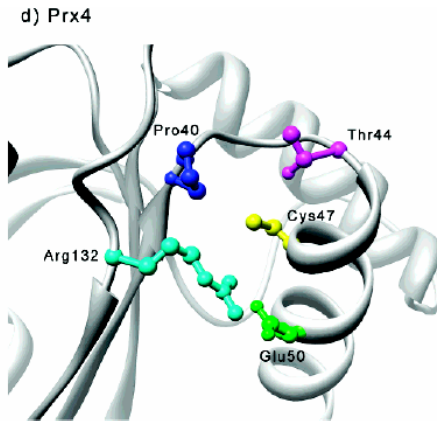
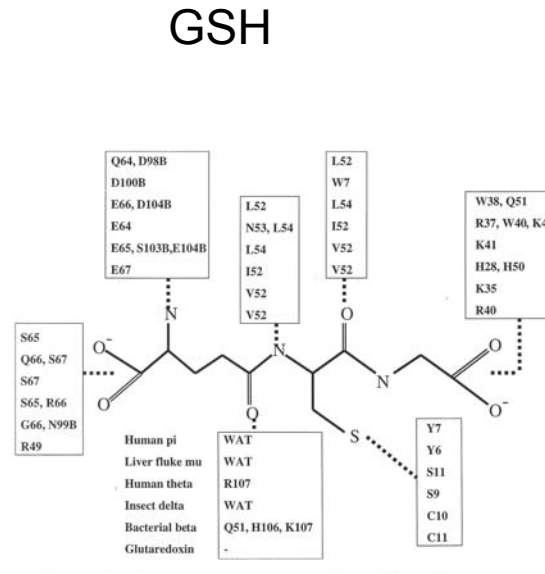


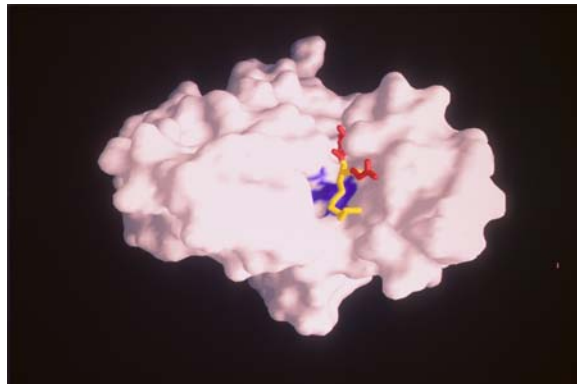
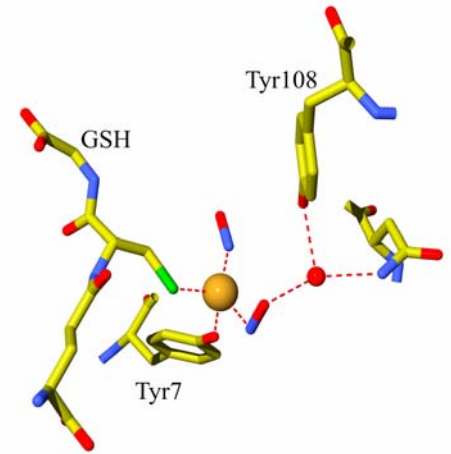
Antioxidant and Detoxificant Defense Systems



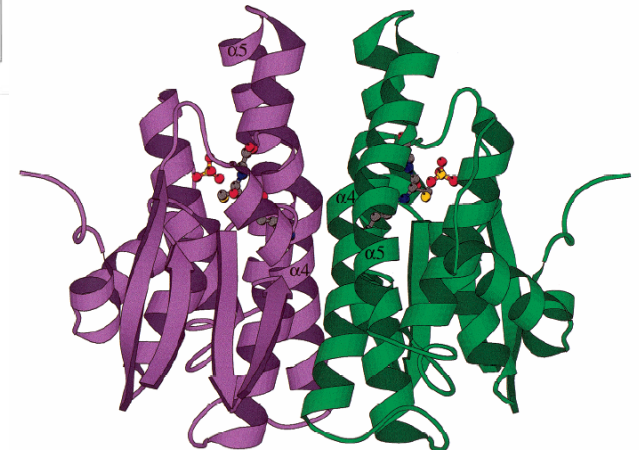
Prx 1



NO donor binding protein

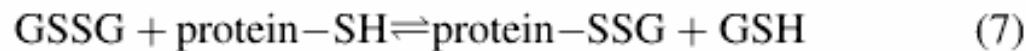
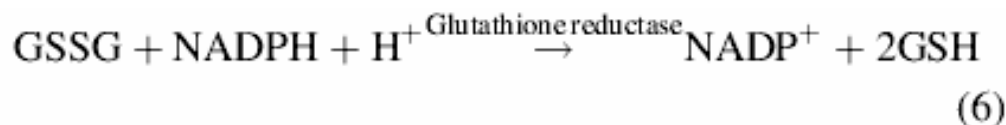
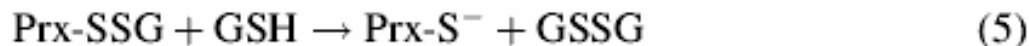
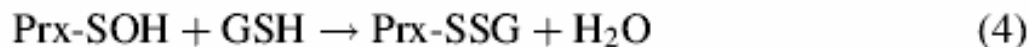
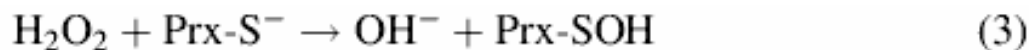
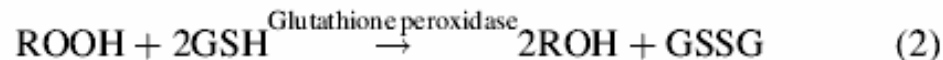
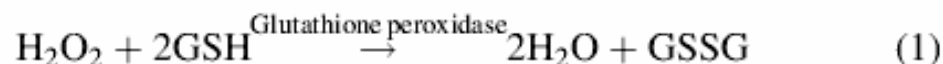


GST P1-1 monomer

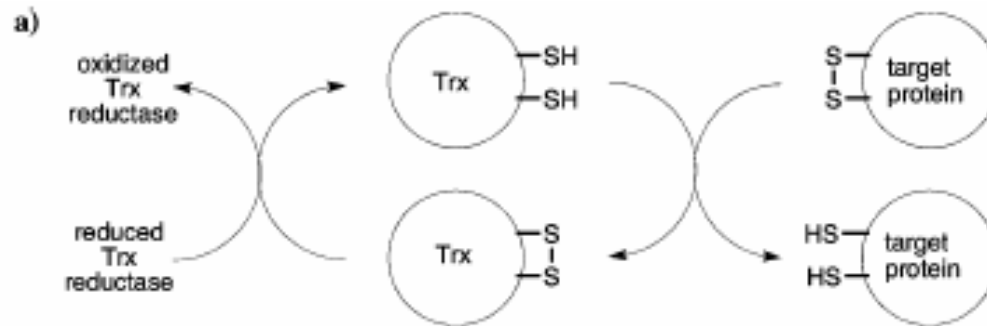


GST Z1-1

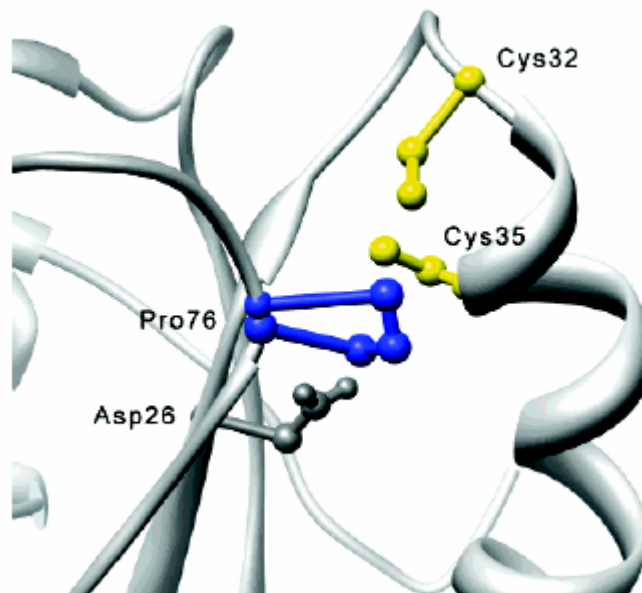
1. GSH functions as substrate in the thiol-disulfide exchange reactions



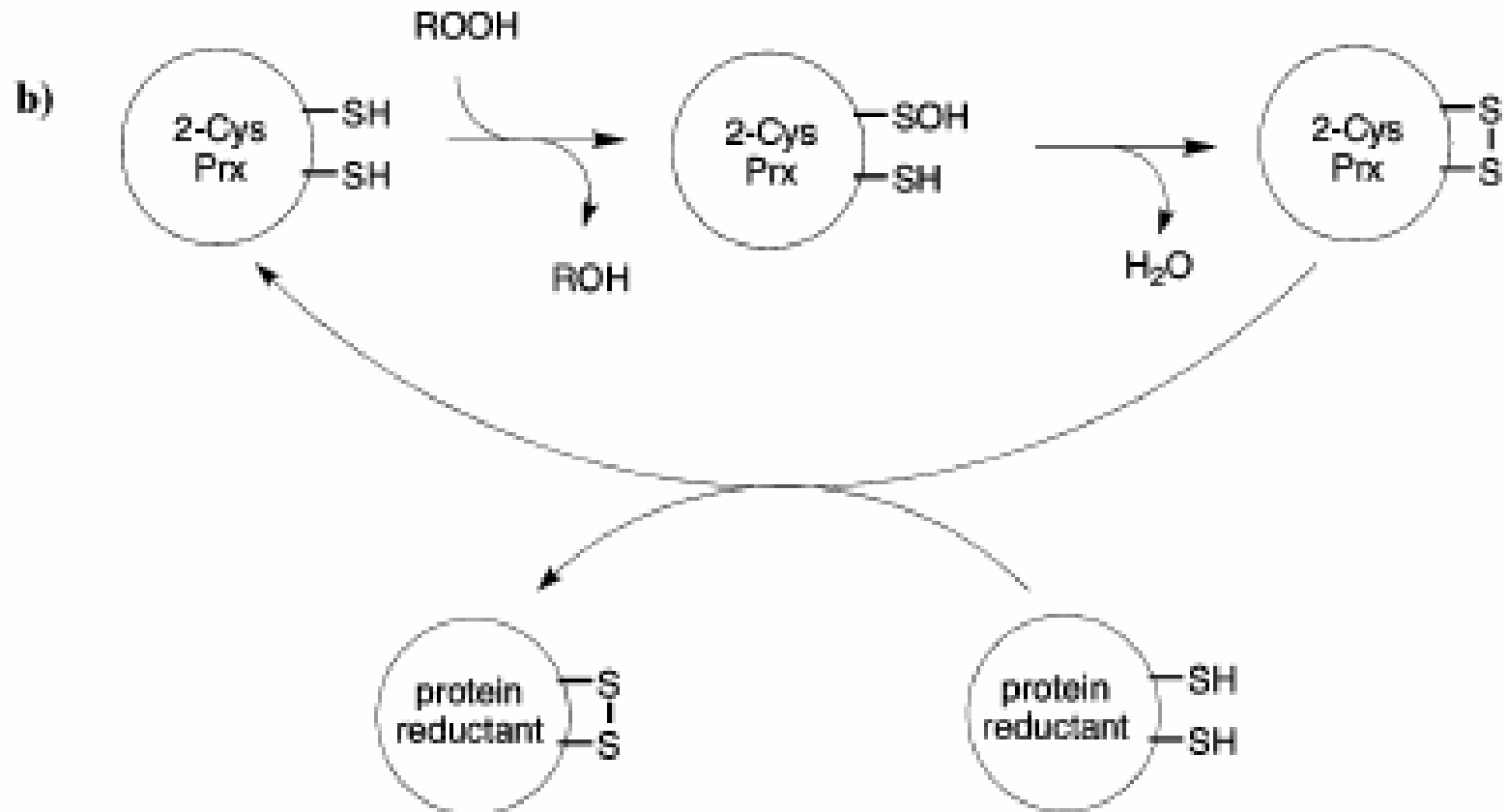
Thioredoxins



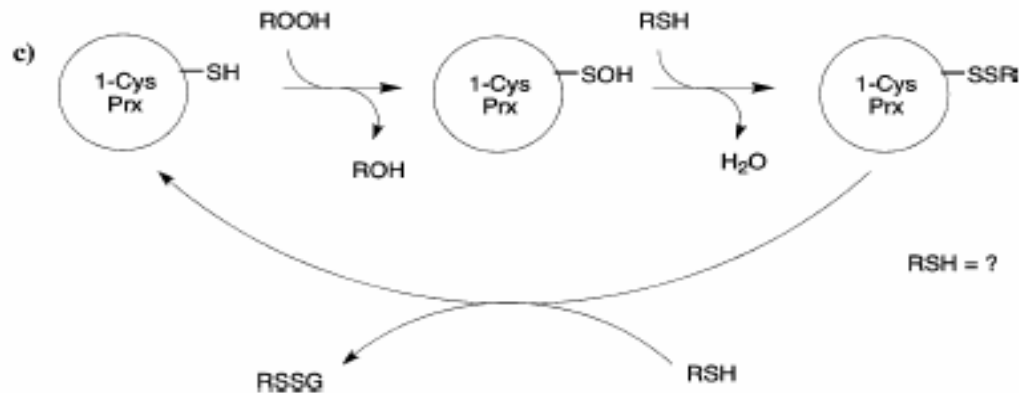
a) Trx



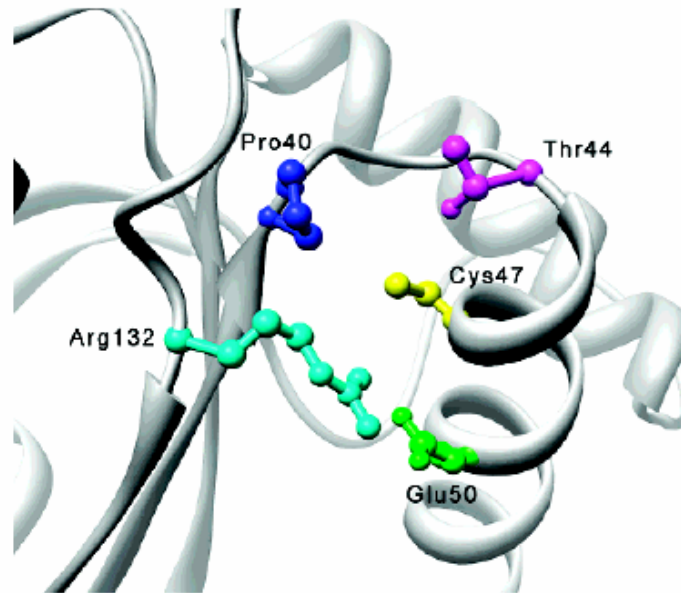
2-Cys peroxyredoxins



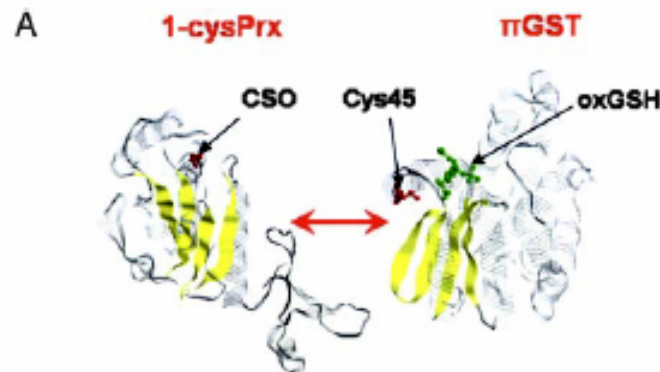
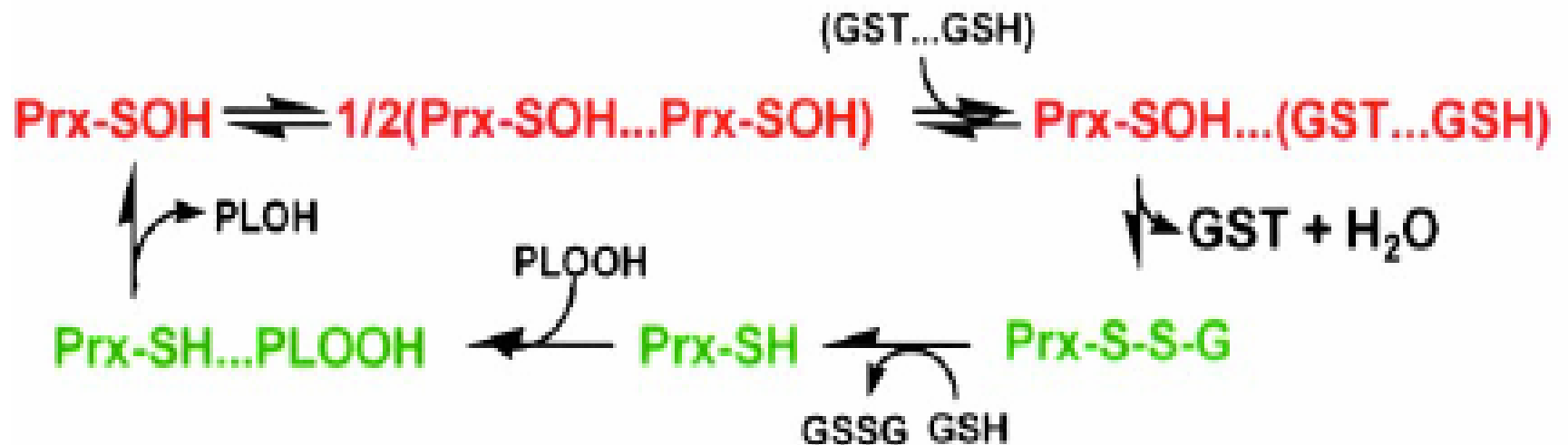
1-Cys peroxyredoxins



d) Prx4



Activation of the antioxidant enzyme 1-CYS
 peroxiredoxin requires glutathionylation mediated
 by heterodimerization with π GST



2. GSH as substrate in conjugating activity of many toxic compounds

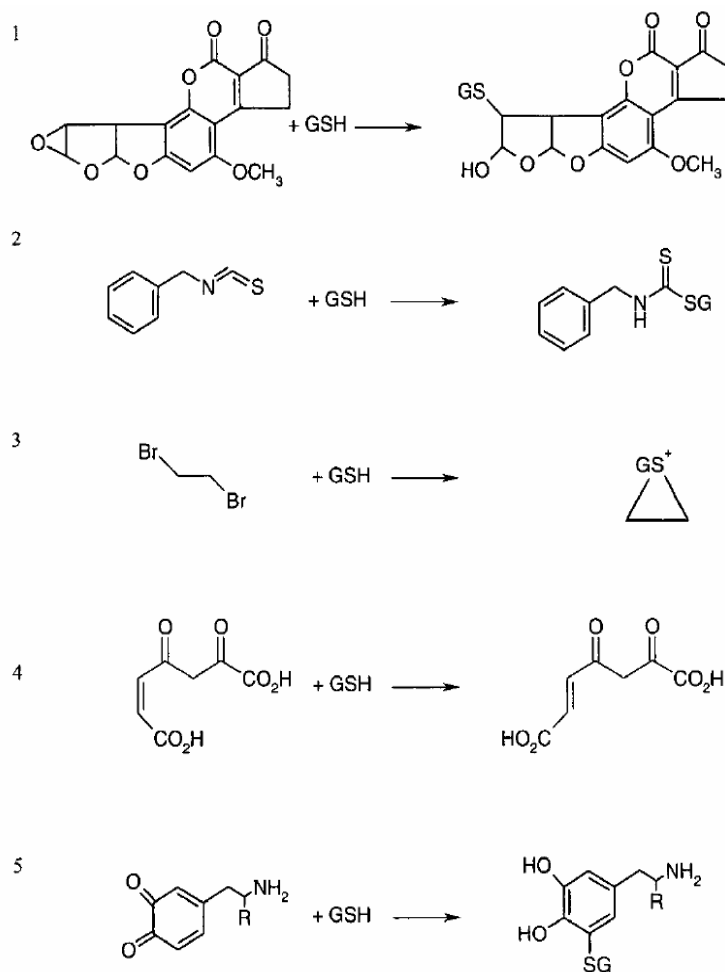


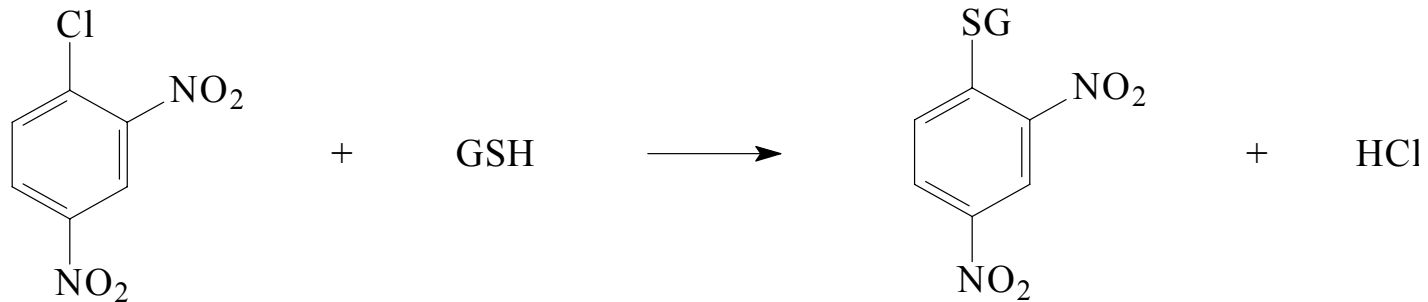
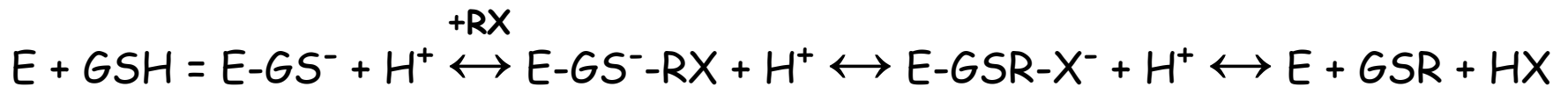
Figure 9.1 Examples of GST catalysed reactions. The GST substrates shown are as follows: 1, aflatoxin B₁-8,9-epoxide; 2, benzylisothiocyanate; 3, dibromoethane; 4, maleylacetoacetate; 5, a model *o*-quinone.

GSTs

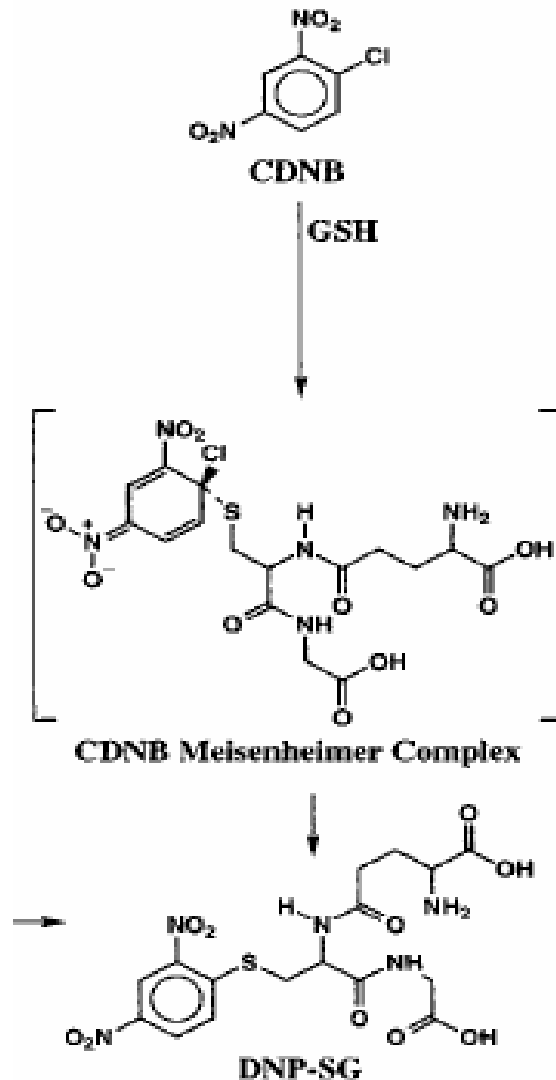


1. A well known function of GSTs is to promote the conjugation of the sulfur atom of glutathione to an electrophilic center of endogenous and exogenous toxic compounds, thereby increasing their solubility and excretion

2. Catalytic mechanism



3. The GSH molecule binds to the active site as thiolate anion



Conjugation of CDNB for glutathione transferase activity

The nucleophilic addition proceeds through the formation of an unstable intermediate: the Sigma complex, or Meisenheimer complex) and then of the stable product dinitrophenylglutathione (DNP-SG).

Multiple levels of defense against reactive oxygen species

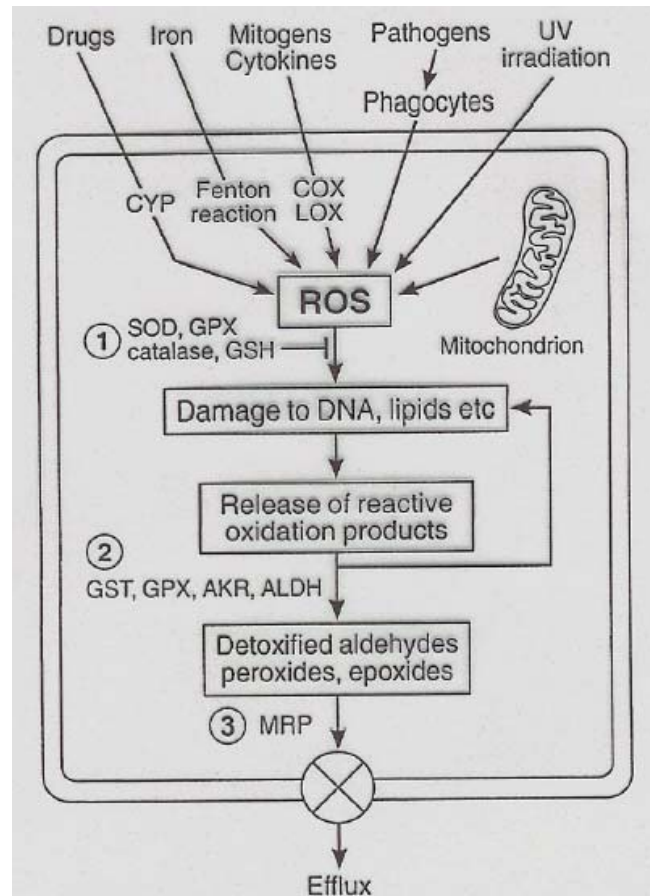
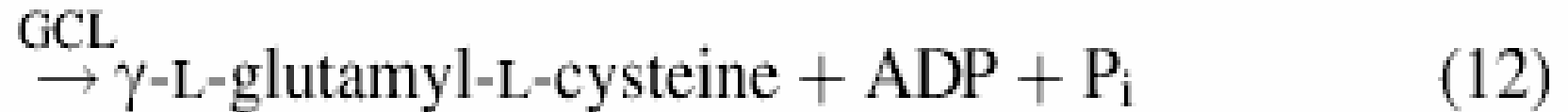


FIGURE 1 Multiple levels of defence against ROS. The cell possesses at least three tiers of defence against ROS. The first of these is provided against the superoxide anion and H_2O_2 by SOD, GPX, catalase and glutathione. The second tier of defence is provided by the detoxication enzymes GST, GPX, AKR and ALDH, and the third tier is provided by the efflux pump MRP. The glutathione-associated components of these defences are inducible and therefore allow adaption to situations in which the cell is subjected to increased levels of ROS.

3. De novo synthesis of GSH

L-glutamate + L-cysteine + ATP



γ -L-glutamyl-L-cysteine + glycine + ATP



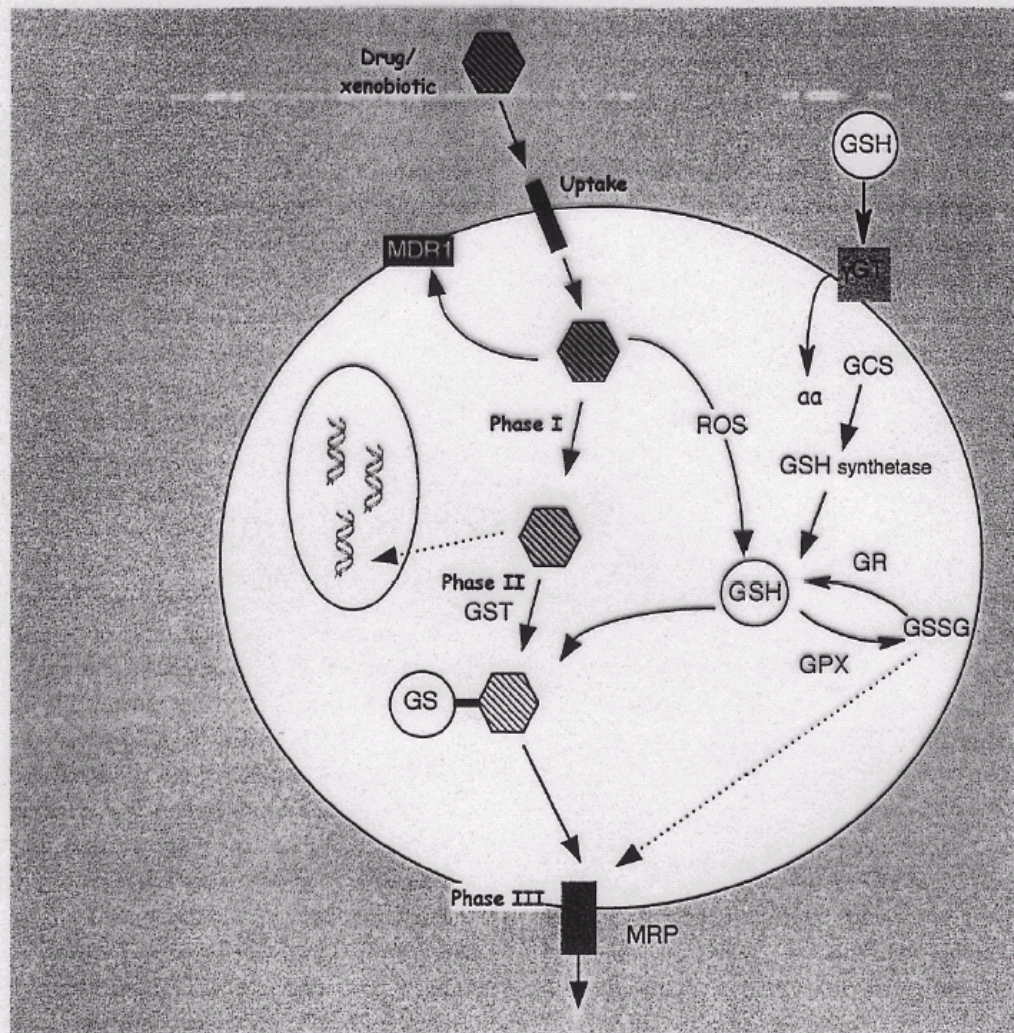


FIGURE 2 *Glutathione-dependent cellular defence mechanisms.* Environmental toxins or drugs can undergo metabolism by cytochrome P450s (phase I) to generate electrophilic cytotoxic intermediates as well as ROS. Glutathione associated detoxification mechanisms play a major role in their detoxification. GST (phase II) catalyse glutathione conjugation reactions, which allows xenobiotics to be recognised by MRP family members which transport conjugates from the cell (phase III), whereupon they can be metabolised further and excreted from the organism. MRP can also transport certain unmodified agents in the presence of glutathione. Glutathione is also important in the reduction of peroxides and the scavenging of free radicals. This can result in the production of GSSG which is either reduced by GR or exported by MRP. Glutathione is synthesised in the cytosol by GCS. γ GT may play a role in salvage of amino acids (aa) from extracellular glutathione.

4. Regulation of the *GSH* synthetic genes

A large number of compounds, including those forming GSH conjugates and those generating oxygen species have been shown to induce GSH biosynthesis through increased transcription of **GCL**, mediated by transcription factors such as:

1. Sp-1, activator protein-1 (TRE)
2. TRE-like (AP-2)
3. Nuclear factor kappa B (kB)
4. Antioxidant responsive element (ARE)

- GCL appears to be rate limiting in GSH biosynthesis and is subjected to feed-back inhibition of GSH
- It is composed by two different subunits: one heavy (73 kDa) (the catalytic subunit and one light (30 kDa) the regulatory subunit.
- The two subunits communicate through hydrophobic and covalent (disulfide bond) interactions.
- The subunits are subject to tissue-specific expression (very high in the kidney)
- There are possible variations in the amounts of either GCL subunit

- GS (glutathione synthetase) is not subject to any regulation but to tissue-specific expression and it has been proposed that in the presence of a high activity of gamma GT (gamma-glutamyl transpeptidase) GSH synthesis can take place without GSL. This would require the transfer of γ -glutamyl to cystine, the import of γ -glutamylcystine, the reduction of disulfide to γ -glutamylcysteine and the ultimate step of the addition of glycine.

Cellular adaptation to environmental factors by variation of GSH content

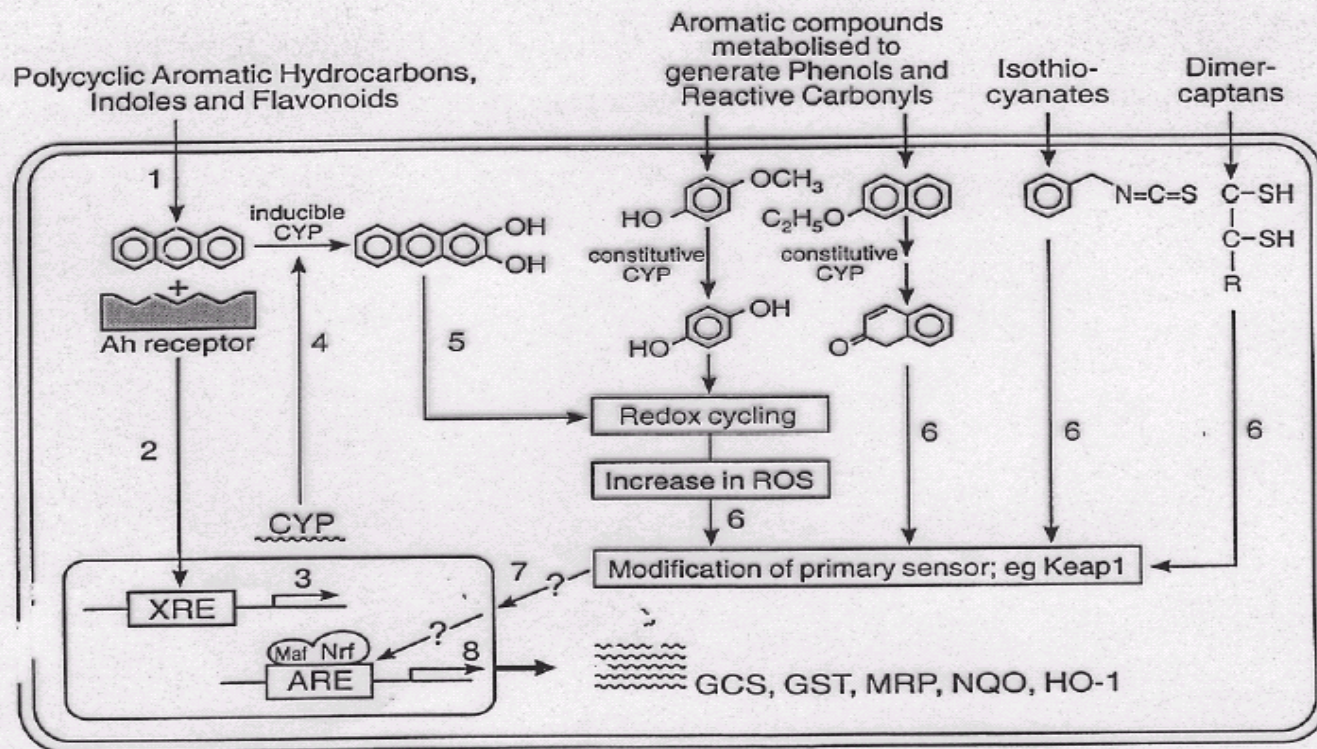


FIGURE 4 Role of intracellular metabolism in gene induction by cancer chemopreventive agents. The chemical structures shown are representative of the types of inducers involved in chemoprevention, and are not intended to represent specific compounds. Inducers which transcriptionally activate genes through the ARE may function directly (e.g. isothiocyanates and dimercaptans). Alternatively, they may require metabolic activation, and are either converted to active inducing agents by CYP isoenzymes that are normally constitutively expressed (e.g. BHA and ethoxyquin), or are converted to active inducing agent by CYP isoenzymes that are not normally expressed and therefore require prior induction themselves (e.g. polycyclic aromatic hydrocarbons, flavonoids and indoles). Chemopreventive agents which require to be oxidised by inducible CYP1A enzymes before they can activate gene expression through the ARE trigger the following steps in order to be effective: 1, binding of chemopreventive agent to the Arylhydrocarbon (Ah) receptor; 2, translocation of the Ah receptor-chemopreventive agent complex to the nucleus; 3, transcriptional activation of xenobiotic responsive element (XRE)-regulated CYP1A genes; 4, oxidation of the agent by CYP1A; 5, redox-cycling of metabolites generated by CYP1A. As shown in step 6, the presence of all inducing agents (whether formed by inducible CYP, constitutive CYP or direct-acting) is detected by one or more intracellular sensors, possibly in a thiol-dependent fashion. The presence of such inducing agents is signalled to the nucleus (step 7) causing Nrf2 and small Maf protein to be recruited to ARE and increased transcription of certain antioxidant and detoxification genes (step 8). The figure is adapted from data presented in Ref. [119].

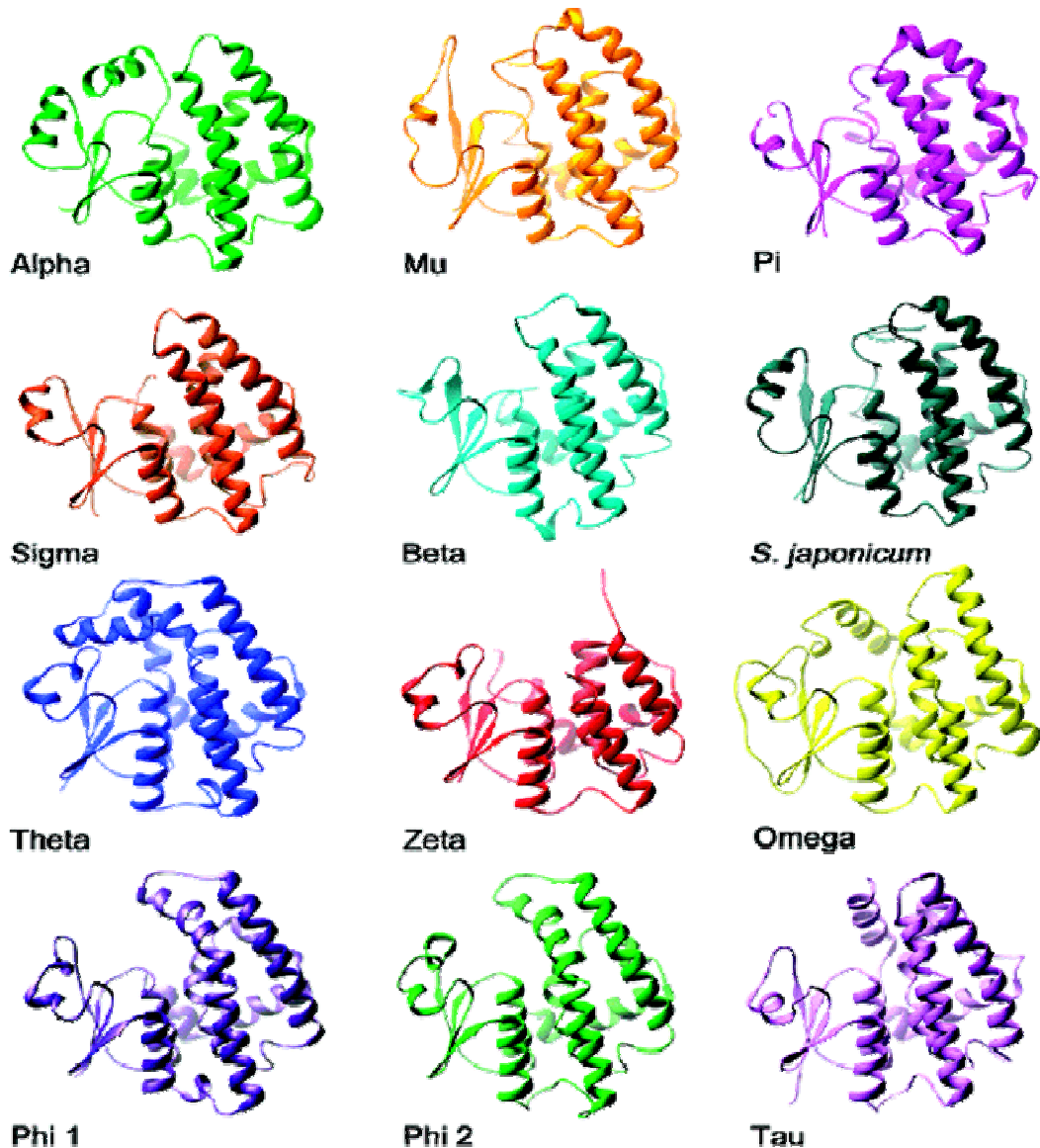
Glutathione transferases (GSTs, EC 2.5.1.18) are grouped in three distinct superfamilies of enzymes:

- 1) The first superfamily is composed of **cytosolic enzymes**, further divided into at least twelve classes. All they share a common fold topology.
- 2) The second is composed of **mitochondrial enzymes** which are only distantly related to the cytosolic enzymes
- 3) The third most recent superfamily is composed of **microsomal enzymes (MAPEG)** with six representative members. They are primarily involved in the biosynthesis of leukotrienes and prostanoids

Different classes of the GST cytosolic superfamily

All soluble GSTs
are dimers
and adopt a
canonical fold
in the monomer

There are, however,
slight differences
which can be
considered the
structural basis for
the substrate
specificity
among the classes



GSTs can be considered as multi-functional enzymes devoted to various aspects of cell defence.

- The Alpha class also displays a peroxidase activity with organic peroxides
- A possible role of "ligandins" (ligand carriers) has been proposed long time ago
- The regulation of Jun kinase protein (a stress-activated protein which phosphorylates c-Jun) may be mediated by GST P1-1
- Certain GSTs (class Theta) may inhibit the proapoptotic action of Bax
- The most recently discovered class Omega, GST O1-1, modulates calcium channels thus protecting mammalian cells from apoptosis induced by Ca^{++} mobilization
- GSTs can be involved also in post translationally modifications of proteins, such as glutathionylation
- A new role has been proposed as NO donor binding proteins

Reactions catalyzed by GSTs

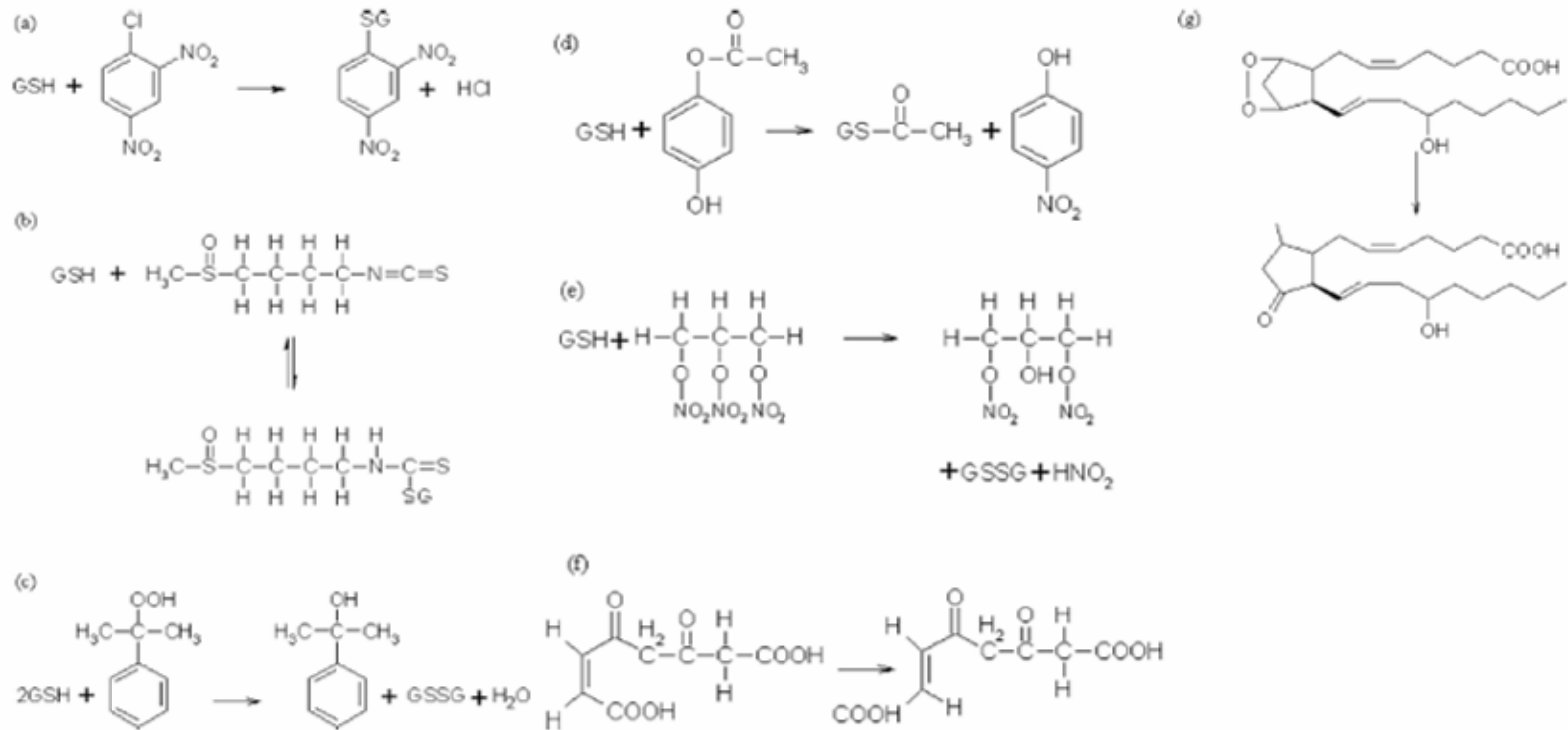


Figure 1 Reactions catalyzed by GST. Example of conjugation, reduction, thiolysis, and isomerization reactions catalyzed by GST. The following substrates are shown: (a) CDNB, (b) sulforaphane, (c) CuOOH, (d) trinitrophenyl acetate, (e) 4-nitrophenyl acetate, (f) maleylacetoacetate, and (g) PGH₂. (conversion to PGD₂ is depicted).

METABOLISM OF XENOBIOTICS BY GST

Detoxification through the Mercapturic Acid Pathway

- 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.
- Exogenous substrates for soluble GST include drugs, industrial intermediates, pesticides, herbicides, environmental pollutants, and carcinogens such as:
- **adriamycin, chlorambucil, *cis*-platin, cyclophosphamide, ethacrynic acid, melphalan, mitozantrone, and thiotepa are detoxified by GST**
- **Environmental chemicals and their metabolites detoxified by GST include acrolein, atrazine, DDT, inorganic arsenic, lindane, malathion, methyl parathion, muconaldehyde, and tridiphan.**

Detoxification through the Mercapturic Acid Pathway

- 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 B1, 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 B1-8,9-epoxide** with GSH is a major mechanism of protection against the mycotoxin, at least in rodents.
- **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 .

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.
- **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. Following export from cells via MRP1 or MRP2, thiocarbamates spontaneously degrade to their isothiocyanates, liberating GSH.

Table 9.1 Compounds activated by glutathione *S*-transferases

Type of activation	Class of compound	Examples
Direct metabolism to reactive intermediate	Halogenated alkanes Oxidised alkenes	Dichloromethane, dibromoethane, trichloropropane, butadiene diepoxide, isoprene diepoxide
Reversible reactions	Isothiocyanates Isocyanates	Benzyl isothiocyanate, phenethyl isothiocyanate methylisocyanate, <i>N</i> -(1-methyl-3,3- diphenylpropyl)isocyanate
β -Elimination of oxidised sulfhydryl of GSH-conjugate	Latent pro-drugs	TER 286
β -Lyase-dependent activation of cysteinyl- conjugates derived from GSH conjugates	Halogenated alkenes	Hexachlorobutadiene, tetrachloroethene

TER 286: γ -glutamyl- α -amino- β [(2-ethyl-*N,N,N',N'*-tetrakis(2-chloroethyl)phosphorodiamidate)-sulphonyl]propionyl-(*R*)-(-)-phenylglycine

METABOLISM OF ENDOGENOUS COMPOUNDS BY GST

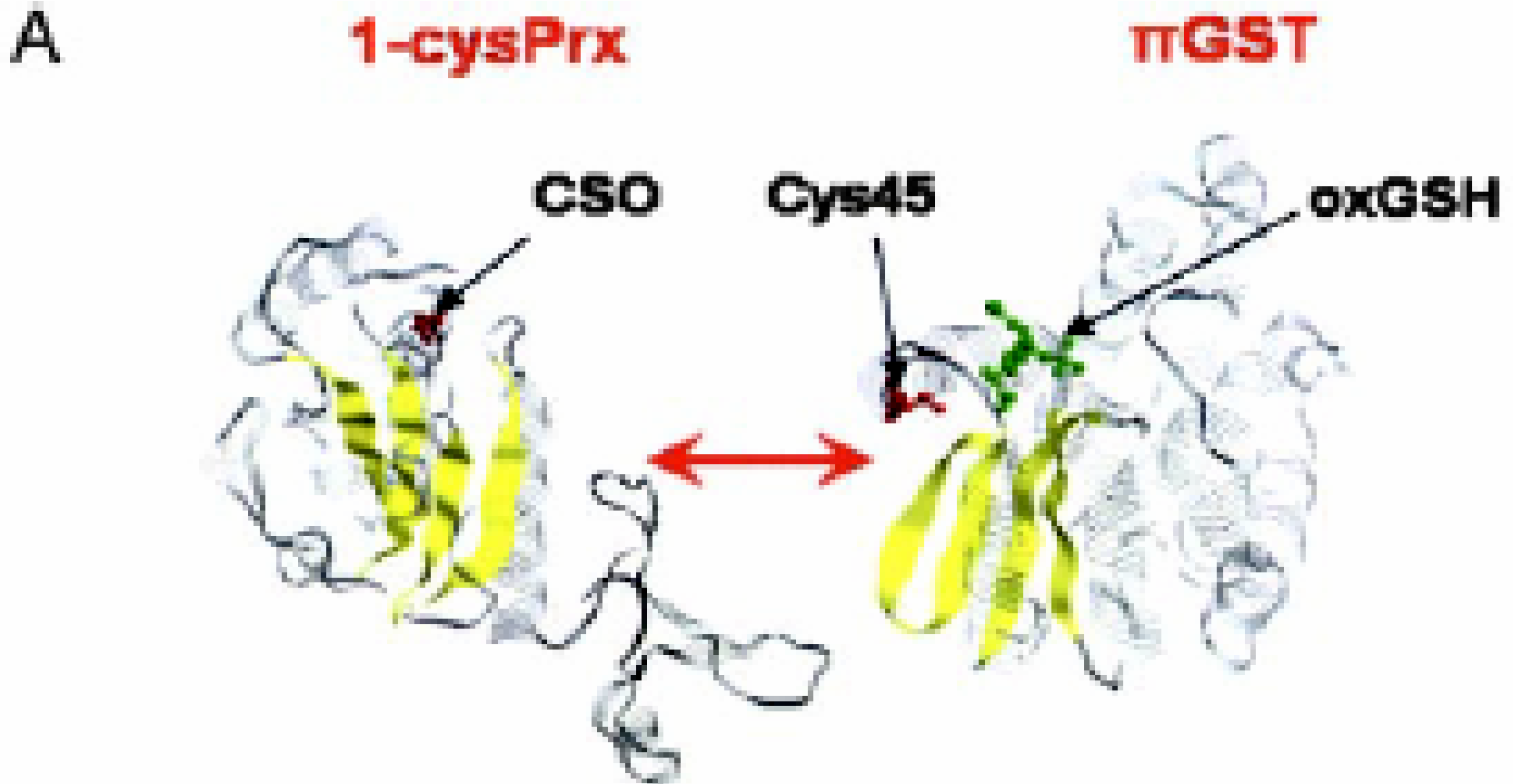
Detoxification of Products of Oxidative Stress

- The production of **reactive oxygen species**, the superoxide anion O_2^- , hydrogen peroxide H_2O_2 , and the hydroxyl radical $HO^\bullet$, 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 xanthine oxidase-catalyzed reactions are also a source
- **Such species are scavenged by the catalytic activities of superoxide dismutase, catalase, and glutathione peroxidase and non enzymatically 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
- . 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.

Detoxification of Products of Oxidative Stress

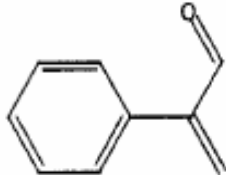
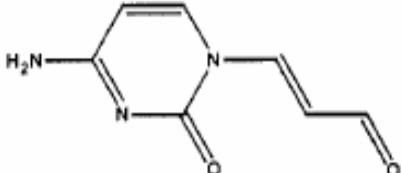
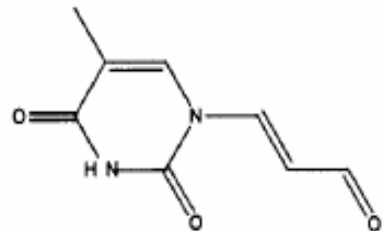
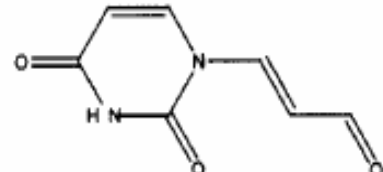
- **Free radical-initiated peroxidation of polyunsaturated fatty acids in membranes** is a particular problem as it results in chain reactions that serve to amplify damage to lipids. The process produces short-lived lipid hydroperoxides that breakdown to yield secondary electrophiles, including epoxyaldehydes, 2-alkenals, 4-hydroxy-2-alkenals, and ketoaldehydes, some of which are genotoxic. Among the end-products of lipid peroxidation, GSTs conjugate GSH with the 2-alkenals **acrolein** and **crotonaldehyde**, as well as 4-hydroxy-2-alkenals of between 6 and 15 carbon atoms in length; conjugation of GSH with the (*S*) enantiomer of 4-hydroxynonenal is favored over the (*R*) enantiomer.
- **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. 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.
- **Oxidation of catecholamines yields aminochrome, dopachrome, noradrenochrome, and adrenochrome** that are harmful because they can produce O₂ by redox cycling.

The 1-cys peroxyredoxin, 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. Through this process, GST may indirectly combat oxidative stress by restoring the activity of oxidized Prx VI.

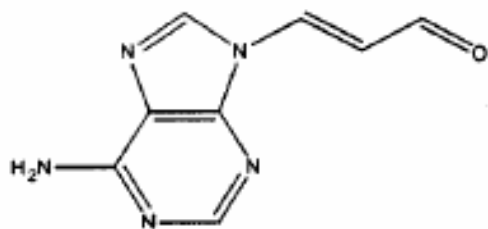


Oxidation of nucleotides yields base propenals and hydroperoxides that are detoxified by GST.

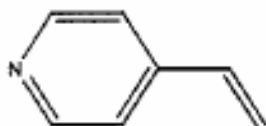
Table 3[~]

Structure	Compound	$k_{cat}/K_m, mM^{-1} \cdot s^{-1}$		
		GSTA1-1	GSTM1-1	GSTP1-1
	2-phenylpropenal	42 ± 5	275 ± 35	164 ± 5
	2-propenal	10	60	350
	crotonaldehyde	<0.1	<0.1	29 ± 0.7
	cytosine propenal	<0.1	<0.1	7 ± 0.8
	thymine propenal	<0.1	<0.1	104 ± 10
	uracil propenal	<0.1	<0.1	222 ± 9

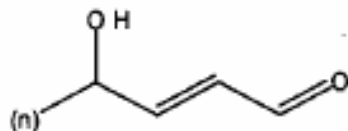
Oxidation of nucleotides yields base propenals, such as adenine propenal, and hydroperoxides that are detoxified by GST.



adenine propenal	6 ± 0.6	46 ± 2	769 ± 54
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4-vinylpyridine	<0.1	<0.1	154 ± 5
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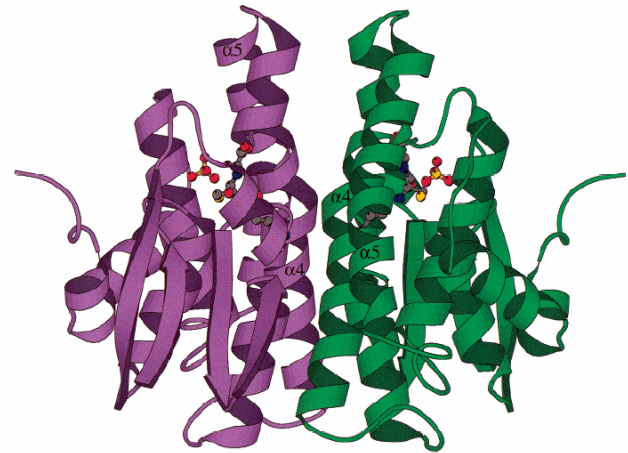


4-hydroxyalkenals

C-5	0.4 ± 0.9	49 ± 14	34 ± 15
C-6	6 ± 5	120 ± 6	19 ± 3
C-7	26 ± 3	401 ± 50	10 ± 3
C-8	76 ± 11	740 ± 13	26 ± 2
C-9	111 ± 21	708 ± 58	39 ± 4
C-10	166 ± 21	839 ± 100	54 ± 7
C-11	227 ± 15	915 ± 125	93 ± 5
C-12	258 ± 20	901 ± 61	93 ± 24
C-14	295 ± 19	786 ± 110	87 ± 8
C-15	197 ± 19	430 ± 79	48 ± 11

Degradation of Aromatic Amino Acids

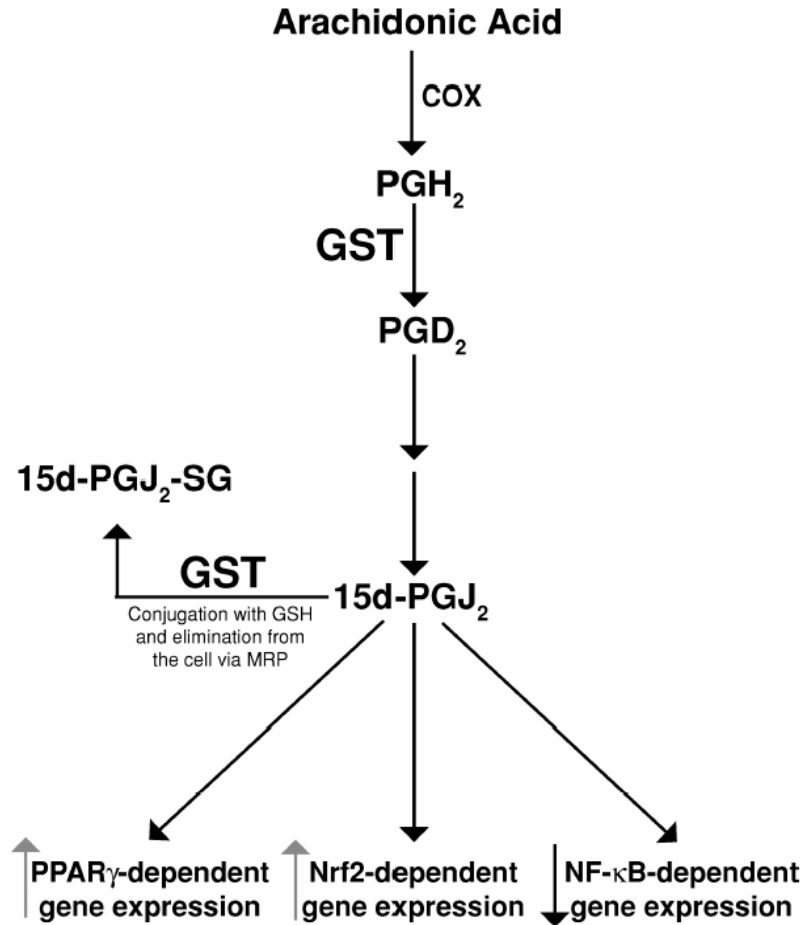
- In mammals, phenylalanine is degraded to acetoacetate and fumaric acid. The five intermediates are tyrosine, 4-hydroxyphenylpyruvate, homogentisate, maleylacetoacetate, and fumarylacetoacetate. The cytosolic **class Zeta GST** has been identified as a maleylacetoacetate isomerase, and therefore catalyzes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



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 PGH₂ to a mixture of PGD₂ and PGE₂, or reduce it to PGF₂ α , it is now clear that certain transferases exhibit remarkable specificity for some of these reactions. The identification of mammalian GSH-dependent prostaglandin D₂ 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 PGD₂ production but also to formation of the downstream cyclopentenone prostaglandin, 15-deoxy- Δ ^{12,14}-prostaglandin J₂ (15d-PGJ₂), which possesses distinct biological activities. Cytosolic transferases expressed in human brain exhibit PGE₂ synthase activity (65). In addition to the cytosolic GST, members of the MAPEG family make major contributions to production of PGE₂ (8), whereas a membrane-bound GSH-activated enzyme has been shown to possess PGF₂ α synthase activity (66). Prostaglandins and isoprostanes containing a cyclopentenone ring also represent GST substrates in glutathione-conjugation reactions (Figure 2) (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 LTC₄.

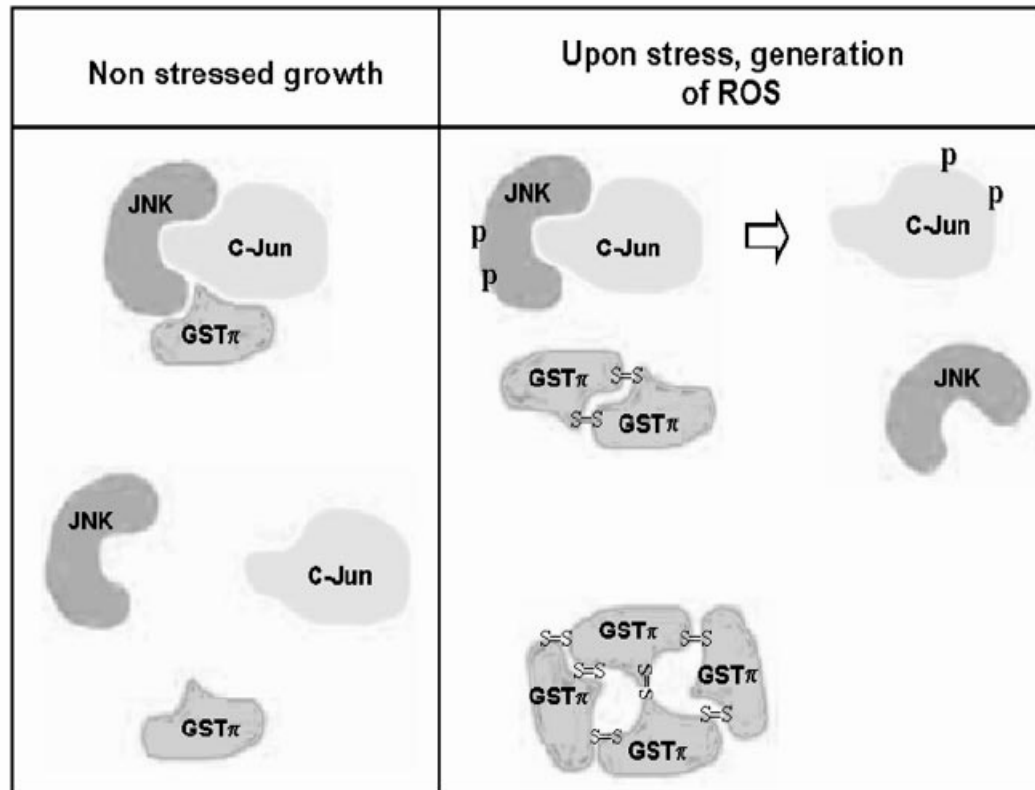
Modulation of Signaling Pathways by GST



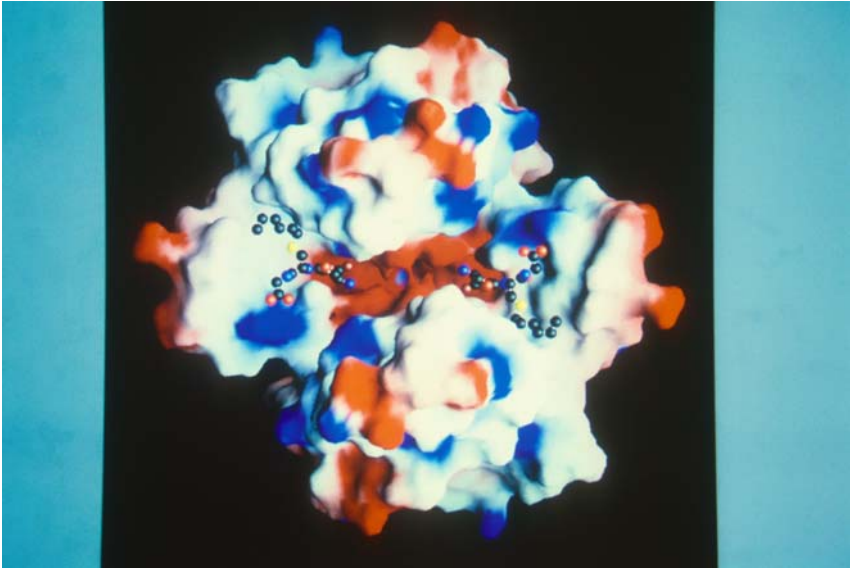
When MCF7 cells were stably transduced with GST A1-1, GST M1-1 and GST P1-1, expression of any of these GSTs significantly inhibited the 15-d-PGJ₂-dependent transactivation of a PPAR γ responsive reporter gene. It was shown that catalysis plays a little, if any, role in GST inhibition of 15-d-PGJ₂-dependent transactivation of a PPAR γ . This strong inhibition was, in contrast, associated with a direct interaction between this terminal metabolite and GST protein. The study suggests a mechanism of inhibition based on sequestering this ligand in the cytosol away from the nuclear target PPAR γ .

Modulation of Signaling Pathways by GST

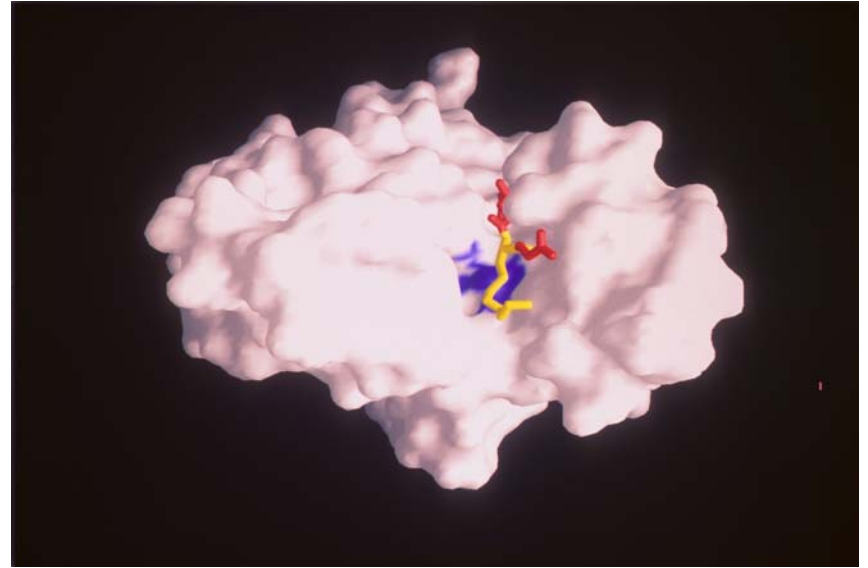
Regulation of JNK signaling through GST P1-1



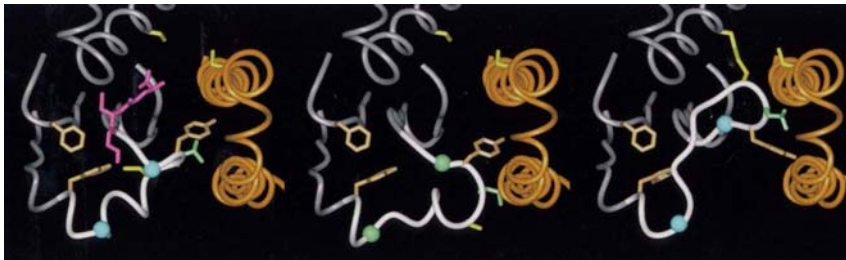
Human Glutathione Transferase P1-1



dimer



monomer



Active site Alpha2 loop

All the ligands bind to the active site in a similar fashion occupying the H-site

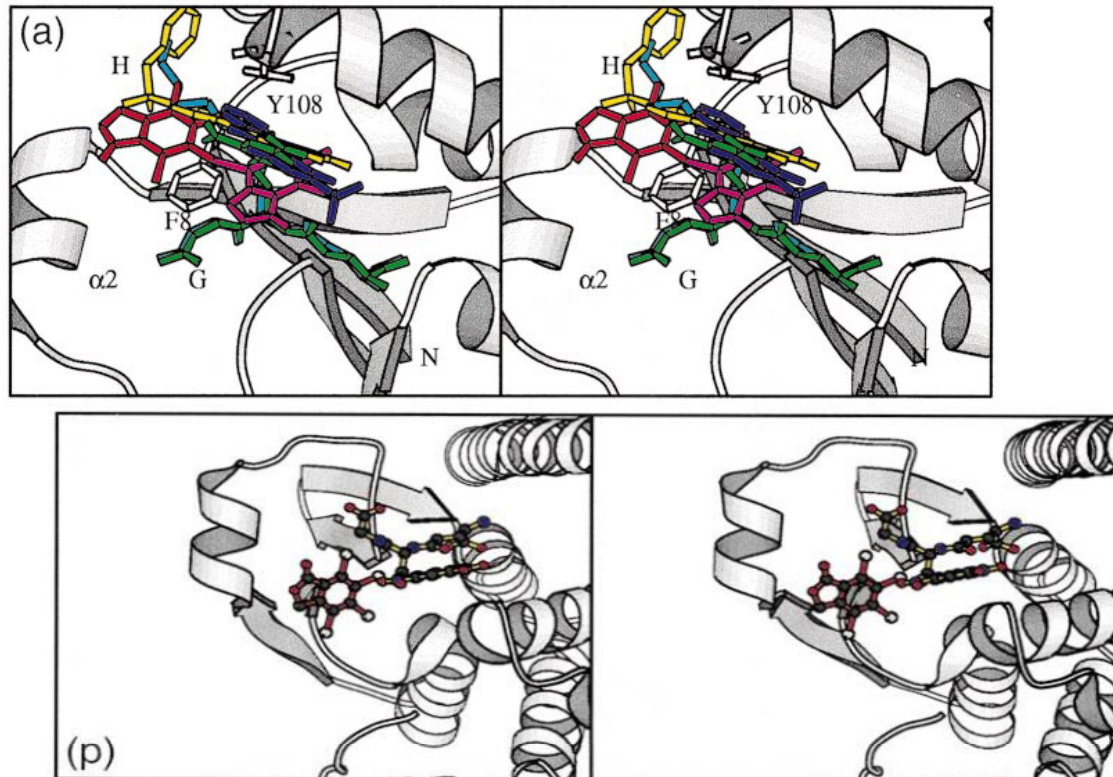


Figure 3. A comparison of the binding locations of GSH conjugates derived from the various crystal structures of pi class GSTs presented here. The superposition was based on overlaying the coordinates of the respective N-terminal domains. The ligands are overlaid on a schematic of the N-terminal domain of the human pi class enzyme. (a) Stereo view showing all inhibitors described in the text. The inhibitors are coloured as follows: SLZ, yellow; S-nonyl GSH, cyan; CB, dark blue; BS no.1, red; BS no. 2, magenta; DNP GSH, green. The side-chains of Phe8 and Tyr108 are shown. (b) Comparison of the binding locations of DNP GSH (denoted by yellow bonds) and BS no. 1 (denoted by red bonds). These pictures were generated with the program MOLSCRIPT (Kraulis, 1991).

SUBSTRATE PREFERENCES OF HUMAN GLUTATHIONE TRANSFERASES**

Family	Class, enzyme	Substrates of reaction
Cytosolic	Alpha, A1-1	Δ 5-ADD, BCDE, BPDE, Busulfan, Chlorambucil, CDNB, Aflatoxin, B1, 8,9-epoxide, DBADE, DBPDE, BPhDE, N-a-PhIP, CDNB, NBD-Cl, CuOOH
	Alpha, A2-2	CuOOH, DBPDE, CDNB, NBD-Cl
	Alpha, A3-3	Δ 5-ADD, Δ 5-pregnene-3,20-dione, DBPDE
	Alpha, A4-4	COMC-6, ethacrynic acid, 4-HNE, 4-hydroxydecenal
	Alpha, A5-5	not done
Cytosolic	Mu, M1-1	CDNB, t-BPO, BPDE, CDE, DBADE, transstilbene oxide, styrene-7,8-oxide
	Mu, M2-2	CDNB, COMC-6, DCNB, aminochrome, dopa O-Quinone, DCNB, PGH2→PGE2
	Mu, M3-3	CDNB, BCNU, PGH2→PGE2, H2O2
	Mu, M4-4	CDNB
	Mu, M5-5	low for CDNB
Cytosolic	Pi, P1-1	acrolein, base propenals, BPDE, CDE, Chlorambucil, COMC-6, EA, Thiotepa, CDNB, benzylisothiocyanate
Cytosolic	Sigma, S1-1	PGH2→PGD2
Cytosolic	Theta, T1-1	BCNU, butadiene epoxide, CH2CL2, EPNP, ethylene oxide, dibromoethane
	Theta, T2-2	CuOOH, menaphtyl sulphate

SUBSTRATE PREFERENCES OF HUMAN GLUTATHIONE TRANSFERASES**

Family	Class, enzyme	Substrates of reaction
Cytosolic	Zeta, Z1-1	dichloroacetate, fluoroacetate, 2-chloropropionate, malelyacetoacetate
Cytosolic	Omega, O1-1	monomethylarsonic acid, Dehydroascorbic acid
Mitochondrial	Omega, O2-2 Kappa, K1-1	dehydroascorbic acid CDNB, CuOOH, (S)-15-hydroperoxy-5,8,11,13-eicosatetraenoic acid
MAPEG	gp I, MGST2	CDNB, LTA4→LTC4, (S)-5-hydroxy-8,11,14- <i>cis</i> -6- <i>trans</i> -eicosatrenoic acid
	gp I, FLAP	non enzymatic binding of arachidonic acid
MAPEG	gp I, LTC4S gp II, MGST3	LTA4→LTC4 CDNB LTA4→LTC4, (S)-5-hydroperoxy-8,11,14- <i>cis</i> -6- <i>trans</i> -eicosatrenoic acid
MAPEG	gp IV, MGST1	CDNB*, CuOOH, hexachlorobuta-1,3-diene, NDBCl
	gp IV, PGES1	PGH2→PGE2
* Activity increased by treating enzyme with <i>N</i>-ethylmaleimide. A systematic study of all these enzymes towards substrates has not been undertaken, and therefore it is not possible to define relative activities towards the compounds listed. These data are taken from papers cited in the text. ** Abbreviations: Δ5-ADD, Δ5-androstesne-3,17-dione; BCDE, benzo[<i>g</i>]chrysene diol epoxide; BCNU, 1,3-<i>bis</i>(2-chloroethyl)-1-nitrosourea; BDPE, benzo[<i>a</i>]pyrene diol epoxide; BPhDE, benzo[<i>c</i>]phenanthrene diol epoxide; COMC-6, crotonyloxymethyl-2-cyclohexenone; DBADE, dibenz[<i>a,h</i>]anthracene diol epoxide; DBPDE, dibenzo[<i>a,l</i>]pyrene diol epoxide; ÉPNP, 1,2-epoxy-3-(<i>p</i>-nitrophenoxy)propane; N-<i>a</i>-PhIP, N-acetoxy-2-amino-1-methyl-6-phenylimidazo[4,5-<i>b</i>]pyridine.		

Table 9.5 Tissue distribution of human glutathione S-transferases

Superfamily	Class	Protein	Organ
Soluble	Alpha	GSTA1 GSTA2	Testis $\approx$ liver $\gg$ kidney $\approx$ adrenal > pancreas Liver $\approx$ testis $\approx$ pancreas > kidney > adrenal > brain
		GSTA3	Placenta
		GSTA4	Small intestine $\approx$ spleen > liver $\approx$ kidney > brain
Soluble	Mu	GSTM1 GSTM2 GSTM3 GSTM4 GSTM5	Liver > testis > brain > adrenal $\approx$ kidney > lung Brain $\approx$ skeletal muscle $\approx$ testis > heart > kidney Testis $\gg$ brain $\approx$ small intestine > skeletal muscle Testis Brain, heart, lung, testis
Soluble	Pi	GSTP1	Brain > heart $\approx$ lung $\approx$ testis > kidney $\approx$ pancreas
Soluble	Sigma	GSTS1	Foetal liver, bone marrow
Soluble	Theta	GSTT1 GSTT2	Kidney $\approx$ liver > small intestine > brain $\approx$ prostate liver
Soluble	Zeta	GSTZ1	Foetal liver, skeletal muscle
Soluble	Omega	GSTO1	Liver $\approx$ heart $\approx$ skeletal muscle > pancreas > kidney
Soluble	Kappa	GSTK1	Liver (mitochondria)
MAPEG	(Microsomal)	MGST-I MGST-I-like I MGST-II MGST-III LTC ₄ S FLAP	Liver $\approx$ pancreas > prostate > colon $\approx$ kidney > brain Testis > prostate > small intestine $\approx$ colon Liver $\approx$ skeletal muscle $\approx$ small intestine > testis Heart > skeletal muscle $\approx$ adrenal gland, thyroid Platelets $\approx$ lung > liver Lung $\approx$ spleen $\approx$ thymus $\approx$ PBL $\gg$ small intestine

Data based on references cited in the text.

PBL: Peripheral blood leukocytes

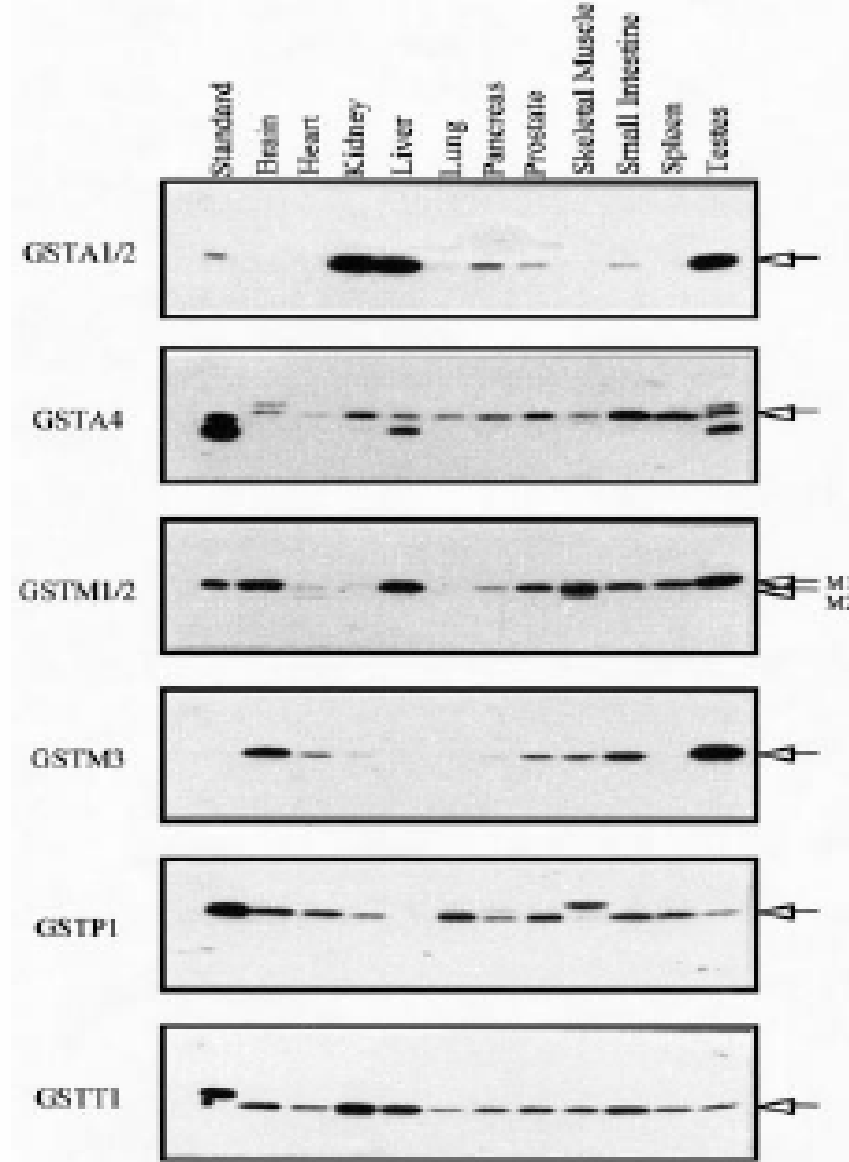


Figure 9.5 Tissue-specific expression of GST isoenzymes in human organs. Extracts from human organs were subjected to immunoblotting as described by Sherratt *et al.* (1997). The identity of the sample loaded in each lane is indicated at the top of the figure. In the left-hand margin, the identity of the anti-serum used to probe the blot is indicated. The horizontal arrows shown in the right-hand margin indicate the mobility of the authentic cross-reacting GST polypeptides.

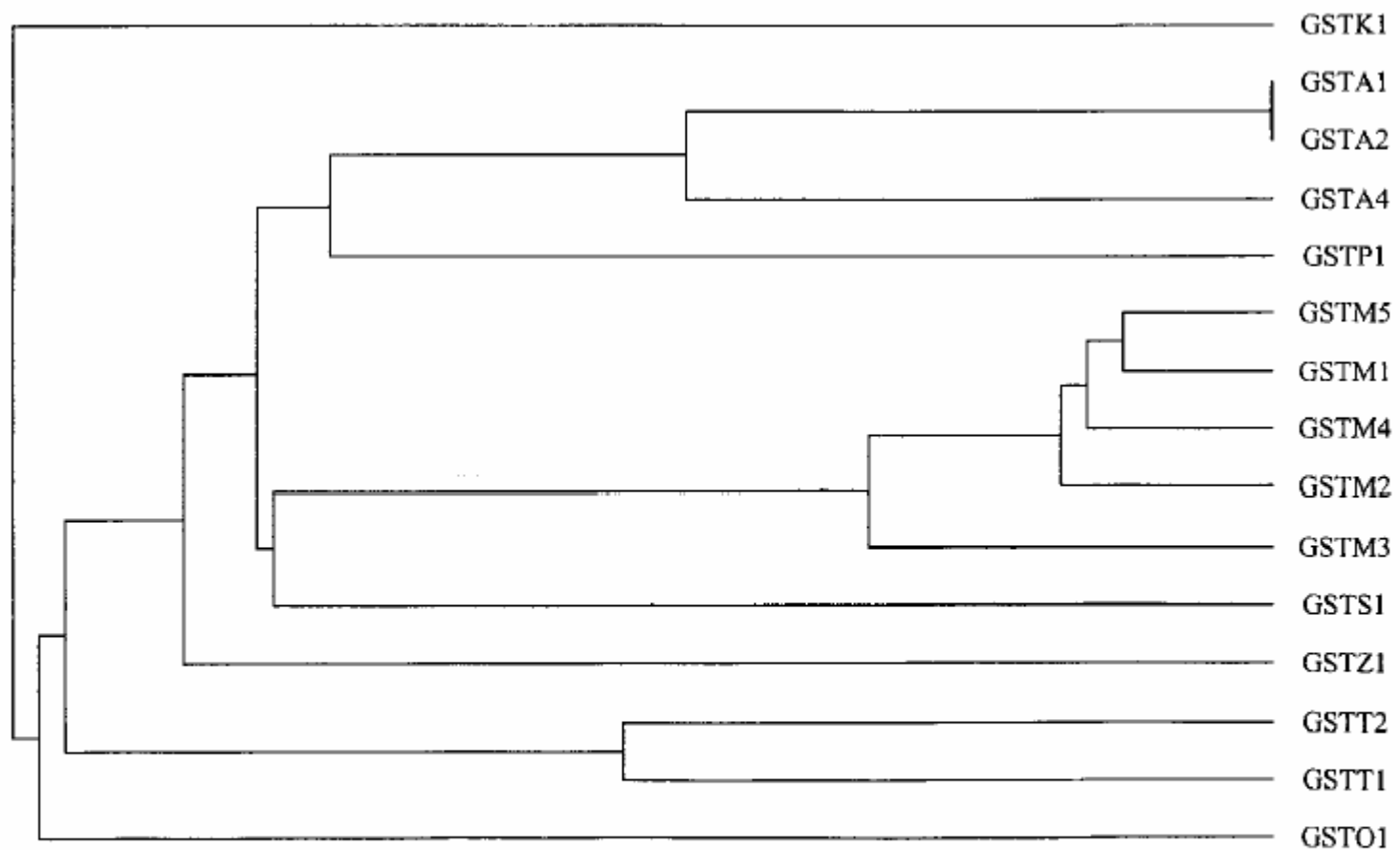


Figure 9.3 An average distance tree of the human soluble GST superfamily. The structural relationship between the different classes of GST was determined by multisequence alignment using Clustalw. The alignment was used to calculate an average distance tree using Jalview. Both applications were accessed through the European Bioinformatics Institute internet server.

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. Molecular cloning and crystallography of the mitochondrial GST have provided definitive evidence that it represents a distinct type of transferase. 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. 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.

Mitochondrial GST

- Although this transferase was originally isolated from mitochondria and is not present in cytoplasm, it has also been shown to be located in peroxisomes. 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, and a possible cleavage site for a mitochondrial presequence exists at the *N*-terminus. A peroxisomal targeting sequence (tripeptide ARL) has been identified in the *C*-terminus of the human GSTK1 subunit.

Evolution of the GST Fold

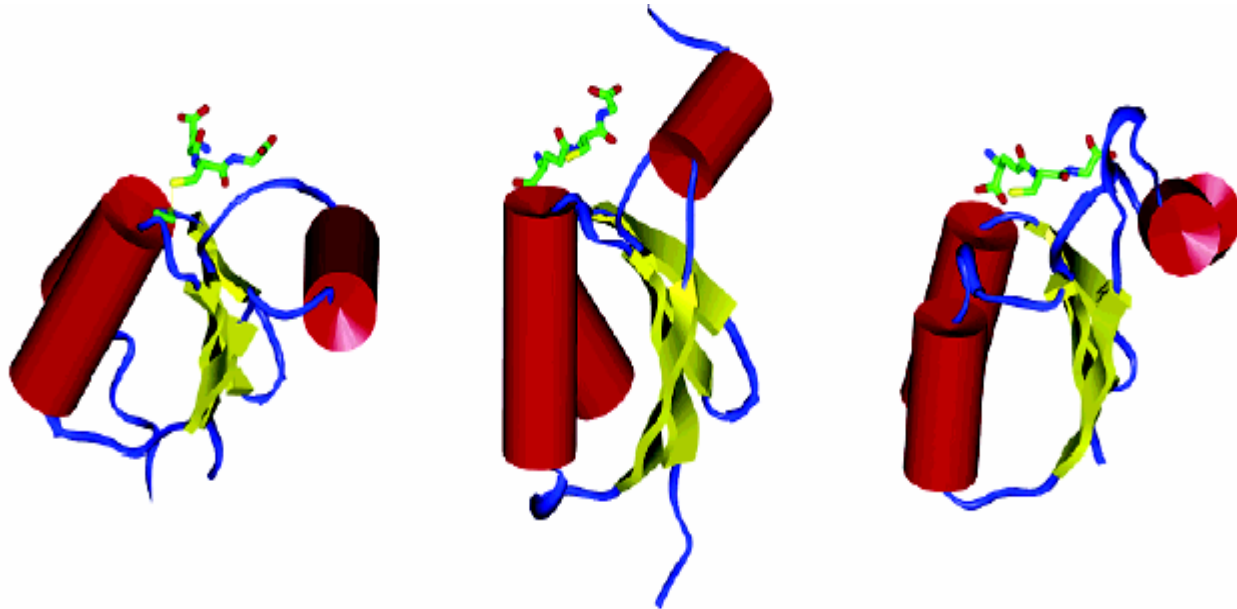


FIGURE 8: Comparison of the thioredoxin domains of glutaredoxin-1(C14S) (left), rGSTK1 (center), and rGSTM1 (right). The glutathione molecules are shown in stick representation.

- 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, 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.
- The resulting Kappa isoform is more similar in its secondary structure organization to the bacterial protein disulphide isomerase DsbA than to the cytosolic isomerases.

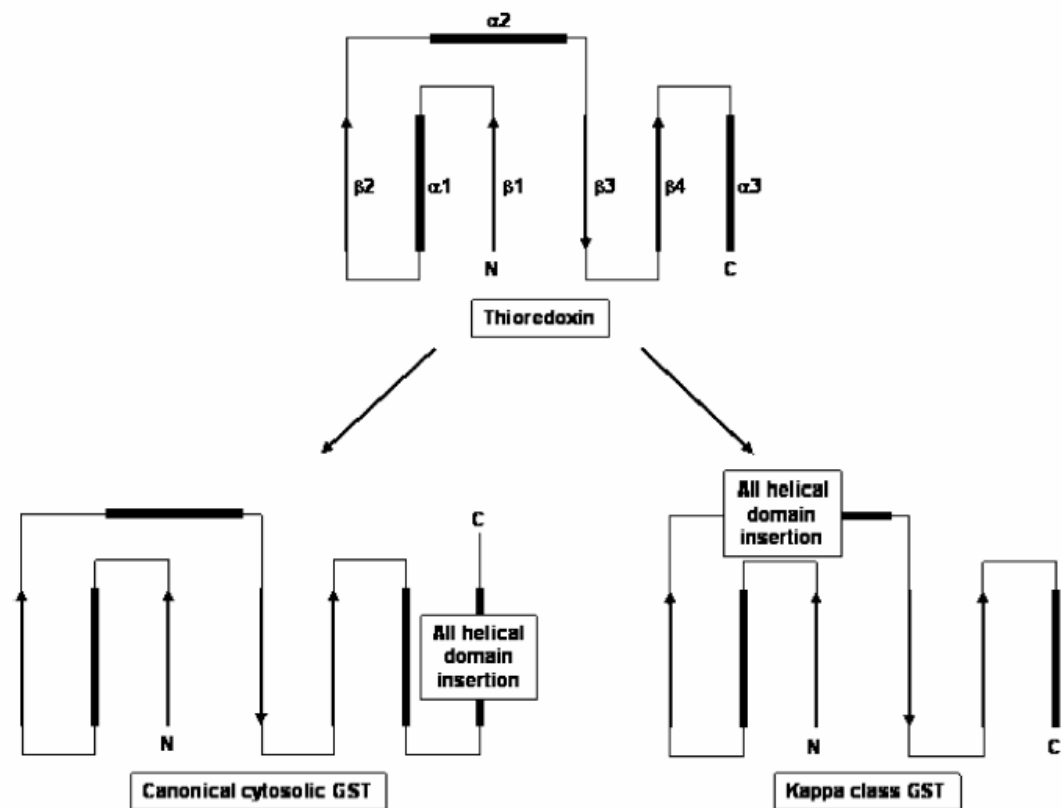


Figure 4 Schematic diagram of evolution of the GST fold. The secondary structure arrangement of thioredoxin, the canonical cytosolic GST fold, and that of GSTK1 and bacterial DsbA is illustrated. Arrows represent β -sheets; rectangles represent α -helices. The regions corresponding to the core thioredoxin 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.

Insertion or addition of all helical domain into the first domain

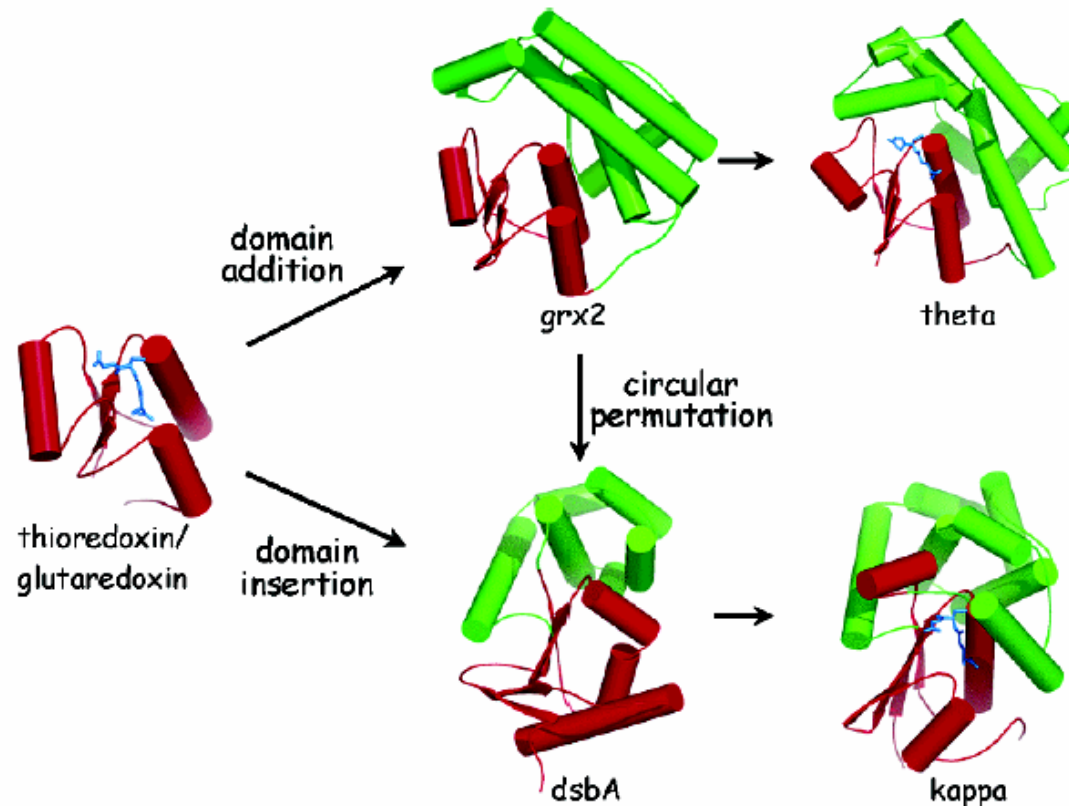


FIGURE 9: Proposed parallel pathways for the evolution of the canonical GSH transferases by domain addition via a grx2-like intermediate (top) and the class kappa GSH transferases by domain insertion or circular permutation of grx2 via a dsbA-like intermediate (bottom). The thioredoxin-like domains are shown in red, the α -helical domains are shown in green, and the glutathione molecules in glutaredoxin-1, the theta and kappa GSH transferases, are shown in blue stick representation.

MAPEG Enzymes

- These members of the GST superfamily constitute a unique branch where most of the members 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. Three further MAPEG members with roles in eicosanoid synthetic pathways were subsequently identified as leukotriene C4 synthase (LTC4S), a microsomal transferase that conjugates leukotriene A4 with GSH; 5-lipoxygenase-activating protein (FLAP), an arachidonic acid-binding protein required for 5-lipoxygenase to exhibit full activity; and prostaglandin E2 synthase 1 (PGES1), which catalyses GSH-dependent isomerization of PGH2 to PGE2.

MAPEG Enzymes

- Evidence suggests MGST1 functions solely as a detoxication enzyme. By contrast, human MGST2 and MGST3 are capable of both detoxifying foreign compounds and synthesizing LTC₄; in the rat, MGST3 is apparently unable to synthesize LTC₄. 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. LTC₄S and PGES1 seem to make no contribution to detoxification, their catalytic actions being restricted to synthesis of LTC₄ and PGE₂, respectively.
- Determination of a 6 °A crystal structure for MGST1 has illustrated the homotrimeric quaternary structure of the enzyme, a quaternary structure also observed for the other subgroup IV enzyme PGES1.

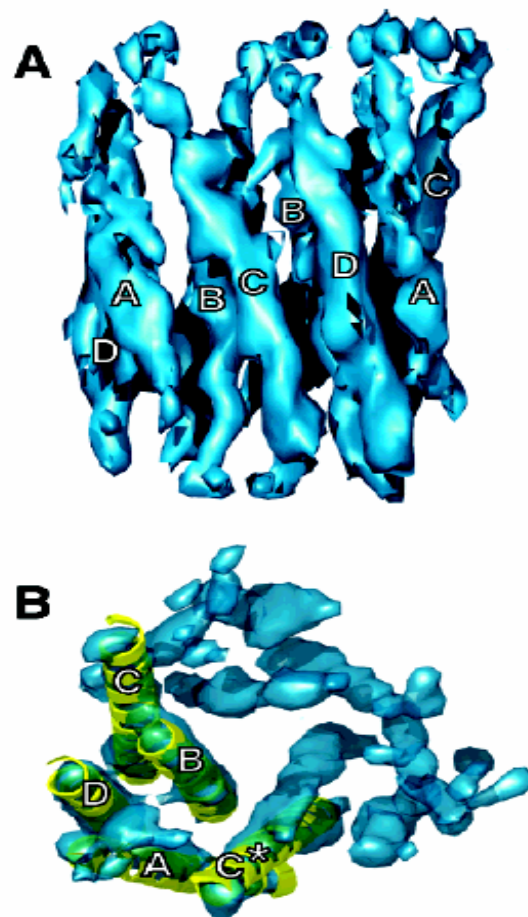


FIGURE 1: Panel A presents a side view of the molecular density map of one trimer of MGST1 at 4.5 Å resolution. The cytosolic domain is on top. A noncrystallographic 3-fold symmetry has been applied as described previously (10). Panel B presents a view of one trimer from the unsymmetrized map looking down the noncrystallographic 3-fold axis from the cytosolic side of the membrane. Two possible four-helix bundles for one monomer are illustrated by grouping helical cylinders ABCD or ABC*D. The map was calculated to 4.5 Å resolution from electron micrographs of plane group $P22_12_1$ two-dimensional crystals (18) tilted between 0° and 53°. The crystal images were processed using electron and X-ray crystallographic software from the MRC (28) and CCP4 (29) program systems and visualized using the *O* (30), MolRay (31) and Povray (32) programs.

Polymorphisms in Cytosolic GSTs

- Cytosolic GSTs display polymorphisms in humans, 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.

Polymorphisms in Cytosolic GSTs

- 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 null 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 homozygous +/+ individuals. An assay has been developed that can identify heterozygotes at the *GSTT1* locus though useful medical applications remain to be established.

Polymorphisms in Cytosolic GSTs

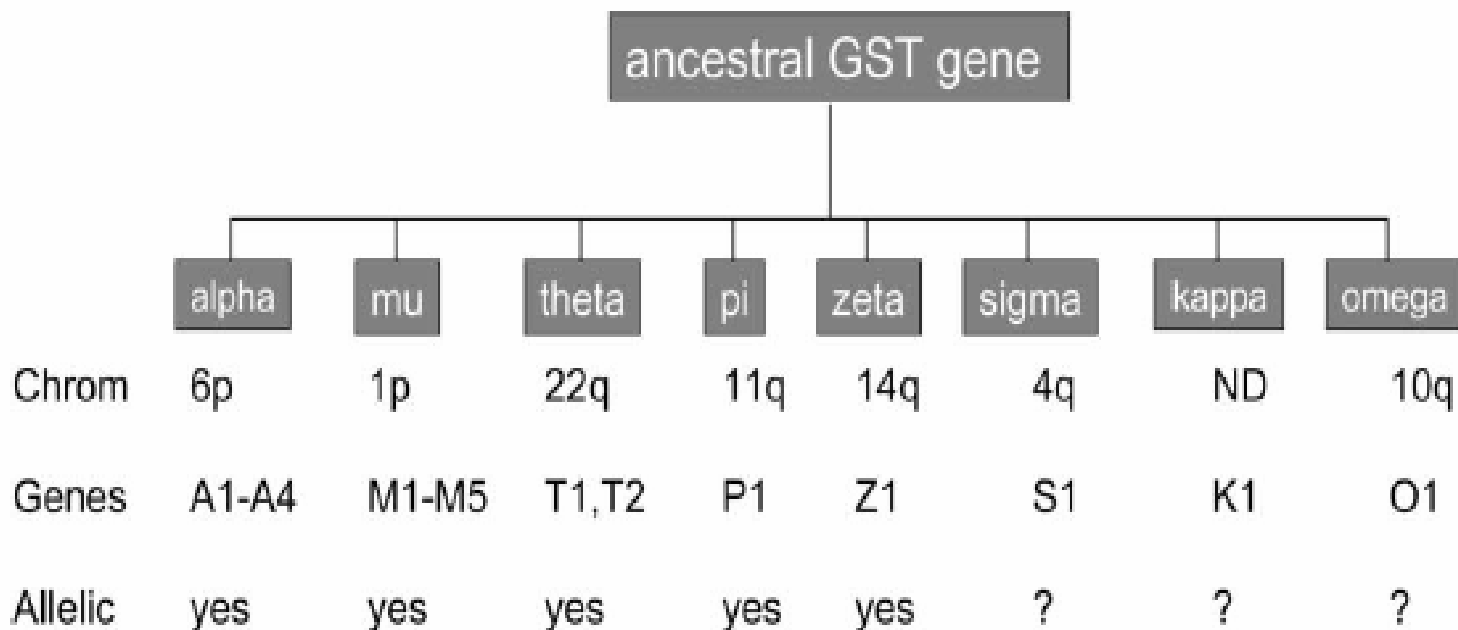


Fig. 1. The glutathione-S-transferase supergene family.

TABLE 2 Polymorphic human cytosolic GST

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

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

Polymorphisms in Cytosolic GSTs

- By contrast with the weak affect 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.
- 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. 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. Further, *GSTM4* and *GSTT2* exhibit promoter polymorphisms that are of functional significance. 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.

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 show altered sensitivity to xenobiotics, and they reveal that loss of certain GST isoenzymes causes an upregulation of the remaining transferases.

CONSEQUENCE OF KNOCKOUT OF GST GENES

- **CLASS ALPHA GST** Homozygous null *GSTA4* mice appear normal but are more susceptible to bacterial infection and display increased sensitivity to paraquat. 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.
- 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 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 and many cancer chemopreventive blocking agents that induce GST can be included in this category of compound.

CONSEQUENCE OF KNOCKOUT OF GST GENES

- 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, olfipraz, and sulforaphane. 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*; 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 synthesis of GSTA4-4 and glutathione levels, resulting in increased capacity to metabolize 4-HNE).
- 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;

CONSEQUENCE OF KNOCKOUT OF GST GENES

- **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.
- **CLASS Pi GST** Mice lacking both *GSTP1* and *GSTP2* have been generated.
- 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. 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. However, livers from *GSTP1/P2-/-* mice have been reported to contain a higher activator protein-1 activity than livers from *GSTP1/P2+/+* mice, a finding that is consistent with the hypothesis that class Pi GST inhibits JNK.
- In a skin tumorigenesis regimen, *GSTP1/P2-/-* mice yielded approximately threefold more papillomas using 7,12-dimethylbenzanthracene as initiator and TPA as promoter, 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.

CONSEQUENCE OF KNOCKOUT OF GST GENES

- **CLASS SIGMA GST** This class of GST encodes the hematopoietic, or GSH dependent, prostaglandin D2 synthase. Knockout of the gene for this enzyme results in generation of mice with an allergic reaction that is weaker than wild-type mice.
- **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. By contrast, under normal dietary conditions, *GSTZ1*^{-/-} mice on a BALB/c background showed enlargement of liver and kidney as well as splenic atrophy. 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. The latter metabolite was also observed in blood of *GSTZ1*^{-/-} mice. 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

CONSEQUENCE OF KNOCKOUT OF GST GENES

- The pathophysiological effects observed in the *GSTZ1*^{-/-} animals were attributed to failure to eliminate either succinylacetone or other MAA-derived metabolites. 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.

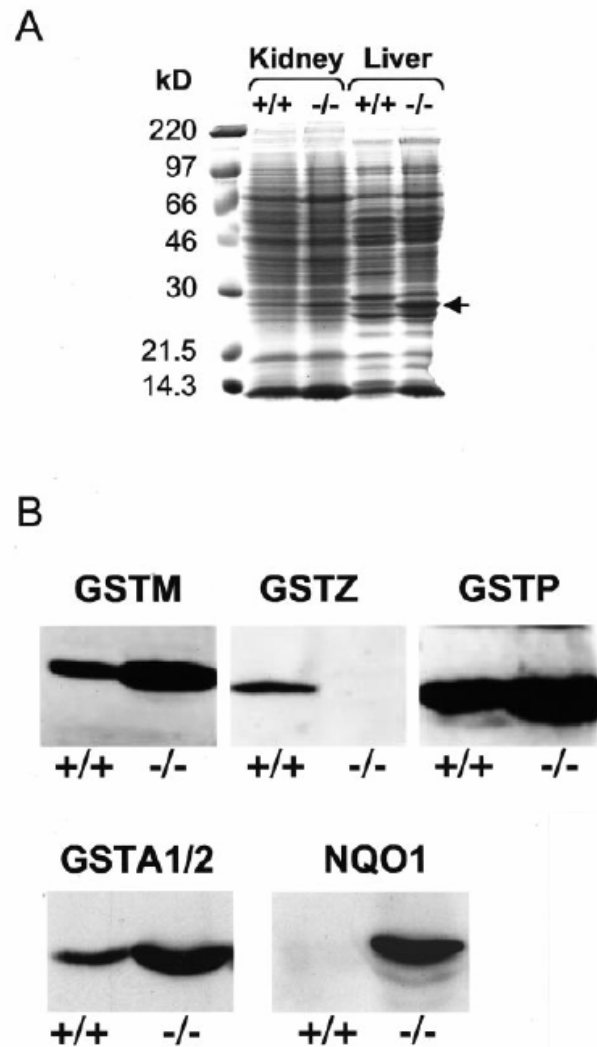
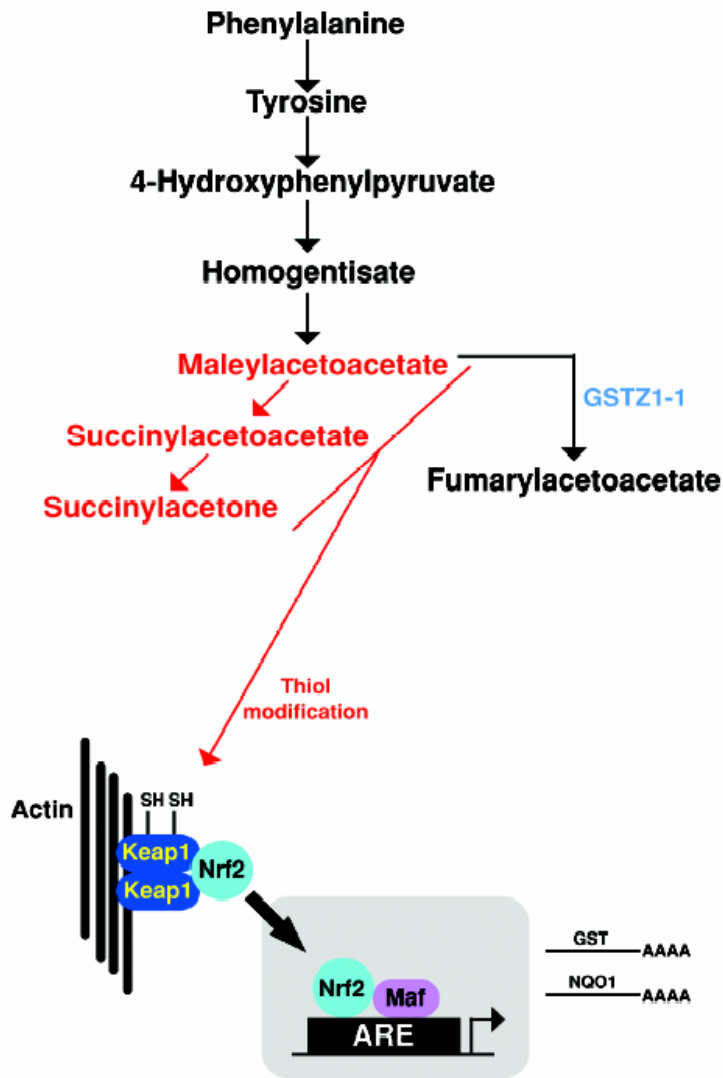


Figure 9. Induction of other GSTs in female *Gstz1*^{-/-} mice. **A:** SDS-PAGE of soluble proteins from the liver and kidney of wild-type (*Gstz1*^{+/+}) and deficient (*Gstz1*^{-/-}) mice. The **arrow** indicates the elevated expression of proteins in the liver of GSTZ1-deficient mice. **B:** Western blots of soluble liver proteins probed with antiserum to hGSTM1-1, hGSTZ1-1, hGSTP1-1, mGSTA1/2, and ratNQO1. The mouse genotype is shown below each track. Each track was loaded with the same amount of total protein.



It appears likely that succinylacetone, and possibly MAA or maleylacetone, 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. This feature infers that enzyme induction is a response to redox stress. It is not known whether the metabolite(s) that affects gene induction is also responsible for the pathological changes.

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 maleylacetone. Certain of these tyrosine metabolites are thiol-active (147) and probably induce gene expression through the Keap1/Nrf2 pathway.

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

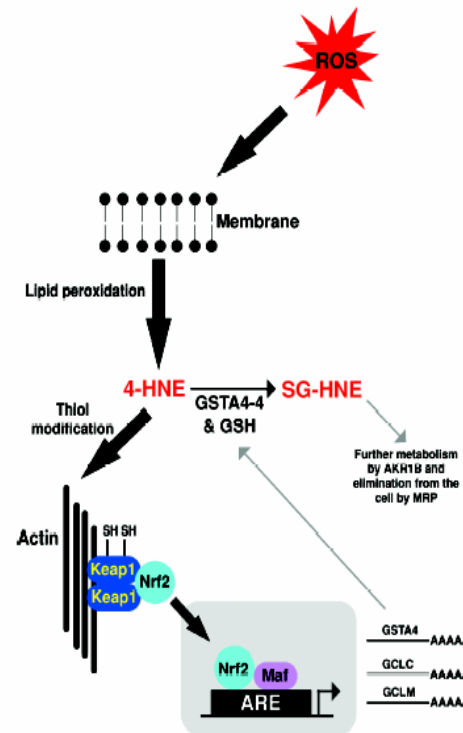


Figure 5 Negative regulation of the ARE-gene battery by GSTA4-4. This cartoon shows how 4-HNE, produced through membrane damage by reactive oxygen species (53), might modify cysteine residues in the cytoskeleton-binding protein Keap1 (73). Such posttranslational modification of Keap1 allows the Nrf2 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.

Conclusions

- These are 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.

Conclusions

- 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 (C273 and C288) 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

GSTs and cancer

Table 1: human tissues

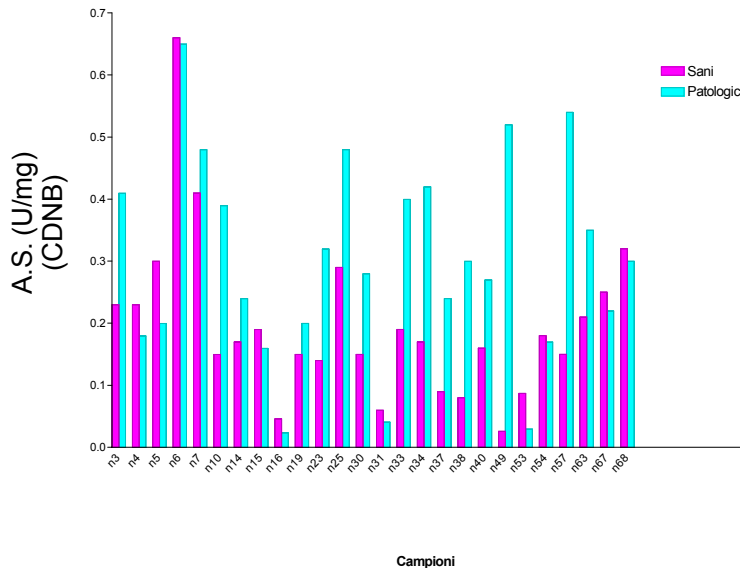
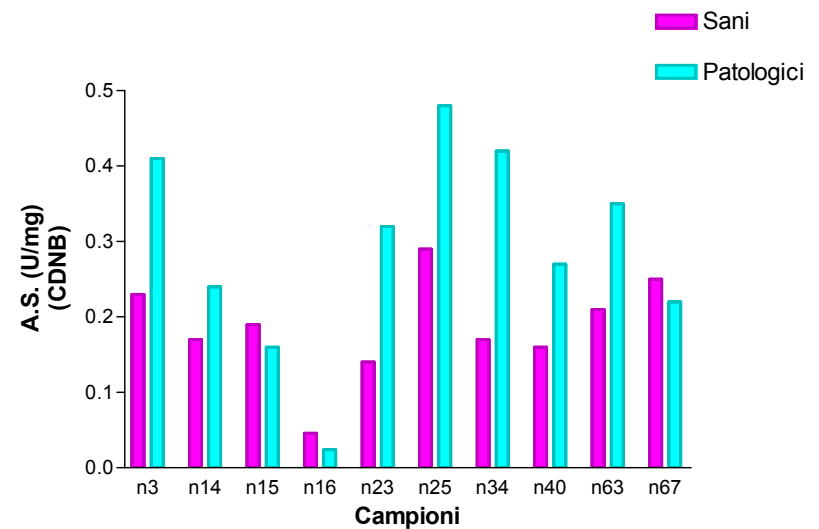
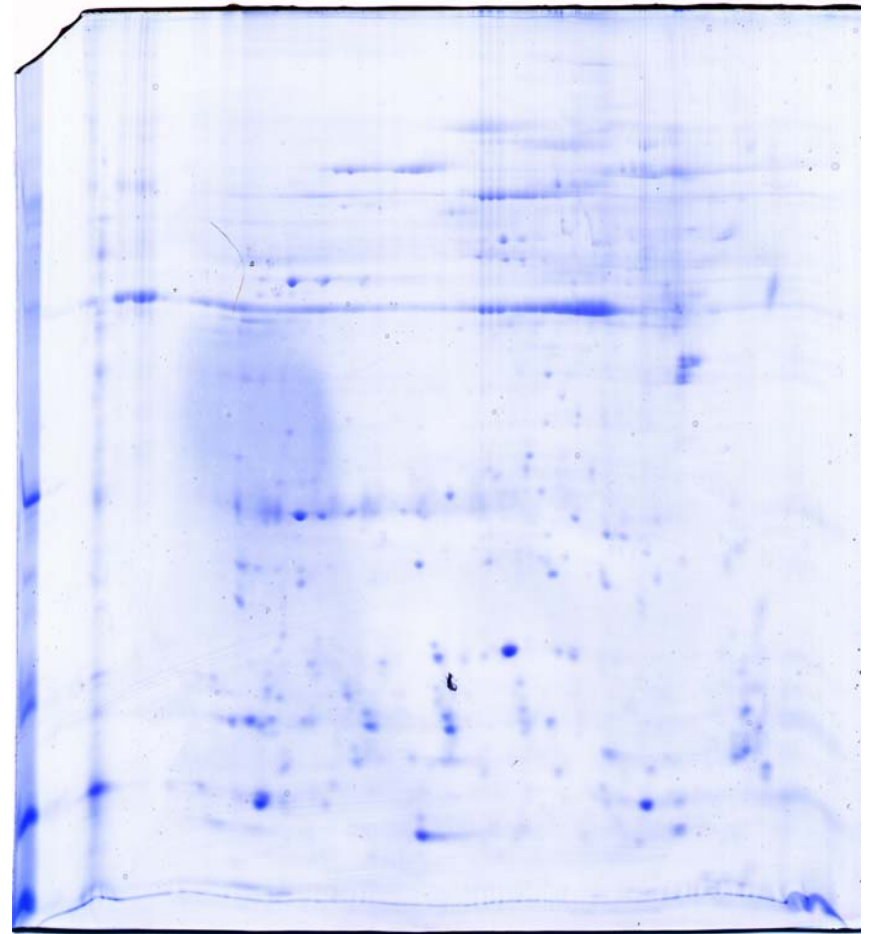
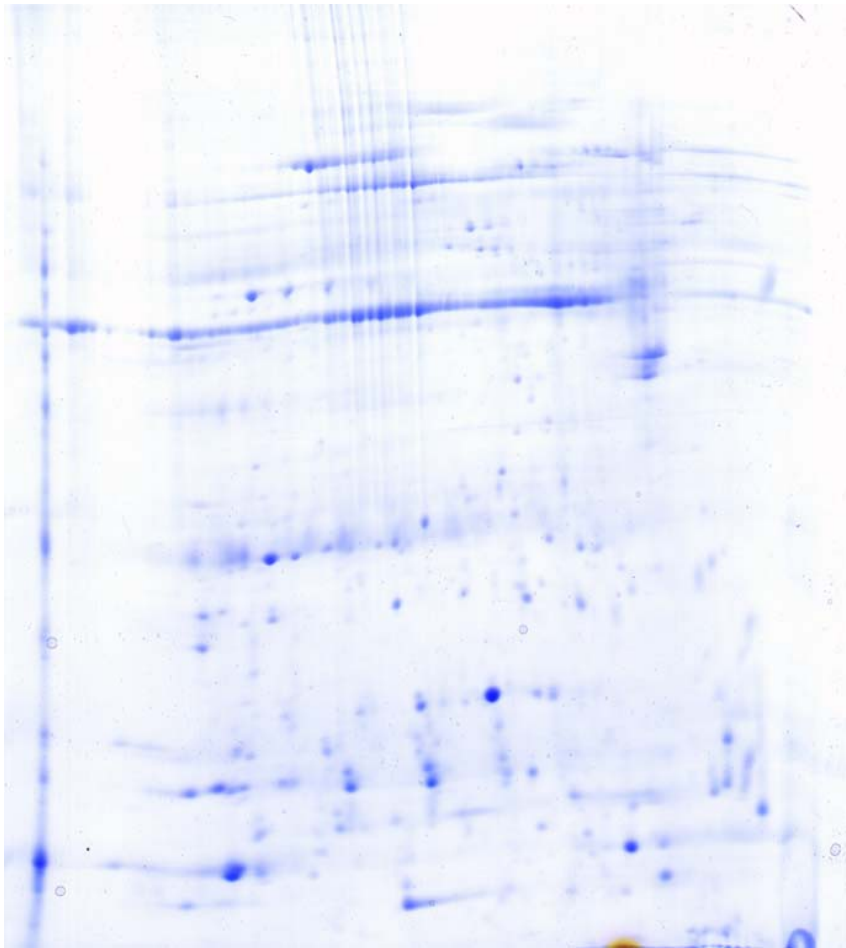


Table 2: human tissues
colon and rectum



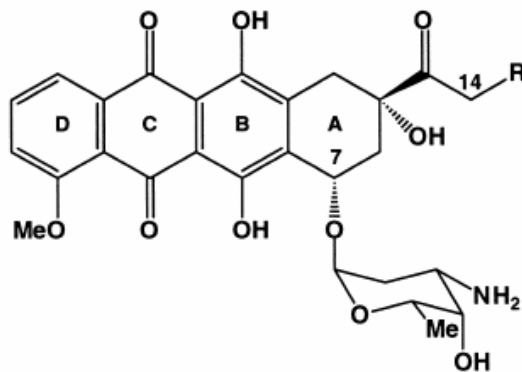
Technical advances in the ability to extract and analyze molecular material have led to an increased interest in and utilization of tissue. However, tissue samples are a precious commodity. The ability to analyze genomic and/or proteomic data derived from archived tissues, for which clinical follow-up is already available, greatly facilitates both diagnostic and therapeutic discovery research. Researchers in pharmaceutical and biotech companies currently utilize tissue for discovery research in genomics, proteomics, experimental medicine, and target validation

2D PAGE of human colon (n. 4)

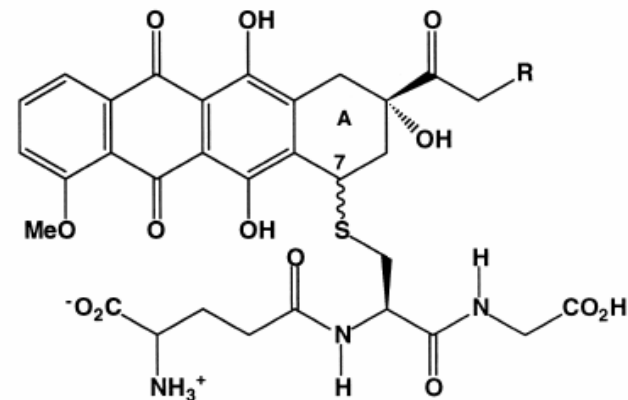


GSTs overexpression and drug resistance

- A significant increase in GST activity in tumor tissues and cell lines has been observed (in particular, for GST P1-1) with respect to normal and preneoplastic tissue and this is often linked to drug resistance. For instance: Adriamycin resistant MCF-7 lines showed a 14-fold increase in GST specific activity in comparison with the parental lines.



1, R=OH, Adriamycin
2, R=H, Daunomycin



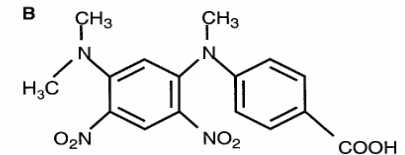
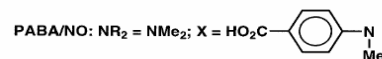
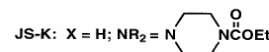
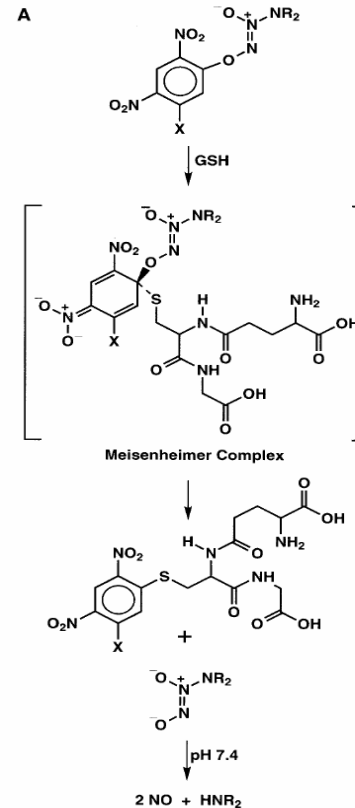
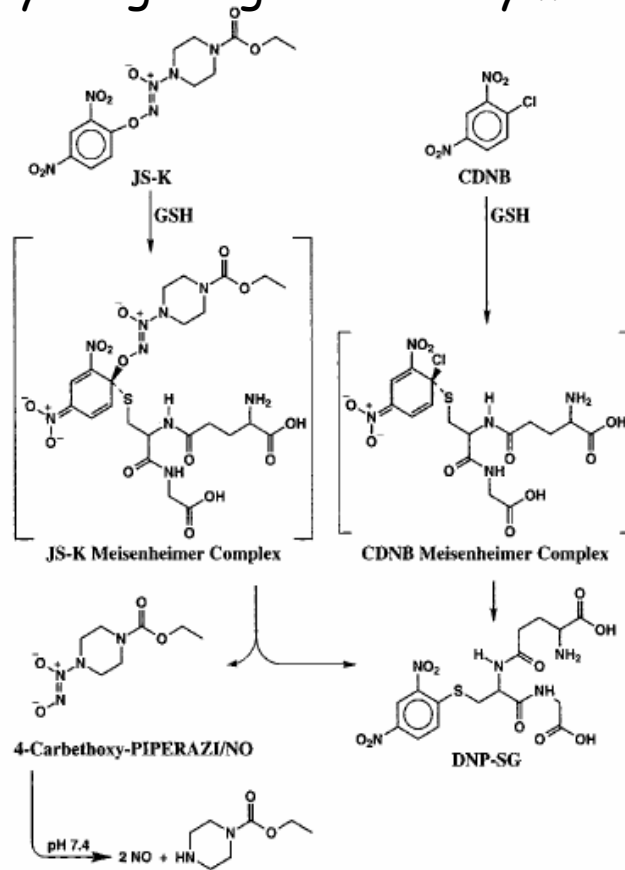
3, R=OH, ADRIGLU
4, R=H, DAUNOGLU

GSTs overexpression and drug resistance

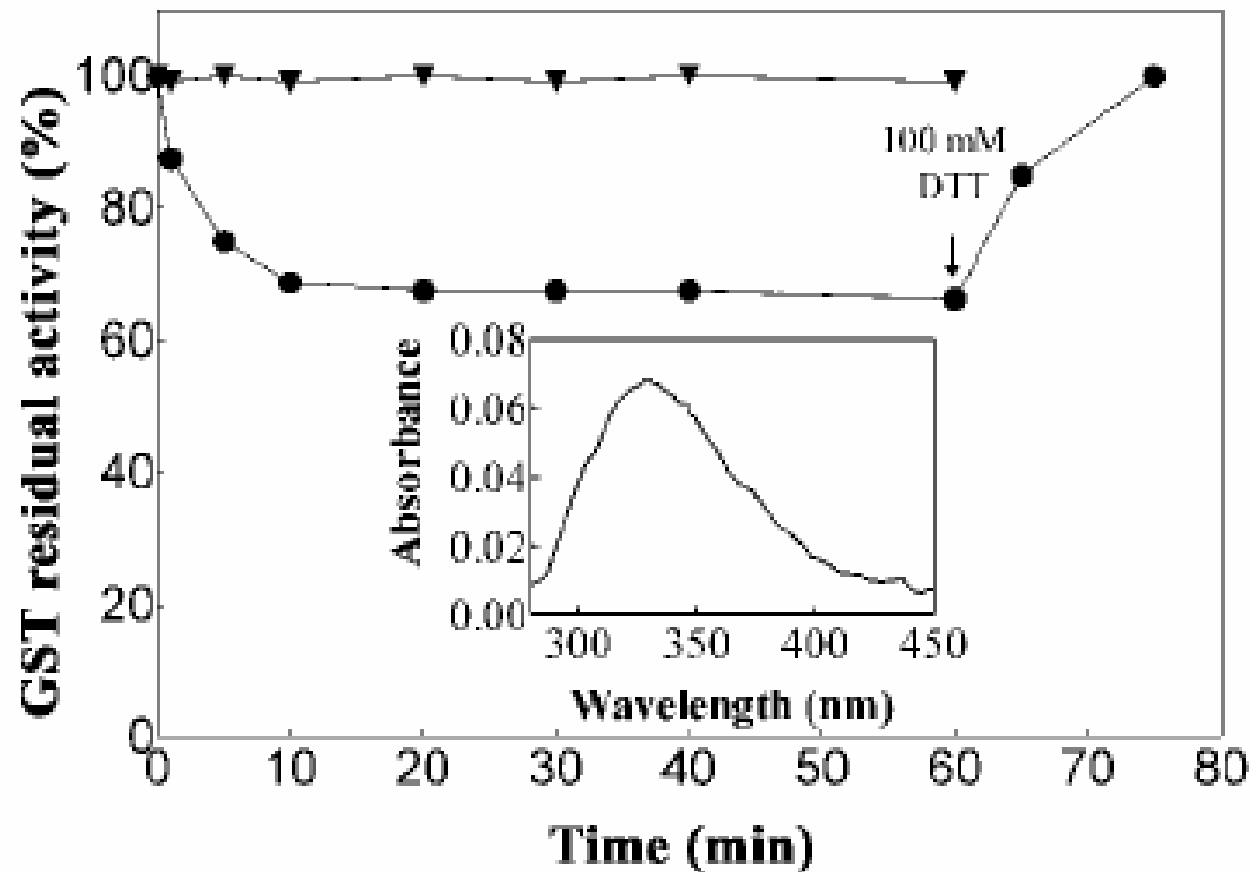
- It has been hypothesized that GST polymorphism (GST M1 and GST T1 null allele and allelic variants of GST P1-1) may confer increased susceptibility to cancer.
- To date there is no conclusive evidence for this. In some cases, this can be true because some carcinogenic compounds cannot be efficiently detoxified in the absence of certain GSTs. In other cases, there is a compensatory response of other antioxidant enzymes which is sufficient to counteract the effect of dangerous compound.
- This is an expanding field and need more data to better evaluate the influence of GST polymorphism.

GSTs overexpression and drug resistance

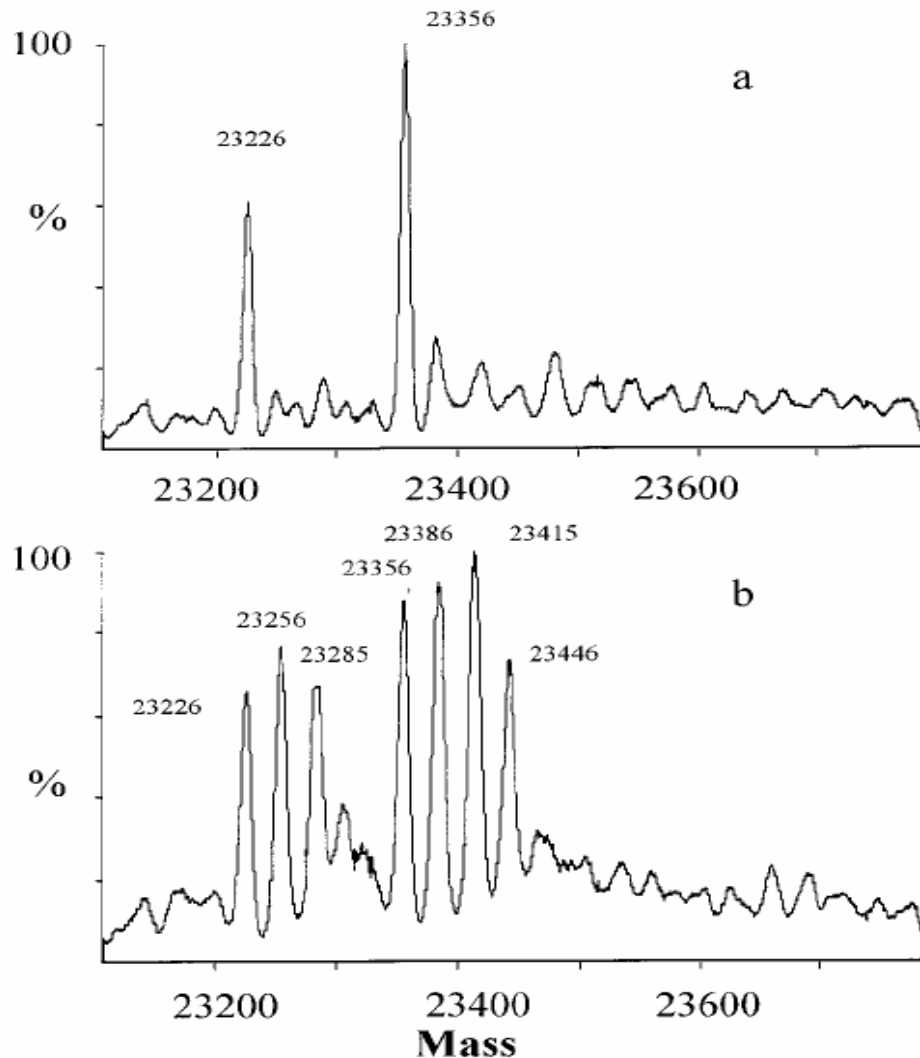
Attempts have been made in order to overtake this chemo-resistance by targeting these enzymes with a number of inhibitors



Inactivation of GST P1-1 by GSNO

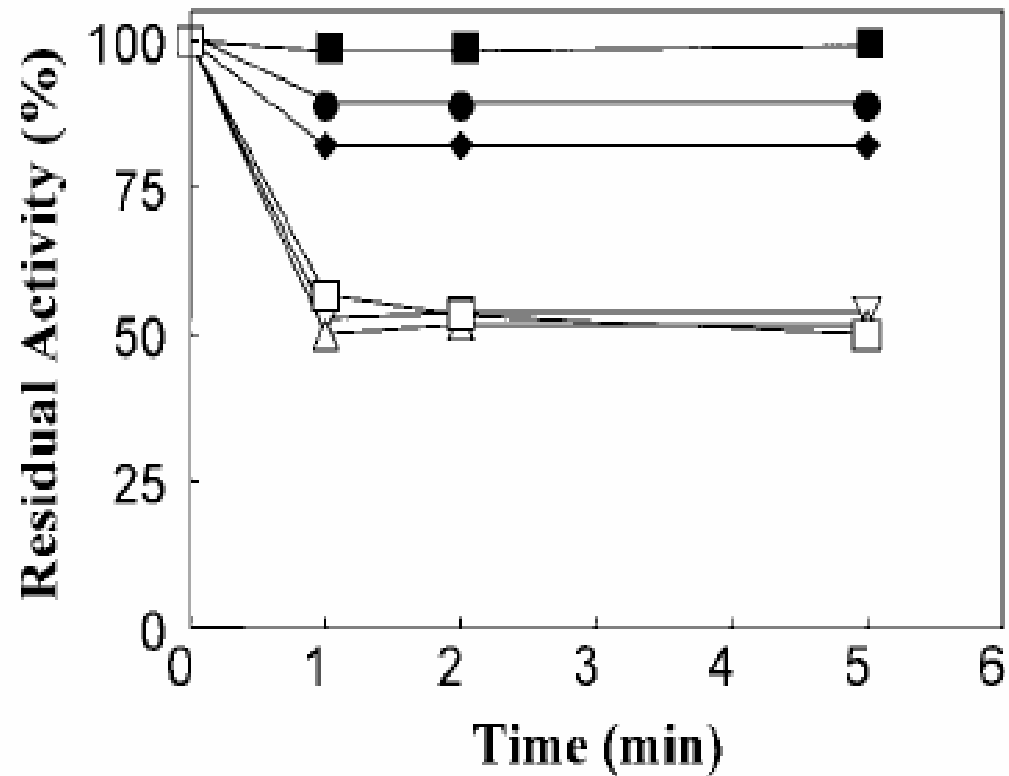


GSNO modification of GST P1-1 produces nitrosylation and not glutathionylation

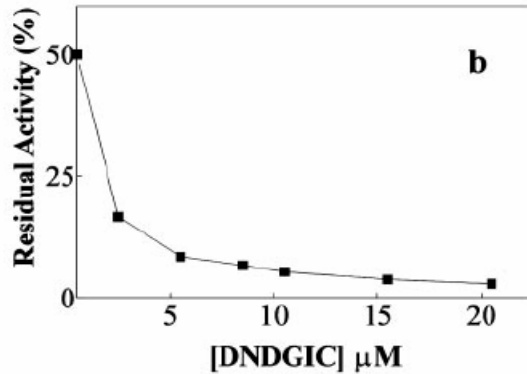
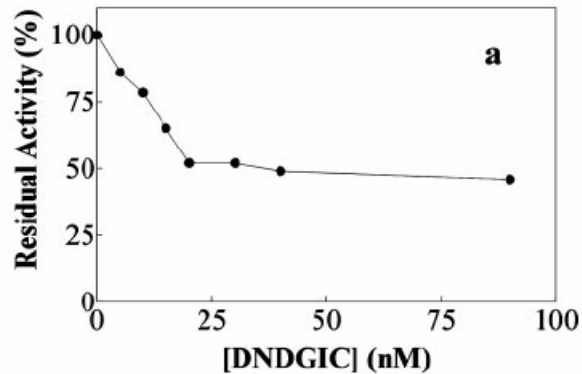


Ion spray mass spectra of GST P1-1 alone (a) and GST P1-1 after GSNO modification (b)

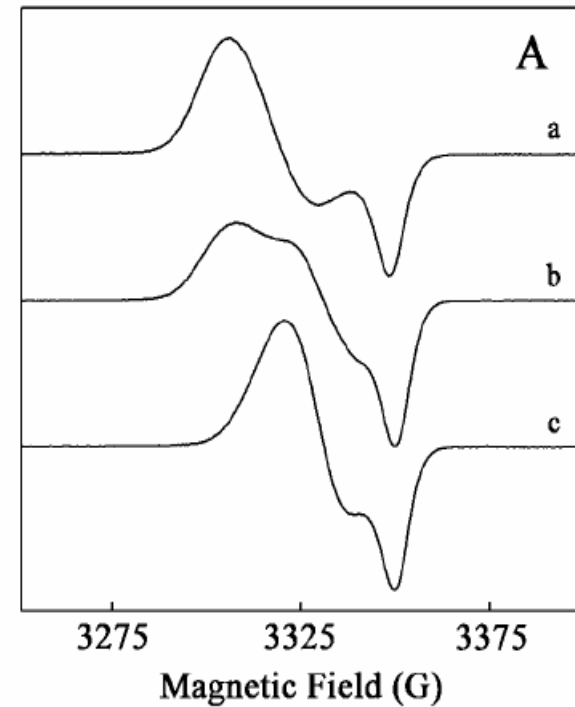
Interaction of GST P1-1 with GSNO in the presence of GSH



Interaction of GST P1-1 with DNDGIC (dinitrosyldiglutathionyl iron complex)



GST P1-1 44 nano moles



EPR spectra of DNDGIC alone (a)
with GST P1-1 before (b)
after G-25 sephadex (c)

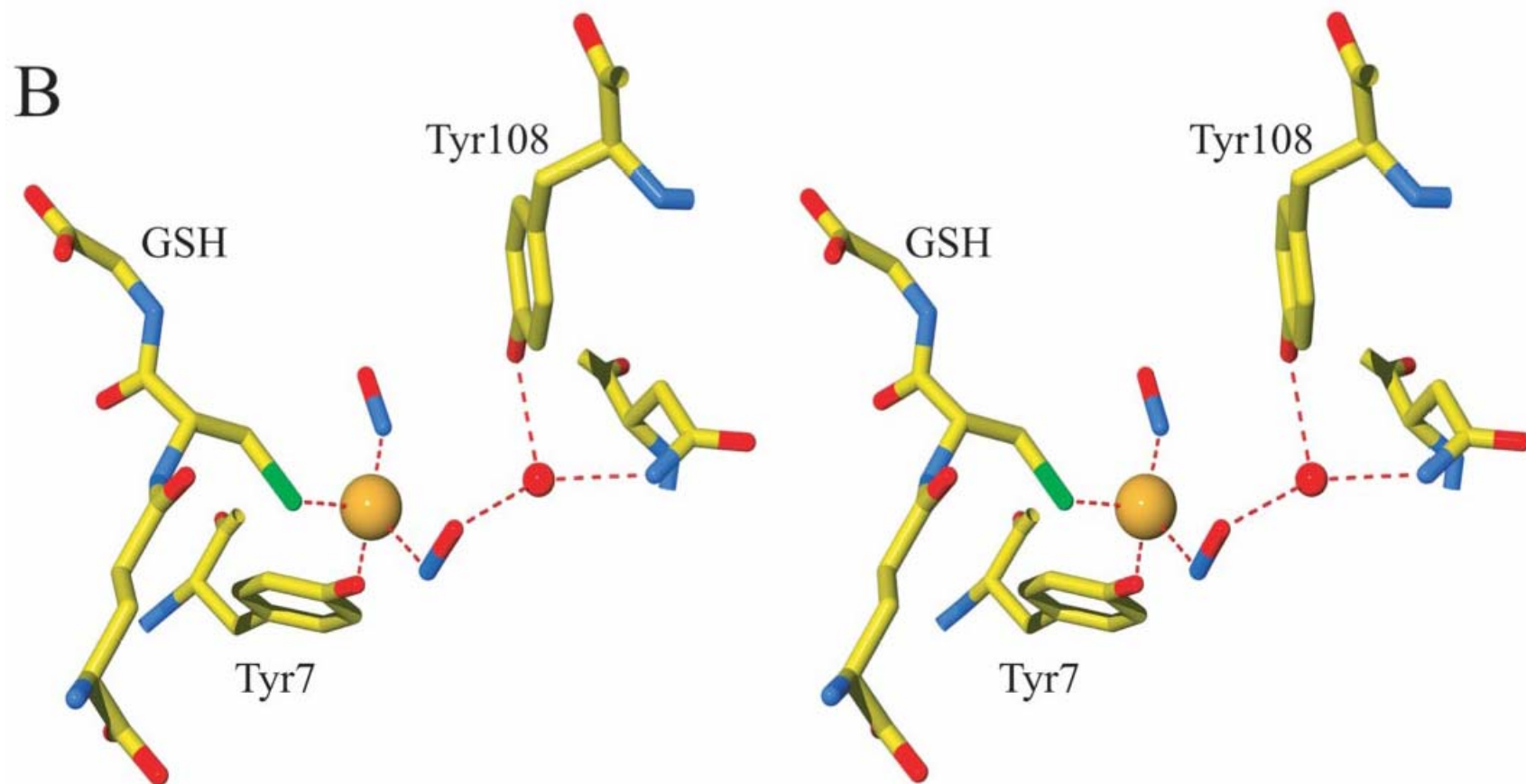
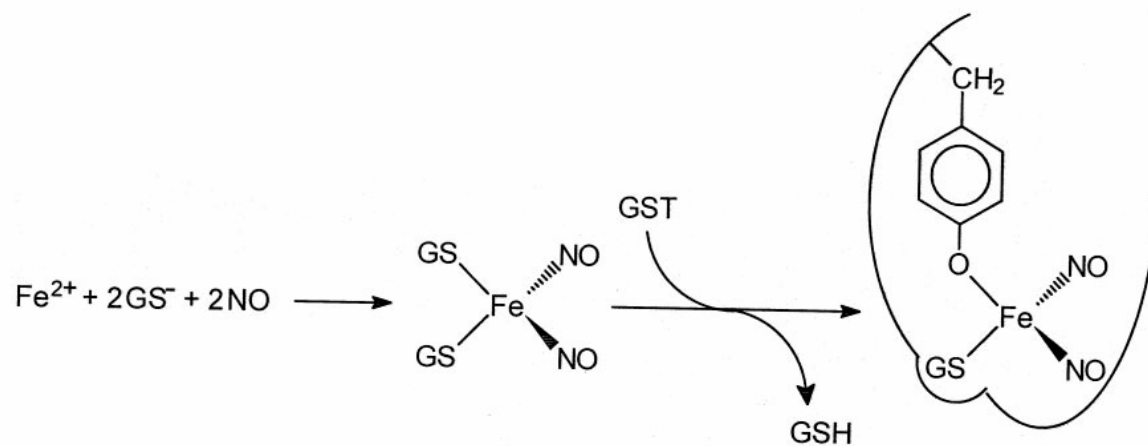
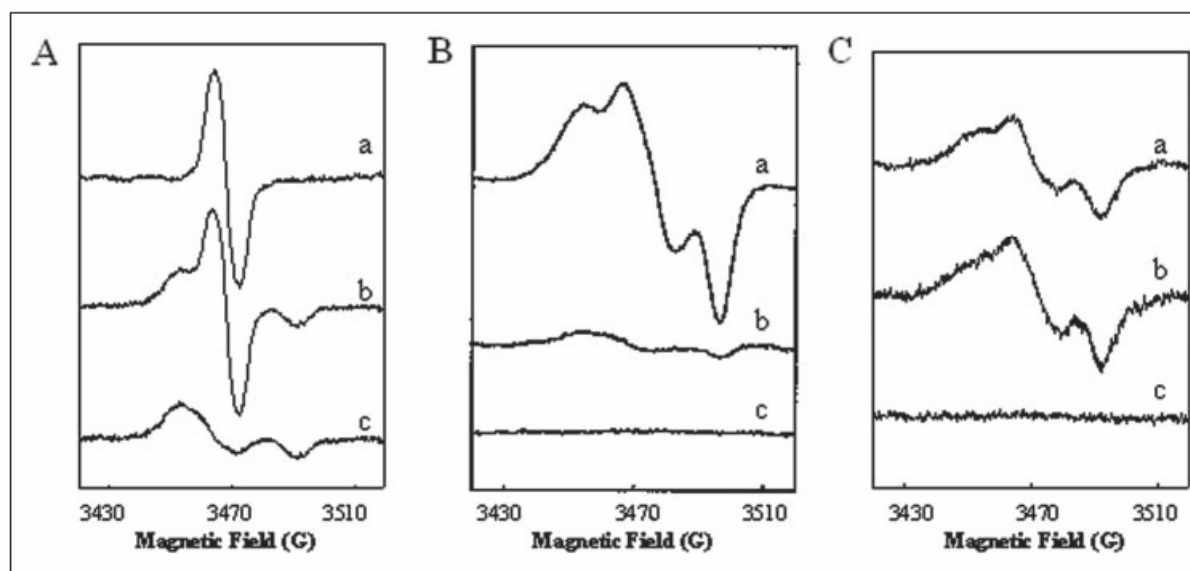


TABLE TWO**Steady-state kinetic properties of wild type and Tyr7 mutant enzymes**

See "Experimental Procedures" for experimental conditions.

Enzyme	K_m^{GSH}	K_m^{CDNB}	k_{cat}	K_i^{DNDGIC}	$\text{p}K_a$
	<i>mM</i>	<i>mM</i>	s^{-1}	<i>M</i>	
WT	0.15 ± 0.04	1.2 ± 0.1	38 ± 2	$<1.5 \times 10^{-9}$	6.2
Y7F	0.16 ± 0.03	1.8 ± 0.1	0.13 ± 0.05	0.94×10^{-6}	8.14
Y7H	0.35 ± 0.08	1.24 ± 0.12	0.08 ± 0.01	7.44×10^{-7}	8.46
Y7C	0.64 ± 0.14	1.11 ± 0.02	0.73 ± 0.12	1.33×10^{-6}	7.74



EPR spectra of (A) Tyr7Phe mutant; (B) intact *E. coli* cells after GSNO exposure; (C) purified GST P1-1