A Novel Glutathione Peroxidase in Bovine Eye

SEQUENCE ANALYSIS, mRNA LEVEL, AND TRANSLATION

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Bovine ciliary body contains a selenium-independent glutathione peroxidase (GPX) with a molecular mass of about 100 kDa that is composed of four identical subunits and exhibits no glutathione S-transferase activity. In this study, we isolated cDNA clones and determined the nucleotide sequence to deduce the primary structure of the enzyme. The cDNA contained 672 base pairs encoding a polypeptide with an estimated molecular mass of 25,064 Da. Translation of bovine ciliary mRNA produced a protein which was immunologically indistinguishable from GPX and showed high enzyme activity. The encoded amino acid sequence of the protein was 95% identical with that of a human keratinocyte gene product expressed in response to keratinocyte growth factor. It also showed sequence identity to bacterial alkyl hydroperoxide reductases and thiol specific antioxidant enzymes. GPX mRNA level was highest in the ciliary body, followed by the retina and iris. In various rat organs, the level of GPX mRNA was highest in the lung, followed by the muscle, liver, eye, heart, testis, thymus, kidney, and spleen. A very low level of mRNA was detected in the brain. Enzyme-linked immunosorbent assay with an antibody raised against the NH2-terminal sequence of GPX detected GPX protein in all rat tissues examined.

The liver is the major site for drug metabolism and detoxification. Growing evidence indicates that these activities are also present in non-hepatic tissues including the eye (1, 2). The incomplete reduction of oxygen by a variety of electron transfer systems gives rise to reactive oxygen species (O2-, H2O2, and HO·) that can cause cellular damage. Drug-metabolizing enzyme systems are such systems. In the eye, vascularized uveal tissues such as ciliary body and iris possess the highest drug-metabolizing activities for detoxification of blood nutrients (3, 4). For protection against the detrimental effects of the toxic oxygen species, the eye tissues, like other mammalian tissues, have both nonenzymatic (ascorbic acid, β-carotene, glutathione, α-tocopherol, etc.) and enzymatic (superoxide dismutase, catalase, and glutathione peroxidase) antioxidant systems. The two-layered epithelium of ciliary body demonstrates fairly high levels of the antioxidant enzyme activities, especially in the nonpigmented epithelium (5). One of the important physiological functions of the ciliary body epithelium is the production of aqueous humor by metabolic ultrafiltration of blood plasma. The aqueous humor provides nutrients to nonvascularized lens and cornea. Destruction of the blood-aqueous barrier in the ciliary epithelium by reactive oxygen species results in the secretion of the aqueous humor containing blood hydrolytic enzymes and other undesirable substances into the anterior chamber of the eye, and may damage the lens and cornea and cause the opacification of these tissues. Protection of the ciliary epithelium from oxidants, therefore, is very important to maintain unimpaired vision.

We have previously investigated the capacity of bovine ciliary body to detoxify peroxides (H2O2, t-butylhydroperoxide) and found that glutathione and its redox cycle play a pivotal role in peroxide reduction (6, 7). Subsequent studies have shown that the glutathione peroxidase activity of this tissue is mainly accounted for by a novel enzyme, which contains no selenium and shows no glutathione S-transferase activity, and hence is different from any known type of glutathione peroxidase (8). There are at least four isoforms of glutathione peroxidase in mammals, all of which are selenium-dependent enzymes: cellular glutathione peroxidase 1 (EC 1.11.1.9) (9), gastrointestinal glutathione peroxidase 2 (10), extracellular glutathione peroxidase 3 (11), and phospholipid hydroperoxide glutathione peroxidase 4 (12). In addition, a glutathione S-transferase isozyme exhibits glutathione peroxidase activity (13). The amino-terminal 27 residue-sequence of the novel enzyme we have purified showed little identity to the amino-terminal sequence of selenium-dependent glutathione peroxidases and glutathione S-transferases.

Frank et al. (14) reported recently that a gene induced in cultured human keratinocytes in response to keratinocyte growth factor encodes a protein that shows a high degree of identity to the amino-terminal sequence of bovine ciliary body glutathione peroxidase we had purified. The finding prompted us to characterize this enzyme further. In this study, we have isolated cDNA clones and analyzed the nucleotide sequence to deduce the primary structure of the enzyme. Using RNA probes prepared from the cDNA clones, mRNA levels in different tissues of bovine eye were determined by RNase protection assay. In addition, enzymatic activities of translation products prepared from bovine mRNA and cDNA clones were investigated. For the sake of convenience, the eye enzyme is designated GPX.1

MATERIALS AND METHODS

Sequencing and Cloning of cDNA—Ciliary epithelium was collected from fresh bovine eyes and immediately frozen in liquid nitrogen.

1 The abbreviations used are: GPX, glutathione peroxidase; ELISA, enzyme-linked immunosorbent assay; PAGE, polyacrylamide gel electrophoresis; RACE, rapid amplification of cDNA ends; PCR, polymerase chain reaction; RT, reverse transcriptase.
Poly(A) RNA was isolated with the Fast Track mRNA isolation kit (InVitrogen) as described previously (15) and analyzed by amplification via reverse transcription PCR using the Superscript preamplification system for the first strand cDNA synthesis (Life Technologies, Inc.) according to the manufacturer's instruction. Sense primer (5'-TCGTA-TCTAGATCGCCATGCCCAGGAGGTCTGCTTC-3') and antisense primer (5'-TGTCACCCGGGAGGCTGGGGTGTAGCGGAGGTA-3') corresponding to the 3' and 5' regions of the cDNA encoding human keratinocyte growth factor-stimulated gene product (12) were used to amplify the cDNA. The PCR product was analyzed by 1% agarose gel electrophoresis.

To determine the sequences corresponding to the 3' and 5' regions of bovine cDNA, 3' and 5' RACE system (Life Technologies, Inc.) was used essentially in the same manner as that described previously (15). Briefly, PCR amplification of the 3' end cDNA was performed using gene-specific primers GSP1 (310–327) and GSP2 (331–348), and universal adapter primer provided with 3' RACE system (the numbering of nucleotide bases of primers corresponds to that of bovine GPX cDNA in Fig. 1). In the 5' RACE, the first strand cDNA was primed with antisense primer GSP1 (327–310). Homopolymeric deoxythymidylated cDNA was amplified by PCR using nested antisense GSP2 (306–292) and sense anchor primer provided with 5' RACE system. RACE products were analyzed by 1% agarose gel electrophoresis, purified by Glass Max spin cartridge system (Life Technologies, Inc.), and sequenced by the dideoxynucleotide chain termination method (16) with sense GSP3 primer (580–591) and antisense GSP3 primer (99–85) using fmol DNA sequencing system (Promega, WI). The BLAST network analysis of the sequence data base at the National Center for Biotechnology Information (NCBI; National Institutes of Health, Bethesda, MD) was used for a search of homologous sequences.

For cloning, the full-length cDNA was prepared by PCR using primers encoding NH2- and COOH-terminal regions of GPX and including BamHI and EcoRI sites. The PCR product was purified, ligated to PCR-Script Amp SK(+) cloning vector (Stratagene, La Jolla, CA) and used to transform the XL1-Blue MRF, Escherichia coli.

RNase Protection Assay—32P-Labeled GPX cRNA was synthesized using a RNA transcription kit (Stratagene) from the plasmid PCR Script Amp SK(+) cloning vector (Stratagene, La Jolla, CA) and used to transform the XL1-Blue MRF, Escherichia coli.

**Fig. 1. Nucleotide sequence of cDNA encoding GPX and deduced amino acid sequence.** Base numbers of the coding region are indicated in small type on the right margin. Amino acid residue numbers are indicated in large type in the center and on both margins.

| Coding Region: 672 bp | Estimated molecular mass for 224 residue polypeptide: 25,064 Da |
|---------------------|---------------------------------------------------------------|
| 5'-noncoding region: 66 bp | |
| 3'-noncoding region: 177 bp | |
FIG. 2. RNase protection assay of mRNA from bovine eye tissues. One μg of mRNA from different eye tissues was analyzed by RNase protection assay as described under “Materials and Methods.” Lane 1, RNA marker (Ambion): 100–500 bases from bottom to top (arrows); lane 2, full-length GPX mRNA (464 bases); lane 3, probe hybridized with yeast RNA, digested with RNase; lanes 4–6, protected fragment from eye tissues (lane 4, ciliary body; lane 5, retina; lane 6, iris).

FIG. 3. Ethidium bromide staining of RT-PCR products. Lane 1, 100-bp DNA ladder (Life Technologies, Inc.); lane 2, iris; lane 3, ciliary body; lane 4, retina. GPX PCR products have 672 bp. Lanes 5 (iris), 6 (ciliary body), and 7 (retina) represent 320-bp β-actin PCR products.

FIG. 4. Immunoblot analysis of GPX in bovine ciliary body. Lane 1, fusion protein; lane 2, in vitro translated product; lane 3, ciliary body partially purified protein. Samples were electrophoresed on 10% SDS-polyacrylamide gel, transferred to nitrocellulose membrane, and treated with anti-GPX peptide antibody. Prestained molecular size standards (low range) (Bio-Rad) are shown on the right.

FIG. 5. RNA blot analysis of GPX mRNA levels in bovine eye tissues. mRNA was isolated from bovine eye tissues, electrophoresed on agarose/formaldehyde gel, transferred to nitrocellulose membrane, and hybridized to 32P-labeled cDNA GPX probe. Panel A, hybridization of GPX mRNA from eye tissues with 32P-labeled cDNA probe. Lane 1, ciliary body, lane 2, iris; lane 3, retina. Panel B, the blot was stripped and hybridized to β-actin as control.

washed in 30 mM sodium chloride, 3 mM sodium citrate, and 0.1% sodium dodecyl sulfate solution at 55 °C and autoradiographed with Kodak XAR film at -70 °C for 16–24 h or stored on phosphor screen for 2–3 h. As a control to normalize for differences in the loading and transfer of RNA, the blot was stripped off and reprobed with labeled cDNA for β-actin. Data were quantitated with an Imaging Densitometer.

RT-PCR of RNA from Various Rat Organs and Southern Blot Analysis—mRNA was extracted from the brain, eye, thymus, lung, liver, muscle, kidney, heart, spleen, and testis of male Lewis rats (5 weeks old, Harlan Sprague-Dawley, Indianapolis, IN) using a Fast Track mRNA isolation kit (InVitrogen). The reverse transcription of mRNA (1 μg) from each sample was carried out by using the Superscript preamplification system (Life Technologies, Inc.) and oligo(dT) primer. PCR primers, sense (1–35) and antisense (672–637), which are specific to 5′ and 3′ ends of bovine ciliary GPX cDNA, were used to amplify the cDNA. However, the amplification products (except for lung and muscle) at 30 cycles could not be visualized on electrophoresed 1% agarose gel stained with ethidium bromide. To detect the amplified

degraded unhybridized probe. The hybridized RNA was separated on a 5% polyacrylamide gel containing 8 μm urea and visualized by autoradiography. The amount of hybridized product was quantitated with an Imaging Densitometer (Bio-Rad).

Partial Sequencing of PCR Products from Different Eye Tissues—The Superscript preamplification system (Life Technologies, Inc.) was used to synthesize the first strand cDNA from 1 μg of mRNA from bovine ciliary epithelium, iris, and retina isolated with the Fast Track mRNA isolation kit (InVitrogen). The PCR mixture (50 μl) contained synthesis buffer (20 m Tris-HCl (pH 8.4), 50 mM KCl, 25 mM MgCl2, 100 μg/ml bovine serum albumin), 100 mM dNTPs, 2.5 units of Taq DNA polymerase, and 20 μmol each of sense and antisense primers. The primers used were the same as those used for cloning. Amplified PCR products were analyzed by 1% agarose gel electrophoresis, purified and sequenced using sense primer (580–591) and antisense primer (99–85).

In Vitro Translation of mRNA Encoding GPX—The TNT® T7 quick coupled transcription/translation system (Promega, Madison, WI) was used for transcription and translation of the GPX gene cloned downstream from T7 RNA polymerase promoter. Approximately 1 μg/2 μl of linear plasmid DNA digested with BsrHII was added to an aliquot of 20 μl of TNT® T7 Quick Master mix (containing TNT® lysate with energy generating system, γNTPs, T7 RNA polymerase, magnesium acetate, potassium chloride, and RNasin® ribonuclease inhibitor, 1 μl of 1 mM methionine and 2 μl of nuclease-free water), and the mixture was incubated for 90 min at 30 °C. The synthesized proteins were analyzed by 10% SDS-polyacrylamide gel electrophoresis and detected by immunoblotting with anti-GPX peptide antibody and horseradish peroxidase-conjugated secondary antibody (17). For Ouchterlony double immunodiffusion assay, agarose immunodiffusion tablets (Bio-Rad) were dissolved in water, boiled for 2–3 min to obtain 1% agarose gel, and cast on glass plates (10 × 10 cm). Immunodiffusion was performed by placing antibody in the center well and synthesized proteins in the surrounding wells at 4 °C for 6–8 h in 100% humidity. Anti-GPX peptide antibody was raised by immunizing rabbits with a 16-amino acid peptide (acetyl-6EAKKLFFPKGVPFTKL-6amide) taken from the carboxyl-terminal region (residues 196–211 in Fig. 1) and purified on a peptide-conjugated Sephadex gel column. The cytoeine residue at the end of the peptide is not in the carboxyl-terminal sequence of GPX. It was included so that the SH group could facilitate conjugation of the peptide to keyhole limpet hemocyanin, which served as a carrier.

Northern Blot Hybridization—mRNA from bovine ciliary epithelium, iris, and retina was electrophoresed through 1.2% formaldehyde-agarose gel and transferred to a nylon membrane. The membrane was hybridized at 65 °C for 2 h with 32P-labeled bovine ciliary cDNA probe using a random-prime labeling kit (Life Technologies, Inc.) in Quick hybridization solution (Stratagene). The hybridized membrane was
PCR product, Southern blot hybridization was performed. In brief, after electrophoresis the PCR products were denatured with 0.5 N sodium hydroxide and 1.5 M sodium chloride for 30 min, followed by neutralization with 1 M Tris (pH 7.4) and 1.5 M sodium chloride for 45 min at room temperature and transferred onto a nylon membrane by capillary action. Hybridization of the membrane was carried out exactly as described for Northern blot. Following hybridization and washing, the membrane was autoradiographed with Kodak XAR film at 270 °C for 48–72 h and relative blot densities were determined.

Immunoblotting of GPX Proteins from Various Sources—Freshly dissected bovine ciliary bodies and rat brain, kidney, liver, lung, heart, eyes, spleen, testis, thymus, and muscle were homogenized in cold Tris buffer as described previously (6). Supernatant fractions from the homogenates were analyzed by 10% SDS-PAGE and detected by immunoblotting with anti-GPX peptide antibody as described for in vitro translation. Ouchterlony double immunodiffusion was performed similarly.

ELISA of GPX and Protein Determination in Various Rat Organs—Immunolabeling of GPX in rat brain, kidney, liver, lung, heart, eyes, spleen, testis, thymus, and muscle with polyclonal peptide antibodies was performed by ELISA (18) in which ELISA plate wells were coated with 100 μl of supernatant per well. Non-specific binding sites were blocked by treating ELISA wells with phosphate-buffered saline/Tween containing 5% bovine serum albumin. Subsequently 1:1000 diluted antibodies in the same buffer were added, and the wells were incubated for 2 h at room temperature. Antibody binding was detected with horseradish peroxidase-conjugated goat anti-rabbit IgG and with o-phenylenediamine and H2O2 as substrates; the extent of reaction was determined at 490 nm in an ELISA plate reader (Bio-Rad).
mid devoid of the GPX insert were stimulated with isopropyl-thio-β-D-galactopyranoside. The cells were disrupted, and the GPX activity of soluble proteins was similarly determined.

**RESULTS**

**Cloning and Sequencing of Bovine GPX cDNA**—The cDNA amplified by PCR, ligated to PCR-Script SK+ (+) plasmid, and cloned in E. coli cells contained 5′ and 3′ sequences derived from 35-mer primers used. Sequences in these regions were determined by means of 5′ and 3′ RACE systems. The complete nucleotide sequence of cDNA encoding bovine ciliary body GPX and a deduced amino acid sequence are shown in Fig. 1. The 915-bp cDNA contained a 672-bp open reading frame with 66 bp in the 5′ non-coding region and 177 bp in the 3′ non-coding region. Translation of the open reading frame from the initiation codon at position 67 would encode a polypeptide of 224 amino acids with a molecular mass of 25,064 daltons.

**RNase Protection Assay**—One µg each of poly(A)+ RNA extracted from iris, ciliary body, and retina and for control 1 µg of yeast RNA (provided with RPA kit) was hybridized with a 32P-labeled 478-base antisense riboprobe and treated with RNase. The results are shown in Fig. 2. Messenger RNA for GPX was expressed by all eye tissues examined, most prominently by the ciliary body, followed by the retina and iris. The relative intensities (% volume) of hybridized bands determined by the imaging densitometer were 3.38, 80.44, and 16.88 for the iris, ciliary body, and retina, respectively.

**Partial Sequencing of PCR Products**—PCR products with an identical mass (672 bp) corresponding to the open reading frame of cDNA were obtained from Poly(A) RNA extracted from bovine iris, ciliary body and retina (Fig. 3). Partial nucleotide sequencing of the PCR products in the 5′ region (64 bases) and 3′ region (71 bases) confirmed that they were identical with the 5′ and 3′ sequences of ciliary body GPX cDNA.

**Synthesis of GPX Protein from Bovine Ciliary Body mRNA**—The protein produced by translation of bovine ciliary body mRNA gave two bands of molecular masses of about 32 and 31 kDa by SDS-PAGE (lane 2 in Fig. 4). Both bands reacted with anti-GPX peptide antibody. The fusion protein had a molecular mass of about 33 kDa. Purified bovine GPX gave a molecular mass of about 29 kDa by high performance liquid chromatography and SDS-PAGE in the previous study (5). The reason for the formation of two translation products is not known. Bands with lower molecular masses observed in all lanes were probably breakdown products.

**Northern Blot Hybridization**—Northern blot analysis was performed with 1 µg of mRNA from bovine ciliary epithelium, iris, and retina. A marked expression of GPX transcript was observed in the ciliary epithelium, retina, and iris (Fig. 5). However, we detected 3.38- and 1.47-kilobase messages in all three mRNA samples. Quantitative data from the imaging densitometer revealed the highest level of mRNA in the ciliary epithelium (92.08%), followed by the retina (23.38), and comparatively low expression in the iris (0.36). We do not know at present why two mRNA species were detected.

**RT-PCR and Southern Blot Analysis of Various Rat Organs**—The level of mRNA encoding GPX gene in various rat organs was determined by RT-PCR and Southern blot analysis. One µg of total mRNA from different organs was used for RT-PCR for comparison. The RT-PCR products were electrophoresed and transferred to a nylon membrane are shown in Fig. 6. Increasing levels of GPX mRNA were observed in the lung, followed by the muscle, liver, eye, heart, testis, thymus, kidney, and spleen (Fig. 6). The very low level of mRNA detected in the brain was arbitrarily taken as unity for comparison.

**Immunoblotting of GPX**—Immunoblot analysis with anti-GPX peptide antibody of the supernatant from homogenates of...
bovine ciliary body and rat organs (lung, muscle, liver, eye, testis, heart, kidney, thymus, spleen, brain) resulted in the detection of a specific band with a mass of about 32 kDa (Fig. 7). The same mobility of the immunoreactive band supported the identity of GPX protein from different sources. Various rat organs showed a number of higher or lower molecular weight bands, probably representing aggregates and breakdown products. Ouchterlony double immunodiffusion of GPX protein from various sources showed a continuous precipitin line (Fig. 8).

ELISA and Protein Determination—The data in Fig. 9A demonstrated that the anti-GPX peptide antibodies recognized the antigenic epitopes in soluble proteins prepared from different sources. Ouchterlony double immunodiffusion of GPX protein from various sources showed a continuous precipitin line (Fig. 8).

**Fig. 11.** Comparison of amino acid sequence between bovine GPX and human keratinocyte protein (KP) and antioxidant proteins. TSA, thiol-specific antioxidant (Onchocerca volvulus) U31052; AH-R (Torlula), alkylhydroperoxidase-rehydrin (Torlula rulalis) U40818; AH-R (Synecho), alkylhydroperoxidase-rehydrin (Synechocytis) D90905. The numbers are accession numbers from BLAST search. The rehydrin sequences represent alkylhydroperoxidase C/thiol-specific antioxidant sequences present in the moss proteins. Identical residues are indicated with dashes and differences with one-letter symbols. The missing residues are shown with asterisks. Residue numbers are given above bovine GPX sequence.
rat tissues; the amount of GPX protein determined by ELISA was highest in the testis and spleen, followed by lung, kidney, eye, brain, thymus, muscle, liver, and heart. Protein content in different rat tissue samples used for ELISA is shown in Fig. 9B.

**GPX Activity**—The GPX activities of *in vitro* translated protein and fusion protein expressed in *E. coli* are shown in Fig. 10. Total GPX activity of translated product was significantly higher than that of the protein expressed in bacterial cells. The difference in total activity between *in vitro* translated GPX and the fusion protein was probably attributed to different purities of samples. Preincubation of both enzyme preparations with anti-GPX peptide antibody (1:10) for 5 min resulted in 50–60% inhibition of their activities. Cellular extracts from *E. coli* cells transformed with blank vectors did not show appreciable GPX activity under the assay condition (not shown).

**DISCUSSION**

Nucleotide sequence analysis indicated that the GPX cDNA contains a single open reading frame, which encodes a polypeptide of 224 amino acids with an estimated molecular mass of 25,064 Da. The NH₂-terminus of GPX purified from bovine eye lacks the methionine residue (6), indicating posttranslational modifications of the enzyme. GPX shows little sequence identity to selenium-dependent glutathione peroxidases and glutathione S-transferases (22–26). The sequence of bovine GPX is 95% identical to that of a protein expressed by human keratinocytes in response to keratinocyte growth factor (12) (Fig. 11). Reactive sulfur- and oxygen-metabolizing enzymes, which are termed thiol-specific antioxidant enzymes and alkyl hydroperoxide reductases, respectively, and constitute a molecular family, have been characterized from a number of microorganisms. Antioxidant sequences are also found in proteins of higher organisms such as rehydrins. Bovine GPX shows a significant sequence identity to the antioxidant proteins of the family (Fig. 11). GPX also shows significant identity to rat heme-binding 23-kDa protein (BLAST accession no. D30035) and mouse thioredoxin peroxidase (BLAST accession no. U51679). Chao et al. (27) compared more than 23 proteins belonging to this family and constructed a consensus sequence. This family of enzymes appears to be subdivided on the basis of the number of conserved cysteine residues. The sequence containing the first cysteine from the NH₂-terminus is PVC in the 1-cysteine members and FVCP in the 2-cysteine members (27). GPX possesses a single cysteine residue in the PVC sequence and belongs to the 1-cysteine group. The conserved VC sequence could be important for the catalytic activity of this family of enzymes. A reaction mechanism involving cysteine SH group(s) was proposed for the active center of antioxidant enzymes (28, 29). The sequences of several bacterial antioxidant enzymes possessing thioredoxin-linked thiol peroxidase activity were recently compared (30). Interestingly, the sequence PVC or GVC appears to be important for antioxidant activities.

Superoxide dismutase, catalase, and glutathione peroxidase are well documented antioxidant enzymes in mammals. However, microorganisms often lack some or all of the antioxidant enzymes. Aerotolerant parasitic protozoa, for example, are deficient in catalase and glutathione peroxidase, although glutathione and NADPH-dependent glutathione reductase are present (31). Glutathione disulfide reduction in these organisms depends upon a thiol-containing cofactor (e.g. trypanothione). Therefore, trypanothione peroxidase is equivalent to glutathione peroxidase in mammals. These findings suggest that diverse antioxidant systems similar to the glutathione peroxidase-glutathione reductase system exist in nature. In view of the sequence similarity of GPX to alkyl hydroperoxide reductases and thiol-specific antioxidant enzymes, it seems likely that GPX we characterized in this work is an evolutionary derivative of the family of antioxidant enzymes that detoxify reactive oxygen and sulfur species.

Selenium-dependent glutathione peroxidases are believed to be critical antioxidant enzymes for the detoxification of hydrogen peroxide and organic hydroperoxides. For example, nutritional studies demonstrated that hyperoxia-induced pulmonary injury is exacerbated in selenium-deficient rats (32). Recently, a mouse mutant deficient in glutathione peroxidase 1, the major selenium-dependent isoform ubiquitously present in all types of mammalian cells, was produced (33). Surprisingly, the knockout mice grew normally, remained healthy and fertile, and showed no increased lung injury to hyperoxia compared with normal mice. Glutathione peroxidase is considered to be essential for ocular lens transparency. The knockout mice, however, showed no sign of opacity development in the lens (34). Since GPX expression in the lung is pronounced, it is of interest to produce a mouse mutant in which GPX is deficient and investigate whether the mutant is highly susceptible to hyperoxia-induced pulmonary injury.

The GPX gene in cultured human keratinocytes was activated by keratinocyte growth factor (i.e. fibroblast growth factor 7) (12). GPX may have a protective role during the skin wound healing process against reactive-oxygen species, which are mediators of tissue injury (12). In the eye, the ciliary body and iris are severely damaged in inflammatory conditions but are regenerated to the original architecture within a few weeks after inflammation (35, 36). GPX may play an important role in the detoxification of reactive oxygen species generated in the inflammatory condition and in facilitating the repair process.

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