Serial Review

Physiological Relevance of Antioxid/Redox Genes; Learning from Genetically Modified Animals
Guest Editor: Junichi Fujii

Unveiling the roles of the glutathione redox system *in vivo* by analyzing genetically modified mice

Junichi Fujii,[1,2,3,*] Jun-itsu Ito,[1,2,3] Xuhong Zhang[1,2,3] and Toshihiro Kurahashi[1,2,3]

[1]Department of Biochemistry and Molecular Biology, Graduate School of Medical Science, Yamagata University, and [2]Respiratory and Cardiovascular Diseases Research Center, Research Institute for Advanced Molecular Epidemiology, Yamagata University, 2-2-2 Iidanishi, Yamagata 990-9585, Japan
[3]Global COE Program for Medical Sciences, Japan Society for the Promotion of Science, Japan

(Received 5 November, 2010; Accepted 31 December, 2010; Published online 3 June, 2011)

Redox status affects various cellular activities, such as proliferation, differentiation, and death. Recent studies suggest pivotal roles of reactive oxygen species not only in pathogenesis under oxidative insult but also in intracellular signal transduction. Glutathione is present in several millimolar concentrations in the cytoplasm and has multiple roles in the regulation of cellular homeostasis. Two enzymes, γ-glutamylcysteine synthetase and glutathione synthetase, constitute the *de novo* synthesis machinery, while glutathione reductase is involved in the recycling of oxidized glutathione. Multidrug resistant proteins and some other transporters are responsible for exporting oxidized glutathione, glutathione conjugates, and S-nitrosoglutathione. In addition to antioxidation, glutathione is more positively involved in cellular activity via its sulfhydryl moiety of a molecule. Animals in which genes responsible for glutathione metabolism are genetically modified can be used as beneficial and reliable models to elucidate roles of glutathione *in vivo*. This review article overviews recent progress in works related to genetically modified rodents and advances in the elucidation of glutathione-mediated reactions.

***Key Words*: glutathione, redox system, knockout mouse, transgenic mouse**

Antioxidative and redox systems constitute defense mechanisms against the oxidation accompanying vital activity and diseased conditions, and their dysfunction causes oxidative stress that exacerbates various injuries. Glutathione is the most abundant nonprotein thiol in cells and plays pleiotropic roles among many low molecular weight antioxidants and redox molecules.[1] Antioxidative functions of glutathione are expressed by either direct interaction with reactive oxygen species (ROS) or the donation of electrons to other redox systems, such as glutathione peroxidase (GPX) and glutaredoxin (Grx).[2] In addition to antioxidation and electron donation, glutathione is required for maintaining homeostasis in animals, such as detoxification, by forming conjugation with toxicants and suppression of apoptosis.[3,4]

Glutathione is present in either the reduced form (GSH) or an oxidized form (GSSG) where two molecules are linked by a disulfide bond. The redox balance of glutathione affects cellular homeostasis. Glutathione is also required for ROS-mediated intracellular signal transduction by maintaining redox potential within cells, because reactive protein thiols that can exist only under a reducing environment are general acceptors of the ROS signal.[5] Levels of glutathione are maintained by *de novo* synthesis from constituent amino acids, Glu, Cys and Gly, and by the recycling of GSSG (Fig. 1). *De novo* synthesis of glutathione is catalyzed by two enzymes, γ-glutamylcysteine synthetase (γ-GCS) and glutathione synthetase (GSS).[1] Oxidized glutathione is either recycled by glutathione reductase (GSR) using NADPH provided largely from the pentose phosphate pathway (PPP) or exported by multidrug resistance protein (MRP).[6] Glutathione conjugates of xenobiotic compounds and S-nitrosoglutathione (GSNO) are also exported out of the cells and ultimately excreted by the kidney. Transnitrosylation from GSNO in blood plasma is involved in vasodilation and cardioprotection.[7]

Knowledge of the roles of glutathione in living animals has been limited due to the difficulty in handling gene expression and, hence, the metabolic process. On the other hand, *in vitro* studies face limitations in understanding the physiologic functions of glutathione because most experiments are performed under a culture with atmospheric oxygen (~21%), which is about one order higher than *in vivo* oxygen conditions (2–5%). Oxidative stress occurs chronically in cells cultured under atmospheric oxygen and, hence, may be a cause for inconsistency from *in vivo* studies.

Advanced technology in establishing genetically modified animals enabled us to elucidate the roles of individual genes in the body. This manuscript is an overview of the progress made thus far in understanding glutathione homeostasis and the *in vivo* roles of the genes involved in glutathione metabolism.

Pleiotropic Functions of Glutathione

Glutathione was discovered more than 100 years ago, but researchers continue to experiment with this tripeptidyl molecule. While intracellular cysteine concentrations are kept low (several μM) due to a cellular toxicity that is caused by the interaction of

*To whom correspondence should be addressed.
E-mail: jfujii@med.id.yamagata-u.ac.jp

doi: 10.3164/jcbn.10-138SR

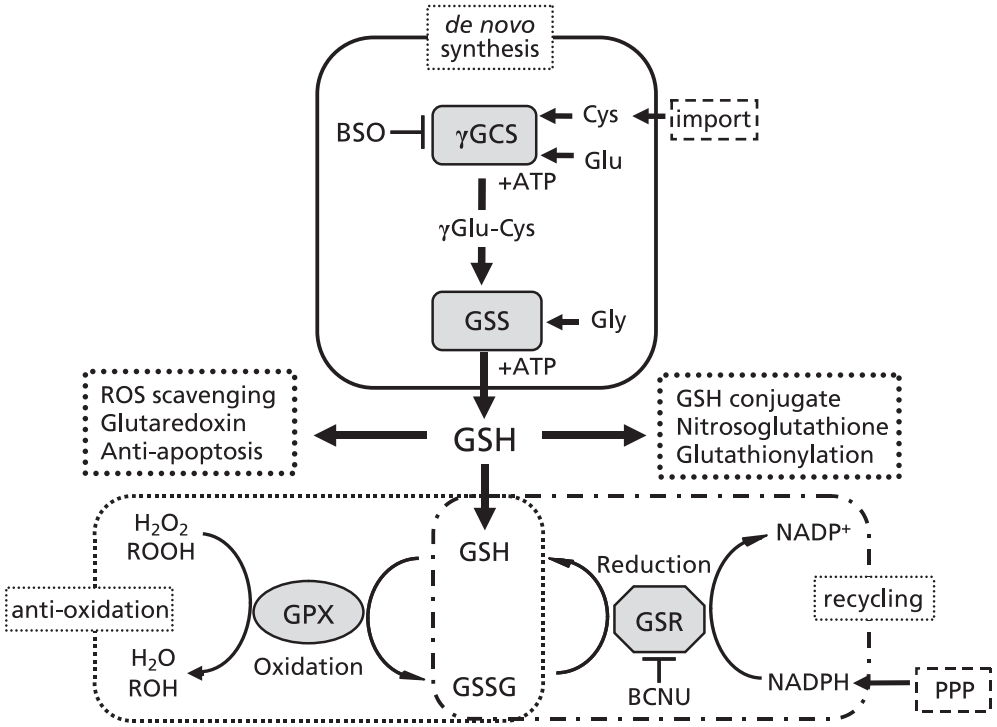

Fig. 1. Roles and metabolism of glutathione. γ-GCS and GSS coordinately catalyze *de novo* synthesis of GSH from three amino acids: Cys, Glu, and Gly. GPX reduces various peroxides, including hydrogen peroxide, by electrons from GSH. The resultant GSSG is reduced back to GSH by GSR via the donation of electrons from NADPH supplied from the pentose phosphate pathway (PPP). GSH not only supplies electrons to peroxides, but also plays multiple roles in cells.

cysteine with pyridoxal-5'-phosphate, a cofactor for amino group-transferring enzymes, glutathione present in the millimolar range is a main compound for the maintenance of redox potential within cells. Glutathione exerts its multiple functions by two means: non-enzymatic and enzymatic reactions. Brief mentions of these follow.

Non-enzymatic reaction of glutathione. GSH and ascorbate are major antioxidants with a strong reducing ability. The direct ROS-scavenging function of GSH can be well characterized by this chemical reaction. In fact, the pKa of a sulfhydryl group of glutathione is comparable to that of cysteine, around 8.5–8.8, and is not a very effective ROS scavenger.[8] Its presence in high concentrations within cells (~10 mM) exerts a potential function as an ROS scavenger and an anti-apoptotic compound in cells by reducing oxidized cytochrome c.[9,10]

The sulfhydryl group of glutathione, as with protein sulfhydryls, suffers from various oxidative and nitrosative modifications. The oxidation of a sulfhydryl to sulfenic acid is fully reversible; that of sulfenic acid to sulfinic acid is partly reversible, as can be seen in the sulfiredoxin system; however, the reaction whereby sulfinic acid converts to sulfonic acid is irreversible (Fig. 2).[11]

Electron donation from radicals, such as a superoxide or a hydroxyl radical, produces a thiyl radical, which is highly reactive to other sulfhydryls, and, consequently, glutathionylates proteins as well as GSSG (Fig. 3).[12] The interaction of glutathione with sulfhydryls in oxidatively modified proteins also causes protein *S*-glutathionylation, a mixed disulfide between Cys-SH of protein and glutathione. In normal liver, around 1% of total glutathione appears to be constantly present as the mixed disulfide with proteins, and the amount of glutathione bound to proteins could increase by 20–50% depending on the cellular redox state.[13,14] While glutathionylation consumes GSH in the cytoplasm and results in a redox imbalance within cells, dysfunction occurs to

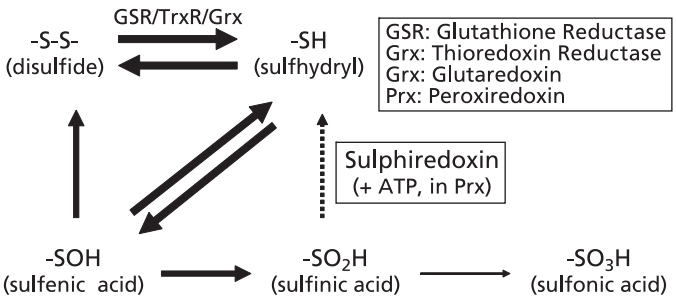

Fig. 2. Oxidative conversion of the sulfhydryl group and its reversibility in cells. Other than disulfide, the sulfhydryl group forms three oxidation states: sulfenic, sulfinic, and sulphonic acid. Sulenic acid can be reduced back to sulfhydryl by reductases but forms a disulfide bond with another sulfhydryl group. It was a general understanding that sulfinic acid could not be reduced back to sulfhydryl until the discovery of sulphiredoxin.

the glutathionylated proteins. A glutathionylated protein can be reduced back to sulfhydryl by Grx, which transiently result in glutathionylated Grx. The glutathione moiety of Grx can be freed by another glutathione to form GSSG, while Grx returns to its initial reduced form.[15] The reaction involving reversible glutathionylation is used for the protective mechanism of essential sulfhydryls in proteins under oxidative stress. This particular protective mechanism by glutathionylation has attracted much recent attention.[16]

The interaction of glutathione with nitric oxide (NO) produces GSNO that constitutes a major nitrosothiol in plasma, as well as in cells. While GSNO exerts multiple functions, such as vasodilatation and signal transduction via transnitrosation, it is also regarded as an effective glutathionylating agent.[17,18] Several

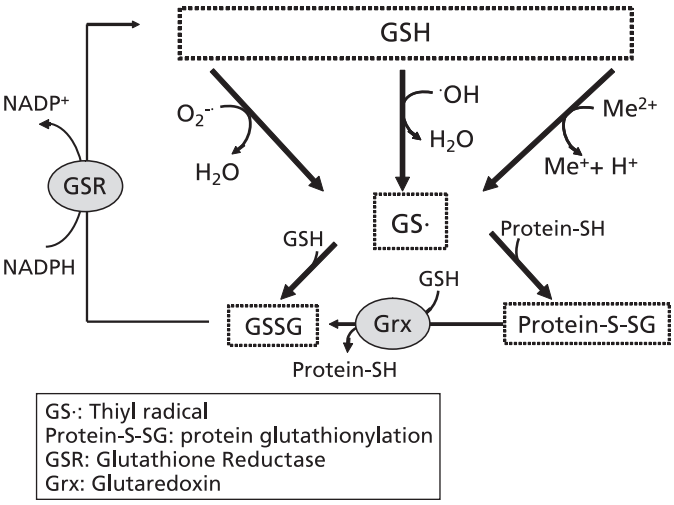

Fig. 3. Reaction pathways of sulfhydryl modification among GSH and proteins by ROS and GSH-mediated recycling.

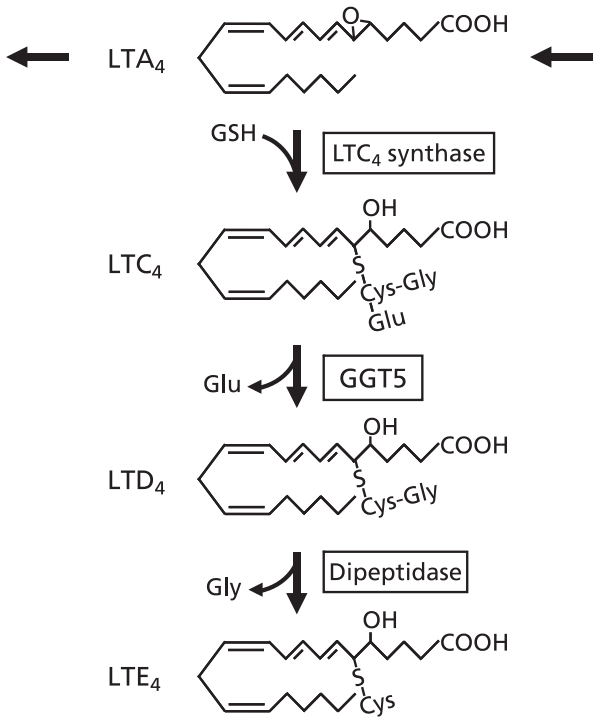

Fig. 4. GGT5-catalyzed LTD_4 formation using GSH followed by conversion to LTE_4.

methods can be used to detect glutathionylation; e.g., radio-labeling with [^{35}S]cysteine, glutathione S-transferase, and anti-glutathione antibody.[19,20] Advances in the proteomic approach that are based upon the separation of proteins by two-dimensional gel electrophoresis followed by identification by mass spectrometry have enabled the efficient detection of glutathionylated proteins with a high degree of reliability. This methodology has made it possible to detect glutathionylated proteins as well as other modifications *in vivo*, and has enhanced the understanding of their roles in the physiological state.

Enzymatic reactions utilizing glutathione as a co-substrate. Glutathione is also an electron donor for enzymatic systems using GPX. Electrons from glutathione are used to reduce various peroxides by GPX.[21] Conventional GPX constitutes 4 members and contains selenocysteine as the catalytic center. Since replacement of the selenocysteine to cysteine shows much lower GPX activity, the high catalytic activity of GPX can be attributed to the selenocysteine residue in the catalytic center. Current understanding of *GPX* family genes is overviewed elsewhere in this serial review.

In addition to conventional GPX, other enzymes such as glutathione S-transferase (GST), also exhibit glutathione-dependent peroxidase activity, albeit with much less efficiency. In addition to ROS scavenging activity, GST catalyzes the formation of glutathione adduct with various biological molecules and functions in the detoxification of xenobiotic compounds.[22] Mice have been generated with deficiencies of *mGSTP1/2*, *mGSTA4-4*, *mGSTZ1-1*, *mGSTM1-1*, *mGSTO1-1* and *mGSTS1-1,6* out of 21 *GST* genes, but the details are not provided here, because an extensive overview of the phenotypes is available in the toxicological report.[23]

Glutathione is also used as a building block of leukotriene (LT), which is a lipid mediator triggering various physiological responses. LTC_4 synthase conjugates LTA_4 with glutathione to form LTC_4,[24] which is then converted to LTD_4, a cysteinyl leukotriene, by hydrolytic removal of glutamate and glycine by a family member of γ-glutamyltransferase, as described below. Thus, in this case glutathione functions as a cysteine donor to form cysteinyl leukotrienes (Fig. 4).

Genes Involved in Glutathione Metabolism

The rate-determining, first step of glutathione synthesis is catalyzed by γ-GCS, which forms γ-glutamylcysteine by ligating glutamate and cysteine using ATP. γ-GCS is a heterodimeric enzyme composed of a catalytic subunit encoded by *GCLC* and a modifier subunit encoded by *GCLM*. Buthionine sulfoximine (BSO) is a specific inhibitor of the enzyme.[1]

Genes involved in glutathione metabolism, *xCT*, *GCLM*, *GCLC*, and *GSR* are induced under oxidative stress in an Nrf2-dependent manner.[25–28] Since disorders related to glutathione synthesis and pathological phenotypes in humans are well documented,[29,30] they are only briefly described here. While deficiencies of γ-GCS and GGT are very rare, GSS deficiency is the more frequent disorder. There are several reports concerning the mutation of *GLCL*.[31–37] Decreased glutathione levels and hemolytic anemia are commonly observed in γ-GCS and GSS deficiencies.

Mouse models for glutathione deficiency have been well documented.[38] However, roles of the genes in glutathione metabolism have been reconsidered by focusing on the progress that is reviewed here, because significant advances have been made in this area.

Import of cysteine and cystine into cells. Since cysteine exerts toxic effects by binding pyridoxial-5-phosphate (PLP), a cofactor for amino group-transferring enzyme reactions, the intracellular cysteine level is restricted (Fig. 5A) and is much less than glutamate and glycine. Thus, cysteine availability determines the synthesis of glutathione in cells. Under oxidative stress caused by acetaminophen-induced hepatotoxicity, the augmented production of ophthalmate—with a concomitant decrease of glutathione—can be observed by metabolomic analysis.[39] Ophthalmate is synthesized when 2-aminobutyrate is used in place of cysteine due to structural similarity through consecutive reactions catalyzed by γ-GCS and GSS (Fig. 5B). Thus, either glutathione or ophthalmate would be an alternative product for γ-GCS and GSS reactions depending on cysteine availability.

Cysteine is synthesized in humans from methionine but is not sufficient for the requirements of glutathione synthesis in most

doi: 10.3164/jcbn.10-138SR

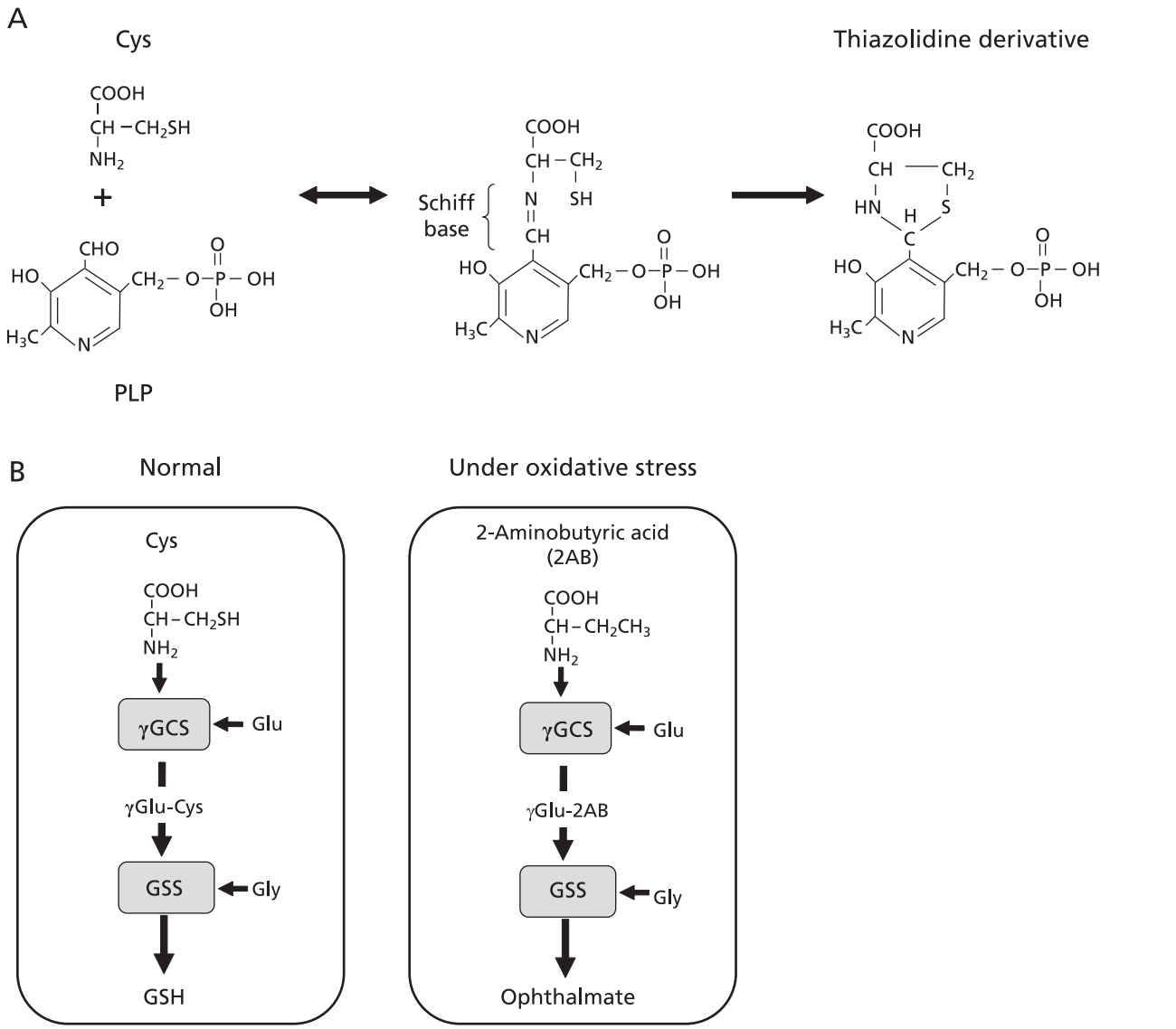

Fig. 5. Schematic representation of the reaction between Cys and PLP and the formation of opthalmate under oxidative stress. (A) The reaction of Cys with PLP forms a Cys-PLP Schiff base, which then cyclizes to form a stable thiazolidine derivative. (B) Under Cys-deficiency by oxidative stress, γ-GCS utilizes 2-aminobutyric acid (2AB) instead of Cys and causes production of ophthalmate instead of GSH. Adopted from reference 39 with modification.

cells, because methionine is an essential amino acid and is present only in a limited amount. Thus, cysteine availability sometimes limits glutathione synthesis, especially under oxidative stress that requires prompt glutathione synthesis. Cysteine and its oxidized form, cystine, are imported into cells via several amino acid transport systems.[40] Neutral amino acid transporters uptake cysteine in either a Na^+-dependent or -independent manner, while cystine is taken up by the cystine/glutamate exchanging transport system, designated as system x_c^-, in exchange for glutamate and by $b^{0,+}$ in a Na^+-independent manner.

In a cell culture system, cysteine is spontaneously auto-oxidized to cystine by oxygen, which is then taken up via system x_c^-. Once cystine is entered into cells, it is simultaneously reduced to cysteine and is utilized for glutathione synthesis as well as protein synthesis. Thus, system x_c^- activity indirectly controls intracellular glutathione availability in various cells in culture.[41] System x_c^- is composed of two proteins, xCT and 4F2hc, and the transport activity of x_c^- is attributed to the xCT protein.[42] The *xCT*$^{-/-}$ mice have higher concentrations of cystine in plasma compared with their wild-type littermates, but are otherwise apparently healthy.[43] A precise description of *xCT* and its physiological relevance is accorded a special section in this serial review.

Deficiency of γ-glutamyl cysteine synthetase (γ-GCS). *GCLC*-deficient mice have been generated by two groups.[44,45] The *GLCL*-null mutant mice are embryonic lethal due to apoptotic cell death.[44] *GCLC*$^{+/-}$ mice exhibit 20% diminution in GSH levels and a compensatory increase (about 30%) in ascorbate.[45] *GCLC*-null cells isolated from embryos die but can survive by supplementation with glutathione (2.5 mM) or N-acetylcysteine (NAC) (1.25–5 mM) in culture media.[44] Rapid onset of steatosis with mitochondrial injury accompanying decreased ATP and increased lipid peroxidation is observed in mice that lack *GCLC* in a hepatocyte-specific manner.[46] *GCLC*-knockdown rats and cells are established using short hairpin RNA against *GCLC*, and are used to evaluate drug-induced hepatotoxicity.[47,48]

GCLM null mice are viable and fertile and have no overt pheno-

type, but levels of glutathione comprise 9–16% of the liver, lung, pancreas, erythrocytes, and plasma of wild-type mice.[49] MEF isolated from *GCLM* null mice are highly sensitive to oxidants such as hydrogen peroxide and arsenic.[49,50] *GCLM* null mice are also sensitive to acetaminophen-induced liver damage and are protected by administered NAC.[51] *GCLM* null mice are sensitive to domoic acid, the causative agent for amnesic shellfish poisoning by mussels, which causes a rapid decrease in cellular glutathione by accelerating efflux from the cells, increases ROS and lipid peroxidation, and induces apoptotic cell death.[52,53]

While compounds that increase cellular glutathione, such as curucumin, quercetin, and tert-butylhydroquinone, induce *GCLM* much more than *GCLC*, they fail to increase γ-GCS activity and glutathione levels in *GCLM* null mice,[54] suggesting an essential role for *GCLM* in the upregulation of γ-GCS activity. Moreover, early onset of senescence, which is characterized by diminished cellular proliferation and increased senescence, which is associated with β-galactosidase activity, is evident in *GCLM*-null fibroblasts.[55] These characteristics are accompanied by an increase in intracellular ROS. Administration of NAC increases glutathione levels and concomitantly prevents premature senescence, suggesting that an increased redox potential attributed to an elevated glutathione can delay cell aging. Moreover, transgenic expression of *GCLM* increases resistance to acetaminophen-induced liver damage in mice[56] and hydrogen peroxide-induced single-strand DNA breakage in cells.[57]

Deficiency of glutathione synthetase. GSS deficiencies are seen more frequently in patients than either *GCLC* or *GCLM* deficiencies, but fewer than 100 have been documented worldwide, which defines them as rare genetic disorders.[29] The patients show 5-oxoprolineuria, hemolytic anemia, and neurological dysfunction.[58,59] About one-third of all patients with this condition die in childhood due to acidosis, electrolyte imbalance, infections, and convulsions. Examination of metabolites in cultured fibroblasts from 9 patients indicates that decreased glutathione and increased cysteine and γ-glutamylcysteine.[60] Since cysteine and γ-glutamylcysteine can partly compensate for the roles of glutathione, this indicates that γ-glutamylcysetine, the product of γ-GCS and a substrate of GSS, accumulates and would compensate redox potential with its sulfhydryl moiety. Hydrolysis of γ-glutamylcysteine by γ-glutamyl cyclotransferase, which was recently identified,[61] produces 5-oxoproline and cysteine. Clarification of the pathogenesis of a GSS deficiency would require the establishment of an animal model and precise analysis.

Recycling oxidized glutathione by glutathione reductase. Glutathione reductase (GSR) is a homodimeric flavoprotein (55-kDa each subunit) that regulates cellular GSH homeostasis by catalyzing the reduction of GSSG to GSH using NADPH as a reducing cofactor (Fig. 1). Anticancer agent 1,3-bis(2-chloroethyl)-1-nitorsourea (BCNU) is a well-known inhibitor for GSR and is used to examine the roles of the enzyme. GSR is predominantly present in liver and erythrocytes and is also expressed at high levels in the epithelia of the lungs[62] and in the reproductive organs of both sexes.[63,64]

Until now, there has been no report regarding the generation of *GSR* knockout mice by a gene-targeting technique. However, Gr1^{a1Neu} mice are a strain that is generated by treating male mice with a mutagen, isopropyl methanesulfonate, that exhibits less than 10% GSR activity in liver compared with control mice.[65] Sequence analysis of the *GSR* shows the deletion involves nucleotides 10,840 through 23,627 of the genomic DNA corresponding to deletion of exons 2 through 5. The deletion also causes a frame shift in exon 6 and introduces premature stop codon in exon 7. Thus, the Gr1^{a1Neu} mouse is incapable of producing a functional GSR protein.[66] Although the mouse does not show hemolytic anemia,[65] which is implicated as a result of a defect in the erythrocyte GSR, proximal tubule injury is induced more severely by a redox cycling toxicant, diquat, compared with wild-type mice.[67] Unexpectedly, however, Gr1^{a1Neu} mice are less susceptible to acute lung injury from continuous exposure to 95% oxygen,[68] although GSR is present predominantly in lung epithelia.[62]

There remains ambiguity concerning what is responsible for the 10% NADPH-dependent GSSG reducing activity in Gr1^{a1Neu} mice. In mammal cells, redox homeostasis of many cellular processes is maintained by thioredoxin (Trx) as well as by glutathione systems.[69,70] NADPH supplies reducing equivalents for these redox systems via pyridine nucleotide disulfide oxidoreductases that include Trx reductase and GSR.[71] These reductases are structurally similar and appear to have evolved from the same ancestral gene, although Trx-reductase has an essential C-terminal extension with a selenocysteine residue at the penultimate position.[72] Pretreatment with aurothioglucose, a Trx reductase inhibitor, in fact exacerbates the effects of hyperoxia on lung injuries in mice. This result suggests that Trx/Trx reductase has a more important protective function than GSR in hyperoxic lung injury.

Recently, a novel pyridine nucleotide disulfide oxidoreductase has been found. The enzyme possesses specificity for both thioredoxin and glutathione and is referred to as thioredoxin/glutathione reductase.[73] This novel reductase appears to have a role in sperm maturation.[74,75] Since this enzyme can reduce oxidized GSSG in a similar manner to GSR, it may at least be partly responsible for the remaining GSSG-reducing activity of Gr1^{a1Neu} mice.

Export of oxidized glutathione and glutathione conjugates via MRP. Depletion of cellular glutathione is mainly caused by exporting GSSG, glutathione S-conjugates, and S-nitrosoglutathione. The export of these compounds is mediated by a subset of proteins belonging to the ATP-biding cassette transporter (ABC transporter) protein superfamily that contains 49 members in humans.[76] Several members of the ABC subfamily C, also known as the multidrug resistance regulator subfamily, appear to mediate the export of them.[6] MRP1 (gene symbol *ABCC1*) has been extensively characterized among the MRP members and provides knowledge of the molecular mechanisms and its physiological functions concerning the transport of glutathione and glutathione conjugates.[77] Overexpression of MRP1 is found in multidrug-resistant cancer cells and causes the failure of chemotherapy. Certain forms of MRP2 (*ABCC2*), MRP4 (*ABCC4*), MRP5 (*ABCC5*), MRP6 (*ABCC6*), MRP8 (*ABCC8*), and CFTR (*ABCC7*; cystic fibrosis transmembrane conductance regulator) also transport some glutathione-related compounds, but different physiological functions from MRP1 are implied. Some glutathione-related compounds can also be transported by members of the solute carrier (SLC) superfamily of proteins.[77]

Extracellular metabolism of glutathione by γ-glutamyl transferase (*GGT*). γ-Glutamyl transferase (GGT) is localized at the cellular surface in the form of a membrane protein and enhances cellular glutathione synthesis by increasing the availability of component amino acids, especially cysteine.[1] Extracellular glutathione is hydrolyzed to its γ-glutamyl moiety and cysteinylglycine, which is further cleaved into cysteine and glycine. The released amino acids are taken up by corresponding transporters and reused by cells. GGT also catalyzes transferring reaction of γ-glutamyl moieties from glutathione and other γ-glutamyl compounds to acceptor molecules.

GGT is one of the most widely used clinical indicators of tissue damage. The best-characterized form is GGT1, which is an extracellular enzyme that is anchored to the plasma membrane of the cells. The human genome contains additional related genes. Systematic designation has been proposed for the human *GGT* family in collaboration with the HUGO Gene Nomenclature Committee.[78] The family includes genes encoding full-length proteins, *GGT1* to *GGT8P*, and light chain only, *GGTLC1* to *GGTLC5P*. Hereafter, this paper will use this nomenclature in reference to the *GGT* family genes.

doi: 10.3164/jcbn.10-138SR

GGT deficiency in humans is rare and has been reported in less than 10 cases worldwide, but no mutations are known either in *GGT1* or in other family members. Other than a low GGT activity, these patients commonly have glutathionuria, increased plasma glutathione levels and the presence of γ-glutamylcysteine and cysteine in urine.[79] Three of the patients show deficiencies in LTD_4 that are generated by a GGT-catalyzed reaction from the primary cysteinyl leukotriene LTC_4.[80]

There are now four mouse models of *GGT* deficiency: GGT^{enu1} mice generated by treatment with *N*-ethyl-*N*-nitrosourea and found to have a point mutation within the protein coding region of *GGT1*;[81] dwarf grey (dwg) and dwg (Bayer) mutations have been identified in *GGT1*;[82] and, two genetically modified mice with targeted disruption of *GGT1*[83] or *GGT5*[84] also have been generated.

In GGT^{enu1} mice, GGT protein synthesis is prematurely terminated and the enzyme is inactive,[85] which causes an altered redox balance and oxidative stress in tissues of the mouse even under normoxic conditions.[86] Total glutathione content in the liver of GGT^{enu1} mice is reduced but can be restored by oral supplementation of the cysteine prodrug L-2-oxothiazolidine-4-carboxyate.[87] GGT^{enu1} mice show an increase in extracellular glutathione in lung lining fluid and are protective against epithelial cell induction of asthma.[88] This suggests that local inhibition of GGT in the lining fluid of lungs may be beneficial in preventing asthma.

The dwg is the result of a spontaneous mutation in mice. Homozygous (dwg/dwg) mice are characterized by a gray coat, a smaller body size, and the development of cataracts by 3–4 weeks after birth.[89] Dwarf grey Bayer (dwg^{Bayer}) mice are also mutants that arise spontaneously from an embryonic stem (ES) cell line during gene targeting of an unrelated gene and show abnormalities similar to those of dwg/dwg mice. The gene locus of dwg^{Bayer} was confirmed as allelic with the dwg mutation. These abnormalities observed in dwg/dwg and dwg^{Bayer}/dwg^{Bayer} mice resemble those of *GGT1*-knockout mice.[83] Analyses of the genomic DNA show that 13 nucleotides on exon 7 of the *GGT1* are deleted in dwg/dwg mice while 46.7 kb containing complete coding sequences of *GGT1*, AI646023 gene, and the first exon of the *GGT5* are deleted in dwg^{Bayer}/dwg^{Bayer} mice.[82]

GGT1 knockout mice have been reported by Lieberman *et al.*,[83] and the roles of GGT1 in individual organs become evident under various pathophysiological conditions using *GGT1*-deficient mice. Observed abnormal phenotypes include growth retardation, sexual immaturity, cataract development, and gray coat color. While expression of genes involved in glutathione metabolism, γ-GCS, GSS, and cystathionase are induced concomitantly with the marked decrease in levels of glutathione in the *GGT1*-deficient mouse liver, antioxidative enzymes, CuZn- and Mn-superoxide dismutase, catalase, and glutathione peroxidase, are not changed.[90] DNA damage accumulates in the organs of mice deficient in *GGT1*, which is due to decreased redox capacity with low cysteine and GSH.[91]

Oral administration of NAC to the *GGT1*-deficient mice partially restores normal phenotypes, suggesting a role of GGT in the supply of cysteine and GSH homeostasis. A decrease in mitochondrial glutathione is correlated with negative effects on mitochondrial respiration with respect to ATP production.[92] Supplementation of NAC again fully restores mitochondrial GSH and respiratory function. The testes and seminal vesicles of *GGT1*-knockout mice are reduced in size, and the mice are infertile.[93] Consistent with the significant role of GSH in fertility, the administration of GSH or NAC to these mice completely restores the testis and seminal vesicle size to values comparable to those of wild-type mice, which renders the mutant mice fertile. Skeletal abnormalities and dwarfism is caused by proliferative defectiveness in *GGT1*-deficient mice. Although cells involved in osteoclast biology do not express *GGT1*, chondrocytes are reversed by NAC administration.[94] Because purified GGT1 protein, even if in an enzymatically inactive form, activates receptor-mediated osteoclast formation,[95] GGT1 may have additional roles other than the well-known enzymatic activity.

When exposed to 80% oxygen, the *GGT1*-deficient mice develop diffuse pulmonary injury and die within 8 days.[96] Investigation of the repair mechanism in bleomycin-induced pulmonary fibrosis suggests participation of increased neutrophils and matrix metalloproteinase-9 in the early inflammatory response.[97] When the nephrotoxic effects of cisplatin are examined, wild-type mice suffer from nephrotoxicity of cisplatin, but *GGT1*-deficient mice show no evidence of nephrotoxicity regardless of NAC supplementation. These results suggest that the nephrotoxicity of cisplatin is caused by metabolic conversion via GGT activity.[98] *GGT* deficiency in human T and B-lymphocytes appears to enhance the lifetime of GSNO,[99] which is a major reaction product of glutathione with reactive nitrogen oxide species in plasma. This results from increased extracellular glutathione concentrations, and decreased intracellular GSNO breakdown. A decrease in GSH levels in *GGT1* deficiency causes a decrease in cytotoxic T lymphocytes and a defect in T cell-dependent immune responses.[100]

A few pathological cases suggest that *GGT1* is not simply advantageous to animals. For example, when the role of GGT1 in the elimination of methylmercury is investigated, the *GGT1*-deficient mice excrete methylmercury more rapidly than wild-type mice, but eliminate inorganic mercury with an efficiency that is similar to wild-type mice.[101] Cataract development in *GGT1*-deficient mice appears to be a consequence of multiple causes, including exogenous damage (exposure to light), decreased lens glutathione levels, and nutritional effects of low cysteine availability.[102]

Other than *GGT1*, a physiological role of *GGT5* is now shown among the *GGT* family. LTC_4 is formed by conjugation of LTA_4 with GSH and is converted to LTD_4 by removing the glutamyl moiety by GGT activity. Substantial conversion of LTC_4 to LTD_4 in *GGT1*-null mice enabled identification of γ-glutamyl leukotrienase, now designated as *GGT5*, that catalyzes the conversion and other glutathione conjugates.[103,104] Analyses of *GGT5*-deficient mice indicate that GGT5 is specifically responsible for LTD_4 formation *in vivo* and attenuation of the acute inflammatory response.[84,105] Interactions between LTC_4 and interleukin 13 signaling pathways are implied using a *GGT5*-deficient mice model of airway diseas.[106]

Perspective

Pleiotropic roles have been established for glutathione in cells, but it is difficult to demonstrate the physiological relevance of them *in vivo*. Technology regarding gene manipulation has enabled analysis of the functions of individual genes *in vivo*. Application of this technique to genes involved in glutathione metabolism has provided us a clear picture of the functions of glutathione. Here, we have reviewed mainly mice models that have been established by either transgenic expression or by targeted disruption of the genes. Despite precious data obtained from these animals, it is sometimes claimed that overexpression or null mutation is far removed from the physiological state. In this regard, the application of small interfering RNA (siRNA) or small hairpin RNA (shRNA) to animal models makes it possible to fine-tune gene expression, and, hence, leads to a better understanding of their functions under more physiologic conditions.

Acknowledgments

Partly supported by the Global COE Program (F03) from the Japan Society for the Promotion of Science (JSPS) and YU-COE Program from Yamagata University.

Abbreviations

2AB	2-aminobutyric acid
ABC transporter	ATP-biding cassette transporter
BCNU	1,3-bis(2-chloroethyl)-1-nitorsourea
BSO	buthionine sulfoximine
γ-GCS	γ-glutamylcysteine synthetase
GGT	γ-Glutamyl transferase
GPX	glutathione peroxidase
Grx	glutaredoxin
GSH	reduced form of glutathione
GSNO	S-nitrosoglutathione
GSS	glutathione synthetase
GSSG	oxidized form of glutathione
GSR	glutathione reductase
GST	glutathione S-transferase
LT	leukotriene
MRP	multidrug resistant proteins
NAC	*N*-acetylcysteine
NO	nitric oxide
PLP	pyridoxial-5-phosphate
PPP	pentose phosphate pathway
ROS	reactive oxygen species
SLC	solute carrier
Trx	thioredoxin

References

1 Meister A. Glutathione biosynthesis and its inhibition. *Methods Enzymol* 1995; **252**: 26–30.
2 Meyer Y, Buchanan BB, Vignols F, Reichheld JP. Thioredoxins and glutaredoxins: unifying elements in redox biology. *Annu Rev Genet* 2009; **43**: 335–367.
3 Awasthi YC, Chaudhary P, Vatsyayan R, Sharma A, Awasthi S, Sharma R. Physiological and pharmacological significance of glutathione-conjugate transport. *J Toxicol Environ Health B Crit Rev* 2009; **12**: 540–551.
4 Franco R, Cidlowski JA. Apoptosis and glutathione: beyond an antioxidant. *Cell Death Differ* 2009; **16**: 1303–1314.
5 Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radic Biol Med* 2010; **48**: 749–762.
6 Ballatori N, Krance SM, Marchan R, Hammond CL. Plasma membrane glutathione transporters and their roles in cell physiology and pathophysiology. *Mol Aspects Med* 2009; **30**: 13–28.
7 Foster MW, Hess DT, Stamler JS. Protein S-nitrosylation in health and disease: a current perspective. *Trends Mol Med* 2009; **15**: 391–404.
8 Winterbourn CC, Hampton MB. Thiol chemistry and specificity in redox signaling. *Free Radic Biol Med* 2008; **45**: 549–561.
9 Suto D, Sato K, Ohba Y, Yoshimura T, Fujii J. Suppression of the pro-apoptotic function of cytochrome c by singlet oxygen via a haem redox state-independent mechanism. *Biochem J* 2005; **392**: 399–406.
10 Vaughn AE, Deshmukh M. Glucose metabolism inhibits apoptosis in neurons and cancer cells by redox inactivation of cytochrome c. *Nat Cell Biol* 2008; **10**: 1477–1483.
11 Janssen-Heininger YM, Mossman BT, Heintz NH, and *et al*. Redox-based regulation of signal transduction: principles, pitfalls, and promises. *Free Radic Biol Med* 2008; **45**: 1–17.
12 Dalle-Donne I, Rossi R, Colombo G, Giustarini D, Milzani A. Protein S-glutathionylation: a regulatory device from bacteria to humans. *Trends Biochem Sci* 2009; **34**: 85–96.
13 Brigelius R, Lenzen R, Sies H. Increase in hepatic mixed disulphide and glutathione disulphide levels elicited by paraquat. *Biochem Pharmacol* 1982; **31**: 1637–1641.
14 Brigeliu R, Muckel C, Akerboom TP, Sies H. Identification and quantitation of glutathione in hepatic protein mixed disulfides and its relationship to glutathione disulfide. *Biochem Pharmacol* 1983; **32**: 2529–2534.
15 Lillig CH, Berndt C, Holmgren A. Glutaredoxin systems. *Biochim Biophys Acta* 2008; **1780**: 1304–1317.
16 Dalle-Donne I, Rossi R, Giustarini D, Colombo R, Milzani A. S-glutathionylation in protein redox regulation. *Free Radic Biol Med* 2007; **43**: 883–898.
17 Klatt P, Lamas S. Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. *Eur J Biochem* 2000; **267**: 4928–4944.
18 Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS. Protein S-nitrosylation: purview and parameters. *Nat Rev Mol Cell Biol* 2005; **6**: 150–166.
19 Gianazza E, Eberini I, Ghezzi P. Detection of protein glutathionylation. *Methods Mol Biol* 2009; **519**: 397–415.
20 Cheng G, Ikeda Y, Iuchi Y, Fujii J. Detection of S-glutathionylated proteins by glutathione S-transferase overlay. *Arch Biochem Biophys* 2005; **435**: 42–49.
21 Flohé L. Selenium in mammalian spermiogenesis. *Biol Chem* 2007; **388**: 987–995.
22 Sau A, Pellizzari Tregno F, Valentino F, Federici G, Caccuri AM. Glutathione transferases and development of new principles to overcome drug resistance. *Arch Biochem Biophys* 2010; **500**: 116–122.
23 Board PG. The use of glutathione transferase-knockout mice as pharmacological and toxicological models. *Expert Opin Drug Metab Toxicol* 2007; **3**: 421–433.
24 Lam BK. Leukotriene C(4) synthase. *Prostaglandins Leukot Essent Fatty Acids* 2003; **69**: 111–116.
25 Wild AC, Gipp JJ, Mulcahy T. Overlapping antioxidant response element and PMA response element sequences mediate basal and β-naphthoflavone-induced expression of the human γ-glutamylcysteine synthetase catalytic subunit gene. *Biochem J* 1998; **332**: 373–381.
26 Wild AC, Moinova HR, Mulcahy RT. Regulation of γ-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. *J Biol Chem* 1999; **274**: 33627–33636.
27 Sasaki H, Sato H, Kuriyama-Matsumura K, and *et al*. Electrophile response element-mediated induction of the cystine/glutamate exchange transporter gene expression. *J Biol Chem* 2002; **277**: 44765–44771.
28 Harvey CJ, Thimmulappa RK, Singh A, and *et al*. Nrf2-regulated glutathione recycling independent of biosynthesis is critical for cell survival during oxidative stress. *Free Radic Biol Med* 2009; **46**: 443–453.
29 Njålsson R. Glutathione synthetase deficiency. *Cell Mol Life Sci* 2005; **62**: 1938–1945.
30 Ristoff E, Larsson A. Inborn errors in the metabolism of glutathione. *Orphanet J Rare Dis* 2007; **2**: 16.
31 Konrad PN, Richards F 2nd, Valentine WN, Paglia DE. -Glutamyl-cysteine synthetase deficiency. A cause of hereditary hemolytic anemia. *N Engl J Med* 1972; **286**: 557–561.
32 Richards F 2nd, Cooper MR, Pearce LA, Cowan RJ, Spurr CL. Familial spinocerebellar degeneration, hemolytic anemia, and glutathione deficiency. *Arch Intern Med* 1974; **134**: 534–537.
33 Beutler E, Moroose R, Kramer L, Gelbart T, Forman L. Gamma-glutamylcysteine synthetase deficiency and hemolytic anemia. *Blood* 1990; **75**: 271–273.
34 Beutler E, Gelbart T, Kondo T, Matsunaga AT. The molecular basis of a case of gamma-glutamylcysteine synthetase deficiency. *Blood* 1999; **94**: 2890–2894.
35 Hirono A, Iyori H, Sekine I, and *et al*. Three cases of hereditary non-spherocytic hemolytic anemia associated with red blood cell glutathione deficiency. *Blood* 1996; **87**: 2071–2074.
36 Ristoff E, Augustson C, Geissler J, and *et al*. A missense mutation in the heavy subunit of gamma-glutamylcysteine synthetase gene causes hemolytic anemia. *Blood* 2000; **95**: 2193–2196.
37 Hamilton D, Wu JH, Alaoui-Jamali M, Batist G. A novel missense mutation in the gamma-glutamylcysteine synthetase catalytic subunit gene causes both decreased enzymatic activity and glutathione production. *Blood* 2003; **102**: 725–730.
38 Dalton TP, Chen Y, Schneider SN, Nebert DW, Shertzer HG. Genetically altered mice to evaluate glutathione homeostasis in health and disease. *Free Radic Biol Med* 2004; **37**: 1511–1526.
39 Soga T, Baran R, Suematsu M, and *et al*. Differential metabolomics reveals ophthalmic acid as an oxidative stress biomarker indicating hepatic glutathione consumption. *J Biol Chem* 2006; **281**: 16768–16776.
40 Kanai Y, Endou H. Heterodimeric amino acid transporters: molecular biology

doi: 10.3164/jcbn.10-138SR

and pathological and pharmacological relevance. *Curr Drug Metab* 2001; **2**: 339–354.
41 Bannai S, Kitamura E. Transport interaction of L-cystine and L-glutamate in human diploid fibroblasts in culture. *J Biol Chem* 1980; **255**: 2372–2376.
42 Sato H, Tamba M, Ishii T, Bannai S. Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. *J Biol Chem* 1999; **274**: 11455–11458.
43 Sato H, Shiiya A, Kimata M, and *et al*. Redox imbalance in cystine/glutamate transporter-deficient mice. *J Biol Chem* 2005; **280**: 37423–37429.
44 Shi ZZ, Osei-Frimpong J, Kala G, and *et al*. Glutathione synthesis is essential for mouse development but not for cell growth in culture. *Proc Natl Acad Sci USA* 2000; **97**: 5101–5106.
45 Dalton TP, Dieter MZ, Yang Y, Shertzer HG, Nebert DW. Knockout of the mouse glutamate cysteine ligase catalytic subunit (Gclc) gene: embryonic lethal when homozygous, and proposed model for moderate glutathione deficiency when heterozygous. *Biochem Biophys Res Commun* 2000; **279**: 324–329.
46 Chen Y, Yang Y, Miller ML, and *et al*. Hepatocyte-specific Gclc deletion leads to rapid onset of steatosis with mitochondrial injury and liver failure. *Hepatology* 2007; **45**: 1118–1128.
47 Morita M, Akai S, Hosomi H, Tsuneyama K, Nakajima M, Yokoi T. Drug-induced hepatotoxicity test using gamma-glutamylcysteine synthetase knockdown rat. *Toxicol Lett* 2009; **189**: 159–165.
48 Hosomi H, Akai S, Minami K, and *et al*. An *in vitro* drug-induced hepatotoxicity screening system using CYP3A4-expressing and gamma-glutamylcysteine synthetase knockdown cells. *Toxicol In Vitro* 2010; **24**: 1032–1038.
49 Yang Y, Dieter MZ, Chen Y, and *et al*. Initial characterization of the glutamate-cysteine ligase modifier subunit Gclm(–/–) knockout mouse. Novel model system for a severely compromised oxidative stress response. *J Biol Chem* 2002; **277**: 49446–49452.
50 Kann S, Estes C, Reichard JF, and *et al*. Butylhydroquinone protects cells genetically deficient in glutathione biosynthesis from arsenite-induced apoptosis without significantly changing their prooxidant status. *Toxicol Sci* 2005; **87**: 365–384.
51 McConnachie LA, Mohar I, Hudson FN, and *et al*. Glutamate cysteine ligase modifier subunit deficiency and gender as determinants of acetaminophen-induced hepatotoxicity in mice. *Toxicol Sci* 2007; **99**: 628–636.
52 Giordano G, White CC, McConnachie LA, Fernandez C, Kavanagh TJ, Costa LG. Neurotoxicity of domoic Acid in cerebellar granule neurons in a genetic model of glutathione deficiency. *Mol Pharmacol* 2006; **70**: 2116–2126.
53 Giordano G, White CC, Mohar I, Kavanagh TJ, Costa LG. Glutathione levels modulate domoic acid induced apoptosis in mouse cerebellar granule cells. *Toxicol Sci* 2007; **100**: 433–444.
54 Lavoie S, Chen Y, Dalton TP, and *et al*. Curcumin, quercetin, and tBHQ modulate glutathione levels in astrocytes and neurons: importance of the glutamate cysteine ligase modifier subunit. *J Neurochem* 2009; **108**: 1410–1422.
55 Chen Y, Johansson E, Fan Y, and *et al*. Early onset senescence occurs when fibroblasts lack the glutamate-cysteine ligase modifier subunit. *Free Radic Biol Med* 2009; **47**: 410–418.
56 Botta D, Shi S, White CC, and *et al*. Acetaminophen-induced liver injury is attenuated in male glutamate-cysteine ligase transgenic mice. *J Biol Chem* 2006; **281**: 28865–28875.
57 Shi S, Hudson FN, Botta D, and *et al*. Over expression of glutamate cysteine ligase increases cellular resistance to H_2O_2-induced DNA single-strand breaks. *Cytometry A* 2007; **71**: 686–692.
58 Dahl N, Pigg M, Ristoff E, and *et al*. Missense mutations in the human glutathione synthetase gene result in severe metabolic acidosis, 5-oxoprolinuria, hemolytic anemia and neurological dysfunction. *Hum Mol Genet* 1997; **6**: 1147–1152.
59 Ristoff E, Mayatepek E, Larsson A. Long-term clinical outcome in patients with glutathione synthetase deficiency. *J Pediatr* 2001; **139**: 79–84.
60 Ristoff E, Hebert C, Njålsson R, Norgren S, Rooyackers O, Larsson A. Glutathione synthetase deficiency: is gamma-glutamylcysteine accumulation a way to cope with oxidative stress in cells with insufficient levels of glutathione? *J Inherit Metab Dis* 2002; **25**: 577–584.
61 Oakley AJ, Yamada T, Liu D, Coggan M, Clark AG, Board PG. The identification and structural characterization of C7orf24 as gamma-glutamyl cyclotransferase. An essential enzyme in the gamma-glutamyl cycle. *J Biol Chem* 2008; **283**: 22031–22042.
62 Fujii T, Endo T, Fujii J, Taniguchi N. Differential expression of glutathione reductase and cytosolic glutathione peroxidase, GPX1, in developing rat lungs and kidneys. *Free Radic Res* 2002; **36**: 1041–1049.
63 Kaneko T, Iuchi Y, Kawachiya S, and *et al*. Alteration of glutathione reductase expression in the female reproductive organs during the estrous cycle. *Biol Reprod* 2001; **65**: 1410–1416.
64 Kaneko T, Iuchi Y, Kobayashi T, and *et al*. The expression of glutathione reductase in the male reproductive system of rats supports the enzymatic basis of glutathione function in spermatogenesis. *Eur J Biochem* 2002; **269**: 1570–1578.
65 Pretsch W. Glutathione reductase activity deficiency in homozygous Gr1a1Neu mice does not cause haemolytic anaemia. *Genet Res* 1999; **73**: 1–5.
66 Rogers LK, Tamura T, Rogers BJ, Welty SE, Hansen TN, Smith CV. Analyses of glutathione reductase hypomorphic mice indicate a genetic knockout. *Toxicol Sci* 2004; **82**: 367–373.
67 Rogers LK, Bates CM, Welty SE, Smith CV. Diquat induces renal proximal tubule injury in glutathione reductase-deficient mice. *Toxicol. Appl. Pharmacol* 2006; **217**: 289–298.
68 Tipple TE, Welty SE, Rogers LK., and *et al*. Thioredoxin-related mechanisms in hyperoxic lung injury in mice. *Am J Respir Cell Mol Biol* 2007; **37**: 405–413.
69 Kondo N, Nakamura H, Masutani H, Yodoi J. Redox regulation of human thioredoxin network. *Antioxid Redox Signal* 2006; **8**: 1881–1890.
70 Meyer Y, Buchanan BB, Vignols F, Reichheld JP. Thioredoxins and glutaredoxins: unifying elements in redox biology. *Annu Rev Genet* 2009; **43**: 335–367.
71 Mustacich D, Powis G. Thioredoxin reductase. *Biochem J* 2000; **346**: 1–8.
72 Fujiwara N, Fujii T, Fujii J, Taniguchi N. Functional expression of rat thioredoxin reductase: selenocysteine insertion sequence element is essential for the active enzyme. *Biochem J* 1999; **340**: 439–444.
73 Sun QA, Kirnarsky L, Sherman S, Gladyshev VN. Selenoprotein oxidoreductase with specificity for thioredoxin and glutathione systems. *Proc Natl Acad Sci USA* 2001; **98**: 3673–3678.
74 Su D, Novoselov SV, Sun QA, and *et al*. Mammalian selenoprotein thioredoxin-glutathione reductase. Roles in disulfide bond formation and sperm maturation. *J Biol Chem* 2005; **280**: 26491–26498.
75 Gerashchenko MV, Su D, Gladyshev VN. CUG start codon generates thioredoxin/glutathione reductase isoforms in mouse testes. *J Biol Chem* 2010; **285**: 4595–4602.
76 Dean M, Allikmets R. Complete characterization of the human ABC gene family. *J Bioenerg Biomembr* 2001; **33**: 475–479.
77 Cole SP, Deeley RG. Transport of glutathione and glutathione conjugates by MRP1. *Trends Pharmacol Sci* 2006; **27**: 438–446.
78 Heisterkamp N, Groffen J, Warburton D, Sneddon TP. The human gamma-glutamyltransferase gene family. *Hum Genet* 2008; **123**: 321–332.
79 Wright EC, Stern J, Ersser R, Patrick AD. Glutathionuria: gamma-glutamyl transpeptidase deficiency. *J Inherit Metab Dis* 1980; **2**: 3–7.
80 Mayatepek E, Okun JG, Meissner T, and *et al*. Synthesis and metabolism of leukotrienes in gamma-glutamyl transpeptidase deficiency. *J Lipid Res* 2004; **45**: 900–904.
81 Harding CO, Williams P, Wagner E, and *et al*. Mice with genetic gamma-glutamyl transpeptidase deficiency exhibit glutathionuria, severe growth failure, reduced life spans, and infertility. *J Biol Chem* 1997; **272**: 12560–12567.
82 Tsuji T, Yamada K, Kunieda T. Characterization of the dwg mutations: dwg and dwg(Bayer) are new mutant alleles of the Ggt1 gene. *Mamm Genome* 2009; **20**: 711–719.
83 Lieberman MW, Wiseman AL, Shi ZZ, and *et al*. Growth retardation and cysteine deficiency in gamma-glutamyl transpeptidase-deficient mice. *Proc Natl Acad Sci USA* 1996; **93**: 7923–7926.
84 Shi ZZ, Han B, Habib GM, Matzuk MM, Lieberman MW. Disruption of gamma-glutamyl leukotrienase results in disruption of leukotriene D(4) synthesis *in vivo* and attenuation of the acute inflammatory response. *Mol Cell Biol* 2001; **21**: 5389–5395.
85 Jean JC, Harding CO, Oakes SM, Yu Q, Held PK, Joyce-Brady M. Gamma-Glutamyl transferase (GGT) deficiency in the GGTenu1 mouse results from a single point mutation that leads to a stop codon in the first coding exon of GGT mRNA. *Mutagenesis* 1999; **14**: 31–36.
86 Jean JC, Liu Y, Brown LA, Marc RE, Klings E, Joyce-Brady M. Gamma-glutamyl transferase deficiency results in lung oxidant stress in normoxia. *Am J Physiol Lung Cell Mol Physiol* 2002; **283**: L766–L776.
87 Held P, Harding CO. L-2-oxothiazolidine-4-carboxylate supplementation in murine gamma-GT deficiency. *Free Radic Biol Med* 2003; **34**: 1482–1487.
88 Lowry MH, McAllister BP, Jean JC, and *et al*. Lung lining fluid glutathione attenuates IL-13-induced asthma. *Am J Respir Cell Mol Biol* 2008; **38**: 509–

516.
89 Harris B, Davisson MT. Dwarf grey (dwg). *Mouse Genome* 1990; **86**: 238.
90 Habib GM, Shi ZZ, Ou CN, Kala G, Kala SV, Lieberman MW. Altered gene expression in the liver of gamma-glutamyl transpeptidase-deficient mice. *Hepatology* 2000; **32**: 556–562.
91 Rojas E, Valverde M, Kala SV, Kala G, Lieberman MW. Accumulation of DNA damage in the organs of mice deficient in gamma-glutamyltranspeptidase. *Mutat Res* 2000; **447**: 305–316.
92 Will Y, Fischer KA, Horton RA, and *et al*. Gamma-glutamyltranspeptidase-deficient knockout mice as a model to study the relationship between glutathione status, mitochondrial function, and cellular function. *Hepatology* 2000; **32**: 740–749.
93 Kumar TR, Wiseman AL, Kala G, Kala SV, Matzuk MM, Lieberman MW. Reproductive defects in gamma-glutamyl transpeptidase-deficient mice. *Endocrinology* 2000; **141**: 4270–4277.
94 Levasseur R, Barrios R, Elefteriou F, Glass DA 2nd, Lieberman MW, Karsenty G. Reversible skeletal abnormalities in gamma-glutamyl transpeptidase-deficient mice. *Endocrinology* 2003; **144**: 2761–2764.
95 Niida S, Kawahara M, Ishizuka Y, and *et al*. Gamma-glutamyltranspeptidase stimulates receptor activator of nuclear factor-kappaB ligand expression independent of its enzymatic activity and serves as a pathological bone-resorbing factor. *J Biol Chem* 2004; **279**: 5752–5756.
96 Barrios R, Shi ZZ, Kala SV, and *et al*. Oxygen-induced pulmonary injury in gamma-glutamyl transpeptidase-deficient mice. *Lung* 2001; **179**: 319–330.
97 Pardo A, Ruiz V, Arreola JL, and *et al*. Bleomycin-induced pulmonary fibrosis is attenuated in gamma-glutamyl transpeptidase-deficient mice. *Am J Respir Crit Care Med* 2003; **167**: 925–932.
98 Hanigan MH, Lykissa ED, Townsend DM, Ou CN, Barrios R, Lieberman MW. Gamma-glutamyl transpeptidase-deficient mice are resistant to the nephrotoxic effects of cisplatin. *Am J Pathol* 2001; **159**: 1889–1894.
99 Henson SE, Nichols TC, Holers VM, Karp DR. The ectoenzyme gamma-glutamyl transpeptidase regulates antiproliferative effects of S-nitrosoglutathione on human T and B lymphocytes. *J Immunol* 1999; **163**: 1845–1852.
100 Lawrence BP, Will Y, Reed DJ, Kerkvliet NI. Gamma-glutamyltranspeptidase knockout mice as a model for understanding the consequences of diminished glutathione on T cell-dependent immune responses. *Eur J Immunol* 2000; **30**: 1902–1910.
101 Ballatori N, Wang W, Lieberman MW. Accelerated methylmercury elimination in gamma-glutamyl transpeptidase-deficient mice. *Am J Pathol* 1998; **152**: 1049–1055.
102 Chévez-Barrios P, Wiseman AL, Rojas E, Ou CN, Lieberman MW. Cataract development in gamma-glutamyl transpeptidase-deficient mice. *Exp Eye Res* 2000; **71**: 575–582.
103 Carter BZ, Wiseman AL, Orkiszewski R, Ballard KD, Ou CN, Lieberman MW. Metabolism of leukotriene C4 in gamma-glutamyl transpeptidase-deficient mice. *J Biol Chem* 1997; **272**: 12305–12310.
104 Carter BZ, Shi ZZ, Barrios R, Lieberman MW. Gamma-glutamyl leukotrienase, a gamma-glutamyl transpeptidase gene family member, is expressed primarily in spleen. *J Biol Chem* 1998; **273**: 28277–28285.
105 Han B, Luo G, Shi ZZ, and *et al*. Gamma-glutamyl leukotrienase, a novel endothelial membrane protein, is specifically responsible for leukotriene D(4) formation *in vivo*. *Am J Pathol* 2002; **161**: 481–490.
106 Chavez J, Young HW, Corry DB, Lieberman MW. Interactions between leukotriene C4 and interleukin 13 signaling pathways in a mouse model of airway disease. *Arch Pathol Lab Med* 2006; **130**: 440–446.

doi: 10.3164/jcbn.10-138SR