether it is more important to defend against oxidative stress.

The level of Q, including mtQ, varies with the type of membrane, tissue and organism [4,103]. It is highest in organs with a high metabolic rate and hence a high energy requirement, such as the heart, muscles, and liver. However, despite high metabolic activity, total Q levels and the QH_2/Q ratio in the human brain are very low compared to

other tissues [4]. The mtQ pool is in excess compared to other components of the respiratory chain [100]. However, with age and in many disease states, including primary and secondary Q deficiencies, associated with increased production and action of mtROS, the level of cellular and thus mitochondrial Q decreases [23,60,71,72,104]. mtQ deficiency may impair oxidative phosphorylation and therefore respiratory chain activity and ATP synthesis [105–108], and may induce oxidative stress (mtROS overproduction) leading to oxidative modification of macromolecules, and ultimately to apoptosis. However, the question of how Q deficiency due to disease or aging affect the redox state of mtQ has not yet been thoroughly investigated.

Increasing mtQ levels must also result in a change in mtQ redox homeostasis and mtROS production. For example, we have observed an increase in mtQ9 content (by ~20%) in skeletal muscle mitochondria after eight-week endurance training [54]. In these mitochondria, H_2O_2 production and mtQ reduction level were elevated under nonphosphorylating conditions, and decreased under phosphorylating conditions, while the efficiency of oxidative phosphorylation increased with unchanged components of the respiratory chain. Recently, it has been shown that elevated levels of mtQ caused by Q10 delivery (via by BPM31510) lead to an increase in ROS generation and consequently, an imbalance in cellular redox homeostasis that activates mtROS-mediated cell death pathways in pancreatic cancer cells [109]. Similarly, cell death activation due to increased mtQ pool was observed *in vitro* in pancreatic cancer cells, and both human patient-derived organoids and tumor xenografts. Interestingly, in other studies, chemotherapy induced increased production of ROS and increased levels of Q10 (especially its reduced form $Q10H_2$) in all cancer cell lines tested [110]. But again, we do not know how mtQ redox state changes under these conditions and what role it plays in mtROS production.

5. mtQ as an Antioxidant

The redox state of the mtQ pool is an important indicator of the bioenergetic and antioxidant status of the mitochondria. The fully reduced form of mtQ ($mtQH_2$) can act as a nonprotein lipophilic antioxidant [111,112]. The level of mtROS depends not only on their production in the mitochondria and thus on the amount of mtQ and its redox state in the inner mitochondrial membrane, but also on the mtROS scavenging by reduced forms of mtQ. Additionally, by acting as an antioxidant, mtQ protects against oxidative damage caused by mtROS. $mtQH_2$ regenerates other antioxidants (vitamin C or vitamin E) as well as reduces and neutralizes oxidants or free radicals, including mtROS [11,22,112]. As an effective free radical scavenger, $mtQH_2$ prevents oxidative modifications of mitochondrial proteins and DNA and inhibits the initiation and propagation of membrane lipid peroxidation. In lipid peroxidation reactions, one-electron oxidation of $mtQH_2$ to semiubiquinone is linked to the reduction of perferryl radicals or lipid peroxyl radicals [12]. Reactive semiubiquinone radicals are neutralized by reduction to QH_2 by α-tocopherol (vitamin E). However, $mtQH_2$ is still a capable antioxidant molecule when vitamin E is absent [113]. On the other hand, the indirect antioxidant effect of $mtQH_2$ can regenerate α-tocopherol from α-tocopheroxyl radicals (α-$TO^{\bullet}$) [114]. Interestingly, in addition to the plasma membrane and intracellular membranes, Q is also a component of low-density proteins (LDL), suggesting its potential antioxidative role along with α-tocopherol in the prevention of atherosclerosis [115].

Thus, as a molecule with antioxidant and prooxidative properties, mtQ contributes both to oxidative damage to mitochondria and to their antioxidant defense. The antioxidant action of $mtQH_2$ is associated with deprotonation (and electron donation), leading to the formation of semiubiquinone radicals and ultimately oxidized mtQ, which is then reduced back to $mtQH_2$ by the mitochondrial respiratory chain [112,116].

It is widely believed that the antioxidant properties of Q may have beneficial effects on health and length of life [117]. Q has been widely introduced as an antiaging supplement as well as a potential treatment for several human diseases related to mitochondrial dysfunction-induced oxidative stress. However, understanding the complex effect of Q on the human body and its relationship to lifespan is still incomplete in view of inconsistent literature reports on Q supplementation, describing its beneficial or nil effects [23,29,60,71,73]. Paradoxically, some Q biosynthesis defects result in an increase in life span in animal models, most likely due to a mild reduction in respiratory chain activity and thus a reduction in mtROS production or due to a physiological adaptation to early mitochondrial dysfunction [118]. On the other hand, Q10 supplementation may have positive effects in the treatment of certain cancers in patients with reduced plasma and tissue Q levels [109]. Similarly, Q10 supplementation may have beneficial effects on aging-related disorders accompanied by a reduced Q content, especially in cardiovascular and metabolic diseases [119–122]. However, effect of Q10 supplementation on reversing functional decline of mitochondrial bioenergetics is unclear. Moreover, it is unclear if this improvement is always related to the increase in mtQ levels and the change in mtQ redox state after Q10 supplementation. Supplementation with high doses of Q10 may increase both circulating and intracellular levels, but there are conflicting results regarding bioavailability [71,123,124]. Although it has been shown that endogenous levels of mtQ in the mitochondria of the rodent brain, skeletal muscle, and heart can be increased after Q10 administration [125,126].

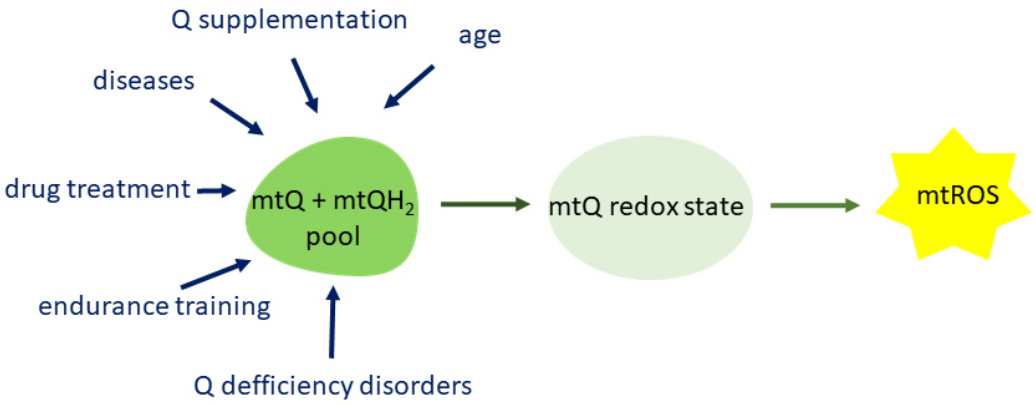

Fig. 6. Factors modulating the mtQ pool content which influences the redox state of mtQ and thus the formation of mtROS. mtQ, mitochondrial coenzyme Q; mtROS, mitochondrial reactive oxygen species.

Nonoxidizable mtQH$_2$ Pool as an Mitochondrial Antioxidant

We have proposed that the antioxidant effect may also be exerted by the reduced mtQH$_2$ pool in the inner mitochondrial membrane, which is not oxidized by the respiratory chain [74]. Interestingly, in mitochondria isolated from different rat tissues with different energy requirements and different mitochondrial oxidative activity heart, brain, liver and lungs, the size of the mtQH$_2$ pool that was not oxidized by the respiratory chain (measured in the absence of respiratory substrates) was associated with the mtROS production capacity during the oxidation of respiratory substrates [74]. Thus, the size of the reduced nonoxidizable pools of mtQ (nonoxidizable mtQH$_2$ pool) may reflect the need for mtQ as a mitochondrial antioxidant. Therefore, it is possible that both the mtQH$_2$ pool, which participates in the mtQ cycle of the electron transport chain, and the mtQH$_2$ pool, which is not oxidized by the chain, contribute to the overall antioxidative activity of mitochondria.

6. Conclusions and Perspectives

As a redox active cofactor of mitochondrial respiratory chain complexes and related enzymes, mtQ influences mtROS formation and contributes to oxidative damage to mitochondria. On the other hand, thanks to its redox properties, mtQ acts as an antioxidant that protects the mitochondria from oxidative modifications. Mitochondria contain the most Q in a cell but are also the most dependent on its content. A number of physiological and pathophysiological factors affect the amount of mtQ and thus its redox state and mtROS production level (Fig. 6), indicating that mtQ homeostasis is essential for metabolic adaptation and the maintenance of mitochondrial function.

The purpose of our review was to highlight the rather little-studied and discussed crucial role of mtQ redox homeostasis in modulating mtROS production. As we proposed, the level of reduction (the endogenous redox state) of mtQ may be a useful marker to assess total mtROS generation. However, there are few studies describing a direct relationship between the mtQ redox state and mtROS production under physiological and pathological conditions. mtQ redox ratio has rarely been measured, especially under conditions of Q deficiency. Undoubtedly, measuring the Q redox state in cells, tissues or isolated mitochondria is technically demanding and not as popular as measuring mtROS formation. Determination of the QH$_2$/Q ratio is more complicated than determining the total Q pool because ubiquinol (QH$_2$) is particularly sensitive to oxidation. The difficulty in determining the redox state of mtQ in biological samples and *in vivo* has been a significant obstacle in characterizing mtQ function in mitochondria under physiological and pathophysiological conditions. An extraction technique followed by high performance liquid chromatography (HPLC) detection [127,128] is usually used to determine the concentration of cellular and mitochondrial Q and QH$_2$, and to calculate the level of Q reduction (QH$_2$/Q$_{total}$) or Q redox state (QH$_2$/Q) in cells/tissues and isolated mitochondria, respectively. However, this technical approach requires a large amount of biological material, is time consuming and, importantly, results in a non-kinetic point measurement. Presumably, the recent advancement in the technique of simultaneous kinetic measurement of oxygen consumption and mtQ redox state by combining a Clark-type oxygen electrode and a Q-type electrode [28,129] will contribute to the widespread measurement of the redox state of mtQ in isolated respiring mitochondria. In addition, a sensitive approach to assess the Q redox state by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in isolated mitochondria, cells and tissues *in vivo* has recently been developed [130]. This technical approach allows the analysis of both the redox state of Q (non-kinetic point measurements) and the size of the Q pool with negligible changes in the redox state due to isolation and extraction from small amounts of biological material. Understanding metabolism linked to the mtQ pool is challenging but new experimental models and methodologies will undoubtedly

help. Determining the redox state of Q under physiological and pathological conditions is of great importance for better understanding how Q contributes to the maintenance of cellular and mitochondrial homeostasis.

Author Contributions

WJ, KD, AB, KW and LG wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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