



Abnormalities of hydrogen sulfide and glutathione pathways in mitochondrial dysfunction

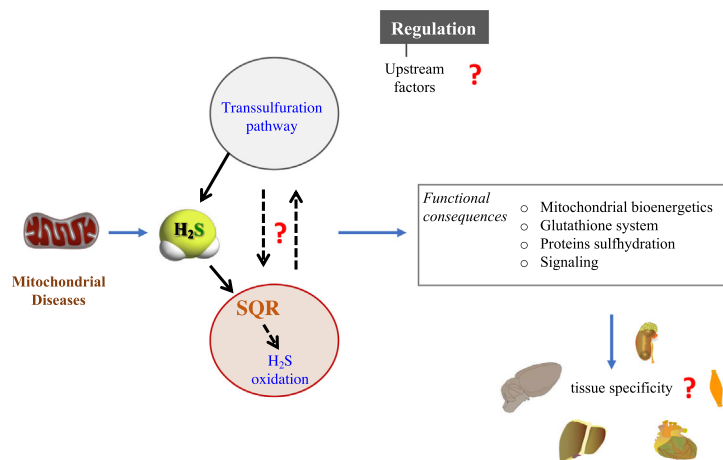
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GRAPHICAL ABSTRACT



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ABSTRACT

Background: Mitochondrial disorders are genetic diseases for which therapy remains woefully inadequate. Therapy of these disorders is particularly challenging partially due to the heterogeneity and tissue-specificity of pathomechanisms involved in these disorders. Abnormalities in hydrogen sulfide (H₂S) metabolism are emerging as novel mechanism in mitochondrial dysfunction. However, further studies are necessary to understand the effects, protective or detrimental, of these abnormalities, and their relevance, in mitochondrial diseases.

Aim of Review: To review the recent evidences of derangement of the metabolism of H₂S, at biosynthesis or oxidation levels, in mitochondrial dysfunction, focusing specifically on the alterations of H₂S oxidation caused by primary Coenzyme Q (CoQ) deficiency.

Key Scientific Concepts of Review: Mitochondria play a key role in the regulation of H₂S and GSH metabolism pathways. However, further studies are needed to understand the consequences of abnormalities of H₂S and GSH synthesis on the oxidation pathway, and *vice versa*; and on the levels of H₂S and GSH, their tissue-specific detrimental effects, and their role the role in mitochondrial diseases. Beside the known

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H₂S pathways, additional, tissue-specific, enzymatic systems, involved in H₂S production and elimination, might exist.

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Introduction

Mitochondrial diseases are metabolic disorders, clinically heterogeneous, and characterized by tissue specificity [1]. The reasons of this tissue-specificity are not completely understood [1], but it may be related to tissue-specific pathological or compensatory mechanisms. Recent findings in *in vitro* and *in vivo* models of mitochondrial diseases indicate that mitochondrial dysfunction alters the metabolism of hydrogen sulfide (H₂S), at biosynthesis or oxidation levels [2–8]. But whether, in the context of mitochondrial diseases, these abnormalities have functional, beneficial or detrimental relevance, and how modifications of the biosynthetic and catabolic pathways affect each other, and H₂S levels, are still unclear. Here, we review these recent findings, focusing specifically on the alterations of H₂S oxidation caused by primary Coenzyme Q (CoQ) deficiency [9,10].

Impairment of H₂S oxidation in CoQ deficiency

CoQ is a critical intermediate in the mammalian respiratory chain located in the mitochondrial inner membrane (MIM), as it transfers electrons from complexes I (CI) and II (CII) to complex III (CIII) in the course of producing ATP via oxidative phosphorylation (OxPhos) (Fig. 1) [11]. [Turunen, 2004 #8] Therefore, it is not surprising that mutations in CoQ synthesis can cause OxPhos disease [9,10], presumably via (a) reduced ATP synthesis as a result of reduced electron flow and/or (b) via increases in CoQ-derived reactive oxygen species (ROS) that damage the OxPhos machinery

[11]. In fact, there are several lines of *in vitro* and *in vivo* evidence that oxidative stress is a deleterious factor in the pathogenesis of CoQ deficiency [12–17].

However, besides its role in the respiratory chain, CoQ participates in other metabolic pathways [11], including the conversion of sulfide (as H₂S) and sulfite (as SO₃) to thiosulfate (as SSO₃) by sulfide-quinone oxidoreductase (SQR; SQOR; gene SQOR) (Fig. 1), the first reaction in the H₂S oxidation pathway (Fig. 2) [18]. Importantly, electrons, in this redox reaction, that pass through CoQ are transferred to CIII and CIV, bypassing CI and CII. The ATP produced via this “SQR-driven respiration” is ordinarily a minor fraction of total ATP synthesis, but becomes significant in reducing environments [19,20].

The initial evidence of this biological function of CoQ was identified in yeast ~ 20 years ago [21,22], when studies in the fission yeast *S. pombe* revealed that strains with defects in CoQ biosynthetic genes, were unable to produce CoQ, accumulated H₂S, and required cysteine and glutathione to grow on minimal medium [21,22]. In these strains, grown in both rich and minimum media, levels of H₂S are decreased by cysteine supplementation, suggesting that cysteine, one of the sulfur amino acids together with methionine, controls the production of H₂S [21,22].

More recent studies in mammalian cells and tissues, showed that CoQ deficiency severely decreases SQR levels and, as a consequence, impairs SQR-driven respiration, and H₂S oxidation, and leads to accumulation of H₂S [7,8]. Specifically, defects of SQR-driven respiration and decreased levels of SQR protein, proportional to the decrease in the levels of CoQ, were found in fibroblasts from patients with CoQ deficiency due to various molecular

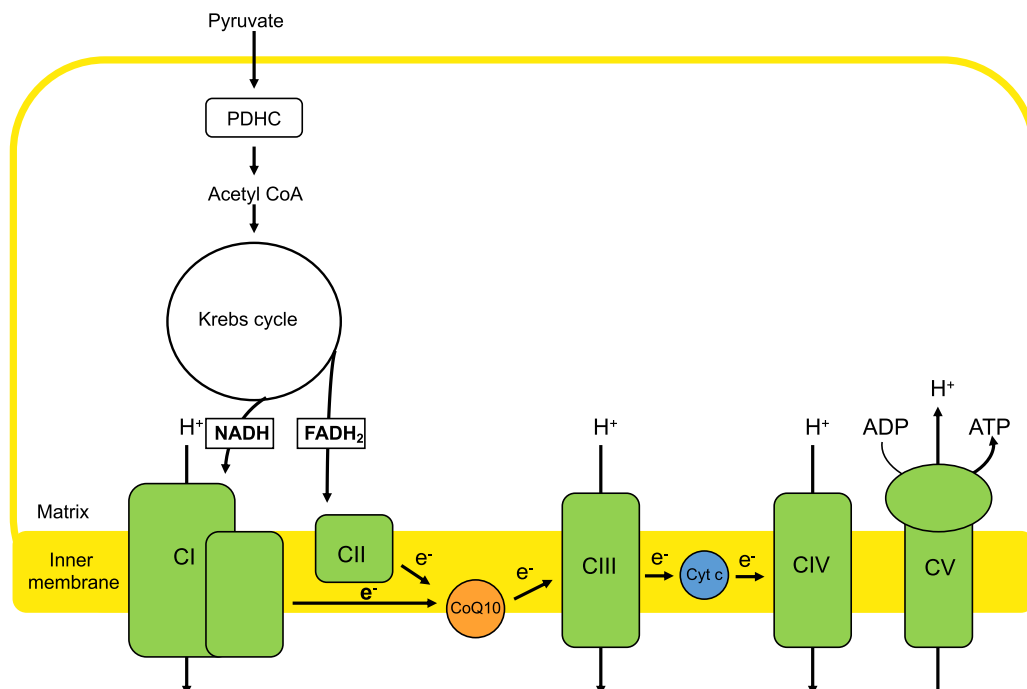


Fig. 1. Schematic representation of CoQ in the mitochondria. CoQ transfers electrons from NADH-ubiquinoneoxidoreductase (CI) to succinate dehydrogenase (CII) and CoQ-cytochrome *c* reductase (CIII) in the mitochondrial respiratory chain.

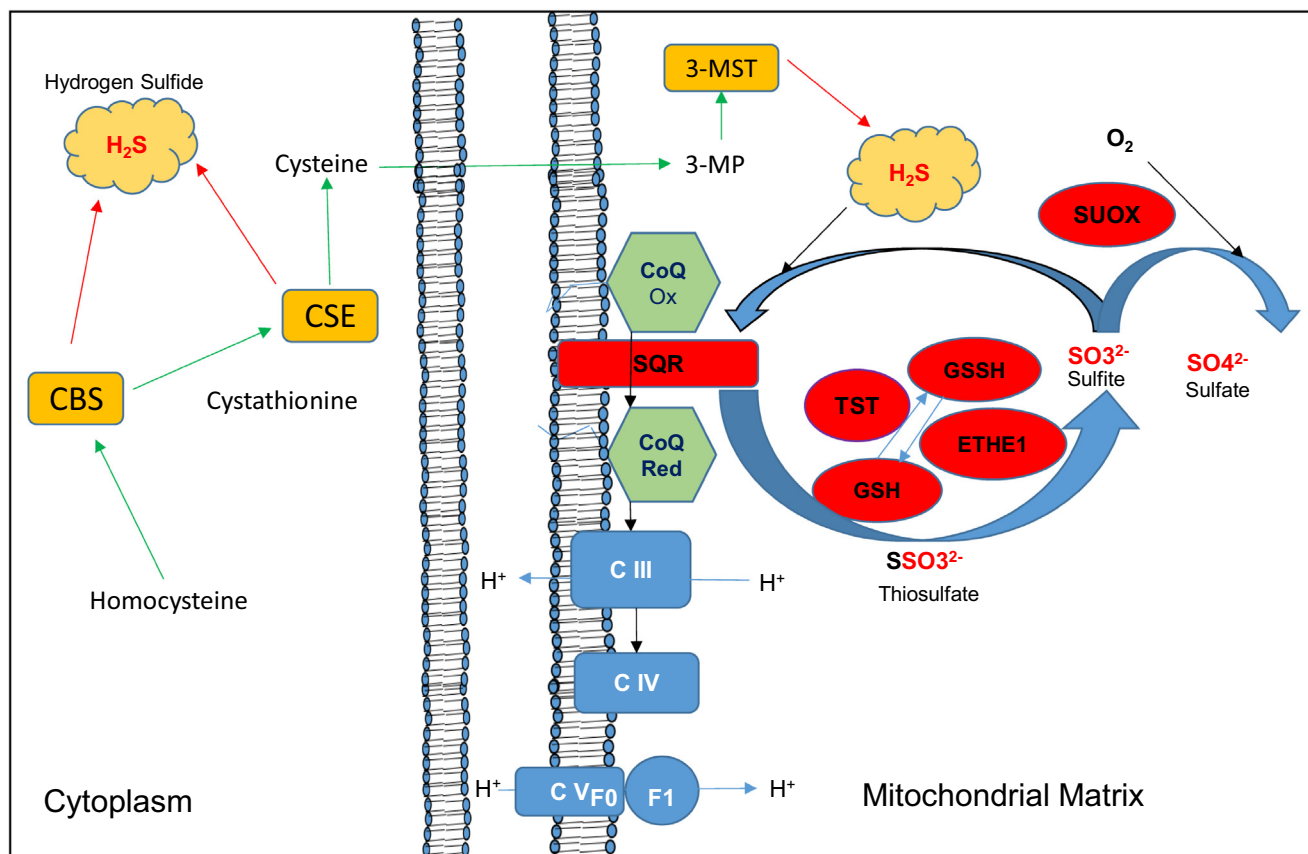


Fig. 2. Transsulfuration and H₂S oxidation pathways. In the transsulfuration pathway (in orange), the enzymes cystathionine β-synthase (CBS) and cystathionine γ-ligase (CSE) use cysteine as a substrate for the synthesis of sulfides (H₂S). The same cysteine is used by glutamate cysteine ligase (GCL) for the synthesis of GSH in the glutathione pathway (not shown). In the mitochondrial H₂S oxidation (in red), sulfide-quinone oxidoreductase (SQR) converts sulfide into thiosulfate by transferring two electrons from H₂S to CoQ. Thiosulfate is then converted into sulfite by thiosulfate sulfurtransferase (TST) and persulfide dioxygenase (ETHE1), a reaction that requires glutathione (GSH) as an electron acceptor. Excess sulfite is converted into sulfate by sulfite oxidase (SUOX). 3-MP = 3-mercaptopyruvate, 3-MST = 3-mercaptopyruvate sulfurtransferase, CoQ Ox = Coenzyme Q oxidized, CoQRed = Coenzyme Q reduced.

defects in enzymes of CoQ biosynthesis [7,8]. These abnormalities were rescued by CoQ supplementation, and recapitulated by genetic and pharmacological inhibition of CoQ synthesis, indicating that CoQ regulates SQR levels. In contrast, SQR, rather than CoQ levels, were responsible for the up-regulation of the downstream H₂S oxidation enzymes (Fig. 2) [7,8].

Importantly, CoQ supplementation also increased levels of SQR in control cells [7], indicating that CoQ regulates H₂S oxidation in physiological conditions, as well as in pathological states. This finding might have therapeutic implications for mitochondrial OxPhos defects other than CoQ deficiency, as deficiencies of CI and CII, which are localized up-stream SQR, and might be bypassed, by using SQR to feed electrons to complex III. For example, it may explain the results obtained by Vafai and colleagues, who developed a chemical screening platform to identify CI by bass factors, and found that naphthoquinones supplementation has therapeutic effects in CI deficient murine myoblasts [23].

H₂S oxidation was found to be impaired also in three mouse models of human primary CoQ deficiency, which manifest clinically with nephrotic syndrome (NS), encephalopathy, and myopathy [7,8], three of the most common phenotypes of human CoQ deficiency [10]. The models showed similarities in the biochemical and molecular phenotype, as well as differences, probably reflective of tissue specific pathomechanisms [7,8].

In the mouse model of CoQ-deficient NS due to a missense mutation in *Pdss2*, the first and rate-limiting step in CoQ biosynthesis [24,25], we observed that all organs had low CoQ levels

and OxPhos deficiency, but remarkably, only kidney also had elevated reactive oxygen species (ROS) and markers of oxidative stress [5,16], decreased levels of H₂S oxidation enzymes, accumulation of H₂S, and GSH depletion [5,7]. *Pdss2^{kd/kd}* mice have also low levels of plasma and urine thiosulfate, and increased blood C4-C6 acylcarnitines [5,7], indicating a defect in short-chain fatty acid oxidation, due to the inhibition of the short-chain acyl-CoA dehydrogenases (SCAD), a known toxic effect of H₂S accumulation [26].

Notably, increased ROS and low SQR were already evident in kidney of *Pdss2^{kd/kd}* mice at age 1 month, before the onset of the disease [5]. Furthermore, administration of CoQ in these *Pdss2^{kd/kd}* mice prolonged survival, from ~ 6 months in untreated mice to > 20 months in the CoQ-treated animals, prevented NS, as well as significantly reversed oxidative stress and sulfide derangement, acetylcarnitine profile, and GSH levels [5].

In *Coq9^{R239X}* and *Coq9^{Q95X}* mice, models of CoQ deficiency due to mutations in *Coq9*, SQR levels and function were decreased proportionally to residual CoQ levels in kidney, brain, and muscle [8]. *Coq9* is required for the stability and function of Coq7, which is responsible for one of the three hydroxylations of CoQ benzoquinone ring [27,28]. *Coq9^{R239X}* mice develop a fatal mitochondrial encephalopathy, associated with oxidative stress and a defect of respiratory supercomplexes assembly in brain, while *Coq9^{Q95X}* mice develop a late-onset mild mitochondrial myopathy in females [27,29]. Supplementation with ubiquinol-10 (the reduced form of CoQ) partially rescues the SQR depletion in muscle and kidney of

Coq9^{R239X}, parallel to increases in CoQ levels on those tissues [8]. In contrast to what observed in *Pdss2*^{kd/kd} mice, tissues of *Coq9*^{R239X} - and *Coq9*^{Q95X} did not show down-regulation of the enzymes downstream of SQR; in fact, TST levels and activity were increased, as observed in CoQ deficient cells [8]. However, similar to *Pdss2*^{kd/kd} mice, H₂S levels were increased in kidney, while levels of total GSH were significantly decreased [8]. The presence of additional H₂S biosynthetic pathways in the kidney, beside the transsulfuration pathway, might explain why H₂S accumulates preferentially in this tissue [30,31].

The consequences of H₂S accumulation found in CoQ deficiency, partially recapitulate the biochemical and molecular abnormalities previously reported in Ethylmalonic aciduria encephalopathy (EE), an autosomal recessive disorder caused by mutations in the gene encoding for the mitochondrial enzyme ETHE1, a persulfide dioxygenase involved in the same H₂S oxidation pathway as SQR (Fig. 2) [32]. Impairment of ETHE1 activity in human and mice causes chronic accumulation of H₂S in tissues, and SCAD inhibition with accumulation of C4- and C5-acylcarnitines in plasma. Furthermore, it causes tissue-specific cytochrome c oxidase (COX) deficiency [32,33], due to the formation of a covalent bond between H₂S and the Fe atom coordinated by heme a. The chronic exposure and binding of H₂S to COX causes accelerated degradation of its protein subunits thus reducing the amount of fully assembled and functionally active enzyme [34,35]. COX activity, but not its protein levels, are decreased also in tissues of the first three patients described carrying mutations in SQR, a novel cause of Leigh syndrome [36]. Interestingly, there is no evidence of COX deficiency in CoQ deficiency, perhaps due to less severe accumulation of H₂S. Fibroblasts from patients with EE have low levels of SQR and GSH, complicating the understanding of the mechanism underlying ETHE1 deficiency [37,38].

Impairment of the glutathione pathway in H₂S oxidation defects

H₂S has been linked to ROS production through different mechanisms [39,40]. One of the mechanisms that links H₂S to oxidative stress is GSH depletion [39,40], which is a well-known cause of ROS and oxidative stress, both present in mitochondrial diseases [41–44], including CoQ and ETHE1 deficiencies [12–16,45]. Studies in yeast indicate that levels of H₂S are tightly regulated by the equilibrium of the transsulfuration and oxidation pathways (Fig. 2). Thus, increased levels of H₂S (e.g. as a result of reduced SQR in CoQ deficiency) might operate in a negative feedback loop on the transsulfuration pathway. Because L-cysteine is also a key precursor of GSH biosynthesis, it is possible that as a consequence of reduced utilization of L-cysteine by the transsulfuration pathway, cysteine for GSH synthesis will also be reduced. Alternatively, H₂S auto-oxidation of transsulfuration enzyme may generate reactive sulfur and oxygen radicals that deplete GSH. Thus, this reduction in the GSH levels could induce a reduction in GPx4 levels and the activities of glutathione peroxidase (GPx) and glutathione reductase (GRD), as observed in *Coq9*^{R239X} mice [8]. Nevertheless, the glutathione system is not globally depleted in CoQ deficient fibroblasts, and *Sqr* depleted Hepa1c1c7 cells [8], indicating that GSH depletion is a tissue-specific, perhaps secondary effect of SQR depletion.

Interestingly, GSH, and GPX4 depletion, together with lipid ROS formation, characterize ferroptosis, a form of regulated non-apoptotic cell death [46]. GPX4 has been known to prevent ferroptosis by converting lipid hydroperoxides into non-toxic lipid alcohols [47]. Recently, also apoptosis-inducing factor mitochondrial 2 (AIFM2) has been identified as a potent ferroptosis-resistance factor, which co-operates with GPX4 and GSH to suppress phospholipid peroxidation and ferroptosis [48,49]. AIFM2,

re-named ferroptosis suppressor protein 1 (FSP1), is recruited to the plasma membrane where it functions as an oxidoreductase that reduces CoQ -using NAD(P)H- which halts the propagation of lipid peroxides [48,49]. However, AIFM2 might block ferroptosis through a mechanism independent of CoQ [50]. CoQ deficiency causes cell death, which correlates with ROS levels, and oxidative stress [12–15], but whether it causes specifically ferroptosis, has never been investigated.

Other mechanisms unrelated to GSH might be responsible for H₂S-mediated oxidative stress; for example, through increased S-sulfhydration of proteins specifically involved in cell redox status. In fact, protein S-sulfhydration, a post-translational modification of protein cysteine residues, is important for regulation of various cell functions [51,52], and is increased in CoQ deficient fibroblasts [7]. Nevertheless, in aging there are increased levels of ROS and oxidative stress, but protein S-sulfhydration is decreased [53].

Up regulation of the transsulfuration and glutathione pathways in mitochondrial DNA defects

H₂S is produced mostly from L-cysteine, that can be taken up with the diet, extracted from endogenous proteins, or synthesized endogenously via trans-sulfuration of serine by L-methionine, through the transsulfuration pathway (Fig. 2), which is expressed in all tissues [30].

Notably, L-cysteine, is also a key precursor of glutathione (GSH) biosynthesis, and its entry into that pathway is rate-limiting. Thus, mechanisms that regulate the availability of L-cysteine, likely affect both pathways, as demonstrated by metabolomics/transcriptomics approaches in different models of mitochondrial dysfunction. For example, studies on the effects of the 1-methyl-4-phenylpyridinium (MPP +), whose neurotoxicity is mediated by different mechanisms, including CI inhibition, revealed increased GSH associated with upregulation of ATF-4 and the transsulfuration enzymes CTH and CBS. In cells stressed with MPP+, knock-down of ATF-4 or CTH, reduced GSH levels [54]. CI deficiency also causes GSH synthesis in a model of renal oncocytoma [55].

Up-regulation of the transsulfuration and GSH synthesis enzymes is found in mitochondrial DNA (mtDNA) depleted HEK-293 cells, and in mice with mtDNA replication defects due to mutations in the mitochondrial helicase Twinkle [3,4]. These alterations are not isolated, but rather represent an aspect of a metabolic switch likely mediated by ATF-4 activation [3,4], and the contribution of every metabolic pathway to the pathogenesis of human mitochondrial disease has not yet been investigated. Evidence of abnormalities of the transsulfuration pathway were also identified in patients with mtDNA maintenance/translation disorders. A similar pattern was observed also in plasma of patients with mitochondrial dysfunction, including mtDNA deletions, secondary to inclusion bodies myositis (IBM) [2,56].

Up-regulation of the transsulfuration pathway and H₂S accumulation are present in a variety of models of longevity and stress resistance associated with dietary restriction [57], and they might contribute to the beneficial effects of hypoxia shown in *Ndufs4*^{-/-} mice [58,59], possibly through SQR-driven respiration, or through protection against oxidative stress mediated by protein S-sulfhydration. *Ndufs4*^{-/-} mice lack NADH: ubiquinone oxidoreductase iron-sulfur protein 4 (*Ndufs4*), and recapitulate the main findings of CI-related Leigh syndrome, the most common infantile mitochondrial encephalopathy. They develop a rapidly progressive encephalopathy, starting at ~ 40 days after birth, with > 90% mortality by 50 days of life [60]. Muscle, brain, and fibroblasts show evidence of oxidative stress and abnormal mitochondria [61,62]. In this model, hypoxia has been shown to prevent and rescue neu-

rological phenotype, and to prolong survival, but the mechanism is still unknown [58,59].

Conclusions

In the last few years, abnormalities of H₂S metabolism have been reported in a variety of models of mitochondrial dysfunction; however, the role of these abnormalities in mitochondrial diseases is still unknown. Further studies are needed to understand the relation of sulfide metabolism and mitochondrial diseases and the consequences of abnormalities of H₂S and GSH synthesis on the oxidation pathway, and *vice versa*; and on the levels of H₂S and GSH, and their tissue-specific detrimental effects. In this regard, we should consider not only the known, additional, tissue-specific, enzymatic pathways, that produce H₂S beside the transsulfuration pathway, but also those other, still unknown systems, involved in H₂S production and elimination, that might exist. Importantly, available data indicate that mitochondria play a key role in the regulation of those H₂S pathways.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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