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Glutathione synthesis and its role in redox signaling

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Abstract

Glutathione (GSH) is the most abundant antioxidant and a major detoxification agent in cells. It is synthesized through two-enzyme reaction catalyzed by glutamate cysteine ligase and glutathione synthetase, and its level is well regulated in response to redox change. Accumulating evidence suggests that GSH may play important roles in cell signaling. This review will focus on the biosynthesis of GSH, the reaction of S-glutathionylation (the conjugation of GSH with thiol residue on proteins), GSNO, and their roles in redox signaling.

Keywords

glutathione; redox signaling; glutathionylation; GSNO; nitrosylation

Introduction

Several reactive species derived from oxygen and nitrogen are produced in cells from a variety sources such as NADPH oxidases (NOX) [1], leaks from the mitochondrial electron transport chain [2], redox cycling of quinones [3], and nitric oxide synthases (NOS) [4]. The principal reactive species produced enzymatically are superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and nitric oxide ($^{\bullet}NO$). These can be involved in cell injury but are also the principal actors in cell signaling, particularly the latter two [5–7]. Other species derived from these, hydroxyl radical ($^{\bullet}OH$) and peroxynitrous acid (ONOOH), are extremely potent oxidants and are more likely involved in cell injury than signaling. Environmental exposure adds additional reactive species such as ozone and nitrogen dioxide [8]. Together these species are often referred to as reactive oxygen (ROS) and nitrogen species [9], terms we will use sparingly as they are not helpful in understanding mechanism. Indeed, an argument has been made for considering only H_2O_2 as the actual species involved among the ROS while $^{\bullet}NO$ and nitrosoglutathione (GSNO), a conjugate of $^{\bullet}NO$ and glutathione (GSH) that is produced by an as yet not definitively demonstrated oxidative mechanism, are responsible for ROS and RNS signaling. Finally, other reactive species, lipid hydroperoxides (ROOH) and α, β-unsaturated aldehydes, that are derived from lipid peroxidation can also participate in both injury and cell signaling [7].

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As mentioned, $O_2^{\bullet -}$, H_2O_2, and $^{\bullet}NO$ can contribute to pathology as well as signaling. Therefore, it is necessary for cells to maintain a narrow rate of production of these reactive species that is not directly harmful yet effective in mediating diverse physiologic functions through signaling. Our focus here will be on the involvement of H_2O_2 and GSNO in signaling. The propensity of H_2O_2 to modify protein function is attributed to its ability to react with particular cysteine residues within proteins [10]. The possible oxidative modification of cysteine residues within proteins includes sulfenic acid (RSOH), intra- or intermolecular disulfide (RSSR), and S-glutathionylation (RSSG), sulfinic acid (RS(=O)OH) and sulfonic acid ($RS(=O)_2OH$) acids, thiyl radicals (RS•), sulfenyl-amides, thiosulfinates, disulfide-S-monoxides; however, physiological signaling probably only involves the first three. Formation of the other species would influence signaling by producing species that are far more difficult to reduce back to a thiol than RSOH, RSSR and RSSG but are also less likely to form at low H_2O_2 concentration. Formation of RSOH, RSSG and RSSG can be transient and reversible, allowing these species to participate in biochemical functions, such as redox sensing and responding, catalysis, and signal transduction [11]. This redox-modification-based mode of signal transduction is called redox signaling. Only some select cysteine residues are involved in reactions with H_2O_2 or ROOH as even the more nucleophilic thiolate ($-S^-$) form is not strong enough to act in the absence of a nearby proton donor or metal to remove the OH^- that would be the leaving group if a thiolate alone reacted with ROOH or H_2O_2. This complex chemistry has been recently reviewed [5].

The concentration of GSH, γ-L-glutamyl-L-cysteinyl-glycine, which is the most abundant non-protein thiol in cells, is in the range of 1–10 mM in most mammalian cells [12]. GSH is also the most abundant antioxidant and a major detoxification agent in cells. Enzymes such as glutathione peroxidases (GPx) and one of the peroxiredoxins (Prdx VI) catalyze the reduction of H_2O_2 (or ROOH) by GSH into H_2O (or the corresponding alcohol (ROH) and GSSG. GSSG is reduced back to GSH by glutathione reductase using NADPH to maintain a steady state GSH/GSSG ratio that is almost all GSH but varies with cell type and disease state [13]. GSH also conjugates with electrophiles and thus participates in the metabolism and detoxification of endogenous compounds and xenobiotic toxicants [14–16]. In addition, GSH is involved in many other metabolic reactions [9, 17, 18]. Therefore, it is not surprising that GSH plays roles in various cellular processes such as cell growth, proliferation, and apoptosis. Thus, it is clear that GSH is also involved in cell signaling through which it affects these important cell functions.

GSH participates in cell signaling through at least two mechanisms, protein S-glutathionylation and cysteine S-nitrosylation through thiol exchange with GSNO. The former is formed when GSH conjugates with reactive cysteine residues within proteins to form protein mixed disulfides (PSSG), and the latter is formed by reaction of a thiolate ($-S^-$) with GSNO. Emerging evidence suggest that both are controlled redox signaling mechanisms. How GSNO is formed has been the subject of intense investigation and several mechanisms have been proposed [6, 19]. Of course, GSH may also indirectly participate in the redox signaling by changing cellular redox homeostasis. The rest of this review will focus on the participation of protein S-glutathionylation and GSNO in signaling and on how cells regulate GSH homeostasis.

Regulation of glutathione content

In most cells, GSH is synthesized *de novo* through a two-step reaction. First γ-glutamylcysteine is formed from glutamate and cysteine catalyzed by glutamate cysteine ligase (GCL). Then glycine is added by glutathione synthetase to form GSH. GCL activity and cysteine availability are two rate-limiting factors in GSH synthesis. It is apparently so

important for cells to maintain redox homeostasis and normal cellular function that both its concentration and GSH/GSSG ratio are tightly regulated. Some authors insist on expressing this as 2GSH/GSSG or as the redox potential ($-RT \ln [GSH]^2/[GSSG]$ where R is the universal gas constant and T is the temperature on the Kelvin scale [13]. First, GSSG is quickly reduced back by GSH reductase or exported to the outside of cells. Second, the import and export of cysteine and export of GSSG are also regulated to maintain the GSH/GSSG ratio. Third, cells can up-regulate the expression of γ-glutamyl transpeptidase (GGT) [20], an enzyme on the outer surface of cells that transfers the glutamate from GSH to an acceptor amino acid. GGT prefers to transfer the glutamate to cystine, the product (γ-glutamylcystine) is transported back into cells where it is rapidly reduced providing both γ-glutamylcysteine and cysteine in what is called the scavenger pathway [21, 22]. More significantly however, the activity of GCL, the rate-limiting enzyme for GSH synthesis, is regulated by GSH through a negative feedback loop [23], by phosphorylation [24], and by protein expression. In response to oxidative stress or other situations when more GSH is required, the expression of GCL is regulated through two redox sensitive signaling pathways comprised of Nrf2-EpRE system and AP-1 [25, 26]. There is more debate regarding a role of a third redox sensitive system, NF-κB [27].

Regulation of GCL expression

The regulation of both subunits of GCL, the catalytic subunit (GCLC) and the modifier subunit (GCLM), has been extensively studied in the past 20 years. A variety of signaling pathways, such as ERK1/2 [28], JNK1/2 [29], p38MAPK [30], PKC [31], and PI3K [32, 33], and transcription factors, such as c-Jun [29], NF-κB [27], JunD [34], and Nrf2 [25], etc., have been found to be involved in the regulation of GCLC and GCLM genes. The most significant and intriguing finding though, is that both genes are regulated via a redox mechanism involving Nrf2-EpRE pathway.

NF-E2 related factor 2 (Nrf2) is a protein that is usually sequestered in the cytosol via associating with Keap1, which is tethered to β-actin. Under unstressed conditions, Nrf2 is rapidly turned over in the cytosol via ubiquitin-dependent degradation mediated by Keap1 [35–37]. Upon exposure to electrophiles or other mediators, Nrf2 dissociates from Keap1 and escapes ubiquitination/degradation [38, 39]. Nrf2 is then translocated to the nucleus, forms heterodimers with other transcription factors such as c-Jun and small Maf proteins (G/F/K), binds to the electrophile response element (EpRE) (also known as the antioxidant response element) in the promoters of GCLC and GCLM, and regulates gene transcription. Studies have demonstrated that Nrf2-EpRE pathway is involved in both the basal and inducible expression of GCL genes [25]. The Nrf2-EpRE pathway is regulated at several levels. 1) The availability of free Nrf2 protein. This is mainly regulated by the interaction of Nrf2 with Keap1 [35–37]. Recently it was found that c-Myc could also decrease Nrf2 half-life via an unidentified mechanism [40]. In addition, it was reported that phosphorylation of Nrf2 by GSK-3β pathway could lead to Nrf2 nuclear transportation and degradation [41–43]. Nrf2 phosphorylation by GSK-3β is also found to lead to Nrf2 degradation through a Keap1-independent manner [42, 44]. 2) Nrf2 partners. Several nuclear proteins are found to be Nrf2 heterodimerization partners, such as c-Jun and small Maf proteins (Maf G/F/K). Our work [45] and that of Jaiswal group [46] suggest that c-Jun is the predominant activating partner and that the small Mafs as well as c-Myc are inactivators of transcription when bound to Nrf2 [40, 47]. How the amount and/or activity of these proteins are regulated under various conditions though, needs to be further determined. 3) Other binding proteins in the Nrf2-EpRE complex. Studies have demonstrated that in addition to Nrf2 and its dimerization partners c-Jun, small Mafs and c-Myc, many other proteins exist in the EpRE complex, including JunD, JunB, Nrf1 [48], Bach1[49], Nrf3 [50], ATF4 [51], CBP/P300 [52], etc. Among them, Bach1 is consistently recognized as a competitor of Nrf2 while the role of

other proteins in Nrf2-EpRE activity though remains obscure and needs further determined. 4) Phosphorylation of Nrf2 and its associated proteins. Although the exact mechanism of how signaling kinases, such as MAPK, PKC, and PI3K, regulate the gene expression of GCLC and GCLM remains unclear, it is generally thought that these kinases act through direct phosphorylation of Nrf2, or other proteins involved in Nrf2-EpRE complex formation.

Another important oxidant responsive cis element regulating GCL genes is the AP-1 binding site (also called the TRE element). There are several TREs in the human GCLC and GCLM promoters. In fact, a TRE is embedded in the EpRE consensus sequence. Studies have shown that TREs are involved in the basal and inducible expression of GCL genes [53]. The main transcription factors binding to TRE are members of the Jun and Fos family [54, 55]. Studies suggest that c-Jun phosphorylation is required for TRE-mediated GCL induction, since inhibiting JNK, which phosphorylate c-Jun, abolished GCL induction and c-Jun binding to TRE [56]. A recent study shows that inactivation of PTP1B may also be involved in the activation of JNK/c-Jun pathway [57].

Protein S-glutathionylation

In a recent review [58], several reaction mechanisms of protein S-glutathionylation (PSSG) formation were proposed. We have added to and modified these in accord with the chemistry of cysteine oxidation in signaling proteins that was discussed in our recent review [5] (Figure 1). Proton catalyzed nucleophilic substitution: To break the O-O bond in ROOH bond (where R is either a H or a lipid) and form a protein-cysteine sulfenic acid and ROH, there must be two conditions met; (a) the cysteine must be in the nucleophilic thiolate form [59] to donate electrons to the O-O bond in ROOH and (b) a hydrogen donor must be close enough to protonate the very poor leaving group RO^- to form ROH, a good leaving group. The sulfenic acid, which is formed would presumably dissociate to the sulfenate (RSO^-) as the pKa of sulfenic acid is lower than the corresponding thiol and the proton would go back to the hydrogen donor. The RSO^- would then react with GSH to form PSSG and return the proton to the proton donor. Metal catalyzed nucleophilic substitution: Here, instead of a proton donor, a metal, which could be Zn^{2+}, binds to the cysteine sulfur giving it a negative charge so it is equivalent to a thiolate. Alternatively, the metal may be in proximity but not bound to a thiolate. When the ROOH is given electron by the thiolate, the RO^- then binds to the metal. ROH is then released by reaction of the metal-OR with water. As in the proton-catalyzed mechanism, RSO^- then reacts with GSH in the second step. Recently it was demonstrated that the sulfenic acid moiety in peroxiredoxin 6 was S-glutathionylated via glutathione S-transferase Pi (GSTP), indicating that a non-enzymatic reaction between sulfenic acid and GSH did not occur [60, 61]; however, in other proteins evidence suggests that non-enzymatic reaction occurs and that RSO^- is a much stronger nucleophile than is RS^- [62]. Other proposals from the Pimental *et al.* review, which we consider less likely under physiological conditions include: PSSG formation from reaction of a PS^- and GS-sulfenic acid (GSO^-); PSSG formation from reaction of PS^- and GSSG via a thiol transferase activity if GSSG concentration is high enough; PSSG formation from condensation of protein sulfenic acid and GSH disulfide S-oxide (GS(O)SG), a dimer of GS(OH) the formation of which is catalyzed by metals [63, 64]; PSSG formation from reaction of GSH and a cyclic sulfenylamide bond, which is generated from reaction of a protein sulfenate and a protein amide under oxidative stress. An example model of this reaction can be demonstrated with PTP1B *in vitro*, although its physiological significance has been questioned due to the reaction being too slow to occur under physiological conditions. Thus, protein S-glutathionylation can be formed through multiple possible reactions. To determine the exact mechanism is experimentally challenging and probably actually varies among target proteins. In keeping with the general rules of physiological signaling in which regulation is an essential requirement in contrast with oxidative stress,

one would expect that glutathionylation associated with signaling is often catalyzed by enzymes that use the target protein cysteine as a substrate replacing one of the GSH in the glutathione peroxidase reaction. Indeed, while there are already some examples in the literature [65], the identification of some of these enzymes is expected in the near future.

S-glutathionylation of cysteine residues on protein needs to be reversed to maintain homeostasis. Accumulating evidence suggests this is mainly catalyzed by a thiol-disulfide oxidoreductase called glutaredoxin (Grx) [66, 67]. There are two isoforms of Grx in mammalian cells: Grx1 in the cytoplasm, and Grx 2 in mitochondria [68]. Evidence for the importance of deglutathionylation by Grx1 has been accumulating [69–71]. In overt oxidative stress however, Grx1 may also promote PSSG formation via a mechanism involving a thiyl radical [72]; however, this is unlikely to be part of signaling. In addition to Grx1, other proteins such as sulfiredoxin (Srx) are also reported to deglutathionylate some specific PSSGs [73, 74]. Thus, as with their formation, many aspects of the exact molecular mechanisms of deglutathionylation remain elusive.

Many proteins have been found to be targets of S-glutathionylation in response to various stimuli [75]. As PSSG may evoke specific changes in the structure and function of target proteins, it can affect many cellular signaling pathways. Table 1 lists some identified signaling proteins that are affected by S-glutathionylation. These targeted signaling molecules are either part of a signaling cascade, or transcription factor, or an enzyme that further generates signaling mediators (e.g. eNOS). Of course, S-glutathionylation of other proteins, such as proteins involved in electron transfer chain, may also indirectly affect signaling pathways by increasing or decreasing H_2O_2 and $O_2^{\bullet -}$ generation from mitochondria [76].

Through regulating the function of signaling molecules (Table 1), protein S-glutathionylation may play important roles in various cell processes and diseases, such as apoptosis, inflammation, mitochondrial function, cancers, and neurodegenerative diseases. As examples of how S-glutathionylation is involved in regulating cell signaling and function, we will now discuss its involvement in inflammation and its regulation of peroxiredoxin I (Prdx I).

The NF-κB pathway controls expression of a variety of genes involved in inflammation. NF-κB is a transcription factor that is normally sequestered in cytosol by IκB. Inflammatory signals activate IκB kinase (IKK), which then phosphorylates IκB leading to IκB degradation and NF-κB activation. Studies have demonstrated that the most common NF-κB form, the heterodimer of p50 and p65, along with IKK-β are targets of S-glutathionylation upon stimulation [69, 77, 78]. S-glutathionylation of p50 at Cys-62 inhibits DNA binding of NF-κB and production of cytokines [77]. In addition, S-glutathionylation of IKK-β at Cys-179 causes inactivation and subsequent attenuation of LPS-induced NF-κB activation and cytokine induction [78]. Other proteins involved in inflammation are also targets for S-glutathionylation. S-glutathionylation of tumor necrosis factors receptor-associated factor 6 (TRAF6) inhibits its function and attenuates NF-κB activation [79]. Although it is generally accepted that oxidative stress can activate NF-κB, it is found that oxidative stress may also decrease the DNA binding activity of NF-κB [80]. Whether S-glutathionylation of p50 and p65 and its inhibitory effect on NF-κB activity is involved in the differences in NF-κB regulation under oxidative stress remains to be determined.

Peroxiredoxin I (Prdx I) is the most abundant and ubiquitously expressed 2-Cys Prdx, a family of peroxidases that are capable of reducing H_2O_2, ROOH and peroxynitrite [81]. Prdx I normally exists in the equilibrium between homodimers and decamers, depending on the oxidation status of the conserved N-terminal low pKa Cys (peroxidatic Cys) in Prdx I

[82]. Under reducing condition, the peroxidatic Cys exists as a thiolate anion and Prdx I forms homodimers predominantly. During reduction of H_2O_2 or ROOH, the peroxidatic Cys is oxidized to sulfenic acid, which will cause formation of intermolecular disulfide bond and Prdx I oligomers. If the sulfenic acid derivative is further oxidized to form sulfinic acid, the Prdx I will form decamers and lose its peroxidases activity. Cells have evolved mechanisms to maintain the structural equilibrium of Prdx I under oxidative stress, these include the reduction of intermolecular disulfide bond by Trx system [83] and reduction of the peroxidatic Cys from sulfinic status back to sulfenic acid by sulfiredoxin (Srx) [84]. Recent studies suggest that glutathionylation is another important mechanism to regulate Prdx structure and function under oxidative stress. Proteomic analysis shows that Prdx I undergoes glutathionylation when cells are treated with low concentration of H_2O_2 [74, 85]. *In vitro* assays found that Prdx1 is glutathionylated at Cys 52, 83, and 173 [74]. Using sedimentation velocity methods, Park *et al* found that glutathionylation of Prdx I could efficiently disrupt the Prdx I oligomerization and shift the equilibrium toward Prdx I dimers [86]. Further study revealed that glutathionylation of single cysteine (Cys 83) was sufficient to convert Prdx I from a decamer to dimer structure. Nevertheless, the significance of glutathionylation of Prdx I remain elusive as it is a less-studied pathophysiologic function of Prdx I. According to a study by Morinaka et al [87], oligomeric structure is essential for Prdx I to bind and activate mammalian ste20-like kinase-1 (MST1) kinase, which should subsequently activate the P53 pathway and cause apoptosis. Therefore, it seems that Cys 83 glutathionylation induced by a low concentration of H_2O_2 would facilitate Prdx I dimer structure and inhibit apoptosis while higher range of H_2O_2 would cause Prdx I oligomerization and induce apoptosis. Cao *et al* also reported that Prdx I could bind and protect phosphatase (PTEN) from oxidation-induced inactivation and thus inhibit cancer cell growth [88], indicating that Prdx I glutathionylation may protect PTEN from inactivation and thus inhibit cell growth.

It should be noted that S-glutathionylation might affect protein function (activity) in diverse manners. For example, S-glutathionylation alters eNOS function and causes it to produce superoxide instead of $^{\bullet}NO$ [89]. In addition, the degree of PSSG formation can also alter protein activities just as do differences in the degree of phosphorylation. For example, low-level S-glutathionylation of ANT increased translocation of ADP/ATP, while higher-level S-glutathionylation of ANT increased ADP/ATP translocation [90]. One must be cautious to separate what happens under physiological redox signaling versus oxidative stress and further what degree of oxidative stress realistically can occur *in vivo* [91].

GSNO: reaction and regulation

$^{\bullet}NO$ is involved in a wide range of biochemical reactions and cell signaling pathways through protein modification. Although $^{\bullet}NO$ can diffuse freely, it is readily oxidized and this limits its function as a second messenger. Formation of S-nitrosothiols (−SNO) from $^{\bullet}NO$ and cysteine can protect $^{\bullet}NO$ from oxidative consumption and thereby extend $^{\bullet}NO$ bioavailability, both temporally and spatially. Protein S-nitrosylation can alter protein function and mediate signaling events and it has been considered as a non-classical mechanism of $^{\bullet}NO$ signaling [92], compared to the classical mechanism. In the classical mechanism $^{\bullet}NO$ activates soluble guanylate cyclase (sGC) by binding to its heme and leading to activation of the cGMP/PKG pathway [93]. Small mass SNOs (products of $^{\bullet}NO$ and cysteine or GSH), are relatively stable and resistant to oxidation and can act as $^{\bullet}NO$ carriers to allow $^{\bullet}NO$ signaling to remote places from $^{\bullet}NO$ synthetases.

The formation of GSNO from $^{\bullet}NO$ or peroxynitrite ($^{-}ONOOH$) and GSH occurs in cells but requires an oxidation step; however, it remains unclear which of several potentially physiologically relevant reactions is responsible [7, 94–96]. GSNO is an intermediate

in •NO metabolism and plays a critical role in fulfilling •NO biofunction and cell signaling either as an •NO donor or through mediating protein S-glutathionylation [97]. The latter however also requires either a reaction of glutathione radical (GS•) with •NO or some other oxidative mechanism. After intracellular formation, GSNO could form GSH and another S-nitrosothiol through transnitrosation, be reduced by GSNO reductase to release •NO and mediate •NO signaling locally [98, 99], or be exported out of the cells via a GSH transporter. Extracellular GSNO can be decreased by a transnitrosation reaction with cystine [100] or by GGT to generate S-nitrosocysteinylglycine that can be cleaved by a membrane dipeptidase to generate S-nitrosocysteine. S-nitrosocysteine can be up taken directly by cells through L-type amino-acid transporter (L-AT) [101]. It appears that S-nitrosocysteinylglycine cannot enter cells directly, but may transfer its •NO to cysteine or cystine through transnitrosation [102, 103]. Upon entering cells, S-nitrosocysteine can be reduced to release •NO or transfer •NO to proteins and thus mediate •NO signaling.

There is no supportive evidence available that extracellular GSNO can enter cells directly. The system composed of GSNO reductase, GGT, dipeptidase, GSNO exporters and importer L-AT, along with cysteine and cystine, play an important role in GSNO metabolism and in regulating its local concentration. It is reported that the extracellular concentration of GSNO in normal brain tissue is 6–8 μM while undetectable in cytosolic extracts of brain tissue [104]. This heterogeneously distribution of GSNO suggests that its metabolism is tightly regulated.

Future direction

After decades of studies, it has been well established that redox-dependent reversible oxidative modification of proteins can occur under various pathophysiologic conditions, and is an important cell-signaling mode analogous to phosphorylation-based cell signaling mechanism. As the most abundant non-protein antioxidant in the cells, GSH is critical to maintain redox homeostasis and thus indirectly involved in redox signaling. Accumulating evidence suggest that GSH may directly participate in signaling process through protein S-glutathionylation and/or GSNO-mediated protein S-nitrosylation. Both S-glutathionylation and S-nitrosylation are reverse post-translational modifications and underlie the functional change of many proteins under various redox conditions. Further research will be needed to elucidate the biochemistry of protein S-glutathionylation, including the contexts, the compartmentalization, catalyzing enzymes, and the selectivity of target proteins and cysteine residues. How Grx1 is involved in deglutathionylation is also required for further study. Additional investigation into the interplay between S-glutathionylation and S-nitrosylation of proteins such as the selectivity of both modifications under conditions with concurrent oxidative and nitrosative stress is also critically needed. Regarding to GSNO, the regulation of GSNO metabolism needs to be elucidated, which include its synthesis, transportation, degradation, and regulating enzymes under various conditions. The elucidation of how protein S-glutathionylation and GSNO are involved in various physiological and pathological processes, remains as a challenging focus of future studies.

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Abbreviations list

ANT	adenine nucleotide translocator
AP-1	activator protein 1

ATF4	activating transcription factor 4
CBP	CREB binding protein
EpRE	electrophile response element
ERK	extracellular signal regulated protein kinase
GSK-3β	glycogen synthase kinase 3β
Grx	glutaredoxin
GSH	glutathione
GSSG	glutathione dioxide
GSNO	nitrosoglutathione
GPx	glutathione peroxidases
GCL	glutamate cysteine ligase
GGT	γ-glutamyl transpeptidase
GCLC	catalytic subunit of GCL
GCLM	modifier subunit of GCL
H_2O_2	hydrogen peroxide
JNK	c-Jun N-terminal kinase
MST1	kinase mammalian ste20-like kinase-1
$^{\bullet}NO$	nitric oxide
NOS	nitric oxide synthases
NOX	NADPH oxidase
Nrf2	nuclear factor erythroid 2-related factor 2
NF-κB	nuclear factor κB
$O_2^{\bullet-}$	superoxide
$^{\bullet}OH$	hydroxyl radical
ONOOH	peroxynitrous acid
OH	sulfinic acid
Prdx	peroxiredoxin
PKC	protein kinase C
p38MAPK	p38 mitogen-activated protein kinase
PSSG	protein mixed disulfides
PI3K	phosphoinositide-3 kinase
PTEN	Phosphatase and tensin homolog
RSOH	sulfenic acid
RSSR	intra- or intermolecular disulfide
RSSG	S-glutathionylation
RS(	$O)_2OH$, sulfonic acid

RS•	thiyl radicals
Srx	sulfiredoxin
-SNO	S-nitrosothiols
sGC	guanylate cyclase
TRE	TBP response element
TRAF6	tumor necrosis factors receptor-associated factor 6

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Highlights

- Glutathione (GSH) is the most abundant antioxidant and a major detoxification agent in cells.
- Glutamate cysteine ligase, which catalyzes the first step in GSH synthetase is regulated by redox signaling.
- Accumulating evidence suggests that GSH plays important roles in cell signaling.
- Protein S-glutathionylation and S-nitrosylation play major roles in redox signaling.

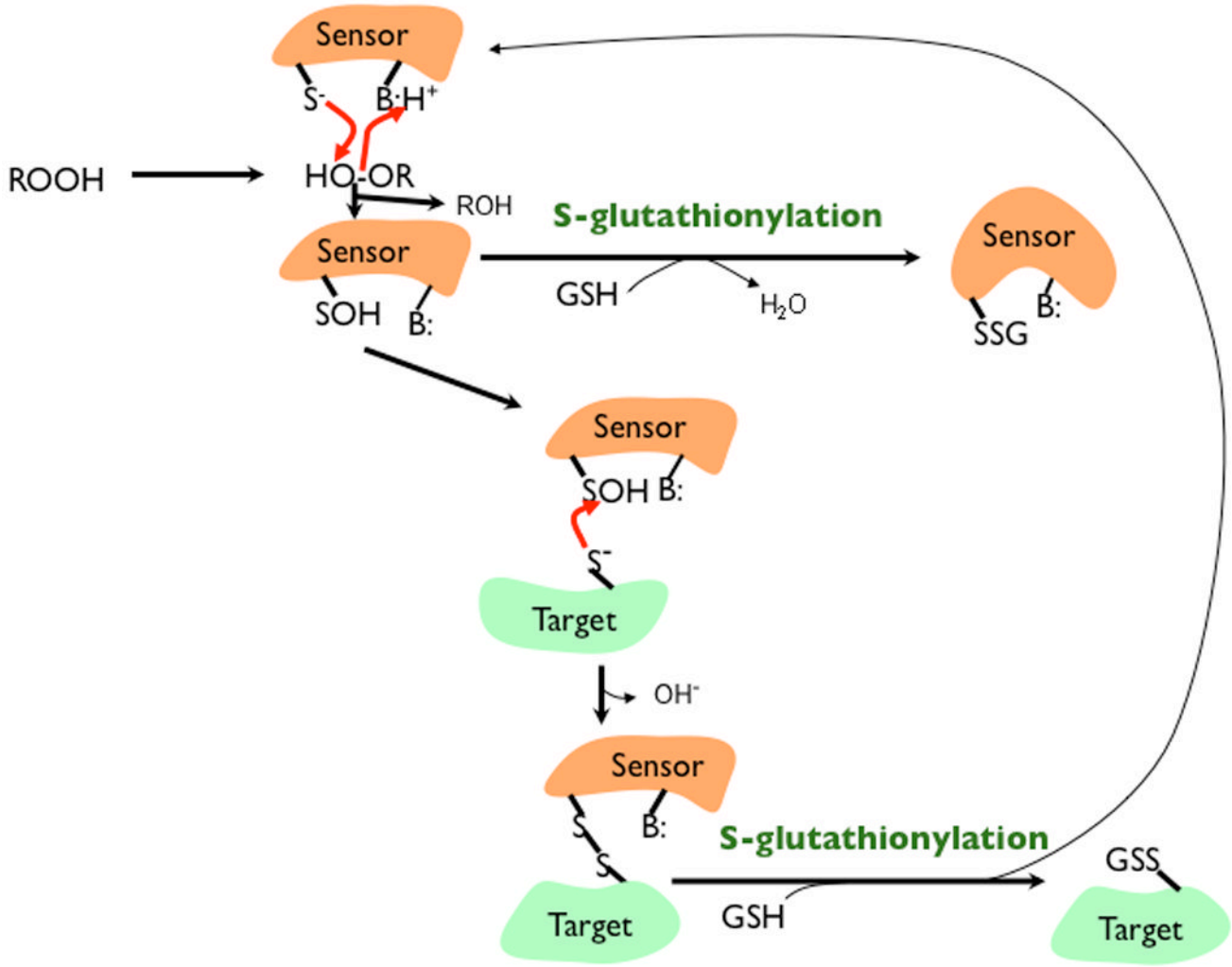

Figure 1.

Glutathionylation in signaling. Some proteins, including peroxiredoxins, may be either sensors or direct targets for glutathionylation. When acting as a direct target, glutathionylation would occur on a protein thiolate (S^-) after it has been oxidized by a hydroperoxide (ROOH) to a sulfenic acid (SOH) with the assistance of a proton donating amino acid (BH^+) that allows ROH to leave. GSH can then react with the SOH; however, either the SOH or GSH would need to be in the anionic form to be a reasonably good nucleophile in that reaction. When acting as a sensor that then assists in glutathionylation, the formation of a disulfide with a target thiolate is a reasonable possibility. This would be followed by disulfide exchange where again the GSH would need assistance from a base (B:) to remove its proton forming GS^-.

Table

Target proteins	Effect on activity	Signaling pathways	References
Ryanodine receptors	increase	Ca^{2+}	[105] [106]
eNOS	inhibition	NO signaling	[89]
Na^{+}-K^{+} ATPase	inhibition	voltage dependend signaling	[107]
p21Ras	increase	p21Ras-MEK-ERK	Clavereul et al [108]
p50	inhibition	NF-κB signaling	[77]
RelA/p65	inhibition	NF-κB signaling	[72]
IKKβ	inhibition	NF-κB signaling	[78]
TRAF 6	inhibition	NF-κB signaling	[79]
c-Jun	inhibition	AP-1 signaling	[109]
GAPDH	inhibition	Ca^{2+}signaling	[110]
p53	inhibition	p53 signaling	[111]
Adenosine transporter (ANT)	varies	mitochondrial function	[90]
Ubiquitin ligases E 1,E2	inhibition	protein degradation	[112]
Rpn2 (regulator of 26 proteosome)	inhibition	protein degradation	[113]
Fas	increase	apoptosis	[114]
caspase 3	inhibition	apoptosis	[115]
PTP1B	inhibition	various signaling pathways	[116]