Reactive oxygen species, especially hydrogen peroxide, are important in cellular signal transduction. However, excessive amounts of these species damage tissues and cells by oxidizing virtually all important biomolecules. Peroxiredoxin 6 (PRDX6) (also called antioxidant protein 2, or AOP2) is a novel peroxiredoxin family member whose function in vivo is unknown. Through immunohistochemistry, we have determined that the PRDX6 protein was widely expressed in every tissue examined, most abundantly in epithelial cells. It was found in cytosol, but not in membranes, organelles, and nuclei fractions. Prdx6 mRNA was also expressed in every tissue examined. The widespread expression of Prdx6 suggested that its functions were quite important. To determine these functions, we generated Prdx6-targeted mutant (Prdx6−/−) mice, confirmed the gene disruption by Southern blots, PCR, RT-PCR, Western blots, and immunohistochemistry, and compared the effects of paraquat, hydrogen peroxide, and t-butyl hydroperoxide on Prdx6−/− and wild-type (Prdx6+/+) macrophages, and of paraquat on Prdx6−/− and Prdx6+/+ mice. Prdx6−/− macrophages had higher hydrogen peroxide levels, and lower survival rates; Prdx6−/− mice had significantly lower survival rates, more severe tissue damage, and higher protein oxidation levels. Additionally, there were no differences in the mRNA expression levels of other peroxiredoxins, glutathione peroxidases, catalase, superoxide dismutases, thioredoxins, and glutaredoxins between normal Prdx6+/+ mice, confirmed the gene disruption by Southern blots, PCR, RT-PCR, Western blots, and immunohistochemistry, and the positions of their Cys residues, their synonyms, and recently re-named PRDX5. To avoid misunderstanding, all PRDX proteins contain a conserved cysteine residue in the NH2 termini of the molecules, corresponding to Cys47 of yeast PRDX. Whereas PRDX1–4 contain an additional conserved Cys in the COOH-terminal region, which is separated by 120–123 amino acids from the NH2-terminal-conserved Cys and corresponds to Cys170 of yeast PRDX, mouse and human PRDX6 contain only the NH2-terminal-conserved cysteine...
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| Mouse gene | Human gene | Conserved Cys | Cellular location | Mouse synonyms | Mouse Chr | Human synonyms | Human Chr | Refs. |
|------------|------------|---------------|------------------|----------------|-----------|----------------|-----------|-------|
| Prdx1      | PRDX1      | 47, 170       | Cytosol          | MSP23/OSF3     | 4–47      | PAG/NKEFA      | 1p94.1    | 43–46 |
| Prdx2      | PRDX2      | 47, 170       | Cytosol          | TSA            | 8–36      | TSA/NKEFB      | 13q12     | 44, 47|
| Prdx3      | PRDX3      | 47, 170       | Mitochondria     | MERS5/AOP1     | 19–50     | MERS5/AOP1     | 10q26.12   | 48, 49|
| Prdx4      | PRDX4      | 47, 170       | Cytosol, extracellular | PRDXIV | X–65.4  | AOE372         | Xp22.11    | 40, 50, 51|
| Prdx5      | PRDX5      | 47, 72, 151   | Mitochondria Peroxisome | PMF20, PRDXV | 19–0.5 | AOE1B166, PRDXV | 11q12.3    | 6–8, 52|
| Prdx6      | PRDX6      | 47            | Cytosol          | AOP2           | 1–83.6    | AOP2, ORF6, PLAβ, NSGP2 | 1q23.3    | 22, 23, 27|

a Gene symbols follow standard nomenclature defined by the International Committee on Standardized Genetic Nomenclature in Mice (www.informatics.jax.org/support/nomen/).
b Gene symbols follow standard nomenclature defined by the Human Genome Organization (HUGO) Nomenclature Committee (www.genenames.org).
c Retrieved from Mouse Genome Database (www.informatics.jax.org), and expressed as Chr–cM.
d Retrieved from NCBI LocusLink (www.ncbi.nlm.nih.gov/LocusLink), and checked for fine position in Ensembl Human Genome Browser (www.ensembl.org/Homo_sapiens/).
e Physical position of this gene was retrieved from the Ensembl Mouse Genome Server (www.ensembl.org/Mus_musculus/). The two flanking markers, DXMit113 and DXMit1178, on either side as shown there both have a genetic position of cM 65.4 as retrieved from Mouse Genome Database (www.informatics.jax.org). This position is therefore used as the genetic position of mouse Prdx6.

(Cys^{47}) and are therefore termed 1-Cys PRDX. The recently identified mouse and human PRDX5s contain three cysteines: 1) Cys^{17} (in humans) or Cys^{36} (in mice, corresponding to Cys^{47} of yeast PRDX), 2) Cys^{27}, and 3) Cys^{151} (6–8). It appears that the Cys^{17}, corresponding to Cys^{47} of yeast PRDX and common to these six PRDX subgroups, is the active site oxidized by H_{2}O_{2} (3). The 2-Cys and 1-Cys PRDXs exist as homodimers, with the two monomers arranged in a head-to-tail manner, as supported by crystal structure analysis (9, 10), and their activity likely depends on quaternary structures (11–13). However, mouse and human PRDX5 do not form dimers (14). When the Cys^{27} (Cys-SOH) of a 2-Cys PRDX is oxidized, it reacts with Cys^{36}-SH of the other subunit to form an intermolecular disulfide, which is then reduced by thioredoxin (3). When human PRDX5 is oxidized, an intramolecular disulfide bond is formed between Cys^{47} and Cys^{151} (14). Probably, an intramolecular disulfide also forms when 1-Cys PRDX (including mouse Prdx1, Prdx2, Prdx3, Prdx4, Prdx5, and Prdx6) is oxidized (3). It is a bipyridyl herbicide that can be reduced by NADPH-cytochrome P-450 reductase in vitro into paraquat radicals, which then react with O_{2} to produce O_{2}^{•−} (26). While excessive O_{2}^{•−} itself is harmful to cells, it can be dismutated into H_{2}O_{2} by SODs. We hypothesized that mouse PRDX6 would eliminate H_{2}O_{2} and reduce the number of OH^{•} produced by Fenton reactions and subsequent tissue injury. Prdx6^{−/−} mutants had more severe tissue damage and a significantly higher mortality rate than did controls, independent of other major antioxidant enzymes. The macrophages from the Prdx6^{−/−} mutants also had more hydrogen peroxide, and were more susceptible to oxidant-induced cell death. Our study provides evidence that, under conditions of excessive oxidative stress, mouse PRDX6 functions in vivo as an antioxidant and non-redundantly to other peroxiredoxins and antioxidant enzymes.

Experimental Procedures

Generation of Prdx6-targeted Mutant Mice—A genomic clone containing the 129/SvJ (129) Prdx6 gene was isolated as previously described (27). The clone was digested with BglII, and a 5094-bp fragment containing the upstream regulatory regions (position 1606–6700) of the gene was isolated. This represented the 5'-arm of Prdx6. The 3'-arm was isolated using a multiple step cloning method. First, the genomic clone was digested with NarI and SacII, which cut into intron 2 and exon 5, respectively. This fragment was isolated and partially digested with NsiI to obtain the correct segment. A 4594-bp fragment corresponding to nucleotides 10,872–13,470 was isolated, representing the 3'-arm of Prdx6. The Litmus 29 cloning vector (New England Biolabs) was digested with NsiI, dephosphorylated, and ligated with the 3'-arm. The ligation products were transformed into CE4 cells, which were screened by restriction digestion. The correct clone (named p182) was obtained, and the 3'-arm was verified by sequencing. To clone the 5'-arm, the p182 plasmid was first digested with EcoRV and ligated with a T4-blunt-ended 5'-arm. The ligation products were transformed into CE4 cells and colonies screened by restriction digestion. A clone (p184) containing the 5'-arm was obtained. The pSABgal plasmid containing the SA splice acceptor from adenovirus, Bgal gene, PGK promoter, neomycin gene, and FA signal from bovine growth hormone was generated using the pXhopKS (−) cloning vector (Stratagene). The 7.9-kb pSABgal plasmid was digested with XhoI, releasing the Bgal-Neo cassette. The p184 plasmid was partially digested with XhoI, and the Bgal-Neo cassette was cloned into the correct XhoI site of the targeted construct. All junctions within the targeting construct were verified by sequencing.
The targeted mutation construct was linearized with SwaI and electroporated into the ES Cell Line R1 (Derived from 129 mice). Using 5'- and 3'-probes, over 100 ES cell clones were screened for the construct by Southern blotting (EcoNI digests). A total of 11 positive clones were identified. These were microinjected into blastocysts from C57BL/6J (B6) mice and implanted into pseudopregnant females. Chimeric mice were crossed to B6 once, and the offspring containing the Prdx6 construct were intercrossed to generate mice homozygous for the targeted and non-targeted Prdx6 alleles, which were screened by PCR. The primers for screening were: 0368 forward, 5'-CAGGATGGAGCCCTCTATGCC-3'; 0369 forward, 5'-TGCGTCTGGAGACCGAAAGA-3'; and 0366 reverse, 5'-CTTGAAGAAGAAGCAGG-3'. Primers 0368 and 0366 are both in intron 2, and amplify a fragment of this intron, giving a 198-bp product from the endogenous Prdx6 gene when the targeting vector is not present. Primer 0369 falls within the inserted Neo gene, and together with primer 0366, they give a 315-bp product when the targeting step at 94 °C followed by 36 cycles of 1) 30 s at 92 °C, 2) 30 s at 55 °C, and 3) 30 s at 72 °C, and a final extension of 3 min at 72 °C.

To generate Prdx6-targeted mutants on 129/SvJ (129) background, the chimeric Prdx6-targeted mutants were crossed to 129 mice, and these offspring heterozygous for the Prdx6-targeted mutation were intercrossed to generate mice homozygous for the targeted Prdx6 gene mutation.

When Prdx6-targeted mutant mice on mixed B6;129 background were used, the controls were the wild-type littermates of the targeted mutants, of the same age and sex. When Prdx6-targeted mutant mice on pure 129 background were used, we used either wild-type 129/SvJ inbred mice (in vivo paragraft studies) or wild-type littermates (for isolation of macrophages), of the same sex and age.

Preparation of Genomic DNA—DNA was extracted from the tail tips of each experimental and control mouse. Approximately 1 cm of each tail tip was placed in 500 μl of tail buffer (50 mM Tris-Cl pH 8.0, 100 mM EDTA, 100 mM NaCl, 1% SDS) and digested overnight with 1 mg/ml proteinase K at 55 °C. DNA was extracted with phenol/chloroform/isomyl alcohol and precipitated from the upper aqueous phase with two volumes of 100% ethanol. The precipitated DNA pellets were dried and resuspended in 1 ml of Tris buffer-EDTA (pH 7.5–8.0).

Southern Blots—Southern blots were used to detect the Prdx6 gene. 10 μg of tail DNA were digested with EcoNI, electrophoresed on a 0.7% agarose gel, and transferred to a Hybond nylon membrane (Amersham Biosciences). A Prdx6-specific probe was generated by PCR amplification of a 530-bp PCR product corresponding to nucleotide positions 663–1193 in the original p165 genomic sequence, which falls near the 5'-end within the 5'-arm of the targeted mutant construct. The following primers were used: forward 5'-GGACCCGCCTTGAGTGAAT-3', reverse 5'-CTCCACCACCTATGTCATTTGA-3'. The probe was labeled with 32P by random priming using the Redi-Prime kit (Amersham Biosciences), and the blots were hybridized using the Rapid-Hybe solution and protocol (Amersham Biosciences).

Reverse Transcription (RT)-PCR and Real Time PCR Analysis—Total RNA from brain and fat tissues was extracted by using RNeasy Lipid Tissue Mini kit, and total RNA from all the other tissues was extracted with the RNeasy Mini kit (Qiagen, Valencia, CA), following the manufacturer's instructions. RT-PCR was performed as follows. cDNA was synthesized by reverse-transcribing 2 μg of total RNA with Omniscript RT kit (Qiagen, Hilden, Germany), using oligo(dT)15 primer (Promega, Madison, WI). The resulting cDNA was used as a template to amplify the Prdx6 transcripts with these primers: forward, 5'-TTGATGATTGAGGGGCGAGGAC3'; reverse, 5'-GTGACCAAGCCTCTGCTTCTGAGGCT-3'. The following PCR condition was used: one 5-min denaturation step at 95 °C followed by 36 cycles of 1) 30 s at 94 °C, 2) 30 s at 55 °C, and 3) 30 s at 72 °C, and a final extension of 3 min at 72 °C.

The final products were separated in 2.5% agarose 3:1 (Amresco) and visualized by ethidium bromide staining.

Quantitative real-time PCR was performed using the ABI Prism 7000 Sequence Detection System (PE Applied Biosystems). Primers were designed using the primer design software Primer Express 2.0 (PE Applied Biosystems). The forward and reverse primers for each pair of primers are located on different exons. To be sure the primers amplified a unique and desired cDNA segment, each potential pair of primers was

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**Table II**

| Gene/Symbol | Gene name | Primers |
|-------------|-----------|---------|
| Prdx1       | Peroxiredoxin 1 | F, 5'-ACCCCAAGAAAGAAAGAGGATT-3', R, 5'-CAACGGGAGGATCTTTGTTTGA-3' |
| Prdx2       | Peroxiredoxin 2 | F, 5'-ACCCCAAGAAAGAAAGAGGATT-3', R, 5'-CAACGGGAGGATCTTTGTTTGA-3' |
| Prdx3       | Peroxiredoxin 3 | F, 5'-AGTCTCCGACTGGTTCGCTAA-3', R, 5'-GGCCCACTGAACACACTACGTG-3' |
| Prdx4       | Peroxiredoxin 4 | F, 5'-TCCTGCTTGGGAGGAGGAAT-3', R, 5'-AGTCTCCGACTGGTTCGCTAA-3' |
| Prdx5       | Peroxiredoxin 5 | F, 5'-GAAAAGAAGCTGGTGGGAGTGT-3', R, 5'-GCCAGGATCCACAAACAAA-3' |
| Prdx6       | Peroxiredoxin 6 | F, 5'-TGTGATGATTGAGGGGCGAGGAC3', R, 5'-CTCAGGAGCTCAGTGAGG-3' |
| Cat         | Catalase   | F, 5'-TCAGGATGGACGTGTGCAGTGA-3', R, 5'-GGGCTAACCACGAGATGACAA-3' |
| Gpx1        | Glutathione peroxidase 1 | F, 5'-ACCATCTACTGCGACTTCTTCTCA-3', R, 5'-GAGGAAACAATCACCGCAGGACTA-3' |
| Gpx2        | Glutathione peroxidase 2 | F, 5'-ACCCCAAGAAAGAAAGAGGATT-3', R, 5'-CAACGGGAGGATCTTTGTTTGA-3' |
| Gpx3        | Glutathione peroxidase 3 | F, 5'-GACCTGCTGAGACGGACTTCA-3', R, 5'-GCCAGGATCCACAAACAAA-3' |
| Gpx4        | Glutathione peroxidase 4 | F, 5'-TGTGATGATTGAGGGGCGAGGAC3', R, 5'-AGTCTCCGACTGGTTCGCTAA-3' |
| Sod1        | Superoxide dismutase 1 | F, 5'-AGTCTCCGACTGGTTCGCTAA-3', R, 5'-GGCCCACTGAACACACTACGTG-3' |
| Sod2        | Superoxide dismutase 1 | F, 5'-AGTCTCCGACTGGTTCGCTAA-3', R, 5'-GGCCCACTGAACACACTACGTG-3' |
| Sod3        | Superoxide dismutase 1 | F, 5'-AGTCTCCGACTGGTTCGCTAA-3', R, 5'-GGCCCACTGAACACACTACGTG-3' |
| Txn1        | Thioredoxin 1 | F, 5'-AGTCTCCGACTGGTTCGCTAA-3', R, 5'-GGCCCACTGAACACACTACGTG-3' |
| Txn2        | Thioredoxin 2 | F, 5'-AGTCTCCGACTGGTTCGCTAA-3', R, 5'-GGCCCACTGAACACACTACGTG-3' |
| Glr1        | Glutaredoxin 1 | F, 5'-AGTCTCCGACTGGTTCGCTAA-3', R, 5'-GGCCCACTGAACACACTACGTG-3' |
| Glr2        | Glutaredoxin 2 | F, 5'-AGTCTCCGACTGGTTCGCTAA-3', R, 5'-GGCCCACTGAACACACTACGTG-3' |
| Bact        | β-Actin    | F, 5'-ACCCCAAGAAAGAAAGAGGATT-3', R, 5'-CAACGGGAGGATCTTTGTTTGA-3' |
gene (centrifugation at 500 g for 60 min). A BglII restriction fragment encompassing about 5100 bp of the upstream regulatory region was isolated as the 5′-arm, and a 4600-bp NsiI restriction fragment spanning introns 2 to 4 was isolated as the 3′-arm. The targeting vector (middle) was generated by cloning each arm into the pSABgal plasmid (middle) containing the SA splice acceptor from adenovirus, BglII gene, PGK promoter, neomycin gene, and PA signal from bovine growth hormone. The final targeted locus is shown at the bottom. Insertion of the construct and disruption of Prdx6 gene was checked by PCR. Genomic DNA was extracted from the tail tips of mice on the 129 background. Without the construct, the wild type (+/+ ) PCR product was 199 bp; homozygous disruption (−/−) of the Prdx6 gene with the inserted construct generated a 315-bp product; heterozygous disruption (+/−) generated both products. C, Southern blot analysis of EcoNI-digested genomic DNA from homozygous Prdx6-targeted mutant mice (−/−) and the littermate wild type controls (+/+ ) with BglI mixed (left two lanes) and 129 (right two lanes) background. Whereas the wild-type gene was recognized as a 7-kb fragment, the targeted mutant gene was recognized as a 20-kb fragment.

checked in the BLAST program in the Ensembl Mouse Genome Server (www.ensembl.org/Mus_musculus) and Celera data base (www.celera.com). The primer sequences for the antioxidant enzymes are shown in Table II. cDNA samples, prepared as in RT-PCR above, were mixed with primers and SYBR Master Mix (PE Applied Biosystems) in a final concentration of 10 mM. The samples were then centrifuged for 10 min at 20,000 g, and homogenized with Dounce homogenizer. An equal volume of 0.5 M sucrose was then added. After centrifugation at 500 × g for 10 min, EDTA was added to the supernatant at a final concentration of 10 mM. The samples were then centrifuged at 16,000 × g for 10 min, after which the supernatant was separated from the pellet and centrifuged at 100,000 × g for 60 min. The resulting supernatant contained the cytosolic fraction, and the pellet was washed in buffer A/0.25 M sucrose and dissolved in lysis buffer (10 mM Hepes-NaOH, pH 7.0, containing 1% v/v Triton X-100, 10% v/v glycerol, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, and 5 μM aprotinin) at the density of 1 × 107 cells/ml, and homogenized with Dounce homogenizer. After centrifugation at 500 × g for 10 min, the supernatant was collected. Protein concentrations in each supernatant were determined by the Bio-Rad dye-binding protein assay. After SDS-PAGE on 12% gel under reduced condition, proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad) in a semi-dry transfer system (Bio-Rad). The membranes were blocked with 5% skim milk in Tris-HCl buffer, and mouse Prdx6 protein was detected using the rabbit anti-mouse PRDX6 peptide antibody (Ab5, 1:2000), horseradish peroxidase-labeled secondary antibody (Santa Cruz) and ECL (Amer sham Biosciences).

Histology and Immunohistochemistry—Mouse tissues were paraffin-embedded and sectioned after being fixed in 4% paraformaldehyde. Sections (4 μm) were stained with hematoxylin and eosin. For immunohistochemistry, endogenous peroxidase activity in the sectioned tissues was blocked with 0.3% H2O2, and nonspecific binding sites were blocked with non-immune goat serum. Subsequently, rabbit anti-mouse PRDX6 (Ab5, 1:2000) was applied. Specific binding was detected by using biotinylated secondary antibody and avidin-biotin-horse radish peroxidase complex (Santa Cruz), and diaminobenzidine as a sub-

Organelle fraction, the pellet from the 16,000 × g centrifugation above was resuspended in buffer A/0.25 M sucrose, centrifuged at 16,000 × g for 10 min, dissolved in lysis buffer, and sonicated. To obtain nuclei, cells were suspended in buffer A containing 0.1% v/v Nonidet P-40 at a density of 1 × 107 cell/ml, homogenized as above, and centrifuged at 500 × g for 10 min. The pellet was suspended in buffer A/0.25 M sucrose, and centrifuged at 500 × g for 10 min, dissolved in 1 ml of lysis buffer and sonicated. To obtain the whole cell lysate, cells were lysed in lysis buffer, and the supernatant was collected after centrifugation for 10 min at 20,000 × g.

Western Blot—Proteins of different cell fractions were prepared as described above. Protein from liver was extracted as follows: liver tissue was homogenized with an extraction buffer containing protease inhibitor mixture (Sigma, cat. no. P8340) in an ice bath. Homogenates were centrifuged for 30 min at 20,000 × g, and the protein-containing supernatants were collected. Protein concentrations in each supernatant were determined by the Bio-Rad dye-binding protein assay. After SDS-PAGE on 12% gel under reduced condition, proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad) in a semi-dry transfer system (Bio-Rad). The membranes were blocked with 5% skim milk in Tris-HCl buffer, and mouse PRDX6 protein was detected using the rabbit anti-mouse PRDX6 peptide antibody (Ab5, 1:2000), horseradish peroxidase-labeled secondary antibody (Santa Cruz) and ECL (Amer sham Biosciences).

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**Fig. 2.** Prdx6 mRNA and PRDX6 protein were not expressed in Prdx6-targeted mutant mice. A, mRNA expression levels were determined by RT-PCR. Total RNA was extracted from livers of B6;129 mixed background female mice at 12 weeks of age (+/+), homozygous wild type; +/-, heterozygous; -/-, homozygous targeted mutant) and reverse-transcribed into cDNA using an oligo(dT)15 primer. The resulting cDNA was used as a template for PCR, which ran for 19, 28, and 36 cycles. The expression level of Gapd mRNA was used as a control. B, PRDX6 protein levels were determined with Western blots. Proteins from either livers or macrophages of either 129 or B6;129 mixed background female mice at 12 weeks of age (+/+), homozygous wild type; +/-, heterozygous; -/-, homozygous targeted mutant) were extracted and run on 10% SDS-PAGE under reducing conditions. After transblot, PRDX6 protein was detected with a polyclonal antibody against mouse PRDX6. C, expression and distribution of PRDX6 protein in the targeted mutant (Prdx6+/−), heterozygous (Prdx6+/-), and wild-type (Prdx6−/−) female mice on 129 background at 15 weeks of age were determined by immunohistochemistry. Tissues from female mice (all littersmates) were fixed in 4% formaldehyde and paraffin-embedded. Expression of PRDX6 protein was detected with a polyclonal antibody against mouse PRDX6. Non-immune rabbit serum was used instead of the anti-mouse PRDX6 antibody in the controls.

Intracellular fluorescence was detected with excitation at 485 nm, and emission at 530 nm, using Victor2 1420 Multilabel Counter.

**Measurement of Enzyme Activities of SOD, CAT, and GPX**—When they were 9 weeks old, Prdx6-targeted mutant mice and their controls were injected (intraperitoneal) with paraquat (methyl viologen; Chem Service) at doses of 25–30 µg in phosphate-buffered saline/g body weight. Mice with mixed B6;129 and pure 129 backgrounds were used. The mice were observed every 12 h (except overnight) for 14 days. Liver, spleen, and kidney tissues from some of the Prdx6-targeted mutant mice and controls were collected at the same times, fixed in 4% paraformaldehyde, and embedded in paraffin. Sections (4 µm) were stained with hematoxylin and eosin, and pathological changes were observed with light microscopy. All animal protocols were approved by the Jackson Laboratory Animal Care and Use Committee.

**Paraoxon-induced Oxidative Stress in Mice**—When they were 9 weeks old, Prdx6-targeted mutant mice and their controls were injected (intraperitoneal) with paraquat (methyl viologen; Chem Service) at doses of 25–30 µg in phosphate-buffered saline/g body weight. Mice with mixed B6;129 and pure 129 backgrounds were used. The mice were observed every 2 h (except overnight) for 14 days. Liver, spleen, and kidney tissues from some of the Prdx6-targeted mutant mice and controls were collected at the same times, fixed in 4% paraformaldehyde, and embedded in paraffin. Sections (4 µm) were stained with hematoxylin and eosin, and pathological changes were observed with light microscopy. All animal protocols were approved by the Jackson Laboratory Animal Care and Use Committee.

**Measurement of Enzyme Activities of SOD, CAT, and GPX**—After the mice were sacrificed by cervical dislocation, they were perfused with 10 mM phosphate buffer saline, pH 7.4, containing 1 mM EDTA. 200-mg liver tissue in 3 ml of homogenizing buffer (50 mM phosphate buffer, pH

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Notes on nomenclature: Gene symbols used in this article follow standard nomenclature for human and mouse genes. As a rule, mouse genes begin with a capital letter and are italicized. Proteins are completely capitalized but not italicized.
7.4, containing 0.1% digitonin, 40 μg/ml phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 7 μg/ml pepstatin, 5 μg/ml aprotonin, 1 mM EDTA, and 40 μM butylated hydroxyl toluene) was thoroughly, gently, and finely minced. This tissue and buffer mixture was centrifuged, and the activities of the enzymes in the supernatant were measured as discussed below.

SOD activity was measured using an SOD assay kit (Calbiochem, La Jolla, CA). The assay uses the accelerated alkaline autoxidation of 5,6,6a,11b-tetrahydro-3,9,10-trihydroxybenzo[c]fluorine by SOD, which yields a chromophore with a maximal absorbance at 525 nm (31). This assay measures the activity of any type of SOD. One unit is defined as the activity that doubles the autoxidation rate of the control blank. Catalase activity was measured based on its reaction with methanol in the presence of H₂O₂ (32). The formaldehyde produced is measured spectrophotometrically with purpald as chromogen, which gives a maximal absorbance at 540 nm (Catalase Assay kit, Cayman Chemical, Ann Arbor, MI). One unit is defined as the amount of enzyme that produces 1.0 nmol of formaldehyde per minute at 25 °C.

GPX activity was measured by a coupled reaction with glutathione reductase (33). The oxidized glutathione produced when GPX reduces hydroperoxide is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to NADP⁺ is accompanied by a decrease in absorbance at 340 nm, which is directly proportional to the GPX activity in the sample (GPX Assay Kit, Cayman Chemical). This assay detects all of the GPX activities in the samples. One unit is defined as the amount of enzyme that oxidizes 1.0 nmol of NADPH to NADP⁺ per minute at 25 °C. Protein concentrations were determined by the Bio-Rad dye-binding protein assay.

Detection of Protein Oxidation and Lipid Peroxidation—As an indicator of protein oxidation, the total number of protein carbonyls in the liver was measured with a spectrophotometric assay (34). Although this assay is not specific for ROS-induced carbonyls, and carbonyls can also
be generated from glucose and aldehyde-dependent pathways, we used this assay because first, it is the most widely used marker of oxidative modification of proteins as shown by many studies, and secondly, in our experiments using Prdx6-targeted mutant mice, we have found a decreased ability of these mice to cope with oxidative stress and increased ROS in them, thus a difference in the amount of carboxyls between Prdx6-targeted mutant and control mice might reflect a difference in oxidative stress in them. Tissues were prepared as were those used for measuring antioxidant enzyme activities (described above). Nuclear acids in the supernatant from the homogenized tissues were precipitated with streptomycin sulfate. Dimethylhydrobenzidine (DNPH) was used to react with protein carboxyls to form protein hydrazones, which give maximal absorbance at 370 nm. Protein concentrations of the samples were quantified by reading the absorption (280 nm) of the samples that went through the same procedures except that DNPH was omitted, using bovine serum albumin as standard.

Results

Generation and Detection of Prdx6-targeted Mutants—We generated mice lacking Prdx6 expression by replacing exons 1 and 2 with neomycin drug resistance and Bsgal genes (Fig. 1A). Insertion of the targeting vector and disruption of Prdx6 gene was verified by PCR. For this purpose, a primer pair designed in the intron 2 of the wild-type Prdx6 gene was used to amplify a 199-bp fragment, indicating that the Prdx6 gene was intact. Using the same reverse primer, and a forward primer designed in the inserted Neo gene, a 315-bp PCR product is obtained, indicating that the targeting vector was inserted into the Prdx6 gene. Thus, a single 199-bp product indicated that the DNA came from Prdx6

Table III

| Tissue                  | Cell type               | Amount | Tissue                  | Cell type               | Amount |
|-------------------------|-------------------------|--------|-------------------------|-------------------------|--------|
| Liver                   | Hepatocytes             | ++     | Encephalus              | Neuron                  | ±      |
|                         | Kupffer cells           | ++     | Astrocytes              | +                       |
|                         | EC                      | +      | Glial matrix            | +                       |
| Lung                    | Bronchiolar epithelium  | ++     | Ependyma epithelium     | ++                      |
|                         | Bronchiolar SMC         | ±      | Cerebellum              | ±                       |
|                         | Alveolar epithelium     | +      | Granular cells          | +                       |
|                         | Alveolar capillary EC    | +      | Purkinje cells          | +                       |
|                         | Small artery SMC        | ±      | Glial matrix            | +                       |
| Kidney                  | Glomerulus              |        |                         |                         |
|                         | Capillary loops EC      | ±      | Spinal cord             | ±                       |
|                         | Mesangial cells         |        | Gray matter matrix      | +                       |
|                         | Epithelium of Bowman’s capsule | ± | White matter matrix | +                      |
| Heart                   | Cardiac myocytes        | +      | Skeleton muscle         | Muscle cells            |
|                         | Intestinal cells        | ±      | Brown Fat               | Fat cells               |
|                         | Small artery EC and SMC | +      | White Fat               | Fat cells               |
| Aorta                   | EC/intima               | +      | Adrenal gland           | Zona glomerulosa        |
|                         | Inner media             | +      | Zona fasiculata         | +                       |
|                         | Rest of media           |        | Zona reticularis        | +                       |
|                         | Adventitia              | +      | Medulla                 | +                       |
| Esophagus               | Squamous epithelium     | ++     | Uterus                  | Gland epithelium        |
|                         | Gland epithelium        |        |                         | Interstitial cells      |
| Stomach                 | Absorptive epithelium   | ++     | Gland epithelium        | +                       |
|                         | Gland epithelium        |        | SMC                     | +                       |
| Intestine and colon     | Absorptive epithelium   | +      | Testis                  | Spermatogenec cells     |
|                         | Gland epithelium        |        |                         |                         |
|                         | Goblet cells            | ±      |                         |                         |


* ±, absence; ++, barely seen; ++ to ++++, from very faint to very strong staining; EC, endothelial cells; SMC, smooth muscle cells.

Prdx6 Was Not Expressed in Homozygous Prdx6-targeted Mutant Mice—RT-PCR revealed that Prdx6 mRNA was absent in livers from homozygous mutants, but present in livers from heterozygous mutants and controls after 19, 28, and 38 cycles of PCR following the reverse transcription (Fig. 2A). Western blot analysis revealed that mouse PRDX6 protein was absent from liver extracts of homozygous mutants but was present in nearly equal amounts in liver extracts from wild-type mice and heterozygous mutants (Fig. 2B, upper panel). Similarly, PRDX6 protein was present in macrophages from Prdx6

PRDX6 Has a Broad Tissue Distribution—The sensitivity and specificity of the antibody against PRDX6 used in this study was described recently (28). In our study, the specificity of the antibody to PRDX6 was clearly shown by the fact that the antibody reacted with antigen from wild-type, but not Prdx6-targeted mutant, mice (in both immunohistochemistry and Western blot), i.e. the antibody reacts only with PRDX6 and is specific for PRDX6 (Fig. 2, B and C). The PRDX6 tissue distribution was assessed immunohistochemically. It was expressed in various amounts in every tissue examined: most abundantly in those with a high proportion of epithelium and epithelium-derived cells, such as those of the liver and kidneys (Fig. 3A, Fig. 2C (panels A and E), and Table III), less in endothelial and interstitial cells, and generally very little in smooth muscle cells, heart muscle, and neurons (Table III). Subcellular fractionation showed that it was present exclu-
To determine if PRDX6 protects macrophages against ROS-induced cell death, we compared the effects of incubating Prdx6−/− and Prdx6+/+ macrophages in DMEM containing various concentrations of paraquat, H₂O₂, and t-butyl hydroperoxide. Data were expressed as mean ± S.E. from three experiments, each with triplicate measurements. *, p < 0.05; **, p < 0.01. *Prdx6−/− macrophages versus Prdx6+/+ macrophages, two-tailed Student’s t test.

**Fig. 4. PRDX6 functioned in macrophages.** A, intracellular hydrogen peroxide levels were higher in Prdx6−/− than in Prdx6+/+ macrophages. Peritoneal macrophages from male Prdx6−/− and male littermate Prdx6+/+ mice on 129 backgrounds at 12 weeks of age were cultivated in 96-well plates. After loading the cells with DCFH-DA, they were incubated in DMEM alone (−), or DMEM containing 100 μM of either paraquat, H₂O₂, or t-butyl hydroperoxide (t-BOOH) for 30 min at 37°C. Intracellular fluorescence was detected with excitation at 485 nm and emission at 530 nm. B, peritoneal macrophages from male Prdx6−/− and male littermate Prdx6+/+ mice on 129 backgrounds at 12 weeks of age were cultivated in 96-well plates in DMEM containing either paraquat (400 μM), H₂O₂ (200 μM) or t-butyl hydroperoxide (t-BOOH) (400 μM) for 24 h. Percent of survival cells was determined with trypan blue exclusion assay. C, peritoneal macrophages from male Prdx6−/− and male littermate Prdx6+/+ mice on 129 backgrounds at 12 weeks of age were cultivated in 96-well plates in DMEM containing various concentrations of paraquat for 24 h. Survival rate of the cells was measured with an MTT assay. D, the same as in B except that paraquat was replaced with H₂O₂. E, the same as in B except that paraquat was replaced with t-butyl hydroperoxide. Data were expressed as mean ± S.E. from three experiments, each with triplicate measurements. *, p < 0.05; **, p < 0.01, Prdx6−/− macrophages versus Prdx6+/+ macrophages, two-tailed Student’s t test.

**PRDX6 Functioned as a Peroxidase in Macrophages**—Inactivation of the peroxidase function of mouse PRDX6 protein in the Prdx6-targeted mutant mice was then studied by DCFH-DA assay. DCFH-DA is a dye that reacts with H₂O₂ to generate fluorescent products that remain trapped inside cells. H₂O₂ levels were significantly higher in Prdx6 homozygous mutant macrophages than in control macrophages, whether in the absence or presence of 100 μM of paraquat, H₂O₂, and t-butyl hydroperoxide (a model organic hydroperoxide) (Fig. 4A), implying that the mutants had abnormally low peroxidase activity and that mouse PRDX6 protein was functional in these cells. However, it is noteworthy that DCF fluorescence is not specific for H₂O₂, and other oxidants such as peroxinitrite, O₅₋ (converted to H₂O₂ by SODs in cells), NO (reacts with O₂ to form peroxinitrite) could also oxidize DCFH₂ into DCF. So DCF fluorescence reflects the overall oxidative stress in cells (35). In this regard, PRDX6 might be able to reduce intracellular oxidative stress.

**PRDX6 Protected Macrophages Against Cell Death in Vitro**—To determine if PRDX6 protects macrophages against ROS-induced cell death, we compared the effects of incubating Prdx6−/− and Prdx6+/+ macrophages in DMEM containing various concentrations of paraquat, H₂O₂, and t-butyl hydroperoxide. In trypan blue exclusion assay, significantly more Prdx6−/− macrophages were killed by either paraquat (400 μM), H₂O₂ (200 μM), or t-butyl hydroperoxide (400 μM) (Fig. 4B). In the MTT assay, incubation of the cells with paraquat at 300–800 μM for 24 h killed significantly more Prdx6−/− than Prdx6+/+ macrophages (Fig. 4C). Similar results were found when the cells were exposed to either H₂O₂ or t-butyl hydroperoxide (Fig. 4, D and E). This suggests that PRDX6 protects macrophages from cell death induced by oxidative stress.

**Mice Lacking PRDX6 Developed Normally**—We found no difference in the age- and sex-matched body weights of adult homozygous and heterozygous mutants and of wild type controls, whether on pure 129 or B6;129 mixed backgrounds (data not shown). The average litter size of homozygous 129 background mice was 2.2 pups/litter (mean ± S.D., n = 11), comparable to the average size of 4.5 pups/litter of normal 129 mice (www.informatics.jax.org. Mouse Genome Informatics). The ratio of 1 female to 1.1 male (n = 58) at birth in the progeny from Prdx6−/− parents with 129 background was normal. The percentage of the 8-week-old Prdx6−/− progeny from Prdx6−/− parents with 129 background was 32% (n = 136), slightly higher than the expected 25%, indicating that neither peri- nor postnatal death rates in the mutants were abnormal.
Similar results were obtained for B6;129 mixed background mutants and their controls. The average litter size of homozygous mutants was 6.9 ± 2.6 pups/litter (mean ± S.D., n = 15), comparable to the average litter size of 6.2 ± 0.1 pups/litter (mean ± S.D., n = 280) of B6 mice and larger than the average size of 4.5 pups/litter of normal 129 mice (www.informatics.jax.org. Mouse Genome Informatics). The ratio of female to male progeny from Prdx6+/− parents with B6;129 mixed background was 1:1.4 (n = 103). The percentage of the 5-week-old Prdx6−/− progeny from Prdx6+/− parents with mixed B6;129 background was 29% (n = 136), slightly higher than the expected 25%, indicating that neither peri- nor postnatal death rates in the these mutants were abnormal.

Macroscopically, no abnormalities were found in any of the organs and tissues (including heart, lungs, liver, spleen, kidneys, brain, skeletal muscle, esophagus, stomach, intestine, thymus, adrenal glands, thyroid gland, bladder, uterus, ovary, testis) of mutants and their controls (data not shown). These tissues were then fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned in 4 μm thickness. Using light microscopy, no abnormality in morphology was found in homozygous or heterozygous Prdx6-targeted mutant mice (data not shown).

**Mice Lacking PRDX6 Were Abnormally Susceptible to Paraquat-induced Oxidative Stress**—To determine if PRDX6 could protect mice against excessive oxidative stress in vivo, we injected (intraperitoneal) mutants and controls (on both mixed B6;129 and B6 backgrounds) with paraquat, an established inducer of oxidative stress in vivo (26). Whereas 75% (9/12) of the mixed background mutants died (most within three and 4 days after being injected), only 8% (1/12) of the controls died (Fig. 5A) (p < 0.01). Whereas half (4/8) of the 129 background mutants died within 3 days of being injected, none of the controls died (Fig. 5B) (p < 0.05). Thus, a lack of endogenously expressed PRDX6 rendered mutants susceptible to paraquat-induced toxicity in vivo.

We collected tissues from some of the mice 3 days after they were injected. Macroscopically, surface and interior lung and liver tissues and peritoneal cavities of mutants were much more severely hemorrhaged (implying abnormally high vascular permeability) than were those of controls.

Microscopically, the most severely damaged tissues in mutants were the lungs, kidneys, and liver (Fig. 6). The lungs of mutants had many collapsed alveoli and alveolar ducts, alveolar edema and hemorrhage, alveolar and bronchiolar epithelial necrosis, thromboses in the bronchial veins, and diffused acute pulmonary necroses. Inflammatory cells had not often infiltrated, implying that the necroses were non-infectious (Fig. 6A). The glomeruli of mutants were swollen and the capillaries congested. The epithelial cells of renal tubules were degenerated, and protein casts were common in the tubules, indicating increased capillary permeability (Fig. 6B). The hepatocytes of mutants were abnormally eosinophilic, implying that many of them were degenerating. Vein thrombi were common (Fig. 6C).

In contrast, the pathological changes in the lungs, kidneys, and livers of controls were much less severe (Fig. 6, D–F).

Surprisingly, even without excessive oxidative stress, protein carbonyl content was higher in Prdx6−/− than in Prdx6+/+ mice (Fig. 7A, left two bars). With oxidative stress, the protein carbonyl content increased in both Prdx6−/− and Prdx6+/+ mice but was still higher in Prdx6−/− mice (Fig. 7A, right two bars). However, there was no difference in the liver S-iso-PGF2α content between Prdx6−/− and Prdx6+/+ mice, whether or not they were exposed to paraquat (Fig. 7B). This was similar to previously published reports that the levels of liver F2-isoprostanes in Gpx1-targeted mutant mice injected with either paraquat or diquat are comparable to those in wild-type controls (36, 37).

![Figure 5](http://www.jbc.org/)  **Fig. 5.** Significantly more Prdx6 mutants than controls died from paraquat. Paraquat (injected intraperitoneal, 30 μg/g body weight) killed significantly more: A, male Prdx6+/− mice on a B6;129 mixed background than male Prdx6−/− controls (p < 0.01, n = 12 of each genotype, χ2 test. All mice were at 11 weeks of age when they were injected with paraquat); and B, male Prdx6+/− mice on 129 background than male 129 controls (Prdx6+/−) (p < 0.05, n = 8 of each genotype, χ2 test. All mice were at 13 weeks of age when they were injected with paraquat). Mice were observed for 14 days after injection, and the percentage surviving was calculated. Shown are the results for the first 7 days; no more mice died for the next 2 months.

**Gene Expression of Other Antioxidant Enzymes**—Although we found that mice lacking PRDX6 were more susceptible to paraquat-induced oxidative stress, we did not know whether oxidative stress in the absence of PRDX6 alters the expression of other antioxidant enzymes and changes the body’s total antioxidant ability. Therefore, by using real time PCR, we measured the mRNA expression levels of all major antioxidant enzymes (Prdx1-5, Gpx 1–4, Cat, Sod1-3, Ttn 1 and 2, and Glrx 1 and 2) in Prdx6−/− and Prdx6+/+ mice, some exposed to paraquat and some not. Because liver, lung, and kidney tissue express abundant PRDX6 (Figs. 2C and 3, A and C) and are most obviously damaged by paraquat (Fig. 6), we measured the expression levels of the enzymes in them (Fig. 8). Not surprisingly, Prdx6 mRNA was not expressed in tissues from Prdx6−/− mice. However, none of the other antioxidant enzymes were expressed differently in Prdx6−/− and Prdx6+/+ mice (p > 0.05), whether or not they were exposed to paraquat. Paraquat did induce the expression of some antioxidant enzymes (Gpx3,
Gpx4, Sod3, Txn2, and Glrx1 in the liver; Gpx2 and Sod3 in the lungs) and suppressed the expression of others (Glrx2 in the liver, Sod1 in the lungs, and Gpx2 in the kidney) in both Prdx6−/− and Prdx6+/+ mice. In any case, our results suggested that PRDX6, not the other antioxidant enzymes tested, protected mice against paraquat-induced oxidative stress.

SOD, CAT, and GPX Enzyme Activities in Prdx6-targeted Mutant and Control Mice—In vivo, hydrogen peroxide can be eliminated by CAT, GPXs and the recently found PRDXs, including PRDX6. On the other hand, paraquat radicals can react with O2 to produce O2•− (26), which can then be eliminated by SODs. Therefore, we determined total SOD, CAT, and total GPX enzyme activities in both Prdx6−/− and Prdx6+/+ mice, some exposed to paraquat and some not. As shown in Table IV, there were no differences in the activities of these three enzymes between Prdx6−/− and Prdx6+/+ mice, suggesting that PRDX6 per se is involved in protecting mice against paraquat-induced oxidative stress, and its function is non-redundant to SODs, CAT, and GPXs.

DISCUSSION

ROS-induced oxidative stress has been implicated in atherosclerosis, diabetes, neurodegeneration, cancer, and other diseases (1). On the other hand, ligand-induced ROS species, especially H2O2, can act as intracellular messenger, and ROS are necessary for maintaining the functions of signaling molecules (such as Src kinases, Janus kinases, MAPKs, NF-xB, AP-1, p53, c-Myb) that regulate physiological and pathophysiological cellular functions (24, 38). Therefore, antioxidants could both protect the body from excessive ROS-induced damage and regulate and fine-tune physiological processes. Both the harmful and beneficial aspects of ROS are controlled by antioxidants. One such antioxidant that could potentially be very important is the relatively novel and little understood PRDX6 (3). Due to its unique one cysteine structure, it is likely playing a different role in the cell than its 2-Cys family members.

PRDX6, a 1-Cys PRDX, exists in the cytosol together with PRDX1, -2, and -4 (3, 39, 40) (PRDX3 exists in mitochondria, Refs. 3 and 39; PRDX6 localizes in mitochondria and peroxisomes, Ref. 6) (Table I). The expression of PRDX6 protein and Prdx6 mRNA had not been carefully studied before. We found that PRDX6 was widely expressed in almost all the tissues examined, suggesting that it had some basic functions for all these tissues. It is abundantly expressed in hepatocytes, implying that it counteracts circulating oxidants, consistent with the function of liver as a detoxifying organ. It is highly expressed in the lungs and gastrointestinal epithelia, suggesting that it is the first line of defense against toxic oxidants in air and food. Its high expression in immature spermatogenic cells implies that it protects them against oxidant-induced mutation and ensures that they mature normally.

It was unknown whether the function of 1-Cys PRDX overlapped with the other three cytosolic PRDXs, and whether one or more of these four PRDXs were redundant, especially in the
FIG. 8. There were no differences in the gene expressions of major antioxidant enzymes except for PRDX6 between Prdx6−/− and Prdx6+/+ mice. Male Prdx6-targeted mutant mice on 129 background (Prdx6−/−) and male wild-type inbred 129 controls (Prdx6+/+) at 14 weeks of age were injected with paraquat (intraperitoneal, 25 μg/g body weight). Forty-eight hours after injection, mice were sacrificed, perfused with phosphate-buffered saline containing 2.5 mM EDTA, and liver, lung, and kidney tissues were collected and stored in RNA later. Total RNA was extracted and reverse-transcribed into cDNA, and real time PCR was performed. mRNA expression of each antioxidant enzyme was adjusted to the mRNA copies of β-actin. Data are expressed as mean ± S.E. of the samples from four mice in each group. Gene expressions of all but the PRDX6 protein were not significantly (p > 0.05) different between Prdx6−/− and Prdx6+/+ mice, whether or not they were injected with paraquat.

Preservation of other antioxidant enzymes such as CAT, GPXs and SODs (41, 42). This question could only be answered by experiments using either targeted or natural mutants. It was also unknown whether 1-Cys PRDX functioned in vivo. Transgenic animals and either targeted or natural mutants could potentially answer this question. Thus, we generated Prdx6-targeted mutant mice, the first and thus far the only reported peroxiredoxin-targeted mutant mice. The disruption of the Prdx6 gene was verified by PCR and Southern blots. The mutant mice did not express Prdx6 mRNA and protein, as demonstrated by RT-PCR, Western blots and immunohistochemical analysis. We compared the expression of mouse PRDX6 and its ability to curtail severe oxidative stress in Prdx6-targeted mutant mice and their controls induced by paraquat, which is widely used as a model compound to produce damaging ROS in vivo (1). Our results clearly showed that endogenously produced mouse PRDX6 functioned in vivo as an antioxidant enzyme, and that its function was not redundant to other PRDXs and antioxidant enzymes, at least under conditions of excessive oxidative stress. We concluded this because: 1) ROS levels in macrophages from Prdx6−/− mutants were higher than those from wild-type mice, 2) a lack of PRDX6 in Prdx6−/− macrophages rendered them more susceptible than controls to ROS and ROS-inducing agents such as paraquat, H2O2, and t-butyl hydroperoxide, 3) PRDX6 protected mice against paraquat-induced tissue protein oxidation and morphological damage, and death, 4) the gene expression of Prdx1-5, Gpx1-4, Cat, Sod1-3, Txn1-2, and Glrx1-2 was not different between Prdx6−/− and
control mice, suggesting that the PRDX6 antioxidant properties in mice are independent of other antioxidant enzymes. We know that non-1-Cys PRDXs can use thioredoxins as physiological electron donors (4, 7, 40); we do not know what 1-Cys PRDXs (including mouse PRDX6) use, though in vitro studies show that cyclophilin (15) can mediate the reduction of H2O2 by recombimant 1-Cys PRDX. Our findings that a lack of PRDX6 leads to high ROS levels in Prdx6−/− macrophages, makes Prdx6−/− macrophages susceptible to ROS-induced cell death, and renders Prdx6−/− mice susceptible to PQ3+ toxicity, indicate that 1-Cys PRDX has antioxidant activity in vivo, though its physiological electron donor(s) is(are) unknown.

Animal models play a unique role in studying gene functions because their genes can be manipulated. So far, gene targeting seems to be the best way: 1) to conclusively determine whether the function of a gene is redundant and 2) to precisely determine the gene role in vivo. Because reactive oxygen species have a variety of functions and are involved in so many diseases, Prdx6-targeted mutant mice can be used to study signal transduction processes and clinically important diseases such as atherosclerosis, diabetes, and neurodegenerative diseases.

Acknowledgment—We thank Ray Lambert for assistance in manuscript preparation.

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Mice with Targeted Mutation of Peroxiredoxin 6 Develop Normally but Are Susceptible to Oxidative Stress
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J. Biol. Chem. 2003, 278:25179-25190.
doi: 10.1074/jbc.M302706200 originally published online May 5, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M302706200

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