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GLUTATHIONE TRANSFERASES

John D. Hayes, Jack U. Flanagan, and Ian R. Jowsey
Biomedical Research Center, Ninewells Hospital & Medical School, University of Dundee, Dundee DD1 9SY, Scotland, United Kingdom; email: john.hayes@cancer.org.uk, j.flanagan@imb.uq.edu.au, i.r.jowsey@dundee.ac.uk

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■ **Abstract** This review describes the three mammalian glutathione transferase (GST) families, namely cytosolic, mitochondrial, and microsomal GST, the latter now designated MAPEG. Besides detoxifying electrophilic xenobiotics, such as chemical carcinogens, environmental pollutants, and antitumor agents, these transferases inactivate endogenous α,β-unsaturated aldehydes, quinones, epoxides, and hydroperoxides formed as secondary metabolites during oxidative stress. These enzymes are also intimately involved in the biosynthesis of leukotrienes, prostaglandins, testosterone, and progesterone, as well as the degradation of tyrosine. Among their substrates, GSTs conjugate the signaling molecules 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) and 4-hydroxynonenal with glutathione, and consequently they antagonize expression of genes trans-activated by the peroxisome proliferator-activated receptor γ (PPARγ) and nuclear factor-erythroid 2 p45-related factor 2 (Nrf2). Through metabolism of 15d-PGJ_2, GST may enhance gene expression driven by nuclear factor-κB (NF-κB). Cytosolic human GST exhibit genetic polymorphisms and this variation can increase susceptibility to carcinogenesis and inflammatory disease. Polymorphisms in human MAPEG are associated with alterations in lung function and increased risk of myocardial infarction and stroke. Targeted disruption of murine genes has demonstrated that cytosolic GST isoenzymes are broadly cytoprotective, whereas MAPEG proteins have proinflammatory activities. Furthermore, knockout of mouse *GSTA4* and *GSTZ1* leads to overexpression of transferases in the Alpha, Mu, and Pi classes, an observation suggesting they are part of an adaptive mechanism that responds to endogenous chemical cues such as 4-hydroxynonenal and tyrosine degradation products. Consistent with this hypothesis, the promoters of cytosolic *GST* and *MAPEG* genes contain antioxidant response elements through which they are transcriptionally activated during exposure to Michael reaction acceptors and oxidative stress.

INTRODUCTION

The glutathione transferases (EC 2.5.1.18) have historically also been called glutathione *S*-transferases, and it is this latter name that gives rise to the widely used abbreviation, GST. These enzymes catalyze nucleophilic attack by reduced glutathione (GSH) on nonpolar compounds that contain an electrophilic carbon, nitrogen, or sulphur atom. Their substrates include halogenonitrobenzenes, arene oxides, quinones, and α,β-unsaturated carbonyls (1–5). Three major families of proteins that are widely distributed in nature exhibit glutathione transferase activity. Two of these, the cytosolic and mitochondrial GST, comprise soluble enzymes that are only distantly related (6, 7). The third family comprises microsomal GST and is now referred to as membrane-associated proteins in eicosanoid and glutathione (MAPEG) metabolism (8). A further distinct family of transferases exists, represented by the bacterial fosfomycin resistance proteins FosA and FosB (9); this family is not discussed further.

Cytosolic and mitochondrial GST share some similarities in their three-dimensional fold (6) but bear no structural resemblance to the MAPEG enzymes (10). However, all three families contain members that catalyze the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) and exhibit glutathione peroxidase activity toward cumene hydroperoxide (CuOOH); these reactions are shown in Figure 1. The cytosolic GST and MAPEG enzymes catalyze isomerization of various unsaturated compounds (8, 11, 12) and are intimately involved in the synthesis of prostaglandins and leukotrienes (4, 8).

Cytosolic GSTs represent the largest family of such transferases and have activities that are unique to this group of enzymes. They catalyze thiolysis of 4-nitrophenyl acetate; display thiol transferase activity; reduce trinitroglycerin, dehydroascorbic acid, and monomethylarsonic acid; and catalyze the isomerization of maleylacetoacetate and Δ^5-3-ketosteroids (Figure 1) (1, 13–17).

Glutathione transferases are of interest to pharmacologists and toxicologists because they provide targets for antiasthmatic and antitumor drug therapies (18–21), and they metabolize cancer chemotherapeutic agents, insecticides, herbicides, carcinogens, and by-products of oxidative stress. Overexpression of GST in mammalian tumor cells has been implicated with resistance to various anticancer agents and chemical carcinogens (2). Furthermore, elevated levels of GST have been associated with tolerance of insecticides and with herbicide selectivity (22, 23).

In microbes, plants, flies, fish, and mammals, expression of *GSTs* are upregulated by exposure to prooxidants (24–30). Increase in transferase activity is also observed in animals that undergo prolonged torpor or hibernation when comparisons are made between their estivated state and their wakeful condition (31). It is similarly observed during transition of the common toad *Bufo bufo* from an aquatic environment to the land (32). Collectively, these findings indicate that induction of GST is an evolutionarily conserved response of cells to oxidative stress.

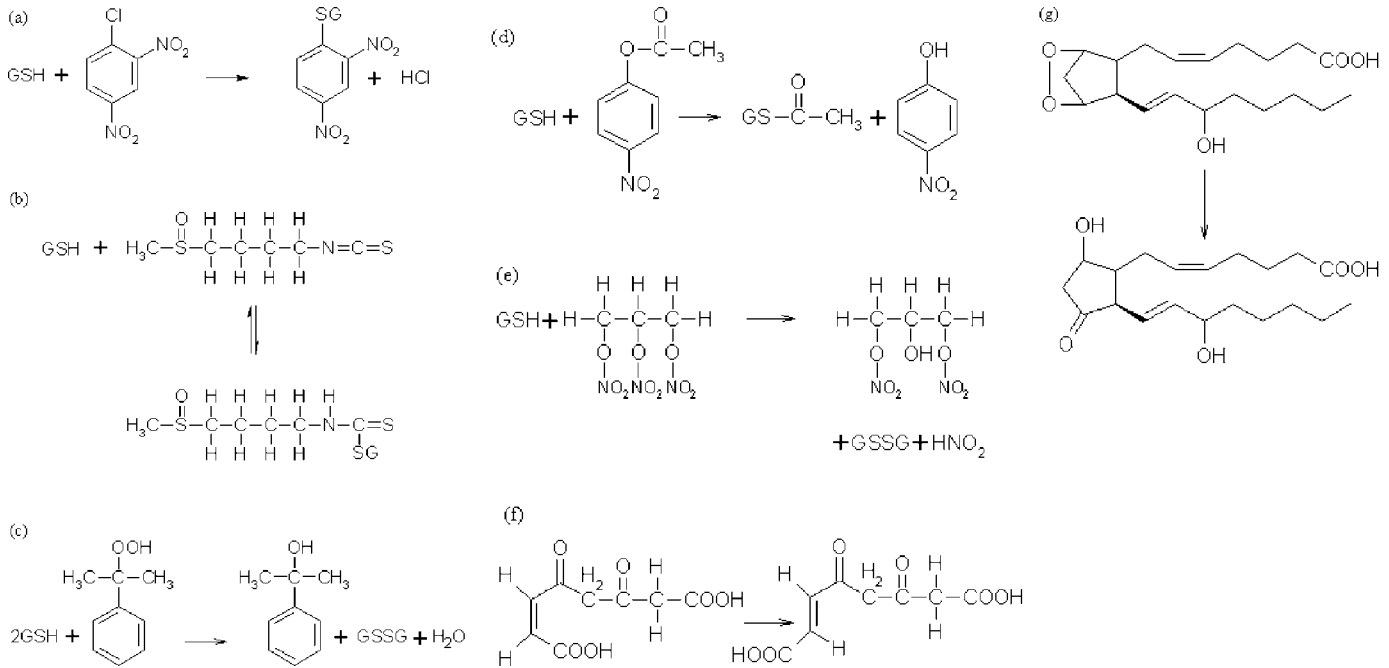

Figure 1 Reactions catalyzed by GST. Example of conjugation, reduction, thiolysis, and isomerization reactions catalyzed by GST. The following substrates are shown: (*a*) CDNB, (*b*) sulforaphane, (*c*) CuOOH, (*d*) 4-nitrophenyl acetate, (*e*) trinitroglycerin, (*f*) maleylacetoacetate, and (*g*) PGH_2 (conversion to PGD_2 is depicted).

METABOLISM OF XENOBIOTICS BY GST

Detoxification through the Mercapturic Acid Pathway

Glutathione transferases catalyze the first of four steps required for the synthesis of mercapturic acids (1). Subsequent reactions in this pathway entail sequential removal of the γ-glutamyl moiety and glycine from the glutathione conjugate, followed finally by *N*-acetylation of the resulting cysteine conjugate. It is important to recognize that GST enzymes are part of an integrated defense strategy, and their effectiveness depends on the combined actions of, on one hand, glutamate cysteine ligase and glutathione synthase to supply GSH and, on the other hand, the actions of transporters to remove glutathione conjugates from the cell (4). Once formed, these conjugates are eliminated from the cell by the trans-membrane MRP (multidrug resistance-associated protein). Nine MRP proteins exist (33), and these are all members of the C family of ABC transporters. Among these, MRP1 and MRP2 can export glutathione conjugates and compounds complexed with GSH (34, 35). The dinitrophenol-glutathione ATPase called RLIP76 promotes efflux of glutathione conjugates from cells (36), but as it is not a trans-membrane protein the mechanism is probably indirect.

Exogenous substrates for soluble GST include drugs, industrial intermediates, pesticides, herbicides, environmental pollutants, and carcinogens. The cancer chemotherapeutic agents adriamycin, 1,3-*bis*(2-chloroethyl)-1-nitrosourea (BCNU), busulfan, carmustine, chlorambucil, *cis*-platin, crotonyloxymethyl-2-cyclohexenone (COMC-6), cyclophosphamide, ethacrynic acid, melphalan, mitozantrone, and thiotepa are detoxified by GST (2, 37, 38). Environmental chemicals and their metabolites detoxified by GST include acrolein, atrazine, DDT, inorganic arsenic, lindane, malathion, methyl parathion, muconaldehyde, and tridiphane (2, 39, 40).

A large number of epoxides, such as the antibiotic fosfomycin and those derived from environmental carcinogens, are detoxified by GST. The latter group includes epoxides formed from aflatoxin B_1, 1-nitropyrene, 4-nitroquinoline, polycyclic aromatic hydrocarbons (PAHs), and styrene by the actions of cytochromes P450 in the liver, lung, gastrointestinal tract, and other organs. Conjugation of aflatoxin B_1-8,9-epoxide with GSH is a major mechanism of protection against the mycotoxin, at least in rodents (41). The PAHs are ubiquitous, found in cigarette smoke and automobile exhaust fumes, and represent an ever-present threat to health. Those that are metabolized by GST include ultimate carcinogenic bay- and fjord-region diol epoxides produced from chrysene, methylchrysene, benzo[*c*]chrysene, benzo[*g*]chrysene, benzo[*c*]phenanthrene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, and dibenzo[*a*,*l*]pyrene (42–44).

Heterocyclic amines, produced by cooking protein-rich food, represent another important group of carcinogens. One of the major heterocyclic amines found in cooked food is 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), and cytosolic GST isoenzymes have been shown to detoxify the activated metabolite, *N*-acetoxy-PhIP (45).

Bioactivation of Xenobiotics by GST

Conjugation of foreign compounds with GSH almost always leads to formation of less reactive products that are readily excreted. In a few instances, however, the glutathione conjugate is more reactive than the parent compound. Examples of this phenomenon are short-chain alkyl halides that contain two functional groups. Conjugation of GSH with the solvent dichloromethane results in the formation of the highly unstable *S*-chloromethylglutathione, which still contains an electrophilic center capable of modifying DNA (46, 47). The 1,2-dihaloethanes are another group of GST substrates that are activated by conjugation with GSH to genotoxic products. However, in this instance, the glutathione conjugate rearranges to form an episulfonium intermediate that is responsible for modifying DNA (47).

Allyl-, benzyl-, phenethyl-isothiocyanates, and sulforaphane are moderately toxic compounds that are formed from plant glucosinolates. They are reversibly conjugated by GST with GSH to yield thiocarbamates (Figure 1). Following export from cells via MRP1 or MRP2, thiocarbamates spontaneously degrade to their isothiocyanates, liberating GSH. Thereafter, the isothiocyanate may be taken up again by the cell and reconjugated with GSH, only to be reexported as the thiocarbamate and revert to the isothiocyanate. This cyclical process results in depletion of intracellular GSH and assists distribution of isothiocynates throughout the body. Should isothiocyanates be taken up by cells that have a low GSH content, they may not be conjugated with GSH, but rather are more likely to thiocarbamylate proteins, a process that can result in cell death (48).

Conjugation of haloalkenes with GSH, which occurs primarily in the liver, can lead ultimately to the generation in the kidney of reactive thioketenes, thionoacylhalides, thiiranes, and thiolactones through the actions of renal cysteine conjugate β-lyase (49).

In cancer chemotherapy, the ability of GST to produce reactive metabolites has been exploited to target tumors that overexpress particular transferases (50). The latent cytotoxic drug TER286 (now called TLK286) is activated by GST through a β-elimination reaction to yield an active analogue of cyclophosphamide (51, 51a). More recently, the prodrug PABA/NO (O^2-[2,4-dinitro-5-(*N*-methyl-*N*-4-carboxyphenylamino)phenyl] 1-*N*,*N*-dimethylamino)diazen-1-ium-1,2-diolate) has been designed to generate cytolytic nitric oxide upon metabolism by GST (52).

METABOLISM OF ENDOGENOUS COMPOUNDS BY GST

Detoxification of Products of Oxidative Stress

The production of reactive oxygen species, the superoxide anion O_2^-, hydrogen peroxide H_2O_2, and the hydroxyl radical HO•, from partially reduced O_2 is an unavoidable consequence of aerobic respiration. Free radicals primarily arise through oxidative phosphorylation, although 5-lipoxygenase-, cyclooxygenase-, cytochrome P450-, and xanthene oxidase–catalyzed reactions are also a source (4). Such species are scavenged by the catalytic activities of superoxide dismutase,

catalase, and glutathione peroxidase and nonenzymatically by α-tocopherol, ascorbic acid, GSH, and bilirubin. Despite these antioxidant defenses, reactive oxygen species inflict damage on membrane lipid, DNA, protein, and carbohydrate. Oxidation of these macromolecules gives rise to cytotoxic and mutagenic degradation products (53). Thus, although O_2^- can damage DNA directly, it can also damage DNA indirectly through the production of these reactive secondary metabolites. Aldehyde dehydrogenase, alcohol dehydrogenase, aldo-keto reductase, GST, and Se-dependent glutathione peroxidase (GPx) are some of the enzyme systems that protect against the by-products of oxidative stress.

Free radical-initiated peroxidation of polyunsaturated fatty acids in membranes is a particular problem as it results in chain reactions that serve to amplify damage to lipids. The process produces short-lived lipid hydroperoxides that breakdown to yield secondary electrophiles, including epoxyaldehydes, 2-alkenals, 4-hydroxy-2-alkenals, and ketoaldehydes, some of which are genotoxic (53). GST isoenzymes exhibit modest Se-independent glutathione peroxidase activity toward 1-palmitoyl-2-(13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoyl)-L-3-phosphatidylcholine and phosphatidylcholine hydroperoxide, indicating they may reduce lipid hydroperoxides within membranes (54–56). The transferases can also reduce cholesteryl hydroperoxides (57) and fatty acid hydroperoxides, including (*S*)-9-hydroperoxy-10,12-octadecadieonic acid and (*S*)-13-hydroperoxy-9,11-octadecadieonic acid (56). Presumably, reduction of phospholipid, fatty acid, and cholesteryl hydroperoxides curtails formation of downstream epoxides and reactive carbonyls arising from oxidation of membranes. Among the end-products of lipid peroxidation, GSTs conjugate GSH with the 2-alkenals acrolein and crotonaldehyde (2, 4), as well as 4-hydroxy-2-alkenals of between 6 and 15 carbon atoms in length (58) (Figure 2); conjugation of GSH with the (*S*) enantiomer of 4-hydroxynonenal is favored over the (*R*) enantiomer (59). Further, GSTs catalyze the conjugation of cholesterol-5,6-oxide, epoxyeicosatrienoic acid, and 9,10-epoxystearic acid with GSH (2). These findings indicate that GST, along with other antioxidant enzymes, such as Se-dependent GPx1, provide the cell with protection against a range of harmful electrophiles produced during oxidative damage to membranes (4).

The 1-cys peroxiredoxin, Prx VI, defends against cellular membrane damage by reducing phospholipid hydroperoxides to their respective alcohols. Reduction of these substrates results in oxidation of Cys-47 in Prx VI to sulfenic acid. It has been proposed that GST reactivates oxidized Prx VI through glutathionylation followed by reduction of the mixed disulfide (60). Through this process, GST may indirectly combat oxidative stress by restoring the activity of oxidized Prx VI.

Oxidation of nucleotides yields base propenals, such as adenine propenal, and hydroperoxides that are detoxified by GST (Figure 2). Oxidation of catecholamines yields aminochrome, dopachrome, noradrenochrome, and adrenochrome that are harmful because they can produce O_2^- by redox cycling. These quinone-containing compounds can be conjugated with GSH through the actions of GST, a reaction that prevents redox-cycling (61). *O*-quinones formed from dopamine can

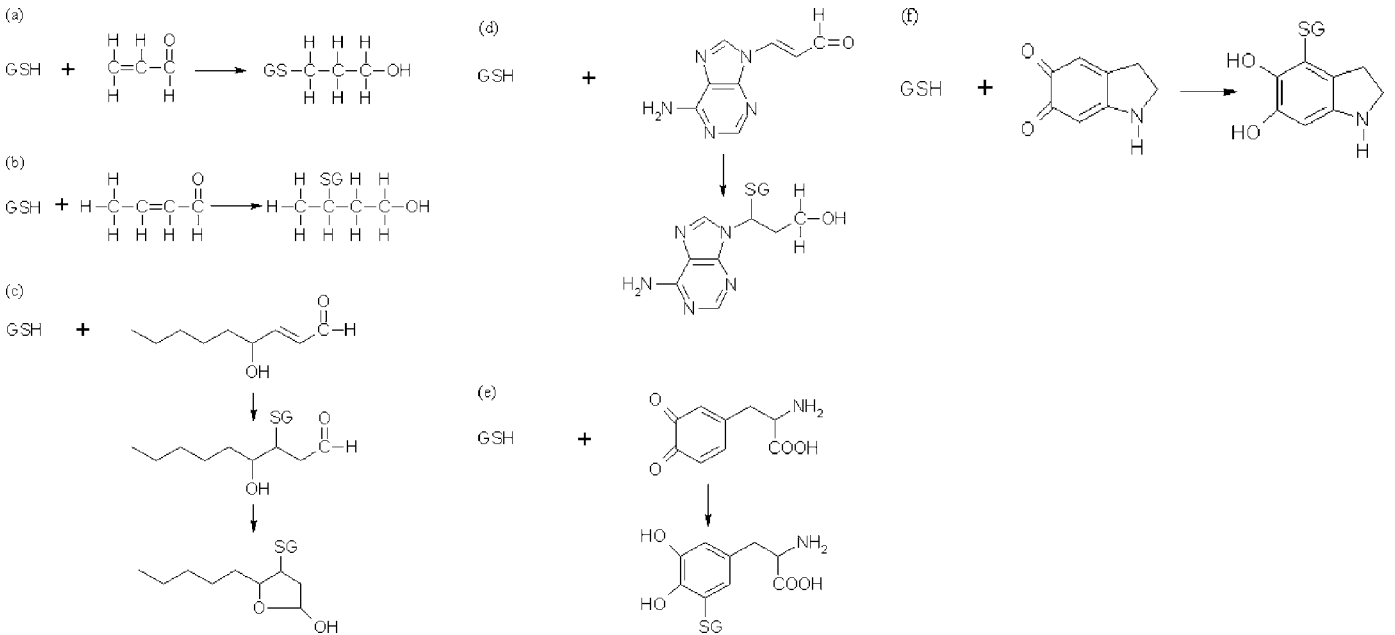

Figure 2 GST-catalyzed conjugation of α,β-unsaturated carbonyls and *o*-quinones with GSH. Reactions catalyzed by GST on the following substrates are shown: (*a*) acrolein, (*b*) crotonaldehyde, (*c*) 4-hydroxynonenal, (*d*) adenine propenal, (*e*) dopa-*o*-quinone, and (*f*) aminochrome.

also be conjugated with GSH by GST, and this reaction is similarly thought to combat degenerative processes in the dopaminergic system in human brain (Figure 2).

Degradation of Aromatic Amino Acids

In mammals, phenylalanine is degraded to acetoacetate and fumaric acid. The five intermediates are tyrosine, 4-hydroxyphenylpyruvate, homogentisate, maleylacetoacetate, and fumarylacetoacetate. The cytosolic class Zeta GST has been identified as a maleylacetoacetate isomerase (14), and therefore catalyzes the penultimate step in the catabolism of phenylalanine and tyrosine (shown in Figure 1).

GST and Synthesis of Steroid Hormones

Both testosterone and progesterone are synthesized from the cholesterol metabolite 3β-hydroxy-5-pregnene-20-one. This compound undergoes side-chain cleavage and oxidation of the 3β-hydroxyl group in the A steroid ring to yield Δ^5-androstene-3,17-dione as an intermediate in the testosterone pathway. Alternatively, it can undergo oxidation of the 3β-hydroxyl to form Δ^5-pregnene-3,20-dione as an intermediate in the progesterone pathway. These two 3-keto-Δ^5-steroids are converted to their 3-keto-Δ^4-steroid isomers by cytosolic GST (62). The 3-keto-Δ^5-steroids are generated by actions of a 3β-hydroxysteroid dehydrogenase that also exhibits keto-steroid isomerase activity and could therefore be responsible for the isomerization step. However, Johansson & Mannervik (62) have shown that a class Alpha GST isoenzyme present only in steroidogenic tissues has a 230-fold higher catalytic efficiency in the isomerization of 3-keto-steroids than the 3β-hydroxysteroid dehydrogenase. It therefore seems most likely that GST catalyzes this step in vivo.

GST and Eicosanoids: Synthesis and Inactivation

Glutathione transferases contribute to the biosynthesis of pharmacologically important metabolites of arachidonic acid. Although early studies suggested that many GST catalyze the isomerization of PGH_2 to a mixture of PGD_2 and PGE_2, or reduce it to $PGF_{2\alpha}$, it is now clear that certain transferases exhibit remarkable specificity for some of these reactions. The identification of mammalian GSH-dependent prostaglandin D_2 synthase as a cytosolic GST serves as an excellent paradigm in this regard (63, 64). This observation is of particular interest as the enzyme contributes not only to PGD_2 production but also to formation of the downstream cyclopentenone prostaglandin, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), which possesses distinct biological activities. Cytosolic transferases expressed in human brain exhibit PGE_2 synthase activity (65). In addition to the cytosolic GST, members of the MAPEG family make major contributions to production of PGE_2 (8), whereas a membrane-bound GSH-activated enzyme has been shown to possess $PGF_{2\alpha}$ synthase activity (66).

Prostaglandins and isoprostanes containing a cyclopentenone ring also represent GST substrates in glutathione-conjugation reactions (67). This modification

facilitates the elimination of these eicosanoids from the cell via MRP1 and MRP3 transporters (68).

Leukotrienes (LTs) are another group of eicosanoids formed from arachidonic acid. MAPEGs are critically involved in their synthesis because one member uniquely activates 5-lipoxygenase, whereas several others catalyze the formation of LTC_4.

Modulation of Signaling Pathways by GST

As endogenous lipid mediators influence diverse signaling pathways, their metabolism by GST has many biological consequences. Although the effects of the classical prostaglandins (PGD_2, PGE_2, and $PGF_{2\alpha}$) are mediated through specific G protein–coupled receptors, cyclopentenone prostaglandins exert their effects through a separate mechanism. Undoubtedly the most widely studied of these is 15d-PGJ_2, a downstream metabolite of PGD_2. The ability of different transferases to affect either synthesis or elimination of this eicosanoid places GST as central regulators in this arena. Perhaps the most significant property of 15d-PGJ_2 is its ability to serve as an activating ligand for the peroxisome proliferator-activated receptor γ (PPARγ). This transcription factor is a critical regulator of adipocyte differentiation and also represents the molecular target of the thiazolidinedione class of insulin sensitizing drugs. Over-expression of GST can diminish transactivation of gene expression by 15d-PGJ_2 mediated by PPARγ through conjugation of the prostanoid with GSH (69).

15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 can stimulate nuclear factor-erythroid 2 p45-related factor 2 (Nrf2)-mediated induction of gene expression through the antioxidant response element (ARE) (70, 71). This occurs because 15d-PGJ_2 is able to modify cysteine residues in the cytoskeleton-associated protein Keap1 (Kelch-like ECH-associated protein 1), and thus overcomes the ability of Keap1 to target Nrf2 for proteasomal degradation (71–73). Conjugation of 15d-PGJ_2 with GSH abolishes its ability to modify Keap1. A similar mechanism appears to underlie the ability of 15d-PGJ_2 to inactivate the β subunit of the inhibitor of κB kinase (IKKβ) and inhibit nuclear factor κB (NF-κB)-dependent gene expression (74). The extent to which GST-catalyzed synthesis and/or metabolism of 15d-PGJ_2 impinges on these signaling pathways is an important area that warrants further study (Figure 3).

The endogenous lipid peroxidation product 4-hydroxynonenal (4-HNE) is believed to act as an intracellular signaling molecule (75–77), and therefore its conjugation with GSH will influence a number of pathways. Like 15d-PGJ_2, this 2-alkenal is an α,β-unsaturated carbonyl that can stimulate gene expression through the ARE (78). In common with 15d-PGJ_2 it is probable that Nrf2 mediates induction of ARE-driven genes by 4-HNE (79, 80). The aldehyde also prevents activation of NF-κB by inhibiting IκB phosphorylation. It has been reported to modulate several cell-surface receptors, activate epithelial growth factor receptor and platelet-derived growth factor-β receptor, and upregulate transforming growth factor receptor β1. Also, 4-HNE stimulates several components in signal

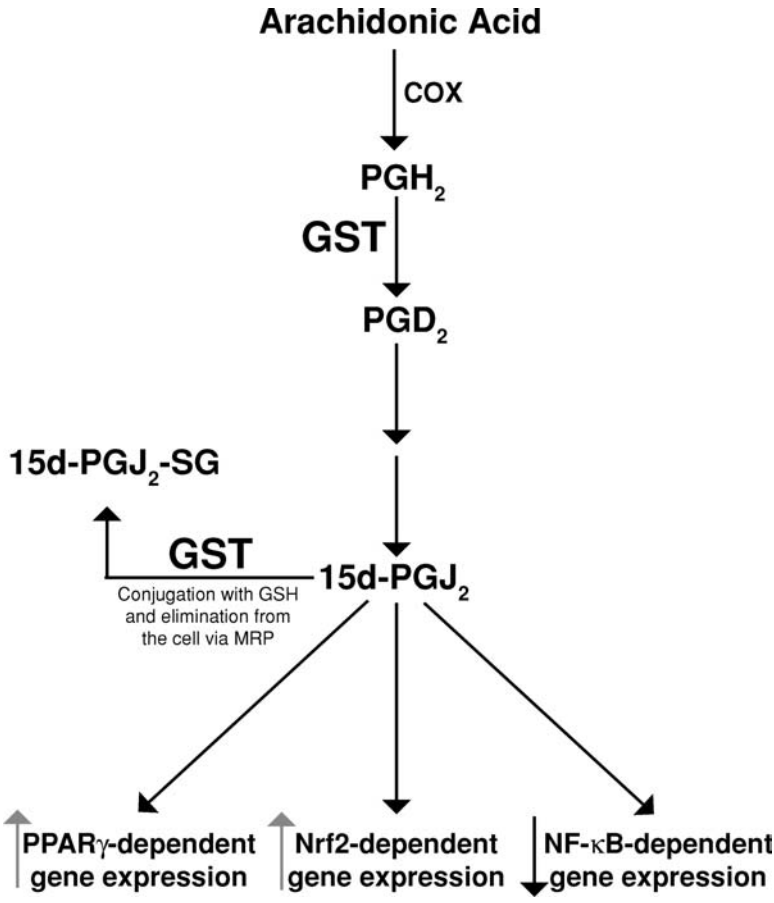

Figure 3 Attenuation of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 signaling by GST. This figure shows the synthesis of 15d-PGJ_2 and the various transcription factors whose activity may be influenced by the prostaglandin (69–74).

transduction pathways, such as JNK, p38, and protein kinase C, as well as increasing p53 protein and promoting apoptosis (77). It is anticipated that conjugation of 4-HNE with GSH will influence many signal transduction pathways and modulate the activity of transcription factors, including NF-κB, c-Jun, and Nrf2.

GST FAMILIES

Cytosolic Enzymes

Mammalian cytosolic GSTs are all dimeric with subunits of 199–244 amino acids in length. Based on amino acid sequence similarities, seven classes of cytosolic GST are recognized in mammalian species, designated Alpha, Mu, Pi, Sigma,

Theta, Omega, and Zeta (2–5). Other classes of cytosolic GST, namely Beta, Delta, Epsilon, Lambda, Phi, Tau, and the "U" class, have been identified in nonmammalian species (5, 23, 81). In rodents and humans, cytosolic GST isoenzymes within a class typically share >40% identity, and those between classes share <25% identity.

At least 16 cytosolic GST subunits exist in the human. As those in the Alpha and Mu classes can form heterodimers (2), a significantly larger number of isoenzymes can be generated from these subunits. The total of 16 homodimers listed in Table 1 includes the relatively poorly characterized GSTM4-4 (82) and GSTM5-5 (83), as well as a transferase, GSTA5-5, that has been identified by genomic cloning but has not been characterized at the protein level (84). An additional human enzyme, hGST5.8, with high activity toward 4-HNE, has been reported and is presumed to be a class Alpha transferase (85). This enzyme seems to be distinct from GSTA1-1, GSTA2-2, GSTA3-3, and GSTA4-4 but it is not included in Table 1 as its primary structure has not been described. The transferases display overlapping substrate specificities, a feature that makes it difficult to identify isoenzymes solely on their catalytic properties. Substrates identified for each of the human cytosolic GST are listed in Table 1 (some examples are illustrated in Figures 1 and 2).

Besides catalyzing conjugation, reduction, and isomerization reactions, cytosolic GST also bind, covalently and noncovalently, hydrophobic nonsubstrate ligands (2). This type of activity contributes to intracellular transport, sequestration, and disposition of xenobiotics and hormones. Such compounds include azo-dyes, bilirubin, heme, PAHs, steroids, and thyroid hormones; it is the nonsubstrate binding activity that led originally to class Alpha GST being called Ligandin (2). Affinity labeling of rat class Alpha GST has revealed a high-affinity nonsubstrate binding site within the cleft between the two subunits (86), indicating that there are two distinct xenobiotic-binding sites in certain isoenzymes. The second nonsubstrate binding site formed in heterodimers will be distinct from those in homodimers, and it may provide an evolutionary reason why it is beneficial for members within the Alpha and Mu classes to heterodimerize.

Class Mu and Pi GST have been reported to inhibit Ask1 and JNK during nonstressed conditions through physical interactions with the kinases (87–89). It has been shown that GSTM1 dissociates from Ask1 by heat shock (88), whereas GSTP1 dissociates from JNK in response to oxidative stress (89). As described above, GSTP1 also physically interacts with Prx VI, a process that leads to recovery of peroxiredoxin enzyme activity through glutathionylation of the oxidized protein (60).

The majority of cytosolic GST isoenzymes are found in the cytoplasm of the cell. However, mouse and human Alpha-class GSTA4-4 can associate with mitochondria and membranes (90–92), as can mouse GSTM1-1 (91). In the case of GSTA4-4, this entails phosphorylation of the transferase, and targeting is dependent on the Hsp70 chaperone (92). Using monkey COS cells, treatment with 4-HNE increases the amount of GSTA4-4 associated with the mitochondria (92). A human transferase that is closely related to GSTA1-1 has been purified from liver microsomes (56), and it appears that certain class Alpha enzymes have a

TABLE 1 Substrate preferences of human glutathione transferases**

Family	Class, enzyme	Substrates or reaction***
Cytosolic	Alpha, A1-1	Δ^5-ADD, BCDE, BPDE, Busulfan, Chlorambucil, DBADE, DBPDE, BPhDE, N-a-PhIP
	Alpha, A2-2	CuOOH, DBPDE, 7-chloro-4-nitrobenz-2-oxa-1,3-diazole
	Alpha, A3-3	Δ^5-ADD, Δ^5-pregnene-3,20-dione, DBPDE
	Alpha, A4-4	COMC-6, EA, 4-hydroxynonenal, 4-hydroxydecenal
	Alpha, A5-5	not done
Cytosolic	Mu, M1-1	*trans*-4-phenyl-3-buten-2-one, BPDE, CDE, DBADE, *trans*-stilbene oxide, styrene-7,8-oxide
	Mu, M2-2	COMC-6, 1,2-dichloro-4-nitrobenzene, aminochrome, dopa *O*-quinone, $PGH_2 \rightarrow PGE_2$
	Mu, M3-3	BCNU, $PGH_2 \rightarrow PGE_2$
	Mu, M4-4	CDNB
	Mu, M5-5	low for CDNB
Cytosolic	Pi, P1-1	acrolein, base propenals, BPDE, CDE, Chlorambucil, COMC-6, EA, Thiotepa
Cytosolic	Sigma, S1-1	$PGH_2 \rightarrow PGD_2$
Cytosolic	Theta, T1-1	BCNU, butadiene epoxide, CH_2Cl_2, EPNP, ethylene oxide
	Theta, T2-2	CuOOH, menaphthyl sulfate
Cytosolic	Zeta, Z1-1	dichloroacetate, fluoroacetate, 2-chloropropionate, malelyacetoacetate
Cytosolic	Omega, O1-1	monomethylarsonic acid, dehydroascorbic acid
	Omega, O2-2	dehydroascorbic acid
Mitochondrial	Kappa, K1-1	CDNB, CuOOH, (*S*)-15-hydroperoxy-5,8,11, 13-eicosatetraenoic acid
MAPEG	gp I, MGST2	CDNB, $LTA_4 \rightarrow LTC_4$, (*S*)-5-hydroperoxy-8,11, 14-*cis*-6-*trans*-eicosatetraenoic acid
	gp I, FLAP	nonenzymatic binding of arachidonic acid
	gp I, LTC_4S	$LTA_4 \rightarrow LTC_4$
MAPEG	gp II, MGST3	CDNB, $LTA_4 \rightarrow LTC_4$, (*S*)-5-hydroperoxy-8,11, 14-*cis*-6-*trans*-eicosatetraenoic acid
MAPEG	gp IV, MGST1	CDNB*, CuOOH, hexachlorobuta-1,3-diene
	gp IV, PGES1	$PGH_2 \rightarrow PGE_2$

*Activity increased by treating enzyme with *N*-ethylmaleimide.

**A systematic study of all these enzymes toward substrates has not been undertaken, and therefore it is not possible to define relative activities toward the compounds listed. These data are taken from papers cited in the text.

***Abbreviations: Δ^5-ADD, Δ^5-androstene-3,17-dione; BCDE, benzo[*g*]chrysene diol epoxide; BCNU, 1,3-*bis*(2-chloroethyl)-1-nitrosourea; BPDE, benzo[*a*]pyrene diol epoxide; BPhDE, benzo[*c*]phenanthrene diol epoxide; CDE, chrysene-1,2-diol 3,4-epoxide; COMC-6, crotonyloxymethyl-2-cyclohexenone; DBADE, dibenz[*a*,*h*]anthracene diol epoxide; DBPDE, dibenzo[*a*,*l*]pyrene diol epoxide; EA, ethacrynic acid; EPNP, 1,2-epoxy-3-(*p*-nitrophenoxy)propane; N-a-PhIP, N-acetoxy-2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.

propensity to associate with membranes. Mouse GSTO1-1 can be targeted to the nucleus following TPA treatment (93), and rat GSTT2-2 can be found in the nucleus following treatment with Oltipraz (4).

In addition to these GST classes, CLICs (chloride intracellular channels) (94, 95) and elongation factor 1Bγ adopt the same crystal structure as cytosolic GST (96). Other proteins, including ganglioside-induced differentiation-associated protein-1 (97), have also been proposed to occupy the GST fold, but this remains to be proven.

Mitochondrial GST

The mammalian mitochondrial class Kappa GST isoenzymes are dimeric and comprise subunits of 226 amino acids. Mouse, rat, and human possess only a single Kappa GST (6, 7, 98, 99). Molecular cloning and crystallography of the mitochondrial GST have provided definitive evidence that it represents a distinct type of transferase (6, 7). The three-dimensional fold of Kappa is more similar to bacterial 2-hydroxychromene-2-carboxylate isomerase, a GSH-dependent oxidoreductase that catalyzes conversion of 2-hydroxy-chromene-2-carboxylate to trans-*O*-hydroxy-benzylidenepyruvate, and to prokaryotic disulfide-bond-forming DsbA and TcpG oxidoreductases, than to any of the cytosolic GST isoenzymes. As such, it has provided a new insight into the evolution of GST.

GST class Kappa has high activity for aryl halides, such as CDNB, and can reduce CuOOH and (*S*)-15-hydroperoxy-5,8,11,13-eicosatetraenoic acid (99). In view of its homology with 2-hydroxychromene-2-carboxylate isomerase, it will be interesting to establish whether GST Kappa can metabolize aromatic hydrocarbons, such as naphthalene.

In the mouse, GST Kappa is present in large amounts in liver, kidney, stomach, and heart, and electron microscopy has confirmed that it is associated with liver and kidney mitochondria (100). Its tissue distribution in the rat seems similar to that in the mouse (98). By contrast, GST Kappa appears to be more widely and uniformly expressed in human tissues (99).

Although this transferase was originally isolated from mitochondria and is not present in cytoplasm (98), it has also been shown to be located in peroxisomes (99). The presence of GSTK1-1 in both organelles suggests it may be specifically involved in β-oxidation of fatty acids, either through its catalytic activity, some transport function, or interaction with a membrane pore. The process of targeting GST Kappa to mitochondria is unclear. It has been reported to associate with the Hsp60 chaperone (7), and a possible cleavage site for a mitochondrial presequence exists at the *N*-terminus (99). A peroxisomal targeting sequence (tripeptide ARL) has been identified in the *C*-terminus of the human GSTK1 subunit (99).

Evolution of the GST Fold

Based on similarity of the tertiary structure of the *N*-terminal domain of cytosolic transferases, the canonical GST fold is thought to have evolved from a thioredoxin/

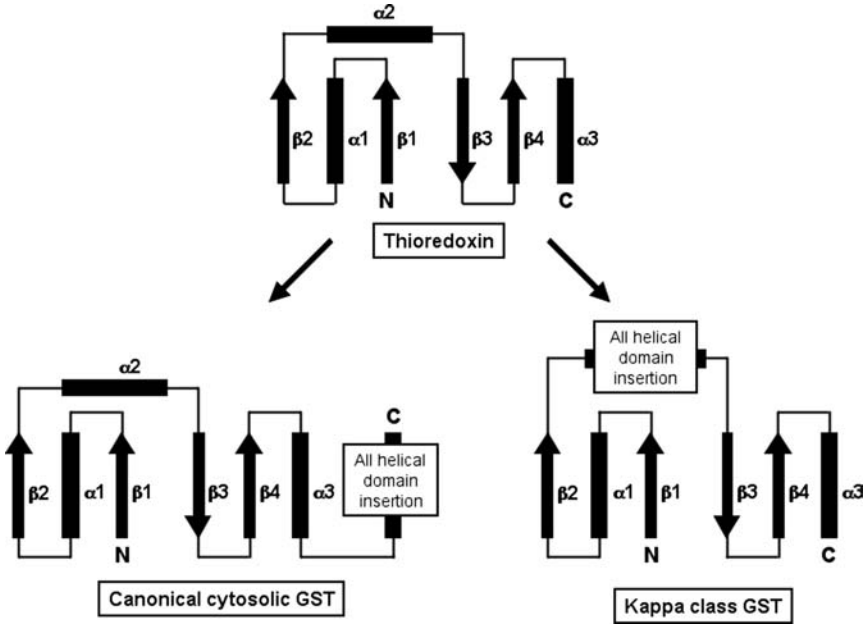

Figure 4 Schematic diagram showing evolution of the GST fold. The secondary structure arrangements of thioredoxin, the canonical cytosolic GST fold, and that of mitochondrial GSTK1 [the latter is predicted to be closely similar to the secondary structure of bacterial DsbA (6, 7)] are illustrated. Arrows represent β-sheets; rectangles represent α-helices. The regions corresponding to the core thioredoxin structure are shown for the cytosolic GST fold and GSTK1. The positions of helical domain insertion that result in either fold are also shown, and they clearly illustrate two sites in the thioredoxin fold that appear to have less evolutionary constraint. The differences in architecture also provide substantial evidence that soluble GSTs have evolved through two differing pathways.

glutaredoxin progenitor (3). Evolution of the cytosolic enzymes appears to be through the addition of an all-helical domain after the thioredoxin $\beta\alpha\beta\alpha\beta\beta\alpha$ structure. By contrast, the crystal structure of the mitochondrial isoform, GSTK1-1, provides clear evidence of a parallel evolutionary pathway (illustrated in Figure 4), as the all-helical domain responsible for binding of the second, electrophilic substrate appears to have been inserted within the $\beta\alpha\beta\alpha\beta\beta\alpha$ core after the $\beta\alpha\beta$ motif (7). The resulting Kappa isoform is more similar in its secondary structure organization to the bacterial protein disulphide isomerase DsbA than to the cytosolic isomerases (6, 7). Moreover, the different mechanisms used to achieve the common *N*- and *C*-terminal domains of cytosolic GST illustrate two regions in the thioredoxin/glutaredoxin fold that are under less evolutionary constraint.

The cytosolic GSTs are catalytically active as dimers, with the dimer interface providing a noncatalytic site for ligand binding. A limited number of studies

indicate that mammalian GSTM1 and GSTP1 can probably exist as monomers through interactions with other proteins, such as Ask1, JNK, and Prx IV (60, 87–89). It is interesting to note that Pettigrew & Colman (101) have reported that heterodimers can be formed between class Mu and class Pi polypeptides in vitro without the need for denaturants, an observation that might reflect some promiscuity in the subunit dimerization in these two classes of GST. Monomeric forms of cytosolic GST have been demonstrated convincingly in nonmammalian species (102). The recent identification of a structural relationship between the cytosolic GSTs and isoforms of the CLICs (94, 95, 102, 103) has revealed the potential for proteins possessing the canonical GST fold to exist as soluble monomers when purified in a functionally active state, in this case forming chloride ion channels. It has also been shown that these monomers can undergo structural rearrangement under oxidizing conditions to form dimers (103). Whether CLIC adopts this form in the membrane is at this point unknown, but it has been proposed that a large conformational rearrangement occurs, facilitating membrane insertion (102).

Identification of the canonical cytosolic GST fold in proteins involved in nondetoxication processes illustrates that this structure is amenable to many different functions, yet it is not clear whether these proteins represent pathways of convergent evolution or the continued evolution of the cytosolic GST.

MAPEG Enzymes

These members of the GST superfamily constitute a unique branch where most of the proteins are involved in the production of eicosanoids. Throughout nature, a total of four MAPEG subgroups (I–IV) have been described, with proteins within a subgroup sharing >20% sequence identity. Six human MAPEGs have been identified, and these fall within subgroups I, II, and IV (8).

The founding member of the MAPEG family, MGST1, was initially identified as a microsomal CDNB-metabolizing enzyme that, in contrast to most cytosolic GST, can be activated by treatment with *N*-ethylmaleimide (2, 8). Three further MAPEG members with roles in eicosanoid synthetic pathways were subsequently identified as leukotriene C_4 synthase (LTC_4S), a microsomal transferase that conjugates leukotriene A_4 with GSH; 5-lipoxygenase-activating protein (FLAP), an arachidonic acid-binding protein required for 5-lipoxygenase to exhibit full activity; and prostaglandin E_2 synthase 1 (PGES1), which catalyses GSH-dependent isomerization of PGH_2 to PGE_2 (8). Following the discovery of MGST1, FLAP, and LTC_4S, bioinformatic approaches were used to isolate cDNAs for MGST2 and MGST3, encoding enzymes that reduce (*S*)-5-hydroperoxy-8,11,14-*cis*-6-*trans*-eicosatetraenoic acid (104). According to sequence-based subdivision of the MAPEG family, subgroup I consists of FLAP, LTC_4S, and MGST2; the only member of subgroup II is MGST3; and MGST1 and PGES1 make up subgroup IV. Subgroup III contains microsomal GST-like proteins from *Escherichia coli* and *Vibrio cholera*.

Evidence suggests MGST1 functions solely as a detoxication enzyme. By contrast, human MGST2 and MGST3 are capable of both detoxifying foreign

compounds and synthesizing LTC_4 (104); in the rat, MGST3 is apparently unable to synthesize LTC_4 (105). FLAP does not have catalytic activity but binds arachidonic acid and appears to be essential for the synthesis of all leukotrienes formed downstream of 5-lipoxygenase. LTC_4S and PGES1 seem to make no contribution to detoxification, their catalytic actions being restricted to synthesis of LTC_4 and PGE_2, respectively (see Table 1).

Determination of a 6 Å crystal structure for MGST1 has illustrated the homotrimeric quaternary structure of the enzyme (10), a quaternary structure also observed for the other subgroup IV enzyme PGES1 (106). By contrast with the trimeric structure of these enzymes, subgroup I contains members that either form monomers or more complex aggregates. For example, FLAP can exist in monomeric, dimeric and trimeric forms, and LTC_4S can similarly form multimeric complexes (107). FLAP and LTC_4S can also form heterodimers and heterotrimers with each other (107). More research is required to understand the stoichiometry and membrane topology of these proteins.

GENETIC VARIATION IN HUMAN GLUTATHIONE TRANSFERASES

Polymorphisms in Cytosolic GST

Cytosolic GST display polymorphisms in humans (Table 2, reviewed in 108–110), and this is likely to contribute to interindividual differences in responses to xenobiotics. The earliest studies in this area addressed the question of whether individuals lacking GSTM1-1 and/or GSTT1-1 (i.e., are homozygous for *GSTM1*0* and/or *GSTT1*0* alleles) have a higher incidence of bladder, breast, colorectal, head/neck, and lung cancer. Following the discovery of allelic variants of *GSTP1* that encode enzymes with reduced catalytic activity, the hypothesis that combinations of polymorphisms in class Mu, Pi, and Theta class GST contribute to diseases with an environmental component was examined by many researchers. In general, it has been found that individual *GST* genes do not make a major contribution to susceptibility to cancer, although *GSTM1*0* has a modest effect on lung cancer, *GSTM1*0* and *GSTT1*0* have a modest effect on the incidence of head and neck cancer, and *GSTP1*B* influences risk of Barrett's esophagus and esophageal carcinoma (111, 112, 112a). It is worth noting that a possible shortcoming of many studies into the biological effects of *GSTM1*0* and *GSTT1*0* is that only individuals who are homozygous nulled for these genes (−/−) have been identified. Invariably, individuals who are heterozygous (−/+) or homozygous (+/+) for the functional allele are not distinguished and analyzed separately. As a consequence, the significance of being homozygous wild type for *GSTM1* and *GSTT1* is seldom addressed. The benefit of such a genotype is probably underestimated in the literature because it is grouped together with the heterozygote genotype. A study that uses a novel assay to distinguish between −/−, −/+, and +/+ genotypes at the *GSTM1* locus has revealed significant protection against breast cancer in

TABLE 2 Polymorphic human cytosolic GST

Class	Allele	Nucleotide(s) in gene at variable position(s)	Protein affected*
Alpha	*GSTA1*A*	−631T/G, −567T, −69C, −52G	"Reference" protein levels
	*GSTA1*B*	−631G, −567G, −69T, −52A	Low protein levels
	*GSTA2*A*	328C, 335G, 588G, 629A	Pro^{110}, Ser^{112}, Lys^{196}, Glu^{210}
	*GSTA2*B*	328C, 335G, 588G, 629C	Pro^{110}, Ser^{112}, Lys^{196}, Ala^{210}
	*GSTA2*C*	328C, 335C, 588G, 629A	Pro^{110}, Thr^{112}, Lys^{196}, Glu^{210}
	*GSTA2*D*	328C, 335G, 588T, 629C	Pro^{110}, Ser^{112}, Asn^{196}, Ala^{210}
	*GSTA2*E*	328T, 335G, 588G, 629A	Ser^{110}, Ser^{112}, Lys^{196}, Glu^{210}
Mu	*GSTM1*A*	519G	Lys^{173}
	*GSTM1*B*	519C	Asn^{173}
	*GSTM1*0*	gene deletion	No protein
	*GSTM1*1x2*	gene duplication	Overexpression of M1 protein
	*GSTM3*A*	wild-type	"Reference" protein levels
	*GSTM3*B*	3 bp deletion in intron 6	Protein unchanged
	*GSTM4*A*	wild-type	"Reference" protein levels
	*GSTM4*B*	T2517C change in intron	Protein unchanged
Pi	*GSTP1*A*	313A, 341C, 555C	Ile^{105}, Ala^{114}, Ser^{185}
	*GSTP1*B*	313G, 341C, 555T	Val^{105}, Ala^{114}, Ser^{185}
	*GSTP1*C*	313G, 341T, 555T	Val^{105}, Val^{114}, Ser^{185}
	*GSTP1*D*	313A, 341T	Ile^{105}, Val^{114}
Sigma	*GSTS1*A*	IVS2 + 11 A	"Reference" protein levels
	*GSTS1*B*	IVS2 + 11 C	Protein unchanged
Theta	*GSTT1*A*	wild-type gene	"Reference" protein levels
	*GSTT1*0*	gene deletion	No protein
	*GSTT2*A*	415A	Met^{139}
	*GSTT2*B*	415G	Ile^{139}
Zeta	*GSTZ1*A*	94A, 124A, 245C	Lys^{32}, Arg^{42}, Thr^{82}
	*GSTZ1*B*	94A, 124G, 245C	Lys^{32}, Gly^{42}, Thr^{82}
	*GSTZ1*C*	94G, 124G, 245C	Glu^{32}, Gly^{42}, Thr^{82}
	*GSTZ1*D*	94G, 124G, 245T	Glu^{32}, Gly^{42}, Met^{82}
Omega	*GSTO1*A*	419C, 464-IVS4 + 1 AAG	Ala^{140}, Glu^{155}
	*GSTO1*B*	419C, 464 deleted	Ala^{140}, Glu^{155} deleted
	*GSTO1*C*	419A, 464-IVS4 + 1 AAG	Asp^{140}, Glu^{155}
	*GSTO1*D*	419A, 464 deleted	Asp^{140}, Glu^{155} deleted
	*GSTO2*A*	424A	Asn^{142}
	*GSTO2*B*	424G	Asp^{142}

*Numbering of amino acids includes initiator methionine. Adapted from Reference 108.

homozygous +/+ individuals (113). An assay has been developed that can identify heterozygotes at the GSTT1 locus (113a) though useful medical applications remain to be established.

Besides influencing susceptibility to carcinogenesis, *GSTP1* polymorphisms are modifiers of response to chemotherapy in patients with metastatic colorectal cancer (114) and those with multiple myeloma (115). It also influences risk of therapy-related acute myeloid leukemia in patients successfully treated for breast cancer, non-Hodgkin's lymphoma, ovarian cancer, and Hodgkin's disease (116).

By contrast with the weak effect that class Mu, Pi, and Theta GST polymorphisms have on tumorigenesis, a number of studies indicate that loss of these genes increase susceptibility to inflammatory diseases, such as asthma and allergies, atherosclerosis, rheumatoid arthritis, and systemic sclerosis (117–119).

In addition to allelic variants in class Mu, Pi, and Theta GST, polymorphisms have also been identified in all the other classes of cytosolic GST (120–122). Class Alpha represents quantitatively a major group of transferases in the liver and these enzymes presumably influence substantially detoxification processes. It has been shown that both *GSTA1* and *GSTA2* are polymorphic, and the various alleles either influence the amount of protein synthesized or the activity of the encoded proteins (84, 123, 124). Further, *GSTM4* and *GSTT2* exhibit promoter polymorphisms that are of functional significance (125). It will be interesting to know whether polymorphisms in these genes influence not only susceptibility to degenerative disease but also efficacy of therapeutic drugs or adverse drug reactions.

Polymorphisms Among MAPEG Members

Several of the *MAPEG* genes have been reported to show variations in the population. As many as 46 single-nucleotide polymorphisms (SNPs) in *MGST1* have been reported in 48 healthy Japanese volunteers (126), and 25 diallelic variants in *MGST3* have been reported in Pima Indians (127); however, the number of true alleles these SNPs reflect, and their biological significance, still requires evaluation in larger populations and in other ethnic groups. Promoter polymorphisms have been reported in the LTC_4S gene, −1072G/A, and −444A/C, and these appear to influence lung function (128). In the *FLAP* gene, also called *ALOX5AP*, 48 out of a possible 144 SNPs have been verified in 186 individuals from Iceland (129). Among a population of 779 Icelandic individuals, a four-SNP haplotype was found to associate with myocardial infarction and stroke, and this was attributed to increased production of LTB_4 (129).

CONSEQUENCE OF KNOCKOUT OF *GST* GENES

Disruption of Mouse Cytosolic *GST* Genes

Table 3 lists the mouse glutathione transferase genes (data taken from 130–132). A number of these have been disrupted by homologous recombination. The gene knockout (KO) mice often show altered sensitivity to xenobiotics, and they reveal

TABLE 3 Mouse GST genes

Class or family	Gene name**	Previous designations for subunits	Accession number*	Chromosomal location
Alpha	*GSTA1*	Ya	pNP_032207	9
	GSTA2	Ya2	pNP_032208	9
	GSTA3	GT10.6, Ya3, Yc	pCAA46155	1
	GSTA4	Yk, GST5.7	pNP_034487	9
	GSTA5	α5	—	—
Mu	*GSTM1*	GT8.7, Yb1	pNP_034488	3
	GSTM2	Yb2	nAF319526	3
	GSTM3	GT9.3, μ4	pP19639	3
	GSTM4	Yb5, μ7	pNP_081040	3
	GSTM5	Fsc2, mGSTM5	pNP_034490	3
	GSTM6	(also called mGSTM5)	nAJ000413	3
	GSTM7	μ3	nAK002213	3
Pi	*GSTP1*	Yf, piB	pNP_038569	19
	GSTP2	Yf, piA	pNP_861461	19
Sigma	*Ptgds2*	—	pNP_062328	6
Theta	*GSTT1*	5	pNP_032211	10
	GSTT2	Yrs	nNM_010361	10
	GSTT3	—	nNM_133994	10
Zeta	*GSTZ1*	MAAI	pNP_034493	12
Omega	*GSTO1*	p28	pNP_034492	19
	GSTO2	—	pNP_080895	19
Kappa	*GSTK1*	—	pAAP20655	6
MAPEG, subgroup I	*MGST2*	—	nBC028535	3
	FLAP	—	nBC026209	5
	LTC_4S	—	nNM_008521	11
MAPEG, subgroup II	*MGST3*	—	nNM_025569	1
MAPEG, subgroup IV	*MGST1*	—	nNM_019946	6
	Ptges1	—	nNM_022415	2

*Superscript prefix n = accession number for nucleotide sequence, superscript prefix p = accession number for protein sequence.

**The genes encoding the cytosolic class Sigma GSTS1 and the MAPEG PGES1 are called *Ptgds2* and *Ptges1*, respectively.

This is adapted from the Web site established by Dr. William Pearson on mouse GST (132). The nomenclature for Mu-class GST differs from that of Andorfer et al. (162): The subunit they called μ3 is GSTM7, the subunit they called μ4 is GSTM3, and the subunit they call μ7 is GSTM4.

that loss of certain GST isoenzymes causes an upregulation of the remaining transferases.

CLASS ALPHA GST Homozygous nulled *GSTA4* mice appear normal but are more susceptible to bacterial infection and display increased sensitivity to paraquat (133). The GSH-conjugating activity toward 4-HNE in this mouse line was

reduced to between 23% and 64% of wild-type levels in the tissues examined, but it was particularly marked in brain, heart, kidney, and lung. Substantial increases in 4-HNE and malondialdehyde were found in the livers of KO animals (133). The livers and brain of *GSTA4*$^{-/-}$ mice contained increases in mRNA for GSTA1/2, GSTA3, GSTM1, catalase, superoxide dismutases 1 and 2, and GPx1. Activation of ARE-driven gene expression (78) appears to be one of the mechanisms by which these genes are upregulated in *GSTA4* KO mice. Certainly, 4-HNE is a Michael reaction acceptor (75) and many cancer chemopreventive blocking agents that induce GST can be included in this category of compound (134). It is therefore presumed that induction of transferases and antioxidant proteins in the mutant mice represents a compensatory response to increases in the intracellular levels of reactive aldehydes resulting from loss of GSTA4-4.

The GSTA4 subunit is induced in mice fed on diets containing the cancer chemopreventive agents α-angelicalactone, butylated hydroxyanisole, ethoxyquin, indole-3-carbinol, limettin, oltipraz, or sulforaphane (135). These data suggest the mouse *GSTA4* gene contains an ARE. Consistent with this hypothesis, we have found, using a bioinformatic search, that the 5′-upstream region of mouse *GSTA4* contains the sequence 5′-TGAGTCAGC-3′. This sequence closely resembles the 5′-TGAGTCGGC-3′ ARE in mouse *NAD(P)H:quinone oxidoreductase 1* (136); both differ from the prototypic core ARE, 5′-TGACnnnGC-3′ (137), in having a G rather than a C at nucleotide position 4 (shown underlined). Assuming this putative ARE in *GSTA4* is functional, induction of the gene by 4-HNE is likely to be mediated by Nrf2. It is envisaged that increased concentrations of 4-HNE lead to modification of cysteine residues in Keap1, stabilization and nuclear accumulation of Nrf2, and increased GSTA4-4 and glutathione levels, resulting in increased capacity to metabolize 4-HNE (see Figure 5, color insert). According to these predictions, mouse GSTA4-4 appears to comprise part of an autoregulatory homeostatic defense mechanism against lipid peroxidation products. Another characteristic of the putative ARE in *GSTA4* is that it contains an embedded 12-*O*-tetradecanoylphorbol-13-acetate (TPA) response element and may therefore also be regulated by the c-Jun transcription factor; for a review of transcriptional regulation of genes through the ARE and related enhancers, see References 138 and 139.

CLASS Mu GST A mouse line lacking *GSTM5*, which encodes the brain/testis-specific transferase, has been generated, but no clear phenotype has been reported to date (140).

CLASS Pi GST Mice lacking both *GSTP1* and *GSTP2* have been generated (141). Under normal conditions, the double gene knockout on 129MF1 or C57/BL6 backgrounds had no obvious phenotype. At a biochemical level, the mutant mice demonstrated a complete lack of transferase activity toward ethacrynic acid in the liver (141). Although GSTP1-1 is quantitatively the principal transferase in male mouse liver, Western blotting failed to demonstrate compensatory increases in expression of hepatic GSTA1/2, GSTA3, and GSTM1 subunits in the double gene

KO animals (141). However, livers from *GSTP1/P2*$^{-/-}$ mice have been reported to contain a higher activator protein-1 activity than livers from *GSTP1/P2*$^{+/+}$ mice (142), a finding that is consistent with the hypothesis that class Pi GST inhibits JNK (21, 89).

In a skin tumorigenesis regimen, *GSTP1/P2*$^{-/-}$ mice yielded approximately threefold more papillomas using 7,12-dimethylbenzanthracene as initiator and TPA as promoter (141), demonstrating a role for GSTP1-1 in xenobiotic defense. Surprisingly, *GSTP1/P2*$^{-/-}$ mice are more resistant than wild-type mice to liver toxicity caused by the analgesic acetaminophen, and this is attributed to faster regeneration of hepatic GSH in the double gene KO animals (143). It was proposed that while Pi-class GST does not catalyze the conjugation of acetaminophen with GSH, it contributes to oxidative stress by facilitating redox-cycling of the acetaminophen metabolite NAPQI, possibly through formation of labile *ipso* adducts with intracellular thiol groups (143). It is postulated that the absence of Pi class GST lessens the ability of NAPQI to redox-cycle and thus deplete GSH.

CLASS SIGMA GST This class of GST encodes the hematopoietic, or GSH-dependent, prostaglandin D_2 synthase. Knockout of the gene for this enzyme results in generation of mice with an allergic reaction that is weaker than wild-type mice (144).

CLASS ZETA GST The murine *GSTZ1* gene, encoding maleylacetoacetate (MAA) isomerase (MAAI) has been disrupted on C57/BL6, 129SvJ, and BALB/c genetic backgrounds. Under normal dietary conditions, the *GSTZ1*$^{-/-}$ mice on C57/BL6 and 129SvJ backgrounds appeared healthy. However, rapid weight loss occurred when the mutant mice were provided with drinking water containing 2% phenylalanine, resulting in death between 5 and 50 days (145). By contrast, under normal dietary conditions, *GSTZ1*$^{-/-}$ mice on a BALB/c background showed enlargement of liver and kidney as well as splenic atrophy (146). When administered 3% phenylalanine in the drinking water, the adult mutant BALB/c mice developed liver necrosis, macrovesicular steatosis, and a loss of circulating leucocytes.

At a biochemical level, livers from *GSTZ1*$^{-/-}$ mice lacked activity toward maleylacetone and chlorofluoroacetic acid, suggesting there is no enzymatic redundancy for GSTZ1-1/MAAI activity. Large increases in fumarylacetoacetate, and modest increases in succinylacetone were observed in the urine of mutant mice (145). The latter metabolite was also observed in blood of *GSTZ1*$^{-/-}$ mice (146). The presence of fumarylacetoacetate in the urine of the KO mice suggests that this MAA metabolite can be formed in extrahepatic tissue by an alternative catabolic pathway (145). The pathophysiological effects observed in the *GSTZ1*$^{-/-}$ animals were attributed to failure to eliminate either succinylacetone or other MAA-derived metabolites (146). The phenotype observed in the mutant mice was exacerbated by inclusion of phenylalanine in the diet.

Hepatic detoxication and antioxidant enzymes are induced as a consequence of perturbations in tyrosine degradation in the *GSTZ1*$^{-/-}$ mice. The GSTA1/2,

GSTM1, and GSTP1/2 subunits, as well as NAD(P)H:quinone oxidoreductase (NQO1), are increased in the livers of *GSTZ1*$^{-/-}$ mice fed on a control diet (145, 146). It appears likely that succinylacetone, and possibly MAA or succinylacetoacetate, are responsible for enzyme induction in these mice. It is noteworthy that certain metabolites that accumulate in the *GSTZ1*$^{-/-}$ mice are capable of modifying protein thiol groups (147). This feature infers that enzyme induction is a response to redox stress (Figure 6). It is not known whether the metabolite(s) that affects gene induction is also responsible for the pathological changes.

Disruption of Mouse *MAPEG* Genes

The *MAPEG* genes in subgroups I and IV have been disrupted. The resulting mice clearly show *MAPEG* genes are involved in allergic and inflammatory processes. No evidence has been reported that they combat oxidative stress in vivo, although this is anticipated from their Se-independent glutathione peroxidase activity.

MAPEG SUBGROUP I Mice lacking the *FLAP* gene are unable to make leukotrienes. Following stimulation with the calcium ionophore A23187, primary cultures of peritoneal macrophages from *FLAP*$^{-/-}$ mice did not synthesize LTC_4 (148). However, production of PGE_2 and thromboxane B_2 was increased by stimulated peritoneal macrophages from *FLAP*$^{-/-}$ mice to a level beyond that seen in wild-type macrophages. In experimental peritonitis affected by Zymosan A, analyses of peritoneal lavage fluid revealed no LTC_4 synthesis in mutant mice but significant amounts of LTC_4 synthesis in wild-type mice. Importantly, no metabolites of the 5-lipoxygenase pathway, such as 5-HETE and LTA_4, were found in lavage of the *FLAP*$^{-/-}$ mice, suggesting FLAP is essential for the synthesis of all leukotrienes. Topical application of arachidonic acid to the ears of mutant mice elicited a reduced inflammatory response as measured by edema.

Mice with the *LTC_4S* gene disrupted develop normally and are fertile. In vitro conjugation of LTA_4 methyl ester with GSH in colon, spleen, lung, brain, and tongue prepared from *LTC_4S*$^{-/-}$ mice was reduced to $\leq$10% of that in wild-type mice (149). By contrast, in testis of the KO animals conjugation of LTA_4 methyl ester with GSH was only reduced to about 65% of the level in wild-type mice, and possibly cytosolic class Mu GST contribute to LTC_4 synthase activity in this organ. Stimulation of LTC_4 production by IgE was abolished in bone marrow–derived mast cells (BMMC) from mutant mice. Also, there was no evidence of production of the LTC_4 metabolites, LTD_4 and LTE_4, in IgE-stimulated BMMC from *LTC_4S*$^{-/-}$ mice. By contrast, LTB_4, 5-HETE, and PGD_2 were produced by BMMC from *LTC_4S*$^{-/-}$ mice (149). Examination of an acute inflammatory response in *LTC_4S*$^{-/-}$ mice by intraperitoneal injection with Zymosan A revealed that protein extravasation was significantly reduced in the mutant mice, and this was associated with failure to produce LTE_4. The ear-swelling anaphylactic response of *LTC_4S*$^{-/-}$ mice was reduced to about 50% of the response seen in *LTC_4S*$^{+/+}$ mice.

MAPEG SUBGROUP IV Mice with disruption of the *Ptges* gene appear normal. Macrophages from *Ptges*$^{-/-}$ mice cultured in the presence of lipopolysaccharide (LPS) for 16 h did not synthesize PGE_2 but did produce IL-6, whereas macrophages from wild-type mice produced both PGE_2 and IL-6 (150). In vivo examination of the arthritic response to immunization with chicken type II collagen showed that the null mouse was protected against fibroplasias, inflammation, proteoglycan damage, cellular infiltration, and cartilage damage associated with the disease (150).

Fever that occurs during inflammatory processes and infection arises in part from PGE_2 synthesis in the brain that acts on EP_3 receptor-expressing neurons in the hypothalamus. Following challenge with LPS, little increase above basal levels of PGE_2 was observed in CSF from *Ptges*$^{-/-}$ mice, whereas substantial increases were observed in CSF from wild-type mice (151). Thus, *Ptges1* partly controls fever that accompanies inflammatory disease.

Knockout of Non-Mammalian GST Genes

In *Proteus mirabilis*, the cytosolic class Beta GST gene has been knocked out, and the resulting bacterial strain was found to be more sensitive to H_2O_2, CDNB, fosfomycin, and minocycline (24). In *Drosophila melanogaster*, a gene encoding a protein homologous to mammalian MGST1 has been disrupted, and the resulting fly line had a reduced life span (152).

REGULATION OF GST BY ENDOGENOUS ELECTROPHILES THROUGH THE Keap1/Nrf2 PATHWAY

The fact that a significant number of cytosolic GST subunits are upregulated in *GSTA4*$^{-/-}$ and *GSTZ1*$^{-/-}$ mice indicates that the expression levels of these transferases is dictated in part by endogenous substrates. This is consistent with the proposal that GST isoenzymes detoxify endogenous carbonyl-containing compounds in vivo. In the case of *GSTA4*$^{-/-}$ mice, the principal regulatory endobiotic is probably 4-HNE (Figure 5). In the case of *GSTZ1*$^{-/-}$ mice, it is likely that upregulation of class Alpha, Mu, and Pi transferases is stimulated by the tyrosine catabolites MAA, succinylacetoacetate, or succinylacetone (Figure 6).

Conditional disruption of the selenocysteine $tRNA^{[Ser]Sec}$ (*Trsp*$^{-/-}$) in the livers of mice, by crossing onto an *albumin-Cre* transgenic background, leads to a loss of the Se-dependent GPx1 and a marked increase in class Mu GST (153). Se-deficient rats, which like *Trsp*$^{-/-}$ mice have an impaired ability to synthesize selenoproteins, possess large increases in hepatic class Alpha, Mu, and Theta GST, as well as aldo-keto reductase 7A1 (154). This observation suggests that the *Trsp*$^{-/-}$ mice almost certainly overexpress many antioxidant enzymes besides class Mu GST. In the mutant mice and Se-deficient rats, the stimulus for GST induction is presumed to be increases in intracellular levels of hydroperoxides and H_2O_2.

It is postulated that as 4-HNE, tyrosine breakdown products, hydroperoxides, and H_2O_2 can all modify protein thiol groups, the Keap1/Nrf2 pathway mediates

induction of *GST* genes in the KO animals described above. According to this proposal, increased levels of 4-HNE, either MAA or its metabolites, and peroxides in the *GSTA4*$^{-/-}$, *GSTZ1*$^{-/-}$, and *Trsp*$^{-/-}$ mice modify Keap1, causing accumulation of Nrf2 and its translocation to the nucleus. Thereafter, Nrf2 is recruited to ARE enhancers in the promoters of inducible genes. A substantial number of GST genes have been found to contain an ARE or related sequences. Table 4 provides a compilation of those *GST*, *NQO1*, and *SOD1* genes that contain such elements (136, 137, 139, 155–162) and could therefore be regulated by this mechanism; it also contains inducible *GST* genes that have ARE-like sequences that have yet to be shown to be functional enhancers (these uncharacterized enhancers are

TABLE 4 Comparison between antioxidant response elements in *GST*, *NQO1*, and *SOD1* genes

Species	Gene	Enhancer	5′-USR	Enhancer	
Rat	*GSTA2*	ARE	gctaa	**TG**g **TGAC**aa**AGCA**	−687
Rat	*GSTA5*	ARE	gacac	g**GC TGAC**ag**AGC**g	−470
Rat	*GSTP1*	GPEI	agtca cta	**TGA**t**TCAGCA**	−2430
Mouse	*GSTA1*	EpRE	gctaa	**TG**g **TGAC**aa**AGCA**	−719
Mouse	*GSTA3*	ARE	ctcag gca	**TGAC**att**GCA**	−138
Mouse	*GSTA4*	n.c.	ctcag	**T**aa **TGA**g**TCAGC**g	−147
Mouse	*GSTM1*	n.c.	tgaac	**T**tg **TGAC**agt**GCA**	−1643
Mouse	*GSTM2*	n.c.	ggagt	**TGC TGAC**a**CAG**gt	−202
Mouse	*GSTM3**	n.c.	tgaac	**T**tg **TGAC**agt**GCA**	−2315
Mouse	*GSTP1*	ARE	caacg	**TG**t **TGA**g**TCAGCA**	−50
Mouse	*GSTP2*	n.c.	caacg	**TG**t **TGA**g**TCAGCA**	−61
Human	*MGST1*	EpRE	ggaca	**T**cg **TGAC**aa**AGCA**	−490
Rat	*NQO1*	ARE	agtca cag	**TGACT**tg**GCA**	−412
Mouse	*Nqo1*	ARE	agtca cag	**TGA**g**TC**g**GCA**	−426
Human	*NQO1*	ARE	agtca cag	**TGACTCAGCA**	−460
Human	*SOD1*	ARE	ataac	**T**aa **TGAC**attt**C**t	−323
		ARE core		**TGAC**nnn**GC**	
		T-MARE		**TGC TGACTCAGCA**	

*The mouse *GSTM3* gene was called *GSTM4* and μ4 in Reference 162.

The core ARE required for gene induction is usually regarded as 5′-TGACnnnGC-3′, based on mutational analysis of the promoter of rat *GSTA2* (137). The nucleotides located in the 5′-upstream region (5′-USR) of the *GSTA2*-ARE have been found to influence basal expression without altering the relative magnitude of induction, and therefore this region is included in the line-up. Nucleotides in capital bold print are those that share identity with the Maf recognition element (MARE); this contains an embedded TPA-response element, denoted by the abbreviation T-MARE (138). The numbering in the right-hand column is the position of the 3′ A nucleotide with respect to the transcriptional start site; in the cases of rat *GSTA5* and mouse *GSTA4* this nucleotide is a G, and in the cases of *GSTM2* and *SOD1* this nucleotide is a T. Data are taken from References 136, 137, 155–162. The abbreviation n.c. stands for not characterized.

indicated by the abbreviation n.c.). The observation that disruption of *GSTA4* and *GSTZ1* genes upregulates the ARE-gene battery supports the hypothesis that the transferases encoded by these genes not only make a major functional contribution to an antioxidant and electrophile defense network but that their substrates are endogenous activators of Nrf2.

The notion that Nrf2 mediates basal expression of GST by endogenous thiol-active endobiotics is supported by the fact that in mice nulled for this transcription factor the normal homeostatic levels of many class Alpha, Mu, and Pi transferases are reduced (163). For example, the levels of mRNA encoding GSTA1, GSTA2, GSTM1, and GSTM3 in the livers of $Nrf2^{-/-}$ mice fed on a normal diet have been reported to be less than 20% of the levels observed in $Nrf2^{+/+}$ mice (131). In addition to changes in expression of cytosolic GST, microarray analyses have shown that expression of *MAPEG* genes is also affected in Nrf2 KO mice (164, 165). Further work is required to establish how important Nrf2 is in regulating GST in species other than the mouse.

It should be appreciated that Nrf2 is not the only transcription factor involved in regulating GST through the ARE. The 5′-upstream region immediately adjacent to the core ARE in genes such as rat *GSTA2*, mouse *GSTA1*, mouse *GSTM2*, mouse *GSTP1*, and mouse *GSTP2* conforms more closely to a TRE-containing Maf recognition element (i.e., T-MARE) than does the same region in rat *GSTP1*, mouse *GSTA3*, or any of the *NQO1* genes; for a review of transcriptional regulation of AREs and MAREs, see Reference 138. It appears that some of these *GST* genes may be regulated entirely by Nrf2-small Maf heterodimers, whereas others may be regulated not only by Nrf2-small Maf heterodimers but also by small and large Maf homodimers. The positive and negative regulation of ARE-driven genes is an area that needs further study.

OVEREXPRESSION OF GSTs DURING TUMORIGENESIS

Expression of GST isoenzymes increases during the development of cancer. The classic Solt-Farber liver chemical carcinogenesis model has been widely studied in this context. This model is established by subjecting rats to the following three-step procedure: (*a*) initiation with diethylnitrosamine, (*b*) selective growth inhibition of noninitiated hepatocytes with 2-acetylaminofluorene, and (*c*) stimulation of liver growth by partial hepatectomy (165a). Examination of this cancer model has revealed that GSTP1 is upregulated >20-fold in both rat preneoplastic nodules and hepatocellular carcinomas (2). This elevation occurs by transcriptional activation through GPEI (155), and recent work has revealed that this is in part mediated by Nrf2 (165b). It appears that sequences immediately 5′ to the GPEI element are required for strong enhancer activity, but the factor(s) involved has not been identified. Members of the ARE-gene battery are often overexpressed during carcinogenesis, and it seems likely that Nrf2 may be responsible for this phenotype.

CONCLUDING REMARKS

This review describes recent advances in knowledge about the transferases. The availability of gene KO models has given unprecedented insights into the in vivo functions of GST and MAPEG proteins. These studies have demonstrated that cytosolic GST are an integral part of a dynamic and interactive defense mechanism that protects against cytotoxic electrophilic chemicals and allows adaptation to exposure to oxidative stress. They have antioxidant and antiinflammatory activities. Similar investigations have shown MAPEG members contribute to inflammatory responses, although it is likely that some are also involved in antioxidant defenses. Further work is required to elucidate the biological functions of the mitochondrial class Kappa GST.

Evidence suggests cytosolic GST metabolize many endogenous and foreign compounds that stimulate expression of the ARE-gene battery. Their catalytic actions therefore negatively regulate Nrf2 by protecting Keap1 from modification of those cysteines (Cys-273 and Cys-288) that are required to capture and destabilize the transcription factor. A most important consequence of this conclusion is that GST indirectly control the levels of other antioxidant and drug-metabolizing enzymes that are regulated through the Keap1/Nrf2 pathway. In addition to phase I, phase II, and phase III detoxication proteins, GST will negatively regulate chaperones, ubiquitin-proteasome components, inflammation-associated proteins, and apoptosis-associated proteins (165, 166).

The gene KO mouse models have revealed the importance of GST in detoxifying 4-HNE and tyrosine catabolites. It is predicted that glutathione transferases similarly contribute to the elimination of 15d-PGJ_2 in vivo. Thus, knockout of certain *GST* genes will cause relative accumulation of 15d-PGJ_2 and constitutive upregulation of PPARγ-driven gene expression and a decrease in expression of NF-κB-driven genes. A possible candidate for this function is GSTA3-3 because its levels increase markedly in mouse 3T3-L1 cells during adipogenesis (70). It can be hypothesized that induction of GSTA3 reflects a cellular response to accumulation of 15d-PGJ_2 designed to metabolize and eliminate the prostanoid.

A possibility that remains to be explored is whether polymorphisms in human *GST* genes influence the activity of Nrf2, PPARγ or NF-κB.

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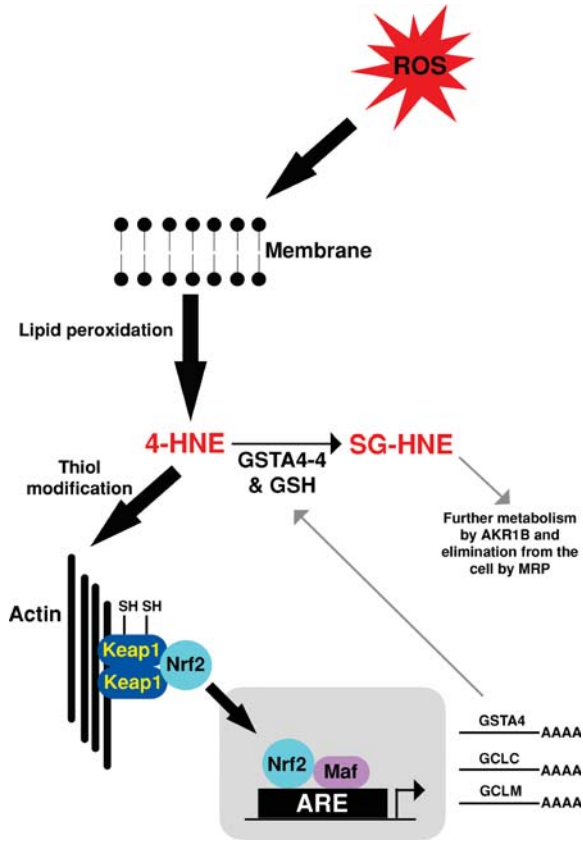

Figure 5 Negative regulation of the ARE-gene battery by GSTA4-4. This cartoon shows how 4-HNE, produced through membrane damage by reactive oxygen species (53), might modify cysteine residues in the cytoskeleton-binding protein Keap1 (73). Such posttranslational modification of Keap1 allows the Nrf2 transcription factor to accumulate and translocate into the nucleus. Once in the nucleus, Nrf2 forms heterodimers with small Maf proteins that are recruited to antioxidant response elements (AREs) in the promoters of antioxidant and detoxication genes. Trans-activation of ARE-driven genes by Nrf2 increases the production of many proteins, including the GSTA4, glutamate cysteine ligase catalytic, and glutamate cysteine modulatory subunits; the latter two comprise the subunits of GCL, which catalyzes the rate-limiting step in the synthesis of GSH. The resulting elevation in amounts of GSTA4-4 and GSH allow increased metabolism of 4-HNE and its elimination from the cell via MRP.

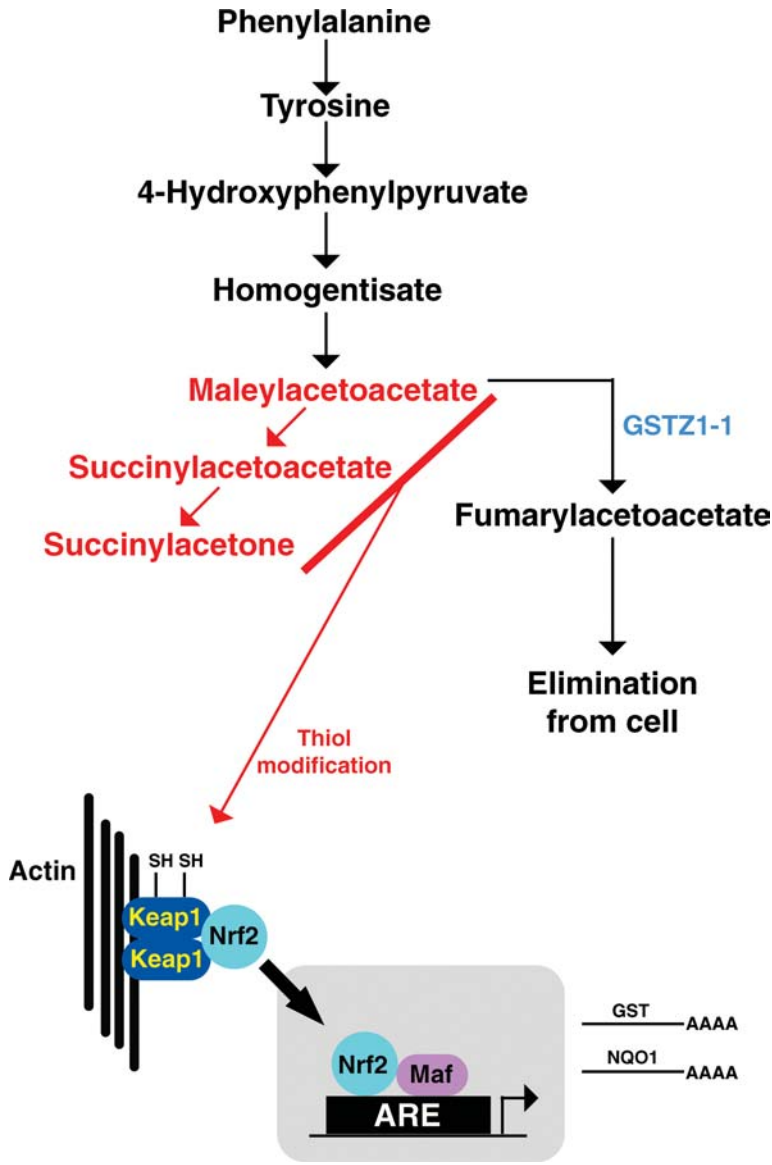

Figure 6 Induction of GST and NQO1 by tyrosine catabolites. Degradation products of tyrosine that accumulate in *GSTZ1* knockout mice stimulate upregulation of class Alpha, Mu, and Pi GST, as well as NQO1 (145, 146). As shown in the figure, the potential inducing agents include maleylacetoacetate, succinylacetone, and succinylacetoacetate. Certain of these tyrosine metabolites are thiol-active (147) and probably induce gene expression through the Keap1/Nrf2 pathway.

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