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Review

Mitochondrial glutathione: Features, regulation and role in disease ☆

Montserrat Marí [a,*], Albert Morales [a], Anna Colell [a], Carmen García-Ruiz [a], Neil Kaplowitz [b], José C. Fernández-Checa [a,b,*]

[a] IDIBAPS, Liver Unit-Hospital Clínic, CIBEREHD, and Department of Cell Death and Proliferation, IIBB-CSIC, 08036, Barcelona, Spain
[b] Research Center for ALPD and Cirrhosis, Keck School of Medicine of the University of Southern California, Los Angeles, CA, USA

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ABSTRACT

Background: Mitochondria are the powerhouse of mammalian cells and the main source of reactive oxygen species (ROS) associated with oxygen consumption. In addition, they also play a strategic role in controlling the fate of cells through regulation of death pathways. Mitochondrial ROS production fulfills a signaling role through regulation of redox pathways, but also contributes to mitochondrial damage in a number of pathological states.
Scope of review: Mitochondria are exposed to the constant generation of oxidant species, and yet the organelle remains functional due to the existence of an armamentarium of antioxidant defense systems aimed to repair oxidative damage, of which mitochondrial glutathione (mGSH) is of particular relevance. Thus, the aim of the review is to cover the regulation of mGSH and its role in disease.
Major conclusions: Cumulating evidence over recent years has demonstrated the essential role for mGSH in mitochondrial physiology and disease. Despite its high concentration in the mitochondrial matrix, mitochondria lack the enzymes to synthesize GSH *de novo*, so that mGSH originates from cytosolic GSH via transport through specific mitochondrial carriers, which exhibit sensitivity to membrane dynamics. Depletion of mGSH sensitizes cells to stimuli leading to oxidative stress such as TNF, hypoxia or amyloid β-peptide, thereby contributing to disease pathogenesis.
General significance: Understanding the regulation of mGSH may provide novel insights to disease pathogenesis and toxicity and the opportunity to design therapeutic targets of intervention in cell death susceptibility and disease. This article is part of a Special Issue entitled Cellular functions of glutathione.

1. Introduction

Despite its exclusive synthesis in the cytosol, GSH is distributed in intracellular organelles, including endoplasmic reticulum (ER), nucleus and mitochondria. The compartmentalization of GSH in separate redox pools is critical to control compartment-specific needs and functions [1,2]. In the nucleus, GSH maintains critical protein sulphydryls that are necessary for DNA repair and expression [3] and functions also as a hydrogen donor in ribonucleotide reductase-catalyzed reduction of ribonucleotides to deoxyribonucleotides, thus playing a contributory role in DNA synthesis [4]. Intracellularly GSH is predominantly found in its reduced form except in the ER, where it exists mainly as oxidized glutathione (GSSG), GSSG being the main source of oxidizing equivalents to provide the adequate environment necessary for disulphide bond formation and proper folding of nascent proteins [5]. In mitochondria, however, GSH is mainly found in reduced form and represents a minor fraction of the total GSH pool (10–15%). Considering the volume of the mitochondrial matrix, the concentration of mitochondrial GSH (mGSH) is similar to that of cytosol (10–14 mM) [1,2,6,7].

Mitochondria are an excellent example of subcellular organelles whose function is closely linked to maintenance of redox balance. The mitochondria are the primary intracellular site of oxygen consumption and the major source of reactive oxygen species (ROS), most of them originating from the mitochondrial respiratory chain. Associated with this constant flow of ROS generation mitochondria are a target for the damaging effects of oxygen radicals [8–10]. Although normal electron transport in mitochondria involves four-electron reduction of molecular oxygen to water, partial reduction reactions occur even under physiological conditions, causing release of superoxide anion ($O^{\cdot 2-}$) and hydrogen peroxide. In accordance with this, it has been estimated that the steady-state concentration of $O^{\cdot 2-}$ in the mitochondrial matrix is five- to tenfold higher than in the cytosol [11].

In addition to the ROS generated under physiological settings, toxic or pathological conditions that lead to an impairment of mitochondrial function can increase the release of ROS. Therefore, although mitochondria are exposed to the constant generation of oxidant species, the organelle remains functional due to the existence of an antioxidant defense system, of which mGSH is a critical component, aimed to prevent

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* Corresponding authors at: Centre Esther Koplowitz, C/ Rosselló 149-153, 08036, Barcelona, Spain. Tel.: +34 93 2275709; fax: +34 93 3129405.
E-mail addresses: monmari@clinic.ub.es (M. Marí), checa229@yahoo.com (J.C. Fernández-Checa).

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or repair oxidative damage generated during normal aerobic metabolism. This review summarizes current knowledge on the physiology and function of mGSH and its role in cell death regulation and pathological states.

2. Mitochondrial oxidative stress and defense

The primary function of mitochondria is to transduce oxygen consumption in the electron transport chain (ETC.) into energy required for myriad cell functions. Although the process is highly efficient, a small fraction of electrons are transferred directly to molecular oxygen, resulting in the generation of $O_2^{\cdot -}$, which can give rise to other potent ROS as well as reactive nitrogen species (RNS). Therefore, a fine equilibrium between ROS production and removal will determine the physiological *vs.* pathological function of ROS. Mitochondria contain an arsenal of antioxidant systems with target specificity [1].

2.1. Superoxide dismutase

The first line of defense against ROS is guaranteed by the presence of Mn^{2+}-SOD (SOD2) in the mitochondrial matrix, which results in superoxide anion dismutation and the subsequent generation of hydrogen peroxide. The relevance of this strategy is illustrated by the fact that global SOD2 deficiency leads to neonatal death in mice. In turn, control of hydrogen peroxide is achieved by the GSH redox system and other defenses such as glutaredoxins and thioredoxins, as depicted in Fig. 1. Hydrogen peroxide can arise through sources other than via superoxide anion dismutation by SOD-2. For instance, $p66^{Shc}$ is a cytoplasmic protein involved in signaling from tyrosine kinases to Ras, which translocates to mitochondria in response to stress contributing to cell death and aging. It has been shown that $p66^{Shc}$ directly stimulates hydrogen peroxide generation, without inhibiting mitochondrial respiration, by transferring electrons to cytochrome c [12].

2.2. Glutathione redox cycle

Although hydrogen peroxide is not a free radical, it is an oxidant and an intermediate in the chain of reactions that generate reactive free radicals, such as hydroxyl radical, which can oxidize mitochondrial components (proteins, lipids, DNA). Since most mitochondria lack catalase, perhaps with the demonstrated exception of rat heart mitochondria [13], the metabolism of hydrogen peroxide is mainly accomplished by GSH, with the participation of either GSH peroxidase or peroxiredoxins. Associated with this function GSH becomes oxidized to GSSG, which is reduced back to GSH by the NADPH-dependent GSSG reductase (GR), as shown in Fig. 1. Among GSH peroxidases (Gpx) that detoxify hydrogen peroxide, Gpx1 is the major isoform localized mainly in the cytosol, with a small fraction also present in the mitochondrial matrix [2,14]. Some potent electrophiles, especially those generated as a consequence of metabolic processes involving both endogenous compounds and xenobiotics, can be readily removed by GSH via catalysis by glutathione transferases (GSTs). GSTs are distributed in mitochondria (GSTA1), cytosol (alpha, mu, pi, and zeta) and membrane-bound (MGST1) isoforms [15,16]. Mitochondrial GSTs display both glutathione transferase and peroxidase activities that detoxify harmful byproducts through GSH conjugation or GSH-mediated peroxide reduction [15,17]. Among human mitochondrial GSTs, the isoforms hGSTA4-4, hGSTA1, hGSTA2, and hGSTP1 showed peroxidase activity, with hGSTA4-4 exhibiting the highest activity [18,19].

mGSH is also the primary defense against oxidative damage to mitochondrial membranes by insuring the reduction of hydroperoxides present on phospholipids and other lipid peroxides. These modified lipids are detoxified by mGSH through the actions of mitochondrial GSTs with modest Se-independent Gpx activity, as well as specific GSH peroxidases, such as Gpx4 which displays high preference for lipid hydroperoxides (Fig. 1). Actually, due to its capacity to reduce hydroperoxide groups on phospholipids, cholesteryl esters and lipoproteins, Gpx4 is considered a critical defense enzyme in protecting membranes against oxidative stress. Gpx4 is synthesized in three forms that arise from the same *Gpx4* gene displaying different translation initiation sites. A short form of Gpx4 is present in somatic tissue mitochondria and is essential for survival and protection against apoptosis in mice, whereas a long form has been shown to be important for male fertility [20–22]. Experiments using cell lines overexpressing Gpx4 have shown its critical role in reducing oxidative stress-mediated toxicity [23]. Interestingly, recent reports also suggest that Gpx4 plays a role in the protection against apoptosis and in maintenance of oxidative phosphorylation complexes in gut epithelial cells [24], by a mechanism involving the protein Apoptosis Inducing Factor (AIF). In line with this, TNFα induced ROS formation, phospholipid peroxidation, mitochondrial damage, and apoptotic death in Jurkat cells, was prevented upon ectopic GPx4 expression [25]. Conversely,

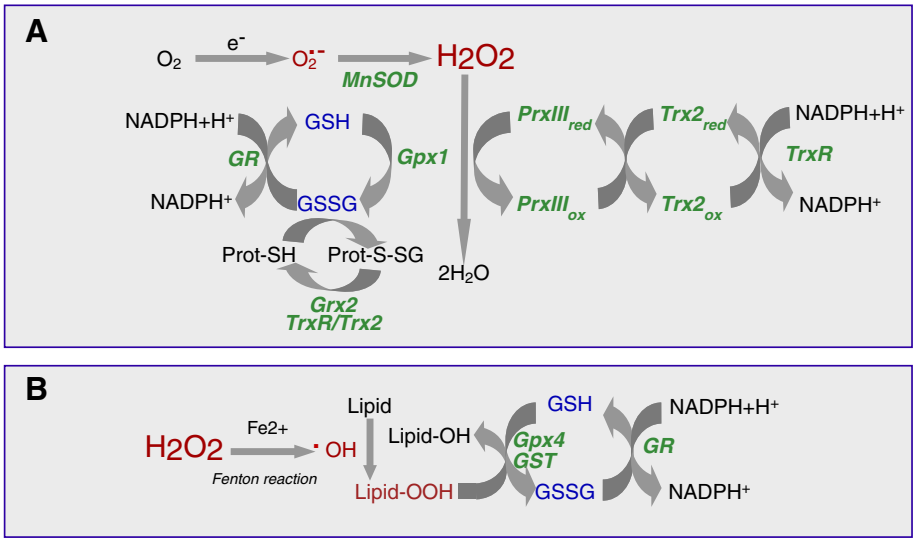

Fig. 1. Mitochondrial antioxidant defense system. Scheme of the different reactions that take place in the mitochondria to cope with the oxidative stress derived from the presence of superoxide anion, hydrogen peroxide, and hydroxyl radical. A, Removal of H_2O_2, and B, elimination of hydroxyl radical generated through the Fenton reaction. GSH peroxidase (Gpx), GSSG-reductase (GR); GSSG, glutaredoxin (Grx), Mn-dependent superoxide dismutase (MnSOD), thioredoxin-2 (Trx2), Trx-Reductase (TrxR), peroxiredoxin III (PrxIII).

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