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Forum Review

Controlled Elimination of Intracellular H_2O_2: Regulation of Peroxiredoxin, Catalase, and Glutathione Peroxidase via Post-translational Modification

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ABSTRACT

The predominant enzymes responsible for elimination of hydrogen peroxide (H_2O_2) in cells are peroxiredoxins (Prxs), catalase, and glutathione peroxidases (GPxs). Evidence suggests that catalytic activities of certain isoforms of these H_2O_2-eliminating enzymes are extensively regulated via posttranslational modification. Prx I and Prx II become inactivated when phosphorylated on Thr^{90} by cyclin B-dependent kinase Cdc2. In addition, the active-site cysteine of Prx I–IV undergoes a reversible sulfinylation (oxidation to cysteine sulfinic acid) in cells. Desulfinylation (reduction to cysteine) is achieved by a novel enzyme named sulfiredoxin. c-Abl and Arg nonreceptor protein tyrosine kinases associate with catalase in cells treated with H_2O_2 by mechanisms involving the SH3 domains of the kinases and the Pro^{293}PheAsnPro motif of catalase and activate catalase by phosphorylating it on Tyr^{231} and Tyr^{386}. Similarily, GPx1 is activated by c-Abl- and Arg-mediated phosphorylation. The tyrosine phosphorylation is critical for ubiquitination-dependent degradation of catalase. *Antioxid. Redox Signal.* 7, 619–626.

INTRODUCTION

Hydrogen peroxide (H_2O_2) has been increasingly recognized as an important component of receptor signaling, which serves as an intracellular messenger mediating various cell functions, including proliferation, differentiation, apoptosis, and senescence (18, 40, 41, 51).

Receptor-mediated production of H_2O_2 has been studied mostly in phagocytic leukocytes (1). In these cells, the one-electron reduction of molecular oxygen by a multicomponent NADPH oxidase system generates superoxide ($O_2\bullet^-$), which is spontaneously or enzymatically converted to H_2O_2. Recently, the intracellular generation of $O_2\bullet^-$ and H_2O_2 has also been detected in various nonphagocytic cells stimulated with various peptide growth factors, cytokines, and agonists of heterotrimeric GTP-binding protein (G protein)–coupled receptors (29, 41). In addition, NADPH oxidase systems similar to that of phagocytic cells were recently identified in nonphagocytes, and some of these enzyme systems were shown to be responsible for H_2O_2 production induced by a wide variety of cell-surface receptors (29). As exemplified in the cells stimulated by angiotensin II (47) or platelet-derived growth factor (PDGF) (2, 37), the production of $O_2\bullet^-$ and H_2O_2 via NADPH oxidase is a highly regulated process involving various protein kinases and phosphatases, adaptor proteins, and heterotrimeric and small-molecular-weight G proteins (29).

Studies of intracellular messengers such as cyclic nucleotides and inositol 1,4,5-trisphosphate indicate that timely elimination of messengers after completion of their functions is critical for cellular signaling. Thus, in general, elimination as well as production of intracellular messengers is a highly controlled process. This would seem especially true for H_2O_2, which is readily converted to deleterious hydroxyl radicals. In mammalian cells, H_2O_2 is mainly eliminated by three types of enzymes: peroxiredoxin (Prx), catalase, and glutathione peroxide (GPx). This review focuses on recent findings that those H_2O_2–eliminating enzymes are subjected to extensive posttranslational regulation.

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CATALYTIC PROPERTIES OF Prx ENZYMES

Prxs are a family of peroxidases that reduce H_2O_2 and alkyl hydroperoxides to water and alcohol, respectively, with the use of reducing equivalents provided by thiol-containing proteins (22, 42). The first Prx proteins to be discovered were a 25-kDa yeast protein, initially called thiol-specific antioxidant (TSA) enzyme (9, 25, 26), and a 21-kDa *Salmonella typhimurium* alkyl hydroperoxide reductase, termed AhpC (48, 50). Subsequently a mammalian homologue of TSA/AhpC was purified and it, together with TSA and AhpC, defined a family of peroxidases that now includes six mammalian isoforms (Prx I–VI) and members identified in organisms from each kingdom (11).

Prx is present in organisms from all kingdoms (11). All Prx enzymes contain a conserved cysteine residue at the NH_2-terminal region, which is the primary site of oxidation by H_2O_2. The six mammalian Prxs exist in at least six isoforms, which can be divided into three subgroups, namely, 2-Cys, atypical 2-Cys, and 1-Cys subgroups (24, 42, 46). All Prx enzymes exist as homodimers. The 2-Cys members, which include Prx I–IV, contain an additional conserved cysteine at the COOH-terminal region, whereas Prx V and Prx VI, the members of the atypical 2-Cys and 1-Cys subgroups, respectively, do not contain this second conserved cysteine. The amino acid sequence identity among the three subgroup members is low (<20%), whereas that among the 2-Cys subgroup members is 60–80%.

The peroxidase reaction mechanisms of Prx enzymes are shown separately for 2-Cys, atypical 2-Cys, and 1-Cys Prx subgroups in Fig. 1. The conserved, H_2O_2-sensitive Cys of 2-Cys Prx, which corresponds to Cys^{51} in mammalian Prx I, is selectively oxidized by H_2O_2 to Cys–SOH, which then reacts with the COOH-terminal conserved Cys-SH (Cys^{172} of Prx I) of the other subunit to form an intermolecular disulfide. The disulfide is subsequently specifically reduced by Trx (Fig. 1A) (10, 12, 42). The oxidized intermediate of the atypical 2-Cys Prx (Prx V) is a monomer containing the intramolecular Cys^{48}-Cys^{152} disulfide and is shown in Fig. 1B (46). However, Prx V activity is also dependent on thioredoxin (Trx). The two disulfide-forming Cys residues of 2-Cys Prx enzymes are separated by 121amino acids, whereas those of the atypical 2-Cys Prx (Prx V) are separated by 104 residues. Moreover, the amino acid sequence surrounding Cys^{152} of Prx V does not resemble that surrounding Cys^{172} of Prx I. In 1-Cys Prx (Prx VI), the NH_2-terminal Cys (Cys^{47}) is the site of oxidation by H_2O_2, but the resulting Cys-SOH cannot form a disulfide because there is no other Cys-SH nearby (23) (Fig. 1C). Although the physiological source of the reducing equivalents for the regeneration of Cys^{47}-SH is not known, dithiothreitol is able to support the regeneration *in vitro.* Whether reduced glutathione (GSH) also can serve as an electron donor is controversial (19, 23, 38).

Prx isoforms are differently distributed in organelles: Prx I and II are in the cytosol; Prx III is expressed with the mitochondrial targeting sequence and exclusively localized in the mitochondria (52); Prx IV contains the NH_2-terminal signal sequence for secretion and is found in the endoplasmic reticulum, as well as in the extracellular space; Prx V is expressed in the long and short forms that are found in the mitochondria and peroxisomes, respectively (27, 46, 56); and Prx VI is found in the cytosol (23, 33).

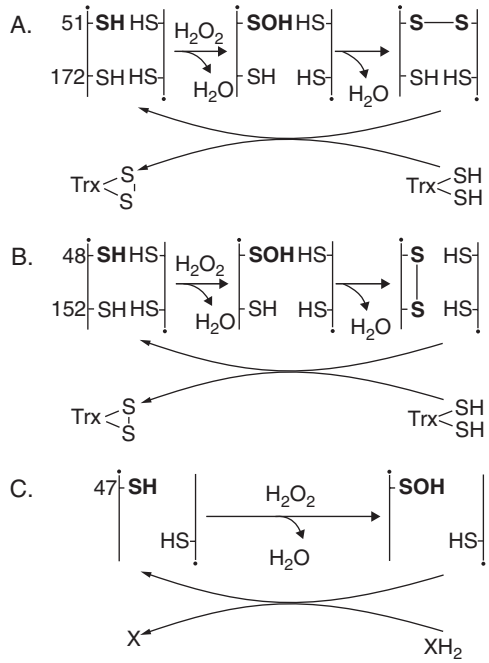

FIG. 1. Peroxidase reaction mechanisms of three subgroups of the Prx family. Mechanisms of 2-Cys Prx (**A**) and atypical 2-Cys (**B**) supported by Trx and the mechanism of 1-Cys Prx (**C**) supported by XH_2, the as yet unidentified electron donor, are shown.

With the V_{max} values of Prx I–III being 6–13 μmol/min/mg at 37°C, Prx enzymes are slow compared with catalase and GPx (13). However, because they exhibit higher affinity toward H_2O_2 (their K_m values for H_2O_2 are <20 μ*M*) and are abundant (the sum of Prx I–III amounts to ~1–10 μg per milligram of soluble protein in most cultured cells) (13, 42), Prx enzymes can be efficient in removing lower levels of H_2O_2. Indeed, when overexpressed in various cells, Prx enzymes efficiently reduced the intracellular level of H_2O_2 produced in the cells stimulated with PDGF or tumor necrosis factor-α, inhibited nuclear factor-κB activation induced by tumor necrosis factor-α, and blocked the apoptosis induced by ceramide (23, 58), indicating that Prx enzymes serve as component of signaling cascades by removing H_2O_2.

DOWN-REGULATION OF Prx I AND Prx II ACTIVITIES VIA CYCLIN B-DEPENDENT KINASE (Cdc2)-DEPENDENT PHOSPHORYLATION

Prx I contains a consensus site (Thr^{90}-Pro-Lys-Lys^{93}) for phosphorylation by cyclin-dependent kinases (Cdks). We showed that Prx I and Prx II can be phosphorylated specifically at Thr^{90} by several Cdks, including Cdc2 kinase (14). Prx I phosphorylation at Thr^{90} caused a decrease in peroxi-

dase activity by >80% (14). To monitor Prx I phosphorylation in cells, we prepared antibodies specific to Prx I phosphorylated at Thr^{90}. Experiments with HeLa cells arrested at various stages of cell cycle showed that Prx I phosphorylation at Thr^{90} occurs in parallel with the activation of Cdc2 kinase. Prx I phosphorylation was observed in cells in mitotic phase, but not in interphase, despite the fact that Prx I can be phosphorylated by other Cdk isoforms *in vitro* (14). This is probably because Prx I, a cytosolic protein, can encounter activated Cdks only after the nuclear envelope breaks down during mitosis and because Cdc2 kinase is the Cdk that is activated in mitotic phase. Both the *in vitro* and *in vivo* phosphorylation of Prx I at Thr^{90} was inhibited by roscovitine, an inhibitor of Cdks. Furthermore, when Cdc2 expression was reduced by RNA interference, Prx I phosphorylation was not observed in the cells synchronized in mitotic phase. Prx II also can be phosphorylated, albeit more weakly than is Prx I, by Cdc2 kinase *in vitro.* Prx II is also a cytosolic protein. Therefore, Cdc2 kinase-dependent phosphorylation and inactivation of Prx II are likely to occur in mitosis.

Inactivation of Prx I and Prx II is expected to result in an increase in the intracellular concentration of H_2O_2. The significance of temporary increase of H_2O_2 concentration during mitosis is not clear. One of the potential target molecules on which H_2O_2 acts to propagate its signal is Cdc25C dual phosphatase, an important regulator of Cdc2 kinase (Fig. 2). Cyclin B, although necessary, is not sufficient to activate Cdc2. Once bound to cyclin B during the G2 phase, Cdc2 is phosphorylated initially on Thr^{14} and Tyr^{15} residues by an inhibitory kinase and then on Thr^{161} by a stimulatory kinase. This triply phosphorylated form of Cdc2 is still inactive. At the onset of mitosis, the Cdc25C phosphatase activates Cdc2 kinase by dephosphorylating Thr^{14} and Tyr^{15} (35, 36) (Fig. 2). Cdc25C phosphatase is weakly active during interphase and fully activated during mitosis. Control of Cdc25C itself is also a highly regulated process, which involves phosphorylation and dephosphorylation: Cdc25C is phosphorylated and activated by several kinases, including Cdc2 kinase, and inactivated by okadaic acid-sensitive phosphatases (21). Therefore, a small amount of active Cdc2 kinase produced by Cdc25C is expected to stimulate further Cdc2 kinase activation, creating a positive feedback effect. Like other protein tyrosine phosphatases, Cdc25 phosphatases contain an essential cysteine residue in the His-Cys-X_5-Arg motif (17). Cdc25C was shown to be sensitive to oxidation and requires the presence of reducing agents for its activity (16, 43). The active-site cysteines of protein tyrosine phosphatases are sensitive to oxidation by H_2O_2 because their ionization is promoted by the conserved histidine and arginine residues. Protein tyrosine phosphatase 1B is reversibly inactivated by H_2O_2 produced in response to PDGF or epidermal growth factor (30, 32, 34). In addition, H_2O_2 may regulate the functions of many transcriptional factors (e.g., activator protein-1, nuclear factor-κB, and p53) (31, 44) and the ring finger protein APC11 (15) during the cell cycle by oxidizing their redox-sensitive cysteines.

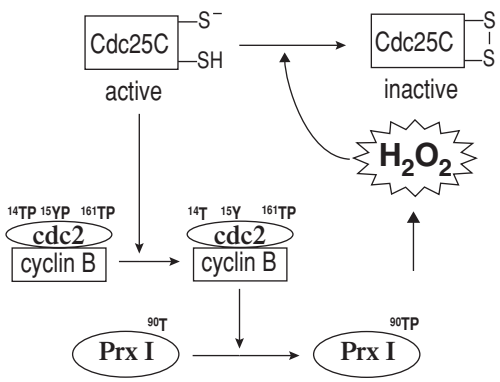

FIG. 2. Inactivation of Prx I by Cdc2-dependent phosphorylation and the potential significance of the resulting H_2O_2 accumulation as a mechanism of inhibition of Cdc25C phosphatase. The dual-specificity phosphatase Cdc25C activates cyclin B-bound, triply phosphorylated Cdc2 kinase by catalyzing the dephosphorylation of Thr^{14} and Tyr^{15}. The activated Cdc2 inactivates Prx I by phosphorylating it on Thr^{90}, probably resulting in an increase of H_2O_2 levels. The resulting H_2O_2 is expected to inactivate Cdc25 phosphatase by oxidizing its catalytic cysteine residue.

On the basis of these various observations, we speculate that as the mitosis progresses to prometaphase, the nuclear envelope breaks down, and Prx I, likely Prx II also, becomes phosphorylated by Cdc2 kinase that has been activated at the stage of G_2 to M transition. As the result of phosphorylation, the two cytosolic Prx enzymes become inactive, allowing accumulation of H_2O_2 in cells. The resulting H_2O_2 causes inactivation of Cdc25C, which in turn serves as a mechanism to halt the positive feedback loop of Cdc25 phosphatase and Cdc2 kinase. Thus, H_2O_2 might serve as an inhibitor of Cdc25C phosphatase throughout later stages of mitosis and cooperate with cyclin B degradation to promote the exit from mitosis (Fig. 2). Irrespective of whether or not Cdc25C is a target of H_2O_2 during the cell cycle, our results clearly demonstrate that peroxidase activity of Prx I is regulated through phosphorylation. This was the first example that any of the H_2O_2-eliminating enzymes, catalase, GPx, and Prx, was regulated through posttranslational modification. The Prx phosphorylation is consistent with the thesis that the intracellular concentration of H_2O_2 is strictly regulated through the fine control of processes involved in the production and elimination of this reagent.

REGULATION OF 2-Cys Prx ENZYMES VIA REVERSIBLE CYSTEINE SULFINYLATION

Because the disulfide-forming Cys residues (Cys^{51} and Cys^{172} in Prx I) of 2-Cys Prx enzymes are situated far apart, with their sulfur atoms separated by ~13 Å (45), formation of the disulfide is a slow process and the sulfenic (Cys-SOH) intermediate is occasionally further oxidized, resulting in inactivation of peroxidase activity (10, 39, 57). By following Prx-dependent NADPH oxidation spectrophotometrically, we observed that peroxidase activities of yeast Prx (also called TSA1) and mammalian PrxI decrease gradually with time (57). The decay in activity was coincident with the conversion

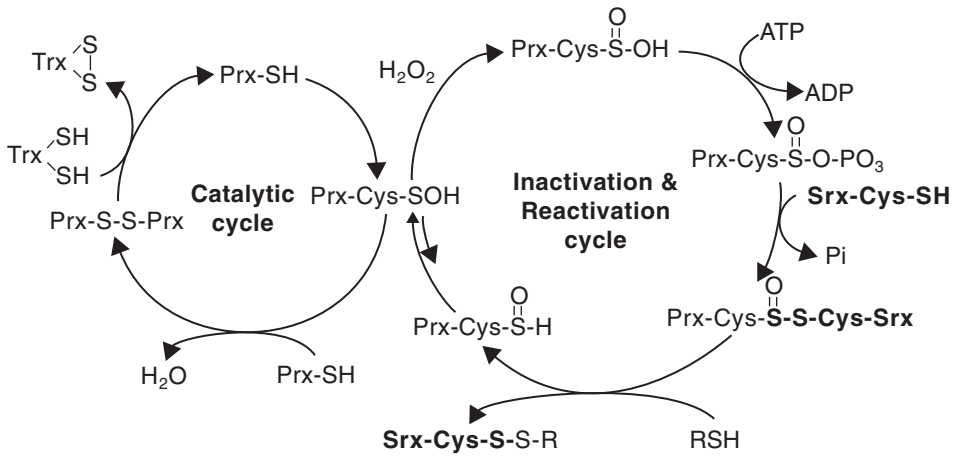

FIG. 3. The catalytic cycle and the inactivation–reactivation cycle of 2-Cys Prx. A 2-Cys Prx reduces H_2O_2 using the reducing equivalents provided by Trx. During the catalytic cycle, a small fraction of 2-Cys Prx becomes inactivated when its active site Cys-SH is hyperoxidized to sulfinic acid (Cys-SO_2H). The sulfinylated enzyme is reactivated by Srx via a mechanism that probably involves phosphotransfer of ATP and thioltransfer of Srx.

of PrxI to a more acidic species as assessed by two-dimensional (2D) gel electrophoresis. Mass spectral analysis and studies with Cys mutants determined that this shift in pI was due to selective oxidation of the catalytic site Cys^{51}-SH to Cys^{51}-SO_2H. Thus, the Cys^{51}-SOH generated as an intermediate during catalysis appeared to undergo occasional further oxidation to Cys^{51}-SO_2H, which cannot be reversed by Trx. Prokaryotic Prx enzymes, which do not contain the COOH-terminal GGLG motif of eukaryotic counterparts, are insensitive to oxidative inactivation (55).

The presence of H_2O_2 alone is not sufficient to cause oxidation of Cys^{51} to Cys^{51}-SO_2H. Rather, the presence of complete catalytic components (H_2O_2, Trx, thioredoxin reductase, and NADPH) is necessary, indicating that such hyperoxidation occurred only when Prx I is engaged in the catalytic cycle (57). Kinetic analysis of Prx I inactivation in the presence of a steady-state, low level (<1 μ*M*) of H_2O_2 indicated that Prx I is hyperoxidized at a rate of 0.072% per turnover at 30°C (57).

Hyperoxidation of 2-Cys Prx enzymes was also detected in various cells treated with H_2O_2. On examination of the redox state of Prx in several mammalian cell lines that had been metabolically labeled with ^{35}S, we observed that, on 2D gels, the ^{35}S-labeled acidic spots corresponding to sulfinylated Prx I and Prx II increased in intensity during exposure of cells to H_2O_2 and then underwent a shift back to the spots corresponding to the respective reduced forms after removal of H_2O_2 in the presence of the protein synthesis inhibitor cycloheximide (53). This observation led us to propose that the sulfinylation reaction is reversible in cells (53).

Given that an acidic shift on 2D gels is also caused by protein phosphorylation, as is the case with Prx I (14), mass spectral analysis of the acidic forms of proteins has been necessary to ascertain the presence of hyperoxidized cysteine residues. To develop an alternative approach to the complex procedure involving isotopic labeling of cells, 2D electrophoresis, and mass spectrometry for the detection of proteins containing hyperoxidized cysteine residues, we prepared rabbit antibodies to a sulfonylated peptide based on the active site sequence (DFTFVCPTEI) that is common to mammalian 2-Cys Prx (54). Although a sulfonylated peptide was used as the immunogen, the resulting antibodies recognized both the sulfinylated and sulfonylated forms of Prx with similar avidity. By using immunoblot analysis with these antibodies, we confirmed the reversibility of sulfinylation of cytosolic Prx isoforms and demonstrated reversibility of the sulfinylation of mitochondrial Prx III (54).

Subsequently, Toledano's laboratory identified the enzyme responsible for the reduction of sulfinylated Prx (3). The enzyme named sulfiredoxin (Srx) was initially identified by its high H_2O_2-induced expression and because the deletion of its gene causes decreased tolerance to H_2O_2. Srx defines a conserved protein family in lower and higher eukaryotes, all sharing a conserved cysteine. Studies with the yeast enzymes showed that the reduction by Srx requires ATP hydrolysis, Mg^{2+}, and a thiol as an electron donor (3). In addition, the conserved cysteine residue of Srx was found to be essential for the reduction activity, probably because the cysteine forms a transient thiosulfinate linkage with the sulfinic acid of Prx. Based on these observations, Toledano and colleagues proposed a mechanism that involves activation of cysteine-sulfinic acid by phosphorylation followed by a thiol-mediated reduction step (Fig. 3).

The fact that Srx is conserved only among eukaryotes is in accord with the observation that eukaryotic, but not prokaryotic, Prx enzymes are susceptible to oxidative inactivation. Therefore, as shown in yeast, Srx likely plays a critical role in supporting peroxidase function of 2-Cys Prx enzymes in higher eukaryotes and thus in signaling pathways involving H_2O_2.

DUAL REGULATION OF CATALASE VIA c-Abl- AND Arg-DEPENDENT PHOSPHORYLATION

Catalase is a dismutase that reduces one H_2O_2 to H_2O and oxidizes a second H_2O_2 to O_2. Being exclusively localized in

the peroxisomes in mammalian cells, a major role of catalase is likely to remove H_2O_2 produced during α-oxidation of fatty acids in peroxisomes. Catalase effects on cytosolic H_2O_2 must rely on the diffusion of H_2O_2 into these organelles. Furthermore, despite its high turnover number, catalase is not efficient in eliminating low levels of H_2O_2 because it is difficult to saturate with H_2O_2 and its catalytic cycle requires the interaction of two H_2O_2 molecules with a single active site, which is less likely at low H_2O_2 concentrations (20). Therefore, catalase is not expected to play a significant role in eliminating low levels of H_2O_2 produced in response to receptor engagement. Nevertheless, Kufe and colleagues have shown recently that catalase is extensively regulated in the responses of cells to extracellular H_2O_2 (4–7, 49).

The Abelson (Abl) family of nonreceptor tyrosine kinases consists of c-Abl and Arg (the product of c-Abl-related gene) (28). The NH_2-terminal regions of c-Abl and Arg are ~90% identical in amino acid sequence and, as found in members of the Src family, contain tandem Src homology 3 (SH3), SH2, and SH1 (kinase) domains (Fig. 4A). Although the COOH-terminal regions of c-Abl and Arg share only 29% amino acid sequence identity, both regions contain three conserved PXXP motifs that can serve as binding sites for SH3 domains. c-Abl and Arg are activated in the response of various types of cells to oxidative stress. Treatment of cells with H_2O_2 induces binding of protein kinase Cδ with c-Abl followed by phosphorylation and activation of c-Abl (49). Thus, in cells, activation of c-Abl by H_2O_2 is attenuated by the protein kinase Cδ inhibitor, rottlerlin, and by overexpression of the regulatory domain of protein kinase Cδ.

Activated c-Abl and Arg appear to regulate catalase (Fig. 4B). In cells treated with H_2O_2, c-Abl and Arg form heterodimers by mechanisms involving a SH3 domain of one kinase and the PXXP motif of the other kinase. The dimeric kinases then associate with catalase through interaction involving the SH3 domain of the kinase dimers and the P^{293}FNP motif of catalase (5, 6) (Fig. 4B). The binding is independent of the c-Abl kinase function as kinase-inactive c-Abl (K→R) or Arg (K→R) mutant also associates with catalase. The bound kinases then phosphorylate catalase at Tyr^{231} and Tyr^{386} and enhance the catalytic activity of catalase by four- to fivefold. Thus, tyrosine phosphorylation of catalase in response to H_2O_2 was attenuated by treating MCF-7 cells with the c-Abl inhibitor STI571 (5). However, the finding that mutation of Tyr^{231} and Tyr^{386} failed to completely abrogate phosphorylation indicates the potential involvement of other tyrosines. The significance of the physical interaction between c-Abl/Arg and catalase and the subsequent phosphorylation of catalase is supported by the demonstration that cells deficient in either c-Abl or Arg exhibit increased H_2O_2 levels (5).

The regulation of catalase by c-Abl and Arg exhibits biphasic response to H_2O_2 concentrations (5). For example, the assocation of c-Abl with catalase in MCF-7 cells is promoted at 0.25–1 m*M* H_2O_2, but not at 2 m*M* H_2O_2. Thus, at lower H_2O_2 levels, c-Abl and Arg phosphorylate and stimulate catalase. In the event that H_2O_2 levels continue to increase, c-Abl and Arg dissociate from catalase and render catalase susceptible to dephosphorylation by tyrosine phosphatases, resulting in decrease of catalase activity.

The biphasic response to H_2O_2 was further enforced by the observation that the association of catalase with c-Abl/Arg and the tyrosine phosphorylation by the kinases alter the stability of catalase (7). Thus, catalase levels in mouse embryo fibroblasts (MEF) deficient in both c-Abl and Arg were approximately three times that in wild-type cells. This elevation was not achieved at the transcriptional level, but was due to altered stability of catalase as demonstrated by pulse-chase experiments: Half-lives of catalase were >24 h and ~10.5 h, respectively, in the *c-abl*$^{-/-}$ *arg*$^{-/-}$ and wild-type MEF cells. Kufe and colleagues further showed that catalase is degraded through ubiquitination-dependent processes and that the rate

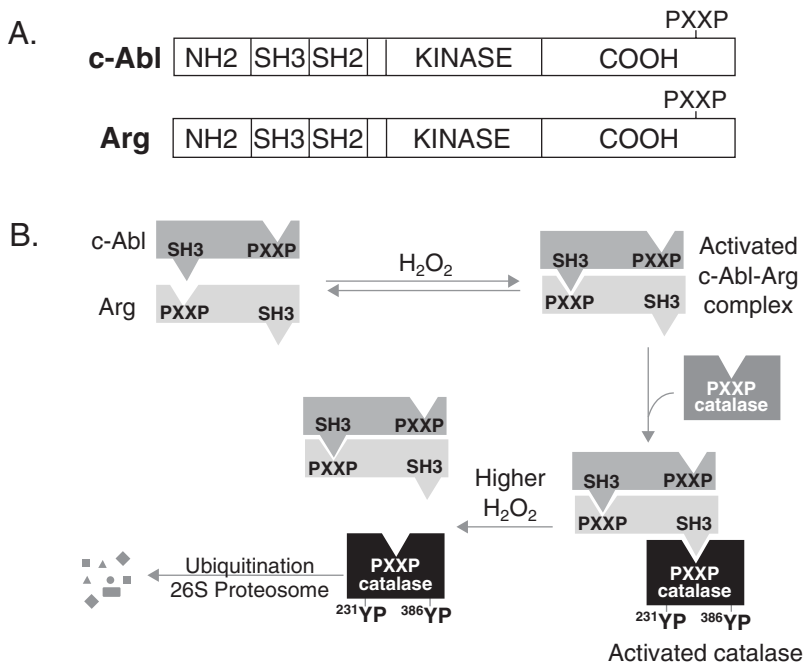

FIG. 4. Dual regulation of catalase by c-Abl and Arg. (**A**) Domain structures of c-Abl and Arg nonreceptor protein tyrosine kinases including their SH domains and proline-rich sequences. (**B**) In cells treated with H_2O_2, c-Abl and Arg form heterodimers by mechanisms involving an SH3 domain of one kinase and the PXXP motif of the other kinase and become activated. The activated kinase dimers then associate with catalase through the interaction between the SH3 domain of the kinases and the PXXP motif of catalase. The bound kinases then phosphorylate catalase at Tyr^{231} and Tyr^{386} and enhance catalase activity. At higher levels of H_2O_2, however, c-Abl and Arg dissociate from catalase and render the phosphorylated catalase susceptible to ubiquitination-dependent protein degradation.

of catalase ubiquitination is dependent on c-Abl- and Arg-mediated tyrosine phosphorylation (7). Ubiquitination of the Tyr → Phe catalase mutants (Y231F and Y386F) is substantially decreased compared with that of wild-type catalase. In concert with these results, human 293 cells expressing Y231F or Y386F exhibit attenuated levels of reactive oxygen species when exposed to H_2O_2 (7).

Based on the observations described above, Kufe and colleagues proposed dual roles of c-Abl and Arg in catalase regulation as shown in Fig. 4. In the model, in addition to stimulating catalase activity by associating with and phosphorylating catalase at lower H_2O_2 levels, c-Abl and Arg promote catalase degradation in the event of uncontrollable H_2O_2 levels. Thus, at lethal concentrations of H_2O_2, c-Abl and Arg function as proapoptotic effectors as indicated by the fact that cells deficient in either kinase exhibit an attenuated apoptotic response (7).

The series of publications from the laboratory of Kufe cited in this review convincingly demonstrated the role of c-Abl and Arg in regulating catalase function. However, one important issue not addressed yet is how the peroxisomal catalase can encounter c-Abl and Arg, which are known to exist in the cytosol, nucleus, and mitochondria, but not in the peroxisomes.

UP-REGULATION OF GPX I ACTIVITY BY C-Abl AND ARG

GPx removes H_2O_2, by coupling its reduction to H_2O with oxidation of GSH. GPxs are obligatory tetramers, each of which contains one selenocysteine (Cys-SeH) at its active site. During the catalytic mechanism of GPx, the Cys-SeH residue reacts with H_2O_2 to produce cysteine selenenic acid (Cys-SeOH). GSH then binds and reduces Cys-SeOH to Cys-SeH. There are at least five types of GPx in mammalian cells. The classical GPx1 is ubiquitous and found mainly in the cytosol and in the matrix of mitochondria, whereas GPx2, GPx4, and GPx5 are found in specific types of cells. GPx3 is a lipid peroxide-specific peroxidase.

Kufe and colleagues extended their studies with c-Abl and Arg to GPx1. They found that c-Abl and Arg bind to GPx1 through interaction between their SH3 domains and a proline-rich region in GPx at amino acids 132–145 (8). GPx1 is then phosphorylated at Tyr^{96} by the kinases and activated. In concert with these findings, inhibition of c-Abl with STI571 in SH-SY5Y cells decreased GPx activity; expression of kinase-active c-Abl increases GPx activity, whereas the dominant-negative c-Abl (K → R mutant) has an inhibitory effect; and GPx activity is decreased in the *c-abl*$^{-/-}$ *arg*$^{-/-}$ MEF cells compared with wild-type cells (8).

CONCLUDING REMARKS

H_2O_2 induces a variety of cellular responses that depend on its concentration. At lower concentrations (the actual concentrations are not known because of lack of good methods to measure intracellular concentrations of H_2O_2), H_2O_2 appears to serve as an intracellular messenger that promotes cell proliferation and regulates cell-cycle progression. Regulation of the activity of Prx enzymes through the phosphorylation by Cdc2 and the reversible sulfinylation might represent the cellular efforts to control the levels of the intracellular messenger H_2O_2. As the concentration of H_2O_2 increases, it begins to exert toxic effects. Thus, the activation of c-Abl and Arg kinases by H_2O_2 and subsequent stimulation of the catalytic activities of catalase and GPx via c-Abl/Arg-mediated phosphorylation provide a mechanism to increase the cellular capacity to remove deleterious H_2O_2. In the event that the concentration of H_2O_2 increases above the levels that can be controlled by cellular antioxidant enzymes, cells become damaged. Under such stressing conditions, cells are likely to undergo apoptosis by targeting phosphorylated catalase for proteosomal degradation and thereby rapidly elevating H_2O_2 levels.

ABBREVIATIONS

Arg, the product of c-Abl-related gene; c-Abl, Abelson nonreceptor tyrosine kinase; Cdc2, cyclin B-dependent kinase; Cdk, cyclin-dependent kinase; 2D, two-dimensional; GPx, glutathione peroxidase; GSH, reduced glutathione; H_2O_2, hydrogen peroxide; MEF, mouse embryo fibroblasts; $O_2^{\bullet -}$, superoxide; PDGF, platelet-derived growth factor; Prx, peroxiredoxin; SH3, Src homology 3; Srx, sulfiredoxin; Trx, thioredoxin; TSA, thiol-specific antioxidant.

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