Mammalian Peroxiredoxin Isoforms Can Reduce Hydrogen Peroxide Generated in Response to Growth Factors and Tumor Necrosis Factor-α*

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Mammalian tissues express three immunologically distinct peroxiredoxin (Prx) proteins (Prx I, II, and III), which are the products of distinct genes. With the use of recombinant proteins Prx I, II, and III, all have now been shown to possess peroxidase activity and to rely on Trx as a source of reducing equivalents for the reduction of H₂O₂. Prx I and II are cytosolic proteins, whereas Prx III is localized in mitochondria. Transient overexpression of Prx I or II in cultured cells showed that they were able to eliminate the intracellular H₂O₂ generated in response to growth factors. Moreover, the activation of nuclear factor κB (NFκB) induced by extracellularly added H₂O₂ or tumor necrosis factor-α was blocked by overproduction of Prx II. These results suggest that, together with glutathione peroxidase and catalase, Prx enzymes likely play an important role in eliminating peroxides generated during metabolism. In addition, Prx I and II might participate in the signaling cascades of growth factors and tumor necrosis factor-α by regulating the intracellular concentration of H₂O₂.

We have previously purified a 25-kDa thioredoxin peroxidase (TPx) from Saccharomyces cerevisiae that reduces hydroperoxides with thioredoxin (Trx) as an immediate electron donor (1–7). A data base search revealed more than 40 proteins from a wide variety of species that show sequence similarity to yeast TPxs (8, 9). These homologous proteins were named the peroxiredoxin (Prx) family (8, 9). These proteins were categorized in a peroxidase (TPx) family because not all members use Trx as the hydrogen donor (10, 11). The Prx family includes 12 mammalian proteins that were identified without reference to peroxidase activity but rather in association with a variety of diverse cellular functions including proliferation, differentiation, and immune response (12–24). Whether any of these mammalian Prx proteins possess peroxidase activity and, if so, the identity of the immediate electron donors both remain unknown. As will be published elsewhere, the mammalian Prx members can be divided into three distinct groups (Prx I, II, and III) on the basis of their amino acid sequences and immunological properties.

We have now shown that recombinant proteins from each of the three groups were able to reduce peroxides with the use of electrons from Trx. However, unlike the situation with other enzymes that use Trx, glutaredoxin (Grx) could not replace Trx as the electron donor. In addition, we showed that the mammalian Prx enzymes are capable of reducing H₂O₂ produced in cells stimulated with growth factors and of inhibiting NFκB activation induced by H₂O₂ or tumor necrosis factor-α (TNF-α), indicating that they function as peroxidases inside cells and possibly serve as components of the signaling cascades by growth factors and TNF-α in which H₂O₂ acts as an intracellular messenger.

EXPERIMENTAL PROCEDURES

Materials—Glutamine synthetase was purified from Escherichia coli as described (1). Rabbit antiserum to human Prx I and Prx II were generated against recombinant proteins prepared as described below. Antiserum to Prx III proteins was generated by immunizing rabbits with a keyhole limpet hemocyanin-conjugated peptide (SPTASKYFERKVIHQ) that corresponds to a sequence in the COOH-terminal region of mouse Prx III.

Cloning and Expression in E. coli of Prx I, II, and III cDNAs—Complementary DNAs corresponding to Prx I, II, and III proteins were obtained by the polymerase chain reaction (PCR) with Taq polymerase (Perkin Elmer). A cDNA library derived from human myelogenous leukemia K562 cells (CLONTECH) served as the template source for amplification of Prx I and II cDNAs. Prx III cDNA was amplified from a cDNA library prepared from mouse erythroleukemia C19 cells (CLONTECH). Because Prx III matured in mitochondria lacks the NH2-terminal 63 amino acids (13, 19), the forward primer was designed to amplify from Pro64 and to include an additional Met at the new NH2 terminus. The forward primers for Prx I (5′-ATACATAATTCCCTCAC-GGAAGTGCTAAAAT-3′), Prx II (5′-ATACATAATTCCCTCAC-GGAAGTGCTAAAAT-3′), and Prx III (5′-ATACATAATTCCCTCAC-GGAAGTGCTAAAAT-3′) contain an NdeI site (underlined) and the initiation codon (italized). The reverse primers for Prx I (3′-CTCCTATTAAAGAG-GTTGCTTCCTACCTCCTAGGTT-5′), Prx II (3′-CTCCTATTAAAGAG-GTTGCTTCCTACCTCCTAGGTT-5′), and Prx III (3′-CTCCTATTAAAGAG-GTTGCTTCCTACCTCCTAGGTT-5′) contain a BamHI site (underlined) and the stop codon (italicized). The PCR products were purified with PCR Select III (5 Prime → 3 Prime, Inc., Boulder, CO) and subcloned into the pGEM-T vector (Promega). The identity of all PCR products was confirmed by sequencing.

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fermed by nucleotide sequencing with an automated fluorescent dye DNA sequencer (Applied Biosystems, model 370A). Each of the Ndel-BamHI fragments from pGEM-T was transferred to the pET-17b plasmid (Novagen) to generate pETprxI, pETprxII, and pETprxIII. An E. coli strain was transfected separately with each of these PCR product into pGEM-T and pET-17b as well as subsequent expression in E. coli were performed basically as described above for the Prx proteins. Recombinant Trx was purified by heat treatment at 65 °C and sequential chromatography on Sephacryl S-100 HR gel filtration and HPLC DEAE-5PW ion exchange columns. The NH2-terminal amino acid sequence of the recombinant protein was confirmed by automated Edman degradation. Grx, Gr, and TR were purified from rat liver as described (26, 27).

Determination of Protein Concentration—The concentrations of Prx I, II, and III and Trx were determined spectrophotometrically with absorbity (A280) at 280 nm values of 0.815, 0.938, 0.825, and 0.738, respectively; these values were calculated on the basis of the amino acid composition of the proteins deduced from the corresponding cDNA sequences. Because the sequences of the proteins were not available, the concentrations of these proteins were determined with the BCA protein assay reagent (Pierce) and bovine serum albumin as standard.

Subcellular Fractionation—Subcellular fractions of A431 cells were prepared by differential centrifugation (28, 29). Briefly, cells were transfected at a density of 3 × 105 cells to a 150-mm dish, allowed to recover for 24 h, and then incubated for 18 h in Dulbecco’s modified Eagle’s medium supplemented with 0.5% fetal bovine serum. Cells were washed with and scraped into ice-cold phosphate-buffered saline, and separated by centrifugation at 500 × g for 5 min. For preparation of organelles, they were then resuspended in buffer A (10 mM Hepes- NaOH (pH 7.0), 5 mM MgCl2, 15 mM KCl, 1 mM phenylmethlysulfonyl fluoride, leupeptin (5 μg/ml), and aprotinin (5 μg/ml)) at a density of 1 × 107 cells/ml and allowed to swell for 10 min at 4 °C. Cells were homogenized gently with a Dounce homogenizer until disruption, which was monitored by assaying the activity of released lactate dehydrogenase, was achieved. The cell homogenate was immediately mixed with an equal volume of buffer A containing 0.5 μL sucrose. Nuclei and unbroken cells were separated by centrifugation at 500 × g for 10 min. After addition of 10% NP-40 to a final concentration of 10 mM, the supernatant was centrifuged at 16,000 × g for 10 min. The resulting particulate fraction was centrifuged in buffer A containing 0.25 μL sucrose, recentrifuged, and dissolved in lysis buffer (20 mM Hepes-NaOH (pH 7.2), 1% Triton X-100, 10% (v/v) glycerol, 1 mM phenylmethylsulfonyl fluoride, leupeptin (5 μg/ml), and aprotinin (5 μg/ml)) to a final volume of 1 mL, and sonicated to obtain the organelle extract. The 16,000 × g supernatant from the previous step was centrifuged at 100,000 × g for 1 h, and the resulting supernatant was designated the cytosolic fraction. The pellet was washed once with buffer A containing 0.25 μL sucrose, dissolved in 1 mL of lysis buffer, and designated the membrane fraction.

For the preparation of nuclei, cells were resuspended at a density of 1 × 107/mL in buffer A containing 0.1% Nonidet P-40, and then homogenized as described above. Nuclei were isolated by centrifugation of the homogenate at 500 × g for 10 min. Contamination of the nuclear pellet by unbroken cells was ruled out by confirming the absence of lactate dehydrogenase. Nuclei were resuspended in 1 mL of buffer A containing 0.25 μL sucrose, recentrifuged at 500× g for 10 min, adjusted to a final volume of 1 mL with lysis buffer, and sonicated to yield the crude nuclear extract.

Portions of each subcellular fraction were separated by SDS-polyacrylamide gel electrophoresis and assayed for Prx and marker proteins (catalase and histone for organelles and nuclei, respectively) by immunoblot analysis.

Construction of Prx I and Prx II Eukaryotic Expression Vectors—Prx I and II coding sequences were prepared by PCR from pETprxI and pETprxII, respectively. The forward primers, which include the initiator codon (italized), were 5’GCGCGCACTTATCTTTCTCCAG-GAATAAGTCTAA-3’ for Prx I and 5’TTCACAAATCTTTGCGCTTTGG-GTACCCCGGC-3’ for Prx II. The reverse primers, which include the stop codon (italicized), were 5’-CAGCTTTCTCCAGAATTCTTTGAGAAAAT-3’ for Prx I and 5’-GCGGGGATTCTTGAATTCAAGTTTGTG- GAGAAAAT-3’ for Prx II. The PCR products were subcloned into pCR3.1β basic vector (Invitrogen) to yield pCRprxI, and pCRprxII. Clones containing the coding sequences in the correct orientation were selected and used for transfection.

Transfection of Eukaryotic Cells—NIH 3T3 and A431 cells were plated at a density of 3 × 105 per 60-mm dish and allowed to recover for 24 h. They were then transfected with 4 μg of appropriate DNA and 20 μL of Lipofectamine (Life Technologies, Inc.) for 5 h, after which 3 mL of Dulbecco’s modified Eagle’s medium containing 20% fetal bovine serum (A431 cells) or calf serum (NIH 3T3 cells) were added to the transfection mixture and the cells were incubated for an additional 18 h. The medium was then aspirated and cells were incubated for 18 h in fresh Dulbecco’s modified Eagle’s medium containing 0.5% propidium iodide and assayed for H2O2 generation. HeLa cells were plated at a density of 4 × 105 per 60-mm dish, and 24 h after plating, cells were transfected with the indicated amount of appropriate DNA using Superfect (Qiagen) for 3 h and further incubated in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum for 24 h before stimulation with TNF-α or H2O2. The efficacy and reproducibility of transfection were confirmed by immunoblot analysis of the target protein in each batch of cells.

Assay of Intracellular H2O2 Generation—Intracellular H2O2 generation was measured with a fluorescent dye, 2’,7’-dichlorofluorescin diacetate (DCFH-DA), as described (30). Briefly, serum-deprived transfected NIH 3T3, A431, and HeLa cells were stimulated for 10 min with platelet-derived growth factor (PDGF)-AB (5 ng/ml), 5 min with epidermal growth factor (EGF) (10 ng/ml), or 5 min with H2O2 (10 μM). The cultures were seeded with paraffin film and placed in a CO2 incubator at 37 °C for 5 min, after which DCF fluorescence was measured with a Zeiss Axiovert 135 inverted microscope equipped with a X20 Neofluor objective and Zeiss LSM410 confocal attachment. To avoid photooxidation of DCFH, we collected the fluorescent image with a single rapid scan (4-line average; total scan time, 4.3 s) with identical parameters such as contrast and brightness for all samples. The cells were then examined by differential interference contrast microscopy. Five groups of 10–20 subconfluent cells or 20–30 confluent cells were randomly selected from the image for each sample, the fluorescence intensity was measured for each group from the fluorescent image, and the relative fluorescence intensity per cell was obtained from the average of the five group values.

Chloroethylene Acetaldehyde Estrogen Extracts were in Cell—HeLa cells that had been plated 24 h prior to transfection at 4 × 105 cells per 60-mm dish were transfected with a reporter plasmid, pNFpECAT (6 μg) plus the indicated amount of either pCR or pCRprxII. The reporter plasmid pNFpECAT containing a CAT reporter gene placed under the control of four NFκB enhancer sites has been described. After stimulation with H2O2 or TNF-α, cells were washed, scraped in phosphate-buffered saline, and disrupted by lysis in 100 μl of 0.25% Triton X-100. The resulting pellet was suspended in 150 μl of 0.25 M Tris-HCl (pH 7.4) and disrupted by repeated freezing and thawing three times. The relative amount of CAT in 20 μg of cell extract was measured using the enzyme-linked immunosorbent assay kit (Boehringer Mannheim).

Gel Mobility Shift Assay—HeLa cell nuclear extracts were prepared as described (31). Nuclear extracts were incubated with a 32P-labeled 34bp DNA probe (106 cpm) in the presence of 0.5 μg poly(dI-dC). After 30 min at room temperature, the bound and unbound oligonucleotides were separated on a 5% polyacrylamide gel. For the competition experiment, 50-fold molar excess of unlabeled oligonucleotides were included. The 32P-labeled and unlabeled oligonucleotides with the 34bp binding site were as described (32) and kindly provided by Dr. Kuan-Teh Jeang (NIAD, NIH).

Data Presentation—With the exception of those shown in Figs. 3 (C and D) and 4B, data presented are from representative experiments that were repeated at least three times with similar results.

RESULTS

Peroxiredoxin Activity of Mammalian Prx Proteins—We have previously shown that yeast Tpx reduces peroxides with the use of electrons provided by the Trx system (Trx, TR, and NADPH) (5). We therefore examined mammalian Prx proteins

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for peroxidase activity toward \( \text{H}_2\text{O}_2 \) in the presence of the Trx system or the Grx system (Grx, glutathione, GR, and NADPH), using recombinant Prx proteins from \( \text{E. coli} \). The rate of \( \text{H}_2\text{O}_2 \) degradation was measured by monitoring the decrease in \( A_{340} \) attributable to the oxidation of NADPH. Prx I, II, and III proteins each catalyzed the \( \text{H}_2\text{O}_2 \)-dependent oxidation of NADPH in the presence of the Trx system (Fig. 1). The oxidation of NADPH required all three protein components (Prx, Trx, and TR), being negligible in the absence of any one of the three. In contrast, none of the three Prx enzymes increased the \( \text{H}_2\text{O}_2 \)-dependent oxidation of NADPH in the presence of the Grx system. Neither increasing the concentrations of Grx from 2.2 to 8.8 \( \mu \text{M} \), glutathione from 2 to 4 \( \text{mM} \), or GR from 46 to 92 \( \text{nM} \), nor varying the pH from 7.0 to 8.0, affected the inability of the Grx system to support the peroxidase activity of Prx I, II, or III. The functional efficacy of the Grx and GR preparations was demonstrated by measuring their glutathione-disulfide transhydrogenase (33) and glutathione peroxidase (34) activities, respectively. Thus, the three mammalian Prx proteins appear to receive reducing equivalents specifically from Trx and TR.

**Subcellular Localization of Prx Isoforms**—Rabbit antibodies were generated against Prx I, II, and III proteins and shown to be specific for the corresponding isofrom (not shown). With these isofrom-specific antibodies, we investigated the subcellular localization of Prx enzymes by immunoblot analysis of nuclear, organelle, cytosolic, and membrane fractions in the absence of \( \text{A431} \) human epidermoid carcinoma cells (Fig. 2). Catalase and histone were chosen as markers of organelle and nuclear fractions, respectively. Prx I and II were detected only in the cytosolic fraction, whereas Prx III was apparent as a strong band in the organelle fraction and as a weaker band in the cytosolic fraction. This weaker band is likely attributable to breakage of organelles because catalase, which is localized in peroxisomes, was also detected in the cytosolic fraction. This result is consistent with the fact that SP22 was identified as a substrate of a mitochondrial protease (19).

**Removal by Prx Enzymes of Intracellular \( \text{H}_2\text{O}_2 \) Generated in Response to PDGF or EGF**—Intracellular generation of \( \text{H}_2\text{O}_2 \) can be monitored with the oxidation-sensitive fluorescent probe DCFH-DA and confocal microscopy. As shown previously (35, 36), exposure of quiescent NIH 3T3 cells to PDGF (5 ng/ml) or A431 cells to EGF (500 ng/ml) resulted in a rapid increase in DCF fluorescence with the maximal effect apparent 10 or 5 min, respectively, after stimulation; fluorescence returned to baseline values after 30 min for both cell types (data not shown). Although many cellular oxidants can oxidize DCFH, \( \text{H}_2\text{O}_2 \) was shown to be primarily responsible for this reaction on the basis that incorporation of catalase into cells prevented the growth factor-induced increase in DCF fluorescence (35, 36).

We investigated whether Prx family members are able to eliminate \( \text{H}_2\text{O}_2 \) produced in response to growth factors by transiently expressing Prx I or II in NIH 3T3 cells and Prx II in A431 cells. The overexpression of the two proteins in transfected cells was confirmed by immunoblot analysis (Fig. 3A). We also attempted to express Prx I in A431 cells, but, for an unknown reason, no substantial increase in the amount of this protein was apparent in transfected cells in three independent experiments. In response to stimulation with PDGF (10 min) or EGF (5 min), DCF fluorescence increased more than 2-fold in NIH 3T3 or A431 cells transfected with vector alone. However, overexpression of Prx I or II prevented the growth factor-induced increase in DCF fluorescence (Fig. 3, B-D).

**Inhibition of NF\( \kappa \)B Activation by Prx II Overexpression**—A large number of extracellular stimuli that activate the transcriptional factor NF\( \kappa \)B are known to do so by producing intracellular \( \text{H}_2\text{O}_2 \) (37–39). In the cytosol, NF\( \kappa \)B is in an inactive form bound to an inhibitory subunit, I\( \kappa \)B. Activation of cells with appropriate stimuli results in phosphorylation of I\( \kappa \)B followed by its selective degradation, thereby allowing free NF\( \kappa \)B to translocate to nucleus (40). Extracellularly added \( \text{H}_2\text{O}_2 \) also results in the activation of NF\( \kappa \)B in HeLa cells (37, 41). We investigated whether overproduction of Prx II can inhibit the \( \text{H}_2\text{O}_2 \)-induced NF\( \kappa \)B activation (Fig. 4). The overproduction of Prx II in HeLa cells transfected with Prx II-expressing vector, pCPRpxII was confirmed by immunoblot analysis (Fig. 4A). The level of PrxII expression was not affected by a 6-h exposure to \( \text{H}_2\text{O}_2 \) (100 \( \mu \text{M} \)). The intracellular peroxidase function of overproduced Prx II was demonstrated by the fact that the \( \text{H}_2\text{O}_2 \)-induced increase in DCF fluorescence was completely prevented by overexpression of Prx II (Fig. 4B). One way to
measure NFkB activity is to assay the expression of CAT reporter gene under the control of NFkB sites. HeLa cells were transiently co-transfected with pCRprxII or control vector pCR and a plasmid (pNFkBCAT) containing four NFkB enhancer sites and a CAT gene. H2O2 increased CAT expression in cells transfected with pCR but not in cells transfected with pCRprxII (Fig. 4C). For an unknown reason, the level of H2O2-induced CAT expression in Prx II-overexpressing cells was lower than that in control cells.

The effect of Prx II overexpression on the activation of NFkB by H2O2 was also analyzed by gel mobility shift assay using radiolabeled DNA containing NFkB-binding sites. The NFkB-specific band was evident when nuclear extracts from HeLa cells were incubated with labeled DNA and separated on a gel, and the same band was eliminated by the addition of an excess of unlabeled NFkB DNA to the binding mixture (not shown). As shown in Fig. 4D, a 30-min treatment of control cells with 100 μM H2O2 resulted in a significant degradation of IκB and the degradation was partly prevented by overexpression of Prx II.

TNF-α is known to activate NFkB in HeLa cells by generating intracellular H2O2 (37–41). We tested the ability of PrxII to block the TNF-α-induced NFkB activation in HeLa cells co-transfected with pNFkB and pCR or pNFkB and a range of different quantities of pCRprxII. We confirmed that we could overexpress Prx II at several different levels by immunoblot analysis (Fig. 5A). TNF-α increased CAT expression in cells transfected with the control vector pCR. But in cells transfected with pCRprxII, the CAT response to TNF-α gradually decreased as the amount of overexpressed Prx II increased (Fig. 5B). We also measured NFkB activity in nuclear extracts of control and Prx II-overexpressing HeLa cells by gel mobility shift assay (Fig. 5C). The intensity of the NFkB-specific band was increased in control cells treated with TNF-α (15 ng/ml) but not in similarly treated Prx II-overexpressing cells.

These results suggest that overexpression of Prx II prevented the TNF-α-induced NFkB activation by removing H2O2.

**Fig. 3. Effect of Prx overexpression on growth factor-induced H2O2 generation.** Panel A, NIH 3T3 and A431 cells were transiently transfected with the indicated expression plasmids, and the extent of Prx expression was measured by immunoblot analysis. Panel B, DCF fluorescence was measured with a confocal microscope after incubation of A431 cells in the presence of EGF for 5 min. Panel C, from the images shown in panel B, relative fluorescence intensity per cell was calculated as describe under “Experimental Procedures.” Panel D, the transfected NIH 3T3 cells were stimulated with PDGF for 10 min, and the DCF fluorescence was measured. Data in panels C and D are means ± S.E. of the values obtained from five groups of 20–30 cells; pCR represents the vector alone without any insert.
DISCUSSION

Our data suggest that like yeast TPx, all three mammalian Prx proteins reduce H$_2$O$_2$ with reducing equivalents provided by the Trx system. Trx and Grx mediate electron transfer through a simple mechanism that includes the reversible oxidation of two vicinal Cys–SH groups to form a disulfide bond (43, 44). Several oxidoreductases, including ribonucleotide reductase, adenosine 3'–phosphate 5'-phosphosulfate reductase, methionine sulfoxide reductase, and glutathione peroxidase, accept electrons from both Trx and Grx (44). However, none of the Prx enzymes are able to receive electrons from Grx. These proteins therefore appear to provide the first instance in which the electron carrier functions of Trx and Grx can be distinguished.

To date, catalase and glutathione peroxidase have been viewed as the major enzymes responsible for removal of cytotoxic H$_2$O$_2$. Our data now suggest that Prx enzymes also might play a role in the removal of H$_2$O$_2$. In rat hepatocytes, catalase is largely or entirely localized to peroxisomes, whereas glutathione peroxidase is present in various intracellular compartments including mitochondria (42%), nuclei (26%), cytosol (21%), peroxisomes (7%), and lysosomes (4%) (45). Our immunoblot analysis indicates that glutathione peroxidase is present in much lower concentrations compared with Prx enzymes in most tissues except liver (data not shown). All three Prx enzymes are abundant in various rat tissues and in cultured cells. The sum of the three Prx enzymes in cultured cells amounts to 2–8 mg/mg soluble protein. Prx III appears to be localized in mitochondria, whereas Prx I and Prx II are cytosolic proteins.

Although H$_2$O$_2$ is generally considered a toxic by-product of respiration, increasing evidence suggests that the production of H$_2$O$_2$ might be an integral component of membrane receptor signaling. In mammalian cells, a variety of extracellular stimuli, including tumor necrosis factor-α (46, 47), PDGF (35), and EGF (36), induces a transient increase in the intracellular concentration of H$_2$O$_2$. Inhibition of this increase by N-acetyl cysteine or catalase prevents the protein tyrosine phosphorylation induced by PDGF or EGF (35, 36) as well as the activation of the nuclear factor NF-κB induced by TNF-α (37).
FIG. 5. Effect of Prx II overexpression on TNF-α-induced NFkB activation. Panel A, HeLa cells were transiently transfected with 5 μg of pCR or the indicated amount of pCRprxII (total amount of plasmid was adjusted to 5 μg by adding pCR) and the extent of Prx II expression was measured by immunoblot analysis. Panel B, after stimulation of the transfected cells (from panel A) with TNF-α (15 ng/ml) for 6 h, relative amounts of CAT were measured in their extracts and expressed as fold activation compared with the amount from pCR-transfected cells. Panel C, HeLa cells were transiently transfected with 5 μg of pCR and pCRprxII and DNA-binding activity of NFkB in the nuclear extract of HeLa cells treated with TNF-α (15 ng/ml) for 30 min was measured by gel mobility shift assay as described in the legend to Fig. 4.

The transient nature of the receptor-mediated increase in intracellular H2O2 suggests that, in addition to its production, the rapid removal of this molecule is important for receptor signaling. Given that we have shown that Prx I and Prx II can remove H2O2 generated in response to growth factors and that Prx II overexpression further increases the rate of H2O2 removal, it seems reasonable to assume that Prx II is likely to participate in the signaling cascade of growth factor and TNF-α. Furthermore, Prx I and Prx II, which are abundant in cytosol, likely play an important role in eliminating H2O2 generated as a by-product of metabolism in the cytosol. These conclusions are consistent with the fact that Prx I and Prx II were discovered in connection with a variety of seemingly unrelated cellular processes such as proliferation, differentiation, natural killer cell activity, the response to oxidative stress, and osteoregulation (12–24).

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