17 Functional Genomics of the Human Glutathione Transferases

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17.1 SUMMARY

The glutathione transferase (GST) superfamily contains a number of enzymes that catalyze the conjugation of glutathione (GSH) to a wide range of compounds of both endogenous and exogenous origin. In addition to catalytic reactions, the GSTs also modulate several cell signaling kinases and ion channels such as Jun N-terminal kinase (JNK) and ryanodine receptors via protein/protein interactions. Pharmacogenetic studies have identified variations in drug response and susceptibility to cancer and other disorders associated with variant GST isoforms. In humans, the cytosolic GSTs have been subdivided into seven distinct classes termed *Alpha, Mu, Pi, Theta, Sigma, Zeta*, and *Omega*, and there are multiple genes in some classes. So far, 48 allelic variants that cause amino acid substitutions have been documented, and in many cases, the variant proteins have been functionally and structurally characterized. Common deletions of the *GSTM1* and *GSTT1* genes have been extensively investigated in relation to cancer susceptibility and drug response, and they have also been the subject of a vast number of disease association studies. Some polymorphisms in promoter regions have been shown to affect the level of GST expression *in vitro* and *in vivo* and this area requires further investigation.

The various members of the GST superfamily are characterized by striking differences in the reactions they catalyze and their substrate specificities. The structures of

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representatives of all the human GST classes have been determined by X-ray crystallography and their differing reaction mechanisms have been investigated by mutagenesis and functional studies.

17.2 INTRODUCTION

The GST superfamily contains a number of enzymes that catalyze the conjugation of GSH to a wide range of compounds of both endogenous and exogenous origin. The GSTs have been intensively studied because of their capacity to protect cellular components from attack by electrophiles, and consequently, they have been implicated in the development of drug resistance and susceptibility to carcinogens. Pharmacogenetic studies have identified variations in drug response and susceptibility to cancer and other disorders associated with variant GST isoforms. A review by Hayes and Pulford [1] provides an excellent summary of the types of environmentally derived compounds that are conjugated or transformed by the action of GSTs. In addition, the Comparative Toxicogenomics Database provides an extensive compilation of publications reporting chemical/GST/disease interactions [2]. GSTs contribute to around 10% of the cytosolic protein in rat liver, and as they can be detected as discrete bands on coomassie blue stained SDS-PAGE gels of liver cytosol, it seems likely that GSTs are involved in additional physiological processes. An early study noted the identity of a rat GST with "ligandin," a protein known for its capacity to bind a range of hydrophobic compounds, including carcinogenic azodyes, corticosteroids, and bilirubin [3,4]. In recent years, it has been shown that the physiological roles of GSTs extend well beyond the disposition of xenobiotics and include both enzymatic and nonenzymatic functions. Some GSTs catalyze cis–trans isomerase reactions that are important in steroid hormone synthesis and the catabolism of tyrosine [5–7], while others protect cells against the products of lipid peroxidation by catalyzing the reduction of lipid hydroperoxides [8] or the conjugation of reactive α,β-unsaturated carbonyl compounds such as 4-hydroxy nonenal [9–11]. In addition, GSTs have been identified, which catalyze thioltransferase reactions and the reduction of biologically significant molecules such as dehydroascorbate [12,13]. Members of the GST superfamily have been shown to engage in protein–protein interactions that can have a direct effect on several signaling pathways. The stress-activated kinase Jun N-terminal kinase, apoptosis-stimulating kinase 1 (ASK1), and tumor necrosis factor (TNF) receptor-associated factor 2 have all been shown to be modulated by GST binding [14–16] and there is evidence that Fanconia anemia group C protein (FANCC) can modulate the activity of GSTP1-1 during apoptosis [17]. A number of studies have shown that the chloride intracellular channel (CLIC) proteins are members of the GST superfamily and can enter membranes to form or modulate ion channels [18–21]. The human muscle-specific GST, GSTM2-2, has been shown to be a potent inhibitor of the cardiac ryanodine receptor Ca^{2+} channels and may play a key role in modulating excitation contraction coupling in the heart [22–24].

GSTs and GST-like proteins are almost ubiquitous in nature and there are close to 30,000 papers concerning GSTs listed in PubMed. Many aspects of this large field have been extensively reviewed [1,8,25–37]. This chapter focuses on the genetic variation in the human GSTs and its impact on their structure and function.

17.3 THE GST GENE FAMILIES

The evolutionary history of the GSTs is complex. Phylogenetic analysis has shown that at least four families of proteins with GST activity have emerged by convergent evolution [38]. In mammals, there are three well-recognized structurally and genetically distinct families of proteins that catalyze GST reactions. These families have been generally termed the *cytosolic, microsomal*, and *mitochondrial GSTs* [8,33,39–41]. The cytosolic GSTs are a large multiclass family of proteins that share a common structural fold and an ability to catalyze a variety of GSH-dependent reactions. Classically, they have been studied as drug and xenobiotic detoxication enzymes, conjugating GSH to small hydrophobic molecules in phase II drug metabolism reactions. However, as the number of members of this gene family has expanded with the genome era, so has the type of reactions and functions associated with these proteins. The membrane-bound microsomal GSTs have also been termed the *membrane-associated proteins in eicosanoid and glutathione metabolism* (MAPEG) [39]. There are at least six members of this family in humans, and although most have the capacity to conjugate GSH to 1-chloro-2,4-dinitrobenzene (CDNB), they appear to play prominent roles in the synthesis of leukotrienes and prostaglandins *in vivo* [39]. Although some cytosolic GSTs are also found within mitochondria, the Kappa class GST is found specifically within mitochondria and peroxisomes [42]. There is a single Kappa class gene in humans, rats, and mice and their amino acid sequences show little identity with the cytosolic or MAPEG GST families. Although the structure of the Kappa class GSTs contains a thioredoxin fold domain that is reminiscent of the cytosolic GSTs, it contains a polyhelical domain embedded in the linear sequence between the N-terminal region β-strands [40,41]. This contrasts with the cytosolic GSTs where the α-helical domain is encoded entirely within the C-terminal sequence (Fig. 17.1). This difference in structure suggests that the Kappa class GSTs also evolved via a distinct but convergent evolutionary path.

17.4 THE CYTOSOLIC GSTs

17.4.1 A Historical Perspective

This chapter focuses primarily on the cytosolic GSTs as they are considered to be of most significance in drug and xenobiotic metabolism. Originally, multiple cytosolic GSTs were identified by the reactions they catalyzed, their elution profile from ion

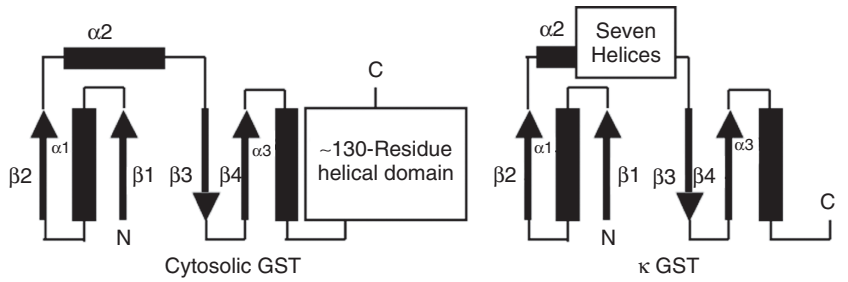

Figure 17.1 Topology diagrams comparing the secondary structural organization of cytosolic GSTs with the Kappa class GST [41,43]. β-Strands are shown as arrows and α-helices are shown as rectangles.

exchange chromatographic columns, their molecular weight, their isoelectric point, and their native electrophoretic mobility [44–47]. These and many other studies led to the development of a range of largely laboratory-specific nomenclature systems that are quite confusing to new readers of the older literature. Although there was early evidence that some GST isoenzymes had other substrate preferences [45,48,49], most GSTs were found to utilize CDNB as a substrate. The discovery of GSH affinity chromatography and the use of CDNB as a substrate allowed the biochemical characterization of many GSTs; however, this focus also delayed the discovery of GSTs that do not bind to GSH affinity matrices and do not use CDNB as a substrate [50–52]. The discovery and characterization of the Theta, Zeta, Omega, and CLIC proteins in mammals came from the utilization of different substrates and the use of bioinformatics to analyze sequence databases [12,18,53,54]. The discovery of novel GSTs by the use of bioinformatics subsequently presented an interesting challenge to identify their substrates.

17.4.2 Classification of the GSTs

The confusion in GST nomenclature was resolved in the early 1990s. The cytosolic GSTs were classified into a number of different classes that are designated by the Greek alphabet. The classes were originally defined in humans, rats, and mice on the basis of their amino-terminal sequence and their antigenic identity. An international nomenclature convention has been established that is theoretically extendable to other species [55,56]. Readers should refer to these reports for a definitive description of the accepted nomenclature system. Seven distinct classes have been well characterized in mammals and have been termed *Alpha, Mu, Pi, Theta, Sigma, Zeta*, and *Omega*. Proteins in each class are termed by the initial letter of the class name. For example, GSTA1-1 specifies the homodimeric product of the Alpha class *GSTA1* gene and GSTA1-2 specifies the heterodimeric product of the *GSTA1* and *GSTA2* genes. Other mammalian proteins that share the same structural fold but have no known enzymatic activity have been identified but not formally classified as GSTs. These include the CLIC family of proteins that have been shown to form chloride channels and to modulate the activity of ryanodine receptor Ca^{2+} channels in muscles [18]. In addition, molecular models of ganglioside-induced differentiation-associated protein 1 (GDAP1) and its paralog GDAP1-like 1 (GDAP1L1) suggest that they have a GST fold, but they appear to be inactive with common GST substrates [57,58]. Although the role of GDAP1 has not been clearly defined, mutations in GDAP1 have been shown to cause a severe recessive form of Charot–Marie–Tooth disease [59,60].

A range of additional classes and GST-like enzymes have been specifically defined in other evolutionary orders, including the Delta and Epsilon classes in insects [61,62]. In plants, Phi, Tau, and Lambda classes have been described together with a dehydroascorbate reductase class that has not been assigned a Greek letter [63]. Numerous GSTs have been identified in different microbial species, and although many show sequence or functional similarities with mammalian GSTs, the class boundaries are indistinct [64–66]. The Zeta class is the most widely conserved class and has been reported in species from bacteria through to plants, fungi, insects, fish, birds, and mammals. The conservation of the Zeta class is probably because of its isomerase activity converting maleylacetoacetate to fumarylacetoacetate in the catabolism of tyrosine [6,54,67]. While the Zeta class has been both structurally and functionally conserved across evolutionary time, some GST classes have developed highly specialized functions while

maintaining structural conservation between species. Possibly the most striking functional divergence has occurred in the Sigma class GSTs that catalyze prostaglandin D_2 synthesis in mammals and act as lens crystallins in squids [68,69].

17.4.3 GST Function and Nomenclature

Historically, and in accordance with the accepted nomenclature system [70], GSTs have been assigned a number within each class (GSTA1-1, GSTA2-2, etc.) in order of their discovery. Since many of the duplication events that have generated the multiplicity of GSTs in specific classes have occurred independently in different species (see below), it unfortunately follows that GSTs that are members of a specific class and have been assigned the same number may have different catalytic functions and show significant differences in their regulation in different species. For example, human GSTA3-3 has specific steroid isomerase activity and potentially plays a significant role in steroid hormone synthesis [5]. However, mouse GSTA3-3 is an efficient conjugator of aflatoxin B1 and has low steroid isomerase activity [71]. This functional difference in the GSTA3-3 isoenzymes in humans and mice accounts for the relative resistance of mice to aflatoxin carcinogenesis compared with humans. In contrast, the GSTA4-4 isoenzymes are paralogous in mice and humans, as they both have high specific activity in the conjugation of 4-hydroxynonenal an endogenous product of lipid peroxidation [10,11]. As a result of these differences, care should always taken in ascribing GST function on the basis of nomenclature.

17.5 CYTOSOLIC GST GENES

A summary of the human cytosolic GSTs and their corresponding genes is provided in Table 17.1. GST classes may include multiple genes and the number of enzymes expressed in each class can vary between species. For example, there are two Pi class and three Theta class GSTs in mice but only one Pi and two Theta class GSTs in humans. In humans, the genes that encode GSTs from each class are grouped into clusters on different chromosomes, indicating the role of gene duplication in generating the diversity that is apparent within many GST classes. A similar arrangement generally occurs in mice and rats but the mouse *GstA3* gene is known to occur on chromosome 1 and has been separated from other Alpha class genes that are located on chromosome 9 (Board and Liu, unpublished data) [72]. The GST genes in humans are relatively small in size most falling between 3 and 17 kb in length and are composed of five to nine exons (Table 17.1).

17.5.1 Human GST Pseudogenes

There are many GST pseudogenes within the human genome. Some are intronless and are derived from reverse transcripts of mRNA that have been inserted randomly in the genome. Intronless GST genes have been reported in *Drosophila melanogaster*, [110] but there is no evidence that any reverse-transcribed GST sequences in the human genome are transcribed and translated into protein. In contrast, there are numerous pseudogenes and gene fragments within the different classes of gene clusters [73,102]. These are further evidence of the on going role of gene duplication and gene conversion

TABLE 17.1 Human Glutathione Transferases and Their Genes

Enzyme					Gene		References
Class and Designation	Accession Number	Locus	Chromosome Band	Exons	Gene Size (kb)	Gene ID	
Class Alpha							
GSTA1-1	NP_665683	*GSTA1*	6p12.2	7	12.5	2,938	73–76
GSTA2-2	NP_000837	*GSTA2*	6p12.2	7	13.5	2,939	73,74,76–79
GSTA3-3	NP_000838	*GSTA3*	6p12.2	7	13.1	2,940	11,73,74
GSTA4-4	NP_001503	*GSTA4*	6p12.2	7	17.4	2,941	11,73,80,81
GSTA5-5	NP_714543	*GSTA5*	6p12.2	7	14.4	221,357	73,82
Class Mu							
GSTM1-1	NP_666533	*GSTM1*	1p13.3	8	6	2,944	47,83–87
GSTM2-2	NP_000839	*GSTM2*	1p13.3	8	16	2,946	86,88,89
GSTM3-3	NP_000840	*GSTM3*	1p13.3	9	7.1	2,947	86,90,91
GSTM4-4	NP_671489	*GSTM4*	1p13.3	8	9.4	2,948	86,92–94
GSTM5-5	NP_000842	*GSTM5*	1p13.3	8	6	2,949	86,95
Class Pi							
GSTP1-1	NP_000843	*GSTP1*	11q13.3	7	3.1	2,950	96–101
Class Theta							
GSTT1-1	NP_000844	*GSTT1*	22q11.23	5	8.2	2,952	53,102–104
GSTT2-2	NP_000845	*GSTT2*	22q11.23	5	3.8	2,953	102,105
GSTT2B-2B		*GSTT2B*	22q11.23	5	3.8	653,689	102,106
Class Zeta							
GSTZ1-1	NP_665877	*GSTZ1*	14q24.3	9	10.9	2,954	6,54
Class Omega							
GSTO1-1	NP_004823	*GSTO1*	10q25.1	6	12.5	9,446	12,107,108
GSTO2-2	NP_899062	*GSTO2*	10q25.1	7	30.6	119,391	107,108
Class Sigma							
GSTS1-1/ HPGDS	NP_055300	*HPGDS*	4q22.3	6	44.3	27,306	109

in the evolution of diversity within the class-specific GST gene clusters in different species. The Alpha class in humans provides an example of this diversity and the problems that can be associated with differentiating genes and pseudogenes. Normally, it is evident that a gene is functional if a corresponding mRNA can be identified. However, if a gene is only expressed transiently in highly specialized cells, it can be difficult to detect the complementary DNA (cDNA). Several studies have noted a number of pseudogenes and gene fragments within the human Alpha class gene cluster on the short arm of chromosome 6 [73,74]. One gene sequence putatively identified as *GSTA5* appeared to be complete with no frame shifts, premature stop codons, deletions or insertions, and acceptable intron/exon boundaries [73]. Although no evidence of the expression of this gene has been found, a recent study has shown that if the gene is driven by a heterologous CMV promoter, fully spliced mRNA and catalytically active enzyme can be expressed in HEK-293 cells [82].

17.5.2 Alternative Splicing of GST Transcripts

Alternative splicing of GST mRNAs is a potential source of variation that is significant in some other drug and xenobiotic metabolizing enzymes such as the UDP-glucuronosyltransferases but does not appear to generate functional diversity among the mammalian GSTs. Although alternative transcripts of human *GSTM4* [92] and GSTT1 [111] and mouse *Gsto1* and *Gsto2* [107] have been reported, and bioinformatics studies on expressed sequence tags readily detect alternative splicing in other GSTs [112], there is no evidence at this stage that alternative transcripts give rise to variant GST proteins in mammals. In mice, several alternative *Gsto2* transcripts containing different 5′ noncoding exons have been detected in EST databases and there are significant differences in the transcript size in different tissues suggesting that alternative splicing in 5′ noncoding regions may be important in regulating expression in particular tissues or conditions [107]. In the parasitic nematode *Onchocerca volvulus*, alternative splicing of the Omega class GST Ov-GST3 may be dependent on a stress response [113]. In contrast to humans and mice, alternative splicing has been exploited extensively in insects to generate functional diversity that has implications in the development of insecticide resistance. Alternative splicing in the GST family has been reviewed recently by Wongsantichon and Ketterman [112].

17.6 GENETIC POLYMORPHISM OF HUMAN GST GENES

In addition to the variation in the GST family inherent in the number of classes and the variable number of gene loci within each class, a further layer of heterogeneity results from deletions and single nucleotide polymorphisms (SNPs). Genetic polymorphism can occur in coding and noncoding regions and can significantly modify gene expression and the function of the expressed enzymes. Genetic polymorphism can therefore result in individual differences that can have an impact on drug and xenobiotic metabolism. Some polymorphisms are common and are found in most populations, while others are rare and can be restricted to specific ethnic groups. A survey of PubMed reveals in excess of one thousand papers investigating the possible association of specific GST polymorphisms with a wide range of disorders that may be influenced by variations in the metabolism and disposition of drugs and other environmentally derived carcinogens and toxins. The validated polymorphisms in human GSTs that alter the amino acid sequence or have a direct impact on the expression, structure, and function of human cytosolic GSTs are provided in Table 17.2.

17.6.1 The Alpha Class

An excellent review of the genetic polymorphisms in the Alpha class GST genes has been published [114].

17.6.1.1 GSTA1. So far there are no validated SNPs in the coding region of *GSTA1* which cause an amino acid substitution. A single SNP (A375G) that is highly polymorphic in Europeans, Africans, and Asians has been detected, but it is synonymous and does not change the K75 residue [128]. Several linked SNPs (G-52A, C-69 T, C-115 T, T-567G, T-631G, and C-1142G) have been validated in the 5′ noncoding region

TABLE 17.2 Characterized Polymorphisms in Human Cytosolic GSTs[a]

Allele/Haplotype	Substitution or Deletion	Gene Frequency				Phenotype	References
		A	B	C	D		
Alpha Class							
GSTA1*A	Promoter	0.65	0.84	0.6	—	Wild-type allele	114
GSTA1*B	Promoter	0.35	0.16	0.4	—	A at −52 causes a significant reduction in hepatic expression	114
GSTA2*A	P110 S112, K196 E210	≈0.3	—	≈0.3	—	Wild-type allele	114
GSTA2*B	P110 S112, K196 A210	0.3	0.18	0.08	—	No observed effect	114
GSTA2*C	P110 T112, K196 E210	0.3	—	0.57	—	Reduced hepatic expression in Caucasians	114
GSTA2*D	P110 S112, N196 A210	0	0	0	—	Not observed so far	114
GSTA2*E	S110 S112, K196 E210	0.01	0.11	0.05	—	Low activity with CDNB and organic hydroperoxides; increased activity with azathioprene	114
GSTA3*A	I71	0.85	1	1	—	Wild-type allele	115
GSTA3*A	L71	0.15	0	0	—	Diminished activity with CDNB; no change in activity with Δ^5-androstene-3-17-dione	115
GSTA4	—	—	—	—		No coding region SNPs validated	—
GSTA5	—	—	—	—		No coding region SNPs validated	—
Mu Class							
GSTM1*A	K173	0.5	0.07	0.16	—	Wild-type allele	47
GSTM1*B	N173	0.04	0.17	0.12	—	No functional difference	47
GSTM1*C	S85	0	0	0.07	—	Not characterized	116
GSTM1*O	Gene deletion	0.46	0.76	0.72	—	Associated with response to chemotherapy and susceptibility to cancer	47
GSTM2	—	—	—	—	—	No coding region SNPs validated	—
GSTM3*A	G147 V224	0.89	0.26	0.66	—	Wild-type allele	117
GSTM3*B	Intron deletion	—	—	—	—	—	118

GSTM3*C	G147 I224	0.11	0.73	0.34	—	Increased catalytic efficiency	117
GSTM3*D	W147, V224	—	—	—	—	—	117
GSTM3*E	W147 I224	—	0.016	—	—	Low specific activity	—
GSTM4	—	—	—	—	—	No coding region SNPs validated	—
GSTM5	—	—	—	—	—	No coding region SNPs validated	—
Pi Class							
GSTP1*I105	I105	0.43	0.81	0.66	—	Wild-type allele	119
GSTP1*V105	V105	0.57	0.19	0.34	—	Difference in stability and activity with carcinogenic diolepoxides	119,120
GSTP1*A114	A114	—	0.99	0.93	—	Wild-type allele	119
GSTP1*V114	V114	—	0.01	0.07	—	—	119
Theta Class							
GSTT1*O	Gene deletion[a]	21.8%	64%	23.7%	9.7%	Associated with carcinogen activation	121
GSTT1*B	P104	—	—	0.01	—	Causes deficiency, occurs in Sweden	122
GSTT1*N43	N43	0.01	0	0	0	Unstable	116
GSTT1*M65	M65	0	0	0	0.008	Unstable	116
GSTT1*N141	N141	—	—	—	—	No effect on activity	123
GSTT1*stop	Nucleotide (G412) deletion	0	0	0	0.008	Deficiency	116
GSTT1*I169	I169	0.13	0	0	0	Not characterized	116
GSTT1*K173	K173	—	—	—	—	Unstable, low activity	123
GSTT2*A	M139	—	—	0.98	—	Wild-type allele	102
GSTT2*B	I139	—	—	0.02	—	Function not studied	102
GSTT2p	Gene deletion	—	—	0.53	—	Lowers GSTT2 transcription	106
Zeta Class							
GSTZ1*A	L8, K32 R42, T82	—	—	0.086	—	Resistant to inactivation by DCA	124
GSTZ1*B	L8, K32 G42, T82	—	—	0.285	—	—	124
GSTZ1*C	L8, E32 G42, T82	—	—	0.473	—	Wild-type allele	124

(*continued overleaf*)

TABLE 17.2 (*continued*)

Allele/Haplotype	Substitution or Deletion	Gene Frequency				Phenotype	References
		A	B	C	D		
GSTZ1*D	L8, E32 G42, M82	—	—	0.156	—	—	124
GSTZ1*E	P8, E32 G42, T82	—	—	—	—	Unstable	124
Sigma Class							
GSTS1	IVS2+11	—	—	—	—	Occurs in Japanese	125
Omega Class							
GSTO1*A	A140 E155	0.92	0.84	0.67	0.77	Wild-type allele	108
GSTO1*B	D140	0.08	0.17	0.34	0.23	No functional change	108
GSTO1*C	ΔE155, K208	0.04	0	0.03	0.03	Unstable protein	13,108
GSTO1*D	ΔE155, E208	—	0.11	0.01	—	Unstable protein	13
GSTO1*E	Y32	0	0	0.01	0	Inactive protein	126
GSTO1*F	V236	0	0	0	0.03	Unstable protein occurs in South America	127
GSTO2*A	N142	0.15	0.73	0.69	0.74	Wild-type allele	108
GSTO2*B	D142	0.85	0.27	0.31	0.26	No functional change	108
GSTO2*C	I41	0.01	0	0	0	Stable	108
GSTO2*D	Y130	0.01	0.02	0	0	Unstable	108
GSTO2*E	I158	0	0	0.01	0	Unstable	108

A, African; B, Asian, predominantly Chinese; C, Caucasian; D, Mexican/South American.
[a]Data shown as percent homozygote nulls.

and analyzed in different haplotypes [129–131]. The most significant 5′ SNP appears to be a G-52A substitution in an SP1 response element where the G allele increases reporter gene expression around fourfold in HepG2, GLC4, and Caco-2 cell lines [73]. The homozygous presence of this allele in the proposed *hGSTA1*A* haplotype (G-52, C-69, and T-567) was associated with fourfold higher levels of GSTA1-1 protein in the liver than in *hGSTA1*B* (A-52, T-69, and G-567) homozygotes [129]. While this finding indicates that the G-52A has a significant effect on GSTA1-1 expression in the liver, similar differences were not found in the pancreas [132], suggesting that the effect of this polymorphism on gene expression may be tissue specific. The frequency of the hGSTA1*A haplotype containing the G-52 allele ranges between 0.6 and 0.85 in African, Asian, and Caucasian populations.

17.6.1.2 GSTA2. A total of four nonsynonymous SNPs that result in the substitutions P110S, S112T, K196N, and E210A have been detected in the coding sequence of *GSTA2* [128,133,134]. These SNPs occur in five haplotypes termed GSTA2*A–GSTA2*E (Table 17.2). However, the K196N substitution that gives rise to the GSTA2*D haplotype has only been observed in two EST database sequences and has not been confirmed in any population studies [133]. Consequently, it is unclear if this variant exists or is the result of a DNA sequencing error.

The E210A and S112T substitutions that are represented in haplotypes *GSTA2*B* and *GSTA2*C* do not appear to have any significant effect on catalytic activity [128]. In contrast, the rare P110S substitution that occurs in haplotype GSTA2*E has diminished activity with several substrates, including CDNB and organic hydroperoxides, but elevated activity toward 4-nitrophenylacetate [133,134]. A recent study has shown that the GSTA2*E variant has very high catalytic efficiency with azathioprene [135]. Azathioprene is used clinically in the treatment of cancer and some autoimmune disorders and a low activity allelic variant of thiopurine methyltransferase has been associated with toxicity resulting from the accumulation of high levels of 6-mercaptopurine [136]. The inheritance of the GSTA2*E variant may also cause the liberation of high concentrations of 6-mercaptopurine and contribute to azathioprene toxicity.

Quantitative studies on GSTA2-2 protein in human liver samples have shown that the presence of the T112 substitution (*GSTA2*C*) causes a significant reduction to levels that are around 25% of those associated with the other haplotypes [134]. The cause of the apparently poor expression of the GSTA2*C subunit is not clear. The possibility that the T112 substitution causes protein instability or sensitivity to degradation seems unlikely but has not been tested. It seems more likely that the SNP is linked to another SNP that directly affects gene expression.

17.6.1.3 GSTA3. GSTA3-3 in humans plays a highly specialized role in the isomerization of Δ^5-3-ketosteroids in the synthesis of testosterone and progesterone. So far only a single SNP in the coding region of GSTA3 has been validated and characterized [115]. This SNP results in an I71L substitution and has only been identified in African subjects. Recombinant GSTA3-3 with the L71 substitution has diminished activity with a number of substrates, including CDNB and 7-chloro-4-nitrobenz-2-oxa-1,3-diazole. The low activity results from a significant increase in the K_m for GSH. Interestingly, there was no difference in the variant enzyme's specific activity toward Δ^5-androstene -3,17-dione and no difference in the K_m for GSH with this substrate. Additional studies revealed that the L71 variant has diminished heat stability.

17.6.1.4 GSTA4. Thus far no SNPs in the coding region of *GSTA4* have been validated and characterized. Several SNPs have been identified in the 5′ noncoding region, but they do not appear to influence function [131].

17.6.1.5 GSTA5. Thus far no SNPs in the coding region of *GSTA5* have been validated and characterized.

17.6.2 The Mu Class

Polymorphisms in the Mu class GSTs have been previously reviewed by Tetlow *et al*. [117]. Because of the similarity of some of the Mu class GST gene sequences, there are a number of alignment errors in SNP databases and the number of real coding region missense SNPs is less than that predicted. For example, there is an erroneous identification of an S210T substitution in both GSTM1 and GSTM2. This has arisen as a result of the misclassification of GSTM1 sequences with S210 and GSTM2 sequences with T210 and vice versa.

17.6.2.1 GSTM1. GSTM1 deficiency is the one most extensively studied GST polymorphisms. This polymorphism was first identified as a genetically determined GST deficiency with a frequency approaching 50% by starch gel electrophoresis [47]. At the same time, ion exchange chromatographic studies on human liver also revealed the absence of a significant GST isoenzyme in some samples [137]. Subsequent research also reported the inability of monocytes from some individuals to conjugate *trans*-stilbene oxide to GSH and this proved to be a result of GSTM1 deficiency since *trans*-stilbene oxide is a specific substrate for GSTM1-1 [138,139]. The original starch gel electrophoretic analysis predicted that GSTM1-1 deficiency was the result of a gene deletion and this was confirmed later by Southern blotting studies [83]. The *GSTM1* null allele is very common and is the most frequent allele in most populations studied so far. An association between GSTM1 deficiency and lung cancer was first reported in a study that was the forerunner of hundreds of investigations into the occurrence of GSTM1 deficiency and a range of different cancers and other disorders [140]. There have been a number of reviews and meta-analyses of these studies [141–152]. Possibly the most clinically significant studies have found that GSTM1 deficiency has a significant impact on survival of childhood leukemia [153–157].

The *GSTM1* null allele appears to have resulted from an unequal crossing over event that deleted an 18-kb region that included the entire *GSTM1* gene [158]. A polymorphic duplication of *GSTM1* has been reported in the Saudi Arabian population [159]. This variation results in an increase in an individual's capacity to conjugate *t*-stilbene oxide and presumably other GSTM1-1 substrates. Another GSTM1 polymorphism also originally identified by starch gel electrophoresis [47] and subsequently shown to result from an N173K substitution where the K173 variant has been termed GSTM1*A and the N173 isoform has been termed GSTM1*B [160] (Table 17.2). Functional studies have indicated that this polymorphism does not significantly affect the activity with a range of substrates [161]. An A85S substitution with a minor allele frequency of 0.068 in Caucasian Americans has been reported [116]. This variant has not been extensively characterized but appears to be stable when expressed in COS-1 cells.

17.6.2.2 GSTM2. Despite extensive studies, no confirmed missense SNPs have been identified in *GSTM2*.

17.6.2.3 GSTM3. Two coding region missense SNPs have been described and characterized [117]. A G147W substitution has been identified as a rare variant in Chinese subjects and a V224I substitution is common in African, Asian, and European subjects. These coding region alleles can be combined into four possible haplotypes (GSTM3*A, G147, V224; GSTM3*C, G147, I224; GSTM3*D, W147, V224; GSTM3*E, W147, I224). The term GSTM3*B has previously been used to describe a deletion in intron 6 (see below). Functional analysis of recombinant enzymes indicates that the presence of I224 in the GSTM3*C haplotype corresponds with a threefold increase in the specific activity with CDNB as a substrate, a reduction in the K_m for GSH, and a large increase in the catalytic efficiency of the enzyme [117].

The term GSTM3*B has previously been used to describe a deletion in intron 6 [118]. GSTM3*B appears to be in linkage disequilibrium with GSTM1*A and it has been suggested that this linked haplotype may include an element that alters the expression of GSTM1 [118].

17.6.2.4 GSTM4. Although the SNP databases predict a large number of missense SNPs, none has been validated as they can be explained as misalignments of other GSTM sequences. A 5′ proximal promoter SNP at nucleotide-79 has been reported to influence expression. The G allele has only 67% of the activity of the C allele [131]. It is not clear if this difference is relevant *in vivo*.

17.6.2.5 GSTM5. No validated SNPs have been characterized in the coding region. Several variant alleles contribute to six haplotypes that have been noted in the 5′ proximal promoter but none has been associated with a significant change in expression [131].

17.6.3 The Pi Class

17.6.3.1 GSTP1. The Pi class is represented in humans by a single functional gene (*GSTP1*). Although the SNP database lists several missense SNPs, only two have been validated and extensively characterized. The Ile105Val and Ala114 Val substitutions were first identified in cDNA clones [96] and later identified as genomic variants [162] (Table 17.2).

GSTP1-1 has been extensively studied in relation to drug resistance [35], and the possibility that the common SNPs at codons 105 and 114 alter drug and xenobiotic metabolism and susceptibility to cancer and other diseases has been the subject of numerous investigations [163–167]. Several studies have noted differences in the activity of GSTP1-1 variants with the anticancer drugs thiotepa and chlorambucil [163,168–170]. Many epidemiological gene association studies have been reviewed elsewhere and have been the subject of extensive meta-analysis [142,143,171–173]. Because of their potential impact on drug metabolism and drug resistance, the catalytic functions of GSTP1 variants have been intensively investigated. Most attention has been focused on the I105V substitution since residue 105 contributes to the architecture of the hydrophobic substrate-binding site (H-site) [174]. GSTP1-1 is active with a range of carcinogenic diol epoxides derived from polycyclic aromatic hydrocarbons [175–179] and differences in the GSH conjugating activity of the V105 and I105 variants have been attributed to the volume of the residue in the H-site and its hydrophobicity. It has been suggested that differences in the catalytic activity of the I105 and

V105 variants toward carcinogenic diol epoxides may underlie the reported associations between these alleles and cancer susceptibility [176], although other reports have implicated other factors [179]. Although most studies have identified significant catalytic and stability differences between the I105 and V105 variants [175,176], there have been contradictory reports. For example, while Zimniak and colleagues [180] reported that the I105 variant was less stable than the V105 variant at 50°C, a separate study indicated that the I105 variant was the more stable at 45°C [181]. Both studies found that the heat stability was differentially affected by the presence of substrates so the extent of GSH binding may explain this discrepancy and suggests that intracellular conditions could differentially affect the stability of the two variant isoenzymes and this could be reflected in different *in vivo* capacities to conjugate certain xenobiotics and their metabolites. Similarly, Johansson *et al*. [181] noted significant structure activity differences that were substrate dependent. For example, with CDNB as a substrate, the I105V polymorphism has significant affect on K_m but not k_{cat}. In contrast, with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole or ethacrynic acid as substrates, the polymorphism affected both parameters. Clearly, further studies are required to fully understand the structure/substrate interactions that underlie the reported associations between *GSTP1* genotype, xenobiotic metabolism, and cancer susceptibility.

17.6.4 The Theta Class

In humans, there are two functional Theta class GST genes (*GSTT1* and *GSTT2*) located on chromosome 22q11 [102,105]. The locus is complicated because of the apparent duplication of *GSTT2* to generate a pseudogene (*GSTT2p*) that is transcribed [102].

17.6.4.1 GSTT1. Although several missense mutations have been predicted in the SNP databases, few have been well characterized. The *GSTT1*A* and *GSTT1*B* alleles result from a T104P substitution that is relatively rare in Scandinavian populations [122]. The insertion of proline in helix 4 appears to destabilize the protein to a level that was below immunological detection and causes GSTT1-1 deficiency [122]. A major resequencing study [116] identified 18 SNPs, including the substitutions D43N, T65M, and V169I as well as a single nucleotide deletion (G412) from exon 4. With the exception of the V169I substitution that occurred at a minor allele frequency of 0.126 in African Americans, the other variants occurred at a frequency of 0.001 or less. The nucleotide 412 deletion causes a frame shift and a premature stop codon and would be expected to cause a deficiency. When transfected into COS-1 cells, the D43N, T65M, and T104P substitutions caused low levels of immunoreactive protein expression [116]. Two other rare variants (D141N and E173K) reported in the NIEHS Environmental Genome Project database have been expressed in *Escherichia coli* and characterized [123]. Although the D141N substitution had little effect on activity with 1,2-epoxy-3-(*p*-nitrophenoxy)propane, ethylenediiodide, and 4-nitrobenzyl chloride, and appeared to have a normal thermal denaturation profile, it had low activity in an ethylene dibromide mutagenicity assay. In contrast, the E173K substitution caused significant instability and had no or low enzymatic activity.

A *GSTT1* null allele (*GSTT1*O*) is common in most populations and results from a gene deletion [102,103,182]. Since it was shown that GSTT1-1 can activate dihalomethanes to mutagenic intermediates [183], there has been considerable interest

in determining if the presence or absence of GSTT1-1 influences susceptibility to cancer and other disorders [184]. Many of the studies have been the subject of review and meta-analysis [143,146–148,150,185,186]. In addition to the potential role of GSTT1-1 deficiency in cancer susceptibility, mismatches in the *GSTT1* genotype between organ donors and recipients are considered to be significant risk factors in transplant rejection and in autoimmune hepatitis [187,188].

17.6.4.2 GSTT2. Although several GSTT2 missense SNPs have been included in the SNP database, close examination reveals that most can be attributed to sequence differences between GSTT2 and *GSTT2p* (also termed *GSTT2b*), a transcribed pseudogene that has been disabled by splice site changes and inframe stop codons [102]. Only one relatively rare missense SNP causing a M139I substitution has been unambiguously identified [102]. This protein has not been functionally characterized, but molecular modeling suggested that the enzyme's function is unlikely to be modified. A relatively common deletion of *GSTT2b* has recently been identified [106]. This deletion appears to diminish the transcription of *GSTT2* and is in linkage disequilibrium with the polymorphic deletion of *GSTT1*. Several SNPs generating at least four observed haplotypes in the 5′ proximal promoter have been reported [131]. Of these, a T-105 to C-nucleotide substitution appears to result in a significant decrease in expression, but it is not clear if this study was able to differentiate between *GSTT2* and *GSTT2p*.

17.6.5 The Sigma Class

The human Sigma class GST is also known as *hemopoietic prostaglandin D synthase* (HPGDS). No functional polymorphisms have been unequivocally identified in the coding sequence but two rare variants in intron 2 (IVS2 + 11A > C) and intron 3 (IVS3 + 13T > C) have been reported [125]. In a survey of asthmatic families, the intron-2 SNP was significantly transmitted to asthma-affected children [125].

17.6.6 The Zeta Class

17.6.6.1 GSTZ1. In humans, there is a single Zeta class GST gene (*GSTZ1*) and it is located at chromosome 14q24.3 [6,54]. GSTZ1-1 is also known as *maleylacetoacetate isomerase* and catalyzes the penultimate step in the degradation of tyrosine [7]. Although deficiencies of other enzymes in this pathway are known and cause disease of varying severity, no cases of GSTZ1-1 deficiency have been unequivocally identified. Several missense SNPs have been identified that result in L8P, K32E, G42R, and T82M substitutions [124,189]. So far five haplotypes have been identified in human population studies (Table 17.2), and characterization of recombinant proteins has revealed a number of significant functional differences [124,189,190]. Notably, the GSTZ1*A haplotype generates a protein with a G42R substitution that has high specific activity with the (R)-enantiomer of 2-chloropropanoic acid and lower specific activity with the (S)-enantiomer. In contrast, the products of the other haplotypes showed no enantiomer selectivity. In addition, the GSTZ1*A protein has low isomerase activity with maleylacetoacetate and is resistant to inactivation by dichloroacetic acid (DCA) compared to the other GSTZ1-1 variants [124,190,191]. The impact of the arginine substitution at codon-42 on the active site and the functional properties of GSTZ1a-1a have previously been discussed in detail [190]. The resistance of the GSTZ1a-1a variant to inactivation

by DCA may be of clinical significance since DCA has been used to treat lactic acidosis [192] and has recently been proposed as a novel anticancer agent [193–195]. The pharmacokinetics of DCA disposition is complex. Although the elimination of DCA is dependent on the activity of GSTZ1-1, DCA also acts as a mechanism-based inactivator of GSTZ1-1 [191,196]. Consequently, the resistance of the GSTZ1a-1a variant to inactivation is likely to shorten the half-life of DCA in individuals with this allele. A cDNA clone with a L8P substitution has been identified in an EST database and although this allele was not identified in a study of 101 Europeans [124], it may be present in other ethnic groups.

A total of 10 SNPs have been identified in a region extending 1.5 kb upstream of the GSTZ1 start of transcription in African and European subjects [197]. Of these SNPs, only two (-1002G >A and −289 C >T) were associated with significant changes in promoter activity. Further studies are required to determine if SNPs influence *GSTZ1* expression *in vivo*.

17.6.7 The Omega Class

In humans, there are two functional Omega class GST genes (*GSTO1* and *GSTO2*) that are located on chromosome 10q24.3 and are separated by about 1.5 kb [13,108]. A review of the structure and function of the Omega class GSTs has previously been published [107].

17.6.7.1 GSTO1. Several studies have reported missense SNPs or deletions in *GSTO1*, including C32Y, A140D, delE155, E208K, and A236V (Table 17.2) [108,126,198,199].

The C32Y substitution is a rare variant reported in Caucasians which is reported to degrade rapidly and has a short intracellular half-life [126]. As C32 is the primary active site residue [12], this substitution might be expected to eliminate the thioltransferase, methylarsonate reductase, and phenacylglutathione reductase activities of GSTO1-1. However, it might be expected to improve the enzyme's capacity to conjugate GSH to compounds such as CDNB since an experimental C32A substitution has been reported to increase activity with CDNB [107]. This activity may be further enhanced because of the substitution to tyrosine which is the active site residue in the Alpha, Mu, and Pi class GSTs that have very high GSH conjugating activity with CDNB.

The A140D substitution is the most common missense polymorphism at this locus. This substitution does not appear to have a significant effect on activity with a range of substrates [13,200].

The deletion of E155 occurs at a low frequency in most populations that have been studied and results from the deletion of AGG from the 5′ splice donor site of exon 4 and the recreation of a new functional splice donor site (AGG*AG*/*G*T to AG/GT) [108,201]. Recombinant protein produced in *E. coli* has increased specific activity with several substrates [13] but low heat stability [108]. Recent data suggest that protein with the E155 deletion may be unstable *in vivo* and the allele could result in GSTO1-1 deficiency in homozygotes. This conclusion is based on the observations that the T47-D breast cancer cell line is hemizygous for the delE155 allele and is devoid of GSTO1-1 protein and activity [201]. In addition, transformed lymphoblastoid cell lines from delE155 heterozygous subjects show only 50% of normal GSTO1-1 enzymatic activity [201]. Given the low frequency of the delE155 allele in Europeans, it is expected

that homozygotes would be rare. However, the frequency of the deletion is more common in Chinese and one apparent homozygote was noted in a small sample of normal Chinese subjects, suggesting that the expected deficiency of GSTO1-1 is not particularly deleterious [108]. The delE155 allele is in strong linkage disequilibrium with an E208K substitution. In Europeans, the delE155 allele is linked to the K208 allele in contrast to Chinese subjects where the delE155 allele is linked to E208 [13]. Studies on recombinant GSTO1-1 with a single E208K substitution without the E155 deletion indicate that this mutation does not significantly affect the enzyme's stability or catalytic activity [13].

An A236V substitution has been reported in population samples from Chile and Mexico [126,127]. Evaluation of the recombinant protein has shown that the A236V substitution causes a marked decrease in the specific activity with all substrates tested and a significant reduction in heat stability [127]. It is likely that this substitution would lead to a deficiency of GSTO1-1 in homozygotes but none has been identified or studied so far.

Linkage disequilibrium has been reported within the *GSTO1* gene and between *GSTO1* and *GSTO2* [126]. Linkage studies have also shown that *GSTO1* modifies the age at onset of Alzheimer's and Parkinson's diseases [202,203] This association has not always been evident in smaller studies [204,205]. GSTO1 polymorphisms have also been associated with vascular dementia and stroke [206,207]. The mechanisms underlying these associations with neurological disease are not clear, but as GSTO1-1 has been implicated in the activation of the proinflammatory interleukin-1 (IL-1) β [208] and as there are numerous reports of IL-1 variants influencing risk of Alzheimer's and Parkinson's diseases, this may be a profitable area for further investigation [209–212].

17.6.7.2 GSTO2. GSTO2-2 is difficult to express in heterologous systems and has not been extensively characterized. The limited studies that have been undertaken have shown that it has very high dehydroscorbate reductase activity [13]. With 66 polymorphisms being detected so far, the GSTO2 locus appears to be very polymorphic [126]. While most variation is in the noncoding regions, four missense SNPs have been characterized (V41I, C130Y, N142D, and L158I) [13,108,126]. The N142D substitution is the most common and is found in all populations studied so far. This substitution does not appear to influence catalytic activity [13] or stability [126]. Similarly, the V41I mutation appears to be relatively stable, but its catalytic activity has not been assessed [126]. In contrast, the C130Y and L158I substitutions are rare and appear to be relatively unstable [126].

17.7 STRUCTURE AND FUNCTION OF THE CYTOSOLIC GLUTATHIONE TRANSFERASES

17.7.1 Crystal Structures

Crystal structures have been determined for representatives of the known mammalian GSTs and many animal, plant, and microbial GSTs. Protein Database (PDB) files for representatives of all the human GST classes and the related CLIC proteins are provided in Table 17.3. Modeling has shown that the human GDAP1 may be a member of the GST structural family, but a definitive crystal structure has not been reported [57]. All

TABLE 17.3 Representative Crystal Structures of Human Cytosolic GSTs and Related Proteins

GST	PDB File[a]	References
GSTA1-1	1GUH	216
GSTA2-2	2WJU	217
GSTA3-3	2VCV	217
GSTA4-4	1GUL	
GSTM1-1	2F3M	218
GSTM2-2	1HNA	219
GSTP1-1	18GS	220
GSTT1-1	2C3N	221
GSTT2-2	3LJR	222
GSTS1-1[b]	2CVD	223
GSTZ1-1	1FW1	67
GSTO1-1	1EEM	12
CLIC-1	1KOM	20
CLIC-2	2 R4V	21
CLIC-3	3FY7	224
CLIC-4	2D2Z	225

[a]These are representative structures and many additional structures with a variety of ligands are available in the PDB.
[b]GSTS1-1 is also commonly known as *hemopoietic prostaglandin D_2 synthase* (HPGDS).

the catalytically active members of the GST family form dimers. While most GSTs form homodimers, there is some evidence that closely related subunits from within a class can form heterodimers. Heterodimerization is particularly evident between GSTA1 and GSTA2 subunits [77] and has been reported to occur between GSTM1 and GSTM2 subunits [213] *in vivo*, dimerization generally occurs within a class as the differences in structure of the dimer interface seems to limit the formation of interclass dimers. However, there is evidence for the formation of Pi–Mu heterodimers *in vitro* under mild nondenaturing conditions, suggesting that heterodimerization may occur *in vivo* in some circumstances [214]. Within the Alpha, Mu, and Pi classes and to a variable extent in other GSTs, the dimers are stabilized by "key and lock" interactions where a Phe or Tyr residue from one subunit is wedged into a hydrophobic pocket in the other subunit. There is evidence that at least in the Pi class, this stabilization indirectly affects the catalytic competence of the enzyme by stabilizing the loop following α-helix 2 that forms the GSH-binding "G"-site [215]. In this light, it is of interest that the Pi–Mu heterodimers reported by Pettigrew and Colman [214] displayed novel catalytic activity.

In vivo, members of the Pi and Mu classes can also occur in a monomer–homodimer equilibrium where the GST Pi or Mu monomers interact with other proteins such as JNK, ASK1, and Prx IV (perioxyredoxin IV) [14,15,226]. The binding of GST monomers to signaling proteins such as JNK and ASK1 highlight some novel noncatalytic roles for the cytosolic GSTs. The CLIC proteins are members of the cytosolic GST family that occur as monomers [18,20,21]. Although these proteins have no known catalytic activity, they can enter membranes and form ion channels [19,21,227,228].

Other studies have shown that they can modulate the activity of ryanodine receptor Ca^{2+} channels that are essential for muscle excitation–contraction coupling [18,229].

The archetypical "cytosolic" GST fold is composed of two domains (Figs. 17.1 and 17.2) [12,67,174,216,222,230]. The N-terminal domain that includes the GSH-binding site appears to have evolved from a thioredoxin/glutaredoxin ancestor and has a typical thioredoxin topology (βαβαββα). The C-terminal domain is composed of a helical bundle that does not appear to have any clear evolutionary progenitor. In contrast, the mitochondrial GSTK1-1 appears to have arisen via a parallel evolutionary pathway, and although it manifests a thioredoxin-like fold, the $\beta\alpha\beta\alpha_n\beta\beta\alpha$ topology, differs significantly as the helical bundle is inserted between β2 and β3 [41,43]. This topology is shared with the bacterial disulfide bond-forming proteins (DsbA).

17.7.2 The Active Site and Catalysis

The active site can be subdivided into the "G"-site or GSH-binding site and the "H"-site that binds the hydrophobic substrate. The G-site primarily involves residues in the N-terminal domain (Fig. 17.2), but in some GSTs, the G-site can also involve residues from the second subunit [216]. The residues involved in binding GSH are generally well conserved across those GSTs that have enzymatic activity. In GSTs from the Alpha, Mu, and Pi classes, the G-site is relatively open and accessible. This has allowed the purification of these isoenzymes by GSH affinity chromatography [50,51]. In the Theta and Zeta classes, the G-site is more buried and this precluded their purification by affinity chromatography and delayed their discovery and characterization. In contrast to the G-site, the residues constituting the H-site are largely within the C-terminal domain (Fig. 17.2) and are variable across the GST structural family reflecting the diversity of the hydrophobic compounds that are substrates for the different isoenzymes. The primary active site residue responsible for catalysis is a tyrosine in the Alpha, Mu, Pi, and Sigma classes, a serine residue in the Theta and Zeta classes and a cysteine in

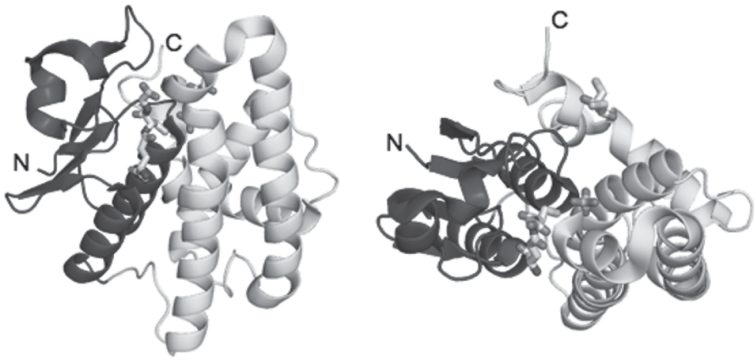

Figure 17.2 Two views of the crystal structure of a human cytosolic glutathione transferase GSTZ1 subunit (PDB code 1fw1) [67]. The N-terminal thioredoxin domain is shown in black, and the C-terminal helical domain is shown in gray. Glutathione, a sulfate ion, and dithiothreitol are represented in CPK stick format. The glutathione is in primary contact with N-terminal domain residues, and its thiol is positioned in the active site at the N-terminal of α-helix 1. The sulfate ion is coordinated by residues in the C-terminal domain and mimics the position of the second substrate. The dithiothreitol molecule forms a disulfide bond with Cys 205. (See color insert.)

the Omega class [12,67,107,216,222,231–233]. Tyr8 of human GSTA1-1 is conserved within the Alpha, Mu, Pi, and Sigma classes and its phenolic hydroxyl group is within hydrogen bonding distance of the GSH thiol. Mutation of Tyr8 to Phe caused a 90% loss of activity but was not absolutely essential for activity [216,231]. Subsequent mutation of Arg15 has confirmed that it also plays an important role in binding and activating GSH in the Alpha class where it is strongly conserved [234]. A conserved Ser residue appears to be important for catalysis in the Theta class GSTs; however, its precise role may vary with different substrates. Mutation of Ser11 in GSTT2-2 abolished its GSH peroxidase activity with cumene hydroperoxide and its GSH conjugating activity with ethacrynic acid but doubled its activity with 1-menaphthyl sulfate [232]. The Zeta class GST GSTZ1-1 is also known as *maleylacetoacetate isomerase* and undertakes the GSH-dependent isomerization of maleylacetoacetate to fumarylacetoacetate, the second last step in the catabolism of tyrosine. In addition, GSTZ1-1 catalyzes the GSH-dependent dehalogenation and oxygenation of α-haloacids such as DCA [190]. There are two adjacent serine residues and a cysteine residue (S_{14}, S_{15}, and C_{16}) in a highly conserved motif in the G-site of all Zeta class GSTs. Mutagenesis of these residues in human GSTZ1-1 found that only Ser14 is specifically required for catalysis despite its less favorable orientation toward the GSH thiol in the crystal structure [67,233]. The Cys16 residue is not essential for activity but does play a role in GSH affinity and binding [235].

The Omega class GSTs catalyze a range of reduction or thioltransferase reactions where cysteine could be expected to play a catalytic role. The determination of the crystal structure of GSTO1-1 shows GSH linked though a disulfide bond to Cys32 in the G-site and mutagenic studies confirmed that the thioltransferase and reductase activities are dependent on Cys32 [12,108,200]. Although GSTO1-1 does not normally catalyze conjugation reactions, mutation of Cys32 to Ala eliminates the thioltransferase and *S*-phenacylglutathione reductase activities but notably generates a novel GSH conjugation activity with CDNB [107].

The substitution reaction catalyzed by many GSTs has been well described [27,30] and is shown in the following equation:

$$\mathrm{GSH} + \mathrm{R{-}X\ (electrophilic\ substrate)} \rightarrow \mathrm{GSR} + \mathrm{H^+} + \mathrm{X^-}$$

The reaction proceeds from the initial lowering of the pK_a of GSH from around pH 9 in solution to around pH 6.5 when it is bound in the G-site. This promotes the deprotonation of the thiol to a highly nucleophilic thiolate anion. While some evidence suggests that the active site tyrosine and serine residues act as hydrogen bond donors that stabilize the GSH thiolate [30,234,236], there is additional evidence that the glutamyl α-carboxyl group of the bound GSH may accept the proton from the thiol group. The use of a decarboxylated analogue of GSH (4-aminobutyric-Cys-Gly) as a substrate for GSTA1-1 supported this proposal as the pK_a of the thiol increased from 6.7 to 9.2 and severely diminished the catalytic activity of the enzyme [237]. In addition, it has also been suggested that water molecules detected in the active site of some GST crystal structures could mediate the proton release [238,239]. A new model for GSH activation in GSTA1-1 has recently been proposed on the basis of QM/MM calculations. In this mechanism, an initial conformational change allows a water molecule to act as a bridge to assist in the transfer of the proton from the GSH thiol to the glutamyl α-carboxylate group [240,241]. Although this mechanism relies

TABLE 17.4 GST Knockout Mouse Strains

GST Gene	Phenotype	References
GstP1 and *GstP2*	Increased skin tumorigenesis	246,247
	Resistant to acetaminophen toxicity	248
	Enhanced lung carcinogenesis	249
	Enhanced colon carcinogenesis	250
	Allergic response in the lung	251
GstA3	Susceptibility to aflatoxin B1	245
GstA4	Susceptible to oxidative stress	252
	Increased susceptibility to CCl_4	253
GstM1	Decreased conjugation of DCNB	254
	Methemoglobinemia	255
GstT1	Model of human GSTT1-1 deficiency	256
GstS1	Delayed hypersensitivity	257
GstO1	Normal phenotype	258
GstZ1	Sensitive to tyrosine/phenylalanine	259,260
	Oxidative stress model	261

on a conformational change that may be specific for the Alpha class GSTs, the original studies with 4-aminobutyric-Cys-Gly showing the absolute requirement of the glutamyl α-carboxylate for catalysis obtained similar results with rat Alpha, Mu, and Pi class GSTs suggesting that the mechanism is not restricted to the Alpha class [242,243].

17.8 GST-DEFICIENT MICE

The physiological role of GSTs and their contribution to the disposition of drugs and carcinogens is increasingly being studied in GST knockout mice and has been recently reviewed [244]. A list of the knockout mice strains and experimental studies is provided in Table 17.4. Although these mice can provide novel insights into drug metabolism, care should be exercised in the interpretation of the data in relation to its relevance in humans as not all human GSTs have exact equivalents in mice [244]. For example, mouse GSTA3-3 has high activity with aflatoxin B1 and protects mice against aflatoxin carcinogenesis [71,245]. In contrast, human GSTA3-3 has low activity with aflatoxin but exhibits a significant steroid hormone isomerase activity that is not present in the mouse enzyme [5]. Inactivation of the *GstA3* gene in mice eliminates their natural resistance to aflatoxin and provides a novel model of human aflatoxin susceptibility [245]. In other cases, such as the Pi and Theta classes, where there are different numbers of isoenzymes in humans and in mice, and because of some redundancy in specificity, elimination of a single isoenzyme may not create an appropriate deficiency model.

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