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Forum Review

Glutathione, Stress Responses, and Redox Signaling in Lung Inflammation

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ABSTRACT

Changes in the ratio of intracellular reduced and disulfide forms of glutathione (GSH/GSSG) can affect signaling pathways that participate in various physiological responses from cell proliferation to gene expression and apoptosis. It is also now known that many proteins have a highly conserved cysteine (sulfhydryl) sequence in their active/regulatory sites, which are primary targets of oxidative modifications and thus important components of redox signaling. However, the mechanism by which oxidants and GSH/protein-cysteine-thiols actually participate in redox signaling still remains to be elucidated. Initial studies involving the role of cysteine in various proteins have revealed that cysteine-SH may mediate redox signaling via reversible or irreversible oxidative modification to Cys-sulfenate or Cys-sulfinate and Cys-sulfonate species, respectively. Oxidative stress possibly via the modification of cysteine residues activates multiple stress kinase pathways and transcription factors nuclear factor-κB and activator protein-1, which differentially regulate the genes for proinflammatory cytokines as well as the protective antioxidant genes. Understanding the redox signaling mechanisms for differential gene regulation may allow for the development of novel pharmacological approaches that preferentially up-regulate key antioxidants genes, which, in turn, reduce or resolve inflammation and injury. This forum article features the current knowledge on the role of GSH in redox signaling, particularly the regulation of transcription factors and downstream signaling in lung inflammation. *Antioxid. Redox Signal.* 7, 42–59.

INTRODUCTION

ALTHOUGH PRIMARILY INVOLVED in gas exchange, the lung is a unique organ in that it has an extraordinary surface exposed to the external environment and also needs to protect itself against infection, large number of particles of various origins in the inhaled air, and oxidative gases. Thus, while preserving its primary function, it must both express and regulate inflammatory pathways that require coordination between different cell types, and anti- and proinflammatory cytokines and chemokines. Excessive inflammatory reactions in airways, vasculature, or alveoli will result in profound adverse effects on lung function and may lead to disease.

The recruitment of inflammatory cells such as neutrophils or monocytes/macrophages in the lungs is thought to occur as a result of a cascade of cellular events initiated by various stimuli synthesized by the resident cells in the lung interstitium. Activation of inflammatory cells and alveolar and bronchial epithelial cells results in the release of a battery of cytokines, including the proinflammatory tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β, both of which cause up-regulation of adhesion proteins on the endothelium, increased permeabil-

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ity, and increased secretion of chemokines such as IL-8, macrophage inflammatory protein-2, or monocyte chemotactic protein-1 (9, 66, 91, 126). Chemokines and cytokines will act in concert to further promote the extravasation and accumulation of leukocytes in the tissues and alveolar space where they become activated to produce reactive oxygen species (ROS), such as superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). The release of ROS is largely believed to contribute to cell and tissue damage associated with many chronic inflammatory diseases (129, 133, 135). Thus, acute and chronic alveolar and/or bronchial inflammation is involved in the pathogenesis of many inflammatory diseases, such as asthma, adult respiratory distress syndrome (ARDS), and chronic obstructive pulmonary disease (COPD). The transcription factors, nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), regulate the transcription of many of the proinflammatory cytokine genes, as well as the up-regulation of protective antioxidant genes. NF-κB and AP-1 activation has been shown to be modulated in response to a plethora of stimuli, including oxidants, antioxidants, and both inflammatory and antiinflammatory agents (130, 131, 133) (Fig. 1).

Reduced glutathione (GSH) is a tripeptide composed of glutamine, cysteine, and glycine. Due to its sulfhydryl group, it functions as an antioxidant, protecting against free radicals and other oxidants, and has been implicated in immune modulation and inflammatory responses (26, 50). Increasing intracellular GSH can decrease the release of cytokines and chemokines from lung cells by decreasing NF-κB activation (9, 10, 66, 126). In addition, GSH is an important regulator of cell proliferation, apoptosis, and gene transcription (11, 115, 131, 132). Thus, GSH is vital in the lung, defending the airspace epithelium from damage in response to oxidants and inflammation (97, 113, 131), and GSH redox status is critical for the transcriptional regulation of these proinflammatory genes. This is illustrated by findings in various pulmonary diseases where decreases in the levels of GSH in the lung lining fluid have been shown to occur in idiopathic pulmonary fibrosis (IPF), ARDS, cystic fibrosis, lung allograft patients, and HIV-positive patients (for reviews, see 131, 132). In contrast, an increase in total GSH concentration, which also includes the oxidized form glutathione disulfide (GSSG), has been reported in the bronchial and alveolar fluid in patients with mild asthma (133). Thus, low levels of GSH in the lung lining fluid of patients with inflammatory respiratory diseases may render them more susceptible to the deleterious effects of subsequent exposure to inhaled toxicants and may also perpetuate the inflammatory response (28, 132, 133). In recent years, it has become evident that small changes in the cellular redox status may alter signaling pathways. This came about at the same time that ROS, previously considered as beneficial only in the context of bacterial killing by phagocytes, became viewed as major modulators of cellular function acting as second messengers to regulate both proliferation and survival (2, 3, 54, 56, 165, 168). The aims of this forum article are (a) to discuss the mechanism of redox signaling and (b) to describe the regulation of cellular GSH redox status and its role in gene transcription under conditions of oxidative stress and in inflammation.

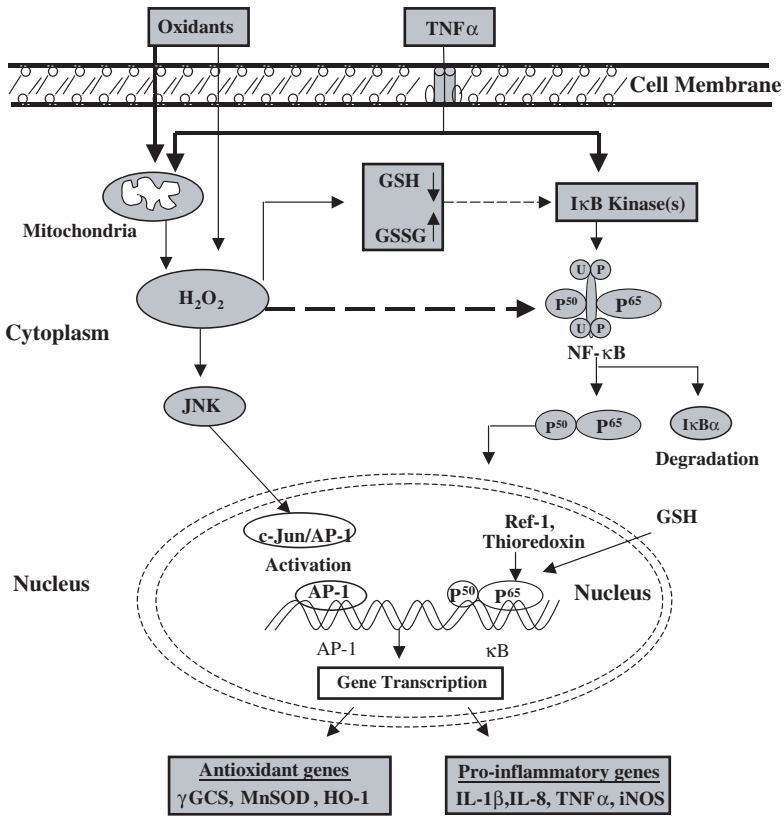

FIG. 1. Model for the mechanism of NF-κB and AP-1 activation leading to gene transcription. TNF-α/oxidants act on mitochondria to release H_2O_2 (and possibly $ONOO^-$), which is involved in the activation of NF-κB and AP-1. $O_2^{\bullet -}$ does not leave mitochondria unless generated by an outer mitochondrial membrane oxidoreductase. H_2O_2 can leave mitochondria as enough is generated to overcome the mitochondrial peroxidase activity. Activation of NF-κB involves the phosphorylation, ubiquitination, and subsequent proteolytic degradation of the inhibitory protein IκB. Free NF-κB then translocates into the nucleus and binds with its consensus sites. Intracellular redox ratio of GSH/GSSG levels and intranuclear presence of Ref-1 and Trx can modulate AP-1 and NF-κB activation. Similarly, AP-1 [either c-Jun–c-Jun (homodimer) or c-Fos–c-Jun (heterodimer)] is activated by the phosphorylation of the JNK pathway leading to the activation of AP-1, which binds with its TRE consensus region. Activation of NF-κB/AP-1 leads to the coordinate expression of protective antioxidant and proinflammatory genes.

ROS

The term "reactive oxygen species" (ROS) encompasses $O_2^{\bullet -}$, hydroxyl radical ($^{\bullet}OH$), singlet oxygen ($^1O_2$), and H_2O_2. Only $^{\bullet}OH$ and $O_2^{\bullet -}$ are free radicals, *i.e.,* they contain an unpaired electron, whereas $^1O_2$ represents an excited state of oxygen and H_2O_2 is not a radical and does not spontaneously react with carbon-centered molecules or with thiols. Divalent reduction of O_2 to form H_2O_2 (half life 1 ms) being spin-restricted, one-electron reduction is favored and $O_2^{\bullet -}$ (half-life 1 μs) is readily formed. Therefore, the major source of H_2O_2 in cells arises from the dismutation of $O_2^{\bullet -}$. At physiological pH, nonenzymatic dismutation is rapid, but dismutation is markedly accelerated by the superoxide dismutases (105), a reaction that occurs at near diffusion-limited rate (Fig. 2). As a result, the steady-state concentration of $O_2^{\bullet -}$ is estimated to be ~10^{-11} *M* (23). This suggests that $O_2^{\bullet -}$ interacts with other free radicals or other molecules within a very short radius of its site of production. H_2O_2 can diffuse freely through membranes, probably through a water channel. Catalase, which is generally found only in peroxisomes, catalyzes the very rapid dismutation of H_2O_2 into water, whereas glutathione peroxidases, which are selenoproteins found in the cytosol and mitochondria, catalyze the reduction of H_2O_2, using GSH and producing GSSG (Fig. 2). These antioxidant enzymes can also reduce lipid hydroperoxides, as well as peroxynitrite ($ONOO^-$). Peroxiredoxins are another class of enzymes that can reduce H_2O_2 using thiols (145) in a reaction similar to that catalyzed by the selenium in glutathione peroxidases, some using thioredoxin (Trx) as a cosubstrate and at least one using GSH (32). Interestingly, they contain reactive cysteine residues in their unprotonated form, *i.e.,* thiolate (S^-), and the catalytic reaction includes several steps, one of which is the formation of a sulfenate intermediate (PSO^-), which then reacts with a thiol that can be the second reactive thiol present in most 2-Cys peroxiredoxins (183) or GSH (32). Peroxiredoxins might play an important role in controlling redox signaling pathways (82, 184).

$^{\bullet}OH$ is a very reactive oxygen species that has a lifetime of ~2 ns in aqueous solution and a radius of diffusion of ~20 Å. Thus, it induces peroxidation only when it is generated in close proximity to its target. It can be generated when H_2O_2 collides with a reduced transition metal, such as ferrous iron in a Fenton reaction. It is the most reactive radical known to chemistry. It can attack and damage almost every molecule found in living cells at a diffusion-controlled rate, *i.e.,* $^{\bullet}OH$ reacts as soon as it comes into contact with another molecule in solution. $^{\bullet}OH$ is so reactive that, when generated *in vivo,* it does not persist for even a microsecond and rapidly combines with molecules in its immediate vicinity. The principal reaction of $^{\bullet}OH$ with biologic molecules, almost all of which are nonradicals, is hydrogen abstraction, creating a radical of the target molecule that has a number of possible chemical fates (13). This can include chain reactions, such as in lipid peroxidation. Abstraction from thiols results in formation of thiyl radicals ($RS^{\bullet}$), which have many interesting chemical properties (161). They can combine with oxygen to generate oxysulfur radicals, such as RSO_2 and $RSO^{\bullet}$, a number of which damage biological molecules (2–4). Thus, $^{\bullet}OH$ may play a significant role in the tissue damage associated with acute or chronic inflammation, but it is unlikely to act specifically on any particular target.

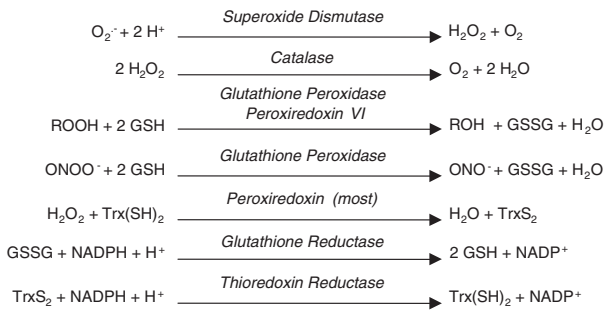

FIG. 2. Enzymatic reactions of GSH and Trx redox systems.

ROS are produced as inevitable by-products of energy production within mitochondria during the course of normal metabolism. The production of $O_2^{\bullet -}$ by the mitochondrial respiratory chain occurs continuously during normal aerobic metabolism. It has been estimated that a small, but significant, percentage of all the electrons traveling down the mitochondrial respiratory chain never make it to the end, but instead form $O_2^{\bullet -}$. The autoxidation of ubisemiquinone in mitochondria and metal-catalyzed autoxidation of molecules generate $O_2^{\bullet -}$ through one-electron O_2 reduction. In addition to mitochondria, cytochrome P450s and their reductases, the xanthine/xanthine oxidase system, and nitric oxide synthase have been suggested to generate ROS. Under normal metabolic conditions, each cell in the body is exposed to ~10^{10} molecules of $O_2^{\bullet -}$ each day. The other abundant source of $O_2^{\bullet -}$ and H_2O_2 is the professional phagocytes, *i.e.,* neutrophils and macrophages, which have long been known to express an NADPH oxidase that requires stimulation for assembly of its cytosolic components with the two subunits of the membrane flavocytochrome ($gp91^{phox}$ and $p22^{phox}$) to generate high levels of $O_2^{\bullet -}$ (17).

The recent recognition that many cells express homologues of the catalytic subunit of the NADPH oxidase, $gp91^{phox}$, now collectively known as NOX for NADPH oxidase and DUOX for dual oxidase, has raised questions about their mode of regulation to produce ROS either constitutively or in response to cytokines, growth factors, and calcium signals. All NOX enzymes contain a structure similar to the phagocyte $gp91^{phox}$ (NOX2) but, in some, additional domains are present, which are the basis for the classification into three categories (92). NOX1, 3, and 4 are the closest in structure to NOX2, having a similar electron transfer center and using NADPH as the electron source for $O_2^{\bullet -}$ production. NOX5 is distinguishable by its N-terminus that contains three EF-hands that confer a requirement for Ca^{2+} for activation. The regulatory N-terminus and the catalytic C-terminus of NOX5 were recently shown to interact in a Ca^{2+}-dependent manner (18). DUOX1 and 2 build up on the structure of NOX5 and contain, in addition, at the N-terminus a peroxidase-homology domain with a sequence similar, but not identical, to myeloperoxidase. DUOX1-dependent H_2O_2 release by cultured human bronchial epithelial cells was recently detected (60). It is interesting to note that many bar-

rier cells/cell linings have the ability to express at least one of the NOX isoforms in response to various inflammatory mediators and lipopolysaccharide via Toll-like receptor 4, *i.e.,* NOX1 in colon epithelium and keratinocytes, NOX4 in the kidney epithelium, and DUOX1 in lung epithelium (143). The expression of DUOX1 and/or DUOX2 by the tracheal, bronchial, and salivary epithelial cells is of particular interest. DUOX generates H_2O_2 via direct/indirect dismutation of $O_2^{\bullet -}$ and therefore serves as a source of H_2O_2 in these fluids, mediating antimicrobial activities along with lactoperoxidase (60). It has been suggested that H_2O_2 produced by DUOX might act as substrate for the lactoperoxidase in the bronchial and salivary fluids (60). Recently, homologues of the $p47^{phox}$ and $p67^{phox}$, the cytosolic components of the phagocyte NOX2, have been isolated and termed NOXO1 and NOXA1, respectively (166). The production of $O_2^{\bullet -}$ by NOX1 appears to increase in response to stimulation and in the presence of NOXO1 and NOXA1, although this is observed mostly in transfected cells.

The role of the novel NOX in innate immunity, modification of the extracellular matrix, and signal transduction pathways leading to cell proliferation or apoptosis is under intense investigation (92). Nevertheless, the discovery has significantly altered the belief that ROS are detrimental and has given additional support to the concept that ROS can be produced deliberately and act on specific targets to modulate signaling pathways. In fact, the role of ROS as second messengers, in particular that of H_2O_2, is gaining acceptance, as the four characteristics of classical second messengers, *i.e.,* regulated enzymatic production, degradation by specific enzymes, presence at low concentrations that can be transiently elevated to measurable amounts upon stimulation, and (at least for H_2O_2) ability to react at specific sites, such as metals and thiolates, are now getting support from studies with protein tyrosine phosphatases (PTPs) and other thiolate-containing proteins (see below). The question of specificity is critical as it differentiates redox signaling from oxidative stress. Redox signaling entails at least one reaction where reversible oxidation of a signaling molecule by a reactive species occurs that purposely modulates a signaling pathway. Oxidative stress is often characterized as an increase in the antioxidant/oxidant ratio, tilted toward a higher proportion of oxidants. Depending on the cell type and the extent of stress, oxidative stress can elicit responses ranging from severe oxidative damage, loss of cell function and viability, to apoptosis and ultimately necrosis, but may also elicit other responses ranging from cell differentiation to cell cycle progression (3, 23, 67, 93, 115, 164). During different phases of inflammation (initiation, resolution, or excessive and chronic inflammation), both redox signaling and oxidative stress may occur.

GSH AND CELLULAR REDOX REGULATION

The control of the intracellular redox environment is vital for proper cellular function. The cells, in particular in the lungs, have well developed defense mechanisms that insure the proper balance between the prooxidant and antioxidant molecules and protect themselves from the constant oxidative challenge. GSH is the predominant nonprotein sulfhydryl in the cells and is a key player in the maintenance of the cellular redox status, defined as the ratio of the concentration of oxidizing equivalents to that of reducing equivalents (55). Glutathione exists primarily in two redox forms, *i.e.,* GSH and GSSG, the latter representing a negligible $1/100^{th}$ of the total GSH pool. The normal GSH content of a cell ranges from 1 m*M* to 10 m*M*, levels that are imperative for a cell to maintain and that are a function of the balance between depletion and synthesis. Cells can excrete GSSG or reduce it back to GSH at the expense of NADPH through the action of glutathione reductase. However, *de novo* synthesis of GSH from its amino-acid constituents is essential for the elevation of GSH that occurs as an adaptive response to oxidative stress. GSH synthesis involves two enzymatic steps catalyzed by glutamylcysteine ligase (GCL; formerly called γ-glutamylcysteine synthetase) and glutathione synthetase (73, 106). The enzyme GCL controls the rate of GSH synthesis (73) because the rate-limiting step for *de novo* synthesis of GSH is the cellular levels of the amino acid cysteine. In that regard, the plasma membrane ectoenzyme γ-glutamyltranspeptidase, which is the only enzyme that can break the γ-linkage found in GSH and GSH-conjugates, is essential in providing cysteine. It metabolizes the extracellular GSH and preferentially forms γ-glutamylcysteine, which is taken up by cells, thus bypassing its production by GCL. GCL is composed of a heterodimer containing a 73-kDa heavy catalytic subunit (GCLC) and a 30-kDa light modifying subunit (GCLM) (73). Although the heavy subunit contains all of the catalytic activity, the association of the heavy subunit with the regulatory light subunit can modulate GCL activity. The ratio of the two subunits for physiological function has long been assumed to be 1:1; however, in tissues the ratio varies significantly, and usually GCLC/GCLM is significantly greater than 1:1 (89). GCL is regulated by GSH through feedback inhibition. Regulation of GCL at the gene level is considered below.

The GSSG/2GSH ratio can serve as a good indicator of the cellular redox state (149). This ratio results primarily from a combination of the rates of H_2O_2 removal by glutathione peroxidase and GSSG reduction by glutathione reductase, regulating the GSH concentration. Thus, antioxidant enzymes play a critical role in the maintenance of the cellular reductive potential, and the genes for manganese superoxide dismutase (MnSOD), GCLC, glutathione peroxidase, Trx, reductase, and metallothionein are induced by modulation of cellular GSH/GSSG levels in response to various oxidative stresses, including hyperoxia and inflammatory mediators such as TNF-α and lipopolysaccharide in lung cells (41, 42, 132). The intracellular redox status of lung epithelial cells has been shown to be a critical factor in determining cell susceptibility or tolerance to oxidative insults. We have shown that depletion of GSH with buthionine sulfoximine sensitizes both A549 and 16-HBE cells to the injurious effects of hyperoxia and H_2O_2, resulting in increased membrane permeability and activation of NF-κB (139). In contrast, pretreatment of these cell lines with hyperoxia prior to H_2O_2 exposure protects against the cytotoxic effects of H_2O_2, as well as preventing NF-κB activation. These protective effects were achieved by enhancing the levels of

GSH in response to pretreatment with hyperoxia. Thus, modulation of intracellular GSH can either increase or decrease tolerance to subsequent oxidant exposure.

Whereas GSH is crucial in redox control, the Trx system, *i.e.,* Trx and Trx reductase, which is ubiquitously expressed, also plays important roles in scavenging ROS and, along with GSH and glutaredoxin, modulates the thiol/disulfide status of many signaling proteins (72). Trx is a 12-kDa protein disulfide reductase that catalyzes protein reduction via an NADPH-dependent reaction in conjunction with Trx reductase. Members of the Trx family have a conserved redox-active center, often referred to as the Trx fold, with two cysteine residues (Cys-Gly-Pro-Cys) that undergo reversible oxidation to form a disulfide bridge through transferring of reducing equivalent to a disulfide substrate. Trx then reverts to the reduced form through the action of Trx reductase, an NADPH flavoprotein.

INCREASED SYNTHESIS OF GSH THROUGH UP-REGULATION OF GCL

The synthesis of GSH is up-regulated during oxidative stress and inflammation. Thus, an increase in GCL expression would be expected under oxidative stress, and the first demonstration of that was with the redox cycling and GSH conjugating quinone, menadione (157).

Exposure of alveolar epithelial cells *in vitro* to oxidants, oxidant-generating systems, and lipid peroxidation products, such as H_2O_2, hyperoxia, ozone, menadione, and 4-hydroxy-2-nonenal, leads to short-term falls in intracellular GSH associated with increased GSSG levels, followed by increases in GSH levels or up-regulation of GCLC mRNA in alveolar epithelial, endothelial and other cells *in vitro,* and in rats *in vivo* (47, 57, 58, 97, 99, 131, 136, 138, 156, 170).

The GCLM is also concomitantly induced in response to oxidants and phenolic antioxidants in rat lung epithelial L2 cells, suggesting that concomitant induction of both subunits may be a potential mechanism to enhance cellular GSH synthesis, and so develop cellular tolerance to oxidative stress (114, 170). Although the mRNAs for both subunits increase in response to oxidative stress, there may be a disproportional increase in the proteins (89). Thus, the short-term effects of various oxidants and oxidant-generating systems appear to up-regulate the gene for GSH synthesis, possibly providing a protective/adaptive mechanism against subsequent oxidative stress.

Identification and characterization of the types of diverse stimuli that act as potent inducers of GCL should aid in the development of effective pharmacological strategies for antioxidant treatment involving GSH regulation in inflammatory lung diseases. To this effect, several studies have been directed toward understanding and elucidating the molecular mechanisms of GSH synthesis and regulation in type II alveolar epithelial cells in response to various environmental, oxidant, antioxidant, and inflammatory stimuli. Our groups and other investigators have reported that the promoter (5'-flanking) region of human GCLC gene is regulated by a putative c-Jun homodimeric complex-AP-1 sequence (1, 47, 48, 134, 138, 154, 167, 172). This sequence is located at the proximal region of the GCLC TATA box in various cell lines, including human alveolar epithelial cells (167, 172).

Mulcahy and co-workers (111, 114), however, have reported a distal antioxidant response element (ARE) containing an embedded 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-responsive element (TRE/AP-1) and an electrophile response element (EpRE or its functional equivalent, ARE), which play a key role in the regulation of the GCLC and GCLM, respectively, in response to a planar aromatic xenobiotic, the phenolic antioxidant β-naphthoflavone specifically in a liver cell line (HepG2 cells) (52). They further suggested a differential induction of mafF, mafG, and mafK expression by EpRE activator in the regulation of GCLC regulation in a variety of cell lines (112). It has been shown recently that H_2O_2-dependent activation of GCLC-ARE4 reporter occurs via the mitogen-activated protein kinase (MAPK) pathways without oxidation of cellular GSH or Trx-1, suggesting that redox GSH status of the cells is not required for regulation of GCLC or ARE (64). Mulcahy and colleagues also showed that the internal AP-1 site is important for the constitutive expression of the GCLM gene (111). However, Galloway and co-workers (57, 58) were unable to demonstrate a role for ARE in the induction of GCLM by oxidants such as *tert*-butylhydroquinone in HepG2 cells. They suggested that an AP-1 site was the critical element for the basal regulation of this subunit. Therefore, it is likely that the expression of the GCL subunit genes is regulated by the different regulatory signals in response to diverse stimuli in specific cells.

Exposure to phenolic antioxidants, such as dietary 2(3)-*tert*-butyl-4-hydroxyanisole and butylated hydroxytoluene, as well as the synthetic indolic antioxidant, 5,10-dihydroindeno(1,2-b) indole and pyrrolidine dithiocarbamate, a sulfhydryl-modifying antioxidant compound, up-regulates GCLC and GCLM in human endothelial cells and other cell lines (58, 99, 173, 180). Similarly, curcumin, a dietary phenolic compound (diferuloylmethane), induced GCL induction that was associated with increased DNA binding of transcription factor complexes to TRE and EpRE elements (48). Furthermore, curcumin increased JunD and c-Jun content in AP-1 complexes and increased JunD while decreasing MafG/MafK in EpRE complexes. Thus, the changes in the pool of transcription factors of EpRE and AP-1 complexes may affect the expression of GCL. These effects of phenolic antioxidants are associated with AP-1/ARE transactivation (21, 109, 128). Taken together, antioxidants protect cells from oxidants by either scavenging these molecules directly or by regulating intracellular GSH levels through the induction of GCL. It should, however, be kept in mind that recent studies have shown that many compounds characterized originally as antioxidants have direct effects upon several signal transduction enzymes independent of their antioxidant function. For example, vitamin E has significant effects upon signaling independent of its lipid peroxidation-inhibiting role (16).

Transforming growth factor-β1 (TGF-β1) is a multifunctional growth factor that modulates cellular proliferation and induces differentiation and synthesis of extracellular matrix proteins, including collagens and fibronectin, in many types of lung cells. Recent studies have shown increased expression of TGF-β1 in bronchiolar and alveolar epithelium in IPF and COPD patients, and higher levels in bronchoalveolar lavage fluid of atopic asthmatics as compared with healthy subjects

(43, 142). TGF-β1 also down-regulates GCLC mRNA and GSH synthesis in human alveolar epithelial cells and pulmonary artery endothelial cells *in vitro* (12, 178). The decrease showed decreased GSH biosynthesis. Both we and other workers have shown that GCLC mRNA expression is under the control of the AP-1 transcription factor (134, 137, 138, 172), and that TGF-β1 may decrease GCLC gene expression via an AP-1 mechanism (80). Recently, Jardine *et al.* showed that down-regulation of GCLC in response to TGF-β1 in lung epithelial cells was mediated via an AP-1 heterodimer consisting of c-Jun and Fra-1 (80). Thus, higher levels of TGF-β1 may down-regulate GSH synthesis in lungs of patients with inflammatory diseases such as IPF and COPD. Moreover, decreased GSH levels may also have direct functional consequences leading to inflammation. *In vitro* studies showed that GSH (in the concentration range normally found in epithelial lining fluid) suppressed fibroblast proliferation (29). In addition, depletion of GSH in response to TGF-β1 appears to be a key requirement for subsequent collagen I mRNA expression in murine embryo fibroblasts (100). This induction was attenuated by pretreatment with *N*-acetyl-L-cysteine (NAC), GSH, or GSH ester. The relevance of GSH regulation and subsequent tolerance/susceptibility in lung epithelial cells in response to pro-/antiinflammatory mediators and/or oxidants under chronic inflammation *in vivo* is not known.

Glucocorticoids (GC), such as dexamethasone, are antiinflammatory agents used to manage inflammatory lung diseases. These agents act by interacting with GC receptors (GR), thereby interfering with the binding of transcription factors to the GR and subsequent nuclear translocation and activation of proinflammatory genes. GC also regulate posttranslational modification of nucleosomes, which increase or decrease accessibility of transcription factors to gene promoters. Inhaled GC target the airway epithelium to control the inflammatory events. Dexamethasone has been demonstrated to decrease both basal and TNF-α-stimulated GSH levels in the lung epithelial cell line, A549 (136, 138). A decrease in GSH in response to dexamethasone is due to inhibition of AP-1 activity and down-regulation of GCLC gene expression (138). Thus, one of the side effects of using dexamethasone in patients with inflammatory lung diseases may be to enhance levels of oxidative stress by inhibiting the synthesis of the protective antioxidant GSH.

ROLE OF THIOLS IN REDOX SIGNALING AND STRESS RESPONSES

Proteins bearing cysteine-SH residues in the thiolate form (S^-) are considered prone to oxidative modification, which may interfere with biological functions either as "damage" or in context to oxidant-dependent signal transduction. Cysteine thiolates (Cys-S^-), but not cysteine thiol (Cys-SH), can be readily oxidized to a sulfenic acid (−SOH), which is a relatively reactive form that can quickly form a disulfide with a nearby thiol. Strong oxidants will oxidize either Cys-S^- or Cys-SH to sulfinic (Cys-SO_2H) and sulfonic (Cys-SO_3H) acid derivatives. This difference provides much of the basis for the difference between redox signaling and oxidative stress, in which the latter involves nonspecific oxidation, whereas the former involves oxidation of only those cysteines in environments promoting the dissociation of the thiol. The higher oxidation states have essentially been considered as irreversible modifications and would likely be associated with oxidative injury. However, a recent work by Biteau *et al.* revealed that cells might contain a specific enzyme capable of reducing sulfinic acid derivatives (22). They isolated a protein in yeast that can reduce the sulfinic derivative of yeast peroxiredoxin Tsa1. This protein is highly conserved in eukaryotes and was named as sulfiredoxin. They propose that sulfiredoxin catalyzes the multistep reduction process by acting as both a specific phosphotransferase and a thioltransferase. Sulfiredoxin apparently is able to overcome the energy barrier that normally prevents the reduction of protein-Cys-SO_2H by introducing a phosphate group in the peroxiredoxin-sulfinate moiety to make sulfinic phosphorylester in the presence of ATP and Mg^{2+} (Fig. 3). A thiolsulfinate disulfide is formed

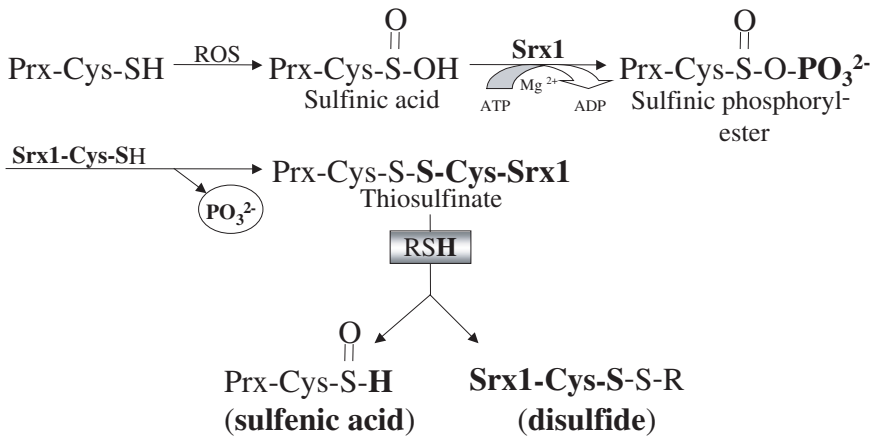

FIG. 3. The mechanism of peroxiredoxin sulfinic acid reversal by sulfiredoxin. The thermodynamic energy barrier for reduction of sulfinic acid species can be overcome by the help of a novel class of dual enzyme sulfiredoxin (Srx). This enzyme first catalyzes a phosphate group transfer from ATP to peroxiredoxin (Prx)-sulfinate (Prx-Cys-SOH) as a part of its phosphotransferase activity to form an intermediate disulfide (Prx-Cys-S-S-Cys-Srx). The latter is then reduced to a sulfenate and a corresponding disulfide, depending on the reducing group involved. RSH, thiol donor.

with another sulfiredoxin molecule and then reduced forms by replacing the phosphate group. Finally, the thiolsulfinate can be reduced due to peroxiredoxin-sulfenate and a sulfiredoxin-disulfide by reducing agents such as dithiothreitol or Trx. Thus, peroxiredoxin inactivation may facilitate H_2O_2 signaling, whereas its reverse activation by sulfiredoxin may add a new dimension in the regulation of such a signaling. In contrast, disulfide bonds and protein sulfenic acid moieties can be easily reduced and are often considered as the mediators of redox signaling (38, 163, 165). Nonetheless, it is essential to the understanding of redox signaling to remember that all cysteine residues are not equal. GSH and most protein cysteines cannot react at a biologically significant rate with H_2O_2 unless they are in close association with a metal (182) or exist in the form of a thiolate anion ($-S^-$). Indeed, the glutathione peroxidase and peroxiredoxin reactions that appear to involve H_2O_2 reacting with GSH never involve that actual interaction. Instead, the glutathione peroxidase reaction involves the interaction of H_2O_2 with the selenocysteine of that enzyme, whereas the peroxiredoxin reactions involve interaction of H_2O_2 with a cysteine-thiolate residue of the peroxiredoxin. When ionized to the thiolate form, cysteine then reacts quite rapidly with H_2O_2. As the pK_a of cysteine is normally ~8.3, its ionization will only occur in unusual environments as a function of the surrounding residues. For example, when a cysteine is in the vicinity of a positively charged amino acid, its pK_a is lowered to below 5.0. Such a cysteine is deprotonated and becomes a prime target for H_2O_2 oxidation. Thus, only cysteine-containing proteins providing this environment will be affected by H_2O_2 directly.

Cysteine in its thiolate form could also participate in thiol–disulfide exchange provided that there is no interference by steric hindrance:

$$RS^- + R'SSR'' \rightleftarrows RSSR'' + R'S^-$$

In contrast, exchange between thiols and disulfides is very slow and must be catalyzed by enzymes such as glutaredoxin or other protein disulfide isomerases that have a Trx-like structure in their active site. Such disulfide exchange is a potential signaling mechanism because of its capacity for modifying cysteine residues in enzymes. The relationship to redox signaling is provided by the production of GSSG during the enzymatic reduction of hydroperoxides or $ONOO^-$ by glutathione peroxidase. Normally, GSSG represents < 1% of the total glutathione pool. When H_2O_2 or $ONOO^-$ is transiently elevated, an elevation in GSSG, also transient, can occur, providing a possible mechanism for signaling by means of thiol–disulfide exchange. In this scenario, signaling is indirectly dependent on ROS generation. Nonetheless, as this mechanism requires a change in GSSG that is usually only observed during oxidative stress, such signaling is more likely an oxidative stress response rather than physiologic redox signaling. In physiological redox signaling, disulfides are more likely formed by reaction of the thiolate with H_2O_2, forming a sulfenic acid, followed by reaction with GSH to form the mixed disulfide. Apart from providing the proper cellular reducing environment, there is growing evidence that the GSH redox couple dynamically regulates protein function by the reversible formation of mixed disulfides between protein cysteines and GSH (14, 40, 62, 169). The term *S*-thiolation refers to the phenomenon wherein a mixed disulfide is formed between a protein and a cysteine or other nonphysiological thiols. A specific mixed disulfide formation in conjunction with GSH is termed *S*-glutathiolation. Protein *S*-glutathiolation has been implicated in redox buffering of oxidative stress, extracellular protein stabilization, protection of proteins against irreversible oxidation of their critical cysteine residues, and regulation of enzyme activity (6, 40, 169). ROS, reactive nitrogen species (RNS), and alterations in intracellular redox potential have been reported to induce protein *S*-glutathiolation.

The strongest consensus in redox signaling is that the small thiols and thiol-containing proteins play an essential role in regulation of the cell redox status. Reversible changes in the redox status of protein thiols leading to formation of mixed protein-GSSG or intramolecular disulfides, and the direct oxidative modification of the cysteine residues to form a sulfenic acid or *S*-nitroso adduct, have been characterized (5, 25, 27, 46, 61). These 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 Trx. Numerous proteins with cysteine residues that have been shown to exhibit redox sensitivity to enter in redox signaling include transcription factors, caspases, PTPs, small GTPases, and cytoskeletal components (see below). A recent review has emphasized that a loss of function by disulfide formation yet remains to be firmly established as a regulatory mechanism in light of the findings that disulfide formation, at least in certain cases, may actually enhance the function of a given protein (15). The finding of an oxidative activation of bacterial oxyR transcription factor supports the later hypothesis of protein activation by disulfide formation (188). Other examples of such disulfide-dependent activation are chaperone hsp33 (78), the bacterial sigmaR, RsrA system that controls expression of Trx (125), and a family of receptor-type tyrosine kinases encoded by c-RET proto-oncogene (85). H_2O_2 is now recognized to be the most important oxidant to have signaling properties in view of its longer half-life and diffusibility and specificity toward protein cysteine residues found in a particular environment that facilitates their deprotonation to a sulfenate (144).

ACTIVATION OF REDOX-SENSITIVE TRANSCRIPTION FACTORS IN INFLAMMATION

Activation of intracellular signaling pathways culminates with the transcription of genes. Early on, transcription factors were among the first signaling components to be identified as redox-sensitive signaling proteins. The DNA binding activity and possibly the transactivation potential of Sp1, AP-1, and p53 were shown to be regulated through specific cysteine motifs that needed to be reduced for activity (103, 108, 130). During oxidative stress/inflammation, NF-κB and AP-1 were activated in epithelial cells and inflammatory cells, leading to the upregulation of a number of proinflammatory genes (11, 108,

130). Here, we will concentrate on the mechanisms involved in the redox regulation of these two transcription factors.

Redox regulation of NF-κB

The members of the NF-κB/Rel family of transcription factors activate the transcription of many genes involved in lung inflammation and in the antioxidant response, including inducible nitric oxide synthase, proinflammatory cytokines, IL-1β, TNF-α, IL-6, the chemokine, IL-8, E-selectin, vascular cell adhesion molecule-1, intercellular adhesion molecule-1, granulocyte-macrophage colony-stimulating factor, and MnSOD (6, 46, 67, 87, 116, 130). NF-κB exists as a heterodimeric complex usually of p50 and p65/RelA subunits. In the absence of stimulation, NF-κB is found in the cytoplasm as an inactive, non-DNA binding form in association with inhibitory κB (IκB), an inhibitor protein that masks the nuclear translocation signal, thereby sequestering NF-κB in the cytoplasm. A large number of diverse agents have been shown to induce the activation of NF-κB through a similar mechanism, *i.e.,* the rapid phosphorylation by specific IκB kinases (83) of the inhibitory subunit, IκB (at Ser32 and Ser36 for IκBα), targeting IκB for ubiquitination and proteolytic degradation by the E3 ubiquitin-ligases ($E3RS^{I\kappa B}$) and the 26S proteasome, respectively (108, 130). This results in release of NF-κB, which can then freely translocate to the nucleus where it binds to cognate DNA elements. New data also reported IκB-independent mechanisms of activation of NF-κB where phosphorylation of p65 NF-κB by various kinases had an effect on the transactivation activity of NF-κB, independently of nuclear translocation (151).

Several years ago, it was proposed that activation of NF-κB was redox-sensitive based on studies with antioxidants suggesting that agonists activating NF-κB, in particular TNF-α, could stimulate the production of ROS (153). Exogenous H_2O_2 was also shown to induce NF-κB activation; however, it became clear later that this effect was cell-specific and that the requirement for intracellular generation of ROS was not universal, but cell- and stimulus-specific (8, 24, 69). Lipid peroxidation products (24, 79) or depletion of GSH and subsequent increase in cytosolic GSSG in response to oxidative stress were shown to induce rapid phosphorylation, ubiquitination, and degradation of IκB (63, 79). In addition, several novel mechanisms have been proposed for H_2O_2-induced activation of NF-κB, which include tyrosine phosphorylation of IκB (30, 152) and, more recently, activation of IκB kinase by H_2O_2 (133). However, it is not yet known whether oxidative stress and/or an imbalance in GSH redox status may directly stimulate the activity of IκBα kinase or elevated GSH levels inhibit IκBα kinase activity. It is also possible that changes in intracellular GSH redox status during oxidative stress may affect the proteasome enzymatic activity that leads to the activation of NF-κB (8, 174).

Nevertheless, other studies reported opposite effects. *In vitro* studies demonstrated inhibition of NF-κB DNA-binding activity by oxidant or alkylating agents such as diamide and *N*-ethylmaleimide, respectively (171), probably through oxidation of the cysteine residue at position 62 in p50 NF-κB, which has been shown to be essential for DNA-binding activity and is the targeted site for enhancement of activity by Trx (24). Increasing intracellular GSH following treatment with NAC resulted in inhibition of the phosphorylation of serine residues on IκBα following TNF-α treatment, leading to the down-regulation of NF-κB DNA-binding activity and expression of NF-κB subunits in endothelial cells (34). Overexpression of GCL, which resulted in increased GSH levels in rat hepatic cells, inhibited TNF-α-mediated NF-κB activation and NF-κB-dependent gene transcription through inhibition of IκBα degradation (104), possibly via inactivation of ubiquitin-conjugating enzymes, which are known to be redox-sensitive (77, 121). Pretreatment of airway epithelial cells with NAC or GSH inhibited NF-κB and subsequent IL-8 expression in response to TNF-α, whereas elevated levels of Trx protein enhanced these responses (68). Interestingly, accumulation of GSH occurred primarily in the cytoplasm, whereas Trx accumulated in the nucleus in response to TNF-α stimulation. These results suggest that the compartmentalization of thiols may be a key determinant in the regulation of redox-sensitive transcriptional activities. Schubert *et al*. showed that NAC and pomegranate wine (PW), a natural antioxidant-resveratrol, prevented NF-κB activation in bovine artery endothelial cells in response to TNF-α by blocking NF-κB nuclear translocation, without interfering with either IκB phosphorylation or degradation (159). Furthermore, rapid serine phosphorylation of IκB at sites other than Ser32/36 was observed in response to NAC and PW. NAC was also able to prevent phosphorylation of p65 on Ser536 following TNF-α exposure, an effect not observed with preincubation with PW. In PW-treated cells, IκBα resynthesis was not detected, whereas this *de novo* synthesis was observed with NAC and TNF-α. Taken together, the data suggest that thiols and antioxidants may inhibit NF-κB by regulating phosphorylation at multiple serine residues in both IκB and the p65 subunit of NF-κB. However, a new study provided evidence supporting a lack of role for ROS in NF-κB activation, arguing that NAC mediates its inhibitory effects in response to TNF-α through mechanisms independent of its effects on GSH levels (68). In fact, they showed that NAC inhibited TNF-α-stimulated signal transduction in L929 cells, a murine tumorigenic fibroblast cell line, by lowering the affinity of TNF receptor 1 for TNF-α and thereby preventing recruitment of RIP and TRAF2 to the receptor. Activation of NF-κB in response to TNF-α was also shown to play a critical role in protecting cells from TNF-induced cell death using cells lacking the p65 NF-κB subunit or other components of the pathway (84). Using murine embryonic fibroblasts deficient in TRAF2/TRAF5 or in p65, a recent study demonstrated that the pro-survival functions of NF-κB were to inhibit the TNF-induced intracellular accumulation of ROS that was responsible for the prolonged MAPK activation and necrotic cell death (146). Hence, the exact role of H_2O_2 or alterations in intracellular GSH redox status in the NF-κB pathway and downstream gene expression remains to be elucidated using novel tools and cellular models, and the contradictory effects may reflect distinct mechanisms at multiple points of activation in different cells types (24).

Redox regulation of AP-1

AP-1 is a leucine-zipper transcription factor composed of dimers that contain members from the JUN (c-Jun, JunB,

JunD), FOS (c-Fos, Fra1, Fra2), ATF, and MAF protein families, and has been shown to be critical in the regulation of genes involved in cell proliferation, differentiation, stress, apoptosis, and tumor promotion. AP-1 binds to consensus sequences in the TRE, which is present in the promoter region of a wide variety of genes implicated in cell proliferation and tumor promotion. This binding requires that the single conserved cysteine in the DNA-binding domain of each subunit that is required for electrostatic interactions with a zinc atom to form a "zinc finger" be maintained in its reduced state (1). AP-1 activity can be regulated at various levels, *i.e.,* dimer composition, posttranslational modifications, transcription, and interaction with other proteins (101).

Modulation of intracellular thiol levels in epithelial HeLa cells with NAC enhanced both untreated and TPA-stimulated AP-1 DNA binding and transactivation (21, 109), probably through regulation of this cysteine. The ratio of thiol to disulfide forms of glutathione, protein disulfide formation, and *S*-glutathiolation have been shown to be involved in the redox-regulation of c-Jun DNA binding (87). Oxidative stress imposed by endogenous generation of H_2O_2 or exogenous H_2O_2 treatment, UV irradiation, TNF-α, depletion of intracellular GSH with buthionine sulfoximine or increase in the GSH/GSSG ratio by diamide treatment of HepG2 cells may also increase AP-1 DNA binding (109). This may be due in part to the redistribution of GSH into the nucleus during these processes, in addition to the presence of Ref-1 and Trx, which facilitate AP-1 DNA binding (87, 109, 176, 185). In contrast, oxidized GSSG interfered with this binding, suggesting that the formation of the disulfide bond between cysteine residues inhibits AP-1 DNA binding (59, 70). In addition, Trx and Ref-1 are themselves regulated by redox cycling and could be the actual target of ROS. In particular, Trx has a cysteine that is in the thiolate form and is therefore a suitable target for H_2O_2. The neighboring thiol in Trx rapidly reacts with the sulfenic acid intermediate to produce an intramolecular disulfide. Although Ref-1 Cys64 was previously regarded as the redox catalytic site based on *in vitro* studies, an *in vivo* mutant where a cysteine to alanine point mutation was introduced showed normal levels of endogenous AP-1 DNA binding, indicating that this cysteine is not essential for redox regulation of Ref-1 and suggesting that other redox-active residues or another mechanism might be responsible for Ref-1 redox regulatory function (123).

It is important to note that this type of redox regulation of AP-1 occurs in the nucleus (150). In contrast, activation of AP-1 in the cytosol occurs through oxidative mechanisms, which involve oxidation and the activation of upstream cytosolic signaling pathways, including kinases as the activity of AP-1 is controlled by phosphorylation (181). Increased binding and transcriptional activation are both signaled by phosphorylation of c-Jun (122) by members of the MAPK family, which also control their transcription (39). Adler *et al.* showed that glutathione *S*-transferase (GST) pi regulates c-Jun N-terminal kinase (JNK) activity by the formation of a GSTpi–JNK complex (2), which prevents JNK activation and is maintained by free radical scavengers. Following exposure to an oxidative stress, GSTpi dissociates from JNK, allowing c-Jun phosphorylation to occur, which in turn culminates in AP-1 activation (7, 186). Further studies will be needed to understand better the redox regulation of AP-1, in particular the respective role of phosphorylation versus transcription and the composition of the active dimers.

Redox regulation of ARE (Nrf and Maf proteins)

It has been recognized recently that transcription factor Nrf2 is essential for the ARE-mediated induction of phase II detoxifying and GCL genes in response to electrophiles and phenolic antioxidants in HepG2 cells (70, 74, 180). It is now known that Nrf proteins bind to the ARE consensus sequence, which shows striking similarity to a binding motif referred to as the Maf recognition element (MARE), also known as the erythroid transcription factor (NF-E2) binding sequence. NF-E2 or MAREs are specifically recognized by either homodimers of small Maf family members (MafK, MafG, MafF) or by heterodimeric proteins composed of the Cap'n'Collar subfamily of basic leucine zipper transcription factors and small Maf partners, which mediate expression through AP-1/EpRE sequences. The NF-E2/AP-1 element and the ARE, which has been identified in several phase II genes, possess striking similarity. A protein Kelch-like ECH-associated protein1 (Keap1), which plays a role in the ARE-mediated signaling, has been identified as a negative regulator of Nrf2 that also acts as a possible intracellular sensor for thiol-active xenobiotics in HepG2 cells (75). Keap1 binds and retains Nrf2 in the cytoplasm where it is unable to function as a transcription factor. During transition to oxidizing conditions, Nrf2 dissociates from Keap1, allowing it to translocate to the nucleus where it heterodimerizes with small Maf proteins and activates ARE-driven transcription (49). The actual mechanism of dissociation of Nrf2 from Keap1 is currently unknown, but it is thought to involve thiol modifications or phosphorylation of Keap1, Nrf2, or both. Indeed, there is evidence in a cell-free system that cysteine thiols of Keap1 are reactive to inducers of phase 2 genes (49). Four of the most reactive cysteines reside in the region required for binding to Nrf2. Exposure of the Keap1/Nrf2 complex to the inducers resulted in the dissociation of the complex. Thus, the sulfhydryl groups of Keap1 are sensors regulating the induction of phase 2 enzymes (177). Therefore, under the conditions of initial depletion of intracellular GSH, Trx and/or production of ROS may lead to the dissociation of the complex and activation of Nrf2 (86). Furthermore, it has been shown recently that scaffolding of Keap1 to the actin cytoskeleton controls the function of Nrf2, and under oxidative stress and electrophilic stress disruption of the actin cytoskeleton promotes nuclear entry of an Nrf2 reporter protein (81). It has been also demonstrated that mice with site-directed mutation of the Nrf2 gene (Nrf2−/−) were more susceptible to the injurious effects of hyperoxia and cigarette smoke as noted by marked increase in pulmonary hyperpermeability, macrophage inflammation, and epithelial injury compared with the wild-type mice (70). A significant reduction in the mRNA expression of ARE-responsive lung antioxidant and phase 2 enzymes, some of which included heme oxygenase-1, glutathione peroxidase-2, and NAD(P)H:quinone oxidoreductase, was also observed in Nrf2−/− mice compared with normal mice. Thus, Nrf2 appears to protect against pulmonary hyperoxia injury and cigarette smoke in mice, presumably by up-regulating the transcription of lung antioxidant defense en-

zymes. However, the role of Keap1 and Nrf2, if any, in the induction of phase II genes, in particular GCL, in lung cells in response to oxidative stress remains to be explained. Keap1 also appears to regulate the degradation of Nrf2 via a proteasome-dependent pathway, and in mouse macrophages deficient in Keap1, Nrf2 accumulates in the nucleus (76, 90, 187). Electrophilic compounds have also been shown to cause Nrf2 nuclear translocation, concomitant with protein stabilization. Furthermore, electrophilic lipids, like prostaglandins, isoprostanes, and 4-hydroxynonenal, also cause the dissociation of Nrf2 from Keap1, resulting in the activation of the ARE.

REDOX REGULATION OF PTPS

The regulation of tyrosine phosphorylation represents a key mechanism in various cellular processes, and the role of PTPs in maintaining the low levels of tyrosine phosphorylation in quiescent cells and as critical components of signaling pathways has recently taken center stage. The PTP superfamily consists of close to 70 enzymes that all share the conserved signature motif CX_5R (X = amino acid) and have been grouped into four categories based on their functions, sequences, and structures: (a) tyrosine-specific phosphatases, (b) VH-1-like dual-specificity PTPs, (c) Cdc25, and (d) low-molecular-weight (LMW) phosphatases (53). Studies on the mechanism of catalysis shared by all PTPs showed that, due to the unique environment in the active site, the cysteine residue in the conserved motif has an unusually low pK_a and exists predominantly as a thiolate anion ($Cys\text{-}S^-$) at physiological pH. This thiolate is critical for the activity, as its mutation to a serine results in total loss of phosphatase activity (45). The thiolate anion could act as a nucleophile to attach the phosphoryl group of the phosphorylated amino acid, leading to the formation of a thiol phosphate intermediate (45, 53).

As large increases in cellular tyrosine phosphorylation were brought about by treatment of various cells with vanadate, a well known inhibitor of PTPs (65), or with oxidants or thiol-directed agents, the concept that the activity of PTPs might be modulated by ROS was introduced several years ago. It was hypothesized that the essential thiolate could react with H_2O_2 to form a sulfenic acid (Cys-SOH) intermediate, which is catalytically inactive (45) (Fig. 4). This intermediate quickly reacts with a thiol such as GSH to produce a disulfide in the PTP, a form that is also catalytically inactive. Importantly, the inhibited PTP could be fully reactivated by LMW thiols such as dithiothreitol and GSH or by Trx and glutaredoxin (20, 94).

PTP1B, which is involved in insulin signaling, was the first PTP to be shown to undergo oxidative regulation *in vivo* after treatment with epidermal growth factor (94) or insulin (102). $O_2^{\bullet -}$, probably in its protonated, hydroperoxyl radical ($HO_2^{\bullet}$) form (pK_a ~4.7), may also react with the thiolate anion in the microenvironment of a PTP. It has been reported that PTP1B may be more efficiently regulated by $O_2^{\bullet -}$ than by H_2O_2, with a calculated rate constant *in vitro* ($\sim 3 \times 10^2\ M^{-1}s^{-1}$) about eight times faster for $O_2^{\bullet -}$ than for H_2O_2 (20). PTP1B was also found to be glutathionylated at the active-site cysteine, a process that is thought to protect the Cys-SOH from further oxidation to $Cys\text{-}SO_2H$/$Cys\text{-}SO_3H$ during oxidative stress (20).

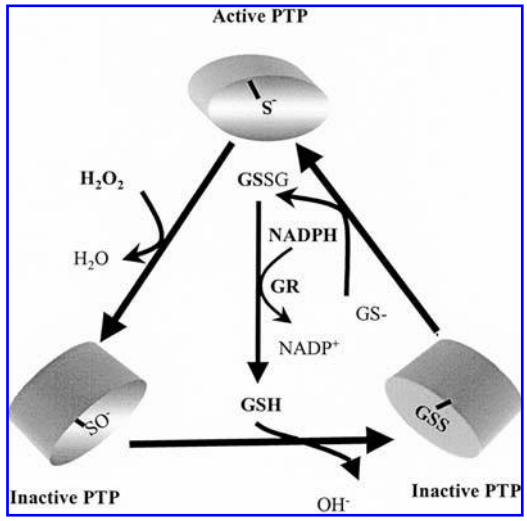

FIG. 4. The active-site thiolate in PTPs can react with H_2O_2 to form a sulfenate. This form of the enzyme is catalytically inactive. GSH can react with the sulfenate to form a mixed disulfide that can then react with another GSH to form GSSG and restore the active form of the PTP.

A recent report demonstrated that the Cys-SOH in PTP1B could be rapidly converted into a sulfenyl-amide intermediate in which the sulfur moiety of the catalytic cysteine is linked to the main-chain nitrogen of an adjacent residue (147). This modification, which causes large changes in the PTP1B active site, could also be a protective intermediate and might facilitate reactivation of PTP1B by biological thiols (175). The SH3-containing PTP, SHP-2, which is involved in platelet-derived growth factor (PDGF) signaling, was recently shown to be reversibly inactivated *in vivo* by H_2O_2 produced by PDGF stimulation (107). Interestingly, only SHP-2 associated with the autophosphorylated receptor was susceptible to transient inhibition by H_2O_2, emphasizing an additional level of specificity imparted by subcellular localization. Furthermore, a good correlation existed between the time course of inhibition of SHP-2 and that of activation of extracellular signal-regulated kinase (ERK)1/2, indicating that the sensitivity of the ERK pathway to H_2O_2 was due to targeting upstream of the ERK module.

Inactivation of some PTPs, such as PTEN (phosphatase and tensin homologue), Cdc25, and LMW-PTP, may involve the formation of a disulfide bond between the active-site cysteine and a neighboring thiol/cysteine (36). PTEN is a tumor suppressor that belongs to the PTP family despite having negligible PTP activity and predominantly functioning as a lipid phosphatase that removes the 3'-OH group from the phosphoinositide inositol ring in its substrate phosphatidylinositol trisphosphate (PIP3), thereby reversing the activity of phosphatidylinositol 3-kinase (PI3K) and modulating that of downstream molecules such as Ser/Thr protein kinase AKT, which can be activated by ROS (36). *In vitro* studies showed that Cys124 in the active site of PTEN was the target of oxidation by H_2O_2 and formed a disulfide with Cys71 during oxidation. This oxidation was reversible, predominantly through the action of

Trx, which was found to associate with PTEN in HeLa cells (95). Recently, Leslie *et al.* reported that oxidative inactivation of PTEN by exogenous H_2O_2 led to increased accumulation of PIP3 and activation of protein kinase B/AKT (96). In addition, endogenous production of H_2O_2 by lipopolysaccharide- or TPA-stimulated macrophages also inactivated a fraction of PTEN. Interestingly, the mammalian homologue of the forkhead transcription factor, FKHRL1, which is a target for the PI3K/AKT pathway, was shown to be regulated by a redox-dependent mechanism in p66Shc-null cells (118), which might be the result of the inhibition of PTEN by increased ROS production in these cells.

The LMW-PTPs are a family of 18-kDa enzymes involved in cell-growth regulation. Although they have limited sequence homologies with other PTPs, they share the signature motif; however, in addition to the catalytic Cys in position 12, another cysteine is present in position 17 within the active site, which is a unique feature among PTPs. During oxidation by PDGF-induced H_2O_2, Cys17 forms an intramolecular disulfide bond with Cys12, protecting the catalytic cysteine from further oxidation and facilitating GSH-dependent recovery of the activity (33). This family of phosphatases acts on growth factor-induced mitosis by dephosphorylating activated growth factor receptors (37) and on cytoskeletal rearrangement through dephosphorylation of p19RhoGAP and downstream regulation of the small GTPase Rho (33, 141). It was further shown that Rac-mediated ROS production resulted in down-regulation of Rho activity through reversible inhibition of LMW-PTP and increased tyrosine phosphorylation and activation of p190Rho-GAP (119).

The Cdc25s are a family of dual-specificity phosphatases that are critical components of cell-cycle progression and checkpoint control, and comprise Cdc25A, which regulates the G_1/S transition, and Cdc25B/Cdc25C, which are involved in G_2/M progression (20). The regulation of the activity of the Cdc25 is complex and involves phosphorylation, creating a binding site for 14–3-3 proteins, which may increase the cytoplasmic localization of Cdc25C. Their crystal structure showed that the reactive cysteine in the active site could form an intramolecular disulfide bond with another conserved cysteine in the molecule, suggesting a possible regulation by ROS. Such interaction was demonstrated on exposure to H_2O_2 of Cdc25C where Cys377 in the active site could undergo oxidation and a disulfide be formed between the Cys377-sulfenate and Cys330 in the invariant region, possibly rescuing the protein from irreversible oxidative damage (148). In fact, *in vitro* studies showed that the Cdc25s are highly susceptible to oxidation, and the rate of thiolate conversion to sulfenic acid by H_2O_2 for Cdc25B was 15-fold and 400-fold faster than for PTP1B and GSH, respectively. The intramolecular disulfides could not be reduced by GSH, but were rapidly and efficiently reduced by Trx (162). Thus, these rapid kinetics support a physiological role for reversible redox regulation of the Cdc25s during the cell cycle. However, several studies have reported cell-cycle arrest under oxidative conditions (19, 124). Cytosolic peroxiredoxin I and II can be phosphorylated on a threonine at position 90 by the CDK Cdc2, resulting in inhibition of its peroxidase activity and increased levels of H_2O_2 in HeLa cells during the cell cycle (31). It was suggested that this mechanism may be responsible for the oxidation of Cdc25C. Additional studies with antioxidants such as Trx (117) and GSH have revealed a reversal in mitotic arrest and phase-specific modulation (19). Thus, redox regulation of Cdc25 phosphatases may be an important component of the cell-cycle regulation machinery. The VH-1 group of dual-specificity phosphatases that control the activity of the MAPK have not been shown yet to undergo oxidation on their essential cysteine in the signature motif.

Hence, evidence is accumulating for a central role for the reversible oxidation and inhibition of PTPs during intracellular redox signaling for growth and apoptosis, and more PTPs are being identified that can undergo such changes. Nevertheless, further studies will be needed to understand better how specificity is achieved and whether subcellular colocalization of the source of peroxide and the phosphatase is required to achieve a physiologically significant rate of interaction between the thiolate and peroxide. Furthermore, the mechanisms of rereduction, although hypothesized, have not been clearly established. The role of Trx or glutaredoxin, in particular, will need to be further assessed.

CONCLUSIONS

The GSH redox status plays an important role in the protein modifications and signaling pathways, including a dual effect on redox-sensitive transcription factors. Protein *S*-glutathiolation as a candidate mechanism for protein regulation during intracellular redox changes has gained a renewed impetus in view of the involvement of oxidants/antioxidants in various disease processes. This mechanism has been rediscovered as an adaptive cellular response protecting critical regulatory molecules from permanent loss of function due to either oxidative or nitrosative stress. Emerging roles of ROS/RNS-mediated oxidative stress include cellular resistance to such oxidative stress, in addition to the modulation of signal transduction pathways, tyrosine phosphorylation/dephosphorylation, transcriptional regulation, posttranslational protein modification, proteolytic processing, ubiquitination, and proteolysis.

The upstream regulation of the MAPKs, transcriptional regulation of expression of various inflammatory mediators, regulation of expression and activity of PTPs, involvement of the oxidative species in cell proliferative mechanisms, induction of a multitude of antioxidant genes, and transient silencing of various protein functions by sulfenic-species formation are all important indicators as to how thiol-dependent reactions involving GSH, cysteine, methionine, and other LMW thiols may determine the overall outcome of an oxidative stress. GSH, being the most abundant intracellular thiol and by its ability of nuclear translocation, obviously plays a central role in the above-mentioned processes. Therefore, a cell uses every possible strategy to maintain GSH levels at optimum. The cell has evolved to sense the redox changes by sensing the balance between GSH and GSSG and deploys specific sensor molecules depending on the thermodynamic and redox potential at a given GSH/GSSG ratio.

GSH is also an important protective antioxidant in the lungs and is altered in the extracellular lining fluid in several inflammatory diseases. The GSH level is regulated by oxidants, antioxidants, proinflammatory mediators, and antiinflamma-

tory agents and growth factors in lung cells. Study of the mechanisms and critical levels of GSH present in various intracellular organelles required for the amplification of signal transduction and gene transcription in inflammation is an important area of further research. Various redox-sensitive mechanisms in the form of Trxs, peroxiredoxins, sulfiredoxins, and disulfide isomerases are involved in the oxidative signaling process with an ultimate aim to protect and modulate cell proteins and hence function during oxidative stress. Further research is required to elucidate the cellular and molecular redox-regulating mechanisms in inflammation that regulate proinflammatory mediators. Understanding of such events may lead to the design of novel antioxidant therapeutic strategies for the treatment and halting of the progression of various inflammatory diseases.

The current interest in protein-function regulation by protein *S*-thiolation will lead to the identification and characterization of an increasing number of novel targets of ROS/RNS-induced thiolated proteins and unravel the molecular mechanisms and posttranslational structural alterations. Such knowledge may provide new insights for the development and design of novel and effective therapeutic agents not only for a wide variety of chronic inflammatory lung disease, but also for other dreaded diseases, such as cancer and cardiovascular and neurodegenerative disorders.

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ABBREVIATIONS

AP-1, activator protein-1; ARDS, adult respiratory distress syndrome; ARE, antioxidant response element; COPD, chronic obstructive pulmonary disease; DUOX, dual function oxidase; EpRE, electrophilic response element; ERK, extracellular signal-regulated protein kinase; GC, glucocorticoid; GCL, glutamylcysteine ligase; GCLC, GCL catalytic subunit; GCLM, GCL modifying subunit; GR, glucocorticoid receptor; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione *S*-transferase; H_2O_2, hydrogen peroxide; IκB, inhibitory κB; IL, interleukin; IPF, idiopathic pulmonary fibrosis; JNK, c-Jun N-terminal kinase; LMW, low molecular weight; MAPK, mitogen-activated protein kinase; MARE, Maf recognition element; MnSOD, manganese superoxide dismutase; NAC, *N*-acetyl-L-cysteine; NF-κB, nuclear factor-κB; NOX, NADPH oxidase; $O_2^{\bullet -}$, superoxide anion; $^{\bullet}OH$, hydroxyl radical; $ONOO^-$, peroxynitrite; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol trisphosphate; PTEN, phosphatase and tensin homologue; PTP, protein tyrosine phosphatases; PW, pomegranate wine; RNS, reactive nitrogen species; ROS, reactive oxygen species; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; TRE, TPA-responsive element; Trx, thioredoxin.

REFERENCES

1. Abate C, Patel L, Rausher FJ, and Curran T. Redox regulation of fos and jun DNA-binding activity in vitro. *Science* 249: 1157–1161, 1990.
2. Adler V, Yin Z, Fuchs SY, Benezra M, Rosario L, Tew KD, Pincus MR, Sardana M, Henderson CJ, Wolf CR, Davis RJ, and Ronai Z. Regulation of JNK signaling by GSTp. *EMBO J* 18: 1321–1334, 1999.
3. Adler V, Yin Z, Tew KD, and Ronai Z. Role of redox potential and reactive oxygen species in stress signaling. *Oncogene* 18: 6104–6111, 1999.
4. Aikawa R, Komuro I, Yamazaki T, Zou Y, Kudoh S, Tanaka M, Shiojima I, Hiroi Y, and Yazaki Y. Oxidative stress activates extracellular signal-regulated kinases through Src and Ras in cultured cardiac myocytes of neonatal rats. *J Clin Invest* 100: 1813–1821, 1997.
5. Akerboom TPM and Sies H. Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. *Methods Enzymol* 77: 373–382, 1981.
6. Akira S and Kishimoto A. NF-IL6 and NF-κB in cytokine gene regulation. *Adv Immunol* 65: 1–46, 1997.
7. Allen RG and Tresini M. Oxidative stress and gene regulation. *Free Radic Biol Med* 28: 463–499, 2000.
8. Anderson M, Stall E, Gitler C, and Herzenberg L. Separation of oxidant-initiated and redox-regulated steps in the NF-κB signal transduction pathway. *Proc Natl Acad Sci U S A* 91: 11527–11531, 1994.
9. Antonicelli F, Parmentier M, Rahman I, Drost E, Hirani N, Donaldson K, and MacNee N. Nacystelyn inhibits hydrogen peroxide mediated interleukin-8 expression in human alveolar epithelial cells. *Free Radic Biol Med* 32: 492–502, 2002.
10. Aoki T, Suzuki Y, Suzuki K, Miyata A, Oyamada Y, Takasugi T, More M, Fujita H, and Yamaguchi K. Modulation of ICAM-1 expression by extracellular glutathione in hyperoxia-exposed human pulmonary artery endothelial cells. *Am J Respir Cell Mol Biol* 15: 319–327, 1996.
11. Arrigo A-P. Gene expression and the thiol redox state. *Free Radic Biol Med* 27: 936–944, 1999.
12. Arsalane K, Dubois CM, Muanz T, Begin R, Boudreau F, Asselin C, and Cantin AM. Transforming growth factor-1 is a potent inhibitor of glutathione synthesis in the lung epithelial cell line A549: transcriptional effect on the GSH rate-limiting enzyme γ-glutamylcysteine synthetase. *Am J Respir Cell Mol Biol* 17: 599–607, 1997.
13. Aruoma OI, Halliwell B, and Dizdaroglu M. Iron ion-dependent modification of bases in DNA by the superoxide radical-generating system hypoxanthine/xanthine oxidase. *J Biol Chem* 264: 13024–13028, 1989.
14. Aruoma OI, Halliwell B, Hoey BM, and Beutler J. The antioxidant action of *N*-acetyl-L-cysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide and hypochloric acid. *Free Radic Biol Med* 6: 593–597, 1989.

15. Aslund F and Beckwith J. Bridge over troubled waters: sensing stress by disulfide bond formation. *Cell* 96: 751–753, 1999.
16. Azzi A, Aratri E, Boscoboinik D, Clement S, Ozer NK, Ricciarelli R, and Spycher S. Molecular basis of alpha-tocopherol control of smooth muscle cell proliferation. *Biofactors* 7:3–14, 1998.
17. Babior BM. NADPH oxidase. *Curr Opin Immunol* 16: 42–47, 2004.
18. Banfi B, Tirone F, Durussel I, Knisz J, Moskwa P, Molnar GZ, Krause KH, and Cox JA. Mechanism of Ca^{2+} activation of the NADPH oxidase5 (NOX5). *J Biol Chem* 279: 18583–18591, 2004.
19. Barnouin K, Dubuisson ML, Child ES, Fernandez de Mattos S, Glassford J, Medema RH, Mann DJ, and Lam EW. H_2O_2 induces a transient multi-phase cell cycle arrest in mouse fibroblasts through modulating cyclin D and p21Cip1 expression. *J Biol Chem* 277: 13761–13770, 2002.
20. Barrett WC, DeGnore JP, Konig S, Fales HM, Keng YF, Zhang ZY, Yim MB, and Chock PB. Regulation of PTP1B via glutathionylation of the active site cysteine 215. *Biochemistry* 38: 6699–6705, 1999.
21. Bergelson S, Pinkus R, and Daniel V. Intracellular glutathione levels regulate Fos/Jun induction and activation of glutathione *S*-transferase gene expression. *Cancer Res* 54: 36–40, 1994.
22. Biteau B, Labarre J, and Toledano MB. ATP-dependent reduction of cysteine-sulfinic acid by *S. cerevisiae* sulfiredoxin. *Nature* 425: 980–984, 2003.
23. Boveris A and Cadenas E. Cellular sources and steady-state levels of reactive oxygen species. In: *Oxygen, Gene Expression, and Cellular Function, Lung Biology in Health and Disease,* edited by Clerch LB and Massaro DJ. New York: Marcel Dekker, 1997, pp. 1–25.
24. Bowie A and O'Neill LAJ. Oxidative stress and nuclear factor-κB activation: a reassessment of the evidence in the light of recent discoveries. *Biochem Pharmacol* 59: 13–23, 2000.
25. Bradshaw RA, Kanarek L, and Hill RL. The preparation, properties, and reactivation of the mixed disulfide derivative of egg white lysozyme and L-cystine. *J Biol Chem* 242: 3789–3798, 1967.
26. Brown LAS. Glutathione protects signal transduction in type II cells under oxidant stress. *Am J Physiol* 266: L172–L177, 1994.
27. Bump EA and Reed DJ. A unique property of fetal bovine serum: high levels of protein-glutathione mixed disulfides. *In Vitro* 13: 115–118, 1977.
28. Cantin AM, North SL, Hubbard RC, and Crystal RG. Normal alveolar epithelial lining fluid contains high levels of glutathione. *Am J Physiol* 63: 152–157, 1987.
29. Cantin AM, Larivee P, and Begin R. Extracellular glutathione suppresses human lung fibroblast proliferation. *Am J Respir Cell Mol Biol* 3: 79–85, 1990.
30. Canty TG Jr, Boyle EM Jr, Farr A, Morgan EN, Verrier ED, and Pohlman TH. Oxidative stress induces NF-κB nuclear translocation without degradation of IκBα. *Circulation* 100: 11361–11364, 1999.
31. Chang TS, Jeong W, Choi SY, Yu S, Kang SW, and Rhee SG. Regulation of peroxiredoxin I activity by Cdc2-mediated phosphorylation. *J Biol Chem* 277: 25370–25376, 2002.
32. Chen JW, Dodia C, Feinstein SI, Jain MK, and Fisher AB. 1-Cys peroxiredoxin, a bifunctional enzyme with glutathione peroxidase and phospholipase A2 activities. *J Biol Chem* 275: 28421–28427, 2000.
33. Chiarugi P, Cirri P, Taddei L, Giannoni E, Camici G, Manao G, Raugei G, and Ramponi G. The low M(r) protein-tyrosine phosphatase is involved in Rho-mediated cytoskeleton rearrangement after integrin and platelet-derived growth factor stimulation. *J Biol Chem* 275: 4640–4646, 2000.
34. Cho S, Urata Y, Iida T, Goto S, Yamaguchi M, Sumikawa K, and Kondo T. Glutathione downregulates the phosphorylation of IκB: Autoloop regulation of the NF-κB-mediated expression of NF-κB subunits by TNF-α in mouse vascular endothelial cells. *Biochem Biophys Res Commun* 253: 104–108, 1998.
35. Cho S, Hazama M, Urata Y, Goto S, Horiuchi S, Sumikawa K, and Kondo T. Protective role of glutathione synthesis in response to oxidized low density lipoprotein in human vascular endothelial cells. *Free Radic Biol Med* 26: 589–602, 1999.
36. Cho SH, Lee CH, Ahn Y, Kim H, Kim H, Ahn CY, Yang KS, and Lee SR. Redox regulation of PTEN and protein tyrosine phosphatases in H_2O_2-mediated cell signalling. *FEBS Lett* 560: 7–13, 2004.
37. Cirr P, Chiarugi P, Taddei L, Raugei G, Camici G, Manao G, and Ramponi G. Low molecular weight protein-tyrosine phosphatase tyrosine phosphorylation by c-Src during platelet-derived growth factor-induced mitogenesis correlates with its subcellular targeting. *J Biol Chem* 273: 32522–32527, 1998.
38. Claiborne A, Yeh JI, Mallett TC, Luba J, Crane EJ 3rd, Charrier V, and Parsonage D. Protein-sulfenic acids: diverse roles for an unlikely player in enzyme catalysis and redox regulation. *Biochemistry* 38: 15407–15416, 1999.
39. Cobb MH. MAP kinase pathways. *Prog Biophys Mol Biol* 71: 479–500, 1999.
40. Cotgreave IA and Gerdes RG. Recent trends in glutathione biochemistry: glutathione–protein interactions: a molecular link between oxidative stress and cell proliferation. *Biochem Biophys Res Commun* 242: 1–9, 1998.
41. Das KC. c-Jun NH_2-terminal kinase-mediated redox-dependent degradation of IκB: role of thioredoxin in NF-κB activation. *J Biol Chem* 276: 4662–4670, 2001.
42. Das KC, Lewis-Molock Y, and White CW. Activation of NF-κB and elevation of MnSOD gene expression by thiol reducing agents in lung adenocarcinoma (A549) cells. *Am J Physiol* 269: L588–L602, 1995.
43. De Boer WI, van Schadewijk A, Sont JK, Sharma HS, Stolk J, Hiemstra PJ, and van Krieken JHJM. Transforming growth factor β1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 158: 1–7, 1998.
44. Denu JM and Dixon JE. Protein tyrosine phosphatases: mechanisms of catalysis and regulation. *Curr Opin Chem Biol* 2: 633–641, 1998.

45. Denu JM and Tanner KG. Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry* 37: 5633–5642, 1998.
46. Desai A, Huang X, and Warren JS. Intracellular glutathione redox status modulates MCP-1 expression in pulmonary granulomatous vasculitis. *Lab Invest* 79: 837–847, 1999.
47. Dickinson DA, Iles KE, Watanabe N, Iwamoto T, Zhang H, Krzywanski DM, and Forman HJ. 4-Hydroxynonenal induces glutamate cysteine ligase through JNK in HBE1 cells. *Free Radic Biol Med* 33: 974–987, 2002.
48. Dickinson DA, Iles KE, Zhang H, Blank V, and Forman HJ. Curcumin alters EpRE and AP-1 binding complexes and elevates glutamate-cysteine ligase gene expression. *FASEB J* 17: 473–475, 2003.
49. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, Yamamoto M, and Talalay P. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc Natl Acad Sci U S A* 99: 11908–11913, 2002.
50. Droge W, Schulze-Osthoff K, Mihm S, Galter D, Schenk H, Eck H., Roth S, and Gmunder H. Functions of glutathione and glutathione disulfide in immunology and immunopathology. *FASEB J* 8: 1131–1138, 1994.
51. Eaton DL and Hamel DM. Increase in γ-glutamylcysteine synthetase activity as a mechanism for butylated hydroxyanisole-mediated elevation of hepatic glutathione. *Toxicol Appl Pharmacol* 126: 145–149, 1994.
52. Erickson AM, Nevarea Z, Gipp JJ, and Mulcahy RT. Identification of a variant antioxidant response element in the promoter of the human glutamate-cysteine ligase modifier subunit gene. Revision of the ARE consensus sequence. *J Biol Chem* 277: 30730–30737, 2002.
53. Fauman EB and Saper MA. Structure and function of the protein tyrosine phosphatases. *Trends Biochem Sci* 21: 413–417, 1996.
54. Finkel T. Signal transduction by reactive oxygen species in non-phagocytic cells. *J Leukoc Biol* 65: 337–340, 1999.
55. Forman HJ and Dickinson DA. Oxidative signaling and glutathione synthesis. *Biofactors* 17: 1–12, 2003.
56. Forman HJ, Torres M, and Fukuto J. Redox signaling. *Mol Cell Biochem* 234–235: 49–62, 2002.
57. Galloway DC and McLellan LI. Inducible expression of the gamma-glutamylcysteine synthetase light subunit by *t*-butyl hydroquinone in HepG2 cells is not dependent on an antioxidant-responsive element. *Biochem J* 336: 535–539, 1998.
58. Galloway DC, Blake DG, Shepherd AG, and McLellan LI. Regulation of human γ-glutamylcysteine synthetase: co-ordinate induction of the catalytic and regulatory subunits in HepG2 cells. *Biochem J* 328: 99–104, 1997.
59. Galter D, Mihm S, and Droge W. Distinct effects of glutathione disulphide on the nuclear transcription factors κB and the activator protein-1. *Eur J Biochem* 221: 639–648, 1994.
60. Geiszt M, Witta J, Baffi J, Lekstrom K, and Leto TL. Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense. *FASEB J* 17: 1502–1504, 2003.
61. Gilbert HF. Biological disulfides: the third messenger? Modulation of phosphofructokinase activity by thiol/disulfide exchange. *J Biol Chem* 257: 12086–12091, 1982.
62. Gilbert HF. Redox control of enzyme activities by thiol/disulfide exchange. *Methods Enzymol* 107: 330–351, 1984.
63. Ginn-Pease ME and Whisler RL. Optimal NF-κB mediated transcriptional responses in Jurkat T cells exposed to oxidative stress are dependent on intracellular glutathione and costimulatory signals. *Biochem Biophys Res Commun* 226: 695–702, 1996.
64. Go YM, Gipp JJ, Mulcahy RT, and Jones DP. H_2O_2-dependent activation of GCLC-ARE4 reporter occurs by mitogen-activated protein kinase pathways without oxidation of cellular glutathione or thioredoxin-1. *J Biol Chem* 279: 5837–5845, 2004.
65. Gordon JA. Use of vanadate as protein-phosphotyrosine phosphatase inhibitor. *Methods Enzymol* 201: 477–482, 1991.
66. Gosset P, Wallaert B, Tonnel AB, and Fourneau C. Thiol regulation of the production of TNF-α, IL-6, IL-8 by human alveolar macrophages. *Eur Respir J* 14: 98–105, 1999.
67. Gotoh Y and Cooper JA. Reactive oxygen species- and dimerization-induced activation of apoptosis signal-regulating kinase 1 in tumor necrosis factor-alpha signal transduction. *J Biol Chem* 273: 17477–17482, 1998.
68. Harper R, Wu K, Chang MMJ, Chang MJ, Yoneda K, Pan R, Reddy SPM, and Wu R. Activation of nuclear factor-κB transcriptional activity in airway epithelial cells by thioredoxin but not by *N*-acetyl-cysteine and glutathione. *Am J Respir Cell Mol Biol* 25: 178–185, 2001.
69. Hayakawa M, Miyashita H, Sakamoto I, Kitagawa M, Tanaka H, Yasuda H, Karin M, and Kikugawa K. Evidence that reactive oxygen species do not mediate NF-κB activation. *EMBO J* 22: 2356–3366, 2003.
70. Hayes JD and McLellan LI. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free Radic Res* 31: 273–300, 1999.
71. Hirota K, Matsui M, Iwata S, Nishiyama A, Mori K, and Yodoi J. AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. *Proc Natl Acad Sci U S A* 94: 3633–3638, 1997.
72. Holmgren A. Thiols of thioredoxin and glutaredoxin in redox signaling. In: *Signal Transduction by Reactive Oxygen and Nitrogen Species: Pathways and Chemical Principles,* edited by Forman HJ, Fukuto J, and Torres M. Dordrecht and Boston: Kluwer Academic Publishers, 2003, pp. 33–52.
73. Huang CS, Chang LS, Anderson ME, and Meister A. Catalytic and regulatory properties of the heavy subunit of rat kidney γ-glutamylcysteine synthetase. *J Biol Chem* 268: 19675–19680, 1993.
74. Ishii T, Itoh K, Takahashi S, Sato H, Yanagawa T, Katoh Y, Bannai S, and Yamamoto M. Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. *J Biol Chem* 275: 16023–16029, 2000.

75. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, and Yamamoto M. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev* 13: 76–86, 1999.
76. Itoh K, Wakabayashi N, Katoh Y, Ishii T, O'Connor T, and Yamamoto M. Keap1 regulates both cytoplasmic-nuclear shuttling and degradation of Nrf2 in response to electrophiles. *Genes Cells* 8: 379–391, 2003.
77. Jahngen-Hodge J, Obin MS, Gong X, Shang F, Nowell TR Jr, Gong J, Absasi H, Blumberg J, and Taylor A. Regulation of ubiquitin-conjugating enzymes by glutathione following oxidative stress. *J Biol Chem* 272: 28218–28226, 1997.
78. Jakob U, Muse W, Eser M, and Bardwell JC. Chaperone activity with a redox switch. *Cell* 96: 341–352, 1999.
79. Janssen-Heininger YMW, Macara I, and Mossman BT. Cooperativity between oxidants and tumor necrosis factor in the activation of nuclear factor-kappa B. *Am J Respir Cell Mol Biol* 20: 942–952, 1999.
80. Jardine H, MacNee W, Donaldson K, and Rahman I. Molecular mechanism of transforming growth factor (TGF)-β_1-induced glutathione depletion in alveolar epithelial cells. Involvement of AP-1/ARE and Fra-1. *J Biol Chem* 277: 21158–21166, 2002.
81. Kang MI, Kobayashi A, Wakabayashi N, Kim SG, and Yamamoto M. Scaffolding of Keap1 to the actin cytoskeleton controls the function of Nrf2 as key regulator of cytoprotective phase 2 genes. *Proc Natl Acad Sci U S A* 101: 2046–2051, 2004.
82. Kang SW, Chang TS, Lee TH, Kim ES, Yu DY, and Rhee SG. Cytosolic peroxiredoxin attenuates the activation of Jnk and p38 but potentiates that of Erk in HeLa cells stimulated with tumor necrosis factor-alpha. *J Biol Chem* 279: 2535–2543, 2004.
83. Karin M and Delhase M. The IκB kinase (IKK) and NF-κB: key elements of proinflammatory signalling. *Semin Immunol* 12: 85–98, 2000.
84. Karin M and Lin A. NF-kappaB at the crossroads of life and death. *Nat Immunol* 3: 221–227, 2002.
85. Kato M, Iwashita T, Takeda K, Akhand AA, Liu W, Yoshihara M, Asai N, Suzuki H, Takahashi M, and Nakashima I. Ultraviolet light induces redox reaction-mediated dimerization and superactivation of oncogenic Ret tyrosine kinases. *Mol Biol Cell* 11: 93–101, 2000.
86. Kim YC, Yamaguchi Y, Kondo N, Masutani H, and Yodoi J. Thioredoxin-dependent redox regulation of the antioxidant responsive element (ARE) in electrophile response. *Oncogene* 22: 1860–1865, 2003.
87. Klatt P, Molina EP, De Lacoba MG, Padilla CA, Martinez-Galisteo E, Barcena JA, and Lamas S. Redox regulation of c-Jun DNA binding by reversible *S*-glutathiolation. *FASEB J* 13: 1481–1490, 1999.
88. Kokura S, Wolf RE, Yoshikawa T, Granger DN, and Aw TY. Molecular mechanisms of neutrophil-endothelial cell adhesion induced by redox imbalance. *Circ Res* 84: 516–524, 1999.
89. Krzywanski DM, Dickinson DA, Iles KE, Wigley AF, Franklin CC, Liu RM, Kavanagh TJ, and Forman HJ. Variable regulation of glutamate cysteine ligase subunit proteins affects glutathione biosynthesis in response to oxidative stress. *Arch Biochem Biophys* 423: 116–125, 2004.
90. Kwak MK, Wakabayashi N, Greenlaw JL, Yamamoto M, and Kensler TW. Antioxidants enhance mammalian proteasome expression through the Keap1-Nrf2 signaling pathway. *Mol Cell Biol* 23: 8786–8794, 2003.
91. Lakshminarayanan V, Drab-Weiss EA, and Roebuck KA. Hydrogen peroxide and tumor necrosis factor-alpha induce differential binding of the redox-responsive transcription factors AP-1 and NF-κB to the interleukin-8 promoter in endothelial and epithelial cells. *J Biol Chem* 273: 32670–32678, 1996.
92. Lambeth JD. NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol* 4: 181–189, 2004.
93. Lee AC, Fenster BE, Ito H, Takeda K, Bae NS, Hirai T, Yu ZX, Ferrans VJ, Howard BH, and Finkel T. Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species. *J Biol Chem* 274: 7936–7940, 1999.
94. Lee SR, Kwon KS, Kim SR, and Rhee SG. Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. *J Biol Chem* 273: 15366–15372, 1998.
95. Lee SR, Yang KS, Kwon J, Lee C, Jeong W, and Rhee SG. Reversible inactivation of the tumor suppressor PTEN by H_2O_2. *J Biol Chem* 277: 20336–20342, 2002.
96. Leslie NR, Bennett D, Lindsay YE, Stewart H, Gray A, and Downes CP. Redox regulation of PI 3-kinase signalling via inactivation of PTEN. *EMBO J* 22: 5501–5510, 2003.
97. Li XY, Donaldson K, Rahman I, and MacNee W. An investigation of the role of glutathione in the increased permeability induced by cigarette smoke in vivo and in vitro. *Am Rev Respir Crit Care Med* 149: 1518–1525, 1994.
98. Liu RM, Vasiliou V, Zhu H, Duh JL, Tabor MW, Puga A, Nebert DW, Sainsbury M, and Shertzer HG. Regulation of [Ah] gene battery enzymes and glutathione levels by 5,10-dihydroindeno[1,2-b]indole in mouse hepatoma cell lines. *Carcinogenesis* 15: 2347–2352, 1994.
99. Liu RM, Gao L, Choi J, and Forman HJ. γ-Glutamylcysteine synthetase: mRNA stabilisation and independent subunit transcription by 4-hydroxy-2-nonenal. *Am J Physiol* 275: L861–L869, 1998.
100. Liu RM, Liu Y, Forman HJ, Olman M, and Tarpey MM. Glutathione regulates transforming growth factor beta stimulated collagen production in fibroblasts. *Am J Physiol Lung Cell Mol Physiol* 286: L121–L128, 2004.
101. Macian F, Lopez-Rodrigues C, and Rao A. Partners in transcription: NFAT and AP-1. *Oncogene* 20: 2476–2489, 2001.
102. Mahadev K, Zilbering A, Zhu L, and Goldstein BJ. Insulin-stimulated hydrogen peroxide reversibly inhibits protein-tyrosine phosphatase 1b in vivo and enhances the early insulin action cascade. *J Biol Chem* 276: 21938–21942, 2001.
103. Mallis RJ, Buss JE, and Thomas JA. Oxidative modification of H-ras: *S*-thiolation and *S*-nitrosylation of reactive cysteines. *Biochem J* 355: 145–153, 2001.
104. Manna SK, Kuo MT, and Aggarwal BB. Overexpression of γ-glutamylcysteine synthetase suppresses tumor necro-

sis factor-induced apoptosis and activation of nuclear transcription factor-kappa B and activator protein-1. *Oncogene* 18: 4371–4382, 1999.
105. McCord J and Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* 244: 6049–6059,1969.
106. Meister A and Anderson ME. Glutathione. *Annu Rev Biochem* 52: 711–760, 1983.
107. Meng TC, Fukada T, and Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo. *Mol Cell* 9: 387–399, 2002.
108. Mercurio F and Manning AM. NF-κB as a primary regulator of the stress response. *Oncogene* 18: 6163–6171, 1999.
109. Meyer M, Schreck R, and Baeuerle PA. Hydrogen peroxide and antioxidants have opposite effects on activation of NF-κB and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J* 12: 2005–2015, 1993.
110. Moellering D, McAndrew J, Jo H, and Darley-Usmar VM. Effects of pyrrolidine dithiocarbamate on endothelial cells: protection against oxidative stress. *Free Radic Biol Med* 26: 1138–1145, 1999.
111. Monova H and Mulcahy RT. An electrophile response element (EpRE) regulates β-naphthoflavone induction of the human γ-glutamylcysteine synthetase regulatory subunit gene: constitutive expression is mediated by an adjacent AP-1 site. *J Biol Chem* 273: 14683–14689, 1998.
112. Moran JA, Dahl EL, and Mulcahy RT. Differential induction of mafF, mafG and mafK expression by electrophile-response-element activators. *Biochem J* 361: 371–377, 2002.
113. Morrison D, Rahman I, Lannan S, and MacNee W. Epithelial permeability, inflammation and oxidant status in the airspaces of chronic smokers. *Am J Respir Crit Care Med* 159: 473–479, 1999.
114. Mulcahy RT, Wartman MA, Bailey HH, and Gipp JJ. Constitutive and β-napthoflavonone-induced expression of the human γ-glutamylcysteine synthetase heavy subunit gene is regulated by a distal antioxidant response element/TRE sequence. *J Biol Chem* 272: 7445–7454, 1997.
115. Nakajima Y, Aoshiba K, Yasui S, and Nagai A. Hydrogen peroxide induces apoptosis in bovine tracheal epithelial cells in vitro. *Life Sci* 64: 2489–2496, 1999.
116. Nathens AB, Bitar R, Watson RW, Issekutz TB, Marshall JC, Dackiw AP, and Rotstein OD. Thiol-mediated regulation of ICAM-1 expression in endotoxin-induced acute lung injury. *J Immunol* 160: 2959–2966, 1998.
117. Natsuyama S, Noda Y, Yamashita M, Nagaham Y, and Mori T. Superoxide dismutase and thioredoxin restore defective p34cdc2 kinase activation in mouse two-cell block. *Biochim Biophys Acta* 1176: 90–94, 1993.
118. Nemoto S and Finkel T. Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway. *Science* 295: 2450–2452, 2002.
119. Nimnual AS, Taylor LJ, and Bar-Sagi D. Redox-dependent downregulation of Rho by Rac. *Nat Cell Biol* 5: 236–241, 2003.
120. Obaya AJ and Sedivy JM. Regulation of cyclin-Cdk activity in mammalian cells. *Cell Mol Life Sci* 59: 126–142, 2002.
121. Obin M, Shang F, Gong X, Handelman G, Blumberg J, and Taylor A. Redox regulation of ubiquitin-conjugating enzymes: mechanism insights using the thiol-specific oxidant diamide. *FASEB J* 12: 561–569, 1998.
122. Okazaki K and Sagata N. The Mos/MAP kinase pathway stabilizes c-Fos by phosphorylation and augments its transforming activity in NIH 3T3 cells. *EMBO J* 14: 5048–5059, 1995.
123. Ordway JM, Eberhart D, and Curran T. Cysteine 64 of Ref-1 is not essential for redox regulation of AP-1 DNA binding. *Mol Cell Biol* 23: 4257–4266, 2003.
124. O'Reilly MA. Redox activation of $p21^{Cip1/WAF1/Sdi1}$: a multifunctional regulator of cell survival and death. *Antioxid Redox Signal* 7: 108–118, 2005.
125. Paget MS, Kang JG, Roe JH, and Buttner MJ. Sigma R, an RNA polymerase sigma factor that modulates expression of the thioredoxin system in response to oxidative stress in Streptomyces coelicolor A3(2). *EMBO J* 17: 5776–5782, 1998.
126. Parmentier M, Hirani N, Rahman I, Donaldson K, and Antonicelli F. Regulation of LPS-mediated IL-1β by *N*-acetyl-L-cysteine in THP-1 cells. *Eur Respir J* 16: 933–939, 2000.
127. Pietarinen-Runtti P, Raivio KO, Saksela M, Asikainen TM, and Kinnula VL. Antioxidant enzyme regulation and resistance to oxidants of human bronchial epithelial cells cultured under hypoxic conditions. *Am Respir Cell Mol Biol* 19: 286–292, 1998.
128. Pinkus R, Weiner LM, and Daniel V. Role of oxidants and antioxidants in the induction of AP-1, NF-κB and glutathione *S*-transferase gene expression. *J Biol Chem* 271: 13422–13429, 1996.
129. Rahman I and MacNee W. Role of oxidants/antioxidants in smoking-induced airways diseases. *Free Radic Biol Med* 21: 669–681, 1996.
130. Rahman I and MacNee W. Role of transcription factors in inflammatory lung diseases. *Thorax* 53: 601–612, 1998.
131. Rahman I and MacNee W. Lung glutathione and oxidative stress: implications in cigarette smoke-induced airway disease. *Am J Physiol* 277: L1067–L1088, 1999.
132. Rahman I and MacNee W. Regulation of redox glutathione levels and gene transcription in lung inflammation: therapeutic approaches. *Free Radic Biol Med* 28: 1405–1420, 2000.
133. Rahman I and MacNee W. Oxidative stress and regulation of glutathione synthesis in lung inflammation. *Eur Respir J* 16: 534–554, 2000.
134. Rahman I, Bel A, Mulier B, Lawson MF, Harrison DJ, MacNee W, and Smith CAD. Transcriptional regulation of γ-glutamylcysteine synthetase-heavy subunit by oxidants in human alveolar epithelial cells. *Biochem Biophys Res Commun* 229: 832–837, 1996.
135. Rahman I, Morrison D, Donaldson K, and MacNee W. Systemic oxidative stress in asthma, COPD, and smokers. *Am J Respir Crit Care Med* 154: 1055–1060, 1996.
136. Rahman I, Bel A, Mulier B, Donaldson K, and MacNee W. Differential effects of oxidants and dexamethasone on γ-glutamylcysteine synthetase and γ-glutamyl transpeptidase in alveolar epithelial cells. *Am J Physiol* 275: L80–L86, 1998.

137. Rahman I, Smith CAD, Antonicelli F, and MacNee W. Characterisation of γ-glutamylcysteine-heavy subunit gene promoter: critical role for AP-1. *FEBS Lett* 427: 129–133, 1998.
138. Rahman I, Antonicelli F, and MacNee W. Molecular mechanism of the regulation of glutathione synthesis by tumour necrosis factor-α and dexamethasone in human alveolar epithelial cells. *J Biol Chem* 274: 5088–5096, 1999.
139. Rahman I, Mulier B, Gilmour PS, Watchom T, Donaldson K, Jeffery PK, and MacNee W. Oxidant-mediated lung epithelial cell tolerance: the role of intracellular glutathione and nuclear factor-κB. *Biochem Pharmacol* 62: 787–794, 2001.
140. Rahman I, Gilmour PS, Jimenez LA, and MacNee W. Oxidative stress and TNF-alpha induce histone acetylation and NF-kappaB/AP-1 activation in alveolar epithelial cells: potential mechanism in gene transcription in lung inflammation. *Mol Cell Biochem* 234–235: 239–248, 2002.
141. Raugei G, Ramponi G, and Chiarugi P. Low molecular weight protein tyrosine phosphatases: small, but smart. *Cell Mol Life Sci* 59: 941–949, 2002.
142. Redington AE, Madden J, Frew AJ, Djukanovic R, Roche WR, Holgate ST, and Howarth PH. Transforming growth factor-beta 1 in asthma: measurement in bronchoalveolar lavage fluid. *Am J Respir Crit Care Med* 156: 642–647. 1997.
143. Reeves EP, Lu H, Jacobs HL, Messina CG, Bolsover S, Gabella G, Potma EO, Warley A, Roes J, and Segal AW. Killing activity of neutrophils is mediated through activation of proteases by K^+ flux. *Nature* 416: 291–297, 2002.
144. Reth M. Hydrogen peroxide as second messenger in lymphocyte activation. *Nat Immunol* 3: 1129–1134, 2002.
145. Rhee SG, Kang SW, Netto LE, Seo MS, and Stadtman ER. A family of novel peroxidases, peroxiredoxins. *Biofactors* 10: 207–209, 1999.
146. Sakon S, Xue X, Takekawa M, Sasazuki T, Okazaki T, Kojima Y, Pioao JY, Yagita H, Okumura K, Doi T, and Nakano H. NF-κB inhibits TNF-induced accumulation of ROS that mediate prolonged MAPK activation and necrotic cell death. *EMBO J* 22: 3898–3909, 2003.
147. Salmeen A, Andersen JN, Myers MP, Meng TC, Hinks JA, Tonks NK, and Barford D. Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. *Nature* 423: 769–773, 2003.
148. Savitsky PA and Finkel T. Redox regulation of Cdc25C. *J Biol Chem* 277: 20535–20540, 2002.
149. Schafer FQ and Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med* 30: 1191–1212, 2001.
150. Schenk H, Klein M, Erdbrugger W, Droge W, and Schulze-Osthoff K. Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-kappaB and AP-1. *Proc Nat Acad Sci U S A* 91: 1672–1676, 1994.
151. Schmitz ML, Bacher S, and Kracht M. IκB-independent control of NF-κB activity by modulatory phosphorylations. *Trends Biochem Sci* 26: 186–190, 2001.
152. Schoonbroodt S, Ferreira V, Best-Belpomme M, Boelaert JR, Legrand-Poels S, Korner M, and Piette J. Crucial role of the amino-terminal tyrosine residue 42 and the carboxyl-terminal PEST domain of IκBα in NF-κB activation by an oxidative stress. *J Immunol* 164: 4292–4300, 2000.
153. Schreck R, Rieber P, and Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-κB transcription factor and HIV-1. *EMBO J* 10: 2247–2258, 1991.
154. Sekhar KR, Meredith MJ, Kerr LD, Soltaninassab SR, Spitz DR, Xu ZQ, and Freeman ML. Expression of glutathione and γ-glutamylcysteine synthetase mRNA is Jun dependent. *Biochem Biophys Res Commun* 234: 588–593, 1997.
155. Shertzer HG, Vasiliou V, Liu RM, Tabor MW, and Nebert DW. Enzyme induction by L-buthionine (*S,R*)-sulfoximine in cultured mouse hepatoma cells. *Chem Res Toxicol* 8: 431–436, 1995.
156. Shi MM, Iwamoto T, and Forman HJ. γ-Glutamylcysteine synthetase and GSH increase in quinone-induced oxidative stress in BPAEC. *Am J Physiol* 267(4 Pt 1): L414–L421, 1994.
157. Shi MM, Kugelman A, Iwamoto T, Tian L, and Forman HJ. Quinone-induced oxidative stress elevates glutathione and induces γ-glutamylcysteine synthetase activity in rat lung epithelial L2 cells. *J Biol Chem* 269: 26512–26517, 1994.
158. Shimizu M, Hochadel JF, Fulmer BA, and Waalkes MP. Effect of glutathione depletion and metallothionein gene expression on arsenic-induced cytotoxicity and c-myc expression in vitro. *Toxicol Sci* 45: 204–211, 1998.
159. Shubert SY, Neeman I, and Resnick N. A novel mechanism for the inhibition of NF-κB activation in vascular endothelial cells by natural antioxidants. *FASEB J* 16: 1931–1933, 2002.
160. Singh I, Pahan K, Khan M, and Singh AK. Cytokine-mediated induction of ceramide production is redox-sensitive. *J Biol Chem* 273: 20354–20362, 1998.
161. Slater TF. Free radical mechanisms in tissue injury. *Biochem J* 222: 1–15, 1984.
162. Sohn J and Rudolph J. Catalytic and chemical competence of regulation of cdc25 phosphatase by oxidation/reduction. *Biochemistry* 42: 10060–10070, 2003.
163. Stamler JS and Hausladen A. Oxidative modifications in nitrosative stress. *Nat Struct Biol* 5: 247–249, 1998.
164. Suh YA, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D, Chung AB, Griendling KK, and Lambeth JD. Cell transformation by superoxide generating oxidase MOX-1. *Nature* 401: 79–82, 1999.
165. Suzuki YJ, Forman HJ, and Sevanian A. Oxidants as stimulators of signal transduction. *Free Radic Biol Med* 22: 269–285, 1997.
166. Takeya R, Ueno N, Kami K, Taura M, Kohjima M, Izaki T, Nunoi H, and Sumimoto H. Novel human homologues of p47phox and p67phox participate in activation of superoxide-producing NADPH oxidases. *J Biol Chem* 278: 25234–25246, 2003.

167. Tanaka T, Uchiumi T, Kohno K, Tomonari A, Nishio K, Saijo N, Kondo T, and Kuwano M. Glutathione homeostasis in human hepatic cells: overexpression of γ-glutamylcysteine synthetase gene in cell lines resistant to buthionine sulfoximine, an inhibitor of glutathione synthesis. *Biochem Biophys Res Commun* 246: 398–403, 1998.
168. Thannickal VJ and Fanburg BL. Reactive oxygen species in cell signaling. *Am J Physiol Lung Cell Mol Physiol* 279: L1005–L1028, 2000.
169. Thomas JA, Poland B, and Honzatko R. Protein sulfhydryls and their role in the antioxidant function of protein *S*-thiolation. *Arch Biochem Biophys* 319: 1–9, 1995.
170. Tian L, Shi MM, and Forman HJ. Increased transcription of the regulatory subunit of γ-glutamylcysteine synthetase in rat lung epithelial L2 cells exposed to oxidative stress or glutathione depletion. *Arch Biochem Biophys* 342: 126–133, 1997.
171. Toledano MB and Leonard WJ. Modulation of transcription factor NF-κB binding activity by oxidation-reduction in vitro. *Proc Nat Acad Sci U S A* 88: 4328–4332, 1991.
172. Tomonari A, Nishio K, Kurokawa H, Arioka H, Ishida T, Fukumoto H, Fukuoka K, Nomoto T, Iwamoto Y, Heike Y, Itakura M, and Saijo N. Identification of cis-acting DNA elements of the human gamma-glutamylcysteine synthetase heavy subunit gene. *Biochem Biophys Res Commun* 232: 522–527, 1997.
173. Tu Z and Anders MW. Up-regulation of glutamate-cysteine ligase gene expression by butylated hydroxytoluene is mediated by transcription factor AP-1. *Biochem Biophys Res Commun* 244: 801–805, 1998.
174. Ukada K, Wangpoengtrakul C, Osawa T, Toyokuni S, Tanaka K, and Uchida K. 4-Hydroxy-2-nonenal-mediated impairment of intracellular proteolysis during oxidative stress. *J Biol Chem* 274: 23787–23793, 1999.
175. van Montfort RL, Congreve M, Tisi D, Carr R, and Jhoti H. Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. *Nature* 423: 773–777, 2003.
176. Voehringer DW. Bcl-2 and glutathione: alterations in cellular redox state that regulate apoptosis sensitivity. *Free Radic Biol Med* 27: 945–950, 1999.
177. Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, Kang MI, Kobayashi A, Yamamoto M, Kensler TW, and Talalay P. Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. *Proc Natl Acad Sci U S A* 101: 2040–2045, 2004.
178. White AC, Das SK, and Fanburg BL. Reduction of glutathione is associated with growth restriction and enlargement of bovine pulmonary artery endothelial cells produced by transforming growth factor-beta 1. *Am J Respir Cell Mol Biol* 6: 364–368, 1992.
179. Wild AC and Mulcahy RT. Pyrrolidine dithiocarbamate up-regulates the expression of the genes encoding the catalytic and regulatory subunits of γ-glutamylcysteine synthetase and increases intracellular glutathione levels. *Biochem J* 338: 659–665, 1999.
180. Wild AC, Moinova HR, and Mulcahy RT. Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. *J Biol Chem* 274: 33627–33636, 1999.
181. Wilhelm D, Bender K, Knebel A, and Angel P. The level of intracellular glutathione is a key regulator for the induction of stress-activated signal transduction pathways including Jun N-terminal protein kinases and p38 kinase by alkylating agents. *Mol Cell Biol* 17: 4792–4800, 1997.
182. Winterbourn CC and Metodiewa D. Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide. *Free Radic Biol Med* 27: 322–328, 1999.
183. Wood ZA, Schroder E, Robin HJ, and Poole LB. Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem Sci* 28: 32–40, 2003.
184. Wood ZA, Poole LB, and Karplus PA. Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. *Science* 300: 650–653, 2003.
185. Xanthoudakis S and Curran T. Identification and characterization of Ref-1, a nuclear protein that facilitates AP-1 DNA-binding activity. *EMBO J* 11: 653–665, 1992.
186. Yin Z, Ivanov VN, Habelhah H, Tew K, and Ronai Z. Glutathione *S*-transferase p elicits protection against H_2O_2-induced cell death via coordinated regulation of stress kinases. *Cancer Res* 60: 4053–4057, 2000.
187. Zhang DD and Hannink M. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. *Mol Cell Biol* 23: 8137–8151, 2003.
188. Zheng M, Aslund F, and Storz G. Activation of oxyR transcription factor by reversible disulfide bond formation. *Science* 279: 1718–1721, 1998.

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2. Longze Zhang , Junjing Zhang , Baolu Zhao , Xi Zhao-Wilson . Quinic Acid Could Be a Potential Rejuvenating Natural Compound by Improving Survival of Caenorhabditis elegans under Deleterious Conditions. *Rejuvenation Research*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
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4. Rui-Ming Liu, Praveen Kumar Vayalil, Carol Ballinger, Dale A. Dickinson, Wen-Tan Huang, Suqing Wang, Terrance J. Kavanagh, Qiana L. Matthews, Edward M. Postlethwait. 2012. Transforming growth factor # suppresses glutamate–cysteine ligase gene expression and induces oxidative stress in a lung fibrosis model. *Free Radical Biology and Medicine* **53**:3, 554-563. [CrossRef]
5. Akinori C. Nagato, Frank S. Bezerra, Manuella Lanzetti, Alan A. Lopes, Marco Aurélio S. Silva, Luís Cristóvão Porto, Samuel S. Valença. 2012. Time course of inflammation, oxidative stress and tissue damage induced by hyperoxia in mouse lungs. *International Journal of Experimental Pathology* **93**:4, 269-278. [CrossRef]
6. Saibal Biswas, Ian Megson, Catherine Shaw, Irfan RahmanCigarette Smoking, Inflammation, and Obesity 85-112. [CrossRef]
7. Su Jin Kang, Young Joon Lee, Eun-Kyung Lee, Mi-Kyoung Kwak. 2012. Silver nanoparticles-mediated G2/M cycle arrest of renal epithelial cells is associated with NRF2-GSH signaling. *Toxicology Letters* **211**:3, 334-341. [CrossRef]
8. Irfan Rahman, Vuokko L Kinnula. 2012. Strategies to decrease ongoing oxidant burden in chronic obstructive pulmonary disease. *Expert Review of Clinical Pharmacology* **5**:3, 293-309. [CrossRef]
9. Se-Ran Yang, Irfan Rahman, James E. Trosko, Kyung-Sun Kang. 2012. Oxidative stress-induced biomarkers for stem cell-based chemical screening. *Preventive Medicine* **54**, S42-S49. [CrossRef]
10. James T. Handa. 2012. How does the macula protect itself from oxidative stress?. *Molecular Aspects of Medicine* . [CrossRef]
11. Omca Demirkol, Nuran ErcalGlutathione 69-86. [CrossRef]
12. Livan Delgado Roche, Emilio Acosta Medina, Ángela Fraga Pérez, María A. Bécquer Viart, Yosdel Soto López, Viviana Falcón Cama, Ana M. Vázquez López, Gregorio Martínez-Sánchez, Eduardo Fernández-Sánchez. 2012. Lipofundin-Induced Hyperlipidemia Promotes Oxidative Stress and Atherosclerotic Lesions in New Zealand White Rabbits. *International Journal of Vascular Medicine* **2012**, 1-7. [CrossRef]
13. Cheng-Ying Ho, Yu-Ting Cheng, Chi-Fai Chau, Gow-Chin Yen. 2011. Effect of Diallyl Sulfide on in Vitro and in Vivo Nrf2-Mediated Pulmonic Antioxidant Enzyme Expression via Activation ERK/p38 Signaling Pathway. *Journal of Agricultural and Food Chemistry* 111213160717002. [CrossRef]
14. Gangduo Wang, Jianling Wang, Xiuzhen Fan, G.A.S. Ansari, M. Firoze Khan. 2011. Protein adducts of malondialdehyde and 4-hydroxynonenal contribute to trichloroethene-mediated autoimmunity via activating Th17 cells: Dose– and time–response studies in female MRL+/+ mice. *Toxicology* . [CrossRef]
15. Hans Hoppeler, Oliver Baum, Glenn Lurman, Matthias MuellerMolecular Mechanisms of Muscle Plasticity with Exercise . [CrossRef]
16. J. Vidya Sarma, Peter A. WardOxidants and Redox Signaling in Acute Lung Injury . [CrossRef]
17. Silvia Diabaté, Britta Bergfeldt, Diana Plaumann, Caroline Übel, Carsten Weiss. 2011. Anti-oxidative and inflammatory responses induced by fly ash particles and carbon black in lung epithelial cells. *Analytical and Bioanalytical Chemistry* . [CrossRef]
18. Michael Muller. 2011. Glutathione modulates the toxicity of, but is not a biologically relevant reductant for, the Pseudomonas aeruginosa redox toxin pyocyanin. *Free Radical Biology and Medicine* **50**:8, 971-977. [CrossRef]
19. Si-Yen Liu, Wei-Zhi Wang, Chia-Liang Yen, Ming-Yi Tsai, Pei-Wen Yang, Jiu-Yao Wang, Chun-Yi Ho, Chi-Chang Shieh. 2011. Leukocyte nicotinamide adenine dinucleotide phosphate-reduced oxidase is required for isocyanate-induced lung inflammation. *Journal of Allergy and Clinical Immunology* **127**:4, 1014-1023. [CrossRef]

20. Eun-Jung Park, Jinkyu Roh, Younghun Kim, Kwangsik Park, Dae-Seon Kim, Seong-Do Yu. 2011. PM 2.5 collected in a residential area induced Th1-type inflammatory responses with oxidative stress in mice. *Environmental Research* **111**:3, 348-355. [CrossRef]
21. Karla Maria Pereira Pires, Frank Silva Bezerra, Mariana Nascimento Machado, Walter Araújo Zin, Luís Cristóvão Porto, Samuel Santos Valença. 2011. N-(2-mercaptopropionyl)-glycine but not Allopurinol prevented cigarette smoke-induced alveolar enlargement in mouse#. *Respiratory Physiology & Neurobiology* **175**:3, 322-330. [CrossRef]
22. U. Van der Velden, D. Kuzmanova, I. L. C. Chapple. 2011. Micronutritional approaches to periodontal therapy. *Journal of Clinical Periodontology* **38**, 142-158. [CrossRef]
23. Adam CsordasInterfering with Smoking-Induced Pathophysiology 329-353. [CrossRef]
24. Young Sook Kim, Jun Ho Jeon, Jintaek Im, Seok-Seong Kang, Jung Nam Choi, Hyang Ran Ju, Cheol-Heui Yun, Chang Gue Son, Choong Hwan Lee, Seung Hyun Han. 2011. Induction of intercellular adhesion molecule-1 by water-soluble components of Hericium erinaceum in human monocytes. *Journal of Ethnopharmacology* **133**:2, 874-880. [CrossRef]
25. Sundararajan JayaramanAssessment of Beta Cell Viability . [CrossRef]
26. Svetlana N. Radyuk, Igor Rebrin, Vladimir I. Klichko, Barbara H. Sohal, Katarzyna Michalak, Judith Benes, Rajindar S. Sohal, William C. Orr. 2010. Mitochondrial peroxiredoxins are critical for the maintenance of redox state and the survival of adult Drosophila. *Free Radical Biology and Medicine* **49**:12, 1892-1902. [CrossRef]
27. Eun-Jung Park, Jin-Kyu Roh, Young-Hun Kim, Kwang-Sik Park. 2010. Induction of Inflammatory Responses by Carbon Fullerene (C60) in Cultured RAW264.7 Cells and in Intraperitoneally Injected Mice. *Toxicological Research* **26**:4, 267-273. [CrossRef]
28. Mohd Javed Akhtar, Maqusood Ahamed, Sudhir Kumar, Huma Siddiqui, Govil Patil, Mohd Ashquin, Iqbal Ahmad. 2010. Nanotoxicity of pure silica mediated through oxidant generation rather than glutathione depletion in human lung epithelial cells. *Toxicology* **276**:2, 95-102. [CrossRef]
29. Jimmie Colon, Nelson Hsieh, Amber Ferguson, Patrick Kupelian, Sudipta Seal, D. Wayne Jenkins, Cheryl H. Baker. 2010. Cerium oxide nanoparticles protect gastrointestinal epithelium from radiation-induced damage by reduction of reactive oxygen species and upregulation of superoxide dismutase 2. *Nanomedicine: Nanotechnology, Biology and Medicine* **6**:5, 698-705. [CrossRef]
30. Eun-Jung Park, Hero Kim, Younghun Kim, Jongheop Yi, Kyunghee Choi, Kwangsik Park. 2010. Inflammatory responses may be induced by a single intratracheal instillation of iron nanoparticles in mice. *Toxicology* **275**:1-3, 65-71. [CrossRef]
31. K. Maghni, J.-L. Malo, J. L'Archevêque, L. Castellanos, D. Gautrin. 2010. Matrix metalloproteinases, IL-8 and glutathione in the prognosis of workers exposed to chlorine. *Allergy* **65**:6, 722-730. [CrossRef]
32. Xiao Chu, Keji Song, Kan Xu, Xiaozhe Zhang, Xuemei Zhang, Yu Song, Dacheng Wang, Songcai Liu, Xuming Deng. 2010. Ceftiofur attenuates lipopolysaccharide-induced acute lung injury. *International Immunopharmacology* **10**:5, 600-604. [CrossRef]
33. Eun-Jung Park, Hero Kim, Younghun Kim, Kwangsik Park. 2010. Intratracheal instillation of platinum nanoparticles may induce inflammatory responses in mice. *Archives of Pharmacal Research* **33**:5, 727-735. [CrossRef]
34. Dan Jia, Nathan A. Koonce, Robert J. Griffin, Cassie Jackson, Peter M. Corry. 2010. Prevention and Mitigation of Acute Death of Mice after Abdominal Irradiation by the Antioxidant N-Acetyl-cysteine (NAC). *Radiation Research* **173**:5, 579-589. [CrossRef]
35. Run-Hua LIU, Wen-Bo JIANG, Di-Qiu YU. 2010. The Response of AtCER to Oxidative Stress in Arabidopsis thaliana. *Acta Botanica Yunnanica* **31**:4, 326-334. [CrossRef]
36. Young Sook Kim, Jintaek Im, Jung Nam Choi, Seok-Seong Kang, Yeo Jin Lee, Choong Hwan Lee, Cheol-Heui Yun, Chang Gue Son, Seung Hyun Han. 2010. Induction of ICAM-1 by Armillariella mellea is mediated through generation of reactive oxygen species and JNK activation. *Journal of Ethnopharmacology* **128**:1, 198-205. [CrossRef]
37. R.-M. Liu, K.A. Gaston Pravia. 2010. Oxidative stress and glutathione in TGF-#-mediated fibrogenesis. *Free Radical Biology and Medicine* **48**:1, 1-15. [CrossRef]
38. Xianchun LiGlutathione and Glutathione-S-Transferase in Detoxification Mechanisms . [CrossRef]
39. Immacolata Castellano, Francesca Cecere, Alberto De Vendittis, Roberta Cotugno, Angela Chambery, Antimo Di Maro, Andzelika Michniewicz, Giuseppe Parlato, Mariorosario Masullo, Enrico Vittorio Avvedimento, Emmanuele De Vendittis, Maria Rosaria Ruocco. 2009. Rat mitochondrial manganese superoxide dismutase: Amino acid positions involved in covalent modifications, activity, and heat stability. *Biopolymers* **91**:12, 1215-1226. [CrossRef]

40. Malcolm J. Jackson. 2009. Redox regulation of adaptive responses in skeletal muscle to contractile activity. *Free Radical Biology and Medicine* **47**:9, 1267-1275. [CrossRef]
41. Vignesh Muthuvijayan, Jun Gu, Randy S. Lewis. 2009. Analysis of functionalized polyethylene terephthalate with immobilized NTPDase and cysteine. *Acta Biomaterialia* **5**:9, 3382-3393. [CrossRef]
42. Tomohiro Shibasaki, Yoshihito Iuchi, Futoshi Okada, Kazuho Kuwata, Takuya Yamanobe, Shiro Bannai, Yoshihiko Tomita, Hideyo Sato, Junichi Fujii. 2009. Aggravation of ischemia–reperfusion-triggered acute renal failure in xCT-deficient mice. *Archives of Biochemistry and Biophysics* **490**:1, 63-69. [CrossRef]
43. Kyle R Gibson, Ilene L Neilson, Fiona Barrett, Tim J Winterburn, Sushma Sharma, Sandra M MacRury, Ian L Megson. 2009. Evaluation of the Antioxidant Properties of N-acetylcysteine in Human Platelets: Prerequisite for Bioconversion to Glutathione for Antioxidant and Antiplatelet Activity. *Journal of Cardiovascular Pharmacology* **54**:4, 319-326. [CrossRef]
44. Feng Zhang, Xinying Wang, Liya Pan, Weiya Wang, Ning Li, Jieshou Li. 2009. Glutamine attenuates lipopolysaccharide-induced acute lung injury. *Nutrition* **25**:6, 692-698. [CrossRef]
45. Kristin L. Aillon, Yumei Xie, Nashwa El-Gendy, Cory J. Berkland, M. Laird Forrest. 2009. Effects of nanomaterial physicochemical properties on in vivo toxicity. *Advanced Drug Delivery Reviews* **61**:6, 457-466. [CrossRef]
46. Srikanth Pendyala , Peter V. Usatyuk , Irina A. Gorshkova , Joe G.N. Garcia , Viswanathan Natarajan . 2009. Regulation of NADPH Oxidase in Vascular Endothelium: The Role of Phospholipases, Protein Kinases, and Cytoskeletal Proteins. *Antioxidants & Redox Signaling* **11**:4, 841-860. [Abstract] [Full Text PDF] [Full Text PDF with Links]
47. C.J. Harvey, R.K. Thimmulappa, A. Singh, D.J. Blake, G. Ling, N. Wakabayashi, J. Fujii, A. Myers, S. Biswal. 2009. Nrf2-regulated glutathione recycling independent of biosynthesis is critical for cell survival during oxidative stress. *Free Radical Biology and Medicine* **46**:4, 443-453. [CrossRef]
48. Christopher C. Franklin, Donald S. Backos, Isaac Mohar, Collin C. White, Henry J. Forman, Terrance J. Kavanagh. 2009. Structure, function, and post-translational regulation of the catalytic and modifier subunits of glutamate cysteine ligase. *Molecular Aspects of Medicine* **30**:1-2, 86-98. [CrossRef]
49. Adel Boueiz, Paul M. Hassoun. 2009. Regulation of endothelial barrier function by reactive oxygen and nitrogen species. *Microvascular Research* **77**:1, 26-34. [CrossRef]
50. Elizabeth H. Jeffery, Marcela Araya. 2009. Physiological effects of broccoli consumption. *Phytochemistry Reviews* **8**:1, 283-298. [CrossRef]
51. Michael D. Mattie, Matthew K. McElwee, Jonathan H. Freedman. 2008. Mechanism of Copper-Activated Transcription: Activation of AP-1, and the JNK/SAPK and p38 Signal Transduction Pathways. *Journal of Molecular Biology* **383**:5, 1008-1018. [CrossRef]
52. Hyang-Rim Lee, Jeong-Min Cho, Dong-ha Shin, Chul Soon Yong, Han-Gon Choi, Nobunao Wakabayashi, Mi-Kyoung Kwak. 2008. Adaptive response to GSH depletion and resistance to l-buthionine-(S,R)-sulfoximine: involvement of Nrf2 activation. *Molecular and Cellular Biochemistry* **318**:1-2, 23-31. [CrossRef]
53. Feng Zhang, Xinying Wang, Weiya Wang, Ning Li, Jieshou Li. 2008. Glutamine Reduces TNF-# by Enhancing Glutathione Synthesis in Lipopolysaccharide-Stimulated Alveolar Epithelial Cells of Rats. *Inflammation* **31**:5, 344-350. [CrossRef]
54. Gaurav Kaushik, Toshi Kaushik, Suchit Khanduja, Chander Mohan Pathak, Krishan Lal Khanduja. 2008. Cigarette smoke condensate promotes cell proliferation through disturbance in cellular redox homeostasis of transformed lung epithelial type-II cells. *Cancer Letters* **270**:1, 120-131. [CrossRef]
55. Malcolm J. Jackson. 2008. Redox regulation of skeletal muscle. *IUBMB Life* **60**:8, 497-501. [CrossRef]
56. Sundararajan Jayaraman. 2008. A novel method for the detection of viable human pancreatic beta cells by flow cytometry using fluorophores that selectively detect labile zinc, mitochondrial membrane potential and protein thiols. *Cytometry Part A* **73A**:7, 615-625. [CrossRef]
57. K CALFEEMASON, E LEE, B SPEAR, H GLAUERT. 2008. Role of the p50 subunit of NF-#B in vitamin E-induced changes in mice treated with the peroxisome proliferator, ciprofibrate. *Food and Chemical Toxicology* **46**:6, 2062-2073. [CrossRef]
58. Emilie Boncoeur, Telma Roque, Elise Bonvin, Vinciane Saint-Criq, Monique Bonora, Annick Clement, Olivier Tabary, Alexandra Henrion-Caude, Jacky Jacquot. 2008. Cystic Fibrosis Transmembrane Conductance Regulator Controls Lung Proteasomal Degradation and Nuclear Factor-#B Activity in Conditions of Oxidative Stress. *The American Journal of Pathology* **172**:5, 1184-1194. [CrossRef]
59. Immacolata Castellano, Maria Rosaria Ruocco, Francesca Cecere, Antimo Di Maro, Angela Chambery, Andzelika Michniewicz, Giuseppe Parlato, Mariorosario Masullo, Emmanuele De Vendittis. 2008. Glutathionylation of the iron

superoxide dismutase from the psychrophilic eubacterium Pseudoalteromonas haloplanktis. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1784**:5, 816-826. [CrossRef]

60. Katri Koli , Marjukka Myllärniemi , Jorma Keski-Oja , Vuokko L. Kinnula . 2008. Transforming Growth Factor-# Activation in the Lung: Focus on Fibrosis and Reactive Oxygen Species. *Antioxidants & Redox Signaling* **10**:2, 333-342. [Abstract] [Full Text PDF] [Full Text PDF with Links]
61. 2008. CrossRef Listing of Deleted DOIs. *CrossRef Listing of Deleted DOIs* . [CrossRef]
62. Ren-Feng Guo , Peter A. Ward . 2007. Role of Oxidants in Lung Injury During Sepsis. *Antioxidants & Redox Signaling* **9**:11, 1991-2002. [Abstract] [Full Text PDF] [Full Text PDF with Links]
63. O YILMAZ, S KESER, M TUZCU, B CETINTAS. 2007. Resveratrol (trans-3,4#,5-trihydroxystilbene) decreases lipid peroxidation level and protects antioxidant capacity in sera and erythrocytes of old female Wistar rats induced by the kidney carcinogen potassium bromate. *Environmental Toxicology and Pharmacology* **24**:2, 79-85. [CrossRef]
64. Saibal Biswas, Irfan Rahman, Ian Megson, Catherine ShawCigarette Smoking, Inflammation, and Obesity 43-61. [CrossRef]
65. Peter Kovacic. 2007. Protein electron transfer (mechanism and reproductive toxicity): Iminium, hydrogen bonding, homoconjugation, amino acid side chains (redox and charged), and cell signaling. *Birth Defects Research Part C: Embryo Today: Reviews* **81**:1, 51-64. [CrossRef]
66. Iain L. C. Chapple, John B. Matthews. 2007. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontology 2000* **43**:1, 160-232. [CrossRef]
67. R. Franco, O. J. Schoneveld, A. Pappa, M. I. Panayiotidis. 2007. The central role of glutathione in the pathophysiology of human diseases. *Archives Of Physiology And Biochemistry* **113**:4-5, 234-258. [CrossRef]
68. Peter Kovacic, Robert S. Pozos. 2006. Cell signaling (mechanism and reproductive toxicity): Redox chains, radicals, electrons, relays, conduit, electrochemistry, and other medical implications. *Birth Defects Research Part C: Embryo Today: Reviews* **78**:4, 333-344. [CrossRef]
69. Ulysses J. Magalang , Ramya Rajappan , Melissa G. Hunter , Vijay K. Kutala , Periannan Kuppusamy , Mark D. Wewers , Clay B. Marsh , Narasimham L. Parinandi . 2006. Adiponectin Inhibits Superoxide Generation by Human Neutrophils. *Antioxidants & Redox Signaling* **8**:11-12, 2179-2186. [Abstract] [Full Text PDF] [Full Text PDF with Links]
70. R VILELA, L LANDS, B MEEHAN, S KUBOW. 2006. Inhibition of IL-8 release from CFTR-deficient lung epithelial cells following pre-treatment with fenretinide. *International Immunopharmacology* **6**:11, 1651-1664. [CrossRef]
71. Susanne Fritsch, Silvia Diabaté, Harald F. Krug. 2006. Incinerator fly ash provokes alteration of redox equilibrium and liberation of arachidonic acid in vitro. *Biological Chemistry* **387**:10_11, 1421-1428. [CrossRef]
72. Savita Khanna, Sashwati Roy, Narasimham L. Parinandi, Mariah Maurer, Chandan K. Sen. 2006. Characterization of the potent neuroprotective properties of the natural vitamin E #-tocotrienol. *Journal of Neurochemistry* **98**:5, 1474-1486. [CrossRef]
73. J VITECEK, J PETRLOVA, J PETREK, V ADAM, D POTESIL, L HAVEL, R MIKELOVA, L TRNKOVA, R KIZEK. 2006. Electrochemical study of S–nitrosoglutathione and nitric oxide by carbon fibre NO sensor and cyclic voltammetry – possible way of monitoring of nitric oxide. *Electrochimica Acta* **51**:24, 5087-5094. [CrossRef]
74. Cheol-Heui Yun, Dae Kyun Chung, Kristopher Yoon, Seung Hyun Han. 2006. Involvement of reactive oxygen species in the immunosuppressive effect of 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), a food-born carcinogenic heterocyclic amine. *Toxicology Letters* **164**:1, 37-43. [CrossRef]
75. Kazuhiko Koike. 2006. Oxidative stress and apoptosis in hepatitis C: the core issue. *Journal of Gastroenterology* **41**:3, 292-294. [CrossRef]
76. Mee-Hyang Kweon, Young In Park, Ha-Chin Sung, Hasan Mukhtar. 2006. The novel antioxidant 3-O-caffeoyl-1-methylquinic acid induces Nrf2-dependent phase II detoxifying genes and alters intracellular glutathione redox. *Free Radical Biology and Medicine* **40**:8, 1349-1361. [CrossRef]
77. Dr. Irfan Rahman , Se-Ran Yang , Saibal K. Biswas . 2006. Current Concepts of Redox Signaling in the Lungs. *Antioxidants & Redox Signaling* **8**:3-4, 681-689. [Abstract] [Full Text PDF] [Full Text PDF with Links]
78. Saibal K. Biswas, David E. Newby, Irfan Rahman, Ian L. Megson. 2005. Depressed glutathione synthesis precedes oxidative stress and atherogenesis in Apo-E-/- mice. *Biochemical and Biophysical Research Communications* **338**:3, 1368-1373. [CrossRef]
79. Fernando Holguin, Sumita Khatri, David M Guidot. 2005. Antioxidant treatment for lung diseases. *Expert Opinion on Therapeutic Patents* **15**:12, 1711-1725. [CrossRef]

80. E. M. Erin, H. Neighbour, A. J. Tan, O. Min Kon, S. R. Durham, T. T. Hansel. 2005. Nasal testing for novel anti-inflammatory agents. *Clinical <html_ent glyph="@amp;" ascii="&"/> Experimental Allergy* **35**:8, 981-985. [CrossRef]

81. Irfan Rahman . 2005. Redox Signaling in the Lungs. *Antioxidants & Redox Signaling* **7**:1-2, 1-5. [Citation] [Full Text PDF] [Full Text PDF with Links]

82. Rachel Nadif, Margaret Mintz, Anne Jedlicka, Jean-Pierre Bertrand, Steven R. Kleeberger, Francine Kauffmann. 2005. Association of CAT polymorphisms with catalase activity and exposure to environmental oxidative stimuli. *Free Radical Research* **39**:12, 1345-1350. [CrossRef]