Enzyme Systems that Metabolise Drugs and Other Xenobiotics.
Edited by Costas Ioannides

ISBNs: 0-471-894-66-4 (Hardback); 0-470-84630-5 (Electronic)

9 Glutathione *S*-transferases

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Introduction

Glutathione *S*-transferase (GST; EC 2.5.1.18) isoenzymes are ubiquitously distributed in nature, being found in organisms as diverse as microbes, insects, plants, fish, birds and mammals (Hayes and Pulford 1995). The transferases possess various activities and participate in several different types of reaction. Most of these enzymes can catalyse the conjugation of reduced glutathione (GSH) with compounds that contain an electrophilic centre through the formation of a thioether bond between the sulphur atom of GSH and the substrate (Chasseaud 1979; Mannervik 1985). In addition to conjugation reactions, a number of GST isoenzymes exhibit other GSH-dependent catalytic activities including the reduction of organic hydroperoxides (Ketterer *et al*. 1990) and isomerisation of various unsaturated compounds (Benson *et al*. 1977; Jakoby and Habig 1980). These enzymes also have several non-catalytic functions that relate to the sequestering of carcinogens, intracellular transport of a wide spectrum of hydrophobic ligands, and modulation of signal transduction pathways (Listowsky 1993; Adler *et al*. 1999; Cho *et al*. 2001).

Glutathione *S*-transferases represent a complex grouping of proteins. Two entirely distinct superfamilies of enzyme have evolved that possess transferase activity (Hayes and Strange 2000). The first enzymes to be characterised were the cytosolic, or soluble, GSTs (Boyland and Chasseaud 1969; Mannervik 1985). To date at least 16 members of this superfamily have been identified in humans (Board *et al*. 1997, 2000; Hayes and Strange 2000). On the basis of their degree of sequence identity, the soluble mammalian enzymes have been assigned to eight families, or classes, designated Alpha (α), Mu (μ), Pi (π), Sigma (σ), Theta (θ), Zeta (ζ), Omega (ω) and Kappa (κ) (Mannervik *et al*. 1985; Meyer *et al*. 1991; Meyer and Thomas 1995; Pemble *et al*. 1996; Board *et al*. 1997, 2000). Four additional classes of this superfamily, called Beta (β), Delta (δ), Phi (ϕ) and Tau (τ) are represented in bacteria, insects and plants (Hayes and McLellan 1999), but discussion of these non-mammalian GSTs is beyond the scope of this chapter. The second, more recently defined superfamily is composed of microsomal transferases, and has been designated *m*embrane-*a*ssociated *p*roteins in

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*e*icosanoid and *g*lutathione metabolism, or MAPEG for short (Jakobsson *et al*. 1999a). In humans, the MAPEG superfamily has at least six members (Jakobsson *et al*. 2000).

Evolution of a large number of soluble GST and MAPEG members has allowed diversification of function, regulation and subcellular localisation in the two superfamilies. The soluble GSTs appear to be involved primarily in the metabolism of foreign chemicals, such as carcinogens, environmental pollutants and cancer chemotherapeutic drugs, as well as the detoxication of potentially harmful endogenously derived reactive compounds (Hayes and Pulford 1995). Many endogenous GST substrates are formed as a consequence of modification of macromolecules by reactive oxygen species, and the transferases are therefore considered to serve an antioxidant function (Mannervik 1986; Hayes and McLellan 1999). A few soluble GSTs are also involved in the synthesis and inactivation of prostaglandins. By contrast, MAPEG members are not principally involved in detoxication reactions, but are rather involved in the biosynthesis of leukotrienes and prostanoids, endogenous lipid signalling molecules (Jakobsson *et al*. 1999a). Thus, collectively, the catalytic actions of GST isoenzymes contribute to cellular detoxication processes and to autocrine and paracrine regulatory mechanisms.

Historical perspective of the research field

The transferases were first studied because of their involvement in the metabolism of xenobiotics rather than because of their contribution to the biosynthesis of leukotrienes or prostaglandins. Specifically, in 1961, extracts from rat liver were reported to catalyse the conjugation of GSH with either 1,2-dichloro-4-nitrobenzene (DCNB) (Booth *et al*. 1961) or with bromosulphophthalein (Coombes and Stakelum 1961). Once protein purification schemes were devised, it became apparent that one subunit belonging to class Mu of the soluble GST superfamily was responsible for both of these two activities (Habig *et al*. 1974; Mannervik and Jensson 1982). A separate line of investigation into cytosolic proteins in rat liver that bind carcinogens, steroids and bilirubin, led to the first description of class Alpha GST, though at the time the enzyme(s) was called 'ligandin' (Ketterer *et al*. 1967; Litwack *et al*. 1971; Hayes *et al*. 1979). Recognition that 1-chloro-2,4-dinitrobenzene (CDNB) is a more general transferase substrate facilitated identification of Alpha-, Pi-, Sigma- and Kappa-class GST, as well as other members of the Mu-class family (Habig *et al*. 1976; Kitahara *et al*. 1984; Urade *et al*. 1987; Harris *et al*. 1991). Class Theta transferases were first purified using 1-menaphthyl sulphate and 1,2-epoxy-3-(*p*-nitrophenoxy)propane as substrates (Fjellstedt *et al*. 1973; Gillham 1973; Hiratsuka *et al*. 1990; Meyer *et al*. 1991). More recently, the class Zeta and Omega GST were characterised using a bioinformatics approach (Board *et al*. 1997, 2000).

The first evidence for the existence of distinct membrane associated transferases was provided some twenty years after the original demonstration of soluble GST activity in rat liver. The membrane-associated enzyme was initially designated microsomal GST, and it was recognised to be functionally unique because its ability to conjugate CDNB with GSH is increased by covalent modification of the protein with the thiol agent *N*-ethylmaleimide (Morgenstern *et al*. 1979, 1980). The activation of the CDNB-GSH-conjugating activity of the rat microsomal GST occurs through alkylation of Cys-49

(DeJong *et al*. 1988), though it can also be achieved by limited proteolysis at either Lys-4 or Lys-41 (Morgenstern *et al*. 1989). Importantly, molecular cloning of the enzyme demonstrated that it shares no sequence identity with soluble GST (DeJong *et al*. 1988). McLellan *et al*. (1988) first reported the purification of human microsomal GST, now called MGST-I. A further human MAPEG member, called FLAP (5-lipoxygenase activating protein), was discovered through isolation of a membrane protein required to allow 5-lipoxygenase to convert arachidonic acid to 5-hydroperoxy-8,11,14-*cis*-6-*trans*-eicosatetraenoic acid and leukotriene A_4 (Dixon *et al*. 1990; Miller *et al*. 1990). Others were discovered through the characterisation of microsomal transferases responsible for leukotriene C_4 synthesis (Lam *et al*. 1994; Welsch *et al*. 1994; Jakobsson *et al*. 1996, 1997; Scoggan *et al*. 1997). The final member, identified because of its regulation by the p53 tumour suppressor protein, has been variously called *p*53 *i*nducible *g*ene 12, or PIG12 (Polyak *et al*. 1997), MGST-I-like I (Jakobsson *et al*. 1999a), or prostaglandin E synthase (Jakobsson *et al*. 1999b).

Functions of glutathione *S*-transferases

CATALYTIC PROPERTIES OF GST

Classically, GST enzymes have been considered to play a major part in phase II of drug-metabolism where they contribute to cell survival by detoxication of foreign compounds. In this role, GST action follows phase I of drug-metabolism which is often catalysed by members of the cytochrome P450 (CYP) supergene family (Klaassen 1996). The CYP enzymes catalyse the introduction of a functional group, such as an epoxide, into an otherwise chemically inactive xenobiotic. This functional group offers an electrophilic centre that is attacked by reduced glutathione (GSH), the incoming nucleophile, in a reaction catalysed by GST (Figure 9.1). The addition of GSH to the molecule gives it a molecular 'flag' which allows the xenobiotic-conjugate to be removed from the cell during phase III of drug-metabolism, a process which requires the participation of drug transporters such as multi-drug resistance associated protein (MRP) (Hayes and McLellan 1999). Once transported out of the cell, the peptide portion of the GSH-conjugate is subjected to peptidase attack, by γ-glutamyltransferase and either aminopeptidase M or cysteinylglycine dipeptidase, to yield a cysteinyl conjugate which is in turn N-acetylated to form a mercapturic acid (Boyland and Chasseaud 1969; Jakoby and Habig 1980). It is the mercapturic acid that is typically the final metabolite that is eliminated from the body in urine.

Compounds that undergo GST-catalysed conjugation with GSH include epoxide-containing compounds, alkyl- and aryl-halides, isothiocyanates, α,β-unsaturated carbonyls and quinones (Hayes and Pulford 1995). Not all xenobiotics that are substrates for GST require to be activated by CYP. It is likely that some are activated by cyclooxygenases (Marnett 1994) or by interaction with free radicals during oxidative stress (Trush and Kensler 1991). Others, such as isothiocyanates, may either be formed during digestion of vegetables (Verhoeven *et al*. 1997) or are generated as products of combustion (Klaassen 1996).

In addition to synthesising glutathione *S*-conjugates, GSTs catalyse the reduction of peroxide-containing compounds that may otherwise be toxic to the cell (Mannervik

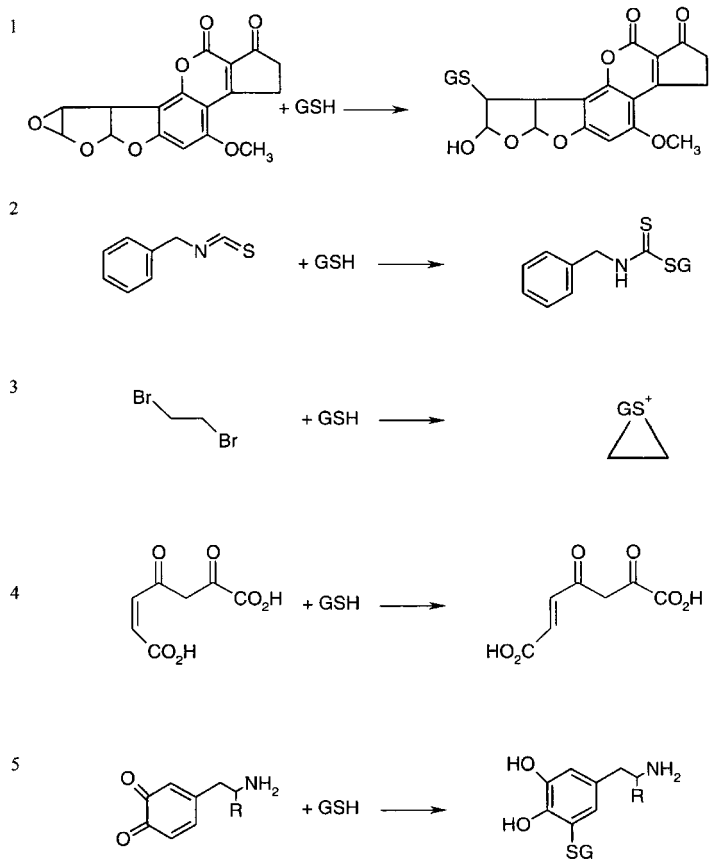

Figure 9.1 Examples of GST catalysed reactions. The GST substrates shown are as follows: 1, aflatoxin B_1-8,9-epoxide; 2, benzylisothiocyanate; 3, dibromoethane; 4, maleylacetoacetate; 5, a model *o*-quinone.

1986). The peroxidase activity of GST is not dependent on selenium but it does require GSH. It is a two-step reaction. The first step is an enzymic reduction of the peroxide to an alcohol, with the concomitant production of hydroxylated glutathione (GSOH). The second step entails the spontaneous reaction of GSOH with a molecule of GSH to yield water and oxidised glutathione (GSSG). Examples of this type of substrate include hydroperoxides of fatty acids and phospholipids. A related reaction is the GSH-dependent reduction of organic nitrate esters to alcohols and inorganic nitrite (Jakoby and Habig 1980).

GSTs also serve an important role in the isomerisation of many biologically important molecules. The transferases can catalyse *cis–trans* isomerisation reactions or the movement of a double bond within a polycyclic molecule (Benson *et al.* 1977;

Keen and Jakoby 1978). Physiological examples include the conversion of 13-*cis*-retinoic acid to all-*trans*-retinoic acid, a reaction that results in an increase in affinity of the retinoid for its receptor (Chen and Juchau 1997). The GSH-dependent conversion of prostaglandin (PG) H_2 to either PGD_2 or PGE_2 are other examples of isomerisation reactions in which GSTs are involved (Urade *et al*. 1995; Jakobsson *et al*. 1999b). Also, the isomerisation of maleylacetoacetate to furmaylacetoacetate, a step in the degradation of tyrosine, represents a GST-catalysed reaction (Fernández-Cañón and Peñalva 1998) (Figure 9.1).

BINDING OF NON-SUBSTRATE LIGANDS BY GST

In their non-enzymic ligand-binding capacity, GST isoenzymes serve a variety of functions involved in carcinogen-detoxication and intracellular transport of a wide spectrum of substances. It has been recognised for many years that a number of class Alpha GSTs can bind covalently reactive metabolites formed from 3-methylcholanthrene and azo-dye carcinogens (Litwack *et al*. 1971; Coles and Ketterer 1990). In these instances, GST is thought to sequester genotoxic compounds in a suicide type of reaction that prevents xenobiotics from interacting with DNA.

The soluble transferases bind many compounds in a non-covalent fashion. These ligands, which are typically lipophilic in nature and are not substrates for a conjugation reaction, include steroid hormones, thyroid hormones, bile acids, bilirubin, free-fatty acids and numerous drugs (Listowsky 1993). The biological consequence of these interactions between GST and non-substrate ligands is for the most part uncertain but the transferases may assist other enzymes in catalytic reactions, they may influence nuclear hormone receptor activity, or they may act to transport small molecules around the cell for excretion.

The finding that class Alpha, Mu and Pi GSTs have a high affinity for many glutathione *S*-conjugates is consistent with the theory that these enzymes do not release xenobiotics to diffuse across the cell once catalysis has occurred (Meyer 1993). However, it remains to be demonstrated whether either non-substrate ligand or glutathione *S*-conjugates are released by GST following an interaction with a drug-transporter protein such as MRP at the plasma membrane. GSTs are known to occupy a number of compartments within the cell including the nucleus, cytoplasm, mitochondrion and endoplasmic reticulum. Furthermore, drug treatments can cause GST to move between the cytoplasm and the nuclear compartments (Sherratt *et al*. 1998). It therefore appears possible that these enzymes could control the movement of xenobiotics within the cell, or the passage of xenobiotics across cells.

Among the MAPEG superfamily, FLAP appears to act as a carrier protein for arachidonic acid (Mancini *et al*. 1993). Although FLAP does not appear to exhibit catalytic activity, it acts to facilitate the conversion by 5-lipoxygenase of arachidonic acid to leukotriene A_4. FLAP serves as a useful reminder that the binding of non-substrate ligands by GST, and subsequent interaction with other proteins, may be essential for the efficient function of enzyme systems that are unrelated to the transferases.

INVOLVEMENT OF GST IN PROTEIN-PROTEIN INTERACTIONS

Recent studies have shown that soluble GST can bind to other intracellular proteins and modulate their function. In a model presented by the research groups of Ronai and Tew, it has been proposed that class Pi GST subunits form a complex with c-Jun N-terminal Kinase (JNK), and in so doing maintain it in an inactive form (Adler *et al*. 1999). It is believed that under non-stressed conditions, the binding of GSTP1 to JNK helps to block signalling along the stress kinase pathway. According to this hypothesis, when cells are subjected to oxidative stress, class Pi GST subunits dissociate from the kinase and allow JNK to become activated.

Using a tet-off-inducible system to control levels of class Pi GST in NIH 3T3 cells, Yin *et al*. (2000) confirmed that expression of the transferase could reduce the extent of JNK phosphorylation. In these experiments, expression of class Pi GST also caused a reduction in the phosphorylation of mitogen-activated protein (MAP) kinase kinase 7, but an increase in the phosphorylation of MAP kinase kinase 4, p38, extracellular receptor kinase and inhibitor of κ-kinase. The expression of the transferase in 3T3 fibroblasts made the cells more resistant to death caused by exposure to H_2O_2, and this is suggested to be attributable to modulation of stress kinase responses (Yin *et al*. 2000).

Class Pi GST is not unique in modulating signal transduction. A class Mu transferase can bind and inhibit apoptosis signal-regulating kinase 1 (Cho *et al*. 2001)

The human Omega class GSTO1-1 has been shown by Board and his colleagues to interact with the calcium channel ryanodine receptors that are found in the endoplasmic reticulum of various cells (Dulhunty *et al*. 2000). These workers have suggested that this transferase may protect cells against apoptosis in cardiac muscle that contains the ryanodine receptor 2 channel.

This represents an emerging and potentially exciting area of research that adds to the diverse roles played by GST within the cell. Importantly, it suggests that certain transferases can act as sensors of oxidative stress within cells and help coordinate the regulation of stress kinases. Future research is likely to reveal the existence of additional interactions between GST and cellular proteins, as well as details about the putative role of GST as a sensor of oxidative and chemical stress.

Metabolism of xenobiotics and endobiotics by GST

GST AND DETOXICATION

The ability of GSTs to inactivate potentially cytotoxic and genotoxic compounds has been the most thoroughly studied aspect of their function within the cell. The transferases play an important role in the detoxication of a broad spectrum of noxious chemicals that may lead to mutagenic events or cytotoxicity (Coles and Ketterer 1990; Hayes and Pulford 1995). Examples of such compounds include the ultimate carcinogens produced from aflatoxin B_1 and benzo(*a*)pyrene that are formed as a consequence of phase I drug metabolism (Figure 9.1). These are examples where conjugation of the compound with GSH leads to the production of a harmless metabolite that is readily eliminated from the cell. GSTs can also detoxify a number of man-made compounds that are common environmental pollutants. Examples are

pesticides widely used in agricultural farming. The transferases are capable of metabolising dichlorodiphenyltrichloroethane (DDT), atrazine, lindane and methyl parathion either by catalysing formation of GSH-conjugates or by dehalogenation activity.

The detoxication reactions listed above are of benefit to the host, and remove potentially toxic chemicals from the cell. In cancer therapy, however, the defence provided by GST within a tumour cell is a potential problem to the host rather than a benefit. Certain anticancer drugs such as 1,3-*bis*(2-chloroethyl)-1-nitrosourea (BCNU), chlorambucil, cyclophosphamide, melphalan and thiotepa are detoxified by GST (Tew 1994; Hayes and Pulford 1995). A large body of literature suggests that GST overexpression in tumours is an important mechanism of acquired resistance to cancer chemotherapeutic agents, particularly if it is associated with overexpression of MRP (Morrow *et al*. 1998).

Apart from detoxication of foreign compounds, there are a number of harmful endogenous compounds formed as by-products of normal metabolism that are GST substrates. The process of aerobic respiration can lead to the production of reactive oxygen species (the superoxide anion O_2^-, hydrogen peroxide H_2O_2, and the hydroxyl radical •OH) (Finkel and Holbrook 2000). Attack of membrane lipids by free radicals leads to the formation of lipid peroxidation products which can propagate a chain reaction of lipid peroxidation in an aerobic environment, that will ultimately end in membrane destruction (Slater 1984). GST from both the soluble and MAPEG supergene families have the capacity to reduce these compounds rendering them harmless. They also act to detoxify downstream products of oxidative damage such as the reactive aldehydes 4-hydroxynonenal and acrolein (Hayes and McLellan 1999). Furthermore, GST can reduce DNA hydroperoxides such as thymine peroxide (Bao *et al*. 1997), an event that may be important *in vivo* as GSTs appear to be present within the nucleus of a cell. Another important endogenous function of GST is in the detoxication of *o*-quinones derived from catecholamines (Baez *et al*. 1997) (Figure 9.1). A member of the class Mu GST gene family has been shown to provide a neuroprotective metabolic pathway for dopamine and dopa *o*-quinones. This metabolism prevents the formation of aminochrome and dopachrome respectively that can lead to neurodegeneration.

GST AND BIOACTIVATION

Not all reactions catalysed by GST lead to the formation of a less toxic product. In a number of instances, the action of GST has been shown to lead to a more reactive and potentially carcinogenic intermediate. Compounds that undergo bioactivation are synthetic and do not occur naturally within the environment. This is best exemplified by the case of short-chain alkyl-halides. During the industrial age, a number of such chemicals have been developed that have various useful properties, in particular that of being excellent solvents. The American NTP report in 1986 that dichloromethane (DCM), a solvent used extensively by both the consumer and industry, is carcinogenic in mice but not in other rodents led to one of the first toxicology investigations to entail detailed mechanistic studies (Green 1997). The studies revealed that in the mouse a member of the class Theta GST gene family was capable of metabolising

DCM to form a highly reactive *S*-chloromethylglutathione conjugate. This intermediate still contains an electrophilic centre with the remaining chloride and the addition of GSH is thought to facilitate its ability to move around the cell. Methanes that contain only a single halide substituent undergo the same conjugation reaction catalysed by class Theta GST but are not toxic to the cell (Chamberlain *et al*. 1998). This is not an isolated incidence, as another commonly used dihaloalkane, dibromoethane, is also activated through substitution of thiolate for halide (van Bladeren 1980) (Figure 9.1). The intermediate formed from this compound, and similar compounds, spontaneously cyclises to form an episulphonium anion that is an even more potent mutagen than the dihalomethane metabolites. These dihaloalkanes are carcinogenic in all rodent model systems. The man-made bifunctional electrophiles that are activated by GST are listed in Table 9.1. Certain alkenes undergo more classical metabolism requiring activation by CYP to introduce the electrophilic centre before their mutagenic potential is increased further by GST. Examples of such compounds include butadiene and isoprene, both of which are used in the rubber industry (Guengerich *et al*. 1995).

There are also examples of compounds that may initially be detoxified by GST-catalysed conjugation but then undergo spontaneous reversal of the initial conjugate to regenerate the toxic xenobiotic (Baillie and Slatter 1991; Baillie and Kassahun 1994). Isothiocyanates are a group of xenobiotics that undergo a GST-catalysed reversible conjugation with GSH to form thiocarbamates (Kolm *et al*. 1995). Following conjugation, the thiocarbamate is secreted from the cell of synthesis, but as it is labile and the reaction is reversible, the parent isothiocyanate can be regenerated. If this occurs at a site that lacks GST, toxicity may result (Bruggeman *et al*. 1986).

The conjugation of haloalkenes with GSH can also lead to toxicity (Anders *et al*. 1988; Dekant *et al*. 1994). In this case the toxicity is not due to the glutathione *S*-conjugate itself, but results from the formation of reactive metabolites produced from the cysteine *S*-conjugate. Specifically, unstable thiols can be formed by the action of cysteine conjugate β-lyase (see Chapter 1). Since this enzyme is found in high amounts in the kidney, renal damage is usually observed when unstable thiols are produced.

The ability of GST to activate compounds to a cytotoxic intermediate may be beneficial in certain cases. This property of GST can be turned to therapeutic advantage for the treatment of malignant disease. A number of soluble GSTs, in particular class-Pi transferase, are overexpressed in many human tumours and may contribute to drug resistance. One approach to circumvent this problem is the development of modulators of GST activity, such as specific enzyme inhibitors, or strategies to deplete GSH as methods of reducing GST activity in cancer cells. Another approach has been to use the increase in GST activity in cancer cells to achieve enhanced activation of cytotoxic prodrugs within the tumour. TER 286 [γ-glutamyl-α-amino-β((2-ethyl-*N*,*N*,*N*′,*N*′-tetrakis(2-chloroethyl)phosphorodiamidate)-sulphonyl)-propionyl-(*R*)-(-)phenylglycine] is a latent drug that is activated to a nitrogen mustard alkylating agent by a reaction catalysed by GST (Morgan *et al*. 1998). The nitrogen mustard then spontaneously yields an aziridium ring compound that can alkylate DNA (Figure 9.2). This compound has been found to be effective against drug-resistant breast and colon cancer cells and has entered clinical trials.

In their activities with respect to toxicity, GSTs can be considered to be both an

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, *N*-(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

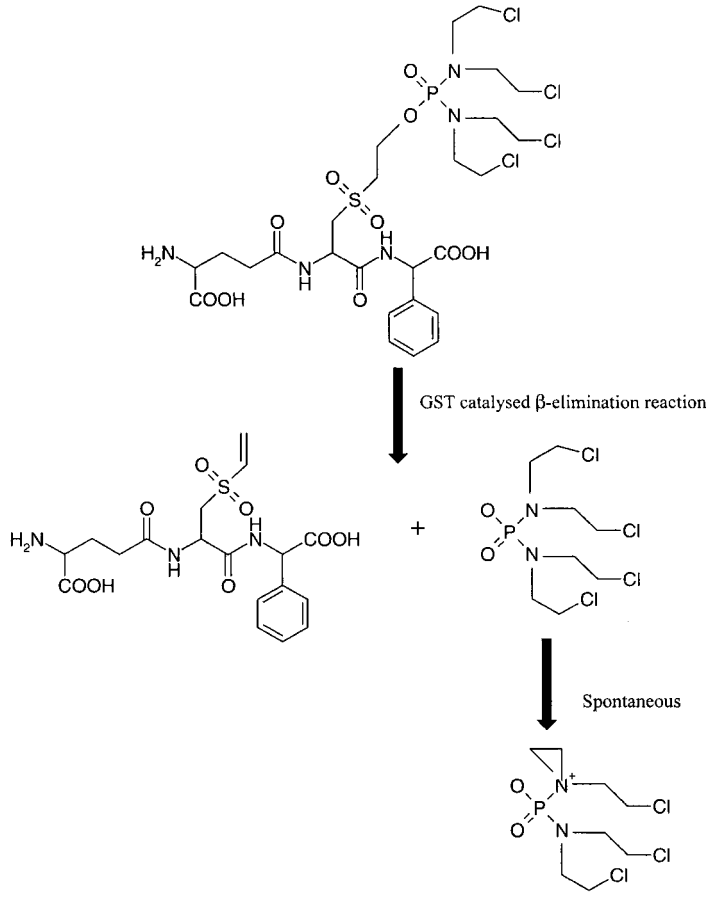

Figure 9.2 Bioactivation of TER 286. TER 286: γ-glutamyl-α-amino-β-((2-ethyl-*N*,*N*,*N*′,*N*′-tetrakis (2-chloroethyl)phosphorodiamidate)-sulphonyl)propionyl-(*R*)-(-)phenylglycine.

advantage and a disadvantage. Thus, an in-depth knowledge of the activity of these enzymes would be helpful during early development of modern drug-treatment strategies. An example of this detailed foreknowledge would have been of value before the use of troglitazone, a member of the thiazolindinedione family of peroxisome proliferator activated receptor-γ (PPARγ) agonist insulin-sensitisers that is used to treat non-insulin-dependent diabetes mellitus. Extended clinical use of this compound can lead to rare instances of hepatic damage where occasional fatalities occur due to liver failure (Kohlroser *et al*. 2000). Early suggestions are that a reaction involving CYP isoenzymes can lead to the formation of functionalised intermediates that can be conjugated with GSH (Baillie and Kassahun 2000; Kassahun *et al*. 2001). Which of the intermediates formed during the metabolism of troglitazone is responsible for the toxic effects is uncertain. As our understanding of GST and other drug-metabolising

enzymes increases at the mechanistic level, so our ability to develop safe and effective therapeutics will be enhanced.

GST AND BIOSYNTHETIC METABOLISM

Apart from detoxifying chemicals in the defence of the cell, GSTs play a crucial part in the synthesis of biologically important endogenous molecules. Transferases in the MAPEG superfamily act as prostaglandin E_2 and leukotriene C_4 synthases, and therefore play a central role in the production of mediators of inflammation and hypersensitivity. Members of this superfamily possess some substrate overlap that gives rise to a degree of degeneracy (Table 9.2). In instances where a particular leukotriene C_4 synthase is expressed at low levels in a given tissue, another MAPEG enzyme may compensate for its absence. Certain members of the soluble GST supergene family are also involved in prostanoid biosynthesis (Table 9.2). Class Alpha GST catalyse production of prostaglandin $F_{2\alpha}$. The class Sigma transferase is also known as a GSH-dependent prostaglandin D_2 synthase and initiates the pathway that leads to the eventual formation of the J_2-series of prostaglandins that are important signalling molecules as they are ligands for PPARγ.

In addition to synthesising prostaglandins, class Alpha, Mu and Pi GST catalyse the conjugation of PGA_2 and PGJ_2 with GSH (Bogaards *et al*. 1997). The prostaglandin conjugates are then eliminated from the cell by the MRP transporter. It is therefore envisaged that GST isoenzymes regulate the half-life of certain prostanoids in cells thereby attenuating their biological actions.

Apart from their involvement in prostanoid biology, GSTs are known to contribute to the metabolism of other endogenous compounds. The class Zeta transferase plays an important role in the degradation of tyrosine. It has been identified as the maleylacetoacetate isomerase that is responsible for the conversion of maleylacetoacetate to furnarylacetoacetate (Fernández-Cañón and Peñalva 1998). In humans, malfunction of this pathway can lead to serious pathological changes in the liver, kidney and peripheral nerves.

Genetic and biochemical properties of the glutathione *S*-transferase system

GST SUPERGENE FAMILIES

Glutathione *S*-transferase enzymes have evolved on at least two separate occasions. Two distinct multi-gene families exist, the soluble GST superfamily and the MAPEG superfamily. Transferases from the soluble GST and MAPEG families have no similarity at the level of primary structure. There are significant differences in the sizes of the protein subunits in the two superfamilies. The soluble transferases are all dimeric proteins, and those GST subunits that are most closely related can form heterodimers (Hayes and Pulford 1995). By contrast, MAPEG enzymes are trimeric (Hebert *et al*. 1997; Schmidt-Krey *et al*. 2000) and there is no evidence that they can form heterotrimers.

As can be seen from Table 9.3, GST genes are located on a large number of different chromosomes. Genes encoding human soluble GSTs that are members of the same class (i.e. they share greater than 50% sequence identity) all appear to be clustered on

Table 9.2 Involvement of human GST isoenzymes in leukotriene and prostaglandin metabolism

Superfamily	Isoenzyme	Biosynthesis involving isomerisation, reduction, or non-catalytic binding	Conjugation reactions with GSH
Soluble	GSTA1-1	$PGH_2 \rightarrow PGE_2$; $PGH_2 \rightarrow PGF_{2\alpha}$	Conjugation of PGA_2 and PGJ_2
	GSTA2-2	$PGH_2 \rightarrow PGD_2$; $PGH_2 \rightarrow PGF_{2\alpha}$	–
	GSTM1-1	–	Conjugation of PGA_2 and PGJ_2
	GSTP1-1	–	Conjugation of PGA_2 and PGJ_2
	GSTS1-1	$PGH_2 \rightarrow PGD_2$	–
MAPEG	MGST-I-like I	$PGH_2 \rightarrow PGE_2$	–
	MGST-II	Reduction of 5-HPETE	$LTA_4 \rightarrow LTC_4$
	MGST-III	Reduction of 5-HPETE	$LTA_4 \rightarrow LTC_4$
	LTC_4S	–	$LTA_4 \rightarrow LTC_4$
	FLAP	Binding of arachidonic acid	–

5-HPETE, (*S*)-5-hydroperoxy-8,11,14-*cis*-6-*trans*-eicosatetraenoic acid; LT, leukotriene; PG, prostaglandin

Table 9.3 Genetic and biochemical properties of human glutathione *S*-transferases

Superfamily	Class	Chromosomes	Enzyme	Substrates
Soluble	Alpha	6p12	GSTA1-1	CDNB; 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; Δ^5-androstene-3,17-dione
			GSTA2-2	CDNB; 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; cumene hydroperoxide
			GSTA3-3	Not determined
			GSTA4-4	Ethacrynic acid; 4-hydroxynonenal; 4-hydroxydecenal
Soluble	Mu	1p13.3	GSTM1-1	CDNB; *trans*-4-phenyl-3-buten-2-one; aflatoxin B_1-epoxide; *trans*-stilbene oxide
			GSTM2-2	High with CDNB; 1,2-dichloro-4-nitrobenzene; aminochrome
			GSTM3-3	Low towards CDNB; H_2O_2
			GSTM4-4	Not determined
			GSTM5-5	Low towards CDNB
Soluble	Pi	11q13	GSTP1-1	CDNB; acrolein; adenine propenal; BPDE; benzyl isothiocyanate; ethacrynic acid
Soluble	Sigma	4q21-22	GSTS1-1	PGD_2 synthase
Soluble	Theta	22q11	GSTT1-1	1,2-epoxy-3-(*p*-nitrophenoxy)propane; dichloromethane; dibromoethane
			GSTT2-2	1-menaphthyl sulphate; cumene hydroperoxide

continued overleaf

Table 9.3 (*continued*)

Superfamily	Class	Chromosomes	Enzyme	Substrates
Soluble	Zeta	14q24.3	GSTZ1-1	Dichloroacetate; fluoroacetate; malelyacetoacetate
Soluble	Omega	10q23-25	GSTO1-1	Thioltransferase (very low with CDNB and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole)
Soluble	Kappa	not determined	GSTK1-1	Not determined
MAPEG	(Microsomal)	12p13.1-13.2	MGST-I	CDNB; 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; 4-nitrobenzyl chloride; cumene hydroperoxide
		9q34.3	MGST-I-like I	PGE_2 synthase
		4q28-31	MGST-II	CDNB; leukotriene C_4 synthase; 5-HPETE
		1q23	MGST-III	Leukotriene C_4 synthase; 5-HPETE
		5q35	LTC_4S	Leukotriene C_4 synthase
		13q12	FLAP	5-lipoxygenase-activating protein (binds arachidonic acid)

BPDE, benzo(*a*)pyrene diol epoxide: CDNB, 1-chloro-2,4-dinitrobenzene; 5-HPETE, (*S*)-5-hydroperoxy-8,11,14-*cis*-6-*trans*-eicosatetraenoic acid; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2

the same chromosome (Pearson *et al*. 1993; Xu *et al*. 1998; Coggan *et al*. 1998). The fact that the Alpha, Mu and Theta GST classes each contain multiple isoenzymes indicates that these families have undergone several relatively recent gene duplication events (Figure 9.3). By contrast, the lack of extensive homology amongst MAPEG members suggests that they have not undergone similar recent gene duplication (Figure 9.4). Possibly the physiological functions of the MAPEG family were largely established, in an evolutionary sense, many years before those of the soluble GST.

At present, the precise relationship between the mitochondrial class Kappa enzyme and other transferases is unclear. Certainly, the dendrogram analysis shown in Figure 9.3 indicates that GSTK1 shares closest homology with the class Omega subunit. As pointed out by Pemble *et al*. (1996), the GSTK1 subunit does not contain a SNAIL/TRAIL motif that is usually present in soluble GST superfamily members, and resides between residues 60–80. Determination of the crystal structure of GSTK1-1, along with identification of the residue involved in forming the glutathione thiolate anion and its catalytic mechanism, will help clarify the evolutionary history of this enzyme.

FUNCTION OF GST ISOENZYMES

Characterisation of the biochemical activities of GST revealed that individual isoenzymes can metabolise a spectrum of electrophilic compounds. In general, individual transferases display overlapping substrate specificities. However, a significant number of GST subunits possess unique catalytic features which supports the notion that each gene evolved to allow detoxication and/or transport of distinct xenobiotics

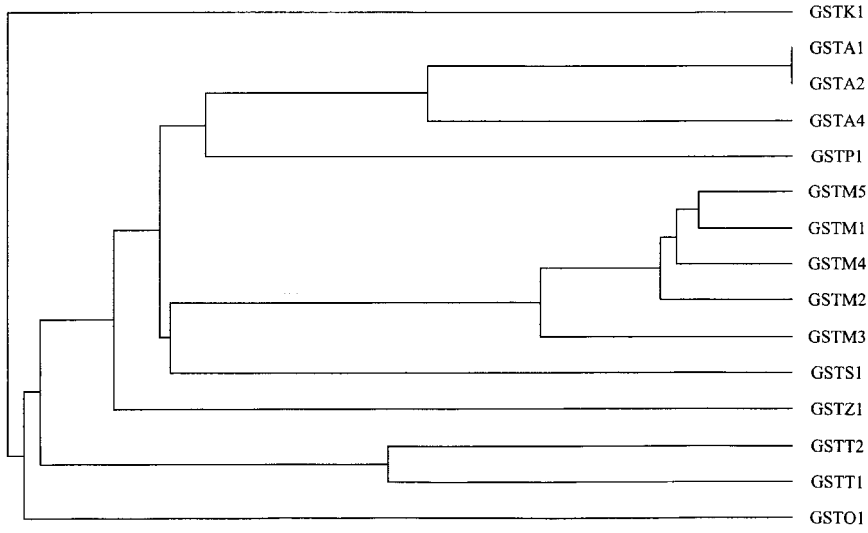

Figure 9.3 An average distance tree of the human soluble GST superfamily. The structural relationship between the different classes of GST was determined by multisequence alignment using Clustalw. The alignment was used to calculate an average distance tree using Jalview. Both applications were accessed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

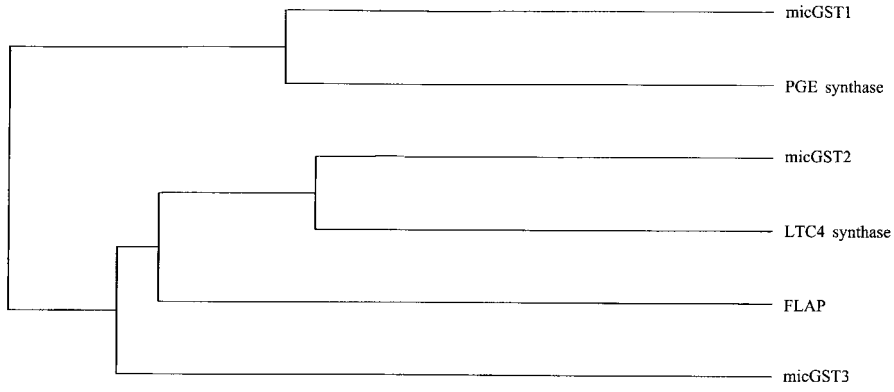

Figure 9.4 An average distance tree of the human MAPEG superfamily. This was constructed as describe in the legend to Figure 9.3. In this dendrogram the following GST abbreviations have been used: micGST1, MGST-I; PGE Synthase, MGST-I-like 1 (or PIG12); LTC4 Synthase, LTC_4S; FLAP, 5-lipoxygenase activating protein; micGST3, MGST-III.

or endobiotics. For example, characteristic activities for a few of the human transferases are as follows: GSTA1-1, isomerisation of Δ^5-androstene-3,17-dione; GSTA2-2, reduction of cumene hydroperoxide; GSTA4-4, conjugation of 4-hydroxynonenal with GSH; GSTM1-1, conjugation of *trans*-stilbene oxide; GSTM2-2, conjugation of DCNB with GSH; GSTP1-1, conjugation of benzo[*a*]pyrene diol epoxide with GSH; GSTS1-1, isomerisation of PGH_2 to PGD_2; GSTT1-1, conjugation of 1,2-epoxy-3-(*p*-nitrophenoxy)propane with GSH; GSTT2-2, conjugation of 1-menaphthyl sulphate with GSH; GSTZ1-1, isomerisation of malelyacetoacetate; MGST-I-like I (or PIG12), synthesis of PGE_2.

It is interesting that even though the two GST superfamilies occupy different subcellular compartments, both contain members that are active towards aryl-halides (CDNB), organic hydroperoxides (cumene hydroperoxide), and prostaglandin H_2 (PGD_2, PGE_2 and $PGF_{2\alpha}$ synthases). This suggests that during evolution both superfamilies have been subject to similar selection pressures.

POLYMORPHIC EXPRESSION OF GST

In the human, a significant number of genetic polymorphisms among the soluble GST have been described (for a review, see Hayes and Strange 2000). Importantly, variation in GST alleles is very common in the population and will presumably make a significant contribution to inter-individual differences in drug metabolism. Gene deletions have been reported for *GSTM1* and *GSTT1*, and alterations in amino acid coding sequences have been demonstrated for *GSTA2*, *GSTM1*, *GSTP1*, *GSTT2* and *GSTZ1* (Table 9.4). One of the allelic forms of *GSTT2* encodes a truncated protein (Coggan *et al*. 1998). An allelic variation occurs in intron 6 of *GSTM3* with one form of the gene lacking a YYI transcription factor binding site (Inskip *et al*. 1995). Allelic variations have also been found among MAPEG members, though these occur in the non-coding regions of *MGST-I, LTC4S* and *FLAP* (Table 9.4).

For the most part, polymorphisms in individual *GST* genes do not obviously confer a markedly increased risk of cancer. Typically, odds ratios associated with any single variant *GST* allele and the development of particular neoplastic diseases are found to be less than 3.0 (Hayes and Strange 2000). However, combinations of variant GST alleles, either with other polymorphic GST or with alleles of other detoxication or antioxidant genes, are likely to have an additive effect in conferring predisposition to

Table 9.4 Polymorphic human glutathione *S*-transferases

Class or superfamily	Gene	Allele	Alterations in gene or in nucleotides	Protein or amino acids affected
Alpha	*GSTA2*	*GSTA2*A*	C335, A629	Thr^{112}, Glu^{210}
		*GSTA2*B*	G335, C629	Ser^{112}, Ala^{210}
Mu	*GSTM1*	*GSTM1*A*	G519	Lys^{173}
		*GSTM1*B*	C519	Asn^{173}
		*GSTM1*0*	Gene deletion	No protein
		*GSTM1*1×2*	Gene duplication	Overexpression
	GSTM3	*GSTM3*A*	Wildtype	Wildtype protein
		*GSTM3*B*	3 bp deletion in intron 6	Primary structure unaltered
	GSTM4	*GSTM4*A*	Wildtype	Wildtype
		*GSTM4*B*	Changes in introns	Unchanged
Pi	*GSTP1*	*GSTP1*A*	A313, C341, C555	Ile^{105}, Ala^{114}, Ser^{185}
		*GSTP1*B*	G313, C341, T555	Val^{105}, Ala^{114}, Ser^{185}
		*GSTP1*C*	G313, T341, T555	Val^{105}, Val^{114}, Ser^{185}
		*GSTP1*D*	A313, T341	Ile^{105}, Val^{114}
Theta	*GSTT1*	*GSTT1*A*	Unique gene	Unique protein
		*GSTT1*0*	Gene deletion	No protein
	GSTT2	*GSTT2*A*	A415	Met^{139}
		*GSTT2*B*	G415	Ile^{139}
Zeta	*GSTZ1*	*GSTZ1*A*	A94; A124; C245	Lys^{32}; Arg^{42}; Thr^{82}
		*GSTZ1*B*	A94; G124; C245	Lys^{32}; Gly^{42}; Thr^{82}
		*GSTZ1*C*	G94; G124; C245	Glu^{32}; Gly^{42}; Thr^{82}
		*GSTZ1*D*	G94; G124; T245	Glu^{32}; Gly^{42}; Met^{82}
MAPEG	*MGST1*	*MGST1*A*	T598 (non-coding 3′)	Wildtype
		*MGST1*B*	G598 (non-coding 3′)	Unchanged
	LTC_4S	*LTC_4S*A*	A-444 (promoter)	Wildtype
		*LTC_4S*B*	C-444 (promoter)	Increase in protein levels
	FLAP	*FLAP*A*	No *Hin*dIII site in intron II	Wildtype
		*FLAP*B*	T → C forming *Hin*dIII site	Unchanged

The nucleotide number quoted is that found in the cDNA. The amino acid number includes the initiator methionine.

degenerative disease. The most dramatic example of this reported to date occurs in breast cancer. Hirvonen and colleagues have found that in premenopausal women, combinations of the *GSTM3*B* allele with the *GSTT1*00* and *GSTP1*AA* genotypes appear to have a twenty-six-fold increased risk of developing advanced breast cancer when compared with other GST genotypes (Hirvonen *et al*. 2001). Substantially increased risk of advanced breast cancer was also seen in *GSTM1*00* individuals with *GSTP1*AA* and either the *GSTM3*B* allele or the *GSTT1*00* genotype (Hirvonen *et al*. 2001).

GST polymorphisms not only influence susceptibility to disease, but they also appear to influence responsiveness to cancer chemotherapeutic agents. In breast cancer, patients that have two copies of *GSTP1*B* and/or *GSTP1*C* have better survival than those with two copies of *GSTP1*A* and/or *GSTP1*D* (Sweeney *et al*. 2000). These authors postulated that the *GSTP1*B* and *GSTP1*C* alleles encode enzymes with less activity towards anti-cancer drugs than the enzymes encoded by *GSTP1*A* and *GSTP1*D*. However, different GSTP1 subunits may show differences in their interaction with stress kinases.

Biological control of GST

TISSUE-SPECIFIC REGULATION

In most species examined to date, the transferases are expressed in an organ-specific fashion. In the rat, class Alpha GSTs are found in largest amounts in liver, kidney and small intestine, class Mu GSTs are found in liver, lung, heart, spleen, thymus, brain and testis, and class Pi GST is present in most extrahepatic tissues (Hayes and Mantle 1986; Li *et al*. 1986; Abramovitz and Listowsky 1987). The rat class Sigma GST is expressed in spleen, class Theta GSTs are found in liver, testis, adrenal gland, kidney and lung, and class Kappa has been identified in liver (Urade *et al*. 1987; Harris *et al*. 1991; Watabe *et al*. 1996). In the mouse, expression of class Alpha, Mu and Pi GSTs has also been shown to differ markedly in liver, lung, kidney, spleen, small intestine, heart, brain, testis and ovary (Pearson *et al*. 1988; McLellan *et al*. 1992; Mitchell *et al*. 1997). Class Theta GSTs are present in mouse liver and lung (Mainwaring *et al*. 1996).

Table 9.5 summarises information about the tissue-specific expression of the human soluble GSTs. In this species, class Alpha are found in substantial amounts in liver, kidney and testis, with some in intestine, pancreas and lung (Mannervik and Widersten 1995; Coles *et al*. 2000). Human class Mu are found primarily in liver, skeletal muscle, heart, brain and testis, but each subunit shows its own distinct tissue-specific pattern of expression (Takahashi *et al*. 1993; Rowe *et al*. 1997). Class Pi and class Theta GSTs are widely distributed in human tissues (Sherratt *et al*. 1997) (Figure 9.5). Interestingly, in the human, GST T1-1 is present in red blood cells where it is postulated to act as a 'sink' for dihaloalkanes that can be bioactivated, thereby possibly preventing genotoxic damage in other cell types.

Less is known about the distribution of the MAPEG enzymes than the soluble GSTs and most of the available data about their expression relates to the human. Among MAPEG members, MGST-I is thought to serve a detoxication role, and it is therefore appropriate that it is present in high amounts in the liver and kidney. It is, however,

Table 9.5 Tissue distribution of human glutathione *S*-transferases

Superfamily	Class	Protein	Organ
Soluble	Alpha	GSTA1	Testis ≈ liver ≫ kidney ≈ adrenal > pancreas
		GSTA2	Liver ≈ testis ≈ pancreas > kidney > adrenal > brain
		GSTA3	Placenta
		GSTA4	Small intestine ≈ spleen > liver ≈ kidney > brain
Soluble	Mu	GSTM1	Liver > testis > brain > adrenal ≈ kidney > lung
		GSTM2	Brain ≈ skeletal muscle ≈ testis > heart > kidney
		GSTM3	Testis ≫ brain ≈ small intestine > skeletal muscle
		GSTM4	Testis
		GSTM5	Brain, heart, lung, testis
Soluble	Pi	GSTP1	Brain > heart ≈ lung ≈ testis > kidney ≈ pancreas
Soluble	Sigma	GSTS1	Foetal liver, bone marrow
Soluble	Theta	GSTT1	Kidney ≈ liver > small intestine > brain ≈ prostate
		GSTT2	liver
Soluble	Zeta	GSTZ1	Foetal liver, skeletal muscle
Soluble	Omega	GSTO1	Liver ≈ heart ≈ skeletal muscle > pancreas > kidney
Soluble	Kappa	GSTK1	Liver (mitochondria)
MAPEG	(Microsomal)	MGST-I	Liver ≈ pancreas > prostate > colon ≈ kidney > brain
		MGST-I-like I	Testis > prostate > small intestine ≈ colon
		MGST-II	Liver ≈ skeletal muscle ≈ small intestine > testis
		MGST-III	Heart > skeletal muscle ≈ adrenal gland, thyroid
		LTC_4S	Platelets ≈ lung > liver
		FLAP	Lung ≈ spleen ≈ thymus ≈ PBL ≫ small intestine

Data based on references cited in the text.
PBL: Peripheral blood leukocytes

also found in pancreas, prostate and brain (Estonius *et al*. 1999; Lee and DeJong 1999). The MGST-I-like I (PIG12) is a prostaglandin E_2 synthase and is found in highest amounts in the testis and prostate, but it is also expressed in the gastrointestinal tract (Jakobsson *et al*. 1999b). The MGST-II, MGST-III, LTC_4S and FLAP isoenzymes are all involved in the synthesis of leukotriene C_4 but display different patterns of expression, being found in varying amounts in platelets, lung, skeletal muscle, adrenal gland, spleen, small intestine and liver (Jakobsson *et al*. 1996, 1997; Scoggan *et al*. 1997).

The information about the two GST superfamilies in rat, mouse and human tissues has been obtained from combinations of enzyme purification, Western Blotting and multiple-tissue Northern Blots. With the exception of class Alpha, Mu and Pi transferases, relatively little is known about the cell types that express the different soluble GST and MAPEG isoenzymes as few immunohistochemical or *in situ* hybridisation studies have been reported. This type of information will be important in efforts to unravel the functions of the two superfamilies.

HORMONAL REGULATION OF GST

In rodents, significant differences in the hepatic expression of class Alpha, Mu and Pi GST have been seen in male and female animals. The livers of male rats contain more

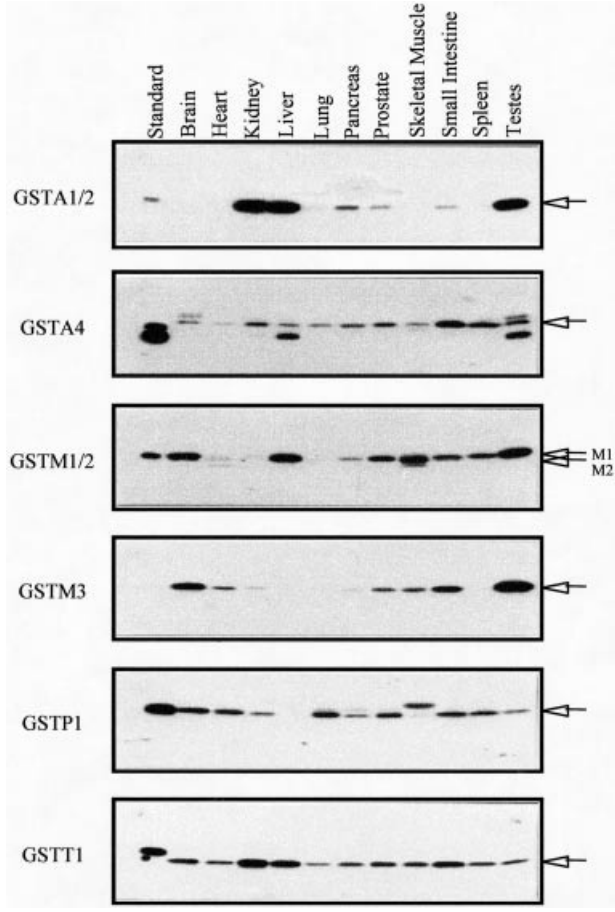

Figure 9.5 Tissue-specific expression of GST isoenzymes in human organs. Extracts from human organs were subjected to immunoblotting as described by Sherratt *et al.* (1997). The identity of the sample loaded in each lane is indicated at the top of the figure. In the left-hand margin, the identity of 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.

rGSTA2, rGSTM1 and rGSTM2 than do livers from female rats, a difference that is dependent on pituitary function (Staffas *et al.* 1992). More recently, it has been found that female rats express a higher level of the rGSTA5 subunit in the liver than do male rats, and that male rats down-regulate the enzyme between week 5 and week 10 of life (Hayes *et al.* 1994). Interestingly, the higher level of expression of rGSTA5 in female rats appears to account for the sex-specific difference in sensitivity to aflatoxin B_1 (for a review on aflatoxin B_1 see Hayes *et al.* 1991). The hepatic differences in expression of class Alpha and class Mu transferases in the rat have been attributed to the sexually dimorphic secretion of growth hormone (Staffas *et al.* 1998).

Unlike rats and humans, mice express class Pi GST in hepatocytes. This class of transferase is found in male mouse liver at substantially greater levels than in female mouse liver (McLellan and Hayes 1987; Mitchell *et al*. 1997). Again, the sex differences in content of class Pi GST in mouse liver appear to be due to growth hormone. Importantly, male Lit/Lit mice, that have a specific defect in the production of growth hormone while still being able to synthesise testosterone, express mGSTP1/2 in the liver at levels that are comparable to that found in the livers of female mice (Dolan 1990). This suggests that it is not testosterone but growth hormone that regulates class Pi GST in mouse hepatocytes.

In rodents, sex-specific expression of GST is not restricted to the liver. Marked differences in the transferases can be observed in the rat adrenal gland. Removal of the pituitary causes a 14-fold increase in rGSTM2 in the adrenal gland of the female rat, whereas the same procedure in male rats produces only a 2.7-fold increase in this subunit. Treatment of the hypophysectomised rats with ACTH largely prevents overexpression of rGSTM2 (Mankowitz *et al*. 1990; Staffas *et al*. 1992). In the mouse, major gender-related differences in the GST content of extrahepatic tissues such as kidney and heart have been reported (Mitchell *et al*. 1997). In the case of cardiac tissue, the mGSTA3 subunit is expressed in the heart of female mice but is absent from that of male mice (Mitchell *et al*. 1997).

Evidence exists for hormonal regulation of GST in humans, with transferase levels generally being higher in the female than the male. The level of hGSTA1-1 in liver from females is higher than in males (Mulder *et al*. 1999). Gender differences have also been reported in human colon (Singhal *et al*. 1992) as well as human renal cortex and lung (Temellini *et al*. 1995). However, such sex differences have not been observed by all investigators (Loguercio *et al*. 1996a, 1996b). There is also a literature showing that class Alpha, Mu and Pi transferases are developmentally regulated in human liver, kidney, lung, spleen and adrenal gland (Fryer *et al*. 1986; Faulder *et al*. 1987).

MODULATION OF GST EXPRESSION BY SELENIUM DEFICIENCY

Selenium is an essential trace element and is incorporated covalently into a number of proteins as selenocysteine. In total there are about 20 selenoproteins including glutathione peroxidase, phospholipid hydroperoxide glutathione peroxidase, type I iodothyronine 5′-deiodinase and thioredoxin reductase. Since these enzymes are dependent on selenium for catalysis, Se deficiency results in a dramatic loss of peroxidase and reductase activities. In contrast, examination of transferase levels in the livers of rats showed that 6 weeks of being placed on a Se-deficient diet is sufficient to approximately double the amount of rGSTA1/2, rGSTA3 and rGSTM1 (Arthur *et al*. 1987). A more recent investigation has demonstrated that Se deficiency preferentially induces rGSTA2 in the liver rather than rGSTA1, and that rGSTA5 and rGSTT1 are induced more markedly than other transferase (McLeod *et al*. 1997). Furthermore, it was found that the extent of GST induction caused by Se-deficiency could be diminished by treatment with the antioxidant N-acetylcysteine, suggesting that oxidative stress is at least partially responsible for induction of GST subunits (McLeod *et al*. 1997).

Hepatic GST activity has also been observed to be elevated in mice placed on a

Se-deficient diet (Reiter and Wendel 1985). However, it is currently unknown which murine genes are induced in this situation. It is not known whether the expression of human GST is influenced by absence of Se in the diet.

REGULATION OF RODENT GST BY FOREIGN COMPOUNDS

Transferase activity in various tissues of rodents can be increased by treatment of the animal by drugs. Figure 9.6 shows induction of GST subunits in rat liver by various xenobiotics including phenolic antioxidants, phenobarbital, β-naphthoflavone and indole-3-carbinol. The organs where GST isoenzymes are inducible typically include liver, small intestine, stomach, oesophagus, kidney and lung. In a review of the literature, more than one hundred xenobiotics have been listed as being capable of inducing GST in rats or mice (Hayes and Pulford 1995). This bewildering number of inducing agents can be simplified by recognising that at the genetic level they influence expression of transferases through a limited number of *cis*-acting elements, usually found in the 5′-flanking region of *GST* genes. At least five enhancers that respond to foreign compounds have been identified that are relevant to induction of GST. Specifically, these are the ARE, GPEI, XRE, PBREM and GRE enhancers, and are described in more detail below.

Metabolisable antioxidants, metabolisable polycyclic aromatic hydrocarbons, diphenols, quinones, isothiocyanates, dithiolethiones and Michael reaction acceptors induce rodent class Alpha, Mu, Pi, Sigma and Theta transferases. Inducible expression of rat *GSTA2* and mouse *Gsta1* occurs through the NF-E2-related factor 2 (Nrf2) transcription factor and the antioxidant responsive element (ARE, 5′-$^{A}/_{G}$TGA$^{C}/_{T}$NNNGC$^{A}/_{G}$-3′) (Rushmore *et al*. 1991; Prestera *et al*. 1993; Itoh *et al*. 1997; Wasserman and Fahl 1997; Hayes *et al*. 2000). This element is also probably responsible for the induction of rGSTA5 by cancer chemopreventive agents (Pulford and Hayes 1996).

The glutathione transferase P enhancer I (GPEI), a palindromic element in which each half comprises an AP-1 site with a single base pair mis-match (Sakai *et al*. 1988; Okuda *et al*. 1989, 1990), has been shown to respond to *tert*-butylhydroquinone *in vitro* (Favreau and Pickett 1995). Despite its similarity to an AP-1 binding site, GPEI-driven gene expression does not appear to be mediated by c-Jun or c-Fos (Morimura *et al*. 1992). It has been speculated that GPEI is involved in induction of the rGSTP1 subunit by coumarin, phenolic antioxidants and *trans*-stilbene oxide in rat liver (Sherratt *et al*. 1998; Kelly *et al*. 2000). Using transgenic rats, Suzuki *et al*. (1996) have shown that GPEI is responsible for the induction of rGSTP1 in the liver by lead. It is also responsible for overexpression of rGSTP1 in hepatic preneoplastic nodules produced by chemical carcinogenesis (Suzuki *et al*. 1995).

Planar aromatic compounds and indoles transcriptionally activate *rGSTA2* through the aryl hydrocarbon (Ah) receptor and the single xenobiotic responsive element (XRE) in the gene promoter (Rushmore and Pickett 1990). Figure 9.6 shows that indole-3-carbinol does not induce rGSTP1 in rat liver.

Glucocorticoids, such as dexamethasome, can either induce or repress GST expression in rodent liver (Dolan 1990; Waxman *et al*. 1992; Prough *et al*. 1996). The variable effect of dexamethasone on GST expression depends on the age of the experimental animal, with hepatic GST in younger animals being inducible by

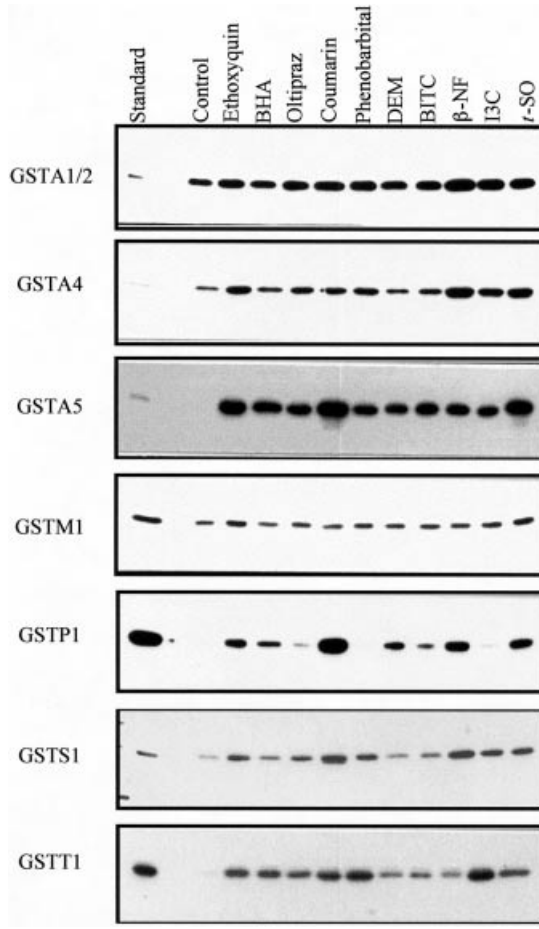

Figure 9.6 Regulation of GST isoenzymes in rat liver. Twelve-week-old male Fischer rats were placed on either a control diet, or a diet containing a xenobiotic. The treatments have been described by Sherratt *et al*. (1998). Briefly, they were as follows: 0.5% ethoxyquin for 14 days; 0.75% butylated hydroxyanisole (BHA) in diet for 14 days; 0.075% oltipraz in diet for 14 days; 0.5% coumarin in diet for 14 days; 0.1% phenobarbital in drinking water for 7 days; 0.5% diethylmaleate (DEM) in diet for 14 days; 0.5% benzylisothiocyanate (BITC) in diet for 14 days; 200 mg/kg β-naphthoflavone (β-NF) intraperitoneal injection daily for 7 days; 0.5% indole-3-carbinol (I3C) in diet for 14 days; 400 mg/kg *trans*-stilbene oxide (*t*-SO) intraperitoneal injection daily for 3 days. Samples of liver cytosol were prepared from these animals and portions (4 µg protein) were subjected to Western Blotting. The xenobiotic treatments are shown at the top of the figure. The antibodies against rat class Alpha, Mu, Pi, Sigma and Theta that were used to probe the blots are shown in the left-hand side of the figure.

glucocorticoids. In the adult rat, dexamethasone is able to inhibit induction of GSTA2 by the planar aromatic hydrocarbon benzanthracene (Falkner *et al*. 1998). Presumably regulation of GST by dexamethasone is mediated by the glucocorticoid and pregnane X receptors, and occurs through the glucocorticoid responsive element (GRE). Examination of the 5′-flanking region of the rat *GSTA2* gene has revealed the presence of a palindromic GRE and several GRE half sites (Falkner *et al*. 1998). Furthermore, the negative effect of dexamethasone on the expression of *GSTA2* in the rat involves the palindromic GRE.

Phenobarbital and 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene induce a number of GST isoenzymes in rat and mouse liver (Hayes *et al*. 1979; Di Simplico *et al*. 1989). Phenobarbital induces rGSTA1/2, rGSTA4, rGSTA5, rGSTS1 and rGSTT1 but not rGSTP1 in rat liver (Figure 9.6). In the case of induction of cytochrome P450 genes, induction by these compounds occurs through the nuclear orphan receptors CAR (constitutive active receptor) and RXR (retinoid X receptor) and the phenobarbital responsive enhancer module (PBREM) (Honkakoski *et al*. 1998). It is probable that phenobarbital induction of GST is mediated by a similar mechanism.

REGULATION OF HUMAN GST BY FOREIGN COMPOUNDS

In the human, the regulation of GST by xenobiotics has been studied in colon and by cell culture techniques. Quantification of the transferases in the rectum of human volunteers who were placed on a diet containing 300 g daily of Brussels sprouts revealed a modest 15% increase in class Alpha GST and 30% increase in class Pi GST (Nijhoff *et al*. 1995). Using primary human hepatocytes treated with various xenobiotics, Northern Blotting has shown that dithiolethiones, 3-methylcholanthrene and phenobarbital can increase steady-state mRNA levels for hGSTA1/2 (Morel *et al*. 1993). Treatment of human primary hepatocytes with the isothiocyanate sulphoraphane has also been shown to increase the level of mRNA for hGSTA1/2 (Mahéo *et al*. 1997). The notion that GSTs are inducible in the liver is supported by the work of Dierickx (1994) who demonstrated increased levels of hGSTA1 in HepG2 cells treated with picolines. Similarly, in HT29 human colon carcinoma cells, GST activity towards CDNB can be increased by treatment with allyl sulphide and benzyl isothiocyanate (Kirlin *et al*. 1999) and in MCF7 breast cancer cells following treatment with catechol (Sreerama *et al*. 1995) or 2,5-bis(2-hydroxybenzylidene)cyclopentanone (Dinkova-Kostova *et al*. 2001). Also, GST activity is elevated in HT29 cells following treatment with aspirin (Patten and DeLong 1999). The above data indicate that human GST genes may be regulated by foreign compounds in the liver and gastrointestinal tract. However, it remains to be demonstrated whether enhancers such as the ARE, GPEI, XRE, PBREM or GRE are involved.

REGULATION OF GST BY ENDOBIOTICS

In rat liver RL34 cells, the rGSTP1 subunit is inducible by the cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-PGJ_2 (Kawamoto *et al*. 2000). This prostanoid is an α,β-unsaturated carbonyl and its induction of rGSTP1 appears to occur through GPEI and may involve c-Jun. The cytotoxic product of lipid peroxidation, 4-hydroxynone-

nal, is also an α,β-unsaturated carbonyl and it too is an excellent inducer of rGSTP1 in RL34 cells (Fukuda *et al*. 1997).

The fact that GPEI and the ARE share sequence identity (see Hayes *et al*. 1999) raises the important question of whether either 15-deoxy-$\Delta^{12,14}$-PGJ_2 or 4-hydroxynonenal are endogenous compounds that stimulate ARE-driven transcription as well as GPEI-driven transcription. Certainly, Prestera *et al*. (1993) have shown that 1-cyclopenten-2-one is a reasonably good inducer of ARE-driven reporter gene expression in HepG2 cells.

Future research directions

Over the past ten years, improvements in protein purification, molecular cloning and bioinformatics have lead to our current understanding of the diversity of the soluble GST and MAPEG superfamilies. However, much remains to be learnt about the *in vivo* functions of the transferases. To date, only one mouse with targeted disruption of a GST gene(s) has been reported. This animal lacks *Gstp1* and *Gstp2*, and though phenotypically normal, it is more sensitive to skin carcinogenesis when treated with 7,12-dimethylbenzanthracene and 12-*O*-tetradecanoylphorbol-13-acetate (Henderson *et al*. 1998). Interestingly, the mutant mouse has proved to be more resistant than the wild-type mouse to acetaminophen (paracetamol) poisoning, presumably because of failure to deplete hepatic GSH pools when it is treated with high doses of the drug (Henderson *et al*. 2000). It is anticipated that the generation of additional gene knockout murine lines will help clarify the biological functions of both GST superfamilies. Clearly, substantially more work is required in this area in order to elucidate the contribution that individual transferases make to the metabolism and disposition of drugs.

A significant number of soluble GST have been crystallised and their three-dimensional structures determined (Reinemer *et al*. 1991; Mannervik and Wildersten 1995; Armstrong 1997). With a better knowledge of the mechanisms responsible for catalysis and the active site residues involved in substrate binding, it has become possible to engineer these proteins to exhibit novel properties and unique specificities. For example, Gulick and Fahl (1995) employed forced evolution to select a rat class Alpha GST with increased activity for the nitrogen mustard mechlorethamine. A phage display approach has been used to obtain a mutant of a human class Alpha GST with increased activity towards ethacrynic acid (Hansson *et al*. 1997). More recently, Mannervik and his colleagues have been able to confer the ability to metabolise 4-hydroxynonenal on an enzyme with little activity for this α,β-unsaturated carbonyl by mutating residues associated with the β-α1 loop, the α4 helix and the α9 helix (Nilsson *et al*. 2000). Clearly, this approach has numerous applications in medicine and biotechnology.

Concluding comments

This chapter provides an overview of the functions, genetics and regulation of the two GST superfamilies. Recent studies have begun to address the endogenous functions of GST within the cell, such as their contribution to leukotriene and prostaglandin

biosynthesis. Emerging evidence suggests that at least two soluble GST subunits may modulate signal transduction pathways and another influences ryanodine receptor function. This represents a departure from the classical view that GSTs are solely enzymes involved in phase II of drug-metabolism, and indicates they are intimately involved in cellular stress responses. The polymorphic expression of GST represents a significant risk factor in the development of malignant disease, in particular breast cancer, and influences responsiveness to chemotherapy. Over the past 10 years enormous advances have been made in understanding how xenobiotics regulate gene expression. Data are accumulating that induction of GST genes is mediated by Nrf2, c-Jun, the Ah receptor, CAR and possibly the glucocorticoid receptor. Although GSTs have been the subject of scientific research for about forty years, and great advances have been made in our knowledge of their structure, it is clear that much remains to be learnt about the biological functions of these enzymes. Establishing the role of GST in the response of the host to drugs remains a challenge for pharmacologists and toxicologists alike.

Acknowledgements

We are indebted to Professor Philip G. Board and Dr Jack Flanagan for their helpful advice. We also thank the Biotechnology and Biological Sciences Research Council (94/F08200) for financial support.

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