Peroxiredoxin Ahp1 Acts as a Receptor for Alkylhydroperoxides to Induce Disulfide Bond Formation in the Cad1 Transcription Factor*$$^a$$

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Reactive oxygen species (ROS) generated during cellular metabolism are toxic to cells. As a result, cells must be able to identify ROS as a stress signal and induce stress response pathways that protect cells from ROS toxicity. Recently, peroxiredoxin (Prx)-induced relays of disulfide bond formation have been identified in budding yeast, namely the disulfide bond formation of Yap1, a crucial transcription factor for oxidative stress response, by a specific Prx Gpx3 and by a major Prx Tsa1. Here, we show that an atypical-type Prx Ahp1 can act as a receptor for alkylhydroperoxides, resulting in activation of the Cad1 transcription factor that is homologous to Yap1. We demonstrate that Ahp1 is required for the formation of intermolecular Cad1 disulfide bond(s) in both an in vitro redox system and in cells treated with alkylhydroperoxide. Furthermore, we found that Cad1-dependent transcriptional activation of the HSP82 gene is dependent on Ahp1. Our results suggest that, although the Gpx3-Yap1 pathway contributes more strength to stress than the Ahp1-Cad1 pathway, the Ahp1-induced activation of Cad1 can function as a defense system against stress induced by alkylhydroperoxides, possibly including lipid peroxides. Thus, the Prx family of proteins have an important role in determining peroxide response signals and in transmitting the signals to specific target proteins by inducing disulfide bond formation.

Oxygen serves as an electron acceptor, enabling efficient production of ATP. However, oxygen can also be converted into toxic reactive oxygen species (ROS), such as superoxide and hydrogen peroxide (H$_2$O$_2$); ROS can damage a variety of cellular components, including proteins and unsaturated lipids (1).

Toxicity often arises from lipid peroxidation as well as production of alkoxy/peroxy radicals and aldehyde compounds, which are products of radical lipid reactions triggered by ROS. Additionally, ROS are important contributors to cellular metal toxicity (2).

Detection of ROS (peroxides) is an important step in the oxidative stress response. In bacteria, sensing of H$_2$O$_2$ involves a transcription factor OxyR (3–5), whereas transcription factors Yap1 and Pap1 have this role in yeast. These transcription factors activate the transcription of genes encoding enzymes that mitigate oxidative stress in response to H$_2$O$_2$. OxyR reacts directly with H$_2$O$_2$ to form a sulfenic acid at Cys$^{199}$ of the transcription factor. The sulfenic acid rapidly forms a disulfide bond with Cys$^{208}$, activating the transcription factor, which then triggers expression of the target genes. By contrast, H$_2$O$_2$ receptors are required for activation of the Yap1 transcription factor in Saccharomyces cerevisiae (budding yeast) (6) as well as its Schizosaccharomyces pombe ortholog, Pap1 (fission yeast) (7, 8). Glutathione peroxidase (Gpx)-like factor Gpx3 (also known as Orp1) is the H$_2$O$_2$ receptor involved in Yap1 activation in budding yeast. The presence of H$_2$O$_2$ is first detected in yeast when a catalytic cysteine of Gpx3 is directly oxidized by H$_2$O$_2$ to form sulfenic acid. This reaction is followed by the transient formation of an intermolecular disulfide bond with a cysteine residue in the Yap1 transcription factor. A thiol-disulfide exchange reaction results in the formation of an intramolecular disulfide bond in Yap1, which triggers activity of the transcription factor. In the case of Pap1, the H$_2$O$_2$ receptor is Tpx1 in fission yeast. Whether molecular mechanisms other than those involving specific peroxide receptors participate in H$_2$O$_2$ sensing remains to be determined. The fact that both Gpx3 and Tpx1 have thioredoxin (Trx)-dependent peroxidase (Txp) activity is potentially important for developing a better understanding of H$_2$O$_2$ sensing.

Prx is a family of ubiquitous peroxidases found in species ranging from Escherichia coli to humans (9). In many cases, Prx can reduce H$_2$O$_2$ and/or alkylhydroperoxides at the expense of electrons from NADPH through the Trx-dependent redox system (Trx activity) (see Fig. 3A). The catalytic cysteine (Cys$^+$) residue of Prx is directly oxidized by hydroperoxides (see Fig. 1D). This oxidation event is followed by the formation of a disulfide bond linkage with a resolving Cys of the same molecule of Prx or with a resolving Cys of another Prx molecule (dimer formation). The disulfide bond can be reduced by Trx. Five Prx family proteins (Tsa1, Tsa2, Prx1, Dot5, and Ahp1)
Ahp1 as Receptor for Peroxide to Activate Cad1

(10) and two Gpx-like proteins (Gpx2 and Gpx3) display Tpx activity in budding yeast (6, 11). Differential expression, localization, and specificity of each of these Prx family proteins (12) suggest that they may have different spatial-temporal roles. By contrast, only Tpx1 and Gpx1 have Tpx activity in fission yeast (13). Multiplicity of Prx enzymes in budding yeast is analogous to that in the mammalian system, as mammalian cells express six conserved Prx family proteins. Thus, functional studies on the different Prx proteins in budding yeast may provide an understanding of the role of these proteins in a broad range of eukaryotes.

Recently, we have suggested (14, 15) that, in addition to their function as peroxidases, Prx family proteins may serve as intrinsic receptors of \( \text{H}_2\text{O}_2 \). Additionally, Prx proteins may relay information about the presence of hydroperoxides to the independent target proteins of each Prx; however, the contribution of the Prx family members in sensing the presence of hydroperoxides is yet to be determined. Furthermore, the putative alkylhydroperoxide-sensing mechanism is entirely unresolved. It is possible that multiple sensing mechanisms exist that are determined by unique specificities of each Prx protein for different hydroperoxides and to their respective target proteins. In the present study, we addressed whether the Prx Ahp1 is responsible for hydroperoxide sensing.

EXPERIMENTAL PROCEDURES

Media and Strains—Yeast cells were grown in synthetic dextrose (SD) medium supplemented with amino acids (SD dropout) (16) or in YPAD medium (1% peptone, 0.5% yeast extract, 2% glucose, 0.08 mg/ml adenine sulfate) at 30 °C. We used the BY4742 parent strain of \( \text{S. cerevisiae} \) (MATa his3Δ1 leu2Δ2 his3Δ1 ura3Δ0; EUROSCARF, Frankfurt, Germany). For genetic epistasis analysis of resistance to tert-butylhydroperoxide (tBOOH) (see Fig. 2D), we used a Yap1 disruption derivative of the W303–1b (W303 yap1Δ::URA3) as a parent strain.

Oxidation Reaction of Cad1—for the \textit{in vitro} assay of the oxidation reaction of Cad1, the carboxyl-terminal region of Cad1 (nucleotides 332–1230 corresponding to amino acids 111–409), in which the amino-terminal bZIP region was deleted, was cloned between BamHI and SalI sites in pGEX-6P-2 (GE Healthcare). To construct the HA-Cad1 expression plasmid, the HA tag was inserted between the BamHI site and the amino terminus of the above Cad1 sequence. We purified the Cad1 (111–409) protein and reduced it with DTT. The amino terminus of the above Cad1 sequence. We purified the Cad1 (111–409), in which the amino-terminal bZIP region was deleted, was cloned between BamHI and SalI sites in pGEX-6P-2 (GE Healthcare). To construct the HA-Cad1 expression plasmid, the HA tag was inserted between the BamHI site and the amino terminus of the above Cad1 sequence. We purified the Cad1 (111–409) protein and reduced it with DTT. The oxidation process was examined as described previously (14).

Oxidation was initiated by the addition of 0.4 mM tBOOH. Aliquots of the oxidation reaction were withdrawn at various times and analyzed with anti-Ahp1 antibodies, are described in the supplemental Experimental Procedures.

RESULTS

Requirement of Ahp1 for Resistance to Alkylhydroperoxide—Ahp1 was previously identified as an antioxidant to alkylhydroperoxides (20). We compared the requirement of \( \text{GPX3}, \text{ TSA1}, \text{ and AHP1} \) genes for resistance to \( \text{H}_2\text{O}_2 \) and to the alkylhydroperoxide tBOOH (Fig. 1A). Disruption of \( \text{GPX3} \) affected cell sensitivity to these hydroperoxide stresses, suggesting that Gpx3-induced transcriptional activation of Yap1 is an important detoxifying system in response to \( \text{H}_2\text{O}_2 \), tBOOH and methyl linolenic acid (an unsaturated lipid that induces lipid peroxidation stress). \( \text{TSA1} \) encodes a major Prx family protein; Tsa1 and Ahp1 consist of 91% and 3.9% of total Tpx, respectively (14, 21), and disruption of the \( \text{TSA1} \) gene resulted in a modest increase in sensitivity to tBOOH and methyl linolenic acid. Disruption of the \( \text{AHP1} \) gene significantly increased sensitivity only to methyl linolenic acid. Our results suggested that Ahp1 contributes resistance more to unsaturated lipid than hydroperoxides (Fig. 1A). Next, we compared the resistance of \( \text{GPX3} \) and \( \text{GPX3} \) and \( \text{GFX3} \) cells to tBOOH. We employed a low concentration of tBOOH in this case because disruption of \( \text{GPX3} \) induced the sensitivity of yeast cells to hydroperoxides (0.05 mM; Fig. 1B). Sensitivity to tBOOH was significantly increased when \( \text{GPX3} \) and \( \text{AHP1} \) were simultaneously disrupted (\( \text{GPX3 AHP1} \)). To examine the possibility that Ahp1 could act as a tBOOH receptor and activate Yap1-dependent transcription (analogous to the mechanism by which Gpx3 responds to peroxide and activates Yap1), we compared Yap1-dependent transcription in an \( \text{AHP1} \) disruption mutant with Yap1-dependent transcription in the wild-type strain. Our findings suggest that Ahp1 is involved in reducing intracellular peroxide levels because disrupting \( \text{AHP1} \) increased Yap1-dependent transcriptional levels in unstressed cells (Fig. 1C). By contrast, hydroperoxide-induced Yap1-dependent transcription was unaffected by disruption of the \( \text{AHP1} \) gene. Furthermore, disruption of \( \text{AHP1} \) did not affect basal transcription levels in \( \text{GPX3} \) cells (Fig. 1C, inset). These results suggest that Ahp1 does not function as a peroxide receptor upstream of Yap1-dependent transcription.

Cad1 Is a Candidate Target Protein of Ahp1—We pursued the possibility that Ahp1 acts as an alkylperoxide receptor and transmits the peroxide signal by introducing disulfide bond(s) in target proteins other than Yap1. Our previous study (14) demonstrated that substitution of a resolving cysteine in Tsa1 stabilizes the formation of a transient disulfide bond between a
catalytic cysteine of Tsa1 and Yap1. Therefore, we aimed to isolate proteins that could interact with the catalytic cysteine of an Ahp1 mutant containing a substitution of the resolving Cys at position 120 (Ahp1C120T) (see Fig. 1D). Using the two-hybrid system, we identified Cad1 (also known as Yap2) as a protein that could specifically interact with Cys62 of Ahp1 (Fig. 1E). To confirm this interaction, we simultaneously expressed Ahp1C120T and HA-tagged Cad1 in yeast cells. Using this system, we found that binding of Ahp1C120T to Cad1 was strongly induced in response to tBOOH exposure (Fig. 1F). We observed both monomeric and dimeric Ahp1C120T, even under nonreducing conditions in SDS-denatured samples analyzed by PAGE (lane 10). In addition, we observed the presence of slower migrating complexes, likely corresponding to Cad1-bound to Ahp1C120T via a disulfide bond. It is possible that dimeric and monomeric Ahp1 are released from slower migrating complexes after immunoprecipitation. We also observed dimeric Ahp1 (wild-type) in Cad1 immunoprecipitated samples (Fig. 1F).
Ahp1 as Receptor for Peroxide to Activate Cad1

A

Primer sets for ChIP assay

Control

Cad1 binding region

HSE: Hsf1 binding site

F (–671) ➔ R (–510)

F (–233) ➔ R (–35)

B

ChIP assay (HSP82 promoter)

Primer sets: Control (–500) ➔ Binding region (–200)

IP: HA-Cad1
tBOOH (mM) 0 0.6 1.2 0 0.6 1.2

C

Expression of HSP82

WT ahp1Δ cad1Δ ahp1Δ cad1Δ

FIGURE 2. Ahp1 is required for Cad1-specific transcriptional induction. A, schematic illustration of the promoter region of HSP82 and the position of primers used for chromatin immunoprecipitation (ChIP) analysis is shown. B, input DNA and immunoprecipitated (IP) DNA were used as templates for PCR using either a control primer set or a primer set specific for the Cad1 binding region. C, induction of HSP82 transcription was dependent on both Ahp1 and Cad1. Quantitative PCRs were performed using RNA prepared from the indicated yeast cell line after treatment with 0.6 mM tBOOH for 20 min. D and E, both Ahp1 and Cad1 are required for maximum resistance to tBOOH. The indicated yeast cells, derived from W303 yap1ΔLEU2 (parent) (D) and BY4742 (yap1Δ) (E), were spotted onto YPAD plates without peroxide containing 5 and 20 μM tBOOH as described in the legend to Fig. 1.

W303 (yap1Δ)

tBOOH (μM) 0 5 20

Parent

ahp1Δ
cad1Δ

ahp1Δ cad1Δ

BY4742 (yap1Δ)

tBOOH (μM) 0 20

Parent

ahp1Δ
cad1Δ

ahp1Δ cad1Δ

tate of lysate from cells treated with tBOOH (lane 8). We reported a similar phenomenon in the case of Tsa1-Yap1 association (14).

Requirement of Ahp1 for Cad1-dependent Activity—We investigated whether Ahp1 might induce transcriptional activity of Cad1, analogous to the mechanism whereby Gpx3 induces transcriptional activity of Yap1 in response to H₂O₂ exposure. Interestingly, Cad1 is a Yap1 family transcription factor and contains two domains that are similar to domains in Yap1: a bZIP domain and a carboxyl-terminal cysteine-rich domain (cCRD) (22) (see Fig. 1G). Consistent with homology to the bZIP domain, Cad1 exhibits a binding specificity similar to Yap1 and can activate some of the same target genes when it is overexpressed (23). However, Cad1 can also activate independent target genes, including HSP82, an inducible HSP90 family protein (24). Based on these data, we utilized chromatin immunoprecipitation followed by PCR to examine whether Cad1 binds to the promoter region of HSP82 (Fig. 2A). We determined that Cad1 can bind to the −510 to −671 region of the HSP82 promoter but could not bind to the −35 to −233 region of the HSP82 promoter. The amount of Cad1 bound to the former region increased in response to tBOOH exposure (Fig. 2B). The Cad1-bound region of the HSP82 promoter does not contain any consensus sequences for Yap1 or Cad1. However, this region does contain one heat shock factor binding consensus sequence. We did not identify the exact Cad1 binding site on the HSP82 promoter. As a control, we performed a similar assay using PCR probes corresponding to a region of the TRX2 promoter that contains two Yap1 binding sites. This experiment demonstrated that exposure to H₂O₂ enhanced binding of Yap1. In addition, this assay showed that exposure to tBOOH increased binding of Cad1 to the same promoter region (see supplemental Fig. 1). Consistent with the binding activity of Cad1, transcriptional activation of HSP82 was induced by tBOOH exposure in an Ahp1- and Cad1-dependent manner (Fig. 2C). Together, these results suggest that Ahp1 acts as receptor for tBOOH and activates binding of Cad1 to the promoter region of HSP82.

We next examined the importance of Cad1 to Ahp1 activity in promoting resistance to tBOOH. Because disruption of neither AHP1 (Fig. 1) nor CAD1 in wild-type cells resulted in tBOOH-sensitive phenotypes (data not shown), we utilized yap1Δ disruption mutants as the parent strains. As shown in Fig. 2D, disruption of AHP1 and CAD1 resulted in a more sensitive phenotype than observed in parent cells (yap1Δ) derived from W303. Although AHP1 disruption resulted in a more sensitive phenotype than disruption of CAD1, we found that the phenotype of the AHP1/CAD1-double mutant was similar to that of the AHP1 disruption mutant (Fig. 2D, 50 μM). These results suggest that Ahp1-dependent up-regulation of Cad1 determines the requirement of Ahp1 for resistance to tBOOH at least in the specific condition. We could not observe the above phenotype with BY4742 (yap1Δ) by disruption of CAD1 (Fig.
Ahp1 as Receptor for Peroxide to Activate Cad1

**Ahp1-induced Oxidation of Cad1 in Vitro**—A characteristic feature of Cad1 is a cluster of cysteine-rich regions. Specifically, the protein contains both a unique domain composed of seven cysteine residues and cCRD (Fig. 1G) similar to Yap1. The presence of this domain prompted us to investigate whether Cad1 is oxidized by the redox cycle of Ahp1 in the presence of tBOOH, as we demonstrated for Yap1 (14, 17). First, we evaluated the suitability of tBOOH as a substrate for Ahp1 using the Trx reduction system (Fig. 3A; Trx2/Trx1/NADPH). As shown in Fig. 3B, both tBOOH and H2O2 efficiently oxidized Ahp1. By contrast, oxidation of Tsa1 by tBOOH was inefficient. Gpx3 was efficiently oxidized by both H2O2 and tBOOH in a manner similar to Ahp1 (see supplemental Fig. 2). These results suggest that Tsa1 is primarily responsible for reduction of H2O2, whereas Gpx3 and Ahp1 are also responsible for the reduction of alkylhydroperoxide.

We next examined Cad1 disulfide bond formation by assaying its mobility in nonreducing SDS-PAGE. We successes to express a truncated Cad1 protein (bZIP region was removed) in E. coli, but not full-length Cad1 protein. Direct treatment of the truncated Cad1 with tBOOH for 30 min did not significantly affect Cad1 mobility (Fig. 3C). This was also the case in the presence of the Trx reduction system (Fig. 3D). By contrast, the combination of Ahp1 and the Trx redox system (Fig. 3E and F) yielded a slower migrating Cad1 (Cad1ox2; Fig. 3F; 0.5 min) once Ahp1 was fully oxidized (Fig. 3E). Oxidized Cad1 (Cad1ox2; Fig. 3F; 0.5 min) once Ahp1 was fully oxidized (Fig. 3E). Oxidized Cad1 (Cad1ox2; Fig. 3F; 0.5 min) once Ahp1 was fully oxidized.

**Cad1 Is Oxidized in Yeast Cells in Response to tBOOH**—We next examined the oxidation state of Cad1 in cells treated with tBOOH. As shown in Fig. 4A and supplemental Fig. 3A, Cad1 formed slower migrating complexes, corresponding to
molecular masses of 110–60 kDa, in wild-type cells within 0.5 min of tBOOH exposure. Complexes lingered for 10 min but were no longer observed after 15 min. Because the complexes were sensitive to DTT treatment (Fig. 4 A, right, and supplemental Fig. 3 A), it is likely that slower migrating complexes contained possible dimers of Cad1 linked by disulfide bonds. We did not observe the oxidized form of Cad1 in AHP1 disruption mutant cells (Fig. 4 B), suggesting that the cysteine residue of Cad1 may attack the disulfide bond between Cad1 and Ahp1 to form a Cad1 dimer linked by a disulfide bond or an intramolecular disulfide bond in Cad1.

FIGURE 4. Ahp1-dependent Cad1 oxidation in cells treated with tBOOH. A–C, HA-Cad1-expressing cad1Δ cells (A and C) and ahp1Δcad1Δ cells (B) were treated with 0.6 mM tBOOH and fixed in trichloroacetic acid at each time point (minutes) as described under “Experimental Procedures.” We treated lysates with N-ethylmaleimide to block free cysteine -SH groups and fractionated by SDS-PAGE (10% for A and B, 15% for C) after treatment with DTT (right panels). We detected Cad1 (A and B) and Ahp1 (C) proteins by immunoblotting with an anti-HA antibody and anti-Ahp1 serum, respectively. Arrows indicate oxidized Cad1 complex (Cad1ox-complex), reduced Cad1 (Cad1red), oxidized Ahp1 (Ahp1ox), and reduced Ahp1 (Ahp1red). Positions of molecular mass markers (kDa) in each panel are indicated by arrowheads.

D Dimer-dependent Cad1 oxidation in cells treated with tBOOH. Even in an unstressed steady state, Ahp1 is continuously oxidized by direct reaction with intracellular peroxide, inducing formation of the dimer. Dimerized Ahp1 is also continuously reduced by Trx. The dimer/monomer ratio may be determined by the peroxide level and redox state of Trx. When a catalytic cysteine in the Ahp1 dimer reacts with peroxide, the resulting sulfenic acid (S-OH) attacks Cad1 to form a transient complex linked by disulfide bonds. A cysteine residue of Cad1 may attack the disulfide bond between Cad1 and Ahp1 to form a Cad1 dimer linked by a disulfide bond or an intramolecular disulfide bond in Cad1.

It is worth noting that the oxidation process of Cad1 in vitro (Fig. 3F) was somewhat different from that observed in cells (Fig. 4A). In cells, the possible Cad1 dimer, linked via a disulfide bond, formed directly without the need for intramolecular disulfide bonds. Our use of a truncated Cad1, devoid of the bZIP region, for the in vitro oxidation study might explain the initial induction of possible intramolecular disulfide bonds. Nevertheless, our results strongly suggest that Ahp1 is required for disulfide bond formation in Cad1 in response to tBOOH exposure.

Unsaturated Lipid Can Oxidize Ahp1—Our results suggested that Ahp1 might act as a receptor for lipid peroxide induced in cells treated with methyl linolenic acid. Thus, we examined oxidation of Ahp1 in the in vitro Trx reduction system and in yeast cells treated with methyl linolenic acid. We found that methyl linolenic acid could oxidize Ahp1 (Fig. 5A).
Ahp1 but not Tsa1, and Gpx3 was oxidized in cells in response to methyl linolenic acid (Fig. 5, B–D).

**DISCUSSION**

The Prx family of proteins possess the ability to reduce peroxide at the expense of their free cysteine residues, resulting in the formation of disulfide bonds or peroxidation to sulfinic acid. In addition to this defined function, our previous data indicate that a major Prx, Tsa1, can induce disulfide bridges to Yap1, implying an alternative function for Prx proteins. Here, we show that the Prx Ahp1 can catalyze formation of disulfide bonds to Cad1 in response to alkylhydroperoxide tBOOH both in vitro reconstitution system and in yeast cells. Furthermore, we show that Ahp1 is required for the up-regulation of Cad1-dependent transcriptional activity. Because Ahp1 is responsible for resistance to unsaturated lipid (Fig. 1) and Ahp1 is oxidized...
Ahp1 as Receptor for Peroxide to Activate Cad1

![Diagram](image)

FIGURE 6. Ahp1-dependent Cad1 activation and Gpx3-dependent Yap1 activation to protect from alkylhydroperoxide and lipid peroxide toxicity. The Gpx3/Yap1 system may be required for sensing and signal transduction in response to a broad range of peroxides, whereas the Ahp1/Cad1 system may have an important function in response to stresses induced by alkylperoxide and lipid peroxide exposure. Tsa1 can confer Yap1 activation in ybp1Δ cells (W303). Both Yap1 and Cad1 may have both shared and unique target genes (24). It is also suggested that Gpx3 is responsible for detoxification of lipid peroxides (27).

in cells in response to unsaturated lipid (Fig. 5). Ahp1 might act as both a receptor and a peroxidase for alkylhydroperoxides, including lipid peroxides (Fig. 4D). These results bolster our hypothesis that Prx proteins can act as intrinsic receptors of hydroperoxides.

We found that Gpx3 is also reactive to alkylhydroperoxide (supplemental Fig. 2) and is required for resistance to alkylhydroperoxide and methyl linolenic acid (Fig. 1), although apparent oxidized form of Gpx3 was not observed (Fig. 5D). In addition, we demonstrated that Ahp1 is required for maximum resistance of BY4742 (gpx3Δ) cells to tBOOH (Fig. 1) and that both Cad1 and Ahp1 are required for W303 (yap1Δ) (Fig. 2). These findings suggest that both Ahp1-dependent Cad1 activation and Gpx3-dependent Yap1 activation are required for protection against alkylperoxide and lipid peroxide toxicity (Fig. 6). It is clear, however, that the Gpx3-Yap1 pathway contributes more strongly to resistance than the Ahp1-Cad1 pathway.

Similar to the mechanism of Yap1 activation, tBOOH and cadmium inhibit Cad1 nuclear export by effecting structural changes through oxidation of cysteine residues (22, 26). Prior studies have also demonstrated that direct binding of cadmium to cysteine residues in cCRD is responsible for inhibiting its nuclear export signal (22). Ahp1 may be unnecessary for activation in the case of the direct binding mechanism.

This study indicates that Ahp1 should be categorized as a Prx family member that induces disulfide bond formation in its target molecules. Our results further support the hypothesis that Prx proteins are generally able to act as receptors for peroxides and as transmitters of redox signals to other sensor/transducer molecules. We have chosen to call this redox signal transduction “Prx-dependent peroxide signal transmission.” Current efforts are under way to identify similar systems of mammalian Prx-dependent sensing and transduction of peroxide signals.

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