Glutathione Intracellular Compartmentalization

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Reduced glutathione (GSH) is the most abundant non-protein endogenous thiol. It is a ubiquitous molecule produced in most organs, but its synthesis is predominantly in the liver, the tissue in charge of storing and distributing it. The conservation of hepatic glutathione levels is a dynamic process resulting from the balance between the synthesis rate, transport, use and removal of such thiols. Its synthesis takes place only in the cellular cytosol since all the necessary enzymes for its synthesis are found there. Nevertheless, within the cell, glutathione is compartmentalized into different cell organelles and ratios.

glutathione S-glutathionylation transporters

1. Introduction

Oxidative stress is one of the main causes of the development of different types of diseases, such as cancer [1][2], neurodegenerative pathologies [3][4], liver [5][6], cardiac [7][8], pulmonary [9][10] and renal diseases [11][12]. Therefore, strategies have been developed to reduce its effects, such as modifying the lifestyle of patients, that is, changes in diet and physical activity; abolishing any habit that generates oxidizing molecules (such as smoking or drinking alcohol) is also important. With such measures, it is sought to strengthen the antioxidant systems of the patient, for prevention of disease or to decrease its effects [13][14][15][16].

Regarding oxidative stress, the enzymatic systems that contribute the most to the generation of ROS include the proteins that are bound to the plasma membrane, such as the family of NADPH oxidases [17][18], the enzymatic systems that participate in the lipid metabolism within peroxisomes and the activity of various cytosolic enzymes such as cyclooxygenases. Although all these sources contribute to the increase in the oxidative state of the cell, the vast majority of cellular ROS (approximately 90%) originates from the mitochondria [19][20].

To counteract the effect of ROS, the cell has a series of antioxidant compounds. One of the most important antioxidant molecules in cellular systems is reduced glutathione (GSH). This tripeptide (glutamate, cysteine and glycine) [21][22] is the most abundant non-protein thiol in cells, with concentration reaching up to 15 mM [20]. Most of this glutathione is in a reduced state (about 99%), the remaining 1% being oxidized glutathione (GSSG) [23][24]. The concentration of glutathione is regulated by different processes, such as its own synthesis, its re-oxidation, its use for the detoxification of diverse substances (such as alcohol and drugs), and its transport to the different intracellular and extracellular compartments. [25][26]. Glutathione, through the multiple activities and functions in which it participates (neutralization of free radicals, donor of reducing equivalents, coenzyme, elimination of

xenobiotics and other endogenous metabolites, etc.), is important for cellular homeostasis, since it is involved in the dynamic balance that the organism requires for its proper functioning and morphological integrity [27][28][29].

2. Glutathione Intracellular Compartmentalization

The conservation of hepatic glutathione levels is a dynamic process resulting from the balance between the synthesis rate, transport, use and removal of such thiols [26]. Its synthesis takes place only in the cellular cytosol since all the necessary enzymes for its synthesis are found there [30]. Nevertheless, within the cell, glutathione is compartmentalized into different cell organelles and ratios. A concentration of 1–15 mM is found in the cell cytosol [20][29]. GSH is also present in the endoplasmic reticulum, nuclear matrix and peroxisomes, but at concentrations that need to be determined [27][31].

Mitochondria lack the enzymes needed for GSH biosynthesis, therefore the mitochondrial GSH pool must be imported from the cytoplasm [26]. This tripeptide is mainly found in mitochondria in its reduced form. It represents 10–15% of total cellular GSH, with a concentration range of 5–10 mM [30]. Glutathione cannot freely cross a lipid bilayer because it is negatively charged at physiological pH, so the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM) must be equipped with transporters or channels to facilitate the entry of GSH. The OMM is rich in porins that form aqueous channels through the lipid bilayer and allow diffusion between the intermembrane space (IMS) and the cytosol of molecules smaller than ~5 kDa, including glutathione [26]. Kojer demonstrated that glutathione pools in the IMS and the cytosol are linked by porins [32]. The inner membrane (IMM) is where, in mammalian cells, the dicarboxylate carrier (DIC) and the oxoglutarate carrier (OGC) were described to carry most of the GSH [33]. On the other hand, it has been reported that DIC and OGC together represent only 45–50% of the total glutathione uptake in hepatic mitochondria, so it has been proposed that the glyoxalase system contributes to mitochondrial GSH supply. This metabolic pathway is widespread in all biological systems and is involved in the cellular detoxification of α-ketoldehydes produced during glycolysis; it catalyzes the conversion of 2-oxaldehyde to 2-hydroxyacid, through the intermediate S-2-hydroxyacylglutathione. The glyoxalase system consists of two enzymes, glyoxalase I (Glo I) and glyoxalase II (Glo II) and GSH as a cofactor. In the cytosol, Glo I catalyzes the formation of S-D-Lactoylglutathione (SLG) from hemithioacetal (MeCOCH(OH)-SG) generated from methylglyoxal (MG) and GSH. The SLG can enter the mitochondria and through Glo II is hydrolyzed into D-lactate and GSH; this represents a complementary mechanism for the supply of GSH to the mitochondria [34].

The concentration of GSH present in the mitochondria is kept constant due to the transport of GSH from the cytosol, through two GSH transportation systems, one of high-affinity, stimulated by ATP, and one of low-affinity, stimulated by ATP and ADP [35]. In the case of endoplasmic reticulum, evidence suggests the presence of a transportation system that allows the selective passage of GSH onto GSSG [36]. In this organelle, GSH contributes to the reduction of protein-disulfide isomerase (PDI), responsible for catalyzing the formation of disulfide bonds in proteins [31][36]. The use of GSH to maintain oxidoreductases in their reduced form leads to a constant production of GSSG in the lumen of the endoplasmic reticulum. GSSG is transported to the cytosol with facilitation of diffusion through the Sec61 protein-conducting channel [37], where it is reduced by the enzyme glutathione reductase [30][36][37].

The mechanisms of nuclear glutathione transport and sequestration are under discussion [38]. Certainly, the synthesis of GSH does not take place in the nucleus because, like mitochondria, it lacks the enzymes required for GSH biosynthesis [26]. Bcl-2 proteins possess a BH-3 domain where GSH binds and since its presence seems to be correlated to the increase of the GSH pool in the nucleus, it is possible that Bcl-2 proteins are involved in GSH translocation into the nucleus through Bcl-2 associated athanogene pores (BAG) [38][39][40]. Diaz Vivancos et al. (2010) proposed a model for the glutathione cycle in the nucleus [27]. In this model, GSH is recruited and directed to the nucleus in the early G1 phase of cellular division; thus, GSH increases in the nucleus while cytosolic GSH is depleted. The altered cytosolic redox environment promotes the synthesis of new GSH, whereby the overall glutathione pool significantly increases; the nuclear envelope dissolves so that there is a rebalancing between cytosolic and nuclear GSH during G2 and M phase. During telophase, the nuclear membrane reassembles, the cell divides and the total GSH pool is allocated equally among the daughter cells (**Figure 1**) [27].

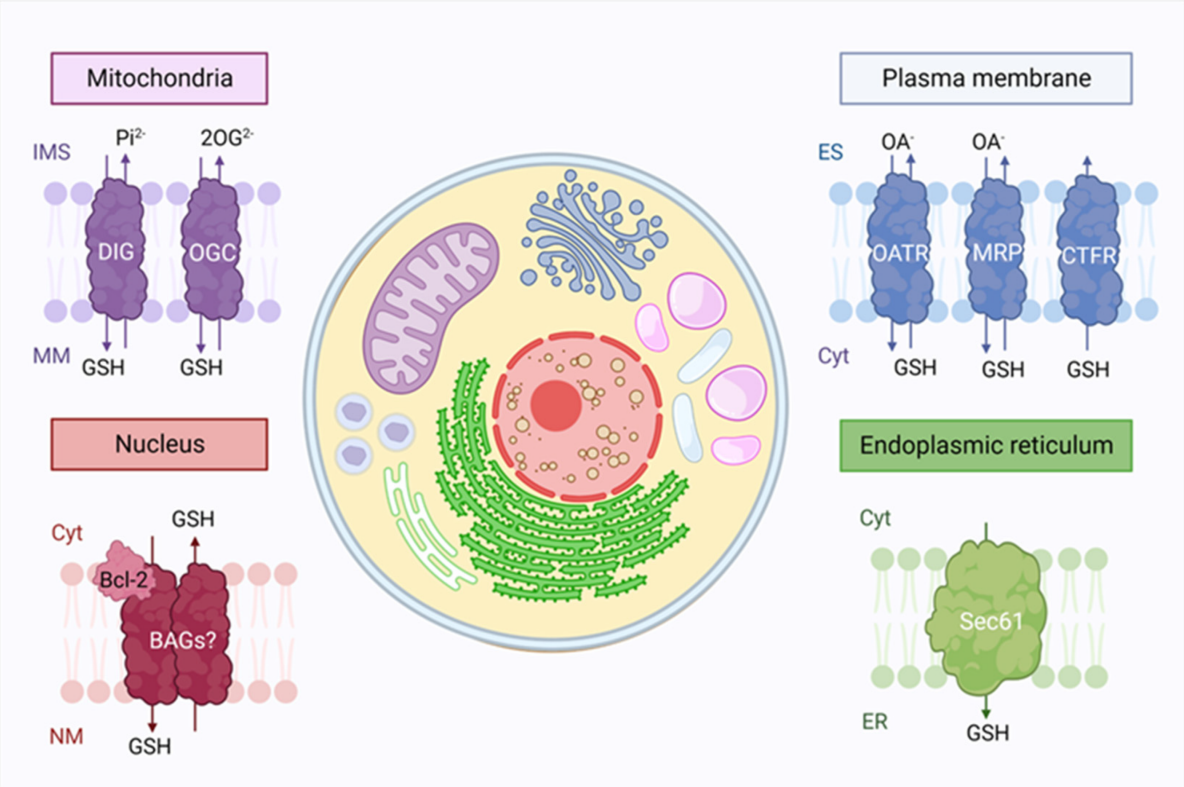

Figure 1. Glutathione intracellular compartmentalization. Glutathione synthesis takes place only in the cytosol (cyt), but it is distributed to many organelles due to the presence of transporters. In mitochondria, the outer membrane contains a large amount of porins, which allow glutathione transport, while dicarboxylate (DIG) and the oxoglutarate (OGC) transporters are present in the inner membrane. In the nucleus, Bcl-2 proteins are believed to be involved in the GSH translocation through Bcl2-associated athanogene pores (BAG). Glutathione is also found in the endoplasmic reticulum (ER), where its facilitated diffusion occurs through the Sec61 protein-conducting channel. Finally, the exchange between extracellular and intracellular glutathione in the plasma membrane occurs through the functioning of three families of transporters: the organic-anion-transporting polypeptide (OATR), the drug resistance-associated proteins (MRP) and cystic fibrosis transmembrane conductance regulator (CTRF). IMS: Intermembrane space, MM: Mitochondrial matrix, NM: Nuclear matrix, ES: Extracellular space.

The redox state of GSH/GSSG in plasma is controlled by multiple processes, including the synthesis of GSH from its constitutive amino acids, cyclic oxidation and reduction involving GSH peroxidase and GSSG reductase, protein S-glutathionylation, transport of GSH into plasma, and degradation of GSH and GSSG by γ-glutamyltranspeptidase [30][41].

GSH is present in all mammalian cells in a constant state of metabolic recirculation (synthesis, degradation, and irreversible loss of GSH). Its half-life is 4 days in human erythrocytes, 2 to 4 h in the cytosol of rat hepatic cells and 30 h in the mitochondrial lumen [42]. Many different conditions affect the intracellular GSH contents, some of them being the presence of heavy metals, high glucose concentrations, heat shock, exposure to reactive oxygen and nitrogen species including H_2O_2 and nitric oxide, ozone exposure, ionizing radiation, cigarette smoke [25][43][44][45]. Differences between GSH content in some mammalian cells are listed in **Table 1**.

Table 1. Glutathione distribution and homeostasis in different cell types.

Cell Type	GSH Cytosolic Concentration	GSH Homeostasis	References
Astrocytes	8–10 mM	Generate GSH conjugates exported from the cells by MRPs. Protect brain cells from ROS and xenobiotics	[46][47]
Neurons	0.2–2 mM	Lack of cystine transportation system, synthesis depends on cystine uptake via the cystine/glutamate exchange transporter	[48][49]
Hepatocytes	5–10 mM	Synthesis of GSH protects against oxidative stress, about 10% of total cytosolic GSH is transported to mitochondria	[50][51][52][53]
Erythrocytes	2.3–3 mM	Its levels are influenced by the environment. In addition, erythrocytes have the enzymatic machinery for the synthesis of GSH and the release of its derivates	[54][55][56]
Pneumocyte	400 µM in epithelial lining fluid	GSH protects lungs against oxidative damage. Type II pneumocytes contain more γ-glutamyl transferase than type I	[57][58][59]
Cardiomyocyte	2 mM	The insulin-signaling cascade regulates GSH concentration in ventricular myocytes by PI 3-kinase and MAP kinase pathways for controlling redox state	[60][61]

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