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Review

Mitochondrial respiratory chain complexes as sources and targets of thiol-based redox-regulation ☆

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ABSTRACT

The respiratory chain of the inner mitochondrial membrane is a unique assembly of protein complexes that transfers the electrons of reducing equivalents extracted from foodstuff to molecular oxygen to generate a proton-motive force as the primary energy source for cellular ATP-synthesis. Recent evidence indicates that redox reactions are also involved in regulating mitochondrial function via redox-modification of specific cysteine-thiol groups in subunits of respiratory chain complexes. Vice versa the generation of reactive oxygen species (ROS) by respiratory chain complexes may have an impact on the mitochondrial redox balance through reversible and irreversible thiol-modification of specific target proteins involved in redox signaling, but also pathophysiological processes. Recent evidence indicates that thiol-based redox regulation of the respiratory chain activity and especially S-nitrosylation of complex I could be a strategy to prevent elevated ROS production, oxidative damage and tissue necrosis during ischemia–reperfusion injury. This review focuses on the thiol-based redox processes involving the respiratory chain as a source as well as a target, including a general overview on mitochondria as highly compartmentalized redox organelles and on methods to investigate the redox state of mitochondrial proteins. This article is part of a Special Issue entitled: Thiol-Based Redox Processes.

1. Introduction

Mitochondria are extraordinary organelles holding key positions in a number of fundamental cellular processes including ATP-synthesis, biosynthetic pathways, ion homeostasis, oxygen sensing and apoptosis. All of these pathways encompass redox-reactions as central elements. In addition, mitochondria contain major cellular generators of reactive oxygen species (ROS) that include components of the respiratory chain and a number of other redox enzymes [1–4], as well as powerful antioxidative defense systems [5–8] making mitochondria also a central player in cellular redox homeostasis. Elevated mitochondrial ROS production has been associated with a number of pathophysiological processes [9] including neurodegenerative diseases like Morbus Alzheimer and Morbus Parkinson [10], cancer [11,12] and oxidative damage during ischemia–reperfusion injury [13,14]. In addition, recent findings and novel concepts imply mitochondrial ROS as regulatory agents in a number of signal transduction pathways [15–17]. Hence, the functions of mitochondrial ROS seem to be highly ambivalent, deleterious and disease-causing on one side, taking part in physiological redox-regulation on the other. To unravel this 'ROS paradoxon' one faces two major challenges concerning the biochemistry of the elusive agents involved: (1) the sources and underlying molecular mechanisms of mitochondrial ROS production and their control under different physiological and pathophysiological circumstances have to be elucidated and (2) the ROS targets under these conditions have to be identified.

Since oxidation and reduction of thiol proteins are thought to be the major mechanisms by which reactive oxidants integrate into cellular signal transduction pathways [18,19], recent research has focused on thiol-based redox modifications [20]. The processes and mechanisms

Abbreviations: A/D-transition, 'active/deactive-transition' of mitochondrial complex I; Gpx1 and 4, glutathione peroxidases 1 and 4; DIGE, difference gel electrophoresis; GELSILOX, gel-based stable isotope labeling of oxidized cysteines; GR, glutathione reductase; GSH, glutathione; Grx2, glutaredoxin-2; GSNO, S-nitrosoglutathione; GSSG, glutathione-disulfide; IAA, iodoacetic acid; IAM, iodoacetamide; ICAT, isotope-coded affinity tag; IEF, isoelectric focusing; IMS, intermembrane space; LC, liquid-chromatography; MS, mass spectrometry; NEM, N-ethyl maleimide; SDS, sodium dodecylsulfate; NNT, nicotinamide nucleotide transhydrogenase; Δp, proton-motive force; Prx 3, peroxiredoxin 3; RET, reverse electron transfer; RNS, reactive nitrogen species; ROS, reactive oxygen species; TCA cycle, tricarboxylic acid cycle; TCEP, tris(2-carboxyethyl)phosphine; TOM, translocases of the outer membrane; Trx2, thioredoxin 2; TrxR2, thioredoxin reductase 2; VDAC, voltage-dependent anion channel

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that regulate such processes in mitochondria are largely unknown. However, it seems plausible that the respiratory chain should play a central role, since it comprises the major mitochondrial ROS generators complex I (NADH:ubiquinone oxidoreductase) [21–26], complex II (succinate:ubiquinone oxidoreductase) [27–29] and complex III (cytochrome bc_1 complex; ubiquinol:cytochrome *c* oxidoreductase) [30–35]. On the other hand, specific cysteine thiols of respiratory chain complexes have been identified as targets of ROS and reactive nitrogen species (RNS) during oxidative stress [8,36–41]. Taken together these observations suggest a feed-back loop that uses reversible redox modifications of respiratory chain complexes to avoid irreversible oxidative damage caused by an elevated mitochondrial ROS production. A recent example is the reversible S-nitrosylation of complex I that is protective against myocardial ischemia/reperfusion damage [42,43].

This review intends to highlight different aspects of the intertwined relation between the respiratory chain and mitochondrial thiol-based redox processes: respiratory chain complexes as sources and targets of thiol-based redox-regulation, the influence of respiratory chain activity on the 'general' redox environment and redox-status of antioxidative defense systems. Relevant methods for the analysis of thiol-based redox processes in mitochondria will be discussed and the mitochondrial disulfide relay that facilitates the import of proteins into the intermembrane space including some respiratory chain complex subunits [44] will be briefly mentioned.

2. Mitochondria are highly compartmentalized redox organelles

It is evident from electron microscopy and especially cryo-electron tomography that mitochondria are highly compartmentalized organelles [45,46]. The mitochondrial matrix is surrounded by an outer membrane (OM) and an inner membrane (IM) separated by the intermembrane space (IMS; Fig. 1). The IM forms large invaginations into the matrix forming the so-called cristae that can develop into complex networks with different shapes [46]. The cristae are functionally separated from the inner boundary membrane by cristae junctions that limit the diffusion of IM proteins and IMS proteins [47]. The majority of respiratory chain complexes is localized in the cristae membranes which are shaped by highly organized respiratory chain supercomplexes [48] and rows of complex V (ATP synthase) oligomers [49–51]. The redox milieu – mainly determined by the redox status of glutathione – differs substantially between the mitochondrial compartments [52], thus placing the respiratory chain complexes at the boundary of two quite different redox environments: a reducing matrix and a relatively oxidizing IMS and cristae lumen [47] (Fig. 1). This 'boundary effect' has fundamental consequences for the distribution, reactivity and functionality of surface exposed cysteine thiols of mitochondrial proteins. As a result, the mitochondrial sub-compartments represent distinct reaction rooms that allow compartmentalized redox processes including redox signaling [53]. The different redox environments are governed by the distribution

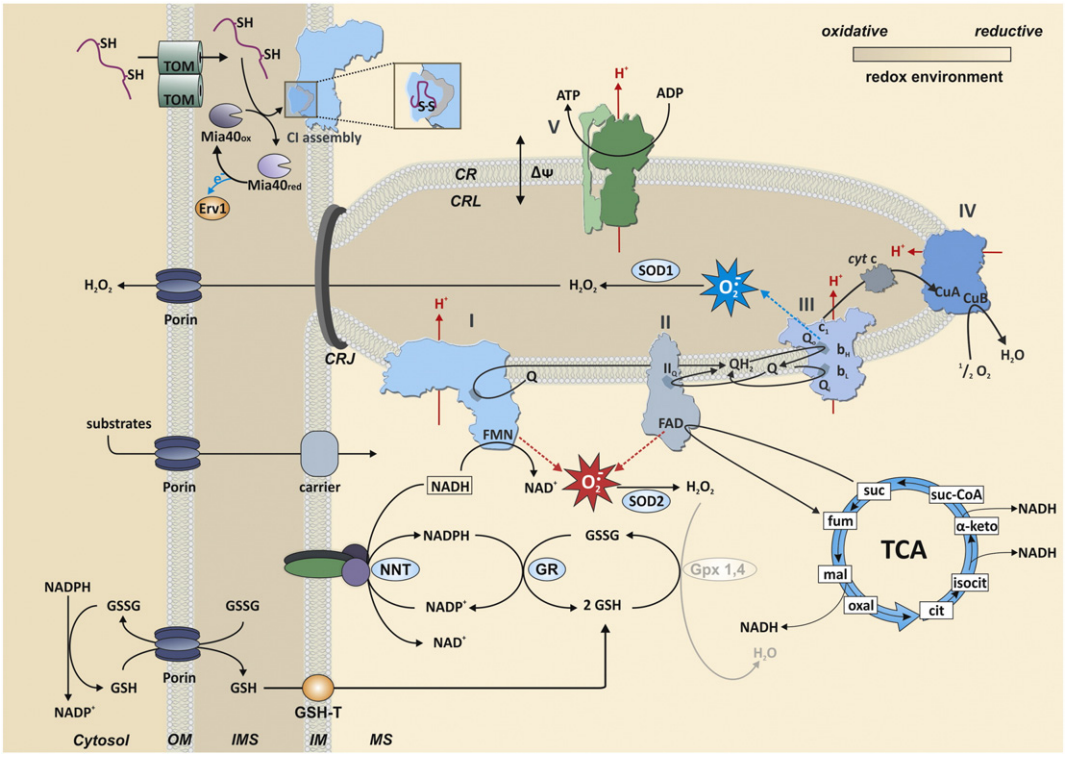

Fig. 1. *Mitochondrial redox compartmentalization.* The redox environment of the mitochondrial compartments is influenced by the distribution of ROS sources (only complexes of the oxidative phosphorylation – indicated by Roman numerals I–V – are shown), components of the antioxidative defense (only GSH-related processes are shown) and their regenerating (reducing) systems. GSH is transported into the matrix by a not yet unambiguously identified transporter (GSH-T). In general, the intermembrane space (IMS) is more oxidizing than the cytosol and the matrix space (MS). It is not clear whether the redox environment of the cristae lumen (CRL) – separated by cristae junctions (CRJ) – differs from the peripheral IMS located between the inner boundary part of the inner membrane (IM) and the outer membrane (OM). Importantly, the regeneration of the GSH-pool in the IMS relies on activities of cytosolic enzymes and the cytosolic NADPH-pool, while the regeneration of the matrix GSH-pools and other components of the antioxidative defense (not shown) depends on activities of mitochondrial enzymes including nicotinamide nucleotide transhydrogenase (NNT) and the matrix NADPH-pool. The latter is eventually controlled by the combined activities all NADH-generating and -consuming processes (e.g. respiratory chain and TCA cycle, fueled by the substrates succinate (suc), fumarate (fum), malate (mal), oxaloacetate (oxal), citrate (cit), isocitrate (isocit), α-ketoglutarate (α-keto) and succinyl-CoA (suc-CoA)). Superoxide produced by complexes I and II is released into the matrix, while superoxide produced at the Q_o-site of complex III is mainly released into the IMS. This primary ROS is converted into H_2O_2 by the activities of the superoxide dismutases SOD1 and SOD2, respectively. The relatively oxidizing environment in the IMS is important for the Mia40-Erv1 pathway that is also essential for the correct folding and assembly of respiratory chain complex subunits. For a detailed description of all aspects see text. TOM, translocases of the outer membrane; GR, glutathione reductase; Gpx1,4, glutathione peroxidases 1 and 4; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; Q, ubiquinone; QH_2, ubiquinol; II_Q, Q-binding site of complex II; Q_o and Q_i, ubiquinol oxidation and ubiquinone reduction centers of complex III; b_H and b_L, high potential and low potential cytochrome *b*, c_1, cytochrome c_1; CuA and CuB, copper A and copper B of complex IV.