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Forum Review

The Regulation and Role of Extracellular Glutathione Peroxidase

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ABSTRACT

Reactive oxygen species and reactive nitrogen species are mediators of lung tissue damage. To minimize the effect of oxidative stress, the lung is well equipped with an integrated antioxidant system. In some circumstances, antioxidants increase in response to oxidants and reduce tissue injury. The lung is somewhat unique in that it has an extracellular surface, which is often directly exposed to oxidative stresses. In this context, the extracellular antioxidant system, comprised primarily of glutathione and glutathione peroxidase, is especially important in protecting against oxidant injury. Induction of extracellular glutathione peroxidase occurs in airway inflammation and undoubtedly plays an important defense against oxidative injury to the airway surface. *Antioxid. Redox Signal.* 7, 72–79.

INTRODUCTION

LUNGS are unique in having a large epithelial surface area that is at risk for oxidant-mediated attack. The tracheobronchial tree and the alveolar space are exposed to reactive oxidizing species in the form of inhaled airborne pollutants, tobacco smoke, and products of inflammation. The lung, therefore, requires additional antioxidant resources to prevent airway-borne oxidant injury (29). The major airways contain high-molecular-weight mucopolypeptide glycoproteins synthesized by lining epithelial cells and glands that increase mucus production in the presence of inflammation (29). The lung cells contain intracellular antioxidant enzymes to maintain a normal redox state. The alveolar space can recruit additional antioxidant activity from the epithelial lining fluid (ELF). This fluid contains large amounts of glutathione (GSH; 100-fold higher than in plasma), 90% of which is in the reduced form (8, 50, 51). The ELF also contains catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) (8, 15, 20, 50, 51). Additional antioxidants contained in ELF include ceruloplasmin, transferrin, ascorbate, vitamin E, ferritin, other serum proteins, and small molecules such as bilirubin (29). The multiplicity of the antioxidant systems available to the lung and their overlapping specific activities suggest that a good control of redox balance is critically important to maintain normal pulmonary cellular function. A large portion of oxidative stress occurs on the extracellular surface of the lung epithelium. Therefore, critical first-line antioxidant defenses are located in the ELF. Interestingly, bronchial epithelium is the first target of concentrated inspired oxygen, and epithelial damage is a typical feature in human airway diseases, such as asthma, chronic obstructive pulmonary disease, and emphysema. Disequilibrium, either through increased oxidant stress or decreased antioxidant resources, can result in a series of pathophysiologic events in the lung that culminate in cellular death and pulmonary dysfunction (29). In this context, extracellular antioxidants may be the main protective mechanism of the lung against oxidant-mediated lung diseases. Here, the role and function of extracellular GPx (eGPx) is reviewed.

REDOX STATE OF THE LUNG

The airway epithelium is an important cellular barrier between the lung parenchyma and the surface ELF. Therefore, these cells are immediately and directly exposed to any change in the redox environment on the airway surface, which makes them especially susceptible to environmental oxidative dam-

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age. Redox reactions have attracted attention as important chemical processes that regulate signal transduction (52). The redox state of a compound can be defined as the tendency to accept or donate electrons (24). As reactive oxygen species (ROS) and reactive nitrogen species (RNS) are potent oxidizing agents, they can affect the local or general cytosolic balance of oxidation/reduction (redox state). *In vitro*, under defined conditions, this can be measured (24). However, in intact cells with a multitude of pathways that can accept and/or donate electrons, it is much more difficult to define this term. Under physiological conditions, the cellular redox state is characterized by a reducing cytosol (24). The major redox "buffer" in the cytosol is GSH, and the vast excess of reduced substances over oxidized ones is largely responsible for the reducing potential of the cytosol (19). Other "redox buffers" include NAD/NADH and NADP/NADPH (24).

THE GLUTATHIONE SYSTEM: GSH AND GPX

The glutathione system is a central mechanism for reducing hydrogen peroxide (H_2O_2). The key enzyme in the redox cycle responsible for the reduction of H_2O_2 is GPx (EC 1.11.1.9), which is a group of antioxidant enzymes that catalyze the reduction of H_2O_2 and/or lipid hydrogen peroxides by the oxidation of GSH or *S*-nitroso-L-glutathione (GSNO) and function in protecting the cell from oxidative damage (26, 30 40, 49). The reducing capacity of GPx enzymes is based on high levels of GSH (L-γ-glutamyl-L-cysteinylglycine), a ubiquitous cellular nonprotein sulfhydryl antioxidant, which is a small molecule that plays key roles in basic metabolic and cell cycle-related processes. Among its many functions, this molecule detoxifies free radicals and exogenous toxins and is important in maintaining intra- and extracellular redox balance (19, 39). The glutathione disulfide (GSSG) that is formed in the course of the reaction is subsequently reduced back to GSH by glutathione reductase, an intracellular enzyme that uses NADPH generated from the hexose monophosphate shunt system as an electron donor (19, 39). Subsequently, GSSG breaks down to its amino acid components for cellular uptake and recycling (Fig. 1).

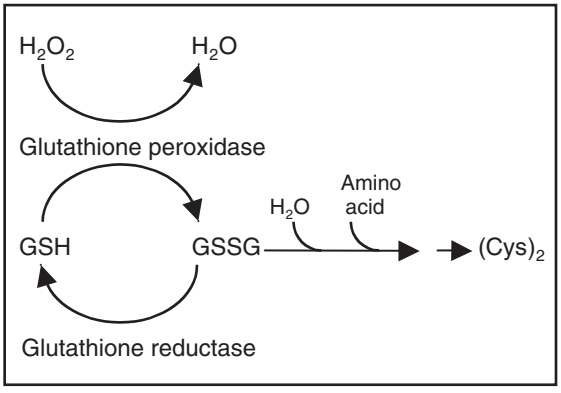

FIG. 1. Oxidation and reduction of glutathione. GSH, reduced glutathione; GSSG, oxidized glutathione.

Healthy, nonstressed cells maintain a high intracellular GSH/GSSG ratio to ensure the availability of GSH and thereby promote active reduction of H_2O_2 through the glutathione system (19, 39). Exposure to oxidative stress leads to rapid changes in GSH and GSSG in cells and in the overlying supernatant, verifying alterations in the redox environment. We and others have reported similar alterations of GSH and GSSG in asthmatic airways (15, 17, 20, 32, 50). Rapid increase of intracellular GSH is a response to oxidative stress (17, 44) and a critical determinant of cellular tolerance to oxidizing environments (45). Exposure to pyrogallol causes a transient depletion of GSH followed by a prolonged elevation in intracellular GSH levels (17). γ-Glutamyl cysteine synthetase (γ-GCS) is an enzyme that determines the rate of GSH synthesis and consists of γ-GCS-HS (heavy subunit) and γ-GCS-LS (light subunit) (28). Other studies have shown that ROS increase GSH through induction of γ-glutamylcysteine synthetase, the rate-limiting enzyme of GSH biosynthesis (43). Other protective responses to oxidative stress include uptake of GSH into cells (19, 39) and export of the oxidized form to overcome an accumulation of GSSG within the cytosol. Bronchial epithelial cells in culture appear to use similar protective strategies against oxidative injury (17).

Free GSH can also function as a water-soluble antioxidant by interacting directly with radical intermediates in nonenzymatic catalyzed reactions. Scavenging of superoxide anion ($O_2^{\bullet -}$) by GSH leads via several steps to the formation of thiyl radicals ($GS^{\bullet}$) and H_2O_2, which is a radical propagation reaction (41, 56, 60, 61). This reaction leading to the formation of thiyl radicals and H_2O_2 can occur in physiologically relevant concentrations (41, 56, 60, 61). Hence, a substance generally accepted to be an antioxidant may possess prooxidant activity under certain conditions (6, 21).

eGPx

There are four forms of GPx, which vary in their localization, structure, and enzymatic nature: (a) the classical cellular GPx (cGPx), which was the first mammalian selenoprotein to be identified (7, 26, 40, 47); (b) the phospholipid hydroperoxide GPx (PHGPx) (57); (c) the gastrointestinal form of GPx (giGPx) (12); and (d) the extracellular GPx (eGPx) (53). The existence of multiple forms of GPx is due to the expression of different genes (12, 23, 54).

Characteristics

eGPx, first identified as a distinct enzyme in human plasma, is located at chromosome 5 band q32 (64). The nucleotide sequence data revealed that eGPx gene is composed of five exons spanning ~10 kb (64). eGPx is capable of reducing H_2O_2, organic hydroperoxides, free fatty acid hydroperoxides, and to some extent phosphatidylcholine hydroperoxides (22, 35, 53, 54, 63). The primary structure of human eGPx shows 44% identity with the cGPx, 34% with the giGPx, and 24% with the PHGPx (12, 23, 54). The molecular mass as determined by gel filtration is ~100,000 kDa (53). eGPx exists as a homotetramer with a subunit size of 23 kDa on a denaturing gel electrophoresis and a predicted subunit size of 25.3 kDa based

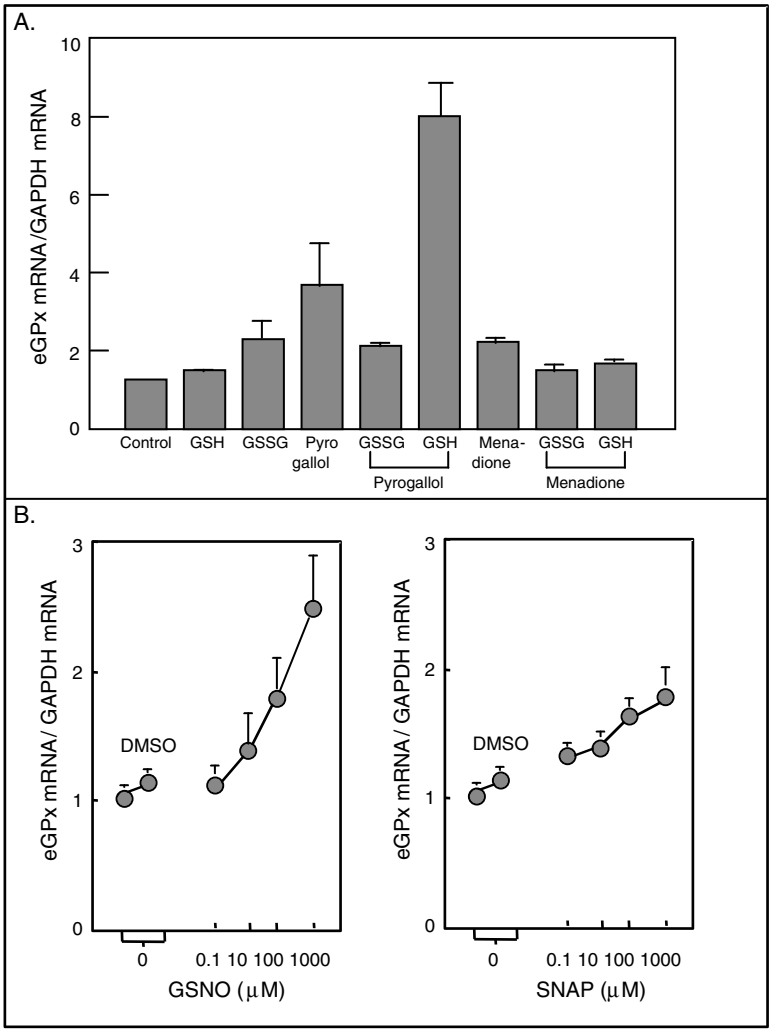

FIG. 2. Oxidative stress leads to eGPx induction. (**A**) Effect of ROS on eGPx induction. BET1A cells were cultured in the presence of combinations of superoxide generating compound pyrogallol (100 μ*M*), the H_2O_2 generating compound menadione (100 m*M*), GSH (10 m*M*), and GSSG (10 μ*M*) for 24 h. Northern blot analysis of total RNA with ^{32}P-labeled eGPx cDNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was performed to quantify changes in mRNA expression. Relative units of eGPx mRNA/GAPDH are summarized in the graph. Results are means ± SD of a minimum of three experiments. (**B**) Effect of RNS on eGPx induction. BET1A cells were exposed to NO-generating compounds, *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) and GSNO, for 48 h. Northern blot analysis of total RNA with ^{32}P-labeled eGPx cDNA and GAPDH cDNA was performed to quantify changes in mRNA expression. DMSO, dimethyl sulfoxide.

on its cDNA sequence (53, 54). The crystal structure of eGPx shows that the subunit structure of eGPx has the typical structure motif of the thioredoxin fold consisting of a central β-sheet and several α-helices (46). The active selenocysteine residue is located in a pocket on the protein surface (46). The overall structure is similar to that of cGPx. The main differences include an extended N terminus and the possible existence of a disulfide bridge in eGPx (46). Most plasma proteins are known to be glycosylated before being secreted into the plasma. eGPx is a glycoprotein indicating that eGPx is not a consequence of passive release of an intracellular enzyme (35, 53). The enzyme is inhibited by β-mercaptosuccinic acid, which is a specific inhibitor for selenium-dependent GPx (10). Copper, mercury, and zinc also strongly inhibit the eGPx enzyme activity (35, 53). Flohe *et al.* (25) first determined that the cGPx K_m for H_2O_2 was variable, or indefinite, because the K_m

TABLE 1. DIFFERENCE BETWEEN EXTRACELLULAR AND INTRACELLULAR GPX

	eGPx	*cGPx*	*Reference*
Activity (U/mg of protein)	20–26	220–260	35, 53
Molecular mass	23 kDa	22 kDa	53
K_m H_2O_2*	3.3 μ*M*	12 μ*M*	35
K_m PPHP*	2.6 μ*M*	10 μ*M*	35
K_m PPHP†	54 μ*M*	25 μ*M*	36
Glycoprotein	Yes	No	53

PPHP, 5-phenyl-4-pentenyl hydroperoxide.
*1 m*M* GSH.
†0.5 m*M* GSH.

TABLE 2. LUNG DISEASES ASSOCIATED WITH eGPx (mRNA OR PROTEIN) EXPRESSION IN HUMAN AIRWAY EPITHELIAL CELLS AND/OR ELF

Disease	*Gene Expression*	*Protein Levels*	*Reference*
Asthma	↑	↑	17
Hyperoxia	No change	N.A.	16
CBD	↑	↑	13, 14
Smoking	↑	↑	14, 16
Ozone exposure	NA	↓	5
NO_2 exposure	NA	No changes	5

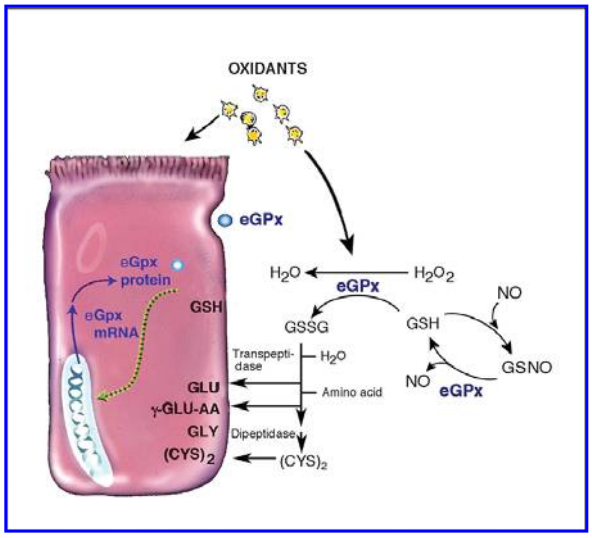

FIG. 3. The role and function of eGPx in the lung. Oxidants are produced in mammalian airways, and increased levels are found in many inflammatory lung diseases, such as asthma and hyperoxia. Inflammation leads to increased levels of ROS. Therefore, induction of eGPx leads to reduction of ROS (*e.g.*, H_2O_2 and $O_2^{\bullet -}$). Nitrosation of GSH by peroxynitrite leads to the formation of GSNO. Recent studies have shown that GPx can protect against NO-mediated protein oxidation and can reduce GSNO. *In vitro* study showed that GPx can protect against NO-mediated protein oxidation by reducing GSNO. Thus, the increased eGPx in lung inflammation may have two functions: reduction of ROS and detoxification and liberation of NO.

for H_2O_2 was dependent on the GSH concentration. For example, a GSH concentration of 2 m*M* leads to the enzyme-H_2O_2 K_m of 8.8 μ*M*, whereas GSH at 4 m*M* leads to a K_m of 17.8 μ*M* (25). The K_m values of both eGPx and cGPx for all hydrogen peroxides are dependent on the concentration of GSH available to the reaction and are in the low micromolar range, which makes GPxs very effective hydroperoxide scavengers even at relatively low concentrations of GSH (25, 35, 53) (Table 1). Whereas the cGPx does not show saturation with respect to GSH (9, 58), eGPx enzyme definitely shows saturation with respect to GSH (35). The K_m of eGPx for GSH ranges between 4.3 m*M* and 5.3 m*M* (35, 53). The eGPx ac-

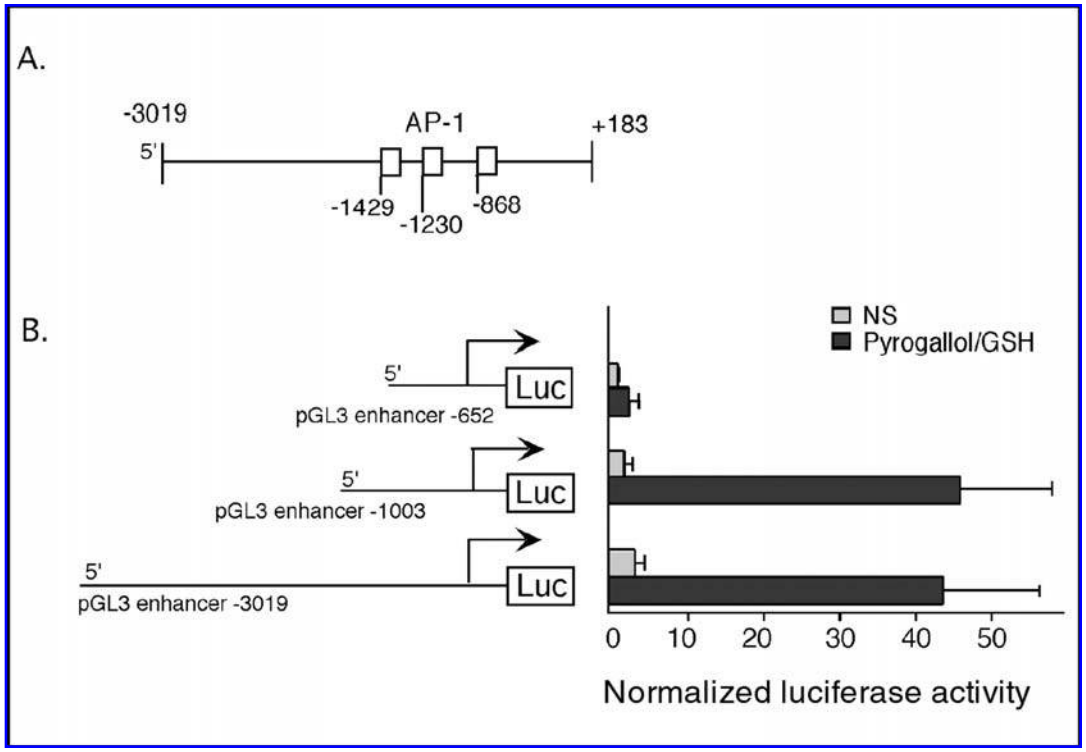

FIG. 4. Transcriptional activity of the eGPx gene 5'-flanking region. (**A**) Consensus sequence for AP-1 binding is present in the eGPx gene 5'-flanking region. The numbers in the figure represent the relative nucleotide positions to the transcriptional start site of the eGPx gene. (**B**) Transcriptional activity was determined in BET1A cells incubated with and without pyrogallol/GSH as described in Fig. 2A. Levels of firefly luciferase expression by fusion gene constructs of the eGPx 5'-flanking region and a luciferase reporter gene are shown relative to the expression of the Renilla luciferase reporter gene. The histograms represent the means ± SEM of three separate experiments.

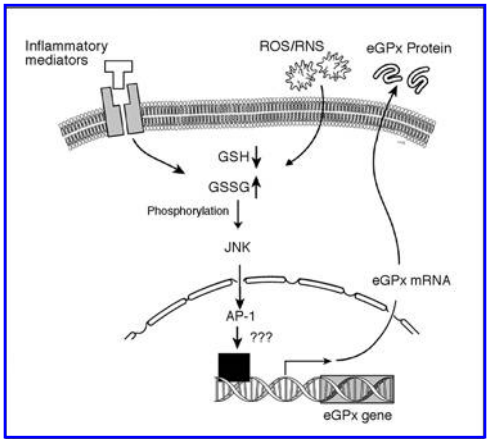

FIG. 5. Model showing the possible mechanism of eGPx induction by oxidative stress. Oxidative stress or inflammatory mediators can modulate the redox state (GSH/GSSG ratio) in the cell, which leads to activation of transcription factor AP-1. The 5'-flanking region of eGPx shows a consensus sequence for AP-1. Activation of AP-1 and binding to the eGPx promoter form a possible mechanism for eGPx gene and protein induction.

tivity is optimum at pH 8.9, which is similar to other GPxs (36). The differences between eGPx and cGPx in physical and kinetic properties are compared in Table 1.

Expression

Northern blot analysis shows that human kidney, liver, eyes, heart, lung, breast, skeletal muscle, pancreas, brain, gastrointestinal tract, thyroid, and placenta contain detectable eGPx mRNA (2–5, 11, 14, 17, 31, 33, 38, 55, 59). Avissar *et al.* have demonstrated by the ratio of eGPx/cGPx that the kidney has the highest potential to be the main source of eGPx in the human body (3). eGPx expression is restricted and developmentally regulated, which suggests that it may serve as an antioxidant at the embryo–maternal interface (33). In the human kidney, eGPx mRNA is predominantly localized to the proximal tubules and to the parietal cells of Bowman's capsule (3, 59). In the human lung, alveolar macrophages and bronchial epithelial cells are positive for eGPx mRNA (5, 16, 17). eGPx is easily detected in blood plasma, breast milk, amniotic fluid, exocoelomic fluid, and ELF (2, 4, 14, 33, 35, 53).

Role and function of eGPx in the lung

eGPx expression is found in healthy lungs within bronchial epithelial cells and alveolar macrophages, which indicates that eGPx synthesis and secretion into ELF occurs in part by these cells (4, 5, 15, 17). eGPx is an important enzymatic component of the mechanisms for detoxifying ROS in the lung and may play a significant role in preventing oxidant-mediated lung diseases. Given the fact that eGPx is up-regulated in ELF obtained from lungs of individuals with asthma or chronic beryllium disease (CBD) or exposed to exogenous oxidants, the airway has the capacity to an increased eGPx in response to increase of ROS (Table 2) (13, 14, 16, 17). Furthermore, the striking increase of eGPx mRNA in asthmatic, CBD, and smokers bronchial epithelial cells provides clear evidence that these cells are one source of the increased eGPx in ELF (13, 14, 17). Parallel to *in vivo* findings, bronchial epithelial cells (BET1A) significantly increase eGPx mRNA expression in response to increased intracellular or extracellular ROS *in vitro* (Fig. 2A) (16, 17). Previous reports have shown that the *S*-nitrosated (GSNO) form of GSH is an equivalently effective cosubstrate (27, 30). The observation that GSNO concentration decreases in the presence of GPx suggests that GPx increases the availability of nitric oxide (NO) from GSNO (27, 30). Interestingly, GNSO also induces the eGPx gene (Fig. 2B) (16, 17). Overexpression of SOD prevents the induction of eGPx, suggesting the importance of superoxide in eGPx induction (17). Not all oxidative stress will lead to an increase of eGPx; for example, exposure to ozone decreases levels of eGPx protein and activity, whereas no change is detected with exposure to NO_2 (5). On the basis of our studies and others, we propose that up-regulation of eGPx in the lung is likely an important defense mechanism against ROS and RNS (Fig. 3).

Transcriptional regulation of eGPx

In general, ROS and RNS regulate the expression of numerous genes via signaling mechanisms. Redox-sensitive transcription factors, such as signal transducers and activators of transcription (STAT), nuclear factor-κB, and transcription activator protein-1 (AP-1), are regulated and influenced by the redox status and are implicated in the transcriptional regulation of a wide range of genes, such as proinflammatory and antioxidant genes (18, 34, 37, 48). AP-1 is a protein dimer, composed of a heterodimer of Fos and Jun proteins, which are protein products of c-Fos and c-Jun proto-oncogenes (1, 62). These gene products can form homodimeric (Jun–Jun) or heterodimeric (Jun–Fos) complexes. Studies from a number of laboratories have demonstrated that oxidant stress, such as cigarette smoke, induces the expression of c-Fos and c-Jun in epithelial cells (1, 43). Cigarette smoke increases AP-1-DNA binding in human epithelial cells *in vivo* (42). *In vitro* studies of the 5'-flanking region of the eGPx promoter demonstrate that the consensus element for AP-1 is exquisitely ROS-inducible with the redox-sensitive portion within –1,003 bp of the 5' starting point (17) (Fig. 4). Interestingly, GPx and GSH levels are highly correlated, suggesting that induction of GPx may be mediated through redox mechanisms similar to GSH (14). This suggests that increased formation of ROS and RNS leads to alterations in the redox system in the lung, which can modulate AP-1 activation and result in the induction of the eGPx gene (Fig. 5).

CONCLUSION

Collectively, the eGPx up-regulation in respiratory epithelial cells by ROS *in vitro*, together with the finding of increased eGPx expression in oxidant-related lung diseases, provides strong support for eGPx gene as a major inducible defense in the airway epithelium against oxidative injury. Increased ROS formation by inflammatory and epithelial cells in the lung leads to alterations in the intracellular and extracellular reducing-oxidizing environment, *i.e.,* GSH/GSSG levels. Loss of antioxidant activity, such as SOD in asthma, also con-

tributes to redox alteration. The high level of oxidative and nitrosative stress leads subsequently to induction of eGPx mRNA transcription, protein expression, and secretion into ELF. In the context that the susceptibility of cells to ROS depends largely on the ability to up-regulate protective antioxidant systems, increased eGPx is undoubtedly an important defense against oxidative injury to the airway surface.

ACKNOWLEDGMENTS

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ABBREVIATIONS

AP-1, activator protein-1; BET1A, human bronchial epithelial cell line; CBD, chronic beryllium disease; cGPx, cellular glutathione peroxidase; eGPx, extracellular glutathione peroxidase; ELF, epithelial lining fluid; γ-GCS, γ-glutamylcysteine synthetase; giGPx, gastrointestinal glutathione peroxidase; GPx, glutathione peroxidase; GSH, glutathione; GSNO, *S*-nitroso-L-glutathione; GSSG, glutathione disulfide; H_2O_2, hydrogen peroxide; NO, nitric oxide; $O_2^{\bullet -}$, superoxide anion; PHGPx, phospholipid hydroperoxide glutathione peroxidase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase.

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