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Redox modifications of protein–thiols: Emerging roles in cell signaling

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Abbreviations:
AP-1, activator protein-1
ASK-1, apoptosis signaling kinase-1
GSNO, S-nitrosoglutathione
GAPDH, glyceraldehyde-3-P-dehydrogenase
H_2O_2, hydrogen peroxide
$OH^{\bullet}$, hydroxyl radical
$NO^{\bullet}$, nitric oxide radical
NF-κB, nuclear factor-kappa B
$ONOO^{-}$, peroxynitrite
PKC, protein kinase C
PrSH, protein–Cys–SH
PTP-1B, protein-tyrosine phosphatases 1B
$O_2^{\bullet-}$, superoxide radical

ABSTRACT

Glutathione represents the major low molecular weight antioxidant redox recycling thiol in mammalian cells and plays a central role in the cellular defence against oxidative damage. Classically glutathione has been known to provide the cell with a reducing environment in addition to maintaining the proteins in a reduced state. Emerging evidences suggest that the glutathione redox status may entail dynamic regulation of protein function by reversible disulfide bond formation. The formation of inter- and intramolecular disulfides as well as mixed disulfides between protein cysteines and glutathione, i.e., S-glutathiolation, has now been associated with the stabilization of extracellular proteins, protection of proteins against irreversible oxidation of critical cysteine residues, and regulation of enzyme functions and transcription. Regulation of DNA binding of redox-dependent transcription factors such as nuclear factor-κB, p53, and activator protein-1, has been suggested as one of the mechanisms by which cells may transduce oxidative stress redox signaling into an inducible expression of a wide variety of genes implicated in cellular changes such as proliferation, differentiation, and apoptosis. However, the molecular mechanisms linking the glutathione cellular redox state to a reversible oxidation of various signaling proteins are still poorly understood. This commentary discusses the emerging concept of protein-S-thiolation, protein-S-nitrosation and protein–SH (formation of sulfenic, sulfinic and sulfonic acids) in redox signaling during normal physiology and under oxidative stress in controlling the cellular processes.

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1. Introduction

Akin to protein phosphorylation, redox regulation is rapidly evolving as an important metabolic modulator of cellular functions of all living cells and organisms and is being increasingly implicated in many chronic inflammatory and degenerative diseases. Redox alterations of several protein and enzymes have been identified in a wide variety of conditions and the purpose of such regulation has been speculated in the cellular defense mechanism during oxidative stress [1–4]. Protein-S-thiolation and protein-S-nitrosation have emerged to be important mechanisms by which a cell's redox system may be involved in controlling cellular signaling process.

Many proteins and enzymes in a cell consist of cysteine residues which have a sulfhydryl group in their side chain and the proton is labile which makes it a chemical hot spot for a wide variety of biochemical interactions. Such a thiol group provides: sites for formation of co-ordinate bonds to metal ions, site for electrophilic reactions, and an easily reversible site for oxidation/reduction chemistry. Furthermore intracellular proteins contain zinc and iron binding sites co-ordinated by the cysteine sulfur group. Such sites are prominent in DNA binding proteins where structures that interact with DNA are frequently dependent on zinc binding sites. Since oxidized cysteines may be only transiently permitted in the reduced environment inside cells, the complex formed with structural zinc is a very feasible alternative to disulfide protein cross-links. In proteins containing iron–sulfur centres, the participating cysteines probably are quite buried but in the case of aconitase, it has been observed that enough surface exposure exists to permit oxidative modification of the site [5]. The nucleophilic role of Cys–SH in the active sites cysteines for both transfer/addition reactions are well documented for enzymes such as S-transferases, glyceraldehyde 3-P-dehydrogenase, glutathione reductase, thioredoxin/glutaredoxin, and in peptidases such as papain, caspase and calpain. These cysteines may be among the most acidic (lowest pK_a) since the nucleophilicity is enhanced by ionization of the thiol to the thiolate anion, which usually depends on the charge and electron withdrawing properties of the surrounding protein structures [6].

Covalent modifications of proteins at their cysteines may be critical to a particular function of a given protein. When the cysteine–SH is linked by a thioester bond, it may be transitory and have transient regulatory effects on the protein function. ADP ribose is attached to a protein cysteine by S-glycoside bond and this addition is also known to be reversible. Thioether linkages i.e., prenyl anchored protoporphyrin rings, however, are not known to be reversible. On the other hand if farnesyl or geranyl isoprenoid is attached to the C-terminal cysteine, it is thought to bring about membrane association of a protein. More functions of such reactive modifications of cysteines may be further elucidated by new experimental approaches to the study of protein function. The protein cysteine thiols involved in each of the modifications described above, may also react with a host of other reactive oxygen species that are the subject of this commentary.

2. Components of redox regulation

A cell may be challenged with oxidative stress due to generation of several types of oxidants which can be classified as (a) reactive oxygen species such as superoxide radical ($O_2^{\bullet -}$), peroxyl radicals, hypohalite radicals, hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$) and (b) reactive nitrogen species such as nitric oxide radical ($NO^{\bullet}$), peroxynitrite radicals ($ONOO^-$), and a host of other organic species modified by reactive oxygen species and reactive nitrogen species. These reactive oxidants are generated as a result of a cell's normal physiology or due to aging or local or systemic stress [7]. However, cells have developed immaculate line of defenses (which may vary from cell to cell) to nullify the ill-effects of these oxidant species by virtue of the battery of antioxidants they possess (Fig. 1).

The antioxidants are chiefly glutathione (GSH) [8–10], a major redox recycler and ubiquitously distributed molecule, Vitamins C and E, β-carotene, proteins such as albumin to name a few. The activity of antioxidant enzymes like, Cu–Zn or Mn superoxide dismutases, catalase, peroxidases, thioredoxins, glutaredoxins and peroxiredoxins can be regulated according to the need of the cell and the type of oxidant to be dealt with. Although free radicals have been traditionally considered as toxic outcomes of a cell, more recent studies have put forth the notion that these toxic species may actually be generated by a cell on purpose and that these species may be involved in certain key signaling steps in a cell.

2.1. *Protein–thiol modification by S-thiolation and S-nitrosation*

Protein sulfhydryls may be oxidatively modified by S-thiolation and S-nitrosylation, which may significantly modify various cellular processes. Both S-thiolation and S-nitrosation are usually reversible and may be a protective/adaptive strategy of a cell (Fig. 2). Since several proteins in various signaling and metabolic pathways are susceptible to oxidative alterations, the combined effects of oxidation of such proteins may consequently invoke cellular changes that may be necessary for survival during short term oxidative or nitrosative stress. It is therefore necessary to understand these processes on a global metabolic basis in order to fully

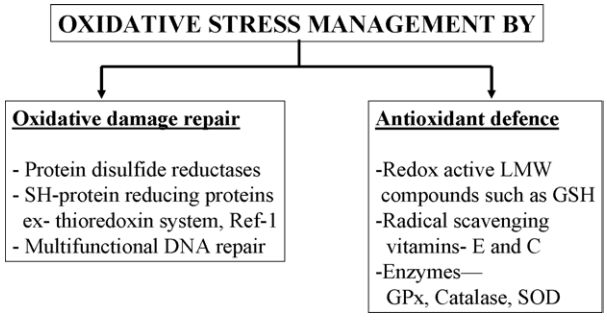

Fig. 1 – Oxidative stress management by a cell. Oxidants may be countered by the cells either by direct scavenging of cellular antioxidants or by repair of the damaged molecules during oxidative stress.

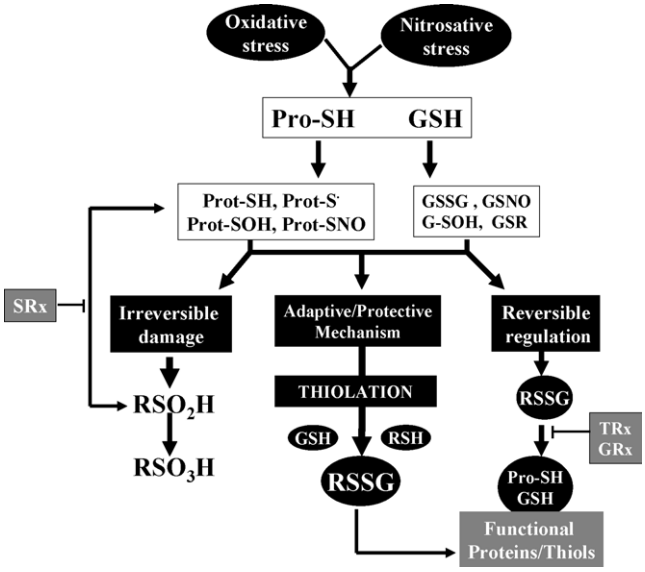

Fig. 2 – Protein-S-thiolation or S-nitrosation although two different processes share common mechanisms. While the reversibly damaged proteins are retrieved by thioredoxins (TRx) and glutaredoxins (GRx), the irreversibly modified molecules are now known to be recovered through a sulfiredoxin (SRx) pathway. Alternatively the proteins may form mixed disulfides (RSSG) and then reduced back to their active forms. Activation of various pathways is shown by forward or downward arrows whereas inhibition of pathways by vertical line.

appreciate the effects of oxidative protein modifications. The exposure of cysteines on the protein surface is a functional necessity to prevent redox changes to spread through the entire protein molecule. The surface oriented cysteine residues are normally kept reduced and may therefore serve as 'redox sensors' of the cells. These residues are associated with their roles in catalysis, regulation, electron transfer, and protein structure and function.

2.1.1. *Protein–thiol alterations due to S-thiolation*

Less than 1% of the total protein is S-thiolated in resting cells. A substantial degree of oxidative stress may therefore be required to alter the resting state redox dynamics as evidenced in studies with leukocytes [11] or monocyte [12]. These studies have revealed that S-thiolation of proteins occurs within minutes after oxidative burst generation. S-thiolation is now believed to take place via two mechanisms: (a) Glutathione disulfide (GSSG) concentration dependent protein disulfide exchange has long been considered to be preponderant mechanism for oxidation [4,5]. The mechanism however, seems unlikely as the cells normally do not attain the required GSSG levels and also due to slow thiol-disulfide exchange reaction rate. (b) GSH-dependent trapping mechanism has been mooted wherein protein–SH are oxidized to a thiyl radical or sulfenic acid and may serve as a primary mechanism for S-thiolation:

$$\text{protein}-\text{S}^- + \text{ROO}^- \rightarrow \text{protein}-\text{S}^\bullet + \text{ROO}^-$$

$$\text{protein}-\text{SH} + \text{R}^\bullet/{}^\bullet\text{OH} \rightarrow \text{protein}-\text{S}^\bullet + \text{RH}/\text{H}_2\text{O}$$

$$\text{protein}-\text{SH} + \text{H}_2\text{O}_2 \rightarrow \text{protein}-\text{SOH} + \text{H}_2\text{O}$$

These activated protein intermediates may then produce a mixed disulfide adduct in conjunction with cellular GSH. Such a mechanism therefore depends on the supply of GSH to effectively neutralize partially oxidized protein cysteines (thiyl radical or sulfenic acid forms). Conversely, in the absence of sufficient glutathione, partially oxidized protein cysteines may react with oxygen or other oxidants to produce irreversibly oxidized sulfinic and sulfonic acid species:

$$\text{protein}-\text{S} + \text{O}_2 \rightarrow \text{protein}-\text{SO}_2\text{H}/\text{protein}\,\text{SO}_3\text{H}$$

The above mechanism might fail if the GSH pool is depleted significantly. Therefore extremely high concentrations of GSH in almost every cell might be the cells', evolutionary weapon to counter oxidative protein damage. Although researchers favour that GSH is relatively more reactive towards oxidants than proteins, the opposite however, appears to gain more grounds. Since most protein S-thiolation studies have been performed with purified proteins, the numerous factors under in situ conditions cannot be ignored for proper interpretation of the obtained results. The S-thiolated proteins represent an inactive state and care should therefore be taken while investigating the effects of antioxidants on a certain metabolic/signaling pathway, as S-thiolated proteins may not respond in an oxidative environment, thus leading to erroneous conclusions.

2.1.2. *Protein–thiol alterations due to S-nitrosylation*

Protein–thiol modification by nitrogen-based reactive species is a less understood phenomenon and appears to have some similarity with S-thiolation process (Fig. 2). Understanding of the two forms of protein–thiol alterations relies much on the chemistry of low molecular weight thiols, such as GSH. Since GSH appears to be a common denominator, the complex chemistry of protein S-thiolation and S-nitrosation with their potential modification abilities may seem to be similar. However, this logic does not always provide explanations for observations that nitrosoglutathione (GSNO) may S-thiolate some cysteines while S-nitrosylate others [13]. In addition, it has been reported that some cysteines resist S-glutathiolation due to charge interactions, and that protein S-nitrosylation requires high GSH concentrations in intact cells add to the complexities of the two processes [14]. The mechanism of protein–NO adduct formation may involve several fundamentally different mechanisms parallel to those proposed for protein S-thiolation. It has been postulated that transnitrosation of proteins by low molecular weight S-nitrosothiols may be a probable mechanism for protein S-nitrosylation:

$$\text{protein}-\text{SH} + \text{RSNO} \rightarrow \text{protein}-\text{SNO} + \text{RSH}$$

The reaction is quite similar to thiol/disulfide exchange and probably is significant at high cellular concentrations of S-nitrosoglutathione. However, transnitrosation could only be significant in causing S-nitrosylation if the concentrations of S-nitrosothiols such as S-nitrosocysteine or S-GSNO are in the millimolar range or greater. Thus, both transnitrosylation and

S-thiolation may be limited by low concentration of available low molecular weight reactants in a cell. GSSG normally does not reach very high concentrations in cells due to redox recycling by glutathione disulfide reductase system and also due to limited uptake of disulfides produced in the extracellular spaces by the cells. In contrast however, S-nitrosothiols are readily taken up across the cell membranes, apparently by transport processes that have not been completely elucidated [14]. Thus, both transnitrosylation and S-thiolation may suffer from the same limitation, of very low concentration of available low molecular weight reactants in a cell. It is however, speculated that S-nitrosylation and S-thiolation may occur at much lower concentrations of GSNO compared to required GSSG concentrations for significant S-thiolation to take place [15]. Transnitrosation offers a likely mechanism for redistribution of S-nitrosothiols among protein–thiols and for denitrosation of protein S-nitrosothiols. Another mechanism in line with that proposed for S-thiolation requires formation of either a reactive protein intermediate or some reactive low molecular weight species other than S-nitrosothiol:

$$\text{protein}-\text{SH} + \text{ONOO}^- \rightarrow \text{protein}-\text{S}^\bullet + \text{HNO}_3$$

$$\text{protein}-\text{S}^\bullet + \text{NO}^\bullet \rightarrow \text{protein}-\text{SNO}$$

$$4\text{NO}^\bullet + \text{O}_2 \rightarrow 2\text{N}_2\text{O}_3$$

$$\text{N}_2\text{O}_3 + \text{protein}-\text{SH} \rightarrow \text{HNO}_2 + \text{protein}-\text{SNO}$$

Since nitrative stress is co-localized in an environment of oxidative stress and may cause oxidative events, the mechanisms may play a critical role in modification of protein–thiols.

3. Thiol modified proteins

A large number of proteins which frequently undergo thiol modifications have now been identified and are important components of cellular housekeeping or are involved in key signaling cascades. The major proteins of the cells that are thiolated during oxidative stress are: actin, creatine kinase, carbonic anhydrase III, glyceraldehyde-3-P-dehydrogenase (GAPDH), glycogen phosphorylase, microsomal glutathione S-transferase (mGST) and hemoglobin.

Actin is a widely distributed cytoskeletal protein having a single reactive cysteine moiety. S-glutathiolation, S-nitrosylation and S-ADP-ribosylation have been reported to alter the functional characteristics of the protein. Since actin is found to shuttle between monomeric and polymeric forms in cells, it has been suggested that oxidative modification of the cysteine may have regulatory consequences on the polymerization/depolymerization process [16].

Creatine kinase is abundant in muscles having one reactive cysteine per subunit. Although the cysteine does not take part in catalysis, it is however, required for the enzyme activity. Oxidative modification of the reactive cysteine is known to completely inhibit enzyme activity. S-thiolation, S-nitrosylation, dethiolation, and irreversible oxidation have been widely explored using this enzyme as a model. Recently, it has been demonstrated that the acidic cysteine moiety of creatine kinase reacts with S-nitroso glutathione to form S-glutathiolated creatine kinase and this reaction is preferred over the formation of the S-nitrosylated form [13]. At decreased GSH levels, irreversibly oxidized sulfinic and sulfonic species of creatine kinase are formed.

Carbonic anhydrase III is one of many isoforms of carbonic anhydrase and is abundantly expressed in some cells. It possesses two reactive cysteines and recently the structure of S-glutathiolated form of this protein was established [17]. Recent evidence suggests that expression of carbonic anhydrase III may be related to oxidative stress. It has been suggested that S-glutathiolated carbonic anhydrase III may be dethiolated by glutaredoxin quite efficiently [18]. GAPDH is an abundant protein in many cells and therefore has been the subject of study for oxidative modification. It has a reactive cysteine that is directly involved in the catalytic mechanism and any modification of this cysteine renders the enzyme completely inactive. Since S-glutathiolation of the protein was also reported in endothelial cells, it was suggested that GAPDH may be S-glutathiolated even in NO-treated cells. Glycogen phosphorylase has two reactive cysteines per subunit. Modification of glycogen phosphorylase-cysteines did not cause any apparent change in the activity of the enzyme. However, the protein's affinity for glycogen particle was reduced in the oxidized state. Microsomal glutathione S-transferase (microsomal GST) is closely associated with a number of membranes. It is the only protein known to be activated by S-glutathiolation [19].

Hemoglobin has at least two reactive cysteines per tetramer from several eukaryotic sources including man [20]. Hemoglobin is susceptible to attack by oxidative mechanisms that result in formation of thiyl radicals. S-glutathiolation or S-nitrosylation of hemoglobin in vitro, has been implicated in transport of protein-bound NO. Thiolated Hb has been clearly demonstrated by electron spin traps [21]. The abundance of hemoglobin in red blood cells therefore makes it an easy target for a wide variety of oxidative modifications and probably serves as a buffer against free radical attack.

Less abundant proteins like protein phosphatases (PTPs), protein kinases, NF-κB and c-Jun/AP-1, p53 and H-ras which are involved in cellular signaling are also S-modified. The effect of oxidative and nitrative stress on protein phosphorylation may occur by oxidative modification of either protein kinases, protein phosphatases, or the phosphoprotein substrate for this modification. Since these proteins are key signal transducing agents, oxidative modifications of such proteins may thus be crucial to a cell's function and survival.

NF-κB and c-Jun/AP-1 are crucial components of redox signaling [1]. In a study involving c-Jun protein, it was recently reported that S-glutathiolation of c-Jun/AP-1 may be an important aspect of this process [22]. S-glutathiolation of c-Jun in presence of an NO donor and GSH, inhibited the DNA binding activity of the protein heterodimer. This was an important finding as to the complex nature of NO as a modifying agent since S-glutathiolation was promoted by the NO donor. More in depth investigations are further warranted to understand the factors regulating such oxidative diversions.

Recent work using pyrrolidine dithiocarbamate has revealed that oxidative modification of p53 can lead to altered

expression of various p53-related gene products [23]. Oxidation of p53 correlated well with a decrease in downstream effector genes of p53 and altered subcellular localization of the protein. The oxidative alterations of p53 were found to be reversed by ref-1 and thioredoxin, thus implying that p53 expression and function may be regulated by the redox status of the cell [24]. Targeted oxidation studies of p53 show that at least one cysteine moiety is modified but, the specific site of oxidation is still to be determined.

4. Protein–thiol modification and cellular consequences

Modification due to thiolation, nitrosation usually being reversible processes in intact cells, generates interest as possible modes of regulatory events that may potentially modify a large number of processes in a cell. To fully comprehend the importance of oxidative protein modifications it is necessary to understand these processes at a global level. Irreversible oxidation of proteins is less appreciated as an important biological event as little data exist as to what extent they occur in vivo. Thus, the rate of degradation and turnover of irreversibly oxidized proteins is largely speculative at present, and accumulation of proteins containing damaged sulfhydryls is an event that has only recently been documented in normal and aged tissue samples. In the ensuing sections various cellular implications of protein–thiol modifications have been brought together to gather the scattered information on a common platform. This is specially intended to provide a holistic view of the overall metabolic alterations due to protein–thiol modifications.

4.1. *Protein–thiol alterations: a novel redox signaling mechanism and adaptive stress response*

4.1.1. *The protein–thiol oxidation*

The first evidence that cells may resist oxidative stress via protein thiolation was provided by Dominici and his coworkers [25]. In an elegant study, they have evidenced S-glutathiolation (addition of GSH to a cysteine–SH of proteins) of γ-glutamyl transpeptidase (γ-GT) on the surface of monocytic U937 lymphoma cells in response to oxidative stress. Initially, S-glutathiolation dependent loss of activity was observed for the enzyme wherein the free surface thiols of the enzyme were S-glutathiolated and may be correlated with a concurrent generation of H_2O_2 which is a by-product of γ-glutamyl transpeptidase-(γ-GT)-function. Oxidation of protein–Cys–SH (PrSH) may interfere with biological functions either as 'damage' or in context to oxidant-dependent signal transduction. Although PrSH behave like non-protein–thiols, their biochemistry is much complicated due to their accessibility, steric interference and charge distribution [26]. The response of PrSH and their reaction mechanisms vary depending upon the source of the PrSH and are influenced by the existing pK_a, disulfide susceptibility/accessibility to oxidants and the conformation of the protein in a given environment. It is to be noted that $NO^{\bullet}$ being scavenged with a variety of reactive oxygen species to form a range of reactive nitrogen species, may lead to enhanced nitration of protein-tyrosine in the lungs and hence may play a pivotal role in airway inflammation [27]. Initially oxidative thiolation of PrSH was recognized as an aftermath of oxidative stress but recent evidences suggest that such transformations may be of greater biochemical consequence, both as a protective and a signaling mechanism. For example, protein-tyrosine phosphatases are inactivated by S-nitrosylation reaction [28]. Although the major mediator of protein thiolation is the thiol antioxidant GSH (which promotes S-glutathiolation), its catabolite dependent metal reduction have recently been identified to act as pro-oxidant capable of modulating redox balance, signal transduction pathways and transcription factors [29]. It was also shown that the antioxidant effects of GSH, N-acetyl-L-cysteine and N-acystelyn have played a role in controlling NF-κB activation or chromatin remodelling through histone deacetylase activity [30].

4.1.2. *Cysteine sulfoxidation as protein function modulator*

Cysteine thiolates (Cys–S^-) but not cysteine thiol (Cys–SH) can be readily oxidized to a sulfenic acid, which is relatively reactive and can quickly form a disulfide with a nearby thiol (Fig. 3). Strong oxidants will oxidize either Cys–S^- or Cys–SH to sulfinic (Cys–SO_2H) and/or sulfonic (Cys–SO_3H) acid derivatives [31]. This difference in the generation of a particular cysteine thiol species provides a basis for distinguishing redox signaling from oxidative stress. While oxidative stress generally involves non-specific oxidation of wide variety of molecules, redox signaling is gradually being recognized to involve oxidation of those cysteines which are located in an environment promoting dissociation of thiols. The higher oxidation states in the form of sulfinic and sulfonic derivatives have essentially been considered as irreversible modifications under biologically relevant conditions and associated with oxidative injury. On the other hand protein–cysteine–sulfenic acid is unstable and may be further oxidized to sulfinic or sulfonic species or scavenged by GSH or vicinal thiols to form intramolecular disulfide or mixed disulfides (Fig. 4). Therefore, it is evident that cysteine may be recycled between a reduced

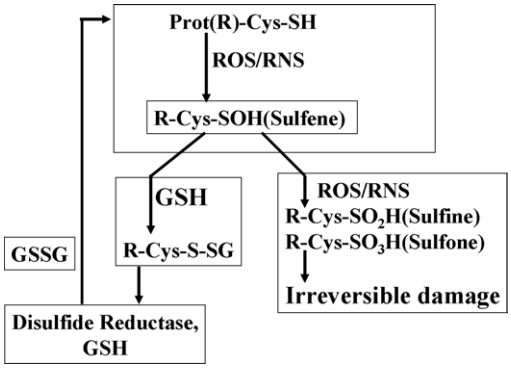

Fig. 3 – Protein cysteines are vulnerable to oxidative modifications. Depending upon the extent of oxidative stress, a cysteine may either form a –sulfene (–SOH), –sulfine ($-SO_2H$) or a –sulfone ($-SO_3H$). While the –sulfene derivative is reversible, the later two derivatives are irreversibly damaged. Recently sulfiredoxin has been implicated in the retrieval of –sulfine derivatives of proteins.

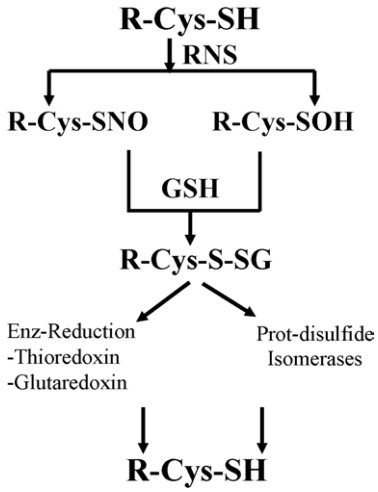

Fig. 4 – Mixed disulfides formation. Mixed disulfides are commonly formed as a result of a reaction with an oxidized protein–Cys–SH and glutathione (GSH). The mixed disulfides are then isomerized by specific isomerases or reduced by thioredoxin (Trx) or glutathione reductase (GRx) to their active reduced forms.

(Cys–SH) and its oxidized forms (sulfinate, sulfinic or sulfonic derivatives). This transition between the reduced and oxidized forms may by itself represent a regulatory mechanism of protein function in a cell adapting or defending against oxidative stress.

Most noteworthy examples of such a regulation are the reactive oxygen species dependent sulfinic acid formation dependent inhibition of PTP-1B and modulation of insulin receptor kinase activity [32]. Recently GSH reductase, cathepsin K, GSNO and other NO donors and GAPDH have been identified as potential inducers of sulfinate species formation. Very recently, Poole et al. have considered the idea that Cys–sulfenic acid might have an important role in the catalytic centres of the respective enzymes [33]. These workers suggested that Cys–sulfenates might be useful as sensors of both oxidative and nitrosative stress which affect enzymes and transcriptional regulators. Since the formation of sulfenic, sulfinic or sulfonic species depend upon the degree of oxidative stress, the presence and stoichiometry of these species may yield useful information regarding the exact status of the prevailing oxidative stress. Much work is needed to emphatically establish protein function modulation via cysteine-sulfoxidation pathways and their relevance in cellular homeostasis, for which new methodologies and detection/analysis techniques have to be developed.

5. Protein function modulation by disulfide/mixed disulfide formation: role of peroxiredoxin, sulfiredoxin, glutaredoxin and thioredoxin

5.1. *Peroxiredoxins*

These are a group of non-seleno-peroxidases which catalyze the breakdown of acid and hydrogen peroxide. Mammalian cells have six isoforms of this family, which are classified on the number and position of cysteine (Table 1) [34]. It was found by Chang et al. that peroxidredoxins inactivation may facilitate H_2O_2 signaling while its reverse activation by sulfiredoxin may add a new dimension in the regulation of such a signaling [35]. In human lungs peroxiredoxins have played a pivotal protective role in exogenous and endogenous attack by oxidants. It has been shown previously by Lehtonen et al. that the augmentation of peroxiredoxins occurs in lung cancer cells [36]. In eukaryotic cells, peroxiredoxins not only act as antioxidants against H_2O_2 and $ONOO^-$, but also influence processes such as apoptosis, cell-differentiation and proliferation. Evidence has been accumulating that eukaryotic peroxiredoxins act as regulators of H_2O_2-mediated cell signaling which is implicated in cancer and neurodegenerative diseases [37].

5.2. *Sulfiredoxin*

The concept that sulfinic and sulfonic acid derivatives of protein–cysteines are irreversibly damaged has been contradicted by the work of Biteau et al. who have recently reported the presence of an enzyme capable of reducing sulfinic acid derivatives [38]. These workers detected an enzyme in yeast that can reduce the sulfinic derivative of yeast peroxiredoxins and termed it as sulfiredoxin. Biteau et al. further proposed that sulfiredoxin may catalyze a multistep reduction process via its intrinsic phosphotransferase and thioltransferase activity. Sulfiredoxin may apparently overcome the energy barrier that normally prevents the reduction of protein–Cys–SO_2H by a transient introduction of a phosphate group in the peroxiredoxin-sulfinate moiety to make sulfinic phosphorylester in the presence of ATP and Mg^{2+}. A thiolsulfinate disulfide is formed with another sulfiredoxin molecule,

Table 1 – Various isoforms of peroxiredoxins and their localization in the lung

Type	MW (kDa)	Location
Peroxiredoxin I	22	Predominantly in bronchial epithelial and alveolar macrophage cells
Peroxiredoxin II	22	Less in alveolar epithelial and vascular endothelial cells
Peroxiredoxin III	28	Predominantly in lungs especially in bronchial epithelium and alveolar macrophage cells
Peroxiredoxin IV	31	Scarce in lung cells
Peroxiredoxin V	17	Predominantly in lungs especially in bronchial epithelium and alveolar macrophage cells
Peroxiredoxin VI	25	Predominantly in lungs especially in bronchial epithelium and alveolar macrophage cells

Six isoforms of peroxiredoxins (I–VI) have been characterized in human lungs. They have a diverse relative abundance in different areas of lungs. The molecular weights are in the range of 17–31 kDa.

followed by replacement of the phosphate group to form reduced and stabler forms of the enzyme. The thiolsulfinate can also be reduced to peroxiredoxin-sulfenate and a sulfiredoxin-disulfide by reducing agents such as dithiothreitol or thioredoxin. Cysteine in its thiolate form could also participate in thiol-disulfide exchange provided that there is no interference by steric hindrance:

$$R_1S^- + R_2SSR_3 \leftrightarrow R_1SSR_3 + R_2S^-$$

5.3. *Glutaredoxin*

Exchange between thiols and disulfides are very slow and must be catalyzed by enzymes such as glutaredoxin or other protein disulfide isomerases that have a thioredoxin-like structural motif. Such disulfide exchange is a potential signaling mechanism due to its capacity for modifying cysteine residues in enzymes. Glutaredoxin have profound antioxidant capacity and is abundantly present in lungs [39]. Glutaredoxin catalyze the reduction of protein disulfide to their respective sulfhydryls by donating reducing equivalents to the oxidized proteins. The oxidized glutaredoxin in turn gets reduced by transfer of reducing equivalents from GSH as shown below:

$$\text{protein}-\text{S}-\text{S}-\text{protein} + 2\,\text{glutaredoxin SH}_{(\text{red})} \rightarrow 2\,\text{protein}-\text{SH} + 2\,\text{glutaredoxin S}_{(\text{oxd})}$$

$$2\,\text{glutaredoxin S}_{(\text{oxd})} + 2\,\text{GSH} \rightarrow 2\,\text{glutaredoxin SH}_{(\text{red})} + \text{GSSG}$$

Mitochondrial glutaredoxin 2 is a small redox protein and exhibit a typical thioredoxin-fold, and Cys–X–X–Cys as active site motif [40]. The N-terminal of the mitochondrial glutaredoxin 2 contains a mitochondrial localization signal, which is cleaved off after transport into the mitochondria. Human glutaredoxin 2 has been found to be expressed in several tissues including heart, skeletal muscle, kidney, lung, and liver [41,42]. The other human glutaredoxin, glutaredoxin 1 is localized in cytosol, has an additional cysteine and influences apoptosis signaling by binding to apoptosis-signaling kinase-1 (ASK-1) [43]. Absence of the additional cysteine in glutaredoxin 2 may be associated with negative regulation of ASK-1. The fact that glutaredoxin 2 contains Cys–Ser–Tyr–Cys, and glutaredoxin 1 a Cys–Pro–Tyr–Cys classical active site motif, imply differences in their function and localization. Glutaredoxin 2 has a greater specific reactivity towards glutathionylated proteins, which may influence mitochondrial reactive oxygen species production as well as cellular reactive oxygen species scavenging ability. A recent study by Beer et al. [44], demonstrated a glutaredoxin 2 dependent catalysis of glutathionylation/deglutathiolation of mitochondrial proteins at a wide range of GSSG/GSH levels. Previous studies have revealed that mitochondria are more efficient in maintaining its GSH level during oxidative stress than the cytosol, where GSH is more easily depleted [45]. Recently glutaredoxin 2 overexpression, especially mitochondrial glutaredoxin 2 but not truncated glutaredoxin 2, in 2-deoxy-D-glucose and doxorubicin induced apoptosis was found to exert pronounced protective effect on mitochondria mediated apoptosis [46]. The anti-apoptotic effect of glutaredoxin 2 was attributed to the prevention of oxidation of cardiolipin, cytochrome c release, and caspase activation, the key features during the process of apoptosis. It was proposed that glutaredoxin 2 may facilitate maintenance of cellular/mitochondrial redox homeostasis, thereby preventing oxidation of cardiolipin and cytochrome c release.

5.4. *Thioredoxin*

The redox status of proteins outside and within the cells is controlled by different mechanism. Proteins present on the extracellular face of a cell are stabilized by disulfide bonds (S–S) between two protein molecules and the proteins within the cells are redox stabilized by free sulfhydryl moieties. Regulation of redox states of proteins is carried out by a special class of proteins known as thioredoxins, which evolved as chaperones (MW 10–12 kDa) [47]. Thioredoxin are dithiol $[(SH)_2]$–disulfide oxidoreductases and catalyze reduction of disulfide to their corresponding sulfhydryls. The thioredoxin system comprises of thioredoxin and thioredoxin reductase and need NADPH for their function. Mammalian thioredoxin are seleno-enzymes that reduce oxidized-thioredoxin and other protein disulfides. Thioredoxin of lower and higher animals are quite different, mammalian thioredoxins being closely related to the enzyme glutathione reductase.

The antioxidant activity of all forms of thioredoxin can be directly attributed to their thiol groups at the active site. These activities are most likely by the virtue of thioredoxin acting as a cofactor for the peroxiredoxins [48]. Thioredoxin-1 may also function as a cofactor in secondary antioxidant repair systems along with GSH and glutaredoxin. However, evidences have emerged wherein thioredoxin has been reported to introduce disulfides into proteins during oxidative stress [49]. At the present juncture this later mechanism does not seem to be physiologically significant since such a reaction would possibly be inhibited by the presence of another dithiol motif at Cys-62 and Cys-69 of the thioredoxin-1. In a study by Meuillet et al. it was shown that thioredoxin-1 inhibits its PtdIns-3-phosphatase activity by binding in a redox sequence to PTEN [50]. Thioredoxins are involved in a wide variety of cellular phenomena such as cell proliferation, reduction of ribonucleotide reductase, thioredoxin peroxidase, thiol-dithiol exchange between cysteine residues of key transcription factors and protection against exogenous oxidants [51] (Fig. 5).

5.4.1. *Thioredoxin and transcription factors*

Many transcription factors have critical thiol moieties and are known to be regulated, at least in part, by the thioredoxin system and has been discussed in detail elsewhere [52]. It has been shown that GSH levels play an important part in the regulation of transcription factors leading to proinflammatory and antioxidant gene transcription [53]. Of particular note are p53, NF-κB, AP-1, Nrf2, each of which is modulated by its thiols and has been implicated in cell proliferation and apoptosis (Fig. 5). For instance, NF-κB must be reduced in order to bind to DNA at the nuclear level. Thus, the redox status of specific subcellular sites is crucial for determining the activation state of NF-κB [54]. Although GSH was considered necessary for NF-κB reduction, it now appears that thioredoxin-1 is the proximate factor for such a reduction to take place [55]. In

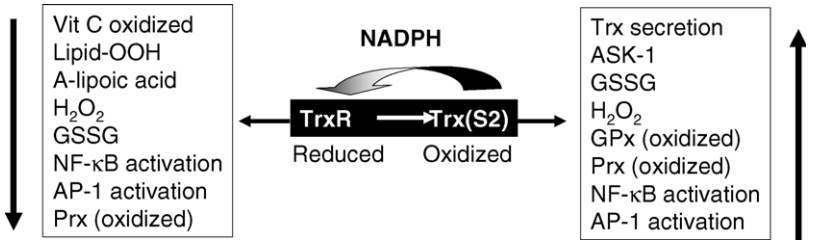

Fig. 5 – Involvement of thioredoxin reductase (TrxR) in oxidative stress management and transcription. Oxidation of TrxR will increase production of reactive oxygen species (ROS), GSSG level, secretion of thioredoxin (Trx), expression of NF-κB dependent protein, expression of apoptosis signaling kinase (ASK-1) and activate the oxidation of glutathione peroxidase (GPx) resulting in oxidative stress mediated damage. Reduced TrxR on the other hand will decrease the levels of these harmful factors.

light of the knowledge that numerous xenobiotics alter transcription factors such as NF-κB and AP-1, and considering the myriad effects of these transcription factors on cellular growth and death, thioredoxin-1 may most likely exert at least some of its effects through actions on redox-regulated transcription factors. Suggestions have recently been forwarded that thioredoxin-1 may also activate NF-κB by affecting the degradation of IκB mediated through the JNK-signaling. A link between NF-κB and JNK-signaling was suggested, based on the findings that overexpression of MEKK1 (a MAPKKK upstream of JNK) was involved in NF-κB activation [56]. Several antioxidant and xenobiotic sensitive genes involved in cytoprotection contain within their promoter an antioxidant response element (ARE). Transcriptional regulation of these genes are effected via Nrf2 binding to the ARE sequences, which form a heterodimer with small MafK proteins [57]. Under non-oxidative states Nrf2 is normally located in the cytosol through its association with Keap1. During oxidative stress, cysteine residues within Keap1 are oxidized, dissociating Nrf2 from Keap1. This allows nuclear translocation of Nrf2 where it binds to genes containing ARE elements [58] (Fig. 6). It is interesting to note that although oxidative stress situations of the cytosol promote Nrf2 activation, oxidizing conditions in the nucleus inhibit Nrf2 binding to the ARE. Therefore location of the oxidative stress by itself may play a regulatory role in deciding the ultimate response by the cell.

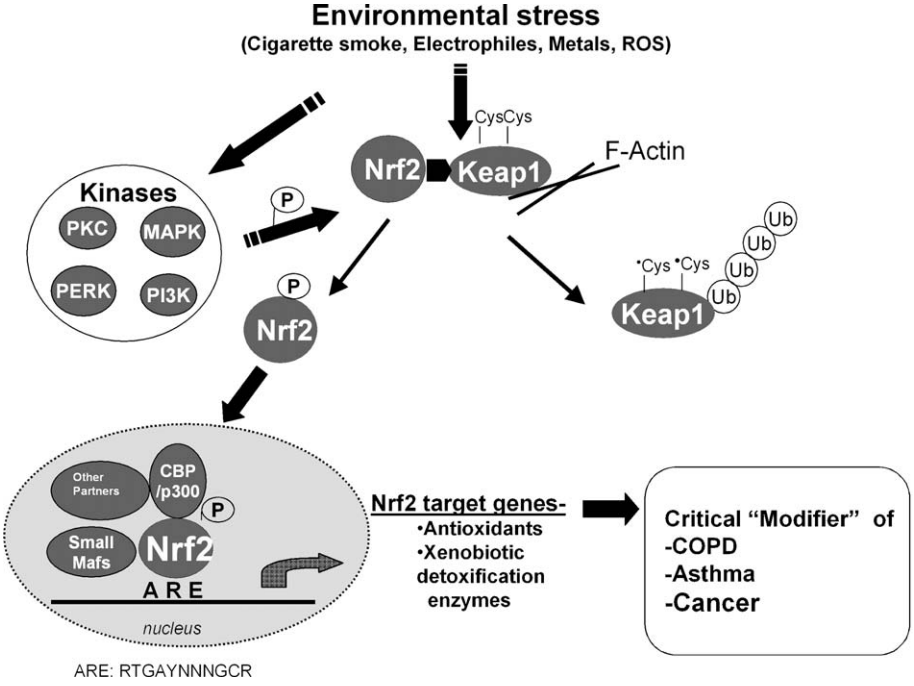

Fig. 6 – Tentative model for redox-mediated activation of Nrf2 leading to induction of phase II genes (Courtesy by Dr. Shyam Biswal from Johns Hopkins University). Oxidants and electrophiles oxidize thiol groups present on cytoplasmic Kelch-like ECH-associated protein (Keap1) which allows Nrf2 to become separated and translocated into the nucleus. Nrf2 forms heterodimers with small Maf proteins that bind to the antioxidant response element (ARE) regions of phase II genes and promote transcription.

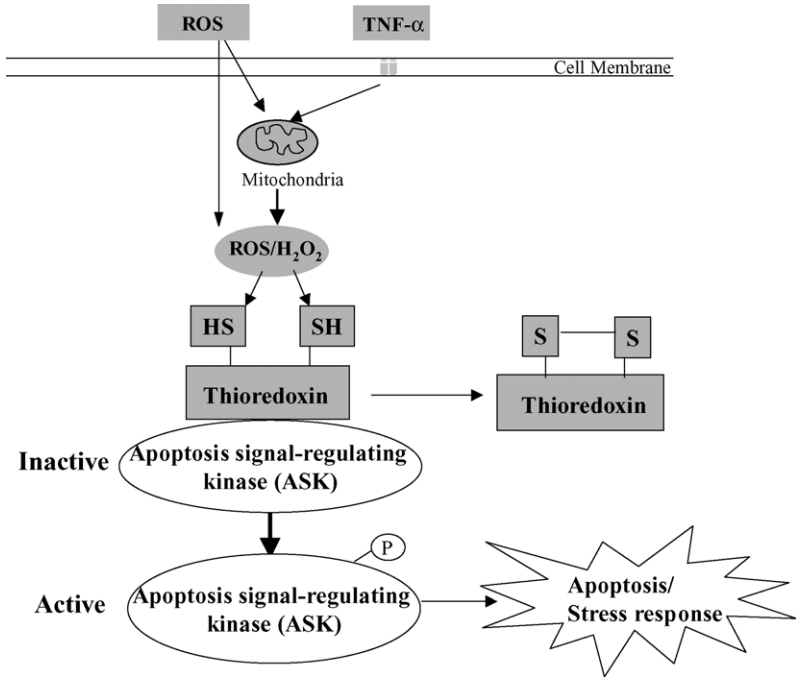

Fig. 7 – Model for the mechanism of redox-mediated apoptosis signaling kinase (ASK-1) activation in stress-induced apoptosis. TNF-α/oxidants act on mitochondria to generate ROS which are involved in the oxidation of reduced thioredoxin. Thioredoxin is bound to ASK-1 and upon oxidation of thioredoxin ASK-1 is oxidized and activated.

5.4.2. *Thioredoxin and apoptosis*

Another aspect where thioredoxin may play a critical regulatory role is apoptosis. An important finding regarding the role of thioredoxin in control of apoptosis is the binding of thioredoxin-1 with ASK-1 to form an inactive complex [59] (Fig. 5). Certain apoptosis-inducing conditions (particularly oxidative stress) can dissociate the thioredoxin-ASK-1 complex, activating ASK-1 leading to the activation of c-Jun amino-terminal kinase (JNK)/p38 (MAP) kinases, which in turn lead to apoptosis [60] (Fig. 7). ASK-1 was initially identified as a MAPKKK through a demonstration that it activated SAPK-JNK and MKK3/MKK6-p38 signaling cascades [61]. Thioredoxin-1 acts by binding to the N-terminal part of ASK-1 thus inhibiting ASK-1 activity and hence ASK-1-dependent apoptosis. This was further supported by the finding that down-regulation of thioredoxin-1 levels lead to activation of ASK-1. Furthermore, it has been reported that HIV-1 Nef protein inhibited ASK-1 activity by blocking the dissociation of the thioredoxin-1-ASK-1 complex [62]. Recently it has been reported that thioredoxin-1 could promote ASK-1 ubiquitination and further proteasomal degradation in endothelial cells [63]. As stated earlier, the inhibition of ASK-1 by thioredoxin-1 depends on the oxidation state of thioredoxin-1. Both single mutants, but not the double mutant at the redox-active site of thioredoxin (C32S, C35S) retain binding activity for ASK-1 and an ability to induce ASK-1 ubiquitination/degradation [63], suggesting that thioredoxin-1 may form a stable complex with ASK-1 through either of its Cysteine (Cys) residues. Such a complex formation has been shown between the single Cys-containing thioredoxin-1 and thioredoxin-1 reductase (through Cys32) or NF-κB (through Cys35) [64].

Since thioredoxin is present in very low concentrations as compared to other cellular antioxidants, it appears that thioredoxin may act mainly via its influence on signaling pathways rather than direct scavenging mechanisms observed for other antioxidants [51]. Interestingly, thioredoxin activity can be ablated by a self-thiolation mechanism via introduction of a GSH dependent disulfide bond at its Cys72 position [65]. Glutathiolated-thioredoxin may then regain its activity by an auto-deglutathiolation mechanism. Thus a reversible disulfidation mechanism is evident which might be an important adaptive and signaling cellular response during oxidative stress.

6. Protein–S glutathiolation and cell signaling

The term S-thiolation is often referred to the phenomena wherein a mixed disulfide is formed between a protein and a cysteine or other non-physiological thiols. The adaptive response of a cell to an oxidative environment is reflected by the modulation of structure and functions of its proteins. In addition, to counter a general stress situation, a cell may also employ a variety of other adaptive mechanisms such as acetylation, acylation, proteolytic processing, allosteric modulation, phosphorylation, alkylation and a host of other mechanisms [1]. However, the mechanisms as to how proteins are protected and modulated during an oxidative or a nitrative stress require deeper understanding. A large body of work in the past two decades has amply demonstrated the formation and accumulation of protein-mixed disulfides both, in intact tissues and cell cultures challenged with oxidative stress [66].

In view of the fact that GSH is the major thiol antioxidant of all cells and also the fact that GSH is a major component in many protein–thiol modifications, some of the S-glutathiolation dependent redox-adaptive and signaling mechanisms will be described in the following sections.

6.1. *Role of S-glutathiolation in cellular resistance to oxidative stress*

Physiological redox signaling disulfides are more likely formed by reaction of the thiolate with H_2O_2 to form a relatively unstable sulfenic acid intermediate followed by conjugation with GSH to form a mixed disulfide. These normally reversible chemical modifications of the thiols can result in a conformational change that may affect DNA binding of transcription factors or enzymatic activities or the formation or release of protein complexes. In addition, these changes are transient with the duration of the intermediate determined by the ratio of GSH/GSSG and reduced/oxidized thioredoxin.

Classically, *o*-phosphorylation of proteins has been considered to be the major mechanism of cellular homeostasis. An analogous mechanism, termed as S-glutathiolation has now been recognized as a potential modulator of redox-sensitive thiol proteins, especially those involved in signal transduction and protein translocation pathways. The role of cellular redox alterations is increasingly being recognized in the control of transcriptional factor binding to DNA [22]. Actin, PTP-1B, Ras and several other proteins are now known to be regulated by S-glutathiolation. Decomposition of GSNO formed as a consequence of nitrosative stress, has been found to induce S-glutathiolation of various proteins such as human Cu,Zn SOD, rabbit muscle GAPDH and bovine serum albumin, creatine kinase, carbonic anhydrase, actin and glycogen phosphorylase [67]. Peroxynitrite anion a toxic product of NO has recently been implicated in inducing S-glutathiolation of proteins. It was observed that sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA or Ca pump) activity was increased by S-glutathiolation of the enzyme due to $ONOO^-$ derived from NO [68]. This is an interesting observation especially in the light that NO is a known smooth muscle relaxant. Involvement of $ONOO^-$ dependent S-glutathiolation in muscle relaxation is therefore an important indication of the role of S-glutathiolation in oxidative stress response and cell signaling.

6.2. *S-glutathiolation and phosphorylation/dephosphorylation: a possible cross-talk*

Protein-tyrosine phosphatases play an important role in the dynamics of cell regulation due to phosphorylation-dephosphorylation mechanisms during extracellular signaling [69,70]. Phosphorylation of tyrosine residues of various target proteins have been recorded in response to cytokines and growth factors and have been found to be at least partly mediated by the generation of reactive oxygen species [71]. A recent report has suggested that PTP-1B is oxidized at Cys215 in a redox dependent manner [72]. This suggestion was later confirmed in a study wherein purified PTP-1B when treated with H_2O_2 was irreversibly inactivated and reversibly inhibited when treated with O_2^- [73]. It was observed during these experiments that PTP-1B was oxidized at its Cys215 to sulfenic acid which reacted with GSH to form a stable mixed disulfide [74]. Furthermore, the S-glutathiolated PTP-1B thus formed was found to undergo reduction to an active form when reacted with glutaredoxins. Importantly, S-glutathiolation of PTP-1B has also been demonstrated in intact cells. Interestingly not only reactive oxygen species but also GSSG was found to induce PTP-S-glutathiolation [75]. Overall, S-glutathiolation appears to be a protective/adaptive mechanism during oxidative stress and may also modulate signal transduction through the tyrosine phosphorylation pathway via modulation of the PTP-1B activity status. Several signal transduction pathways involving cell cycle progression, growth and differentiation, cytoskeletal function has been found to be modulated by S-glutathiolation of proteins. A redox dependent β2-integrin (CD11b/CD18 or Mac-1) mediated H_2O_2 and TNF-α promoted activation of neutrophil adhesion and recruitment was found to involve S-glutathiolation as a component of this signaling pathway [76]. Therefore inactivation of phosphotyrosine phosphatase-1B by S-glutathiolation might be an important point of cellular signaling mechanism where protein-phosphorylation and oxidative stress may cross-talk.

Another prominent and important phosphorylation mechanism in a cell is mediated by protein kinase C (PKC), a family of ten isozymes that play distinct and in some cases opposing roles in cell growth, survival and differentiation [77]. PKC activation involves a phosphatidylserine-dependent binding of the second messenger *sn*-1,2-diacylglycerol to tandem Cys-rich binding regions in kinase regulatory domain. In addition, pro-oxidant conditions or oxidative stress situations have been known to support PKC regulation by redox signaling [78]. H_2O_2 for instance, activates PKC isozymes in COS7 cells via a lipid-independent mechanism involving stabilization of the phosphotyrosine moieties of the isozymes at conserved sites in the catalytic domain [79]. Human PKC isozymes have been found to contain a very high ratio of cysteine residues (16–28 Cys residues). This includes one or two Cys-rich regions (six cysteines per region) in the regulatory domain, and 5–8 Cys in the catalytic domain. In a study involving seven PKC isozymes it was observed that a thiol-specific oxidant diamide, was able to S-glutathiolate all the isozymes, i.e. there was a formation of disulfide linkage of glutathione (GSH) to PKC-Cys residues (PKCS-SG) [80]. S-glutathiolation at any 1–3 redox-sensitive Cys residues is sufficient to inactivate fully the kinase activity of most of the isozymes [81]. In view of the large cysteine content of the enzyme and the isozymes, it is probable that PKC may be easily amenable to redox regulation by mechanism akin to S-glutathiolation.

6.3. *S-glutathiolation and the proteasome pathway*

Critical cellular processes including the cell cycle, cytokine-induced gene expression, differentiation, cell death, and the stress response are known to be under regulation by the ubiquitin–proteasome pathway [82]. Classically, ubiquitinylation subjects a protein selectively to rapid degradation by the 26 S-proteasome, however, recent advances in the field suggest the presence of other important non-proteolytic

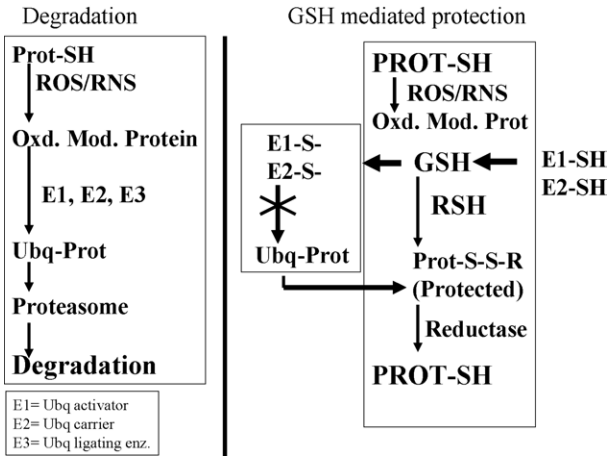

Fig. 8 – Regulation of ubiquitin-proteasome pathway. Ubiquitination of a protein via E1, E2 and E3 component of the ubiquitin-proteasome pathway subjects a protein to degradation through the 26 proteasomal mechanisms. S-glutathiolation of the E1 and E2 components renders the enzyme inactive and hence protects the loss of critical proteins.

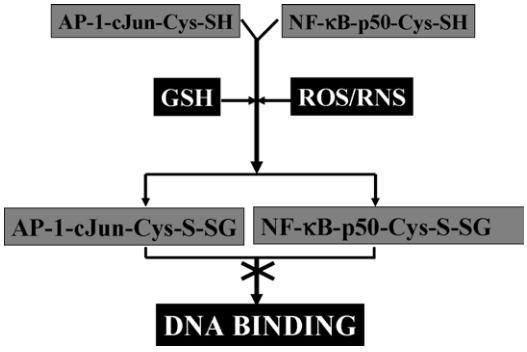

Fig. 9 – Transcriptional regulation by glutathiolation. Transcription factors such as NF-κB, AP-1/c-Jun require an active cysteine–SH for their binding to the DNA. S-glutathiolation of these cysteines prevents DNA binding of these transcription factors and hence obliterates inflammatory/apoptotic responses due to oxidative stress.

functions of ubiquitin–proteasome pathway [83]. It is now accepted that ubiquitination is regulated by oxidative stress and has been shown to be redox modulated by GSH in a study done on neuronal cells using cadmium as an agent for oxidative stress [84]. Proteins once ubiquitinated, are directed to the proteasomes for further proteolytic cleavage in a cascade involving the action of three enzymes E1, E2 and E3 (the proteolytic enzyme components of the proteasomes). It is now understood that ubiquitination of proteins protect the cell from accumulation of oxidatively damaged molecules via their degradation through this pathway. It has been demonstrated that E1 and E2 components of the ubiquitin pathway are reversibly inhibited by S-glutathiolation during oxidative stress [85], a mechanism which may prevent loss of reversibly oxidized and reparable proteins (Fig. 8).

Reversible thiolation/dethiolation of ubiquitin-conjugating enzymes may serve several potentially adaptive functions in cells including thiolation/dethiolation dependent protection of E1 and E2 from permanent oxidative damage and promote their reactivation when oxidant stress has abated [86]. Once oxidative stress diminishes, oxidatively altered proteins can regain functional conformations through the actions of a variety of dethiolases, reductases, and chaperonins [87]. It is therefore conceivable that down-regulation of ubiquitin dependent proteolysis protect potentially repairable proteins from untimely and wasteful degradation. Upon cellular recovery from stress, re-establishment of the GSSG/GSH ratio and ubiquitin–proteasome activity is expected to restore normal proteolytic regulation of cell cycle effectors.

6.4. *S-glutathiolation modulation of transcription factors*

The evidence of S-glutathiolation involvement in transcription was first obtained from the study wherein it was shown that binding of the nuclear factor-1 to DNA required a particular ratio of GSH/GSSG and that oxidative inactivation of nuclear factor-1 due to mixed disulfide formation was reversed by glutaredoxin [88]. This was corroborated by the observation that binding of AP-1-c-Jun subunit to the DNA depended on the cellular GSH/GSSG ratio [22]. The GSH/GSSG ratio provided a redox potential which determined the oxidation of c-Jun via the formation of a mixed disulfide as well as its S-glutathiolation at the conserved cysteines of the dimerization domain of the DNA binding site. Interestingly, since various transcription factors such as NF-κB, members of Jun/Fos, ATF/CREB and c-Myb exhibit a common putative GSH binding domain, it appears that S-glutathiolation may represent a general mechanism of redox-signal transduction leading to suppression of gene expression [89]. The NF-κB subunit-p50 has further been shown to be modulated by the GSH/GSSG ratio by a mixed disulfide formation at the cysteine residue of the DNA binding domain. This observation was found to be in agreement with an in vitro finding of an inhibition of AP-1 activation due to decrease in the GSH/GSSG ratio (Fig. 9). The finding of a nuclear glutaredoxin has further emphasised the importance of a reversible and enzymatic modulation of mixed disulfide-dependent alterations of nuclear protein–thiols.

7. Conclusions

The consequences of oxidative damage include altered cell signaling, proliferation and apoptosis. Many proteins are targets of such oxidative attack due to the presence of reactive cysteine residues in their primary structures. Protein-S-glutathiolation and S-nitrosation have been identified as major mechanisms of protein–thiol modification. Thioredoxins, peroxiredoxins, glutaredoxins, in addition to the general antioxidants of the cells form a powerful combination not only to prevent irreversible damage to crucial proteins but also take part in redox recycling of oxidized thiol molecules. Thus protein function modulation by S-glutathiolation and/or S-nitrosation spans a wide variety of cellular functions ranging from resistance to oxidative stress, phosphorylation depen-

dent signal transduction, post-translational protein modification and clearance via proteasomes to transcriptional activation and inhibition. Pivotal to all such oxidative modifications is the central role of GSH whose availability and redox status appears to determine the fate of an oxidatively modified protein. Thus more intense and focused investigation into the regulation and function of GSH is immediately warranted rather than labeling it as a mere antioxidant. Formation of protein-S-thiolation, protein-S-nitrosation and protein–SH (formation of sulfenic, sulfinic and sulfonic acids) represent a state of cellular adaptation to oxidative stress and renders such proteins inactive, which may be missed during in vitro experimentation. These processes were once considered irreversible, but recent data indicate that they are in fact reversible by various redox regulating enzymes. Many clinical trials with antioxidants have either proved ineffective or inconclusive and this may be due to a possible administration of the antioxidants during an oxidatively compromised phase of the cells. Better and in depth understanding of the implications of redox alterations will open new vistas into the understanding of yet improperly understood disease processes. The new and refined knowledge in turn, would open up new frontiers in therapeutic approaches and drugs targeted at specific molecules. Understanding of several downstream transient redox alterations will give a new insight into the myriad ways a cell maintains homeostasis under adverse conditions, failure of which will lead to a particular cellular anomaly.

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