Redox Biology 38 (2021) 101772

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Short Review

Effects of hydrogen sulfide on mitochondrial function and cellular bioenergetics

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ARTICLE INFO

Keywords:
Mitochondria
Hydrogen sulfide
Sulfide oxidation
Bioenergetics
Cell signaling
Sulfhydration/persulfidation

ABSTRACT

Hydrogen sulfide (H_2S) was once considered to have only toxic properties, until it was discovered to be an endogenous signaling molecule. The effects of H_2S are dose dependent, with lower concentrations being beneficial and higher concentrations, cytotoxic. This scenario is especially true for the effects of H_2S on mitochondrial function, where higher concentrations of the gasotransmitter inhibit the electron transport chain, and lower concentrations stimulate bioenergetics in multiple ways. Here we review the role of H_2S in mitochondrial function and its effects on cellular physiology.

1. Introduction

H_2S was believed to be a noxious molecule and an environmental hazard until it was discovered to be produced endogenously [1]. H_2S is generated in mammals by three enzymes: cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS) and 3-mercaptopyruvate sulfur transferase (3-MST). CSE and CBS are key enzymes in the reverse transsulfuration pathway leading to the transfer of sulfur from homocysteine to cysteine (Fig. 1). CBS condenses serine with homocysteine to form cystathionine, which is then acted on by CSE to produce cysteine. CSE and CBS produce H_2S by several different reactions [2]. CSE can use either cysteine or homocysteine in the presence of its cofactor, pyridoxal 5-phosphate (PLP) to produce H_2S. CBS does not produce H_2S from cysteine alone and prefers a combination of cysteine and homocysteine to produce H_2S; CBS also utilizes homocysteine to produce H_2S, although when homocysteine levels are high, the enzymatic activity of CBS is inhibited. 3-MST on the other hand produces H_2S in conjunction with cysteine amino transferase (CAT) to produce H_2S. H_2S participates in a wide spectrum of physiological processes in every tissue in the body, functioning as a gaseous signaling molecule or gasotransmitter [3–5]. H_2S levels are tightly regulated in cells as either excess or scarcity of the gaseous signaling molecule is detrimental. The mitochondria play a central role in the catabolism of H_2S, which regulates its steady state levels.

1.1. Hydrogen sulfide and mitochondrial bioenergetics

The mitochondria are the powerhouses of cells and the sites of aerobic respiration, generating ATP via oxidative phosphorylation (OXPHOS), accounting for about 80% of the energy requirements, the remaining 20% being met by glycolysis [6]. The mitochondrial OXPHOS system is composed of five multiprotein complexes (designated complex I–V) [6]. Electrons are transferred from NADH, an intermediate of the Krebs cycle, to NADH coenzyme Q reductase (complex I), which relays them to ubiquinone or coenzyme Q. Coenzyme Q also receives electrons from succinate dehydrogenase (SDH; complex II) and passes them to complex III (cytochrome bc1), which transfers them to cytochrome c, which relays them to complex IV (cytochrome c oxidase) that in turn uses these electrons to reduce molecular oxygen to water (Fig. 2A). The

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https://doi.org/10.1016/j.redox.2020.101772
Received 5 September 2020; Received in revised form 17 October 2020; Accepted 26 October 2020
Available online 28 October 2020

Abbreviations

3-MST	3-mercaptopyruvate sulfur transferase
AMPK	AMP-activated protein kinase
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
CAT	cysteine amino transferase
CBS	cystathionine β-synthase
CS	citrate synthase
CSE	cystathionine γ-lyase
DNMTA	DNA methyltransferase
DS	Down syndrome
IDH2	isocitrate dehydrogenase 2
IRF-1	interferon regulatory factor 1
LCAD	long chain acyl-CoA dehydrogenase
mPTP	mitochondrial permeability transition pore
OXPHOS	oxidative phosphorylation
PTEN	lipid phosphatase and tensin homolog
PLP	pyridoxal 5-phosphate
sAC	soluble adenylyl cyclase
SPRC	S-propyl-L-cysteine
SQR	sulfide quinone oxidoreductase
SDH	succinate dehydrogenase
TR	thiosulfate reductase
Trx	thioredoxin
TrxR	thioredoxin reductase
TST	thiosulfate sulfurtransferase
TFAM	mitochondrial transcription factor A
USP8	ubiquitin specific peptidase 8

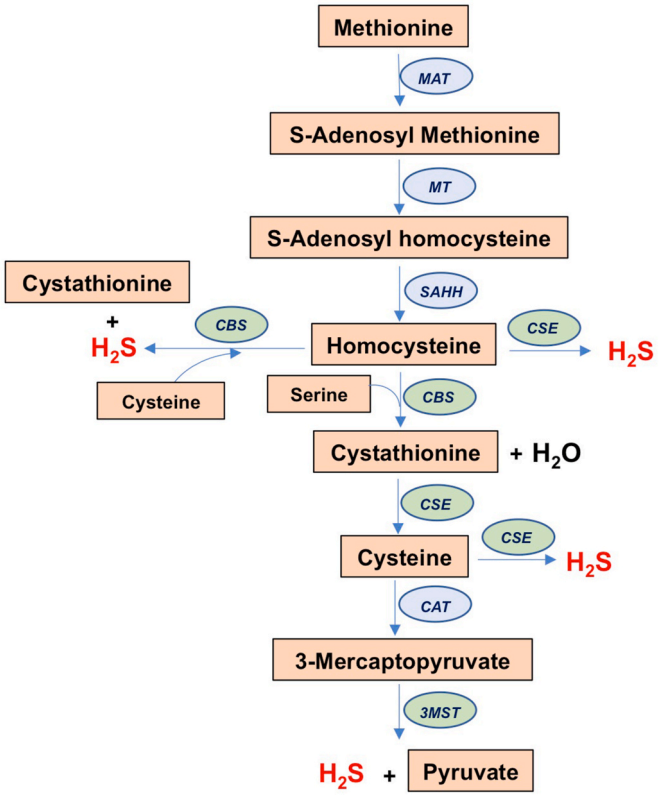

Fig. 1. Biosynthetic pathway leading to hydrogen sulfide production. Shown is the transsulfuration pathway, which involves transfer of sulfur from homocysteine to cysteine. Homocysteine is derived from dietary methionine in mammals by the action of methionine-adenosyltransferase (MAT) and methyltransferase (MT) and S-adenosylhomocysteine hydrolase (SAHH). Homocysteine condenses with serine in a condensation reaction catalyzed by cystathionine β-synthase (CBS) to produce cystathionine and water. Cystathionine is acted on by cystathionine γ-lyase (CSE) to generate cysteine. Cysteine is used as a substrate by CSE to generate H_2S. CBS prefers a combination of cysteine and homocysteine to produce H_2S and cystathionine. Cysteine is utilized by cysteine amino transferase (CAT) to produce 3-mercaptopyruvate, which is the substrate for 3-mercaptopyruvate sulfur transferase (3-MST) to produce hydrogen sulfide (H_2S). Homocysteine can also be utilized as a substrate by CSE and CBS to generate H_2S.

biological effects of H_2S follow a bell shaped or biphasic dose-response curve. In the case of mitochondrial function, lower doses of H_2S are beneficial, whereas higher doses are inhibitory. One of the earliest reported effects of H_2S on mitochondrial function involved an induction of a state of suspended animation, which involved the inhibition of cytochrome c oxidase (Complex IV) of the electron transport chain in the mitochondria [7–10]. H_2S binds the copper center of cytochrome c oxidase to inhibit its activity [10,11]. The toxic effects of H_2S that led to its classification as an environmental toxin or pollutant was primarily attributed to this property [12]. Another example of excess H_2S production is Down syndrome (DS), which is caused by the trisomy of chromosome 21, which causes aberrant expression of genes on the chromosome, causing mental retardation along with vascular and metabolic abnormalities. As CBS is localized on chromosome 21, excess production of H_2S was proposed to mediate these abnormalities in DS [13]. In postmortem samples of brains from DS patients, overexpression of CBS was observed which fits in with theory [14]. Similarly, overexpression of 3-MST was also observed in DS fibroblasts, which could contribute to excess H_2S and mitochondrial dysfunction [15]. Inhibiting H_2S production in fibroblasts derived from DS patients restored mitochondrial bioenergetics [16]. Excess H_2S production was also reported in amyotrophic lateral sclerosis (ALS), a disease affecting motor neurons of the brain and spinal cord, leading to paralysis [17,18].

H_2S was first linked to oxidative phosphorylation in 1986, when it was discovered that *Solemya reidi*, a gutless clam found in sulfide-rich habitats, oxidized H_2S in its tissue mitochondria [19]. Two decades later, H_2S was shown to be the first inorganic donor for energy production by mitochondria at low micromolar concentrations [20]. The donation of electrons occurs at the level of coenzyme Q by the action of sulfide quinone oxidoreductase (SQR) on H_2S. Coenzyme Q also receives electrons from complex I by oxidation of NADH and Complex II by oxidation of succinate [21]. In addition to these parallel pathways, other oxidation reactions that donate electrons to coenzyme Q are FADH2 generated during fatty acid oxidation, or the oxidation of L-α glycerophosphate in muscle.

1.2. *Mitochondrial localization of H_2S enzymes*

At low concentrations, H_2S has beneficial effects on mitochondrial function. Several reports indicate that the biosynthetic enzymes for H_2S may be present within the mitochondria. While 3-MST is present in both the cytoplasm and the mitochondria, CSE and CBS, are predominantly cytosolic, but they do translocate to the mitochondria as well. In vascular smooth muscle cells, calcium influx triggers mitochondrial translocation of CSE, a process dependent on translocase of the outer membrane 20 (Tom20) to generate H_2S in the mitochondria [22]. The existence of CSE in the mitochondrial compartment was also suggested by earlier studies which report an increase in cystathionine content in rat liver mitochondria treated with propargylglycine, an inhibitor of CSE [23]. CBS, too is associated with mitochondria, and has been reported to be associated with the outer mitochondrial membrane in colon cancer

cells, and stimulates mitochondrial bioenergetics [24]. Thus, all the three H_2S enzymes modulate mitochondrial function and energetics. Mitochondrial homeostasis is intimately linked to almost all aspects of cellular physiology, several of which are regulated by H_2S, hence it is not surprising that effects of the gasotransmitter on mitochondria are pivotal in the cellular functioning of the heart. The effects of H_2S on mitochondrial functioning are discussed below.

1.3. The catabolism of H_2S in mitochondria

In mammals, H_2S is produced by both their own cells and by the intestinal flora [25]. As anaerobic metabolism by resident microbiota in the colon produce significant levels of H_2S, the cells of the intestine must defend themselves, by either utilizing or detoxifying excess sulfide. H_2S is oxidized to thiosulfate and sulfate in the mitochondria and its rate varies in different tissues [26]. The sulfide oxidation pathway is highly active in cells of the colon. SQR, part of the sulfide oxidation unit (SOU) catalyzes the first step in mitochondrial sulfide oxidation (Fig. 2B) [21,27]. Colon epithelial cells are exposed to high H_2S levels and thus these cells harbor an efficient mitochondrial H_2S oxidation pathway. In the human colon, the sulfide oxidation pathway enzymes exhibit an apical localization aligned with the host-microbiome interface [28]. H_2S is oxidized by SQR, which forms a persulfide and releases two electrons, which are transferred by flavin adenine dinucleotide to CoQ, which then relays them to the ETC. The persulfide is then transferred to an acceptor such as GSH to form GSH persulfide (GSSH) or sulfite (SO_3^{2-}), which is oxidized to sulfate, as shown in Fig. 2B. GSSH is then oxidized by persulfide dioxygenase (ETHE or SDO (sulfur dioxygenase) to sulfite. Sulfite can be oxidized to sulfate by sulfite oxidase (SO) or reduced by thiosulfate sulfurtransferase (TST/rhodanese) to form thiosulfate (SSO_3) by the addition of a persulfide. The sulfane sulfur from thiosulfate can be acted on by another sulfurtransferase called thiosulfate reductase (TR) to form GSSH and SO_3^{2-} [27]. Thus, in short, steady state levels of H_2S are kept in control by the opposing actions of H_2S biosynthetic enzymes and H_2S degrading enzymes. Suboptimal SQR activity has also been suggested to be a cause of mitochondrial dysfunction in Leigh's disease, where inactivating mutations in SQR can cause an increase in H_2S levels, which can then inhibit complex IV [29].

1.4. H_2S and second messenger signaling

Similar to NO and CO, H_2S influences second messenger signaling involving cyclic nucleotides. H_2S is an endogenous inhibitor of phosphodiesterases (PDEs) which degrade cGMP and cAMP to mediate vasorelaxation [30]. Subsequent studies revealed that H_2S not only inhibits PDEs, but also stimulates activation of soluble guanylyl cyclases, which synthesize cGMP from GTP, by altering the redox state (reduction of Fe^{3+} to Fe^{2+}), facilitating its activation by NO [31]. ATP production in

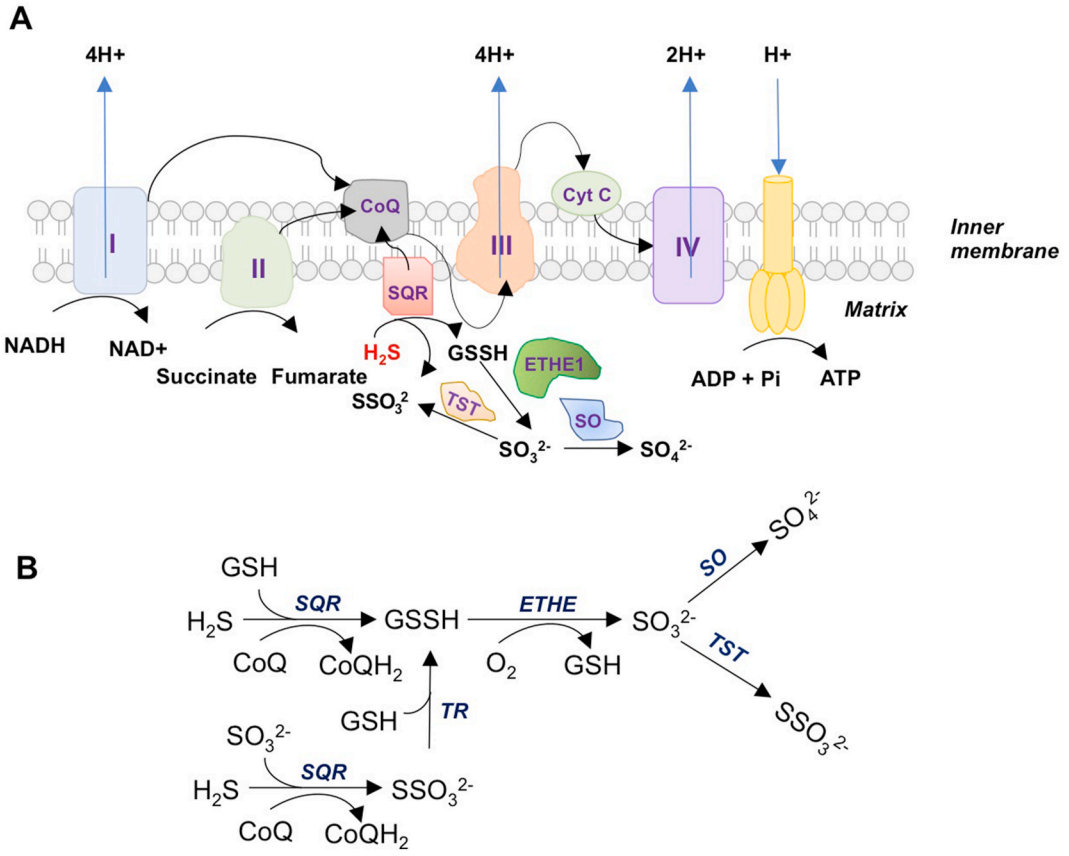

Fig. 2. Effects of H_2S on mitochondria. A) The mitochondrial electron transport chain (ETC) and H_2S. The ETC comprises five complexes, designated I through V. Complex I and complex II also donate electrons to CoQ, by oxidation of NADH and succinate, respectively. The electrons are further relayed to complex III and then to complex IV (cytochrome c oxidase) via cytochrome c (cyt c). Cytochrome c oxidase transfers electrons to oxygen (which is the terminal electron acceptor and is reduced to water), while pumping protons across the membrane. The proton motive force is utilized by the F_0F_1 ATP synthase complex (often referred to as complex V) to catalyze the formation of ATP from ADP. H_2S also donates electrons to the ETC to stimulate mitochondrial energetics. The donation of electrons by H_2S occurs at the level of coenzyme Q (CoQ) through sulfide quinone oxidoreductase (SQR), forming sulfite (SO_3^{2-}), sulfate (SO_4^{2-}) and thiosulfate ($S_2O_3^{2-}$, denoted as SSO_3^{2-}) in the process. **B)** The H_2S oxidation pathway. SQR oxidizes H_2S using SO_3^{2-} or glutathione (GSH) as electron acceptors, converting them to $S_2O_3^{2-}$ and glutathione persulfide (GSSH). Thiosulfate reductase (TR) converts $S_2O_3^{2-}$ to GSSH using GSH. Persulfide dioxygenase (ETHE1) in the mitochondrial matrix oxidizes GSSH to SO_3^{2-}. Sulfite is further oxidized to SO_4^{2-} by sulfite oxidase (SO). Thiosulfate sulfur transferase (TST), a rhodanese, converts SO_3^{2-} to $S_2O_3^{2-}$. Thus, oxidation of H_2S in the mitochondria yields SO_4^{2-} and $S_2O_3^{2-}$.

mitochondria is regulated by mechanisms involving protein kinase A (PKA), which phosphorylates mitochondrial proteins, including subunits of cytochrome c oxidase. PKA is activated by mitochondrial soluble adenylyl cyclase (sAC) in response to metabolically generated carbon dioxide [32]. The mitochondria too possess PDEs that regulate cyclic nucleotide levels, such as PDE2a, which degrades mitochondrial cAMP and NaHS was reported to inhibit its activity and elevate mitochondrial cAMP levels to augment mitochondrial respiration [32,33]. It should be noted that the first report of sulfide oxidation linked to ATP synthesis in any organism not specifically adapted to a sulfide-rich environment was by Yong and Searcy [34] who showed that chicken liver mitochondria consumed O_2 at an accelerated rate when supplied with low concentrations of H_2S and that sulfide oxidation was coupled to ATP synthesis.

1.5. H_2S and NAD^+ metabolism

NAD^+ is a cofactor required for several enzymes involved in maintenance of mitochondrial function [35]. In addition to its essential role in the mitochondrial ETC at complex I, as a hydride acceptor to form NADH, which furnishes electrons to the ETC, NAD^+ is consumed by enzymes such as the sirtuins and poly ADP ribosyl polymerases (PARPs) to regulate various aspects of cellular physiology such as mitochondrial biogenesis and DNA repair. Decrease in sirtuin activity and NAD^+ levels have been linked to aging [36,37]. H_2S has been reported to increase NAD^+ levels in the vascular endothelium and H_2S itself, associated sulfhydration and NAD^+ are decreased during aging [38,39]. The sirtuins, SIRT1 and SIRT3 are sulfhydrated, which enhances their activity [40,41]. Accordingly boosting NAD^+ levels may improve overall health and lifespan [37,42].

1.6. H_2S and oxygen sensing

H_2S plays important roles in maintenance of bioenergetics during hypoxia. H_2S produced during normoxic conditions is oxidized in the mitochondria, while during hypoxia; this degradation is decreased, leading to an increase in its levels. Oxygen-sensitive H_2S metabolism occurs in the mitochondria, which may balance energy requirements [43]. More recently, using a mitochondria-targeted mass spectrometry probe MitoA, it was shown that hypoxia increases mitochondrial H_2S in cardiomyocytes, suggesting a role for the gasotransmitter in oxygen sensing [44]. In addition to short-term oxygen sensing during acute hypoxia, H_2S is also involved in long-term oxygen sensing or chronic hypoxia. Decreasing oxygen from 21% to 1% progressively increased H_2S production in HEK 293 cells, which was concentrated in the mitochondria [45]. Interestingly, concentration of cysteine, the substrate for generation of H_2S, was reported to be about three-fold higher in the mitochondria [22]. The same study also reported mitochondrial translocation of CSE during hypoxia. In addition, during hypoxia, mitochondrial CBS pools are no longer targeted for degradation by the Lon protease due to deoxygenation of its heme group, leading to a six-fold increase in the CBS [21]. Another mechanism involves regulation of protein kinase G (PKG) on oxygen sensing by the carotid body. Under normoxia, PKG, which is stimulated by CO produced by heme oxygenase, phosphorylates CSE at Ser377 inhibiting its activity. During hypoxia, heme oxygenase, whose activity is oxygen dependent, is inactive, leading to decreased phosphorylation of CSE by PKG [46]. Similarly, an interplay of CO and H_2S production during hypoxia was also reported in cerebral microvasculature [47]. Whether mitochondria are involved in the process, remains to be determined.

1.7. Role of sulfhydration in mitochondrial function

Apart from the effects described above, H_2S acts on mitochondrial proteins via a posttranslational modification designated as sulfhydration or persulfidation, wherein the –SH groups of cysteine residues are modified to persulfide or SSH groups [48–51]. We have proposed previously that sulfhydration in addition to regulating signaling pathways, it protects against irreversible oxidation of cysteine residues [49]. This was subsequently demonstrated in the case of the lipid phosphatase and tensin homolog (PTEN), a tumor suppressor, and global protection of proteins during not only aging and neurodegeneration, but also during maintenance of physiological signaling [39,52,53]. This is especially relevant in the case of the mitochondria, as the organelle is constantly exposed to free radicals generated during oxidative phosphorylation. Several mitochondrial proteins have been reported to be sulfhydrated (Table 1). *S*-sulfhydration of the α subunit (ATP5A1) of ATP synthase (F0F1 ATP synthase/complex V) at Cys244 and 294 was reported to increase its activity in HepG2 and HEK293 cell lysates. Sulfhydration of ATP5A1 was upregulated in response to burn injury and decreased in mice lacking CSE implicating a role for CSE-derived H_2S in the process [54]. Sulfhydration also exerts protective roles in mitochondrial function in the cardiovascular system. The Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is associated with heart failure and in the induction of myocardial mitochondrial injury. CaMKII is sulfhydrated at Cys6 in response to treatment with

Table 1
Sulfhydration of proteins involved in mitochondrial function and its effects.

Protein sulfhydrated	Effect on function	Reference
ATP synthase (F0F1 ATP synthase/complex V)	Stimulates enzyme activity and ATP generation	[54]
DJ-1	Sulfhydration prevents the irreversible oxidation of DJ-1. DJ-1 plays critical roles in maintenance of redox balance in mitochondria.	[39]
Interferon Regulatory factor 1 (IRF-1)	Increases binding of IRF-1 at the *Dnmta* promoter and suppresses its expression, which leads to demethylation of *Tfam* promoter leading to increased expression TFAM and mitochondrial biogenesis.	[73]
Lactate dehydrogenase A (LDHA)	Stimulates LDH activity and increases conversion of lactate to pyruvate, generating NAD^+ in the process, and stimulates mitochondrial bioenergetics.	[85]
Protein phosphatase 2A (PP2A)	Inhibits PP2A, a negative regulator of AMP kinase (AMPK) which leads to its activation.	[70]
p66Shc	Prevents PKCβII-mediated phosphorylation of Ser 36 of p66Shc and its translocation to the mitochondria, thereby preventing oxidative stress in the mitochondria.	[86]
Parkin	Activates the E3-ubiquitin ligase activity of parkin, which increases degradation of misfolded proteins.	[58]
Peroxisome proliferator-activated receptor-γ coactivator-related protein (PPRC)	Stimulates mitochondrial biogenesis in mouse hepatocytes.	[71]
Peroxisome proliferator-activated receptor gamma coactivator- 1α (PGC-1α)	Stimulates mitochondrial biogenesis in mouse hepatocytes.	[71]
Sirtuin 1 (SIRT1)	Increases its deacetylase activity and lowers its ubiquitination and reduced its degradation.	[40]
Sirtuin 3 (SIRT3)	Increases its deacetylase activity and protects mitochondria against cisplatin-induced kidney injury. Also protects against paraquat mediated liver injury.	[41,87]
Ubiquitin specific peptidase 8 (USP8)_	Increases association of parkin with USP8, which is a deubiquitination enzyme (DUB), which promotes association of parkin to damaged mitochondria to augment mitophagy.	[60]

S-propyl-L-cysteine (SPRC), in a CSE-dependent manner, which decreases its activity in an isoprenaline-induced heart failure model [55]. The protective effect of H_2S involved decreased oxidative stress, mitochondrial swelling, mitochondrial permeability transition pore (mPTP) opening and apoptosis. Clearance of damaged mitochondria by mitophagy plays a central role in mitochondrial homeostasis and disruption of this process impacts almost all physiological processes, ranging from cardiovascular functions to neuronal homeostasis. Parkin, an E3-ubiquitin ligase is a key protein involved in clearance of misfolded proteins and dysfunctional mitochondria and mutations in the gene encoding parkin, *park2*, are linked to autosomal recessive Parkinson's disease [56,57]. Sulfhydration of parkin enhances its E3-ubiquitin ligase activity and promotes clearance of aggregated proteins and facilitates mitophagy [58] (Fig. 3). The recruitment of parkin to damaged mitochondria requires the action of the deubiquitynating enzyme, ubiquitin specific peptidase 8 (USP8), which removes ubiquitin chains from parkin itself to facilitate recruitment of parkin to the mitochondria [59]. USP8 was also reported to be sulfhydrated in response to treatment with H_2S donors, which facilitated its interaction with parkin and enhanced its mitochondrial docking in a mouse model of diabetic cardiomyopathy [60]. Other modes of sulfhydration mediated by cysteinyl-tRNA synthetase (CARS) was also suggested to play a role in mitochondrial bioenergetics [61,62]. More recently, an alternate mode of sulfhydration involving mitochondrial cytochrome c was discovered [63]. Reduction of ferric cytochrome c to its ferrous form was associated with increased sulfhydration in vitro. Silencing cytochrome c resulted in decreased sulfhydration of mitochondrial proteins. Consistent with these findings, cytochrome c released during apoptosis correlated with sulfhydration of procaspase 9 and loss of its activity. Levels of sulfhydration is also modulated endogenously by the thioredoxin/thioredoxin reductase (Trx/TrxR) system, and several studies have demonstrated the role of this system in signaling cascades ranging from endoplasmic reticulum stress (ER) to apoptosis [64–67]. Thus, the mitochondrial thioredoxin system (TrxR2/Trx2) may also function to regulate sulfhydration, which may play key roles in cellular function via mitochondrial homeostasis. The relative contributions of the different modes of sulfhydration in mitochondrial function during normal conditions and during stress is an area that warrants further investigation.

1.8. H_2S and mitochondrial biogenesis

Besides its effects on mitochondrial bioenergetics, H_2S stimulates mitochondrial biogenesis. Administration of NaHS in a rat model of cardiac arrest and cardiopulmonary resuscitation preserved mitochondrial function and promoted mitochondrial biogenesis in the brain [68]. Similarly, in a model of ischemia reperfusion, genetic and

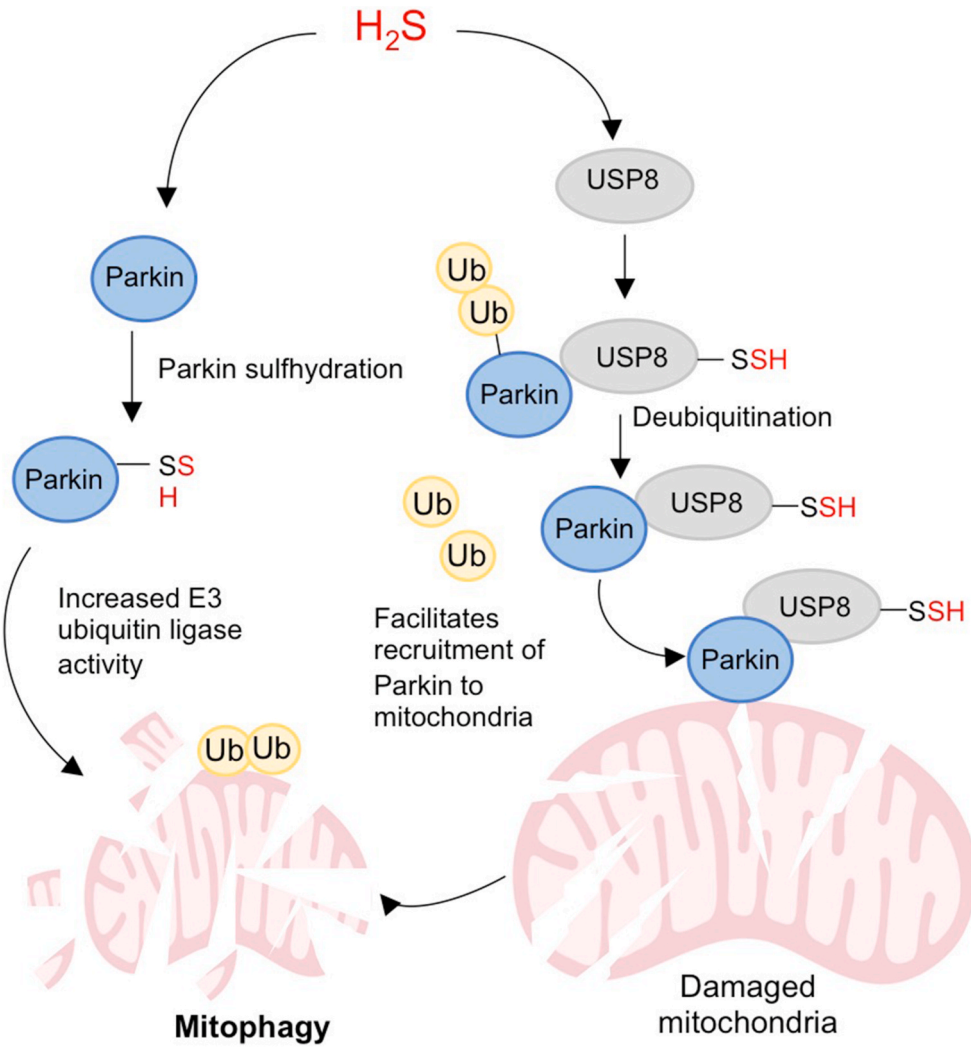

Fig. 3. Role of sulfhydration in mitophagy. The E3 ubiquitin ligase parkin is sulfhydrated which increases the ubiquitination and enhances mitophagy, the clearance of damaged mitochondria. Sulfhydration also facilitates mitophagy by activating the deubiquitinase, ubiquitin specific peptidase 8 (USP8), which removes ubiquitin groups from parkin and promoting its recruitment to damaged mitochondria. Icons of mitochondria generated from BioRender

pharmacologic increases in H_2S levels increased mitochondrial biogenesis in the heart [69]. Mice deficient in CSE had decreased cardiac mitochondrial content as compared to their wild-type controls. By contrast, mice overexpressing CSE and mice administered the orally active H_2S-donor, SG-1002, displayed enhanced cardiac mitochondrial content. In this system, H_2S increased mitochondrial biogenesis by sulfhydrating and inhibiting protein phosphatase 2A (PP2A), which negatively regulates it in an AMP-activated protein kinase (AMPK)-dependent manner [70]. Hepatocytes derived from $CSE^{-/-}$ mice also displayed lower levels of mitochondrial transcription factors and coactivators as compared to wild type [71]. H_2S donors increased the expression of the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), a key player in mitochondrial biogenesis and also caused its sulfhydration [72]. One of the master regulators for mitochondrial DNA replication is mitochondrial transcription factor A (TFAM). Expression of TFAM is negatively regulated by methylation of its promoter by the DNA methyltransferase, DNMTA. Expression of DNMTA is in turn, repressed by interferon regulatory factor 1 (IRF-1). Sulfhydration of IRF-1 enhances its binding to the DNMTA promoter and represses its expression, thereby preventing methylation of the TFAM promoter to increase its expression and thus, mitochondrial copy number [73]. $CSE^{-/-}$ mice exhibit reduced TFAM expression and mitochondrial copy number, confirming the role of CSE in maintenance of mitochondrial DNA copy number. Thus, H_2S donors may alleviate mitochondrial dysfunction caused by inadequate mitochondrial biogenesis.

1.9. H_2S and energy metabolism

Exogenous H_2S switches substrate utilization from fatty acid oxidation to glucose in cardiomyocytes of the obese db/db mice by upregulating the expression and activity of the deacetylase, SIRT3, to cause a decrease in acetylation of fatty acid β-oxidation enzyme long chain acyl-CoA dehydrogenase (LCAD) and the acetylation of glucose oxidation enzymes pyruvate dehydrogenase (PDH), isocitrate dehydrogenase (IDH2), and citrate synthase (CS), which decreased LCAD activity and increased the activities of the glucose oxidation enzymes [74]. The H_2S biosynthetic enzymes also participate in the maintenance of endothelial bioenergetics. 3-MST plays key roles in the energy metabolism of endothelial cells and its silencing reduced mitochondrial respiration and mitochondrial ATP production, and increased glucose uptake as well as fatty acid β-oxidation. 3-MST silencing resulted in increases in metabolites of the oxidative branch of pentose phosphate pathway (PPP) such as 6-phosphogluconate, and sedoheptulose-7-phosphate, but decreased metabolites of the non-oxidative arm of the PPP, such as ribose 1-phosphate, reflecting decreased nucleotide synthesis [75]. 3-MST was proposed to act as a regulator of the complex process that has been defined as the "angiogenic/metabolic switch" by which endothelial cells, switch from a quiescent state to a migratory and proliferative state during angiogenesis [76]. Thus, H_2S generating enzymes regulate various aspects of energy metabolism to maintain mitochondrial homeostasis.

1.10. Mitochondria-targeted H_2S donors

Several diseases are associated with impaired mitochondrial function and H_2S donors can be beneficial in cases where there is a paucity of the gaseous signaling molecule. Several mitochondria-targeted H_2S donors, which include AP39 and AP123, anethole dithiolethione and hydroxythiobenzamide respectively were developed, which improved mitochondrial functions in several cell types [77,78]. For instance, AP39 improved mitochondrial function in renal epithelial cells, endothelial cells and trophoblasts undergoing oxidative stress [77,79,80]. AP39 was also reported to support mitochondrial bioenergetics in the APP/PS1 primary neurons derived from mouse model of Alzheimer's disease and delay disease progression [81]. AP39 was also harnessed for organ preservation. AP39 protected cardiomyocytes from ischemia-reperfusion injury during cardiac transplantation and had a similar effect on renal grafts as well [82,83]. Thus, these donors hold great promise in the treatment of diseases involving suboptimal mitochondrial function.

2. Conclusions and future perspectives

It is becoming increasingly clear that H_2S is both a poison, a fuel and a signaling molecule depending on the context. The deleterious effects of H_2S on Complex IV of the mitochondria can be harnessed in a clinical setting under controlled conditions to induce a state of hypometabolism, which may improve surgical outcomes. Similarly, in colon carcinomas, where excess H_2S is produced by CBS and utilized as a fuel by cancerous cells, inhibition of CBS may be beneficial. Interestingly, in the context of neurodegenerative diseases such as ALS and DS, excess H_2S compromises mitochondrial function and inhibition of H_2S production may be beneficial. A point to be noted is that the sulfide oxidation pathway is highly active in cancer cells and in the colon, while it is almost non-functional in neurons, once again adding an additional layer of distinction between cancer and neurodegeneration at the molecular level [21,84]. Past reports of the actions of H_2S focused on the toxic effects of the gaseous molecule and the studies were conducted using higher doses of H_2S donors. The apparent discrepancy in the effects of H_2S stemmed largely from its biphasic effects and this is especially relevant in the context of mitochondrial function. Thus, use of optimal doses of H_2S donors or its inhibitors as well as timing, duration and routes of delivery should be carefully considered while targeting diseases involving dysregulated H_2S signaling.

Acknowledgements

KK: Supported in part by the National Institutes of Health [R24 DA018055; R01GM123508] and the Professional Staff Congress-City University of New York (PSC-CUNY) [TRADB-49-271]. This work was supported in part by US Public Health Service Grant DA044123 (to S.H. S), the American Heart Association (AHA)-Allen Initiative in Brain Health and Cognitive Impairment (to S.H.S and associates) and the Solve ME/CFS Initiative (SMCI) to BDP.

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