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Forum Review

Heme Oxygenase-1: Redox Regulation of a Stress Protein in Lung and Cell Culture Models

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ABSTRACT

Reactive oxygen species (ROS) may contribute to tissue damage in many pathophysiological conditions and participate in physiological signaling processes. The mechanisms by which cells sense prooxidant states, and activate signaling pathways leading to adaptive responses, remain incompletely understood. Bacteria contain several transcriptional regulators (*e.g.*, OxyR) and a low-molecular-weight heat shock protein (HSP33), whose activity increases upon oxidation of critical sulfhydryl residues. These proteins participate in cellular adaptation to oxidative stress. In higher organisms, heme oxygenase-1 (HO-1) has been widely studied as a model for redox-regulated gene expression. Expression of HO-1 responds to chemical and physical agents that directly or indirectly generate ROS. Depletion of cellular reduced glutathione may act as a signal for HO-1 transcriptional activation. Furthermore, antioxidants and metal-chelating compounds can modulate HO-1 expression. Several signaling molecules (*e.g.*, mitogen-activated protein kinases), transcriptional regulators (activator protein-1, NF-E2-related factor-2, hypoxia-inducible factor-1, Bach-1), as well as two enhancer regions in the *ho-1* 5' regulatory region, participate in the regulation of the *ho-1* gene. HO-1 protein expression can occur in the lung in response to oxidative stress associated with infection, altered oxygen tension, and inflammatory diseases. HO-1 remains widely regarded as a protective mechanism against oxidative tissue injury. *Antioxid. Redox Signal.* 7, 80–91.

INTRODUCTION

Reactive oxygen/nitrogen species (ROS/RNS) have been implicated in tissue damage during the pathogenesis of a number of disease states, including myocardial ischemia/reperfusion (I/R) injury, atherosclerosis, Alzheimer's disease, rheumatoid arthritis, and inflammatory diseases of the lung (8). ROS refers to a group of chemical species derived from the partial reduction of molecular oxygen (O_2) during its metabolism to water by the mitochondrial respiratory chain. ROS include hydrogen peroxide (H_2O_2), (a nonradical oxidant), superoxide anion radical (O_2^-), and the highly reactive hydroxyl radical ($^{\bullet}OH$) (34). RNS refers to the free radical gas nitric oxide (NO) and its reactive derivatives, such as peroxynitrite (NO_3^-) and nitroxyl anion (NO^-) (65). Collectively, ROS/RNS can cause tissue injury as a consequence of the cumulative oxidative or nitrosative modification of vital cellular macromolecules, including lipids, carbohydrate, deoxyribonucleic acid, and protein. Such damage can result from an excessive production of ROS that supercedes an endogenous intracellular antioxidant capacity composed of water or lipid-soluble antioxidant molecules, and detoxifying and repair enzymes (34). In recent years, a hypothesis has emerged that ROS/RNS, at low concentration, may serve physiological and pathophysiological roles by interacting with and influencing the complex signal transduction networks that regulate eukaryotic gene expression. The regulation of gene expression and protein function by ROS/RNS has come to be known collectively as "redox regulation." This term implies a hypothesis that there exists a mechanism(s) whereby a cell "senses" the endogenous concentration of ROS/RNS and "reacts" by altering gene-expression profiles, in the manifestation of an adaptive or survival response.

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The classical definition of redox regulation applies to several well studied examples in bacteria, including the OxyR regulon and the recently discovered redox-regulated heat shock protein (HSP33). The activation of these systems depends on "redox switches" that are turned on or off by the redox state of intrinsic regulatory cysteine sulfhydryl groups (-SH) (76) (Fig. 1).

In mammalian systems, the heme oxygenase-1 (*ho-1*) gene represents one of the most widely studied examples of a redox-regulated gene (94). The activity of this gene product (HO-1), the rate-limiting step in the heme degradation pathway, however, is not known to be directly subject to redox regulation (61). The transcriptional regulation of HO-1, which results in the *de novo* synthesis of active enzyme, is highly inducible by oxidative cellular stress, and involves the participation of a number of upstream transcriptional regulators that are sensitive to oxidative stress or O_2 tension (for reviews, see 80).

This review will introduce the classical bacterial mechanisms of redox regulation, as well as discuss mechanisms of redox regulation in higher organisms with an emphasis on the *ho-1* gene. Redox-sensitive signaling pathways associated with *ho-1* gene expression will be described, including the mitogen-activated protein kinase (MAPK) superfamily of signal transduction proteins, and transcription factors such as the hypoxia-inducible factor-1 (HIF-1), activator protein-1 (AP-1), and NF-E2 related factor (Nrf2). The regulation of HO-1 expression by oxidative stress in the lung, where inflammatory processes may underlie the development of several disease states, is also discussed.

REDOX REGULATION: EXAMPLES FROM BACTERIA AND YEAST

OxyR and SoxRS regulons

A number of bacterial genes associated with antioxidant defense are collectively regulated in the bacterial genome in gene clusters called regulons. The *OxyR* regulon, under control of the OxyR protein, controls the regulation of ~30 genes in *Salmonella typhimurium* and *Escherichia coli*, in response to H_2O_2 stress, including hydroperoxidase-1 and alkylhydroperoxide reductase (18). In the classical model of OxyR activation, Storz and colleagues have identified two critical cysteine residues (Cys^{199} and Cys^{208}) whose reversible oxidation to form an intramolecular disulfide bridge triggers the OxyR protein to assume an activated conformation (99). The

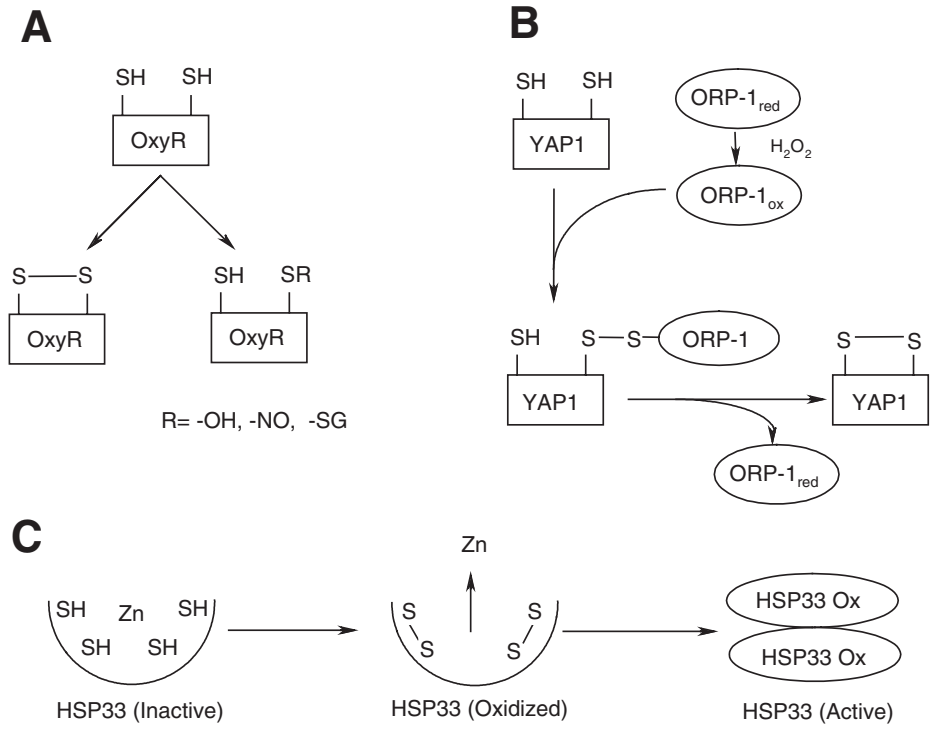

FIG. 1. Redox-regulated factors in lower organisms. The simplified diagrams show several known disulfide switches in lower organisms. (**A**) The bacterial OxyR protein exists in an inactive form when two critical cysteine sulfhydyl residues are reduced. Oxidation of the cysteine residues generates an intramolecular disulfide bridge, leading to an active conformation. On the right, activation of OxyR by oxidative modification of a single critical cysteine has also been proposed. (**B**), the yeast transcription factor yAP-1 is activated by a disulfide relay mechanism. An accessory protein ORP-1, which is oxidized by H_2O_2, in turn forms an intramolecular disulfide bridge with yAP-1. A disulfide exchange reaction generates the reduced form of ORP-1 and the oxidized, activated form of yAP-1. (**C**) The bacterial heat shock protein (HSP33) contains a core of four reduced cysteines that coordinate zinc. Oxidation of the cysteines to two intramolecular disulfide bridges releases the zinc and facilitates the dimerization and activation of the chaperone function of HSP33.

activation of OxyR allows this factor to bind to the bacterial DNA and drive the transcription of OxyR-regulated genes. Recent studies of Stamler and colleagues have proposed that a number of thiol adducts may form at a single critical cysteine (Cys^{199}), including sulfinic acid, nitroso, and mixed disulfide derivatives, which imply more complex modes of activation in response to different types of ROS/RNS (50). The significance of the cysteine adducts of OxyR and the controversy surrounding these alternative activation mechanisms have been elaborately reviewed (76).

Another bacterial regulon, SoxRS, identified by Demple and colleagues, controls the regulation of a second series of genes in *Escherichia coli*, including manganese superoxide dismutase (31). In the SoxRS system, the SoxR factor is activated by a redox switch, involving an iron-sulfur cluster. Activated SoxR drives the transcriptional activation and synthesis of SoxS, which in turn activates the transcription of the downstream genes (6, 71). Unlike OxyR, which responds strongly to H_2O_2 treatment, the SoxRS regulon was originally identified on the basis of its sensitivity to agents that generate O_2^-, such as the redox-cycling compound paraquat (31). A recent microarray analysis of the peroxide response in *Escherichia coli* has confirmed the OxyR regulon, but suggested that some overlap exists between the OxyR and SoxRS regulons in the response to H_2O_2 (99).

HSP33

The low-molecular-weight cytoplasmic heat shock protein (HSP33), first characterized in *E. coli*, exemplifies a protein function directly activated by oxidative modification. Like other members of the heat shock protein family, HSP33 responds to transcriptional activation by heat shock and exhibits chaperone or protein binding activity, which is reversible by the hydrolysis of bound ATP. Unlike all the other known heat shock proteins, the chaperone activity of HSP33 can be regulated by a posttranslational redox mechanism (43). HSP33 contains a core of four highly reactive cysteines that coordinate zinc under reducing conditions and respond to changes in intracellular redox potential (44). Treatment with H_2O_2 oxidizes endogenous cysteine sulfhydryls to form two intramolecular disulfide bridges, with the concomitant release of the coordinated zinc (43, 44). The activation of the HSP33 chaperone function results from the formation of a homodimer of two fully oxidized monomers. HSP33 participates in bacterial adaptation to oxidative stress.

Redox regulated factors in yeast

The yAP-1 regulon regulates the expression of a number of proteins with antioxidant function in yeast (*Saccharomyces cerevisiae*) under the regulation of the transcription factor yAP-1, an analogue of mammalian AP-1 (76). yAP-1 is activated by a disulfide switch (19), but differs from OxyR in that it is not directly oxidized by H_2O_2. Instead, exposure to peroxide causes the formation of an intermolecular disulfide bridge between yAP-1 and an accessory protein: the oxidant receptor peroxidase-1 (ORP-1; formerly Gpx, a thioredoxin-dependent peroxidase) (20). ORP-1 is directly oxidized by H_2O_2 to form a sulfinic acid (S-OH) derivative that reacts with Cys^{598} of yAP-1 to form a disulfide cross-link. An exchange reaction occurs between the yAP-1-ORP complex and Cys^{303} of yAP-1, forming an intramolecular disulfide bridge in yAP-1 and releasing the reduced form of ORP-1 (20). The oxidation of yAP-1 in this way facilitates its nuclear importation, allowing it to express its function as a transcriptional regulator (52).

HO-1: REDOX REGULATION OF TRANSCRIPTIONAL ACTIVATION

HO-1: an oxidant-sensitive gene

The *ho-1* gene, which encodes a low-molecular-weight stress protein (32 kDa in humans and rats, 34 kDa in mice), provides a model system for the study of redox-regulated gene expression in mammalian systems (94). Tenhunen *et al.* first characterized heme oxygenase (HO; E.C. 1.14.99.3) as a microsomal monooxygenase system distinct from cytochrome P450 (91). The HO enzymatic activity uses molecular oxygen and reducing equivalents from NADPH:cytochrome P450 reductase to catalyze the oxidation of heme-b to biliverdin-IXα, which is further converted to bilirubin-IXα by an NAD(P)H-dependent reductase (91). HO activity also releases carbon monoxide (CO) and heme iron as reaction by-products. The expression of HO-1 may occur in most tissues, but increases over background levels in response to extracellular stress. HO-1 has a major genetically distinct isozyme, HO-2, which is constitutively expressed in most tissues. HO-2 does not respond to transcriptional activation by xenobiotics or physical stress and is not known to respond to redox processes. The biology of HO-2 has been reviewed elsewhere (61).

HO-1 induction represents a general transcriptional response to oxidative cellular stress, which can be triggered by stimulation with a large array of chemical and physical agents (83). For the purpose of this review, the spectrum of conditions that induce HO-1 fall into four broad but overlapping categories: oxidative stress, nitrosative stress, thiol-reactive substances, and cytokines (Fig. 2).

HO-1 was originally classified as an oxidant-inducible response by Keyse and Tyrrell (46). Using human skin fibroblasts as a model, they showed that a 32-kDa stress protein, and its corresponding mRNA, accumulated in response to cellular treatment with H_2O_2, sodium *meta*-arsenite ($NaAsO_2$), redox cycling compounds such as menadione, and solar ultraviolet A (UVA; 320–380 nm) irradiation (46). This 32-kDa stress protein was identified as identical to HO-1 by molecular cloning techniques (46). Keyse *et al*. first showed that the induction of HO-1 protein expression by H_2O_2 and UVA radiation was regulated at a transcriptional level in human skin fibroblasts (48). The response could be reproduced in a wide variety of animal and human cell types, and therefore has been used as a general marker of oxidative stress in cell culture models (7). The induction of HO-1 by UVA was associated with the generation of singlet molecular oxygen ($^1O_2$) resulting from the photo-excitation of endogenous chromophores (12). HO-1 also responds to exogenous photosensitizing drugs, such as dihematoporphyrin ether/ester (Photofrin II), which can generate $^1O_2$ and other ROS species upon activation with specific wavelengths of light by a type II photochemical mechanism (29).

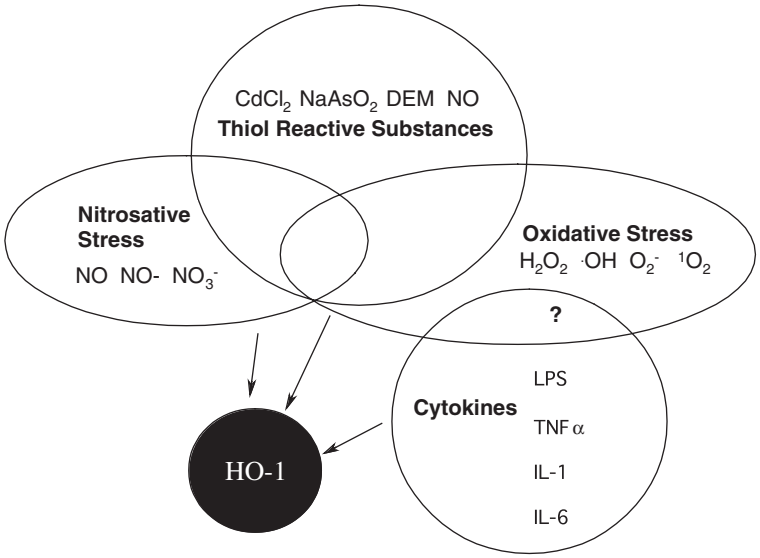

FIG. 2. HO-1 activation by stress. The diagram shows four overlapping classes of HO-1-inducing compounds: (a) oxidative stress, including the generation of ROS, and changes in oxygen tension; (b) nitrosative stress, including the generation of NO and its reactive derivatives; (c) thiol-reactive substances, including agents such as DEM that complex or deplete intracellular GSH; and (d) cytokines, including bacterial endotoxins, and growth factors.

Heme, the natural substrate of HO activity, also potently induces the gene (2). A link between heme and oxidative stress is often inferred from the fact that heme is a biologically relevant iron chelate that participates in many enzyme-directed oxidation reactions. Free heme has been shown *in vitro* to act as a potent catalyst of lipid peroxidation, and to promote oxidative damage to vascular endothelial cells (10, 81).

HO-1 can be induced in cell culture by deviations in oxygen tension above or below the acclimated range (80). Exposure to high oxygen tension (hyperoxia) generates an oxidative stress by increasing the mitochondrial production of ROS (26). Hyperoxia is a potent activator of HO-1 mRNA and protein expression in lung-derived cell lines (59). Hypoxia, or diminished O_2 levels, also triggers the HO-1 response in animal cell types (58, 66, 82). The classification of hypoxia as an oxidative stress remains controversial, although recent studies suggest that hypoxia may increase mitochondrial production of ROS within a critical range of pO_2 (15).

Cytokines

HO-1 is strongly induced by proinflammatory stress. In cell culture models, HO-1 can be activated by bacterial lipopolysaccharide (LPS) and the proinflammatory cytokines, tumor necrosis factor-α (TNFα), interleukin (IL)-1, and IL-6 (14, 92). Proinflammatory cytokines may represent an indirect oxidative stress, in that ROS production can occur as a downstream phenomenon associated with cytokine-dependent receptor activation (62). Several growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor-β (TGFβ) mimic the cytokine response (21, 53).

Nitrosative stress

In addition to oxidative stress, HO-1 responds strongly to RNS. NO, whether applied as gas or as various donor compounds, acts a potent inducer of the *ho-1* gene in a number of cell culture models, involving both transcriptional and post-transcriptional regulation of the gene (for review, see 35, 65). NO and/or its derivative RNS can react with thiol compounds, such as glutathione (GSH), to promote the formation of *S*-nitroso- and *S*-nitro-thiol derivatives (65). Activation of HO-1 by RNS such as NO_3^- and NO^- has been described (25, 68). Motterlini and colleagues suggested that the formation of *S*-nitroso-glutathione during cellular exposure to NO donating compounds or hypoxia may act as an intermediate in HO-1 activation (24, 65). They have recently showed that the induction of HO-1 by heme is potentiated in combination with NO, and postulated the involvement of nitrosylated heme derivatives (69).

Thiol-reactive substances and the role of GSH in redox regulation of HO-1 gene expression

HO-1 activation responds to a number of thiol (-SH)-reactive substances that complex intracellular GSH, including $NaAsO_2$, diethyl maleate (DEM), and $CdCl_2$ (2, 46, 85). Both DEM and $NaAsO_2$ effectively deplete intracellular GSH, through the formation of GSH adducts that cannot be reduced by NADPH:glutathione reductase (85). Heavy metal salts, which induce HO-1 expression *in vivo* and *in vitro*, have long been known to form complexes with thiols (61). The depletion of GSH by conjugation appears to represent a general phenomenon associated with the activation of HO-1 by a number of inducing agents. The chemical depletion of intracellular GSH with buthionine-[*S,R*]-sulfoximine (BSO), an inhibitor of GSH synthesis, strongly enhances the activation of HO-1 by oxidants, such as UVA radiation exposure and H_2O_2 treatment (55). Furthermore, the hepatic induction of HO-1 by LPS and proinflammatory cytokines in rats could be augmented by BSO (79). These observations have collectively pointed to a depletion of cellular reducing equivalents as a general phenomenon associated with the induction of HO-1. The mechanisms by which such changes in cellular redox potential activate downstream signaling events (see below), such as protein kinases and transcriptional machinery, remain an enigma.

Potential roles for iron in redox regulation

Although iron essentially does not exist in biological systems in "free" form, it is thought that potentially reactive iron exists in an intracellular pool in complexes with low-molecular-weight molecules, which is accessible to metal chelators (78). Keyse and Tyrrell showed that the induction of HO-1 by UVA and oxidants could be suppressed by the treatment with metal chelators such as desferrioxamine (DFO) (47). DFO has also been shown to inhibit HO-1 expression in cell culture models of hypoxia and hyperoxia (23, 82). The original interpretation of the phenomenon was that metal-chelating compounds act by removing a pool of potentially reactive iron, which amplifies the effect of oxidative stress conditions (47). In support of this hypothesis, iron loading has been shown to sensitize endothelial cells to peroxide stress (9). Ryter *et al.* showed that iron loading strongly potentiates the HO-1 activation by hypoxia in endothelial cells (82). A second interpretation of this phenomenon is that the chelating compounds remove a pool of iron that is required for gene transcription. Paradoxically, DFO potently activates HIF-1 under normoxic conditions, which is among the factors that govern the transcriptional activation of *ho-1* (58, 88). The prolyl hydroxylase involved in HIF-1α degradation requires iron (42). Recently, it has also been shown that DFO increases the synthesis of the *ho-1* transcriptional repressor Bach-1. Such a mechanism may also account for the inhibitory effects of DFO on *ho-1* activation in a number of systems, although the role of iron is not clear (51). Metal-chelating compounds may be of general use in strategies for the pharmacological manipulation of stress responses *in vivo*.

Modulation of HO-1 expression by antioxidants

Activation of HO-1 is sensitive to a number of natural and synthetic antioxidant compounds in tissue culture models. Whether treatment with antioxidant compounds has an activating or inhibitory effect on HO-1 regulation depends on the compound and model system.

N-*Acetyl-L-cysteine (NAC).* NAC, a precursor of GSH, has been widely used in cell culture as an antioxidant compound. Inhibition of gene expression by NAC, usually at millimolar doses, has often been used to claim the involvement of ROS in the activation process. Early work by Rizzardini *et al.* showed that injection of NAC reversed the HO-1 induction observed during hepatic endotoxinemia in the rat, caused by LPS injection (79). Treatment with NAC inhibited transcriptional activation of *ho-1* by H_2O_2 or LPS treatment, but not by heme (3, 14). Furthermore, in a number of cell culture models, treatment with millimolar concentrations of NAC effectively inhibited the induction of HO-1 by various inducing agents, including PDGF (21), the proinflammatory cytokines TNFα and IL-1 (92), $CdCl_2$ (30), NO/RNS-generating systems (24, 25, 35), diesel exhaust particles (60), and phenolic antioxidants (60). In endothelial cells, NAC also inhibited HO-1 induction in response to hypoxia (82).

α-*tocopherol and ascorbate.* The classical water-soluble (ascorbate) and lipid-soluble (α-tocopherol) endogenous antioxidant compounds have surprisingly not been widely studied in the context of HO-1 activation, having been superceded by NAC as the test compound of choice. The supplementation of human fibroblasts with vitamin E (α-tocopherol) increased HO-1 activation during exposure to UVA radiation (13). Ascorbate supplementation inhibited dopamine-induced HO-1 expression in neuroglial cell culture (87).

Plant derived antioxidants. β-carotene is a lipid-soluble antioxidant that can quench $^1O_2$. Supplementation with all-*trans* β-carotene inhibited the induction of HO-1 in human skin fibroblasts subjected to UVA radiation exposure (93). In the same model, epigallocatechin, a major polyphenolic constituent of green tea, inhibited the UVA-dependent activation of HO-1 (89).

Ginkgo biloba extract (EGb 761) is a standard extract of *Ginkgo Biloba* leaves consisting of a complex mixture of terpenoids and flavonoids. Two recent reports indicate that EGb 761 can activate HO-1 expression (16, 100). EGb 761 treatment protected cultured neurons from ischemic stress and protected endothelial cells from lysophosphatidylcholine (16, 100). The cytoprotection observed in both models was associated with the dose-dependent activation of HO-1. A genetic screen confirmed the elevation of HO-1, as well as several other antioxidant enzymes, after the treatment of bladder carcinoma cells with Ginkgo-derived flavonoids (28). The induction of HO-1 by EGb occurred in the context of increased intracellular GSH content (28). Recently, two additional natural antioxidant compounds, curcumin (a spice-derived antioxidant and antiinflammatory agent) and caffeic acid phenethyl ester, have been shown to induce HO-1 in tissue culture models (86).

Synthetic antioxidants. Diphenyleneiodonium (DPI) is a nonspecific inhibitor of flavoprotein reductases. DPI potently activated *ho-1* gene expression in pulmonary artery endothelial cells (PAEC) under normoxic and hypoxic conditions (84). This potent induction of the response by DPI precluded its use as an inhibitor for studying upstream redox processes involved in *ho-1* gene activation, and could be related to structural similarities of this compound with phenolic antioxidants.

Pyrrolidine dithiocarbonate (PDTC), which can act as a potent inhibitor of nuclear factor-κB, represents another example of a synthetic antioxidant compound that strongly induces HO-1 in cell culture (36).

In conclusion, compounds that classify as "antioxidants" have been shown to either inhibit or induce the activation of *ho-1*. Many antioxidants that activate *ho-1* fall into a class of phenolic compounds that activate the Nrf2 transcription factor which in turn acts on the antioxidant responsive elements (ARE) in the *ho-1* gene (11, 60) (see following section). NAC, which typically inhibits *ho-1* in most systems, acts as a radical scavenger and GSH precursor. In general, antioxidants that scavenge ROS or chelate metal ions without activating the Nrf2 pathway are typically inhibitors of *ho-1* activation.

Role of MAPK in HO-1 gene regulation

Signal transduction in mammalian cells is mediated, in part, by the sequential phosphorylation of effector proteins

belonging to the MAPK superfamily. The MAPKs, a family of Ser/Thr protein kinases, respond to a variety of extracellular stimuli. Three major MAPK signaling pathways, which include extracellular signal–regulated protein kinase (ERK), p38 MAPK, and c-Jun NH_2-terminal protein kinase (JNK), have been identified in mammalian cells (54). The signals generated by kinases are reversed by the action of MAPK phosphatases.

The link between oxidative stress and the regulation of protein phosphorylation/dephosphorylation remains partially understood. In 1993, Guy *et al.* described a general inhibition of protein phosphatase activity by TNFα, leading to a kinase-independent up-regulation of protein phosphorylation, and proposed the involvement of such a mechanism in the oxidant-dependent activation of HO-1 (32). Guton *et al.* demonstrated the direct activation of ERK1/2 and, to a lesser extent, JNK and p38 MAPK after H_2O_2 treatment in several cell lines, including PC12 cells (33). Expression of the wild-type or dominant negative forms of MAPK kinase (MEK) respectively promoted or antagonized cell survival after H_2O_2 treatment (33). Exposure of macrophages to oxidative stress (hyperoxia) activated ERK MAPK (77).

The link between increased intracellular ROS and MAPK activation remains unresolved. Likewise, the upstream molecular signaling pathways specific to *ho-1* gene activation also remain partially understood (Fig. 3). A number of studies using chemical inhibitors (p38 MAPK, SB203580; ERK, PD98059, JNK, SP600125) or genetic tools (*e.g.*, dominant negative mutants of MAPK and genetically deleted MAPK strains) have attempted to delineate the involvement of MAPKs in the activation of *ho-1* in response to specific inducing stresses.

Using such methods, Alam *et al.* demonstrated the role of p38 MAPK, but not ERK, in the *ho-1* transcriptional response to $CdCl_2$ (5). Roles for ERK and/or p38 MAPK (in avian hepatoma cells) and/or JNK (in rat hepatocytes) have been proposed in the activation of *ho-1* by $NaAsO_2$ (22, 49). In the response to NO-donating compounds, both p38 MAPK and ERK were implied (17). Ning *et al.,* using SB203580, as well as overexpression of a dominant negative mutant for p38 MAPK, have demonstrated the critical role for p38 MAPK in the induction of *ho-1* mRNA by TGFβ (70). Kacimi *et al*., using chemical inhibitors, have implicated p38 MAPK, but not ERK

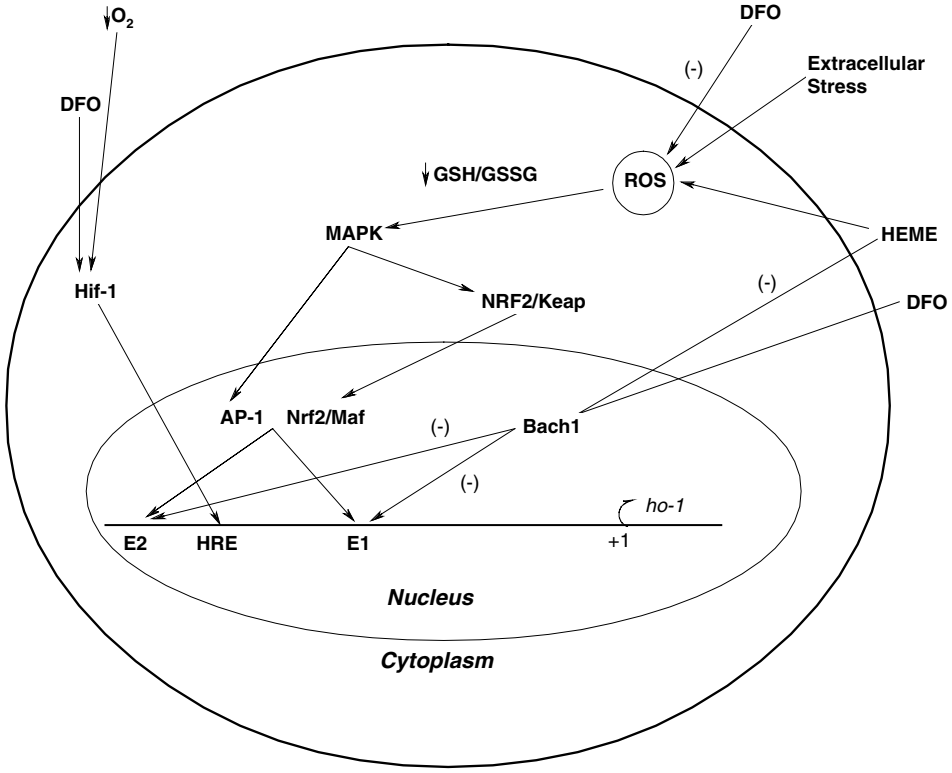

FIG. 3. Redox-dependent pathways in HO activation. The diagram shows the cytoplasmic and nuclear events associated with the redox-dependent activation of the *ho-1* gene. Cytoplasmic events include the elevation of intracellular ROS production by extracellular stimuli, the decrease of reduced gluatathione to oxidized glutathione ratio (GSH/GSSG), and the activation of MAPK. The transcription factor Nrf2 is activated in the cytoplasm by dissociation of the inhibitor Keap-1. Nuclear events in *ho-1* activation include the binding of Nrf2/Maf dimers or AP-1 transcription factors at the −4-kb or −10-kb enhancers (E1, E2) of *ho-1*. The transcriptional repressor Bach-1 antagonizes Nrf2/Maf DNA binding. The activation of *ho-1* by hypoxia involves stabilization of HIF-1α and formation of HIF-1α/β heterodimer. Activated HIF-1 migrates to the nucleus and binds to the *ho-1* gene at the −9-kb HRE. The metal chelator DFO potentially interacts with these pathways at several sites, by inducing HIF-1, by decreasing intracellular ROS, and by inducing the expression of the Bach-1 repressor. Heme, the substrate of HO-1, can inactivate the Bach-1 repressor and promote the formation of ROS. Inhibitory interactions are indicated by (−).

or tyrosine kinase, in the hypoxic *ho-1* activation pathway in cardiomyocytes (45). In contrast, Ryter *et al.* found that SB203580 and PD98059 compound activated *ho-1* in PAEC at 10 μ*M* doses. In the presence of hypoxia (1% O_2), the SB (and to a lesser extent, PD) compound strongly potentiated the hypoxic activation of *ho-1* (84). In an *in vivo* model of lung I/R stress and corresponding cell culture model of anoxia/reoxygenation, all three major MAPKs (p38, ERK, JNK) were implicated in the induction response (97).

Overexpression of JNK, MKK3, and p38γ MAPK augmented, whereas expression of the other p38 isoforms (α, β, δ) inhibited, HO-1 expression in rat hepatocytes (49). Overexpression of several variants of MEKK (MEKK1, TAK, ASK) induced HO-1 in hepatoma cells. This effect was further augmented by coexpression with downstream kinases (MKK4, MKK6, and JNK) and abolished by dominant negative Nrf2 (96).

These studies, taken together, do not provide a unifying signaling pathway for *ho-1* activation. The resolution of these upstream events is complicated by cell type-specific and inducer-dependent variations, and assumes the specificity of chemical inhibitors, which may have further unknown targets. Recent studies point to the importance of p38 MAPK in *ho-1* regulation, at least in those pathways that involve activation of the Nrf2 transcription factor (4, 5) (see following section).

Cis and trans factors in redox regulation of HO-1

Cis factors in HO-1 promoter. The *cis*-acting regulatory regions important for the basal and inducible regulation of the *ho-1* gene have been elucidated by Alam *et al.* Using deletion mutants of the mouse *ho-1* gene 5' regulatory region linked to reporter constructs, Alam *et al*. have discovered two transcriptional enhancer sequences located at –4 kb (E1) and –10 kb (E2) of the transcriptional start site (1, 3). These elements mediate the induction of *ho-1* to a number of inducing agents, including heavy metals, 12-*O*-tetradecanoylphorbol 13-acetate, LPS, heme, and H_2O_2 (1, 3, 14, 39). Both enhancer elements contain repeated essential *cis*-acting stress responsive elements (StRE) with the consensus sequence (T/CGCTGAGTCA). The StRE contains an intrinsic consensus ARE, overlapping an intrinsic Maf recognition element (MARE) and AP-1 binding site (39).

In addition to the distal enhancers, the promoter region of *ho-1* also contains a distinct hypoxia response element (HRE) occurring at –9 kb from the transcriptional start site, which comprises two functional binding sites for the HIF-1, and mediates the *ho-1* response to hypoxia (58).

NrF2. Nrf2, a member of the Cap'n'collar/basic-leucine zipper family, recognizes and binds to consensus ARE sequences found in the promoter regions of several detoxifying enzymes (*e.g.*, NADPH:quinone oxidoreductase, glutathione *S*-transferase, and *ho-1*) (4, 40, 95). Nrf2 forms stable heterodimers with members of the maf (MafK, MafF, MafG) family (40). Transcription factor ATF4 has been suggested by yeast two-hybrid analysis as another possible binding partner to Nrf2 (38). The DNA-binding activity of Nrf2 is markedly induced by electrophilic agents, including polyphenols and plant-derived substances, through a posttranslational mechanism. Under basal conditions, a cytoplasmic factor, Keap-1, inhibits the activity of Nrf2 by binding to the negative regulatory domain of Nrf2. Induction by electrophiles releases the inhibitor, allowing the nuclear translocation of Nrf2 (41). Nrf2 plays a critical role in the induction of *ho-1* by a number of agents, including heme, $CdCl_2$, arsenite, and phenolic compounds (4, 5, 39, 60). The Nrf2-mediated activation of *ho-1* by $CdCl_2$ depended on E1 and was linked to the activation of the p38 MAPK pathway (5). Recent studies have shown the involvement of the Nrf2/Keap1 signaling pathway in the activation of *ho-1* by RNS-related stress, such as nitroxyl heme derivatives (69), by particulate fractions of diesel exhaust, which contain mixtures of phenolic compounds (60), and by the antioxidant curcumin (11).

Bach-1. Bach-1, a transcriptional repressor of *ho-1* that is sensitive to the intracellular concentration of heme, has recently been described (90). Bach-1 potentially serves as a binding partner for maf proteins, and antagonizes the effects of Nrf-2/maf dimers at the StRE (ARE/MARE) sites of E1 and E2. The DNA binding activity of Bach-1 is negatively regulated by heme *in vitro* (72), and this may account for the substrate dependent activation of *ho-1*.

AP-1. The AP-1 family of transcription factors consists of Jun family oncoproteins that homo- or heterodimerize with other members of the Jun or Fos protein families. The treatment of RAW 264.7 cells with LPS increased AP-1 DNA binding activity in nuclear extracts, associated with an increased cellular production of ROS. The transcriptional induction of *ho-1* by LPS required both E1 and E2 enhancers. Deletion of AP-1 binding sites in these enhancers, which inevitably also inactivates the overlapping ARE, attenuated the induction response (14). The activation of *ho-1* transcription by the synthetic antioxidant PDTC involved the activation of AP-1 acting at the E2 distal enhancer (36). Increased AP-1 DNA binding activity has been associated with the endothelial cell-specific induction of HO-1 in PAEC by hypoxia (37, 84). In contrast, the hypoxia inducible factor (see following section) appeared to act as the critical underlying factor in smooth muscle cells (37). In comparison, hyperoxia treatment caused increased DNA binding activity of AP-1 and STAT (signal transducer and activator of transcription; STAT1, STAT3, STAT5) transcription factors in RAW 264.7 cells. The up-regulation of *ho-1* following hyperoxia treatment in this cell line depended on the intrinsic AP-1 elements in the E1 and E2 sites. Furthermore, the induction response required the cooperation of STAT elements located within the proximal promoter region of *ho-1* (59). Although hyperoxia and hypoxia are by definition two opposing states, they both result in the activation of *ho-1* and share transcription factor AP-1 as a common underlying mechanism. However, the two states apparently differ in the accessory transcription factors involved in gene activation.

HIF-1 and the hypoxic response. Hif-1 is a heterodimeric complex consisting of an α-subunit, which is stabilized by hypoxia, and a β-subunit (aryl hydrocarbon nuclear translocator) (88). A recently discovered posttranslational modification (hydroxylation) of HIF-1α by an O_2-dependent prolyl hydroxylase allows recognition by the Von Hippel–Lindau tumor suppressor protein, promoting the rapid ubiquitination and proteolytic degradation of HIF-1α under normoxia (42). The in-

duction of HO-1 by hypoxia in rat aortic vascular smooth muscle was associated with increases in HIF-1α and β protein levels, and required the HRE sequence at –9 kb, but not the E1 and E2 distal enhancers (58). Nakayama *et al.* have described that certain human derived cell lines are refractory to the HO-1 response to hypoxia (67). The transcriptional repression of *ho-1* by hypoxia in human cell lines has been linked to the activation of Bach-1, which binds to the MARE at the E1 (51). A relationship between Bach1 and HIF-1 is unknown.

HO-1 EXPRESSION IN THE LUNG AND ITS FUNCTIONAL SIGNIFICANCE

The lung serves as a portal for the delivery of O_2 to the tissues of the body and, as such, is susceptible to stress related to deviation in O_2 tension. Furthermore, a number of lung disease states are associated with oxidative stress due to inflammatory processes. HO-1 is induced in several animal models of inflammation and oxidative stress in the lung (for review, see 83). The induction of HO-1 in this context protects against tissue damage, by antiinflammatory, antiapoptotic, and antioxidative mechanisms.

Otterbein *et al.* observed increases in pulmonary HO-1 during experimental endotoxemia in rats (73). The induction of HO-1 by hemoglobin pretreatment, protected against subsequent endotoxin stress in this model (73). Otterbein *et al.* further described a mechanism by which HO-1, and its reaction product CO, down-regulated macrophage inflammatory responses. Both HO-1 and HO-derived CO inhibited the LPS-dependent production of proinflammatory cytokines (IL-1β, MIP, and TNFα) in macrophages, while increasing the production of IL-10. The antiinflammatory effects of HO-1/CO in this model depended on up-regulation of p38 MAPK (75).

HO-1 can also be induced in lung tissue by high O_2 tension, which produces an inflammatory stress in the lung similar to acute respiratory distress syndrome (56). HO-1 participates in pulmonary adaptation to high O_2 levels. The adenoviral-mediated gene transfer of *ho-1* into rat lungs protected against the development of lung apoptosis and inflammation during hyperoxia (74). *In vitro* studies showed that the overexpression of HO-1 in lung epithelial cells caused growth arrest and conferred resistance against hyperoxia-induced cell death (57).

Lung I/R represents another model of oxidant-mediated acute lung and vascular injury. HO-1 mRNA was dramatically increased in mouse lung after 30 min of ischemia followed by 2–6 h of reperfusion (97). Homozygous *ho-1* null mice (*hmox-1*$^{-/-}$) displayed increased mortality in a model of lung I/R injury (27). The selective overexpression of HO-1 in the lung of transgenic mice conferred protection against the inflammatory and hypertensive effects of hypoxia (63). These examples, taken together, demonstrate that HO-1 not only is expressed by oxidative injury in the lung, but participates in endogenous adaptive mechanisms in this vital organ.

CONCLUSIONS

In this review, several prokaryotic and eukaryotic mechanisms of redox regulation have been described. Bacteria and yeast contain well defined redox switches for protein function that depend on sulfhydryl oxidation state. The mechanisms of redox regulation in higher organisms remain partially understood. HO-1 represents an example of a mammalian gene product whose transcription is regulated by a multiplicity of redox-related processes. In summary, the regulation of the *ho-1* gene responds to multiple systems that generate intracellular ROS/RNS. This effect is generally inhibitable by thiol antioxidants and metal chelators in a number of systems. Perturbation of GSH/GSSG ratio, leading to a decrease of intracellular reducing capacity, appears to be a general phenomenon associated with *ho-1* activation. The upstream signaling pathways, including MAPK and phosphatases that regulate *ho-1*, are incompletely resolved and vary with experimental system. However, some of these components are responsive to oxidative stress conditions. Furthermore, *ho-1* regulation responds to a general class of phenolic antioxidants that activate the Nrf2/ARE pathway. Other well known redox-activated transcription factors, including AP-1, and the hypoxia-inducible factor have been implicated in *ho-1* activation. A recently characterized inhibitor of *ho-1* transcription, Bach-1, responds to regulation by two classical inducers and inhibitors of the *ho-1* response, heme and DFO, respectively. Further research in defining the upstream events that regulate *ho-1* activation will provide more opportunities for pharmacological intervention.

HO-1 expression can confer cyto- and tissue protection in numerous disease models where inflammation and the generation of ROS are implicated. The mechanisms underlying HO-dependent tissue protection are now the subject of intense investigation and may involve all the HO-1 reaction end products, including CO. Attempts have been made to exploit the therapeutic potential of HO-1 by gene therapy approaches. Reproduction of the effects of HO-1 overexpression has been sought in the pharmacological delivery of HO reaction end products such as biliverdin or CO. The pharmacological modulation of HO-1 by natural nontoxic inducers of HO-1 remains another possible avenue for HO-derived therapies. In this regard, several plant-derived antioxidants described in this review that induce HO-1 without causing toxicity or GSH depletion may show promise. A complete understanding of how protective genes such as *ho-1* are regulated by intricate signal transduction networks during stress and disease states may allow for further drug development.

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ABBREVIATIONS

AP-1, activator protein-1; ARE, antioxidant responsive element; BSO, buthionine-[*S,R*]-sulfoximine; CO, carbon monoxide; DEM, diethyl maleate; DFO, desferrioxamine; DPI, diphenyleneiodonium; ERK, extracellular signal-regulated kinase; GSH, reduced glutathione; GSSG, oxidized glutathione;

HIF-1, hypoxia-inducible factor-1; H_2O_2, hydrogen peroxide; HO-1, heme oxygenase-1; HRE, hypoxia response element; HSP33, heat shock protein 33; IL, interleukin; I/R, ischemia/reperfusion; JNK, c-Jun NH_2-terminal protein kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MARE, Maf recognition element; MEKK, mitogen-activated protein kinase kinase kinase; NAC, *N*-acetyl-L-cysteine; $NaAsO_2$, sodium *meta*-arsenite; NO, nitric oxide; NO^-, nitroxyl anion; NO_3^-, peroxynitrite; Nrf2, NF-E2-related factor-2; O_2^-, superoxide anion radical; $^1O_2$, singlet molecular oxygen; $^{\bullet}OH$, hydroxyl radical; ORP-1, oxidant receptor peroxidase-1; PAEC, pulmonary artery endothelial cells; PDGF, platelet-derived growth factor; PDTC, pyrrolidine dithiocarbamate; RNS, reactive nitrogen species; ROS, reactive oxygen species; STAT, signal transducer and activator of transcription; StRE, stress responsive element; TGFβ, transforming growth factor-β; TNFα, tumor necrosis factor-α; UVA, ultraviolet-A (320–380 nm) radiation; yAP-1, yeast activator protein-1.

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