Review Article

Redox regulation of mitochondrial function with emphasis on cysteine oxidation reactions☆

Ryan J. Mailloux a,b, Xiaolei Jin b, William G. Willmore a,∗

a Institute of Biochemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6
b Toxicology Research Division, Food Directorate, HPFB, Health Canada, Ottawa, Ontario, Canada K1A 0K9

ARTICLE INFO

Article history:
Received 12 December 2013
Accepted 13 December 2013
Available online 19 December 2013

Keywords:
Redox
S-glutathionylation
S-oxidation
S-nitrosylation
Mitochondria

ABSTRACT

Mitochondria have a myriad of essential functions including metabolism and apoptosis. These chief functions are reliant on electron transfer reactions and the production of ATP and reactive oxygen species (ROS). The production of ATP and ROS are intimately linked to the electron transport chain (ETC). Electrons from nutrients are passed through the ETC via a series of acceptor and donor molecules to the terminal electron acceptor molecular oxygen (O2) which ultimately drives the synthesis of ATP. Electron transfer through the respiratory chain and nutrient oxidation also produces ROS. At high enough concentrations ROS can activate mitochondrial apoptotic machinery which ultimately leads to cell death. However, if maintained at low enough concentrations ROS can serve as important signaling molecules. Various regulatory mechanisms converge upon mitochondria to modulate ATP synthesis and ROS production. Given that mitochondrial function depends on redox reactions, it is important to consider how redox signals modulate mitochondrial processes. Here, we provide the first comprehensive review on how redox signals mediated through cysteine oxidation, namely S-oxidation (sulfenylation, sulfinylation), S-glutathionylation, and S-nitrosylation, regulate key mitochondrial functions including nutrient oxidation, oxidative phosphorylation, ROS production, mitochondrial permeability transition (MPT), apoptosis, and mitochondrial fission and fusion. We also consider the chemistry behind these reactions and how they are modulated in mitochondria. In addition, we also discuss emerging knowledge on disorders and disease states that are associated with deregulated redox signaling in mitochondria and how mitochondria-targeted medicines can be utilized to restore mitochondrial redox signaling.

© 2013 The Authors. Published by Elsevier B.V. All rights reserved.

Contents

- Introduction. ....................................................... 124
- Protein cysteine modifications in mitochondria .................. 125
  S-oxidation (sulfenylation and sulfinylation) ...................... 126
  S-glutathionylation ........................................... 127
  S-nitrosylation ................................................ 129
- Regulation of mitochondrial biology by redox switches ....... 129
  Regulation of the TCA cycle .................................. 129
  Regulation of OXPHOS ....................................... 130
  Mitochondrial morphology .................................... 132
  MTP and redox switches ...................................... 132
- Deregulation of mitochondrial thiol reactions in disease .... 133
  Cardiovascular diseases ........................................ 133
  Metabolic syndrome, insulin resistance, obesity and diabetes 133
  Neurodegenerative diseases ................................... 134
  Aging ............................................................ 135

☆This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-No Derivative Works License, which permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

* Corresponding author.
E-mail address: bill.willmore@carleton.ca (W.G. Willmore).

2213-2317/see front matter © 2013 The Authors. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.redox.2013.12.011
Introduction

Mitochondria can be a major source of cellular ROS which inherently depends on the metabolic “state” of mitochondria [1]. However, in order to understand mitochondrial ROS production, it is important to examine aerobic metabolism in detail. Nutrients (e.g. glucose, fatty acids, amino acids) are converted into metabolic intermediates (e.g. pyruvate, acetyl-CoA, oxaloacetate, 2-oxoglutarate) which are then systematically metabolized and decarboxylated by eight different enzymes in the tricarboxylic acid (TCA) cycle (Fig. 1). The decarboxylation steps are coupled to the transfer of electrons to NAD⁺ producing NADH [2]. Complex I (NADH:ubiquinone oxidoreductase) subsequently oxidizes NADH and the liberated electrons are then passed to ubiquinone (Q) generating ubiquinol (QH₂) [3]. Electrons from Complex II (succinate dehydrogenase; SDH), electron transfer flavoprotein oxidoreductase (ETF-QO), dihydroorotate dehydrogenase, and FAD-linked glycerol-3-phosphate dehydrogenase can also feed electrons into the Q pool [4–7]. The electrons are then passed to Complex III through cytochrome C and on to Complex IV which then reduces the terminal electron acceptor O₂ into H₂O (Fig. 1) [8]. The most important aspect of this process is the redox potentials of NADH (E° = −340 mV), and O₂ (E° = +810 mV). Due to this redox difference electron flow from NADH through the well-ordered prosthetic groups located within the individual respiratory complexes to O₂ at Complex IV is an energetically favorable process. According to Peter Mitchell’s Chemiosmotic Theory, the energetically favorable transfer of electrons from NADH to O₂ through the respiratory complexes is coupled to the pumping of protons through Complexes I, III, and IV into the intermembrane space. This creates a temporary form of stored energy, namely, the proton motive force (PMF) which is used by Complex V to produce ATP from ADP and Pᵢ. Hence, the production of ATP from nutrient oxidation in mitochondria depends on a multitude of redox reactions.

Oxygen can only accept one electron at a time during its reduction to H₂O. Electrons from Complexes I and III (and Complex II depending on experimental conditions) can “spin-off” prematurely, univalently reducing O₂ to produce the proximal ROS superoxide anion radical (O₂⁻) [9]. O₂⁻ is then quickly dismutated to H₂O₂ which is considered to be a chief ROS signaling molecule due to its longer half-life and capacity to diffuse through membranes. Complexes I and III remain the most well-characterized sources of O₂⁻ in mitochondria [10]. Other mitochondrial sources of O₂⁻ and H₂O₂ include 2-oxoglutarate dehydrogenase (Odh),...
pyruvate dehydrogenase (Pdh) [11], dihydroorotate dehydrogenase, sn-glycerol-3-phosphate dehydrogenase (mGPDH), electron transfer flavoprotein:ubiquinol oxidoreductase, p66shc/Cytochrome C, Mia40p/Erv1p, and succinate dehydrogenase (Complex II) (reviewed in [10]). These enzymes can contribute significantly to total mitochondrial ROS production and can produce O2− or H2O2 or both which depends on experimental conditions and energetic substrates used [10,12]. H2O2 emitted from mitochondria can also be shown to have a signaling role in cells due to its capacity to dismantle Fe–S clusters [18,19]. It has also recently been shown that O2− increases and decreases “stochastically” with a periodicity of ~20–40 s. The periodicity of O2− “flashes” depends on the metabolic state of mitochondria and has been suggested to play an important role in mitochondrial permeability transition and induction of apoptosis [20]. It is important to note that the existence of periodic O2− “flashes” from mitochondria has recently been challenged since the cyclic permuted YFP (cpYFP) probe used to detect stochastic changes in O2− production by mitochondria is also highly sensitive to changes in pH [21]. Thus, it has been questioned whether the periodic changes in cpYFP are due to changes in O2− production or changes in pH [22]. Indeed, the actual mechanism by which cpYFP interacts with O2− has not been resolved. In addition, it has been suggested that cpYFP relies on protein cysteine thiols to interact with O2− which is unlikely given the slow rate of reaction between O2− and protein cysteine thiols. Thus, the existence of O2− “flashes” and their potential role in signaling needs to be carefully re-examined.

Using oxiradicals as signaling molecules can be dangerous since their accumulation can lead to production of the dreaded hydroxyl radical (OH), a highly reactive species that indiscriminately oxidizes biological macromolecules. But even mitochondrial O2− and H2O2 can have harmful effects if they accumulate to a high enough concentration. At high enough levels, H2O2 can oxidize –SH to sulfenic (–SOH) and sulfonic (–SO3H) acids which renders proteins irreversibly deactivated [23]. Similarly, O2− disassembles Fe–S clusters in a variety of TCA cycle enzymes and in respiratory complexes [24] and can also combine with nitric oxide (NO) to form the highly toxic species peroxynitrite (ONOO−) [25]. Oxidative stress due to H2O2 or O2− is associated with a number of pathologies, neurological disorders, and metabolic diseases. However, despite the ever present threat of oxidative stress, damage, and death, mitochondria continue to employ ROS for intra and intercellular signaling.

As illustrated above, it is very clear that mitochondrial ATP and ROS production are intimately linked [26]. The production of ATP over ROS (or vice versa) ultimately depends on electron transfer through the respiratory chain, nutrient oxidation, nutrient availability, the electronchemical proton gradient across the mitochondrial inner membrane (MIM), the availability of ADP, and even the structure and morphology of mitochondria [1,27–30]. A number of different control mechanisms converge upon mitochondria to adjust the output of ATP and ROS. These include allosteric control, covalent modification, protein synthesis, protein degradation, and formation of respirasomes [31–35]. However, over the past decade, there has been a surge in the number of articles published on the topic of redox control of mitochondrial proteins. Various types of redox modifications and switches, including S-oxidation (sulfenylation and sulfhydration), S-glutathionylation, and S-nitrosylation converge upon mitochondria to regulate a number of processes ranging from OXPHOS to mitochondrial morphology to cell death. However, very few reviews have actually covered the role of redox switches in the control of mitochondrial function in detail [23,36,37]. This is surprising as mitochondria use redox reactions to drive all of its functions. Mitochondria also harbor a micro-environment that favors redox signaling via cysteine oxidation reactions. Thus, mitochondria are also a “hotbed” for regulation by redox. In the following review, what is known about how mitochondrial biology can be regulated by redox switches will be surveyed. We will cover a broad range of subjects including the different types of reactions, why redox signaling is so prevalent in mitochondria, the role of redox switches in controlling mitochondrial function and apoptosis, and the association of deregulated mitochondrial redox signaling in pathologies and how these pathways can be targeted for therapeutic intervention. It is important to note that sources of mitochondrial O2− and H2O2, antioxidant defense systems, and mechanisms that control mitochondrial O2− and H2O2 production will not be discussed since a number of reviews have already covered these very important topics [9,38–40].

### Protein cysteine modifications in mitochondria

Cysteine accounts for ~2% of the amino acid content of cells (protein-bound, peptide-bound, free) making it the least utilized amino acid in biology [41]. Despite its limited use, it is one of the most conserved amino acids in proteins. This can be attributed to its unique physical properties which allow cysteine to fulfill very important functions in cells. Sulfur is large, polarizable, and electron rich and is thus very reactive and able to adopt multiple oxidation states [42,43]. Cysteine residues are used in enzyme active sites and play structural roles which include iron–sulfur (Fe–S) assembly, heme, and zinc finger motifs [44–46]. Cysteine SH can also react with neighboring SH groups to form a disulfide bridge, an important structural component for secreted proteins [47]. Cysteine SH groups are also found on the surface of proteins making contact with the surrounding aqueous environment. This particular group of solvent exposed or “free” cysteine residues can be subjected to a range of redox-sensitive modifications (outlined below). Modification of a solvent exposed SH group depends on its ability to ionize and form a very nucleophilic thiolate anion (S−) [48]. Formation of S− is governed by the pKα of cysteine SH which is influenced heavily by the surrounding microenvironment. At neutral pH, cysteines have a pKα of ~8.3 meaning it will be in a protonated and unreactive state [49]. Exposure to a more alkaline environment can substantially increase the probability that a cysteine SH will adopt a deprotonated state [50]. Adjacent positively charged amino acids can also substantially decrease the pKα of cysteine thiols, rendering them more reactive. The presence of other adjacent positive charges such as the partial positive charge of an α-helix dipole or the nitrogen in the peptide backbone can also decrease the pKα of cysteines [51]. For instance, the catalytic cysteines in glutaredoxin (Grx) isoforms 1 and 2 adopt a very low pKα (Grx1, cystolic isofrom, pKα~3.5 and Grx2, mitochondrial matrix isoform, pKα~4.6) due to close proximity of a lysine residue and the N-terminus of an α-helix [52,53]. Likewise, neighbouring negative charges can increase the pKα which is the case for reduced glutathione (GSH) which has a pKα of ~8.8 [49]. As mentioned SH reactivity also influences whether or not it can adopt other oxidation states; e.g. be reactive enough to sense changes in redox through interactions with H2O2. An excellent example of this is peroxiredoxin (Prx) which uses a catalytic or “peroxidatic” cysteine (CySH), which has an estimated pKα of 5–6, to metabolize H2O2 with exquisite efficiency [49,54]. Steric hindrance also plays a part in cysteine thiol reactivity since cysteine oxidation reactions are nucleophilic substitution (SN2) reactions [55,56].
it is clear that the surrounding microenvironment will dictate whether or not a thiolate will be reactive enough to undergo modification which may also aid in dictating the selectivity for thiol modification.

These basic principles in SH chemistry and reactivity are highly relevant to mitochondria. This is in light of the unique physical properties of mitochondria. As outlined in the introduction, the chief functions of mitochondria are dependent on the pumping of protons from the matrix to the intermembrane space. This effectively alkalinizes the matrix environment which has a tremendous impact on mitochondrial redox potential and thiol ionization. For example, the calculated midpoint potential for GSH to glutathione disulfide (GSSG) ratio in the cytosol is $-240 \text{ mV}$ [57]. Since redox potentials are influenced by pH the alkalinity of the matrix environment lowers the potential even further ($2\text{GSH}/2\text{GSSG}_{\text{mitochondria}} = -280 \text{ to } -340 \text{ mV}$) [58]. Based on this it is very probable that protein cysteine residues in the mitochondrial matrix will exist in an ionized state. The mitochondrial proteome is also very rich in protein thiols. The estimated concentration of exposed protein thiols in mitochondria is $60\text{−90 mM}$ [59]. This actually makes protein cysteine residues the most concentrated thiol in mitochondria. In conjunction with these key properties mitochondria are also a major source for $\text{O}_2^{-}$ and $\text{H}_2\text{O}_2$ and contain high amounts of GSH ($\sim 5 \text{ mM}$) which are both required for redox signaling. Mitochondria are also a major target for nitric oxide (NO) signaling and S-nitrosylation [37,60]. Notably mitochondria have been reported to harbor NO synthase (NOS; specifically neuronal NOS isoform) however; this remains highly contentious (discussed below) [37]. Thus, it can be concluded that mitochondria harbor a unique environment that promotes thiol modification.

**S-oxidation (sulfenylation and sulfinylation)**

In ROS-mediated signaling, the oxidation of protein cysteine SH groups plays a central part in modulating protein function in response to local changes in redox environment. It is important, however, to note that all protein cysteine SH modifications are S-oxidation reactions since they involve changes in valency. Thus, cysteine SH oxidation by ROS is most often described as S-oxidation or thiol oxidation. For our purposes here, we will define S-oxidation specifically as sulfenylation and sulfinylation. It is also important to identify which oxyradical species are responsible for mediating these reactions. H$_2$O$_2$ is the most relevant radical species in redox signaling. This is by virtue of its reactivity with cysteine, capacity to diffuse through membranes, and its prolonged half-life (relative to other oxyradicals). H$_2$O$_2$ signaling is mediated through the formation of SOH [61]. In this reaction, the highly nucleophilic S~ attacks H$_2$O$_2$, which results in formation of SOH and OH~ [48]. Most protein cysteine SH groups react slowly with H$_2$O$_2$ ($\sim 10^8 \text{ M}^{-1} \text{s}^{-1}$) [49]. This rate can be increased to as high as $\sim 10^8 \text{ M}^{-1} \text{s}^{-1}$ which is dependent on protein microenvironment, pH, the pK$_a$ of the SH group, and presence of bulky groups surrounding SH groups [62,63]. Due to its reactivity it has been very difficult to ascertain the pK$_a$ of SOH groups formed in proteins. However, studies with small molecule sulfenic acids have revealed that SOH groups have much lower pK$_a$ values than SH groups [64,65]. Due to their lower pK$_a$, it could be assumed that SOH adopt an ionized state (SO~) which makes them far more reactive. This could account for the labile nature of SOH groups in proteins explaining why they may be so difficult to detect and quantify. In addition, this also explains why SH groups are amenable to undergo a variety of different modifications as outlined in Fig. 2a [66].

SOH groups can participate in a plethora of side reactions (Fig. 2a). For instance, SOH groups can quickly undergo S-glutathionylation if GSSG are in the vicinity. S-glutathionylation of SOH groups is very likely to proceed in mitochondria, given the small volume and high concentration of GSH. Further, S-glutathionylation of SOH has an important biological function in mitochondria, namely, the protection of cysteine residues from further oxidation when H$_2$O$_2$ levels are...
higher than usual. For example, the sulfur residues on lipoate in the E2 subunit of 2-oxoglutarate dehydrogenase (Odh), a TCA cycle enzyme that couples 2-oxoglutarate decarboxylation to NADH production, can be oxidized rapidly by H$_2$O$_2$ [67,68]. To avoid the irreversible oxidation of SOH to SO$_2$H, GSH is conjugated to SOH forming a protein glutathione mixed disulfide (PSSG) which essentially protects lipoate from further oxidation (Fig. 2b) [68]. Grx then catalyzes the deglutathionylation of Odh reactivating the enzyme [68]. Similar observations have been made for other mitochondrial proteins including Complex I and adenine nucleotide translocator (ANT) [69,70]. The absolute number of mitochondrial protein SH groups that can form SOH has yet to be quantified. This may be difficult given that mitochondrial isolation protocols can result in unwanted oxidation of cysteine residues. Oxidation of cysteines during isolation can be prevented or minimized by the addition of N-ethylmaleimide (NEM), an alkylating agent that forms a Michael adduct with SH. Unfortunately, SOH groups are also very labile which also makes them difficult to quantify. Several methods have been used to properly trap and quantify SOH groups. These methods typically involve using dimedone molecules which react selectively with SOH groups tagging them for quantification [71]. Dimedone probes have also been used to measure SH oxidation to SOH in mitochondria [72]. However, it is possible to improve the efficacy of dimedone probes for quantification of mitochondrial SOH levels. For instance, triphenylphosphonium-tagged dimedone (TPP-dimedone) molecules can potentially be developed and used to trap and quantify mitochondrial SOH. TPP-dimedone could be administered in vivo allowing more accurate quantification of mitochondrial SOH. TPP is a lipophilic cation that preferentially accumulates in mitochondria due to large charge difference between the intermembrane space (positive) and matrix (negative) [73]. It is important to point out though that TPP probes can potentially hinder mitochondrial metabolism by uncoupling the proton gradient [74]. It is also important to acknowledge that SOH can form from one electron reduction reactions. These reactions involve the formation of a thyl radical following exposure to radiation [75]. O$_2^-$ has also been shown to catalyze the one electron reduction of low molecular weight thiols with a rate constant of 10$^9$ M$^{-1}$ s$^{-1}$ however this may be insignificant given the rapid kinetics of superoxide dismutase (SOD; 10$^8$ M$^{-1}$ s$^{-1}$) [10].

SOH groups can also react with neighboring SH groups to form intra or intermolecular disulfide bonds (Fig. 2a). This type of reaction is particularly important for antioxidant enzymes like Prx. In mitochondria, Prx3 is a homodimer with both subunits aligned in a head-to-tail fashion with Cys Prx. In mitochondria, Prx3 is a homodimer with both subunits reaction is particularly important for antioxidant enzymes like intra or intermolecular disul

formation is crucial for the modulation of Prx3 and DJ-1 [79,80]. In mitochondria SO$_2$H formation is crucial for the modulation of Prx3 and DJ-1 [79,80]. In the case of the former, SO$_2$H formation is required to render Prx3 inactive for extended periods of time (Fig. 2c). This allows H$_2$O$_2$ to accumulate to sufficient amounts to exert regulatory effects on various proteins, either within or external to mitochondria [81]. Srx has recently been identified in mitochondria indicating mitochondria are equipped with the necessary enzymatic complement to reduce SO$_2$H back to SH [82]. It is important to point out though that unlike cytoplasmic Prx2, Prx3 is more resistant to hyperoxidation [83]. Very little information exists on the importance of SO$_2$H groups in the redox control of proteins, particularly in the mitochondria and which proteins and enzymes might be involved in reduction of SO$_2$H. New tools to properly trap and measure SO$_2$H in cells have been proposed including aryl-nitroso compounds which react with SO$_2$H with high efficiency and specificity [84]. It would be intriguing to determine what percentage of the mitochondrial proteome forms SO$_2$H.

S-glutathionylation

Another important redox switch required to control mitochondrial protein function is S-glutathionylation. In these reactions, GSH is conjugated to an exposed cysteine protein SH generating PSSG. The S-glutathionylation of proteins in mitochondria has been found to be highly sensitive to local changes in redox, specifically through fluctuations in 2GSH/GSSG [16,85,86]. As indicated above, the potential of the 2GSH/GSSG pair in the matrix of mitochondria is estimated to be between −280 to −340 mV [58]. This range has been attributed to the different methodologies used to measure and calculate absolute GSH and GSSG levels. Most measurements of GSH and GSSG levels have been performed using enzyme coupled assays. These types of assays should only be used to measure total glutathione levels and not for determination of 2GSH/GSSG since it is very difficult to accurately quantify the absolute amounts of GSSG. HPLC can also be used which does provide a far more accurate read of 2GSH/ GSSG [86]. This is due to clean separation and resolution of GSH from GSSG which allows easy integration and calculation of absolute GSH and GSSG levels. Using an assumed mitochondrial volume of 0.75 μL (considered to vary between 0.5–1 μL) [81] the molar concentration can be calculated and then subsequently plugged into the Nernst equation to give the potential of the pair. However, isolation of mitochondria and treatment with acids for glutathione extraction can artificially increase GSSG levels decreasing the accuracy of the calculated potential. It is also important to extract mitochondria in the presence of 25 mM NEM to prevent any spontaneous reactions of GSH or GSSG with protein thiols. Samples should also be injected immediately after mitochondrial isolation since GSH can spontaneously oxidize over time. Control experiments should also be performed with mitochondria treated with reducing (DTT) or oxidizing agents (H$_2$O$_2$) [86]. For cells in culture, glutathione pools can be depleted using buthionine sulfoximine (BSO) [87]. Finally, a class of Grx1-GFP probes has been developed that allow the sensitive real-time measurement of 2GSH/GSSG in intact cells or in vivo [88]. These probes are targeted to the mitochondrial matrix using mitochondrial localization sequences. Briefly, the probe works through a series of cysteine disulfide exchange reactions between GSSG and probe itself which ultimately leads to the formation of a disulfide bond on GFP which alters its fluorescence at 408 nm and 488 nm.
The calculated fluorescent ratio can then be used to determine the potential of 2GSH/GSSG. A very small increase in GSSG (10 μM) or H2O2 (12.5 μM), which produces GSSG, can induce a substantial increase in the ratiometric fluorescence of the probe [88]. The fluorescence can then be brought back down to base line with DTT. By virtue of its extreme sensitivity and non-invasiveness (e.g., no need to isolate mitochondria) this stands as the most sensitive and accurate measure for 2GSH/GSSG. It is important to accurately measure the absolute concentrations of GSH and GSSG since GSSG can S-glutathionylate cysteine residues via a simple thiol disulfide exchange reaction. Inaccurate measures of GSSG can lead to false conclusions on the state of the redox environment and whether or not S-glutathionylation reactions will proceed spontaneously. Thus, it is crucial to utilize methods that provide accurate measures of both GSH and GSSG and in this respect, spectrophotometric assays should be avoided.

The S-glutathionylation of proteins can either proceed non-enzymatically or enzymatically and it is thus important to distinguish between the two types. Non-enzymatic S-glutathionylation can proceed via a number of different pathways (Fig. 3a). Reaction 1 simply involves a thiol disulfide exchange reaction between protein cysteine S– and GSSG. The result is the formation of PSSG and GSH [89]. Reaction 1 can only proceed if GSSG has accumulated to a sufficient concentration and the 2GSH/GSSG is ~1 [90]. GSSG reaches this concentration only under oxidative stress. In reaction 2, a protein S– is oxidized to SOH which then reacts with GSSG to yield PSSG. For reaction 3, PSSG formation is initiated by the formation of a thiol radical which interacts with GSH to form a glutathione anion protein thyl radical intermediate [91]. This structure is stabilized by O2 which subsequently yields PSSG. Non-enzymatic glutathionylation reactions are very nonspecific and mostly proceed under oxidative stress. Under oxidative stress proteins in mitochondria can become hyper-glutathionylated which is often considered a marker for oxidative damage [92]. A number of mitochondrial proteins are S-glutathionylated under normal conditions, depending upon the tissue type [86,93]. Further, S-glutathionylation of mitochondrial proteins under normal conditions is cysteine thiol specific, reversible, sensitive to changes in redox environment, and most importantly, enzyme driven [36,90]. Enzyme-driven S-glutathionylation reactions are mostly investigated in the cytosol where Grx1 has been identified as the chief enzyme responsible for catalyzing the deglutathionylation of PSSG. The enzymatic mechanism involves two steps; (1) N-terminal cysteine on Grx deglutathionylates PSSG via a thiol disulfide exchange reaction yielding PSH and a Grx-SSG intermediate, (2) Grx-SSG binds GSH and the glutathionyl moiety is removed regenerating Grx1 and producing GSSG, (3) GSSG is reduced back to 2GSH by glutathione reductase and NADPH (Fig. 3b) [52]. Step 1 of the enzyme mechanism is very rapid with step 2 occurring much more slowly and is thus rate limiting [52]. It is also noted that Grx also has a C-terminal SH that can cross react with Grx-SSG to generate an intramolecular disulfide bridge and GSH. Reversal of the disulfide requires Grx to regenerate Grx1-SSG in the catalytic cycle. Formation of the intramolecular disulfide bridge has been suggested to serve as a site of regulation since it may allow an S-glutathionylation on a protein to persist [10]. The mechanistic details of the catalytic reaction still require clarification however; several catalytic models have been proposed including glutathione scaffold, glutathione activator, and an oxidative half-reaction (reviewed in [94]).

Discussion of the catalytic cycle of Grx1 is critical since mitochondria harbor a Grx1 isozyme, Grx2. Grx2 was only recently identified and is classified as a dithiol Grx since it conforms to the thioredoxin fold (four mixed β-sheets surrounded by α-helices) and contains the CXXC catalytic motif. Intriguingly, Grx2 only has ~34% sequence identity to Grx1 [53,95,96]. However, despite this clear lack of homology, Grx2 has been found to use the precise same catalytic mechanism as Grx1 [52]. The sequence differences between Grx1 and Grx2 are important to the regulation of both proteins. For instance, unlike Grx1, Grx2 has no exposed thiol residues on its surface making it less likely to be deactivated by ROS. Grx2 also forms a homodimer through coordination of a 2Fe–2S cluster which has been shown to be important for its regulation [18]. Grx2 is inactive when complexed with 2Fe–2S.
Disassembly of the 2Fe–2S cluster releases active monomeric Grx2 which is mediated by O$_2^-$. This illustrates that there is a tight link between mitochondrial ROS, redox, and S-glutathionylation reactions (Fig. 3c) [97]. Reassembly of the 2Fe–2S cluster and the Grx2 homodimer requires iron sulfur cluster chaperone IscU [98]. It is also interesting that Grx2 has been found to also catalyze protein S-glutathionylation (Fig. 3d) [85]. This occurs when the 2GSH/GSSG is sufficiently oxidizing [85]. It is important to realize though that precisely how the glutaredoxins, namely Grx1 and Grx2, catalyze S-glutathionylation reactions is unresolved. There is though sufficient evidence to indicate that Grx2 harbors glutathione activity. For instance Complex I has been documented to be S-glutathionylated and de glutathionylated by Grx2 [85]. Grx2 has also been shown to be required for the S-glutathionylation of uncoupling protein-3 (UCP3) [86]. The significance of Grx2-mediated modulation of Complex I and UCP3 by S-glutathionylation is discussed below. In addition, catalysis of S-glutathionylation by Grx2 is feasible from a thermodynamic and redox chemistry perspective given that thiol disulfide exchange reactions are generally reversible. Hypothetically, Grx2 may only harbor glutathionylase activity when Grx2-SSG has accumulated to a sufficient concentration. Indeed, step 2 of the catalytic cycle of Grx2 is rate limiting and, under certain conditions, this may prompt accumulation of a Grx2-SSG intermediate. It is also important to note that glutathione S-transferase (GST; Pi and Mu-family) has recently been found to catalyze the glutathionylation of proteins in the cytosol [99]. Mitochondria have also been reported to contain several GST isozymes including Alpha, Kappa, Mu, Pi and Zeta have been reported suggesting a similar function in mitochondria [100].

S-nitrosylation

Nitric oxide (NO) was among the first gaseous second messengers identified in mammals and plays a central role in vasodilation [101]. Following its production by NOS, NO rapidly diffuses through the endothelial cells, where it was produced, into smooth muscle cells where it activates soluble guanylate cyclase which prompts cyclic guanosine monophosphate (cGMP) production and smooth muscle relaxation. NO is also able to interact with mitochondria. NO binds cytochrome $a_3$ and Cu$^{+}$ prosthetic groups of Complex IV [60]. By binding to the heme or Cu group, NO inhibits Complex IV activity which diminishes OXPHOS. Binding prosthetic groups is not the only way NO can modulate mitochondria. The S-nitrosylation (SNO) of free SH residues in proteins have also been shown to be an important component of NO signaling. A number of different mitochondrial proteins have been shown to be modulated by SNO. The means by which SNO is formed, however, remains elusive. NO is not an oxidant under biological conditions and thus cannot form a SNO via a direct reaction with $S^-$ [102]. However, NO can form a thionitroso group (SNHO) which, following a one-electron oxidation, can possibly generate SNO [75]. Another mechanism for SNO involves the initial reaction of 2 mol of NO with 1 mol of O$_2$ to form nitrogen dioxide (NO$_2$). NO$_2$ is capable of reacting with $S^-$ to form a thyl radical which then reacts with NO to form SNO [103]. Finally, NO$_2$ can react with NO to form dinitrogen trioxide (N$_2$O$_3$) which is able to react directly with S$^-$ to form SNO [104].

Where mitochondrial NO comes from is still enthusiastically debated. First, there have been several reports that NOS isoforms localize to mitochondria [37]. Specifically, it has been frequently reported that nNOS is found in liver and rat brain mitochondria where it actively produces NO following stimulation by calcium [105]. Unfortunately, the presence of mitochondrial NOS remains contentious since other groups have been unable to detect any NOS isoforms in mitochondria [37]. Other sources of mitochondrial NO include NO generated in the cytoplasm or neighboring cells. NO diffuses rapidly through membranes and thus can promptly gain access to the mitochondrial matrix. The issue surrounding external NO is the quenching of NO before it reaches mitochondria. For instance, in normoxia, NO can be scavenged by oxyhemoglobin which produces nitrate (NO$_3^-$) which limits the amount of NO that actually reaches mitochondria [106]. Inside the cell, NO can react to form peroxynitrite (ONOO$^-$) or, following NO$_2$ formation, can react with unsaturated fatty acids to produce nitro-fatty acids [107].

The debate surrounding the source of NO, and which species actually generates SNO, does not diminish the importance of NO in the regulation of mitochondria. SNO has been shown be important for ischemic preconditioning and it has been shown that mitochondrial SNO (mSNO) can be used to greatly reduce cardiac infarct size through reversible SNO modification of Complex I [108]. SNO also modulates ATP synthase, several TCA cycle enzymes, and enzymes involved in β-oxidation [37,109]. NO has also been shown to limit mitochondrial membrane permeability transition (MPT) through SNO formation on cyclophilin D [110]. A number of excellent reviews have been published on the topic NO signaling, SNO, and control of mitochondrial function [37,111,112]. Thus, SNO and the control of mitochondrial functions will not be considered any further in this review.

**Regulation of mitochondrial biology by redox switches**

From the above section it is clear that; (1) redox signaling is very complex, (2) we still understand very little about redox reactions, which can be attributed the lack of sensitive techniques, (3) the importance for regulation of mitochondrial function by redox switches. Mitochondrial ROS production, and how rapidly it is quenched, depends on the tissue and cell type, the energetic state of mitochondria (e.g. respiring under phosphorylating or non-phosphorylating conditions), and which substrate is being metabolized [93,113,114]. Regulation of mitochondria by redox switches would also be dependent upon mitochondrial density, protein content (e.g. thiol availability), antioxidants defense, and glutathione availability. The 2GSH/GSSG ratio is usually in a highly reduced state (in such tissues as heart, muscle, liver, and brain) but the state of this redox pair can change spatially and temporally depending upon ROS concentrations, transport of GSH from the cytosol, export of GSSG, and reduction of GSSG back to GSH by the efficient enzymatic activity of glutathione reductase [86,115,116]. In the following section we will discuss the various mitochondrial proteins and enzymes that are regulated by cysteine oxidation reactions and the impact cysteine oxidation has on enzyme function and activity. We will also highlight the conditions under which proteins in mitochondria are modulated by cysteine oxidation and the impact it has on mitochondrial metabolism and function.

**Regulation of the TCA cycle**

The TCA cycle is the most central metabolic pathway for anaerobic and aerobic organisms. For aerobes, the TCA cycle either serves as an engine that strips electrons from nutrients for production of ATP or provider of the necessary building blocks for the production of biological macromolecules. A number of enzymes in the TCA cycle have been shown to be either sulfenylated or S-glutathionylated. The impact of these modifications on mitochondrial function, and the circumstances under which the modification occurs, is enzyme dependent. Aconitase (Acn) can be reversibly deactivated by H$_2$O$_2$ through sulfydryl group
Regulation of OXPHOS

Most of the information generated in terms of how redox switches work to control mitochondrial proteins has been accrued by studying the impact of S-oxidation reactions on OXPHOS. Most of these studies have been conducted using heart mitochondria of either bovine or mouse origin. Important information concerning the role of redox signaling in the control of mitochondrial bioenergetics has also been generated using cultured cells (lens epithelia, vascular smooth muscle cells, myotubes), isolated liver mitochondria, and isolated skeletal muscle mitochondria [86,125,126]. The impact of chemical-induced S-glutathionylation on mitochondrial bioenergetics has variable effects which are dependent on cell line. Acute treatment of vascular smooth muscle cells (VSMC) with 0.1 mM diamide, an S-glutathionylation catalyst, induces a transient decrease in resting respiration (basal respiration; respiration required to maintain cellular ATP levels under resting conditions) which then subsequently recovers over time [126]. Hill and colleagues also showed that 0.1 mM diamide induced a massive increase in proton leaks in VSMC. Higher diamide concentrations ( > 0.25 mM) induce an irreversible decrease in respiration [126]. These observations indicate that aerobic respiration can indeed be modulated by reversible S-glutathionylation reactions. Different results were obtained with primary mouse myotubes. Treatment with ≤ 0.2 mM diamide did not have any effect on resting respiration; however diamide treatment did induce an increase in PMF [87]. Assessment of state 4 respiratory parameters (proton leaks; non-phosphorylating respiration) revealed that diamide-induced S-glutathionylation inhibited proton leaks through UCP3 [87]. Leaks through UCP3 can be reactivated by a small increase in H$_2$O$_2$ which is required to decrease PMF and diminish mitochondrial ROS emission [87]. In addition, it has recently been shown that Grx2 is required to S-glutathionylate UCP3 [86]. Indeed, UCP3 is in a deglutathionylated and active state in skeletal muscle mitochondria isolated from Grx2 knock-out (Grx2$^{-/-}$) mice [86]. Similar results have been obtained with Min6 insulinoma cells [16]. In this case, chemical induction of S-glutathionylation deactivates leaks through UCP2, a homolog of UCP3, which enhances glucose-stimulated insulin release (GSIS) [16]. The enhancement of GSIS is due to the maintenance of the mitochondrial protonic potential which increases aerobic ATP production for stimulation of insulin release. The progressive oxidation of glucose eventually leads to an increase in mitochondrial O$_2^{-}$ and H$_2$O$_2$, which de glutathionylates UCP2, activates leaks, and desensitizes the GSIS [16]. Chemical induction of UCP2 glutathionylation can also be used to sensitize drug resistant cancer cells to chemotherapeutics [127]. This is due to the fact that certain drug resistant cancer cells overexpress UCP2 which prevents anthracycline-mediated ROS production [128]. Induction of UCP2 S-glutathionylation deactivates proton leaks which enhances drug toxicity through oxidative stress. Thus, reversible S-glutathionylation can control OXPHOS, proton leaks, and mitochondrial ROS emission which can have profound signaling effects in the cell.

Complex I was the first OXPHOS component identified to undergo either S-glutathionylation or sulfenylation. The mechanism of Complex I sulfenylation and S-glutathionylation has mostly been explored in heart mitochondria. In terms of sulfenylation, SOH formation was shown to undergo further oxidation to SO$_2$H and SO$_3$H which irreversibly deactivates Complex I [69]. However, cysteine residues that are oxidized to SOH in Complex I can also be protected from further oxidation by S-glutathionylation [69]. Although S-glutathionylation decreases Complex I activity, the modification can be reversed [85]. Complex I S-glutathionylation is also important since it can diminish O$_2^{-}$ production. In bovine heart mitochondria, Cys$_{531}$ and Cys$_{704}$ on the 75 kDa subunit modification [117]. This reversible inactivation has been shown to occur during ischemia-reperfusion injury. Intriguingly, Acn does not lose its 4Fe–4S cluster due to interactions with Frataxin protein [117]. However, if the stress persists, Acn can be irreversibly deactivated due to 4Fe–4S cluster disassembly [118]. NADP-dependent isocitrate dehydrogenase (Idh) has been reported to be inactivated by S-glutathionylation in HEK293 cells and rabbit heart mitochondria [119]. This is intriguing since this Idh isoform is required to contribute to the NADPH pool in mitochondria, which provides the reducing power necessary to maintain an active antioxidant defense system. The authors reported that half-maximal inhibition occurred at ~ 5 mM GSSG and can be reversed by 10 mM DTT [119]. This would set the 2GSH/GSSG ratio to ~1 and represents a system that is under oxidative stress. It would be important to discern if this inhibition occurs under normal conditions and how rapidly Grx2 (or another enzyme) reverses the inhibition. As mentioned Odh is a major target for redox regulation. Odh has been shown to be targeted by sulfenylation, sulfinylation, and S-glutathionylation; the latter being important in protecting key thiol residues from further oxidation [120]. Odh sits at a major metabolic junction that links carbon catabolism to amino acid production in mitochondria. It has been suggested that reversible oxidation of Odh may be required to modulate the flow and fluxes of metabolites through amino acid metabolism and the TCA cycle. Oxidative deactivation of Odh also has the added benefit of prompting the accumulation of 2-oxoglutarate, which has the capacity to quench H$_2$O$_2$ and would also aid in limiting H$_2$O$_2$ production [121]. Pyruvate dehydrogenase (Pdh), which bears dihydrolipoamide dehydrogenase subunits like Odh, can also be modulated by mitochondrial H$_2$O$_2$ through thiol oxidation [122]. In addition, like Odh, Pdh activity can be recovered with reducing agents, illustrating that this enzyme complex is regulated in manner similar to Odh [122]; SDH has also been found to be controlled by S-glutathionylation [123]. However, the circumstances of its regulation are quite intriguing. In Sprague-Dawley rat heart mitochondria, SDH is maintained in a glutathionylated state [123]. Following ischemia-reperfusion, SDH is deglutathionylated which not only decreases its activity but also induces a burst of O$_2^{-}$ production from SDH [123]; L-carnitine/acyl-carnitine carrier (CAC) has also been found to be S-glutathionylated on Cys$_{136}$ and Cys$_{155}$, respectively [124]. S-glutathionylation state of CAC is sensitive to the potential of 2GSH/GSSG where high GSH leads to deglutathionylation and activation of CAC and high GSSG induces S-glutathionylation and deactivation of the transporter [124]. It was also found that exogenously added Grx1 can reverse S-glutathionylation indicating that CAC may also be targeted by either Grx1 in the intermembrane space or Grx2 in the matrix [124]. This illustrates the complexities surrounding S-glutathionylation and the regulation of protein function in mitochondria.

In the above examples, S-glutathionylation has mostly been shown to proceed under conditions of oxidative stress when GSSG levels are high (Fig. 3a). However, it is also clear that S-glutathionylation is reversible and occurs under normal conditions. For instance, Acn can be reversibly deactivated by S-glutathionylation [117]. It has also been shown that Odh can be deglutathionylated in vitro by purified Grx1 [68]. Also, SDH maintains an S-glutathionylated state in heart mitochondria under normal conditions [123]. The enzyme(s) that actually mediate S-glutathionylation and deglutathionylation under these conditions are still yet-to-be identified. However, Grx2 is a strong candidate for mediating these reactions. In regard to sulfenylation, it is doubtful that SOH formation plays any important regulatory role in mitochondria due to the reactivity of this moiety, especially in an environment rich in ROS, thiols, and glutathione. SOH is most likely a precursor in mitochondria for other reactions such as S-glutathionylation or sulfinylation.
NDUSF1, were identified as S-glutathionylation sites on Complex I [69]. The NDUSF1 subunit sits in the hydrophilic arm of Complex I in proximity of FMN NADH binding site. S-glutathionylation of the solvent exposed Cys331 and Cys704 most likely induces conformational changes in the hydrophilic arm of Complex I which may sterically block the NADH binding site. Thus, preventing NADH oxidation can limit electron flow through the respiratory chain and diminish O$_2^-$ production [129]. However, other studies have shown that S-glutathionylation of Complex I actually increases mitochondrial O$_2^-$ emission [130]. This could be related to maintenance of Complex I in a prolonged S-glutathionylated state. It is important to connect Complex I S-glutathionylation to the S-glutathionylation of Odh since Odh generates NADH for oxidation by Complex I and both protein complexes produce mitochondrial ROS (Fig. 4). Notably, the S-glutathionylation of these two protein complexes diminishes the activity of both protein complexes when H$_2$O$_2$ is high. S-glutathionylation may serve as a mechanism that limits further O$_2^-$ and H$_2$O$_2$ production by a mitochondrion that is already faced with the potential threat of oxidative stress (Fig. 4) [131,132]. Once O$_2^-$ and H$_2$O$_2$ levels are normal, Odh and Complex I are deglutathionylated by Grx2 and OXPHOS resumes unhindered (Fig. 4).

Reversible S-glutathionylation of Complex I is mediated by Grx2. Complex I has been shown to be deglutathionylated by Grx2 in heart mitochondria, lens epithelia, and liver mitochondria [85,86,125]. Grx2 is required to maintain Complex I in an active deglutathionylated state in cardiac mitochondria. For instance, mice treated with doxorubicin display increased Complex I S-glutathionylation and decreased activity which compromises mitochondrial ATP production [129]. Selective overexpression of Grx2 in cardiac tissue maintains Complex I in an active deglutathionylated and active state [129]. Intriguingly, Complex I is not targeted by Grx2 in skeletal muscle mitochondria indicating that regulation of Complex I by Grx2 is tissue-specific [86]. Grx2 is able to catalyze the reversible glutathionylation of Complex I which is dependent on 2GSH/GSSG [85]. A more oxidized 2GSH/GSSG ratio induces Complex I S-glutathionylation whereas a more reduced glutathione pool activates the deglutathionylase activity of Grx2 [85]. Thus, the S-glutathionylation serves various important roles in the control of Complex I which include (1) protection from irreversible oxidation and (2) control of mitochondrial ROS genesis in response to alterations in local redox environment. The fact that Complex I can be controlled by redox switches has made it a pharmacological target for ischemic preconditioning and prevention of heart disease [108].

OXPHOS components have been shown to be modulated by redox switches. ATP synthase (Complex V) has been shown to be S-glutathionylated on Cys294 on its α-subunit which is located in the F1 hydrophilic part of the Complex [133]. This has been shown in heart mitochondria and occurs in dysynchronous heart failure. Cys294 has also been shown to form SOH which reacts with a neighboring Cys103 residue on the γ subunit to form a disulfide bridge [133]. Formation of the disulfide bridge occurs only under chronic heart disease. S-glutathionylation blocks nucleotide binding which diminishes the production of ATP. Garcia et al. also showed that the α-subunit of the F1 complex of ATP synthase is S-glutathionylated in rat brain and liver [134]. Similar to heart mitochondria, S-glutathionylation of ATP synthase in these two tissues diminishes ATP production. Indeed, S-glutathionylation of Complex I and Odh not only limits NADH production but also lowers electron flow through the respiratory chain which would effectively diminish ROS genesis. Temporary S-glutathionylation of Complex I and Odh protects both enzyme complexes from irreversible oxidation when ROS is high but may also hypothetically serve as a mechanism to diminish mitochondrial ROS production (Fig. 4). Temporary S-glutathionylation of the F1 α-subunit of ATP synthase may also be part of this adaptive response to a burst of H$_2$O$_2$ genesis vis a vis the decrease in electron flow which diminishes ATP production. In addition S-glutathionylation mediated decrease in ATP synthase activity would also limit ATP hydrolysis and the pumping of protons back into the intermembrane space which prevents the repolarization of the mitochondrial inner membrane (Fig. 4). The PMF is tightly associated with mitochondrial ROS production [27]. A high protonic potential nonlinearly increases mitochondrial ROS production by the respiratory chain while decoupling the MIM has the opposite effect. Intriguingly, when ROS is high in mitochondria UCP2 and UCP3 become deglutathionylated which activates proton leaks through these two proteins. Proton leaks lower the PMF across the MIM which subsequently diminishes ROS production by mitochondria. Thus, when ROS is high Complex I and Odh are S-glutathionylated to protect from irreversible oxidation which also lowers their activity to diminish electron flow through the respiratory chain which limits ROS production (Fig. 4). At the same time ATP synthase is

---

**Fig. 4.** Hypothetical scheme depicting the regulation of mitochondrial O$_2^-$ production and OXPHOS by S-glutathionylation. When H$_2$O$_2$ is high and 2GSH/GSSG is low in mitochondria, Odh, Complex I, and ATP synthase are temporarily S-glutathionylated to diminish NADH production and limit electron flow through the respiratory chain. Although this may diminish mitochondrial ATP production S-glutathionylation will limit O$_2^-$ production by mitochondria. In addition, S-glutathionylation of ATP synthase may also prevent ATP hydrolysis and the pumping of protons back to the intermembrane space. This would effectively maintain a lower membrane potential and limit ROS genesis. Once ROS levels have decreased and the 2GSH/GSSG ratio is restored Grx2 deglutathionylates Complex I and potentially Odh and ATP synthase which restores mitochondrial ATP production.
S-glutathionylated to lower ATP production and prevent ATP hydrolysis and MIM recoupling. Notably, S-glutathionylation of ATP synthase may also protect cysteine residues from irreversible oxidation. In addition, if UCP2 or UCP3 are present in the tissue, high ROS deglutathionylates these two proteins resulting in their activation and a lowering of mitochondrial PMF which also diminishes ROS production. Thus, reversible S-glutathionylation may serve as an important mechanism for the control of mitochondrial ROS output which can have a profound effect on cellular ROS signaling.

Mitochondrial morphology

Mitochondrial structure and morphology plays a key role in dictating mitochondrial function [135]. The shape of mitochondria has a tremendous impact on mitochondrial metabolism and cell death. Likewise the nutrients being metabolized, the concentration of nutrients in the cell, and the amount of ROS play significant roles in dictating mitochondrial shape and morphology [135]. In mammalian cells, the proteins involved in mitochondrial fusion, are the integral membrane GTPases Mitofusin 1 (Mfn1), Mitofusin 2 (Mfn2) and autosomal dominant Optical Atrophy 1 (Opa1) [136]. Conversely fission is mediated by the dynamin family of GTPases Drp-1. Chronic oxidative stress, high glucose or fatty acids (which increase ROS), or gluco-lipotoxicity are known to induce mitochondrial fragmentation [135]. However, treatment with sub-lethal amounts of H₂O₂ and acute stresses induce a hyperfused state [137]. Co-treatment with antioxidants like Tempol prevents hyperfusion [137]. Recently, Shutt et al. provided evidence that S-glutathionylation of Mfn proteins is required to induce mitochondrial hyperfusion [137]. In vitro determinations showed that addition of GSSG to reaction mixtures induced a hyperfused state which was associated with S-glutathionylation of Mfn2 and formation of Opa1 oligomers. Intriguingly, performing the same reactions in the presence of cystosol fractions from HeLa cells reversed Mfn2 S-glutathionylation. Redpath et al. made similar observations showing that mitochondria in C2C12 cells adopt a hyperfused state when treated with diamide [138]. These results demonstrate S-glutathionylation also plays a key role in mitochondrial morphology and that induction of fusion is sensitive to changes in the 2GSH/GSSG ratio. It has been proposed that mitochondrial hyperfusion may increase resistant towards cell stress and cell death. Hyperfusion may promote protection through the sharing of mitochondrial resources, specifically, anti-oxidant defense enzymes and molecules which could provide more long term protection of mitochondrial proteins, enzymes, DNA, and lipids from acute stressors. Effectively, this protective response would also prevent oxidative stress and induction of intrinsic apoptotic cascades via preservation of mitochondrial redox balance.

MTP and redox switches

Oxyradicals are maintained at low concentrations by antioxidant defense systems. But what happens if oxyradical production cannot be controlled and antioxidant defense systems are overwhelmed? In this scenario, mitochondria induce cell death pathways. The mitochondrial intermembrane space contains several factors, including Smac/DIABLO, apoptosis inducing factor, and cytochrome C, that upon being released into the cytoplasm, activate programmed cell death pathways [139]. The use of cytochrome C as an apoptotic signaling molecule is especially interesting since it is a key component of OXPHOS machinery. This illustrates the tight relationship between the efficiency of electron flow through the ETC and the production of either ATP or ROS.

Release of these apoptogenic factors in the cytoplasm can be mediated by MPT. The mitochondrial permeability transition pore (MPTP) is a non-selective pore that allows the free diffusion of molecules < 1.5 KDa in size [140]. Once open, the MPTP dissipates ion and metabolite gradients and collapses the PMF, which can induce massive swelling of mitochondria and the rupture of the mitochondrial outer membrane [70]. The MPTP is normally closed but can be opened by a number of factors including elevated matrix [Ca²⁺], adenine nucleotide depletion, and increased inorganic phosphate concentration [141]. Notably for the purpose of redox switches, MPT can also be induced by ROS, hydroperoxides (t-butyl hydroperoxide), and chemical toxins (which can induce ROS production) [141]. Antioxidant supplementation prevents MPT. Interestingly, MPT can also be induced by SH blockers and S-glutathionylation catalysts. For instance, induction of MPT can be prevented by incubating mitochondria in N-ethylmaleimide (NEM), a Michael acceptor that covalently binds to SH [70]. Alternatively, MPT can also be induced by diamide [70]. Studies using semiquinone, which actively produces O₂⁻, have found that mitochondrial produced ROS can activate MPT [142,143]. In addition, it has also been shown that H₂O₂ is the oxyspecies that most likely induces MPT since inclusion of catalase and thiol peroxidases to reaction mixtures prevents MPT [143,144]. Thus, the capacity of mitochondria to induce programmed cell death depends on redox switches which, upon sulfenylfication and/or S-glutathionylation, induces pore opening.

The function of redox switches in pore opening has been reviewed recently in [145]. Redox switches most likely serve as the connection between regulation of mitochondrial function and induction of death. The composition of MPTP still remains a contentious issue. MPTP has been shown to be composed of ANT, voltage dependent anion channel (VDAC), P₃ carrier, and cyclophilin D (Cyp-D) [146]. For pore opening to occur, Cyp-D needs to bind to the protein complex. The role of ANT in MPT is enthusiastically debated however; several reports have conclusively shown that oxidation of key cysteine residues on ANT is required for pore opening. Pore opening was shown to require the cross-linking of Cys₁⁶⁰ and Cys₂⁵⁷ (rat) or Cys₂⁵⁶ (bovine) in ANT [70]. This type of cross-linking only occurs if one of the SH groups is oxidized to SOH. Intriguingly, blocking Cys₁⁶⁰ with adenine nucleotides actually prevents pore opening; most likely through prevention of disulfide bond formation [70]. Prolonged exposure to high (> 100 μM) concentrations of H₂O₂ can decrease mitochondrial ATP production which could lead to a decreased amount of adenine nucleotide binding on ANT which would then exposed Cys₁⁶⁰ for oxidation and subsequent disulfide bond formation. It is also important to point out that mitochondrial soluble anion carriers share a high degree of homology including a Cys residue (Cys₂⁵⁶) in the last loop region that contacts the matrix. It was recently reported that ANT can also be S-glutathionylated and maintains an S-glutathionylated state in mitochondria from primary astrocytes and rat cortex [147]. Following oxidative stress, ANT is deglutathionylated which leads to MPT and induction of cell death [147]. Thus, it is possible that S-glutathionylation of ANT may prevent cysteine oxidation and cross-linking of Cys₁⁶⁰ and Cys₂⁵⁶/2⁵⁷. However, several key aspects surrounding ANT, cysteine oxidation, and pore opening need to be resolved such as (1) S-glutathionylation can also potentiate Cys cross-linking and (2) does ANT actually form part of MPT. Indeed, the actual composition of MPTP is still enthusiastically debated and remains a subject of heavy scrutiny. It has been shown that ANT and VDAC are not required for pore formation. Various reports however have shown that P₃ carrier may be a key part of the MPTP [148] but this has also been refuted [149]. It was also recently reported that MPTP is actually composed of ATP synthase dimers [150]. Since ATP synthase is amenable to cysteine oxidation when ROS is high.
it would be intriguing to determine if the oxidation of cysteines on ATP synthase is required to potentiate dimer formation.

Cyp-D has also been found to be modulated by S-oxidation reactions [151]. In this case, S-glutathionylation prevents Cyp-D binding to ANT which blocks MPT [151]. This modification was found to occur on Cys203 [110]. It is intriguing that S-glutathionylation of Cyp-D actually prevents MPTP. As mentioned above, the actual composition of the MPTP is still enthusiastically debated; however; Cyp-D has so far been the only component of MPTP found to be indispensable for pore opening. Cyp-D knockout or inhibition with cyclosporine A prevents MPTP and apoptosis [152,153]. Since S-glutathionylation of Cyp-D prevents pore opening, it is possible that blocking Cys residues on Cyp-D may be a protective response, e.g. when ROS production is transiently highly S-glutathionylation of Cyp-D prevents pore opening. Indeed, a transient burst of H2O2 production by mitochondria also S-glutathionylates Odh and Complex I which may effectively limit mitochondrial ROS production (Fig. 4). In conjunction with this, S-glutathionylation of Cyp-D may be required to prevent pore opening while mitochondria adapt to the transient increase in mitochondrial ROS production [110]. The involvement of ANT in pore opening is more contentious since some studies have shown that knockout of ANT does not diminish or prevent MPT [154]. However, it is possible that other proposed components of MPTP may be required to sense surrounding changes in redox and participate in pore formation following cysteine oxidation.

Deregulation of mitochondrial thiol reactions in disease

Cardiovascular diseases

An increasing number of studies have found that mitochondrial thiol oxidation is involved in cardiac arrest and stroke. Cessation of blood flow to a portion of the heart or brain results in localized hypoxia and often injury is observed during the reperfusion of oxygenated blood (ischemia/reperfusion injury or IRI). Injury mediated through the reperfusion stage is a result of the generation of ROS and RNS, although the sources of these are currently debated [155–157]. A key feature of IRI is mitochondrial energetic dysfunction [158], ROS and RNS, generated during IRI, have been shown to damage components in Complexes I, III, IV and V of the respiratory chain, as well as many Kreb cycle enzymes [159,160]. Short ischemic periods precondition tissues to hypoxic exposure and provide cardioprotective effects (ischemic preconditioning). These effects are mediated through the expression of protective genes and proteins that remain in the cell long after the cessation of ischemic stress. Thiol modifications, particularly of Complex I subunits, have been identified during IRI and results in further ROS generation by this Complex [158]. It was also found that cysteine 90 of the 70 kDa FAD-binding protein of mitochondrial succinate ubiquinone reductase, or Complex II, was glutathionylated and that this modification (as well as electron transfer activity of the respiratory chain itself) was markedly decreased upon reperfusion. It has been previously shown that inhibition of Complex IV by NO can cause the back-up of electrons along the electron transport chain and increase ROS generation at Complex III [167,168]. Since Complex I is the entry point for electrons into the chain, reversible inhibition of Complex I by S-nitrosylation would lower electron flux into the chain and thus lower ROS generation from the chain itself. Although inhibition of Complex I may increase generation of ROS from the complex itself [130], ROS generated from Complex I has been found to be much less than that generated from Complex III [169].

Mitochondrial thiol modification may also alter the timing, or synchrony, of contractions in the heart. Large differences in the timing of contractions can reduce cardiac output and efficiency and result in dysynchronous heart failure (DHF). Mixed disulfide bond formation of mitochondrial F1, F0-ATP synthase subunits have been found to be involved in DHF [133]. Two cysteine disulfide bond oxidations were identified to be involved in DHF. The first was a disulfide linkage between cysteine Cys294 of the α-subunit and Cys103 of the γ-subunit. The second was an oxidation of Cys294 to sulfenic acid. In addition, Cys294 of the α-subunit can also be S-glutathionylated and S-nitrosylated [170]. Either thiol modification led to a significant decrease in ATP synthase activity, resulting in the development of DHF. Glutathionylation of Cys294 of the α-subunit also has the potential to block nutrient binding and thus decrease activity [171].

Metabolic syndrome, insulin resistance, obesity and diabetes

Diabetes is a major health risk, with the global population of diabetics estimated to reach 300 million by the year 2025. Mortality risk is complicated by the association of diabetes mellitus and myocardial infarction. Diabetes is associated with hyperglycemia and this has been shown to have a direct effect on mitochondrial function [172]. The mitochondria from diabetic hearts have been found to be sensitized to mitochondrial permeability transition pore opening, which leads to a propensity for cardiac injury in diabetic patients and in animal models [173,174]. Hyperglycemia leads to high glucose-dependent production of electron transfer donors (NADH and FADH2) and thereby increases electron flux through the mitochondrial electron transport chain. Consequently there is an increase in the ATP/ADP ratio and hyperpolarization of the mitochondrial membrane potential results. This high electrochemical potential leads to a partial inhibition of electron transport at Complex III, leading to an accumulation of electrons on Q. This, in turn, drives the partial reduction of O2 to generate O2-. The generation of O2− from the highly reduced Q, and from Complex III in general, is believed to be

found to have both damaging and protective effects on myocardial tissues. Peroxynitrite-mediated tyrosine S-nitrosylation of the 70 kDa FAD-binding protein of Complex II occurs in the post-ischemic myocardium [165]. S-nitrosylation of Tyr56 and Tyr142 occurred while Cys207, Cys476, and Cys537 were involved in peroxynitrite-mediated Sulfonation. Peroxynitrite-mediated disulfide formation was also found to occur between Cys207,Cys476, Cys439,Cys444, and Cys288-Cys757.

In other reports, a wide variety of nitric oxide donors and S-nitrosylating agents have been found to protect the ischemic myocardium from infarction. In this respect, S-nitrosylation of mitochondrial proteins during ischemia-reperfusion has cardio-protective effects. S-nitrosylation of Cys810 on the NADH-ubiquinone oxidoreductase chain 3 (ND3) subunit of Complex I occurs only after ischemia [108]. Reversible D-nitrosylation of this protein slows the reactivation of mitochondria during the crucial first minutes of reperfusion of heart tissue, thereby decreasing ROS production. Likewise, S-nitrosylation of Cys137 of the 75 kDa Complex I subunit was found to occur [166]. The implications of this are that ROS will decrease upon reperfusion. It has been previously shown that inhibition of Complex IV by NO can cause the back-up of electrons along the chain and increase ROS generation at Complex III [167,168]. Since Complex I is the entry point for electrons into the chain, reversible inhibition of Complex I by S-nitrosylation would lower electron flux into the chain and thus lower ROS generation from the chain itself. Although inhibition of Complex I may increase generation of ROS from the complex itself [130], ROS generated from Complex I has been found to be much less than that generated from Complex III [169].

ROS production during IRI and results in further ROS generation by this Complex [158]. ROS generated from Complex I has been found to be much more damaging and protective effects on myocardial tissues. Peroxynitrite-mediated tyrosine S-nitrosylation of the 70 kDa FAD-binding protein of Complex II occurs in the post-ischemic myocardium [165]. S-nitrosylation of Tyr56 and Tyr142 occurred while Cys207, Cys476, and Cys537 were involved in peroxynitrite-mediated Sulfonation. Peroxynitrite-mediated disulfide formation was also found to occur between Cys207, Cys476, Cys439, Cys444, and Cys288-Cys757. In other reports, a wide variety of nitric oxide donors and S-nitrosylating agents have been found to protect the ischemic myocardium from infarction. In this respect, S-nitrosylation of mitochondrial proteins during ischemia-reperfusion has cardio-protective effects. S-nitrosylation of Cys810 on the NADH-ubiquinone oxidoreductase chain 3 (ND3) subunit of Complex I occurs only after ischemia [108]. Reversible D-nitrosylation of this protein slows the reactivation of mitochondria during the crucial first minutes of reperfusion of heart tissue, thereby decreasing ROS production. Likewise, S-nitrosylation of Cys137 of the 75 kDa Complex I subunit was found to occur [166]. The implications of this are that ROS will decrease upon reperfusion. It has been previously shown that inhibition of Complex IV by NO can cause the back-up of electrons along the chain and increase ROS generation at Complex III [167,168]. Since Complex I is the entry point for electrons into the chain, reversible inhibition of Complex I by S-nitrosylation would lower electron flux into the chain and thus lower ROS generation from the chain itself. Although inhibition of Complex I may increase generation of ROS from the complex itself [130], ROS generated from Complex I has been found to be much less than that generated from Complex III [169].

Mitochondrial thiol modification may also alter the timing, or synchrony, of contractions in the heart. Large differences in the timing of contractions can reduce cardiac output and efficiency and result in dysynchronous heart failure (DHF). Mixed disulfide bond formation of mitochondrial F1, F0-ATP synthase subunits have been found to be involved in DHF [133]. Two cysteine disulfide bond oxidations were identified to be involved in DHF. The first was a disulfide linkage between cysteine Cys294 of the α-subunit and Cys103 of the γ-subunit. The second was an oxidation of Cys294 to sulfenic acid. In addition, Cys294 of the α-subunit can also be S-glutathionylated and S-nitrosylated [170]. Either thiol modification led to a significant decrease in ATP synthase activity, resulting in the development of DHF. Glutathionylation of Cys294 of the α-subunit also has the potential to block nutrient binding and thus decrease activity [171].

Metabolic syndrome, insulin resistance, obesity and diabetes

Diabetes is a major health risk, with the global population of diabetics estimated to reach 300 million by the year 2025. Mortality risk is complicated by the association of diabetes mellitus and myocardial infarction. Diabetes is associated with hyperglycemia and this has been shown to have a direct effect on mitochondrial function [172]. The mitochondria from diabetic hearts have been found to be sensitized to mitochondrial permeability transition pore opening, which leads to a propensity for cardiac injury in diabetic patients and in animal models [173,174]. Hyperglycemia leads to high glucose-dependent production of electron transfer donors (NADH and FADH2) and thereby increases electron flux through the mitochondrial electron transport chain. Consequently there is an increase in the ATP/ADP ratio and hyperpolarization of the mitochondrial membrane potential results. This high electrochemical potential leads to a partial inhibition of electron transport at Complex III, leading to an accumulation of electrons on Q. This, in turn, drives the partial reduction of O2 to generate O2−. The generation of O2− from the highly reduced Q, and from Complex III in general, is believed to be
the primary source of mitochondrial dysfunction during diabetes. Two recent reviews have addressed the role of redox signaling and S-glutathionylation in glucose-stimulated insulin release and how deregulated redox signaling could potentially be related to development of diabetes [175]. However, very few studies have actually addressed the role of redox signaling in modulation mitochondrial function in pancreatic β-cells. A recent report has shown that reversible S-glutathionylation is required to control proton leaks through UCP2 in mitochondria which plays a role in regulating glucose-stimulated insulin release in Min6 cells and pancreatic islets [16].

Nutrient excess leads to excessive levels of energy substrates utilized by the mitochondria with consequential effects on lipid and glucose metabolism. This, in turn, leads to mitochondrial dysfunction and to the development of obesity and obesity-related pathologies such as non-alcoholic fatty acid liver disease (NAFLD) [176]. Few studies have addressed the relationship between deregulated redox signaling and the development of metabolic disease. A recent report has shown that the S-glutathionylated proteome in hepatic and visceral adipose tissue changes drastically upon the onset of obesity in mice [177]. In addition, knock-out of Grx2 leads to a significant decrease in total adiposity in C57Bl6j mice [86]. Intriguingly the decrease in adiposity in Grx2−/− mice correlates strongly with an increase in mitochondrial respiration, nutrient oxidation, and proton leaks through UCP3 in skeletal muscle [86]. It has been suggested that increasing leaks through UCP3 may have a weight loss effect since an increase in leaks would inevitably increase respiration and nutrient oxidation [178]. Thus, the weight loss effect observed in Grx2−/− mice may be due to the maintenance of UCP3 in a de glutathionylated and active state which ultimately leads to increases proton leaks and nutrient metabolism. Previously, it was found that mitochondria from obese rats show an increased capacity for oxidizing succinate, likely due to increased activity of succinate dehydrogenase, the mitochondrial enzyme that oxidizes succinate and reduces Q [179]. Oxidized succinate, or fumarate, can form a Michael addition with thiol groups in proteins [180] and this post-translational modification of proteins has been found to inactivate glyceraldehyde-3-phosphate dehydrogenase. Increased succination of protein thiols in observed during obesity and diabetes [181]. Succination of protein thiols in adipocytes has been found to occur during mitochondrial stress in adipocytes during adipogenesis [182]. S-(2-succinyl)cysteine (2SC), the product of chemical modification of proteins as well as its ability to bind to apoptosis signal-regulating kinase 1 (ASK1) (also known as mitogen-activated protein kinase kinase kinase 5 (MAP3K5)). In a mouse model of PD (acute treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which targets mitochondrial Complex I), Grx was observed to increase in brains [193]. When Grx1 was knocked down by antisense oligonucleotides, inactivation of Complex I by MPTP was not observed. It is also interesting to note that glutathionylation of the tau protein causes it to polymerize and form the neurofibrillary tangles [194] found in AD.

S-nitrosylation of mitochondrial proteins has been shown to be involved in neurodegenerative disorders. Glutaredoxin (Grx) is responsible for the glutathionylation of proteins and has been shown to be decreased in post-mortem AD brains [192]. Loss of Grx would mean loss of the cell’s ability to de glutathionylate proteins as well as its ability to bind to apoptosis signal-regulating kinase 1 (ASK1) (also known as mitogen-activated protein kinase kinase kinase 5 (MAP3K5)). In a mouse model of PD (acute treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which targets mitochondrial Complex I), Grx was observed to increase in brains [193]. When Grx1 was knocked down by antisense oligonucleotides, inactivation of Complex I by MPTP was not observed. It is also interesting to note that glutathionylation of the tau protein causes it to polymerize and form the neurofibrillary tangles [194] found in AD.

Neurodegenerative diseases

The major neurodegenerative diseases, including Alzheimer’s (AD), Friedreich’s (FD), amyotrophic lateral sclerosis (ALS) or Lou Gehrig’s, Huntington’s (HD), Parkinson’s (PD) and multiple sclerosis (MS) diseases, are characterized by a progressive loss of neurons and glutathionylation of proteins have been shown to have a role in this process [185]. All are characterized by either misfolded (e.g. tau), misprocessed (e.g. amyloid precursor protein) or mutated (e.g. α-synuclein) proteins. These proteins (or peptides from proteins) form insoluble aggregates and mitochondrial dysfunction, with corresponding generation of ROS and RNS, has been implicated in the process. The role of mitochondrial protein thiol in the development of neurodegenerative diseases is not fully understood and is an area that requires active investigation.

The levels of GSH have been reported to be reduced in AD [186]. Likewise, similar changes were observed in PD where GSH was reduced and GSSG was increased [187,188]. PD is characterized by early GSH depletion in dopaminergic cells in the substantia nigra (SN). The reasons for depletion of GSH in neurodegenerative diseases are not always clear. In PD, there is no corresponding increase in GSSG [189], so GSH depletion cannot be explained by oxidative stress alone. In this study, there was no increase in glutathione synthase activity in PD, but a large increase in γ-glutamyltranspeptidase, an enzyme that catalyzes the transfer of the γ-glutamyl moiety from GSH or a glutathione conjugate onto an acceptor molecule, was observed. As mentioned previously, mitochondrial GSH is a substrate for mitochondrial Grx. Grx2 has been shown to be involved in mitochondrial iron–sulfur (Fe–S) center biogenesis [190]. Increased cellular iron levels are a hallmark of PD, and decreased levels of GSH in PD results in a depletion of substrate for Grx2. The result is decreased iron incorporation into mitochondrial Complex I and aconitate (Acn) [190]. It also results in significant increases in iron-regulatory protein (IRP) binding, transferrin receptor, decreased ferritin, and subsequent increases in mitochondrial iron levels. Decreases in GSH, and increased levels of GSSG and nitrosothiols, were also observed in MS patients [191].

Glutathionylation of mitochondrial proteins has been shown to be involved in neurodegenerative disorders. Glutaredoxin (Grx) is responsible for the glutathionylation of proteins and has been shown to be decreased in post-mortem AD brains [192]. Loss of Grx would mean loss of the cell’s ability to de glutathionylate proteins as well as its ability to bind to apoptosis signal-regulating kinase 1 (ASK1) (also known as mitogen-activated protein kinase kinase kinase 5 (MAP3K5)). In a mouse model of PD (acute treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which targets mitochondrial Complex I), Grx was observed to increase in brains [193]. When Grx1 was knocked down by antisense oligonucleotides, inactivation of Complex I by MPTP was not observed. It is also interesting to note that glutathionylation of the tau protein causes it to polymerize and form the neurofibrillary tangles [194] found in AD.

S-nitrosylation of mitochondrial proteins has been related to the development of neurodegenerative disorders. S-nitrosylation of mitochondrial Complex I has previously been implicated in the development of PD [195,196]. Protein disulfide isomerase (PDI), a thioredoxin-like domain protein closely related to Grx and Trx, has also been implicated in PD [197]. PDI can become S-nitrosylated (forming SNO-PDI) and this modification disrupts both its chaperone and neuroprotective functions in PD [198] and in AD [199]. The S-nitrosylation of dynamin-related protein 1 (Drp1) may be related to the pathological fragmentation of mitochondria and consequent loss of synapses in neurodegenerative disorders [197]. Drp1 controls mitochondrial fission, and is responsible for proper neuronal development. Drp1 knockout mice exhibit abnormal brain development and die around embryonic day 12. Mitochondrial fusion/fission is essential for maintaining mitochondrial integrity and disruption of these processes leads to mitochondrial dysfunction and the development of PD and AD [200,201]. NO, produced in an inflammatory response to the Aβ peptide, nitrosylates a key cysteine on Drp1 [199], resulting in mitochondrial fission, synaptic loss, and neuronal damage. Prevention of S-nitrosylation of Drp1 by cysteine mutation abrogates these neurotoxic events. However, the role of Drp1 S-nitrosylation in its GTPase activity, mitochondrial fission and neuronal injury in AD remains debated as other reports have found that S-nitrosylation...
of Cys$^{644}$ of Drp1 does not affect its GTPase activity or cause dimerization of the protein [202].

**Aging**

All organisms undergo aging and mitochondrial protein thiol group content may play a role in this process [203,204]. Aging has been associated with more oxidized forms of biomacromolecules, particularly in the mitochondria where the majority of ROS/RNS are produced within the cell [205–207]. Previously, it has been shown that mitochondrial Complex I activity decreases with age and that protection of thiol groups with N-acetylcycteine prevented such decrease [208]. In an interesting study done on one short-lived and one long-lived strain (48% longer) of mouse, mitochondrial thiols (glutathione, reactive protein thiols) were found to be lower in short-lived mice (with corresponding higher levels of GSSG) than in long-lived mice [209]. Peroxiredoxin III, present in the mitochondria, was found to be present in it more oxidized, inactive form in aged rats [210]. Cysteine 109 in the catalytic site was found to be more sulfonated in aged animals. Not all modifications, associated with age, are viewed to be detrimental, with some being beneficial to the aging cell [211].

Age-related declines in GSH result in more oxidizing conditions within plasma[212]. It also means that there may be less available for glutathionylation within mitochondria, and thus proteins would be less glutathionylated during oxidizing conditions. Glutathionylation has been found to have both inactivating effects on its function [213,214], but may also protect the modified protein (due to the reversibility of glutathionylation) from irreversible and permanent damage [68].

The S-nitrosylation of proteins with age has been well studied, but a lack of a focus has been made on the mitochondria. S-nitrosylation of general cellular proteins linked to neurodegenerative diseases [215], cardiovascular diseases [216], obesity and diabetes [217] have all been related to aging. Likewise, general glutathionylation of cellular proteins has been associated with age-related diseases [218]. Succination and disease has not been studied in great detail, but mitochondrial sirtuin 5 has been previously shown to desuccinylate mitochondrial proteins [219]. Such modifications could be elucidated by performing proteomic work on pure isolated mitochondrial preparations. The challenge is that current protocols for such preparations do not yield fully pure mitochondria due to the nature in the cell (mitochondria form a syncytium throughout the cell and not a distinct organelle).

The regulation of mitochondrial abundance and function by thiol modification in disease is an unexplored realm of research that is open for investigation. Nitrosylation, sulfonation, glutathionylation and succination of protein thiols represent rapid, and readily reversed by enzymes and (2) uncontrolled reactions which are nonspecific and associated with oxidative stress and deregulated redox signaling. We also discussed some emerging concepts in terms of mitochondrial medicine and how the concept of mitochondria-targeted pharmaceuticals can potentially be utilized to restore deregulated redox signaling through replenishment of mitochondrial GSH and/or restoring mitochondrial ROS levels. Notably, we overlooked some other important reactions such as formation of persulphydrins (Cys–S–S–) which are the result of H$_2$S signaling. Indeed, mitochondria are not only sensitive to H$_2$S signaling but also important sites for H$_2$S metabolism [220,221]. Much remains to be investigated in terms of redox signaling and the modulation of mitochondrial function. However, based on present knowledge it is clear that redox signaling plays a central role in modulating mitochondrial biology and physiology.

**Conclusions and perspectives**

Mitochondria fulfill a multitude of essential cellular functions which are ultimately dependent on the production of ATP and ROS. ATP is required to drive most cellular processes while ROS can serve as an important signaling molecule. Genesis of either molecule is dependent on the transfer of electrons from nutrients to O$_2$ in the ETC. Mitochondrial dysfunction is related to wide range of diseases and some cases is actually the cause of disease. In the present review we have outlined the importance of redox signals in the modulation of mitochondrial function and how deregulation of the signals can lead to development of various disease states. Key enzymes and proteins involved in nutrient oxidation, metabolism, OXPHOS, solute transport, ROS production, ROS quenching, mitochondrial morphology, and even apoptosis are modulated by S-oxidation, S-glutathionylation, and S-nitrosylation. We illustrated the complexity of these reactions since ROS, GSH, and NO converge on protein cysteine thiols resulting in oxidation and change in protein function. We also showed that these modifications occur under to scenarios (1) controlled reactions which are site specific, rapid, and readily reversed by enzymes and (2) uncontrolled reactions which are nonspecific and associated with oxidative stress and deregulated redox signaling. We also discussed some emerging concepts in terms of mitochondrial medicine and how the concept of mitochondria-targeted pharmaceuticals can potentially be utilized to restore deregulated redox signaling through replenishment of mitochondrial GSH and/or restoring mitochondrial ROS levels.

**Acknowledgements**

This work was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada to WGW.

**References**

[1] G.C. Brown, V. Borutaite, There is no evidence that mitochondria are the main source of reactive oxygen species in mammalian cells, Mitochondrion 12 (2012) 1–4.
[2] A.B. Fernie, F. Carrari, L.J. Sweetlove, Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport, Curr. Opin. Plant Biol. 7 (2004) 254–261.
[3] R. Baradaran, J.M. Berrisford, G.S. Minhas, L.A. Sazanov, Crystal structure of the entire respiratory complex I, Nature 484 (2013) 443–448.
[4] C.L. Quinlan, A.L. Orr, I.V. Perevoschikova, J.R. Treberg, B.A. Ackrell, M. D. Brand, Mitochondrial complex II can generate reactive oxygen species at high rates in both the forward and reverse reactions, J. Biol. Chem. 287 (2012) 27255–27264.
[5] A.A. Khutornenko, V.V. Roudko, B.V. Chernyak, A.B. Vartapetian, P.M. Chumakov, A.G. Evstafieva, Pyrimidine biosynthesis links mitochondrial respiration to the p53 pathway, Proc. Natl. Acad. Sci. USA 107 (2010) 12828–12833.
[6] J. Zhang, F.E. Freeman, J.J. Kim, Structure of electron transfer flavoprotein-ubiquinone oxidoreductase and electron transfer to the mitochondrial ubiquinone pool, Proc. Natl. Acad. Sci. USA 103 (2006) 16212–16217.
[7] A.L. Orr, C.L. Quinlan, I.V. Perevoschikova, M.D. Brand, A refined analysis of superoxide production by mitochondrial sn-glycerol 3-phosphate dehydrogenase, J. Biol. Chem. 287 (2012) 42921–42935.
[8] M.D. Brand, D.G. Nichols, Assessing mitochondrial dysfunction in cells, Biochem. J. 435 (2011) 297–312.
[9] R.J. Mailloux, M.E. Harper, Uncoupling proteins and the control of mitochondrial reactive oxygen species production, Free Radic. Biol. Med. 51 (2011) 1106–1115.
[10] R.J. Mailloux, S.L. McBride, M.E. Harper, Unearthing the secrets of mitochondrial ROS and glutathione in bioenergetics, Trends Biochem. Sci. 38 (2013) 592–632.
[11] A.A. Starkov, G. Fiskurn, C. Chinopoulos, B.J. Lorenzo, S.E. Browne, M.S. Patel, M.F. Beal, Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species, J. Neurosci.: Off. J. Soc. Neurosci. 24 (2004) 7779–7788.
L.A. Sena, N.S. Chandel, Physiological roles of mitochondrial reactive oxygen species, mol. Cell 48 (2012) 158–167.

B. Huang, S.C. Chen, D.L. Wang. Shear flow increases S-nitrosylation of proteins in endothelial cells. Cardiovasc. Res. 83 (2009) 536–546.

M. Lindahl, A. Mata-Cabana, T. Kiesebach, The disulfide proteome and other reactive cysteine proteomes: analysis and functional significance. Antioxid. Redox Signal. 14 (2011) 652–672.

C. Grek, J. Zhang, Y. Manevich, D.M. Townsend, K.D. Wew, Causes and consequences of cysteine S-glutathionylation. J. Biol. Chem. 72 (2013) 2383–2393.

N.L. Cepelev, W.G. Willmore. Regulation of ion channels in response to hypoxia. Free Radic. Biol. Med. 50 (2011) 645–666.

M.D. Stewart, T.J. Igunomewa, Reactive cysteine in the structural Zn(II)-+ site of the C1B domain from PRCAlphal, Biochemistry 51 (2012) 7263–7277.

R. Jill, Function and biogenesis of iron–sulfur proteins, Nature 460 (2009) 831–838.

B. Velam, H. Grosfeld, C. Kromman, M. Leitner, Y. Gozes, A. Lazar, Y. Flasher, D. Marcus, S. Cohen, A. Shafferman, The effect of elimination of intersubunit disulphide bonds on the assembly, secretion and recombination in human acylcoenzyme A. Expression of acylcoenzyme A-580-Ala mutant, J. Biol. Chem. 266 (1991) 23977–23984.

M. Lo Conte, K.S. Carroll, The redox biochemistry of protein sulfenylation and sulfinylation, J. Biol. Chem. 288 (2013) 26480–26484.

C.C. Winterbourn, M.B. Hampton, Thiol chemistry and specificity in redox signaling, Free Radic. Biol. Med. 45 (2008) 549–561.

R.E. Smith, R. MacQuarie, The role of cysteine residues in the catalytic activity of glycerol-3-phosphate dehydrogenase, Biochim. Biophys. Acta 567 (1979) 269–277.

T. Korremme, T.E. Creighton, Ionisation of cysteine residues at the termini of membrane proteins reveals the alpha-helical core of C-140. Resistance to unusual thiol pKa values in proteins of the thioredoxin family, J. Mol. Biol. 253 (1995) 799–812.

M.M. Gallogly, D.W. Starke, J.J. Mieyal, Mechanistic and kinetic details of catalysis of thiol-disulphide exchange by glutathiones and potential mechanistic implications for regulation, Acta Biochim. Biophys. Sin. 11 (2009) 1095–1098.

E. Stroher, A.H. Millar, The biological roles of glutathiones, Biochem. J. 446 (2013) 334–338.

A.G. Cox, C.C. Winterbourn, M.B. Hampton, Mitochondrial peroxiredoxin involvement in antioxidant defence and redox signalling, Biochim. Biophys. Acta 1425 (2001) 313–325.

M. Lo Conte, K.S. Carroll, The redox biochemistry of protein sulfenylation and sulfinylation, J. Biol. Chem. 288 (2013) 26480–26484.

C.E. Paulsen, K.S. Carroll, Cysteine-mediated redox signaling: chemistry, biology, and tools for discovery, Chem. Rev. 113 (2013) 4633–4679.

Y.M. Go, D.P. Jones, Redox compartmentalization in eukaryotic cells, Biochem. Biophys. Acta. 1780 (2007) 1273–1290.

M. Karp, Y.M. Go, D.P. Jones. Conformational thermodynamics of thiol disulfide redox systems: a perspective on redox systems biology, Free Radic. Biol. Med. 44 (2008) 921–937.

R. Requejo, T.R. Hurd, N.J. Costa, M.P. Murphy, Cysteine residues exposed on protein surfaces are the dominant intramitochondrial thiol and may protect against oxidative damage, FEBS J. 277 (2010) 1465–1480.

G.C. Brown, Nitric oxide and mitochondria, Front. Biosci.: J. Virtual Lib. 12 (2007) 1024–1033.

G. Roos, J. Messens, Protein sulfenic acid formation: from cellular damage to redox regulation, Free Radic. Biol. Med. 51 (2011) 314–326.

A.V. Peskin, F.M. Low, L.N. Paton, G.J. Maghzal, M.B. Hampton, C.C. Winterbourn, The high reactivity of peroxiredoxin 2 with H2O2 is not reflected in its reaction with other oxidants and thiol reagents, J. Biol. Chem. 282 (2007) 11885–11892.

A. Hall, D. Parsons, L.B. Poole, P.A. Karplus, Structural evidence that peroxiredoxin catalytic power is based on transition-state stabilization, J. Mol. Biol. 402 (2010) 194–209.

S. Enami, M.R. Hoffmann, A.J. Colussi, Simultaneous detection of cysteine thiolates and sulfenic acids in single mitochondria, Biochim. Biophys. Acta 1557 (2001) 235–242.

J. Chen, R.T. Dirksen, H. Cheng, Superoxide production and the control of glucose-stimulated insulin secretion, J. Biol. Chem. 287 (2012) 503–508.

G.P. McStay, S.J. Clarke, A.P. Halestrap, Role of critical thiol groups on the protein surfaces are the dominant intramitochondrial thiol and may protect against oxidative damage, FEBS J. 277 (2010) 1465–1480.
acid detection methods to visualize and identify labeled proteins, Methods Enzymol. 473 (2010) 95–115.

S.M. Hutson, L.B. Poole, S. Coles, M.E. Conway, Redox regulation and trapping sulfinic acid in the peroxide-sensitive mitochondrial branched chain aminotransferase, Methods Mol. Biol. 476 (2009) 139–152.

H.M. Cocheme, G.F. Kelso, A.M. James, M.F. Ross, J. Trinka, T. Mahendiran, J. Asin-Cayuela, F.H. Blakie, A.R. Manas, C.M. Porteous, V.J. Adlam, R.A. Smith, M.P. Murphy. Mitochondrial targeting of quinones: therapeutic implications, Antioxid. Redox Signal. 12 (2010) 1235–1246.

C. Reily, T. Mitchell, B.K. Chacko, G. Benavides, M.P. Murphy, V. Darley-Usmar, Mitochondrially targeted compounds and their impact on cellular bioenergetics, Redox Biol. 1 (2013) 86–93.

A. Bindoli, J.M. Fukuto, H.J. Forman, Thioly chemistry in peroxidase catalysis and redox signaling, Antioxid. Redox Signal. 10 (2008) 1549–1564.

C.S. Cho, S. Lee, G.T. Lee, H.A. Woo, E.J. Choi, S.G. Rhee. Irreversible inactivation of glutathione peroxidase 1 and reversible inactivation of peroxiredoxin II by H2O2 in red blood cells, Antioxid. Redox Signal. 12 (2010) 915–927.

W.T. Lowther, A.C. Haynes, Reduction of cysteine sulfinic acid in eukaryotic, typical 2-Cys peroxiredoxins by sulfoxidase, Antioxid. Redox Signal. 15 (2011) 99–109.

M. Hamann, T. Zhang, S. Hendrich, J.A. Thomas, Quantitation of protein sulfenic and sulfinic acid, irreversibly oxidized protein cysteine sites in cellular proteins, Methods Enzymol. 348 (2002) 146–156.

M. Chevallet, E. Wagner, S. Luche, A. van Dorsselea, E. Leize-Wagner, T. Vincendeau. Regeneration of peroxiredoxins during recovery after oxidative stress: only some peroxiredoxins can be reduced during recovery after oxidative stress, J. Biol. Chem. 278 (2003) 37146–37153.

R.M. Caner-Aviles, M.A. Wilson, D.W. Miller, R. Ahmad, C. McLeodson, S. Kassahun, K. Baptista, D. Petrosko, R.R. Cookson. M.R. Parkinson’s disease DJ-1 is neuroprotective due to cysteine-sulfenic acid-driven mitochondrial localization, Proc. Natl. Acad. Sci. USA 101 (2004) 9103–9108.

H.A. Woo, S.H. Yim, D.H. Shin, D. Kang, D.Y. Yu, S.G. Rhee. Inactivation of peroxiredoxin 1 by phosphorylation allows H2O2 accumulation for cell signaling. Cell 140 (2010) 517–528.

Y.H. Noh, J.Y. Baek, W. Jeong, S.G. Rhee, T.S. Chang, Sulffired oxin translation into mitochondria plays a crucial role in reducing hyperoxidized peroxi-

A.V. Peskin, N. Dickerhof, R.A. Poynton, L.N. Paton, P.E. Pace, M.B. Hampton, C.C. Winterburn, Hyperoxidation of peroxiredoxin 2 and 3: rate constants for the reactions of the sulfenic acid of the peroxiredoxin cysteine, J. Biol. Chem. 288 (2013) 14170–14177.

M. Lo Conte, K.S. Carroll, Chemoselective ligation of sulfenic acids with aryl-nitroso compounds, Angew. Chem. Int. Ed. Engl. 51 (2012) 6502–6506.

S.M. Beer, E.R. Taylor, S.E. Brown, C.C. Dahn, N.J. Costa, M.J. Runswick, M. P. Murphy. Glutaredoxin 2 catalyzes the reversible oxidation and glutathio-

R. Freiberger, S. Cortassa, C. Maack, B. Ockerman, Y. Kushnareva, A.N. Murphy, A. Andreyev, Complex I-mediated reactive oxygen species generation: modulation by cyclophilin c and NAD(P) oxidation-reduction state. Biochem. J. 368 (2002) 545–553.

T.T. Nguyen, M.V. Stevens, M. Kohn, C. Steenbergen, M.N. Sack, E. Murphy, Cystine 203 of cyclophilin D is critical for cyclophilin D activation of the mitochondrial permeability transition pore, J. Biol. Chem. 286 (2011) 40184–40192.

C.P. Ciardelli, C.G. Austin, A.R. Whorton, Nitric oxide and differential effects of ATP on mitochondrial permeability transition, Nitric Oxide: Biol. Chem. Off. J. Nitric Oxide Soc. 6 (2002) 45–60.

S.J. Chinta, J.K. Andersen, Nitrination and nitration of mitochondrial complex I in Parkinson’s disease, Free Radic. Res. 45 (2011) 53–58.

L.K. Kwong, R.S. Somatic, Substrate and site specification of hydrogen peroxide generation in mouse mitochondria, Arch. Biochem. Biophys. 350 (1998) 318–326.

R. Kushnareva, A.N. Murphy, A. Andrejev, Complex 1-mediated reactive oxygen species generation: modulation by cyclophilin c and NAD(P) oxidation-reduction state, Biochem. J. 368 (2002) 545–553.

C.P. Ciardelli, C.G. Austin, A.R. Whorton, Nitric oxide and differential effects of ATP on mitochondrial permeability transition, Nitric Oxide: Biol. Chem. Off. J. Nitric Oxide Soc. 6 (2002) 45–60.

E. Ford, M.N. Hughes, P. Wardman, Kinetics of the reactions of nitrogen mustard with aryl nitroso compounds, Angew. Chem. Int. Ed. Engl. 51 (2012) 6502–6506.

M.A. Aon, S. Cortassa, C. Maack, B. Ockerman, Y. Kushnareva, A.N. Murphy, A. Andreyev, Complex I-mediated reactive oxygen species generation: modulation by cyclophilin c and NAD(P) oxidation-reduction state. Biochem. J. 368 (2002) 545–553.

I. Gualillo, J.M. Fukuto, H.J. Forman, Thioly chemistry in peroxidase catalysis and redox signaling, Antioxid. Redox Signal. 12 (2010) 915–927.

M. Gutscher, A.L. Pauleau, L. Marty, T. Brach, G.H. Wahnbich, Y. Samstag, M. Johansson, A. Holmgren, Cloning and expression of a novel human glutaredoxin (Grx2), J. Biol. Chem. 276 (2001) 26269–26275.
A.J. Kowaltowski, R.F. Castilho, A.E. Vercesi, Mitochondrial permeability transition pore regulates some necrotic but not apoptotic cell death, Nature 434 (2005) 652–658.

J.E. Kokoszka, K.G. Waymire, S.E. Levy, J.F. Sligh, J. Cai, D.P. Jones, G.R. MacGregor, D.C. Wallace. The ADP/ATP translocator is not essential for cell death: mitochondrial permeability transition pore, Nature 427 (2004) 461–465.

G. Ambrosio, J.L. Zweier, J.T. Flaherty, The relationship between oxygen radical generation and impairment of myocardial energy metabolism following post-ischemic reperfusion, J. Mol. Cell. Cardiol. 23 (1991) 1359–1374.

J.L. Zweier, J.T. Flaherty, M.L. Weisfeldt, Direct measurement of free radical generation following reperfusion of ischemic myocardium, Proc. Natl. Acad. Sci. USA 84 (1987) 1404–1407.

R. Boll, E. Marban, Molecular and cellular mechanisms of myocardial stunning, Physiol. Rev. 79 (1999) 609–614.

A.J. Tompkins, L.S. Burwell, S.B. Digerness, C. Zaragoza, W.L. Holman, P.S. Brooks, Mitochondrial dysfunction in cardiac ischemia-reperfusion injury: ROS from complex I, without inhibition, Biochim. Biophys. Acta 1575 (2002) 223–231.

E.R. Taylor, F. Hurrell, R.J. Shannon, T.K. Lin, J. Hirst, M.P. Murphy, Reversible glutathionylation of adenine nucleotide translocase induced by carbon monoxide decreases the activity of the mitochondrial permeability transition pore, Curr. Med. Chem. 10 (2003) 1507–1513.

C. Brenner, The adenine nucleotide translocase: a central conditioning tachycardia decreases the activity of the mitochondrial permeability transition pore in heart, Biochem. Biophys. Res. Commun. 310 (2003) 916–921.

M. Liesa, O.S. Shirihai, Mitochondrial dynamics in the regulation of nutrient metabolism, EMBO Rep. 13 (2012) 909–913.

M. Baffy, Z. Derdak, S.C. Robson, Mitochondrial recoupling: a novel therapeutic strategy for cancer? Br. J. Cancer. 110 (2013) 5887–5893.

J. Garcia, D. Han, H. Sancheti, L.P. Yap, N. Kaplowitz, E. Cadenas, Regulation of mitochondrial ATP synthase, Trends Cardiovasc. Med. 23 (2013) 14–21.

X. Zhao, Y.R. Chen, G. He, A. Zhang, J.L. Druhan, A.R. Strauch, J.L. Zweier, Endothelial nitric oxide synthase (NOS3) knockout decreases NOS2 induction and contraction in coronary arteries, Proc. Natl. Acad. Sci. USA 110 (2013) 5887–5892.

J.C. Bournat, C.W. Brown, Mitochondrial dysfunction in obesity, Curr. Opin. Endocrinol. Diabetes Obes. 17 (2010) 446–450.

E.J. Anderson, E. Rodriguez, C.A. Anderson, K. Thayne, W.R. Chitwood, Jr., J.L. Zweier, Cardiac resynchronization therapy and reverse remodeling of the failing myocardium: Role of complex II, J. Biol. Chem. 283 (2008) 26316–26326.

A.J. Kowaltowski, R.F. Castilho, M.T. Grimaldi, E.J. Bechera, A.E. Vercesi, Effect of inorganic phosphate concentration on the nature of inner mitochondrial membrane permeability transition, J. Biol. Chem. 276 (2001) 24325–24330.

G. Ambrosio, J.L. Zweier, J.T. Flaherty, M.L. Weisfeldt, Direct measurement of free radical generation following reperfusion of ischemic myocardium, Proc. Natl. Acad. Sci. USA 84 (1987) 1404–1407.

C. Brenner, The adenine nucleotide translocase: a central conditioning tachycardia decreases the activity of the mitochondrial permeability transition pore in heart, Biochem. Biophys. Res. Commun. 310 (2003) 916–921.

J.L. Zweier, C.A. Chen, M.A. Talukder, Cardiac resynchronization therapy and reverse molecular remodeling: importance of mitochondrial redox signaling, Hypertension 59 (2012) 1719–1727.

R.J. Mailloux et al. / Redox Biology 2 (2014) 123–139
